EFFECTS OF INTERSTITIAL LASER PHOTOCOAGULATION AND PHOTODYNAMIC THERAPY ON LUNG PARENCHYMA.

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by
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ABSTRACT

Interstitial Laser Photocoagulation (ILP) and interstitial Photodynamic Therapy (PDT) are minimally invasive treatments for cancer. They use laser light passed directly into the tumour via thin optical fibres to cause tumour necrosis. Some patients with lung cancer have potentially curable tumours but cannot undergo surgery due to medical contraindications. ILP and PDT could potentially be used as an alternative treatment for these patients. Thin laser fibres could be passed into small parenchymal lung cancers (Stage T1) in the manner of a fine needle aspiration biopsy under CT control. To obtain the best chance of local cure of a lung tumour with ILP and PDT, some normal tissue around the tumour should be treated. Tumours are always at least as sensitive to ILP and PDT as the normal surrounding tissue. For these reasons it is essential to know the effects of the treatment on normal parenchyma before applying them to tumours in a new organ.

The aims of this thesis were therefore to see if it was possible to perform these treatments in normal lung parenchyma, and whether after initial necrosis there was safe healing. Initial experiments were on rats. Reproducible well localised necrotic lesions of up to 12 mm diameter could be created with both ILP and PDT using single laser fibres. Differing patterns of histological necrosis were observed, however there was safe healing with both. 3 different PDT photosensitizers were used, with mTHPC giving the largest lesions. Assessments of lung physiology showed no adverse effect, and the pneumothorax rate was low. A possible self-sealing effect on the lung parenchyma was observed where the fibre was inserted.

Subsequent experiments were performed in pigs using multiple laser fibres inserted simultaneously to cause larger lesions of a size which could incorporate a T1 tumour. Lesions of overlapping necrosis of between 3 and 4 cm diameter were made with both treatments and they were well tolerated. The unique features of PDT necrosis were observed in that the initial necrosis only affected epithelial structures and did not affect lung connective tissue. In contrast ILP caused non selective tissue necrosis. With time there was lesion shrinkage due to local fibrosis, however the lung adjacent to these well localised lesions remained normal.

This study has shown the feasibility of performing these techniques on a small group of patients with lung cancer and pilot clinical studies are planned.
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DEDICATION

To my wife Siobhan,
my father G.D. Fielding,
and in memory of my grandfather H.G. Fielding.
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I would like to acknowledge the untiring support and encouragement of my supervisors Professor SG Bown and Dr Martin Hetzel. Their past experience in performing research on new techniques in both the pre clinical and clinical phase was invaluable. Dr Hetzel initially invited me to do this work and funded it, along with the Laser Centre, for which I am very grateful. It was only through Professor Bown's hard work in setting up and maintaining the National Medical Laser Centre with its many facilities and staff that this project was possible.

Many other people at the Laser Centre helped me during this project. In particular Dr Giovanni Buonaccorssi gave much of his time and his knowledge of ILP and PDT in the setting up and performance of these experiments. Dr Sandy McRobert's expert guidance on fluorescence imaging was also greatly appreciated. Alison Curnow's laboratory organisation was extremely efficient. Amanda Jones and Lorraine Aitchison ably assisted me with computing and administration. Others to thank are Mr Alasdair Gordon, Dr Kathy Fan, Mr Douglas Whitelaw, Dr Stanley Chang, Mr Hamid Mumtaz, Mr Colin Hopper, Dr Anshoo Sahota, Brian McElroy and Dr Paul Ripley.

Many staff at the Biological services units (Windeyer, Rockefeller and Royal Veterinary College Potters Bar) are to be thanked for their hard work and excellent care of the animals, particularly Gillian Hughes for anaesthetic assistance with the large animal work. I had many discussions with Dr Mac Johnston at the RVC in the planning of the large animal work, and he was a great resource during the experimental phase. I also gratefully received advice on the small animals from Dr Peter Koder at UCL. The staff at the histology laboratory at the Imperial Cancer Research Fund provided an excellent service. In particular Mr George Elia did the frozen sections and the immunohistochemistry for the fluorescence studies with great skill. Mr Neil Bilby of the histopathology department at University College London Hospitals processed the large animal specimens. I would like to acknowledge the help of a number of histopathologists. They are Dr Andy Hanby of Imperial Cancer Research Fund, and Drs Merry Griffiths and Gerard Cowley of University College London Hospitals. Dr Lucienne Papadarki performed the electron microscopy.

I acknowledge the support of Scotia Pharmaceuticals for providing the mTHPC.
STATEMENT OF ORIGINALITY

To my knowledge this is the first time that the effects of interstitial laser photocoagulation or interstitial photodynamic therapy on the normal lung parenchyma have been studied. The concept and planning of the experiments was through discussions with my supervisors Professor SG Bown and Dr MR Hetzel, and with Dr Sandy MacRobert for the tissue fluorescence studies. I developed the methods for performing the treatments on the lungs myself, however the methods of assessing the tissue responses and the use of the lasers were adapted from work performed by researchers in other organs. After discussions with Dr Hetzel I planned the physiological assessments on rats which I performed in parallel with the other assessments. All of the treatments were performed by myself, with assistance from Dr G. Buonaccorsi with the use of the copper vapour pumped dye laser. I operated the Diode lasers myself apart from the initial experiments where Dr Buonaccorsi assisted me. I cut the sections for the histology assessments and the slides were made by the histology departments at Imperial Cancer Research Fund and at University College London Hospitals. The histological assessment was made by myself, Dr A Hanby, Dr M Griffiths, and Dr G Cowley. It was my idea to perform electron microscopy on the ILP lungs and this was performed by Dr Lucienne Papadarki.

The results of these experiments have expanded the potential applications of interstitial laser treatments. Clinical pilot studies of their use in curative tumour treatments can now include the lung. Also these studies have provided a new model for comparison of the differing tissue responses of ILP and PDT.
ABBREVIATIONS

ALA  Amino Laevulinic acid
ALS2PC  Disulphonated aluminium phthalocyanine
CCD  Charged coupled device
CHART  Combined hyperfractionated accelerated radiotherapy
cm  centimeters
CT  computed tomography
DBPMAF  Vitamin D3 binding protein derived macrophage activating factor
FEV1  Forced expiratory volume in 1 second
FNA  Fine needle aspiration
FVC  Forced Vital Capacity
G  Guage
GY  Gray
H&E  Haematoxyllin and eosin
He Ne  Helium neon
HpD  Hematoporphyrin derivative
ICC  Intercostal catheter
ILP  Interstitial laser photocoagulation
J  joules
Kg  kilogram
LDL  Low density lipoprotein
M  Molar
MAtm  milli atmospheres
mTHPC  meso -tetra -(meta- hydroxyphenyl )chlorin
MIC  Mitomycin C, Ifosfamide, Cisplatin
MRI  Magnetic resonance imaging
MTT DIAPHORASE  3-(4,5-dimethylthiazoyl-2-2,5-diphenyl tetrazolium bromide diaphorase (stain)
MVP  Mitomycin C, Vinblastine, Cyclophosphamide
mW  Milliwatts
ND-YAG  Neodymium Yttrium Aluminium Garnet
nm  nanometers
PDT  Photodynamic therapy
<table>
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<tr>
<td>PpIX</td>
<td>Protoporphyrin 9</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
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<tr>
<td>TNF Alpha</td>
<td>Tumour necrosis factor alpha</td>
</tr>
<tr>
<td>TNM</td>
<td>Tumour node metastasis</td>
</tr>
<tr>
<td>uM</td>
<td>Micrometer</td>
</tr>
<tr>
<td>VO2 Max</td>
<td>Maximal oxygen consumption</td>
</tr>
<tr>
<td>VC</td>
<td>Vital capacity</td>
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OVERALL OBJECTIVE OF THESIS

The overall objective is to determine whether it is technically possible to perform interstitial laser treatments in the lung parenchyma and to compare the healing response and any adverse effects of these treatments, Interstitial Laser Photocoagulation and interstitial Photodynamic therapy. The aim eventually is to apply this information in performing these techniques with curative intent in a select group of patients with lung tumours.
Section A: Background and introduction

CHAPTER 1 : MANAGEMENT OF LOCALISED NON SMALL CELL LUNG CANCER

CHAPTER 2 : INTERSTITIAL LASER PHOTOCOAGULATION

CHAPTER 3: PHOTODYNAMIC THERAPY

CHAPTER 4 : AIMS OF THE THESIS
Chapter 1 The management of localised non small cell lung cancer

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   1.2.2 Preoperative assessment
   1.2.3 Age and lung resection
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1.3 Radiotherapy

   1.3.1 Radical radiotherapy with curative intent
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1.4 Endobronchial laser therapy

1.5 Summary of management of non small cell lung cancer in relation to this thesis
1.1 Introduction

Lung cancer is the commonest cause of cancer related mortality in the Western world accounting for 20-30% of cancer related deaths (Spiro, 1995). It is estimated that by the year 2000 there will be 2 million cases of lung cancer annually worldwide (Stanley and Sternjernsward, 1989). Of all patients presenting with lung cancer only approximately 7-13% will survive 5 years (Capewell and Sudlow, 1990; Rossing and Rossing, 1982).

80-85% of lung cancers are of the non small cell type (squamous cell, adenocarcinoma and large cell) with small cell carcinoma accounting for the remaining 15-20%. At the time of diagnosis in non small cell carcinoma only 25-35% will have localised disease and potentially be curable (Dillman et al., 1991; Melamed et al., 1987) and after staging investigations this figure falls to 15-25%. This figure is much worse in small cell carcinoma with only 5% presenting with localised disease (Spiro, 1990). Cure requires complete removal of the primary tumour when no distal metastatic spread is detectable on screening investigations. Curative treatment is therefore usually only attempted in non small cell lung cancer and the remainder of the discussion will therefore focus on non small cell lung cancer. Surgical resection is the definitive management of these patients. Radical radiotherapy may be offered as an alternative to the subset of patients with poor lung function or other contraindication to surgery, although the potential for the development of radiation fibrosis is a significant disadvantage to this treatment approach. Patients who have disseminated disease are amenable only to palliative treatments. These include radiotherapy, chemotherapy, and endobronchial treatments such as laser bronchoscopy and endobronchial brachytherapy.

This thesis investigates the potential of 2 forms of laser treatment as curative treatments for localised lung cancer, namely Interstitial Laser Photocoagulation (ILP) and Photodynamic Therapy (PDT). These treatments are currently applied in tumours in a variety of different organs. The details of how these treatments are performed are discussed in Chapters 2 and 3. Thin laser fibres are placed directly into the tumour under radiological guidance to cause tumour necrosis. They are minimally invasive and could be used as an alternative to surgery in patients who have curable tumours but who are inoperable due to poor medical condition most commonly due to severe chronic bronchitis and emphysema. Therefore the discussion on the management of lung cancer deals primarily with the existing curative
treatments surgery and radiotherapy. The factors determining operability are also covered in detail since candidates for these new treatments will be those who are unsuitable for surgery. In some centres a small number of patients with very small localised endobronchial primary tumours are treated with endobronchial photodynamic therapy. This will be discussed in the introduction to Photodynamic therapy. Palliative techniques will not be covered apart from a brief discussion on laser bronchoscopy.

1.2 Surgery

1.2.1 The staging of lung cancer

Before a patient is accepted for surgery the tumour must be staged to ensure that it is localised. Lung cancer staging uses the tumour node metastasis (TNM) classification. Mountain developed a staging system in 1972 based on this TNM classification which was subsequently modified in 1986 (Mountain, 1986). Table 1.1 details the descriptors used in this classification system. The system is used primarily to determine the resectability of a particular tumour. It also gives prognostic information. For example, smaller tumours are afforded a lower stage in the 'T' part of the classification as in general these are much more likely to be operable. Also a lower 'T' stage is afforded to tumours which are more than 2 cm from the carina at bronchoscopic examination. Tumours within 2 cm of the carina are usually inoperable and are given the classification 'T3'. However even small tumours which invade the mediastinum or involve the heart or great vessels are inoperable and have the stage 'T4'.

Lymph node metastasis for staging purposes is usually made on the basis of imaging investigations. Patients with no lymph nodes at CT usually proceed to surgery. Usually lymph nodes over 1 cm in diameter are regarded as abnormal, however CT findings should be corroborated by some form of biopsy before a patient is denied potentially curative surgery (Whittlesey, 1988), as large lymph nodes on CT scan of the chest do not always contain metastatic disease. Some centres prefer to stage mediastinal lymph nodes by mediastinoscopy or on biopsy at the time of surgery (Shields, 1993; Fernando and Goldstraw, 1990; Hashim et al., 1982). Despite careful preoperative staging, lymph node dissection at the time of surgery shows that up to 25% of patients have N2 disease with 16% visible macroscopically and 9% on histology of apparently normal lymph nodes (Fernando and Goldstraw, 1990; Seeley et al., 1993). Lymph node enlargement at the hilum
Table 1.1. Descriptors for the TNM classification of lung cancer

<table>
<thead>
<tr>
<th>T Stage</th>
<th>Definition</th>
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<tbody>
<tr>
<td>T0</td>
<td>No evidence of tumour</td>
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<tr>
<td>TX</td>
<td>Tumour proven by the presence of malignant cells in bronchopulmonary secretions but not visualised radiographically or bronchoscopically, or any tumour that cannot be assessed</td>
</tr>
<tr>
<td>TIS</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>T1</td>
<td>A tumour that is 3 cm or less in greatest diameter surrounded by lung or visceral pleura and without evidence of invasion proximal to a lobar bronchus at bronchoscopy</td>
</tr>
<tr>
<td>T2</td>
<td>A tumour more than 3 cm in greatest diameter or a tumour of any size that either invades the visceral pleura or has associated atelectasis or obstructive pneumonitis extending to the hilar region. At bronchoscopy, the proximal extent of demonstrable tumour must be within a lobar bronchus or at least 2 cm distal to the carina. Any associated atelectasis or obstructive pneumonitis must involve less than an entire lung</td>
</tr>
<tr>
<td>T3</td>
<td>A tumour of any size with direct extension into the chest wall (including superior sulcus tumours), mediastinal pleura or pericardium without involving the heart, great vessels, trachea, oesophagus or vertebral body, or a tumour in the main bronchus within 2 cm of the carina without involving the carina</td>
</tr>
<tr>
<td>T4</td>
<td>A tumour of any size with invasion of the mediastinum or involving the heart, great vessels, trachea, oesophagus, vertebral body or carina or presence of malignant pleural effusion</td>
</tr>
<tr>
<td>N Stage</td>
<td>Definition</td>
</tr>
<tr>
<td>N0</td>
<td>No demonstrable metastasis to regional lymph nodes</td>
</tr>
<tr>
<td>N1</td>
<td>Metastasis to lymph nodes in the peribronchial or the ipsilateral hilar region, or both, including direct extension</td>
</tr>
<tr>
<td>N2</td>
<td>Metastasis to ipsilateral lymph nodes and subcarinal lymph nodes</td>
</tr>
<tr>
<td>N3</td>
<td>Metastasis to contralateral mediastinal lymph nodes, contralateral hilar lymph nodes, ipsilateral or contralateral scalene or supraclavicular lymph nodes</td>
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<tr>
<td>M Stage</td>
<td>Definition</td>
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<tr>
<td>M0</td>
<td>No known distant metastasis</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastasis such as scalene, cervical, or contralateral hilar lymph nodes, brain, bones, liver or contralateral lung</td>
</tr>
</tbody>
</table>

Table 1.2. Stage groupings for lung cancer based on the TNM system.

<table>
<thead>
<tr>
<th>Stage</th>
<th>T</th>
<th>N</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occult carcinoma</td>
<td>TX</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage 0</td>
<td>Tis</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage 1</td>
<td>T1</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>N0</td>
<td>M0</td>
</tr>
<tr>
<td>Stage 2</td>
<td>T1</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>N1</td>
<td>M0</td>
</tr>
<tr>
<td>Stage 3A</td>
<td>T1</td>
<td>N2</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>N2</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>N0,N1,N2</td>
<td>M0</td>
</tr>
<tr>
<td>Stage 3B</td>
<td>Any T</td>
<td>N3</td>
<td>M0</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>Any N</td>
<td>M0</td>
</tr>
<tr>
<td>Stage 4</td>
<td>Any N</td>
<td>Any N</td>
<td>M1</td>
</tr>
</tbody>
</table>
ipsilateral to the tumour is afforded a stage of N1. These lymph nodes are contained within the visceral pleural envelope. Mediastinal lymph nodes (N2), and contralateral lymph nodes (N3) are outside the visceral pleural envelope and usually make a tumour inoperable, although as discussed below some N2 tumours are operable.

The M refers to metastases which may occur in the contralateral lung, the liver, bone, adrenal glands, brain and other sites. CT scanning of the liver and adrenal glands is usually done at the time of the chest CT (Shields, 1993). Bone scans can be done where patients have symptomatic bony disease. The best screen for metastatic diseases however is thorough history and physical examination along with routine biochemistry including calcium and alkaline phosphatase with abnormalities followed up by the appropriate scan (Shields, 1993).

Using these TNM descriptors a staging system was developed to simplify and group together different TNM classifications based on the likelihood of surgery being possible. These different stages are shown in Table 1.2. Stage 1 and 2 patients are considered to be operable whereas Stage 3b and 4 are generally regarded as inoperable. The gap in survival between those who are potentially curable at the time of diagnosis and those who have already developed distant metastases is considerable. It is therefore the goal of every physician treating patients with lung cancer to consider the possibility of curative resection in all cases unless there is obvious metastatic disease.

The mean 5 year survival rate taken from 10 studies of patients having resection for stage 1 disease is 65 % (Ginsberg, 1997). There are significant survival advantages for patients presenting in stage 1 and 2 compared to stage 3 and 4 which have 5 year survivals of 3-20%. Survival in stage 3b is significantly worse than stage 3a in terms of long term survival. A recently reported series by Mountain commenced accrual in 1985, when preoperative staging with CT scanning had become standard practice (Table 1.3). Almost 2000 cases with T1 and T2 N0 tumours showed 5 year survival of 61 and 38 % respectively. It was therefore proposed to subdivide stage 1 into stage 1A (T1) and 1B (T2) due to the difference in survival of T1 and T2 tumours (Mountain, CF. Presented at conference of American Joint Committee on Cancer, Phoenix, 1996). Among stage 2 disease there were differences in survivals between T1N1 (34%) and T2N1 (24%) but these were not significant.
There is considerable debate regarding the operability of patients with stage 3A non small cell lung cancer. This is due to the heterogeneity of tumour and nodal disease in this subgroup. For example patients with large mediastinal lymph nodes with a small primary tumour (T1N2) often have a poorer prognosis than patients with local chest wall invasion by the primary tumour but no lymph node metastasis (T3N0). This latter group of patients are conventionally treated with pre-operative radiotherapy and subsequent surgical resection with series reporting survival rates between 25 and 50% at 5 years (Mountain, 1996; Hilaris et al., 1971). Patients with bulky mediastinal lymph nodes (visible on plain chest radiograph) or multilevel nodes have 5 year survival rates of only 7-9% (Naruke et al., 1988; Albain, 1993).

In contrast subtle N2 disease found at the time of surgery has a prognosis of up to 40% at 5 years (Pearson, 1985). Therefore it appears it is not simply the presence of N2 disease that affects surgical resection but the size of these nodes.
Preoperative chemotherapy has been shown in both randomised and non-randomised studies to improve survival in patients with small volume N2 disease. It reduces the size of the primary tumour, however complete responses are infrequent (3-9%, Rosell et al., 1994; Roth et al., 1994). These two randomised studies performed in patients with stage 3A disease showed that preoperative chemotherapy could downstage tumours but this did not improve resection rates. On the other hand downstaging of lymph node metastases was associated with a more favourable prognosis after surgery. Both studies were stopped early due to differences in 3 year survival between the treatment groups and control groups (56% versus 15% in the Roth study). Therefore each arm of these studies had only 30 patients. The study by Roth was criticised as the control group (surgery only) had a poor survival, however most of the patients in this group had bulky N2 disease and the survival rate of 15% for this group was therefore in agreement with previous studies of survival of surgery alone for bulky N2 disease. The study by Rosell was criticised as the control group had more tumours with mutations of the K-ras oncogene on post surgical biopsy specimens, an independent predictor of worse prognosis. However in this particular study the presence of K-ras mutations did not cause differences in observed survival (Rosell et al., 1995). None the less the startlingly good results with both these studies and the small numbers of patients involved mean that further studies should be performed to resolve the issue of the benefit of preoperative chemotherapy.

It seems likely however that any benefit will come from the effect of chemotherapy on distal occult metastases as neither study affected the primary tumour greatly enough to improve surgical resection. If this is the case then further studies should also include stage 1 and 2 tumours since many of these patients are likely to have occult metastatic disease (Geddes, 1979; Pantel et al., 1996). The relapse rate for patients having surgery for stage 1 disease is 35-50% over 5 years with the majority having distal metastases. Micrometastatic disease occurs in lymph nodes in patients with lung cancer including those with early stage disease and can be detected by serial sectioning through lymph node specimens, or immunohistochemical staining for cytokeratins (Passlick et al., 1995).

Once a patient has been designated stage 1 or stage 2 every effort is then made to ensure curative resection is performed as this remains the treatment of choice. This entails detailed pre-operative assessment.
1.2.2 Pre-operative assessment

Many factors determine whether or not a surgeon will operate on a particular patient with early stage lung cancer. These include lung function, general performance status, exercise capacity, age, and other underlying medical conditions, particularly cardiac conditions (Capewell and Sudlow, 1990). Patients with lung cancer, primarily a cigarette related tumour, often have coexistent underlying chronic obstructive airways disease which predisposes to intraoperative gas exchange problems and post operative respiratory failure and lung infections (Legge and Palmer, 1973; Walsh et al., 1994). A major cause of morbidity and mortality following pulmonary resection may be inadequate sputum clearance and spirometry reflects the patients ability to generate high flows within the bronchial tree which are required for expectoration (Goldstraw, 1996).

Preoperative physiological assessment is used firstly to predict the likelihood of perioperative complications and mortality. Secondly patients with compromised pre-operative lung function will inevitably be left with lower lung function after the resections and pre-operative assessment attempts to predict the likelihood of long term respiratory difficulty. This is usually done by simple lung function testing. In borderline cases more complex testing can be used including exercise testing and radioisotopic techniques.

Algorithms such as the one shown in Figure 1.1 give a stepwise approach to physiological assessment (Conrad et al., 1995). Only severely affected patients need undergo the more complex investigations. However there are no universally accepted guidelines for preoperative evaluation (Conrad et al., 1995). Hence there is variability between surgeons in the degree of fitness they regard as acceptable for a patient to undergo lung resection surgery.
Prediction of lung function after lung resection comes from a number of studies which have specifically addressed this issue. Legge and Palmer reported a series of 58 patients with bronchial carcinoma undergoing either pneumonectomy or lobectomy (Legge and Palmer, 1975). FEV1, FVC and TLC fell by 27%, 32% and 28% of predicted respectively. No absolute lung volumes were given in this paper but the mean preoperative FEV1, FVC and TLC were 78, 90 and 100% of predicted. Berend et al reported lung function on a series of patients undergoing lobectomy (Berend et al., 1980). Mean spirometry before and after was 2.5/4.1 and 2.2/3.7 respectively. Total lung capacity fell from 7.1 (+/- 1.1) to 6.2 (+/- 0.9). The operation was tolerated well by all patients and dyspnoea was made worse in only 2 of the 16 patients when assessed at a single time point between 6 weeks and 6 months after the operation. No significant changes in arterial partial pressure of oxygen or carbon monoxide gas transfer were observed.
In 1955 Gainsler reported a correlation between preoperative lung function and post-operative mortality in patients undergoing lung resection for tuberculosis (Gaensler et al., 1955). The important finding was that a maximum voluntary ventilation of less than 50% predicted and FVC of less than 70% predicted had a 50% mortality rate. Current mortality rates are far lower than this (3-5% for pneumonectomy) but postoperative complications still occur in over 25% of patients, mostly pulmonary in nature. These are usually relatively minor and include atelectasis and transient cardiac arrhythmias. The most common cause of death after lung resection is pneumonia and respiratory failure followed by broncho-pleural fistula with empyema, myocardial infarction, and pulmonary embolus (Ginsberg, 1997).

Olsen and Block originally reported criteria for elective pneumonectomy in 1973 (Olsen and Block, 1973). These were an FEV1 of greater than 2 litres, maximum voluntary ventilation of >50% predicted, a ratio of residual volume to TLC of less than 50% predicted and a calculated residual postoperative FEV1 greater than 800 ml. These are traditionally accepted values and are commonly used, however, the absolute value of FEV1 does not take account of differences in height, age, or sex. Therefore an FEV1 of 50% or more of predicted is now the commonly used criterion for pneumonectomy. For lesser operations patients with an FEV1 of less than 35% of the normal predicted value are at high risk of complications (Lockwood, 1973).

More detailed assessment is used if a patient has borderline lung function, including lung perfusion scanning and exercise testing. The lung containing the tumour to be removed may be making little contribution to overall lung function. This can be demonstrated by isotopic lung scanning. Formulae have been developed to combine pre-operative FEV1 with the percentage of regional functioning of the tumour bearing lung and the number of segments to be removed to give an overall assessment of the predicted post-operative FEV1 (Ali et al., 1980). A study by Markos and colleagues assessed the value of pre-operative pulmonary scintigraphy in defining post-operative lung function in 55 consecutive patients (Markos et al., 1989). There were 18 pneumonectomies, 29 lobectomies and thoracotomy without resection in 6. There were cardio-pulmonary complications in 16 patients within 30 days of surgery including 3 deaths, equating to an overall early post-operative mortality of 5.5%. A predicted post-operative FEV1 of less than 40%
predicted was associated with a 50% mortality whereas a value of greater than 40% was associated with no post-operative mortality.

Pre-operative exercise testing assesses both pulmonary and cardiac sufficiency simultaneously, both of these parameters being important in post-operative complications. In 1989 Olsen reported the role of cycle ergometer exercise testing prior to lung resection (Olsen et al., 1989). It was effective in uncovering problems with oxygen transport that determined post-operative outcome and survival. Bechard and Wetstein reported exercise tests in a group of 50 patients who underwent pulmonary resection (Bechard and Wetstein, 1987). In those with a VO2 max of less than 10mls/kg /minute there was a 29% mortality rate and a 43% morbidity rate; in those with a VO2 max between 10 and 20 mls/kg/minute there was a 10% morbidity and no deaths; and in those with a VO2 max greater than 20mls/kg/minute there were no complications.

1.2.3 Age and lung resection

Patients older than 65 years of age account for approximately 62% of lung cancers in males and 57% of lung cancers in females (Yancik and Ries, 1991). In one region in the UK 43 % of patients with lung cancer were aged 75 and over at presentation (Brown et al., 1996). Squamous cell lung cancer increases with age and accounts for 40% of all lung cancers by the age of 80 years. In the Western world the life expectancy of people over the age of 70 is on average 15 years for women and 8-10 years for men (Festen, 1991). Five year survival of patients with lung cancer over the age of 70 years who do not undergo surgery is 7% (Thompson Evans, 1973). Age is not a recognised independent prognostic factor in lung cancer because of the dominance of clinical stage and performance status in this regard. Although long term survival following surgery in elderly patients is similar to younger patients there is a higher peri-operative mortality rate in older patients (Shermon and Guidot, 1987; Yancik and Ries, 1991). The lung cancer study group reported in 1983 that the 30 day mortality for patients over 70 undergoing pneumonectomy was 5.9% and after lobectomy 7.3 % (Ginsberg et al., 1983). This was more than twice that for younger patients. In terms of the likely outcome of not having surgery however, this may be regarded as an acceptable risk by some patients. Lesser resections may be more applicable in the elderly as segmental or wedge resections in one series had a mortality rate of 1.5% and a 5 year survival of 42% (Breyer et al., 1981).
There is a tendency for low operation rates on elderly patients for surgery, even when there are good indications for resection (O'Rourke et al., 1987). Active treatment for lung cancer begins with a tissue diagnosis being made; however in the series by Brown only 66% of those over 75 years had histology obtained compared to 92% in those less than 65 years (Brown et al., 1996).

Therefore the problem of a rising incidence of elderly patients with lung cancer is not being faced by doctors. It is likely that patients who have curable tumours are not being referred even for consideration of surgery (Davison et al., 1997). This may be due to limited resources or to an unwillingness to undertake the higher perioperative risks despite the potential for long term benefit.

1.2.4 Types of lung resection

The surgeon aims to resect all visible tumour at the time of operation. Metastatic disease is more commonly the cause of death in lung cancer than the primary itself. However curing the primary tumour is never the less a prerequisite for cure in patients with lung cancer and to date surgery has remained the gold standard for obtaining local cure. Pneumonectomy is required if the tumour or lymph nodes involve central structures, for example hilar blood vessels or major bronchial structures. Less extensive resection is possible if the lung tumour is situated more peripherally. Lobectomy is the commonest operation performed for lung cancer. It allows removal of the primary tumour and lymph node bearing areas. Survival is equivalent for patients undergoing lobectomy or pneumonectomy when a complete resection is performed (Ginsberg, 1997). A sleeve resection is used when a tumour is confined to a lobar bronchus and re-anastomosis of the bronchus can be performed after the resection (Faber, 1993). It is most commonly used for tumours of the upper lobe. The size of the tumour does not necessarily correlate with the ability to perform smaller degrees of resection. For example tumours in the apical segment of the lower lobe may abut the fissures of the upper lobe and necessitate resection of this lobe as well. If such small tumours also involve the vascular supply to the upper lobe then a pneumonectomy will be required.

Numerous lung sparing procedures are performed in patients who have small peripheral tumours particularly in those with borderline lung function (Jensik et al., 1985; Errett et al., 1985; Perelman, 1986). Lung sparing
procedures include segmentectomy, wedge resection and lumpectomy. A segmentectomy indicates that a distinct anatomic resection is performed including the segmental pulmonary artery, veins and bronchus (Jensik et al., 1985). Segmentectomy was first reported in 1973 by Jensik who performed 73 such resections with similar survival rates to that seen with lobectomy with mortality rate less than 2% (Jensik et al., 1973). A wedge excision is performed without identifying these structures and peripheral lung parenchyma including the tumour is stapled and removed (Errett et al., 1985). It can only be performed on peripheral tumours immediately under the pleura. No attempt is made to encompass all lymphatic drainage areas. Deeper lesions may be removed by precise excision with the use of a cutting Nd-YAG laser or electrocautery. This is known as a lumpectomy (Perelman, 1986). The laser is used because it simultaneously seals resected parenchyma as it cuts. It is not known how much normal lung tissue should be removed although 2cm of uninvolved lung tissue surrounding the tumour is usually recommended.

In 1983 the peri-operative mortality of 2200 resections for lung cancer was analysed (Ginsberg et al., 1983). Of these 1058 were lobectomies, 569 were pneumonectomies, and 143 were lung sparing resections (segmental or wedge resections). There were 81 post-operative deaths during the first 30 days and the rate for pneumonectomy was 6.2% and for lobectomy was 2.9%. Lesser resections had a 1.4% mortality rate which was not statistically different from lobectomy.

Other series demonstrate variations in mortality possibly related to the clinical load of the particular centre. For example in 1993 Miller reported his personal series from 20 years experience (Miller, 1993). 153 patients underwent pneumonectomy with a 5% mortality rate at 30 days post-operatively. 785 patients underwent lobectomy with a mortality rate of 0.4%. 885 patients underwent either wedge or segmental resection and there was only one post-operative death in this group. The surgeon comments however that this was a high volume surgical service, with the majority of the patients screened by chest physicians outside the surgical service implying that there were enough resources to cope with a large number of operative cases. This is in contrast to the series of Markos mentioned above which had a post operative mortality rate of 16.7% for pneumonectomy.

Within the UK there are marked variations in practice between different centres (Muers, 1996). In one study the yearly rate of thoracotomy for lung
cancer in 2 separate districts in 90 and 42 patients over the same 12 month period were 2.6% and 11.2% respectively (Davies, 1994).

A randomized study was recently completed comparing lobectomy to segmentectomy or wedge resection in stage T1N0 patients (Ginsberg and Rubenstein, 1995). The loco-regional recurrence rate for lobectomy was 5%. However the loco-regional recurrence rate in lesser resections was 17.5%. The peri-operative mortality and lung function changes showed no difference between the two groups. Therefore at this time most surgeons believe that lung sparing operations should not be performed if there is adequate pulmonary function for lobectomy. However in compromise situations where no more than minimal functioning tissue can be excised, particularly in very small peripheral tumours, limited resection can be used effectively accepting the fact that there may be an increased chance of loco-regional recurrence (Ginsberg, 1997).

The likelihood of regional lymph node involvement depends on the size of the primary tumour. In a pathological study by Ishida tumours between 1 and 2cm in diameter had lymphatic spread in only 17% of cases (Ishida et al., 1991). Tumours between 2.1 and 3cm in diameter had metastases 38% of the time. Tumours less than 1cm had zero lymph node metastases. Therefore with larger tumours treated by less than a lobectomy there is a greater chance of loco-regional recurrence due to inadequate local control of lymph nodes and vessels at the initial surgery. This form of surgery may therefore eventually only be used with the smallest tumours.

1.2.5 Video-assisted Thoracoscopic lung resection

In the past 2-3 years there have been an increasing number of reports of lung cancer surgery using minimally invasive surgery with video assisted thoracoscopy (Mentzer et al., 1995). Here the surgeon uses 2 or 3 ports for the insertion of the video camera and instruments into the pleural space rather than making a single large thoracotomy incision. Lobectomies, segmentectomies and wedge excisions have been performed with this method. The advantages are reduced post operative pain and chest infections and reduced post operative stay in intensive care units. This implies that surgeons are willing to perform lung resections on patients with worse lung function than would be the case with open thoracotomy. The disadvantage is the loss of tactile assessment of lung tissue which can
influence the extent of resection in some cases and more importantly there are limitations on assessment of the mediastinum at the time of surgery.

The other form of resectional surgery using this method is lung reduction pneumoplasty (also known as lung volume reduction surgery) where the aim is to reduce lung volume in patients with chronic obstructive airways disease to improve dyspnoea (Cooper and Lefrak, 1996; Wakabayashi, 1995). Bullae and other non functioning lung tissue is resected using a combination of standard excision techniques as well as high power YAG laser. In the course of these types of excision some tumours have been detected in the resected lung (Wakabayashi, 1994). By definition these patients have very poor lung function, with FEV1 sometimes as low as 400 ml. Therefore patients with even the most severe lung function abnormalities may be suitable for combined lung volume reduction and lung tumour excision.

Whilst this form of surgery has been demonstrated to be technically feasible there is as yet no long term follow-up of patients who have undergone lung tumour resection by video assisted thoracoscopic surgery. Furthermore although this is described as minimally invasive surgery patients still require intensive preoperative physiotherapy and postoperative care in an intensive care unit including positive pressure ventilation. Also it will be some time before the necessary equipment and specialized training become widely available in thoracic surgical units. Even if this form of surgery does become widely used there are still likely to be some patients (and their referring doctors) who would prefer a truly minimally invasive procedure to obtain local control of their lung tumour, which is the future aim of clinical studies following the animal work reported in this thesis.

1.3 Radiotherapy

1.3.1 Radical Radiotherapy with curative intent

Patients who are considered unsuitable for surgery but who otherwise have potentially curable tumours are either referred for radical radiotherapy or receive expectant management (Haffty et al., 1988).

The basic radiobiological principle is that a constant fraction of cells is destroyed by a given dose of radiation which implies that there is a dose response relationship. To sterilize tumours larger than 6cm in diameter a dose greater than 80 gray is required (Vijayakumar et al., 1991). For
tumours greater than 2 cm in diameter the dose required for control is greater than 70 gray, however doses in this range may be difficult to deliver as they may cause damage to surrounding normal tissues including lung, heart, and spinal cord. In head and neck tumours or breast carcinomas doses of 70 Gy are required to have any chance of controlling tumours 3 cm in size. Therefore with the added risk of damage to the surrounding normal lung tissue which is more sensitive to radiation damage than tumour, it is unlikely that tumours larger than 3 cm can be eradicated (Dosoretz et al., 1992). Therefore the difficulty is in prescribing these higher doses while minimising damage to normal tissue.

There is no general agreement on the volume of lung which should be treated to obtain a cure with radical radiotherapy (Seagren, 1990). Treatment fields are irregularly shaped to give an increased dose to the tumour and spare the normal tissues. There should be a 2 cm margin around the tumour and a 1 cm margin around any electively treated lymph nodes. For tumours in an upper lobe, the treatment field should include both supraclavicular fossae and the lower margin should be about 5 cm below the carina. For tumours in a middle or a lower lobe without positive mediastinal lymph nodes the supraclavicular fossae do not require treatment. If there is mediastinal extension of the tumour based on CT or mediastinoscopy findings, these regions should be treated (Van Houtte and Mornex, 1995).

Radical radiotherapy has been performed for lung cancer with the intent of cure since the 1950's (Seagren, 1990). Treatment has often been to locally advanced disease in inoperable cases due to mediastinal adenopathy. Relatively fewer patients have been treated for small peripheral primary tumours as an alternative to surgery. Results are difficult to interpret in view of the different radiotherapy regimes used and the small number of patients in most series. Table 1.4 is a summary of recent studies adapted from (Van Houtte and Mornex, 1995). These are all retrospective series.

The reported survivals are lower than for surgery of stage 1 disease. Kascowitz showed that the rate of local control with radiation (50%) is significantly less than that for surgery (15%) (Kaskowitz et al., 1993).
<table>
<thead>
<tr>
<th>First Author and year of publication</th>
<th>Number of Patients</th>
<th>T stage</th>
<th>Median survival</th>
<th>5year overall survival</th>
<th>5year disease-free survival</th>
</tr>
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<tr>
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<td>44</td>
<td>T1-T2</td>
<td>not stated</td>
<td>32</td>
<td>not stated</td>
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<td>20 months</td>
<td>15</td>
<td>17%</td>
</tr>
<tr>
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<td>77</td>
<td>T1-T3</td>
<td>18 months</td>
<td>17</td>
<td>not stated</td>
</tr>
<tr>
<td>Haffty 1988</td>
<td>43</td>
<td>T1-T2</td>
<td>28 months</td>
<td>21</td>
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</tr>
<tr>
<td>Noordijk 1988</td>
<td>50</td>
<td>T1-T2</td>
<td>27 months</td>
<td>16</td>
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<tr>
<td>Kaskowitz 1993</td>
<td>53</td>
<td>T1-T2</td>
<td>21 months</td>
<td>6</td>
<td>0 %</td>
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<tr>
<td>Gauden 1995</td>
<td>347</td>
<td>T1-T2</td>
<td>28</td>
<td>27</td>
<td>23%</td>
</tr>
<tr>
<td>Dosoretz 1992</td>
<td>152</td>
<td>T1-T3</td>
<td>17 months</td>
<td>10</td>
<td>15%</td>
</tr>
</tbody>
</table>

Table 1.4 Summary of results of studies of radical radiotherapy with curative intent in patients who are unsuitable for surgery due to medical contraindications.

Results vary in terms of the size of the primary tumour. 3 year disease-specific survival was compared in one series for tumours less than 3cm (30 %) to those 3-6 cm (17 %) (Sandler et al., 1990). All 9 patients with tumours greater than 6 cm were dead after 3 years. It is noteworthy that only 44 % of the 75 patients in this study did not have local progression of their disease so the reported 3 year disease specific survivals cannot be regarded as cures. This is also true in the study by Dosoretz where of 18 patients with T2 tumours treated with more than 65 Gy, 68 % of patients had local failures (Dosoretz et al., 1992) Of 15 patients with T1 tumours treated with the same dose 16 % had local failures. 66 % failed the treatment overall. Of these 70% showed a component of local failure and 45% failed distally. Reports of local failure in these retrospective studies probably underestimate the problem as local relapse of tumour on chest Xray is difficult to differentiate from local fibrosis. Also where distal disease has recurred it is natural for the clinician to pay more attention to this than recurrence at the primary site. It could be argued that this was due to the patients being in worse condition as they had been rejected for surgery. However this problem of local failures after radiotherapy suggests it is the treatment which is ineffective. The
largest study with the best results in terms of recurrence free survival was that by Gauden (Gauden et al., 1995).

Innovations in radiotherapy for lung cancer include the use of improved fractionation schedules and the use of 3 dimensional planning to improve the ability to deliver higher doses to small volumes of tumour (Gregor, 1995). Combined hyperfractionated accelerated radiotherapy (CHART) aims to increase tumour cell kill by interrupting short tumour doubling times as well as reducing the effect on normal tissue (Withers et al., 1988). A pilot study in 76 patients with locally advanced disease using 54 Gy in 36 fractions over 12 days gave improved results at 2 years compared to historical controls (Saunders and Dish, 1990). Preliminary results of a subsequent randomised trial of CHART in lung cancer and head and neck cancer have shown some improvement (30% 2 year survival) compared to conventional radiotherapy (20% 2 year survival) (Saunders et al., 1996). Initial complete lung tumour regression was 34% for CHART and 29% for conventionally treated patients; the trial includes locally advanced disease as well as T1 tumours, however these figures demonstrate the difficulty of achieving local control with radiotherapy. Another important figure from this paper is that of 379 deaths in the lung cancer group only 13% were due to coincidental disease, demonstrating the importance of obtaining cure of lung cancer even in patients who have for example chronic obstructive airways disease, as many of the patients in that study most likely would have. In relation to side effects, dysphagia of mild to moderate degree was observed for up to 6 weeks in 49% of patients after CHART with 30% of all patients requiring analgesia for this at 1 month.

The use of CT scanning is the current standard in localisation of tumours in planning of radiotherapy (Gregor, 1995). Despite this there are still problems with missing the tumour in up to one third of cases. This is because of inexperience in interpreting the often subtle changes seen on CT scans which may require the presence of a diagnostic radiologist in the planning room (Gregor, 1995; Leibel et al., 1991). Further developments have come with 3 dimensional graphics to facilitate conformal planning (Leibel et al., 1991; Armstrong et al., 1995). These techniques which may allow larger tumour doses of radiotherapy are still in the development phase. Problems include the complexity and range of possible treatment geometries and the difficulty of matching these sophisticated plans to the day to day treatment set up (Gregor, 1995; Hazuka et al., 1993; Leibel et al., 1991).
1.3.2 Radiation Pneumonitis

A spectrum of changes can occur following thoracic irradiation. Acute pneumonitis usually occurs from 1-3 months after treatment. With stage 1 involvement there are radiographic changes usually limited only to the radiation port. This is the most common manifestation. In stage 2 there is dyspnoea which gradually improves over several weeks and may require the use of systemic corticosteroids. In stage 3 and 4 there is greater damage requiring, oxygen (stage 3) or assisted ventilation (Stage 4) (Martel et al., 1994). Pulmonary fibrosis in varying degrees develops 6 months after treatment (Van Houtte and Mornex, 1995). It is always seen in the treatment field after curative treatments. Radiation tolerance data of normal lung is scarce (Emami et al., 1991). Only a small number of papers have reported radiation effects on normal lungs after contemporary planning techniques using CT scanning (Armstrong et al., 1995; Martel et al., 1994). Martel reported 42 patients treated with curative intent of whom 9 developed radiation pneumonitis with 3, 4, 1 and 1 patients respectively in each of the stages 1-4. The patient who developed stage 4 toxicity had severe pre-existing obstructive airways disease as is likely to be the case in many patients treated with radiotherapy aimed at cure. Furthermore, although radiation pneumonitis usually occurs in the irradiated lung there have been reports of pneumonitis outside the radiation fields (Mah et al., 1986). Other complications of radiotherapy include transient oesophagitis, pericarditis and myelitis (Van Houtte and Mornex, 1995).

It takes up to 5 weeks of daily treatments to complete standard radiotherapy with curative intent (2 weeks with CHART) along with the planning sessions and therefore this form of treatment for lung cancer constitutes a considerable workload on a radiotherapy department.

Hayman et al have reported results of attempts to locally boost radiation dose to primary lung cancers using brachytherapy delivered percutaneously following external beam radiotherapy (Hayman et al., 1995). This was an attempt to reduce local failure following radiotherapy with curative intent. They treated 9 patients who had residual disease following 60-70 Gy delivered externally. The method of insertion was similar to that proposed in this thesis, using CT guidance to place the thin brachytherapy catheters into the tumour to deliver 10-20 Gy. They reported that local control was obtained in 6 of 9 patients, however there was no histological confirmation.
of this statement, nor was the method of radiological assessment stated although presumably it was contrast CT. There was no effect in 3 patients. This technique can not become a primary treatment modality as it requires 80 - 100 Gy to the whole tumour to ensure tumour sterilization, and it is impractical for the brachytherapy catheters to be able to deliver this dose; to deliver only 10 Gy of brachytherapy percutaneously took up to 2 hours. The practical issues this study raises are discussed in Chapter 12.

1.4 Endobronchial laser therapy

Since the early 1980's this form of palliative therapy has been used to debulk endobronchial tumour with the aim of improving breathlessness, haemoptysis or infections due to airway obstruction (Dumon et al., 1982; Hetzel et al., 1983). Under general anaesthetic usually with rigid bronchoscopy or a combination of rigid and flexible bronchoscopy a fibreoptic laser fibre is passed down the bronchoscope and the laser is fired. Depending on the laser parameters used and the distance of the fibre from the target tumour, tissue is either vapourised completely or thermally coagulated allowing debridement with either forceps or the bronchoscope itself. It allows rapid relief of obstruction with reduced symptoms which is best seen in patients with major bronchus and particularly tracheal obstruction (George et al., 1987). A limited number of studies have assessed objective indices of improvement including lung function and ventilation perfusion scanning (Gilmartin et al., 1987; George et al., 1990). Improvements are most marked with relief of proximal airway occlusion while the benefits of resecting tumours obstructing peripheral lobar bronchi are doubtful except in the case where relief of haemoptysis or drainage of infected secretions is required (George et al., 1990).

The side effects of this treatment include haemorrhage, bronchial perforation, infection and fire due to combustion of laser treated tumour although the risk of fire is very low. All of these complications are preventable by having a sound knowledge of bronchial anatomy and avoiding overuse of the laser which often penetrates deeper into the tissues than the operator suspects. The laser powers used are 30-60 Watts with pulse durations of 1-2 seconds. The following chapter details the use of laser therapy using much lower laser powers (1-2 W) for periods up to 10 minutes, giving slower heating of the tissues for a more controllable and gentle effect. This is known as interstitial laser photocoagulation.
1.5 Summary of management of non small cell lung cancer in relation to this thesis

Surgery remains the definitive management of stage 1 lung carcinoma. The best long term survivals are in patients with small primary tumours (less than 3 cm) with no lymph node metastases. Preoperative assessment of fitness for surgery is comprehensive and the reported series suggest that most patients with localised lung cancer can be offered surgery. However, differences in surgical perioperative mortality between centres imply that surgeons will differ in the type of patient they will be prepared to operate on. Those with the greatest experience in large tertiary referral centres with low mortality rates will operate on patients with poorer lung function than those reporting worse results. Therefore there is always likely to be a subgroup of patients who will be deemed unfit for surgery and potentially suitable for a minimally invasive therapy which will be described in this thesis. The numbers will differ between centres. Even if the proportion of patients treated is small this could potentially amount to a substantial number of patients as lung cancer is such a common disease.

The ongoing problem of poor referral of the large numbers of elderly patients for curative surgery is a complex issue. Factors involved in this may include an incorrect perception in referring doctors that these patients are likely to die with their disease rather than from it. Also although they stand to benefit from surgery in long term survival as much as younger patients, referring doctors, and patients may not be prepared to accept an increased perioperative mortality rate. As the population ages there will be increased demand on surgical services to provide curative resection. A minimally invasive therapy may therefore be a useful alternative in elderly patients who cannot have surgery whether due to limited resources if surgeons are forced only to operate on younger patients or if their physiological assessment is borderline.

Conversely the rise in use of thoracoscopic equipment may render more high risk patients operable. However as yet relatively few centres have large experience with this technique outside of the United States and Japan, and it will be some years before results of long term survival can confirm whether this form of surgery is as effective as conventional surgery. This surgery still requires intensive care monitoring post operatively, and the expense of a general anaesthetic. The results of the randomised study comparing lesser
resections with lobectomy suggest that local recurrence is more common. Given these limitations a minimally invasive therapy could be a better option than thoracotomy and lesser resection, particularly as it would allow all of the tumour to be imaged after treatment to ensure it was necrotic. Wedge excision is only suitable for tumours immediately beneath the pleura, whereas tumours deeper in the parenchyma could be treated by the proposed method, by placing the introducer needles for the laser fibres into the tumour under CT guidance, as is discussed further in Chapter 2.

The main drawback of a minimally invasive treatment would be the inability to surgically stage patients. However the only existing alternative to surgery for these patients is radiotherapy and no surgical staging can be performed in this treatment either. Given the data on the lower incidence of regional lymph nodes with smaller tumours it would be reasonable to only treat patients with tumours less than 3 cm with no mediastinal lymph nodes on CT.

Results of survival after radiotherapy in these patients are difficult to interpret as they often do not include disease free survivals. Also there are problems with localising the tumour for the administration of radiotherapy. The treatments described in this thesis would overcome the latter of these problems since the treatment is delivered into the centre of the tumour directly under CT control. Results of tumour treatments in other organs using the techniques proposed for T1 tumours in the lung will be discussed in the following chapters.
Chapter 2 Interstitial Laser Photocoagulation

2.1 Origins

2.2 Mechanism of action
   2.2.1 Effects of heat on tissues
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2.3 Lasers used for ILP

2.4 Light delivery

2.5 Experimental and clinical work
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   2.5.5 ILP in lung tumours

2.6 ILP treatment monitoring

2.7 Summary of ILP
2.1 Origins

Interstitial laser photocoagulation is a treatment for solid tumours in organs surrounded by normal parenchyma. It was first described in 1983 by S. G. Bown (Bown, 1983). It uses heat derived from the laser to cause tumour necrosis. The main difference from other thermal laser treatments is that it uses low powers to cause gradual tissue effects over a period of minutes, rather than high powers which can instantaneously ablate tumour as is used for example in laser treatments of endobronchial carcinomas. Because the laser light can be delivered through thin flexible optical fibres this treatment can be performed on tumours deep within tissues (Steger et al., 1989).

Tissue hyperthermia has long been known to cause tumour necrosis. Fevers induced by infections sometimes cured sarcomas and other tumours (Coley, 1911). In therapeutic hyperthermia either the whole patient or isolated organs are uniformly heated using electromagnetic waves, ultrasound and extracorporeal means (Hahn, 1982). Temperatures are raised from 42.5-50 °C for up to an hour. Problems include difficulty maintaining the desired therapeutic temperature and inability to reproducibly cause necrosis throughout the tumour. Gaining access to deep seated tumours is also impossible with some methods. Organ dysfunction occurred in regional hyperthermia such as in the liver. The advantage of ILP compared to hyperthermia is that heat is accurately delivered to the tumour itself and to a well defined margin of normal tissue surrounding the tumour (Steger et al., 1989). Systemic heating does not occur.

2.2 Mechanism of action

2.2.1 Effects of heat on tissues

In ILP the tip of the laser fibre acts as a point heat source. The effects are confined to a very small space as the laser fibre is inserted into tissues rather than just acting on the surface (Bown and Hetzel, 1995). Therefore only low powers of laser light are delivered. High powers cause the production of steam which causes excessive local tissue destruction. The lower powers over more prolonged periods give much greater control over the extent and type of tissue damage caused (Masters and Bown, 1990).
With ILP there is a temperature gradient starting at 200-300° C at the centre of the lesion close to the fibre tip, to 45-47° C at the margins of the lesion where a biological effect is still discernable (Matthewson et al., 1987). Temperatures need only exceed 47 °C for cellular necrosis to occur when treatment times are approximately 10 minutes (Bown and Hetzel, 1995). Due to the reduction of light intensity as the distance from the fibre in the tissue is increased biological effects of this light are seldom seen more than 7-10mm from the treatment point (Matthewson et al., 1987).

Thermal effects in the target tissue result from the transformation of absorbed light energy from the laser into heat (Thompsen, 1991). A range of thermal effects can occur in tissues depending on the proximity to the light source. These are more obvious close to the point heat source and are visible with the naked eye immediately after treatment. Removal of small volumes of tissue mass can occur at the fibre tip. The precise mechanisms of this are not fully understood (Boulnois, 1986). Different ablation mechanisms include carbonisation, vapourization, combustion and tissue carmelisation. At temperatures over 100 °C tissue water is vapourised and the effects of heat are due predominantly to tissue dessication. If heat is delivered too rapidly at this temperature tissue may be damaged by rapidly expanding steam vacuoles trapped in the tissue (Bown and Hetzel, 1995). Macroscopically there is whitening of tissues indicating thermal coagulation associated with denaturation of structural proteins. There is shrinkage of cells due to thermal denaturation and contraction of structural proteins and probable collapse of the cytoskeleton. This protein denaturation may be the mechanism of cell death (Steger, 1991). Nuclear pyknosis and cellular hyperchromasia are seen on light microscopy.

Heat effects on tissues further out are more subtle and cannot be seen with the naked eye or light microscopy in lesions immediately after treatment (Thompsen, 1991). Tissue and cellular oedema due to vascular and cell membrane injury may appear up to a few hours after treatment however this may be difficult to distinguish from fixation artefacts. More sophisticated techniques such as transmission electron microscopy demonstrate non-specific reactions including swelling of mitochondria and the endoplasmic reticulum. The first evidence of lethal low temperature injury is tissue necrosis first detected about 24 hours after death of the tissue. In most cases the full extent of the damage cannot be mapped with certainty until 48-72 hours after death. At temperatures of 40-45 °C there is reversible cell injury.
The zones of temperature change give corresponding zones of different histopathology (Matthewson et al., 1987; Bosman et al., 1991). From the hot centre to the cool periphery they are a small central ablation crater with carbonisation, hyalinization of coagulated collagen of reducing intensity, intracellular oedema, then inflammation.

The temperature time thresholds of these changes vary dramatically depending on the experimental environment (Thompsen, 1991). The factors determining the effect of laser light on tissues include the distribution of light within the tissue, the tissue temperature, the time at which the tissue remains at that temperature, and the heat capacity and diffusivity of the tissue. The damage may be accentuated as heat damages tumour vascular supply resulting in tumour hypoxia and acidosis. As tumours are relatively more hypoxic and acidotic than surrounding tissue in any case this may be the reason that tumours are more susceptible to heat in the range 42-45°C (Vaupel and Kallinowski, 1987).

Lethal thermal injury is followed by regeneration of cells and tissues capable of regeneration or wound healing of necrotic and ablated tissues with the formation of scar tissue (Steger et al., 1992b; McNicholas et al., 1993).

2.2.2 Light distribution in tissues

Light of longer wavelength penetrates tissues to greater depth than light at shorter wavelengths (Svaasand, 1985a). It was initially assumed therefore that the best conditions for ILP were to use long wavelength laser light that could penetrate deeply into the tissues and then be absorbed as heat producing large thermal lesions (Svaasand et al., 1985b). Furthermore it was considered important to avoid anything such as tissue charring which interrupted the flow of light from the tip of the laser into the tissues. This would theoretically give rise to a smaller volume of necrosis.

This concept was subsequently challenged by the findings of Amin in 1993 (Amin et al., 1993b). He demonstrated that contrary to previous belief charring of the tip of the fibre was associated with larger and more reproducible sized lesions. It appeared that once tissue charring occurred close to the tip of the laser further light from the laser was immediately absorbed in this region. This caused progressively more charring around the tip of the fibre and a progressive rise in the temperature of this very localised charred piece of tissue. This small volume of charred tissue
therefore acted as a secondary point heat source from which heat radiated into the tissue. The larger and more rapidly this charred heat source developed, the larger and more reproducible the subsequent lesions were found to be. Therefore the ability of the laser light to penetrate normal and tumour tissues was not as important as the ability of the laser to create a small charred volume of tissue which would subsequently act as a local heat source.

2.3 Lasers Used For ILP

The word laser is the acronym of Light Amplification by Stimulated Emission of Radiation (Absten and Joffe, 1993). In lasers a light source is used to energise or pump a medium of gas liquid or crystal. This input of pumping energy raises electrons to higher energy levels in more atoms more quickly than spontaneous decay can return them to their original level. A lasing medium is a medium which allows more atoms or molecules to remain in a high energy state than in their resting state. Once there is a predominance of these higher energy level atoms, spontaneous decay of a single atom emits a photon and this in turn stimulates the emission of sequentially more and more photons with precisely the same wave characteristics and in perfect phase.

The resulting light emitted has the three important characteristics of laser light. These are coherence, collimation and monochromaticity. Coherence implies that the light waves are radiating in a single direction in phase with one another. Collimation means that the beam does not diverge and has minimal loss of power along its length. Such a beam can be focussed to intensify its effects or coupled into a single slender fibre. This allows laser beams to be focused into small spots of high intensity. Monochromaticity means that the light is all of the same wavelength or colour.

A laser is usually named after its active medium. For example the Neodymium Yttrium Aluminium Garnet (NdYAG) laser uses this element as its lasing medium and gives a wavelength of 1064 nanometers (Eckhauser, 1990). NdYAG lasers are most commonly used at high powers for the immediate ablation of tumour tissue for example to recannalise an obstructed oesophagus or bronchus (Bown, 1991; Dumon et al., 1982). These lasers were also the first used for ILP (Hashimoto et al., 1985). However the low powers used in ILP (1-3W) are not easily maintained when using a
clinical NdYAG laser due to instability of the laser output at these low powers (Mills, 1995).

Currently the most commonly used lasers for ILP are the semi-conductor diode lasers (Amin et al., 1993d; Ripley, 1996). Lasing is derived from semiconductors made of Gallium aluminium Arsenide (GaAlAs). Electric current from a mains supply is used to invert populations of electrons within the semiconductor. Light is emitted as these electrons subsequently recombine with positive regions in the semiconductor. These lasers offer advantages over the Nd YAG laser in that they are small, compact, portable and rely only on a standard electrical mains supply. Furthermore they do not require water cooling. The laser beam quality is inferior to the NdYAG laser as it is more divergent when exiting from the laser fibre. Diode lasers produce charring much earlier and more extensively than the Nd-YAG in ILP (Amin, 1993). This is due to absorption of more light close to the fibre tip with diode lasers due to the shorter wavelength.

In 1993 Amin analysed the significance of charring in the treated tissue (Amin et al., 1993b). The livers of normal rats were treated with ILP with either Nd-YAG or 805nm diode lasers. Firstly the diameter of necrosis correlated with the amount of charring at the centre of each lesion. Secondly diameters of necrosis were significantly smaller with the Nd-YAG wavelength using a clean fibre tip than if it was charred. Following ILP with the Nd-YAG laser the tip of the laser fibre appeared relatively clean but it was always charred after ILP with the 805nm diode laser. Thirdly an innovative and simple method to encourage charring was used. A small length of the tip of the fibre was blackened (charred) before treatment by firing it for a few seconds into a pigmented material. With this technique lesion sizes increased by up to 6 fold, depending on the power used. This technique of "pre charring" the fibre is now standard practice in clinical ILP treatments in the UK.

2.4 Light delivery

Light for ILP is delivered from the laser via optical fibres (Amin et al., 1993d). These comprise a small diameter filament of high refractive index glass. This core is coated with a thin layer of lower refractive index polymer known as the cladding. The laser beam is focussed into the core of the optical fibre and by total internal reflection the light is guided along the fibre with very low losses. Since they are small and flexible these optical
fibres may be passed through narrow introducer needles which enables them to be positioned in tumours for ILP (Mills, 1995).

The tip of the laser fibre is usually the plane cut glass end of the fibre revealed by paring back the cladding (Amin et al., 1993b; Steger, 1992a). Alternatively a more sophisticated cylindrical diffuser can be used where light is emitted over a 1-4 cm length (Nolsoe et al., 1992; Van den Bergh et al., 1995). The active length of bare tip fibres is 1-2 mm and causes more focal buildup of heat compared to diffusers theoretically resulting in charring of tissues at this site. Diffusers were developed to provide more even distribution of heat to minimise the risk of charring, since it was initially thought that once charring occurred diffusion of light from the tip of the laser fibre into tissues was no longer possible (Hashimoto et al., 1985). Recent investigations have shown however that to obtain similar volumes of necrosis with diffuser fibres (as for bare tip fibres) the required laser output is high which causes tissue charring (J.Brookes, 1996, personal communication). Furthermore they are slightly more bulky than bare fibres requiring larger introducer needles with the potential for more local trauma. Diffuser fibre tips occasionally break during treatment and the glass from the tips is not retrievable in this situation. Comparatively little in vivo work has been done with diffuser fibres compared to bare fibres and for this and the above reasons only bare fibres were used in this thesis for ILP.

While a small volume of charring is generally desirable in ILP this should be avoided in some tissues such as the brain. In these tissues diffuser fibres would be desirable as on the whole they usually cause less charring at low powers (Nolsoe et al., 1992).

2.5. Experimental and clinical work

The experimental and clinical work with ILP has been performed predominantly in the liver. Other organs treated by this technique include the prostate, breast and pancreas.

2.5.1 ILP of the liver

The first experimental work was performed in rats (Matthewson et al., 1987). Under general anaesthetic the liver was exposed and the laser fibre inserted directly into the organ. Necrotic zone diameters were assessed both macroscopically and histologically at a variety of laser powers 2-3 days after...
treatment. The technique produced necrotic lesions that were well defined and easy to measure macroscopically. For any given power setting the necrotic zone diameter increased with the delivered energy but always plateaued by 1000 joules. The lesion diameters were highly reproducible. For a mean size of necrosis of 12.6 mm from 7 lesions the standard deviation was 1 mm with a coefficient of variation of 8%. There was a gradual reduction in light transmission through the liver during laser exposure. Light intensity measured at the perimeter of the lesion fell to approximately 30% of its initial value. This suggested that during exposure the optical properties of the liver changed particularly at the higher power settings. In retrospect this was probably due to the production of charring which was universal in lesions treated with powers greater than 0.75 watts even for exposures less than 200 seconds.

Histology performed 3 days after ILP showed a small central charred and cavitated area surrounded by necrotic and inflammatory tissue. With time, maturation into a fibrous scar occurred and by 60 days only a small fibrous nodule remained. Much of the necrotic tissue had been replaced by regeneration of normal liver tissue. Larger animal experiments with the use of multiple fibres inserted simultaneously revealed similar results (Steger et al., 1992a; Bosman et al., 1991). It was noted that along with the typical coagulative necrosis around the fibre tip there was relative sparing of tissue around larger blood vessels due to the cooling effect of flowing blood. In pig liver necrotic zone diameters were reported between 15 and 19 mm depending on the powers used.

Because the dimensions of thermal necrosis with a single fibre were too small for general application in the treatment of human tumours the simultaneous use of multiple fibres was assessed in canine liver by Steger et al (Steger et al., 1992a). Four fibres were coupled to the laser by means of a fibre splitter which allowed even distribution of laser light giving the same power from each laser fibre. These four fibres were inserted into dogs undergoing general anaesthetic with the aim of creating overlapping zones of necrosis from each fibre to give an overall larger single lesion. Ultrasound appearances during ILP initially showed four isolated zones of non-overlapping necrosis which subsequently coalesced over 500 seconds. By the end of the treatment there was one large zone of necrosis including four separate charred areas.
Zones of overlapping necrosis were observed on histology at 3 days with diameters of 3-4cm using four fibres separated by 1-1.5cm. There was progressive reduction in lesion size: by one month the mean size was 2.8x2.7cm (n=5) and at 5 months 2.1x1.3cm (n=2). By one year only the four charred areas were visible on histological examination and these were separated by 1.5cm. Therefore most of the lesion had been progressively replaced by ingrowing normal tissue.

Most clinical studies with ILP have been performed in the management of both primary and secondary liver tumours, with the latter the most common current indication (Amin et al., 1993d). Initially the clinical work was performed at laparotomy (Hashimoto et al., 1985) however subsequently percutaneous radiologically guided ILP became more frequently used and is now the method of choice (Amin et al., 1993a; Steger, 1991; Masters et al., 1992).

In the percutaneous technique ultrasound is used to locate the tumours and local anaesthetic is infiltrated into the abdominal wall and liver capsule overlying the tumour. The tumour is biopsied and then the 19G introducer needles containing the laser fibres are inserted into the tumour under ultrasound and or CT guidance so that the needle tips lie 1-1.5cm apart. For tumours 1cm or less in diameter only one or two needles are used and those larger than 3cm require as many as eight needles. Once the needles are in place they are withdrawn a short distance so that 3-4mm of the laser fibre is left exposed within the tumour. Each laser treatment lasts up to 500 seconds after which the needles and fibre tips are carefully repositioned by withdrawing them approximately 1.5cm. The treatment is then repeated. The separation of fibres by 1-1.5cm ensures that necrosis of the intervening tissue occurs (Steger et al., 1992b).

An early series of this technique reported ILP of 55 liver metastases in 21 patients (Amin et al., 1993a). Necrosis as judged by dynamic CT scan appearances was graded from 1 to 3, with Grade 1 representing 100% avascularisation of tumour, Grade 2 more than 50%, and Grade 3 10-50%. Grade 1 necrosis was achieved in 21 of 35 tumours treated with a diameter of less than 4cm, however no tumours larger than 4cm in diameter achieved Grade 1 necrosis. The median diameter of tumours achieving Grade 1 necrosis was 2cm. Overall greater than 50% reduction in tumour volume was achieved in 82% of tumours which compared favourably with other palliative methods used for treating liver metastases. Failure to produce 100%
Avascularity in tumours less than 4cm was due to problems with tumour accessibility and difficult needle placement in some cases. Also inhomogeneity of tumours with variable blood supply as compared to the homogeneous normal liver may have reduced heat absorption by the tumour. Long term survival was not assessed completely due to short follow-up.

ILP treatments were well tolerated by most patients regardless of the number of tumours treated. Mild abdominal discomfort during treatment and for 24-48 hours afterwards was common and for lesions just under the liver capsule additional analgesia was often required either for shoulder pain due to diaphragm irritation or abdominal or back pain probably due to heating of the nearby peritoneum or retroperitoneum. In four cases where tumours were adjacent to the diaphragm or the peritoneum pain resulted in shortening of the treatment time although the treatment was able to be completed in all four cases with intervening gaps rather than a continuous treatment. One patient developed a fluctuant fluid-filled mass immediately beneath the liver capsule following treatment of a superficial liver metastasis; this was managed by antibiotics and this lesion subsequently resolved and was probably a small abscess. Bradycardia occurred in one patient where the lesion was in the tip of the left lobe and was possibly due to stimulation of vagal branches in the adjacent stomach wall. Small subcapsular haematomas were demonstrated on CT scanning in four cases and in one case there was a drop in Hb of 2gms/dL after 2 treatments with ILP. Liver enzymes were elevated to a degree typical of a transient inflammatory response in the liver especially if two or three metastases were treated in one session.

All patients were discharged by 24 hours after ILP although in eleven patients analgesia was required orally for up to ten days. Mild elevation of temperatures was reported 24-72 hours after treatment in several cases which was probably due to the presence of necrotic tissue in the liver.

Since this paper was published CT scanning has become the method of choice for monitoring needle placement. Also the treatments in this paper were performed with the NdYAG laser; however over the past 3 years at the Middlesex Hospital treatments have been performed exclusively with the diode laser. These changes have made the treatment significantly easier and over 100 patients have been treated with this technique. Further improvements are being made in the field of real time monitoring of ILP with MRI as discussed below. Currently it is not known whether ILP to colorectal
liver metastases prolongs survival. This is due to the ongoing evolution of the technique and understanding of appropriate patient selection. Series of patients treated with these improvements are now being treated at the Middlesex Hospital and will provide accurate survival information.

2.5.2 ILP of Prostate

ILP is a potential treatment for malignant prostatic disease with both experimental and clinical treatments being performed to investigate its role in managing local recurrence of tumours after primary treatment. In normal dogs a dose of 1000 joules with ILP produced lesions approximately 1cm in diameter four days after treatment (McNicholas et al., 1993a). At six weeks there was healing by fibrosis surrounded by an area of cystic degeneration. Using a multiple fibre system overlapping areas of necrosis were produced measuring up to 26mm in diameter. ILP of prostatic cancer has also been performed (Amin et al., 1993e). Amin reported a case in 1993 where a localised prostatic carcinoma was treated with three ILP fibres simultaneously. Transrectal ultrasound and CT were used to guide the three needles inserted transperineally into the abnormal area. This patient had recurrence of a localised prostatic carcinoma after initially receiving radiotherapy. Surgery was considered inappropriate because of his previous radical radiotherapy and he was therefore referred for ILP. Ultrasound during ILP showed a gradually enlarging echogenic zone around the fibre tip but within a few minutes of completing treatment the echogenicity and doppler signal decreased. However dynamic CT scan performed 10 days later showed the treated area as a non-enhancing avascular zone and biopsies from this region confirmed the presence of necrosis.

There were no complications during the treatment although mild dysuria occurred afterwards presumably due to some effect on the prostatic urethra. The treated zone included the zone demonstrated on ultrasound prior to the treatment. However in this patient a second treatment was required due to an elevation of Prostate Specific Antigen indicating that there was a more diffuse disease than initially suspected. As with treatments performed in other organs ultrasound gave a guide to the tissue changes taking place but it was not as accurate as subsequent CT scan imaging.
2.5.3 ILP of Breast

ILP has been applied to breast cancers and fibroadenomas (Harries et al., 1994b; Mumtaz et al., 1996). This form of treatment may eventually be shown to spare some patients a surgical procedure on the breast. Harries described the histological results of ILP treatment of breast cancer in 43 patients prior to surgical resection. Local anaesthetic was used and ultrasound located the position of the fibre-tip within the breast tumour. Treatment parameters were 2-3 watts for 500-750 seconds. Necrosis of up to 25mm in diameter occurred in the tumours.

Charring in the tumour around the fibre tip after treatment gave significantly larger diameters of necrosis than when charring did not occur (Median 13 versus 6mm, p=0.002). Twenty-six patients had laser treatment with a clean fibre and 18 had treatment with a pre-charred fibre. This resulted in a more predictable diameter of necrosis with a median of 14mm compared to a median of 8mm for the clean fibres. With single fibres only partial volumes of the tumours were able to be destroyed and for complete treatments multiple fibres will be required.

Ultrasound was used to monitor the treatment effects in real time although there was no correlation between the necrotic diameters measured by ultrasound and histology. However subsequent studies have shown that CT scan and MRI are able to delineate necrotic areas within tumours and subsequent investigations have revealed the excellent discriminatory capacity of MRI to determine the extent of necrosis within the breast tumours (Mumtaz et al., 1996).

2.5.4 ILP in other organs

Colorectal tumours and flank fibrosarcomas have been treated with ILP in experimental animals (Matthewson et al., 1988; Matthewson et al., 1989). Fibrosarcomas were completely destroyed in 10 animals with local recurrence observed in 5 after 2 months. There was a prolonged survival in animals treated with 2 W for 600 seconds compared to controls. Histological changes in the fibrosarcoma showed similar findings to that in normal liver with a well defined margin between viable and necrotic tissue. However whereas the liver healed by fibrous replacement of the necrotic area the necrotic part of the tumour sloughed off.
Dimethylhydrazine induced colorectal cancers in rats were adequately treated by ILP (Matthewson et al., 1988). Necrosis and sloughing of the tumour into the colonic lumen occurred within four days. Treatment parameters of 1 W for 300 seconds was sufficient to cause complete histological necrosis in most tumours 8mm in diameter or less; however, this depended on accurate positioning of the laser fibre in the tumour. Incomplete necrosis was observed with shorter treatment times. With longer treatment times there was increased risk of complications including perforation of the colon wall, suggesting that ILP is not suitable for tumours of hollow organs.

2.5.5 Lung tumours.

There is one report of ILP treatment of a lung parenchymal tumour (Brookes et al., 1996). This was a large secondary from a parotid carcinoma treated with the intent of reducing its volume and possibly therefore controlling haemoptysis. Under local anaesthetic 2 bare fibres were inserted into the tumour under CT control using 19 Guage introducer needles. No discomfort was felt during the treatment. Contrast CT after the procedure showed a 2 cm diameter region of necrosis within the tumour. The normal parenchyma surrounding the tumour had not been treated as tissue responses of the normal lung were unknown at that time, as it was prior to the studies described in this thesis. Nine months after the ILP CT showed only minimal growth of the metastasis whereas other untreated metastases continued to grow. It is well known that for ablation of tumours with ILP, treatment should include all the tumour as well as some surrounding tissue. However this case confirms the feasibility of the technique.

ILP of small endobronchial tumours with curative intent was attempted in 1987 however results were disappointing with little thermal necrosis seen (Bown and Hetzel, 1995). This was performed with a YAG laser before Diode lasers were available and the laser was unstable at the low powers required for ILP. Other reasons for the lack of effect were the considerable movement within the airways and therefore of the fibre within the tumour. Also the cooling effect of the moving column of air and heat loss through major pulmonary vessels may have reduced the thermal effect. Furthermore a bare tip fibre was used with 1 cm of cladding removed. This is longer than the conventional 3-5 mm used now. Shorter lengths of bare tip give a more
concentrated point of light emission and greater capacity to generate a focal heat source. Finally the fibre was not pre-charred.

2.6 ILP Treatment Monitoring

One of the difficulties with ILP is that since tumours are being treated within solid organs it is not possible to assess the results of treatment visually. This is in contrast to the use of lasers with surface application such as in the bronchus where there is immediate visual feedback as to the tissue effects of the laser. This is why it is important to determine the extent of initial damage and healing of thermally mediated laser induced effects that particular treatment parameters will produce. It is a basic prerequisite that the function and structure of the target organ remain acceptable at all stages of healing. These kinds of assessment are best performed in experimental animals. There is however great interest in the ability to monitor the effects of ILP in real time to allow the treating physician immediate feedback as to whether the treatment field has completely encompassed the tumour in question either soon after the treatment is finished or during treatment (Harms et al., 1994; Roberts et al.; 1994, Wyman et al., 1992). ILP treatments of the liver and breast are the two areas where MRI has been used to monitor this treatment. Ultrasound was not effective in delineation of the extent of thermal effects after the procedure was completed (Harries et al., 1994a). Contrast MRI on the other hand was effective in predicting the extent of histological tumour necrosis 48 hours after treatment (Mumtaz et al., 1996).

MRI produces excellent soft tissue images and these can be acquired in multiple planes with potential for three dimensional monitoring (Harms et al., 1994). The MRI signal from tissues is dependant on a number of tissue characteristics one of which is temperature (Parker et al., 1983). Importantly there have been advances in the ability of MRI to deliver fast temperature sensitive images that are acquired on time scales appropriate for essential real time monitoring of ILP (Matsumoto et al., 1994). Some authors consider that MRI may be best used to monitor the outer most periphery of the treatment site or to monitor a leading edge of a temperature gradient moving towards nearby untargeted structures (Fried et al., 1996). During liver tumour treatments MRI images show an enlarging area of low signal with a bright rim which correlates with the CT scan assessment of the treated
area 24 hours later (Roberts et al., 1994). It also shows that tissue destruction of normal liver treated at the edge of the lesion was more extensive than occurred in the tumour for equivalent energy deposition.

2.7 Summary of ILP

Interstitial laser photocoagulation therefore offers a safe, reproducible method of causing necrosis of tumours and some surrounding normal tissues in tumours deep below the surface. The simultaneous use of multiple fibres allows larger areas of necrosis, however smaller tumours are more effectively completely ablated. Healing is usually by regeneration in tissues such as the liver which are capable of this, or by replacement of the tissue with scar. Carbonised tissue is not removed from the treated zone. A further advantage of this technique is that it is repeatable. With diode lasers the technique is now easy to perform with a portable compact laser and the research into monitoring of tissue effects in real time with MRI is continuing to aid the refining of this technique. One of the aims of this thesis is to understand the effects of ILP on normal lung parenchyma so that a rim of normal lung around the tumour could be incorporated in the treatment field with knowledge of its acute effects and healing response. The treatment in the lung would be performed via a percutaneous method as for other organs such as the liver. From its effects in other organs it is a well localised treatment causing no damage to tissues only millimeters outside its zone of necrosis. This would be an important potential benefit in treating tumours in patients with poor lung function.
Chapter 3 Photodynamic therapy

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3.6 Summary of PDT
3.1 Origins and development

Photodynamic therapy is a treatment which has been applied to a wide variety of conditions, both malignant and non-malignant (Dougherty, 1993; Dierickx and Anderson, 1996). It has been used to treat a variety of tumors including bronchogenic carcinoma, head and neck tumors, prostatic carcinoma and skin tumors. Over 3000 patients have now received this treatment worldwide (Kato, 1996) although in many cases the results have been anecdotal. It is a unique treatment requiring the combination of a systemically administered drug with light of a specific wavelength which results in tumor necrosis. It had been known for almost 100 years that when photosensitizing compounds are exposed to light a photochemical reaction occurs (Raab, 1900). Photosensitizers were initially employed for diagnostic purposes when it was noted that tumors preferentially take up some compounds and the fluorescence of those compounds could be employed to detect the margins between tumor and normal tissue. Lipson and Baldes demonstrated the affinity for malignant tissue of hematoporphyrin derivative in 1961 (Lipson and Baldes, 1961). Tumor tissue could be visualised in ultraviolet light because of the porphyrin fluorescence which appeared red. Hematoporphyrin derivative was used in tumor detection during the 1960's particularly in the bronchial tree.

Because it was known that this substance absorbed light in the better tissue penetrating spectrum, that is, in the red part of the spectrum, it seemed logical to try activating this compound within tissues. In 1966 Lipson performed the first tumor treatment with PDT using HPD to treat a patient with metastatic chest wall breast carcinoma (Lipson, RL, presented at Ninth International Cancer Congress, Tokyo, 1966). Experimental work on the use of HPD PDT was first reported by Dougherty in 1974 and subsequently trials on human tumors (metastatic cutaneous carcinoma) were reported in 1977 and 1979 (Dougherty, 1974; Dougherty et al., 1977). Since that time a wide spectrum of tumors has been treated by photodynamic therapy in phase 1 and 2 studies both with surface applications of light on the skin and within hollow viscera as well as deep inside organs by interstitial treatments (Dougherty, 1993). The first phase 3 studies were commenced in 1988 using Photofrin, the purified derivative of hematoporphyrin
derivative (Heier et al., 1995). The first governmental approval for the use of photodynamic therapy in patients came in Canada in 1993. Other countries with approval include Japan, USA and The Netherlands. The conditions currently approved for treatment are early bladder cancer, oesophageal, bronchial, cervical and advanced gastric carcinomas.

The development of PDT was initially empirical with no knowledge of the underlying biological effects of PDT in either tumours or normal tissues (Bown, 1990). Initially it was believed that PDT had the potential to be a truly selective treatment due to the observed preferential uptake of HpD into tumour tissues (Gregorie et al., 1968). With time however it was appreciated that drug was not only also taken up by the normal tissue surrounding the tumour but also by all the major organs, particularly those of the reticuloendothelial system. It is now known that in most organs the specificity of uptake of photosensitizer compared to normal tissue is at best 2-3:1 (Bown and Hetzel, 1995). The exception to this is the brain which has ratios of up to 10-30:1 due to disruption of the blood brain barrier by the tumour.

PDT remains a very specific cancer treatment, but its specificity comes not so much from the distribution of the drug but from the ability to activate the drug in specific areas by the application of light only in the areas where a tissue effect is desired (Bown and Hetzel, 1995). Lasers were an ideal method of doing this because along with their monochromaticity they could be directed with pin point accuracy onto the tumour and a small rim of normal tissue.

Further specificity was achieved by an understanding of the way photosensitizers were distributed within an organ, in particular the time course of concentration in for example the mucosal as opposed to the muscular layer of a particular organ (Bown, 1990). With this knowledge light could be applied to the organ at times after the administration of the drug when it was known the drug would be maximal in the mucosal layer and least in the muscular layer, thereby deriving most effect in the level of interest and sparing the supporting structures. This was demonstrated in the bladder by Pope and Bown in 1991 (Pope and Bown, 1991a). By manipulating the administered dose of photosensitizer they were able to observe uptake of the drug into the mucosa with little in the muscular layer. Treatment of the mucosa, where field change and diffuse malignancy is prone to occur, led to selective necrosis of the mucosa with little functional
effect on the bladder. Prior to these experiments clinical treatments of bladder carcinoma had led to bladder muscle damage with functional effects (Nseyo et al., 1985). The basic experimentation revealed ways in which the unique tissue properties of PDT could be exploited.

It was also gradually appreciated that PDT has unique tissue effects, particularly in hollow organs. Firstly necrosis of normal tissue by PDT heals by regeneration of normal tissue with minimal or no scarring (Barr et al., 1987b). Secondly while tumour is necrosed the supporting connective tissue of an organ is not damaged (Barr et al., 1987a). Even when full thickness necrosis of a hollow viscus has occurred, there is no reduction of the strength of the wall or risk of perforation due to preservation of the supporting connective tissue. Again this understanding only came from experiments on normal tissues. These effects will be discussed in more detail below.

3.2 Mechanism of action of PDT

The end result of the combination of light and photosensitizing drug is the production of an excited species of oxygen known as singlet oxygen (Weishaupt et al., 1976). This is a toxic species and differs from the ground state triplet oxygen in terms of the spin of electrons around its nucleus. It causes peroxidation of membrane and nuclear structures. All cell proteins, membranes, and organelles can be thus peroxidated and destroyed if they are in the vicinity of the singlet oxygen (Henderson and Dougherty, 1992). Singlet oxygen only survives 1-4 microseconds so its effects are only local not systemic (Stewart, 1993).

In addition to this cytotoxic effect PDT also causes thrombosis and occlusion of tumour vessels (Dougherty and Marcus, 1992; Star et al., 1986). This effect starts within 5 minutes of treatment and is thought to be due to the release of thromboxane and other cytokines from endothelial cells and the surrounding tissue (Henderson and Donovan, 1989a). In contrast no immediate effects on tumour cells are seen. Vascular stasis occurs followed by extravasation of red cells and fluid. In tumours this affects the vessels of the supporting stroma, and this is a major cause of tumour cell death with agents such as photofrin.

PDT offers the potential for selective treatment, that is, preferentially more effects being observed in the tumours than the surrounding normal tissue.
(Mlkvy et al., 1996). This is achieved to a small degree by increased uptake of the drug into the tumour as opposed to surrounding normal tissue, but much more effectively by only applying the laser light to the desired treatment area (Bown and Hetzel, 1995). Tissues and organs distant from the site of interest will contain the photosensitizer but a PDT effect will not occur if the laser light is not shone on these areas (Whelpton et al., 1995; Stewart, 1993). The causes of preferential uptake of photosensitizing drug into tissues compared to surrounding tissues are only now becoming understood (Hamblin and Newman, 1994a). There is no difference in the ability of tumour cells themselves in terms of uptake of photosensitizers. Most of the difference comes from the supporting tumour stroma. Concentrations of photosensitizer are up to 5 times more than around malignant cells (Bugelski et al., 1981). Initial theories were that the tumour stroma vessels were relatively leaky and this along with local lymphatic occlusion in the tumour led to concentration of the drug in the tissue stroma (Dougherty and Marcus, 1992). More recently it was found that the inflammatory cells in the tumour stroma, particularly macrophages are major accumulators of photosensitizers (Korbelik and Krosl, 1995a). Using flow cytometry Korbelik and co workers have shown that most of the differences in photosensitizer concentration between tumours can be explained by differences in their macrophage content (Korbelik and Krosl, 1995b). Some populations of macrophages had levels of photosensitizer up to 20 times that of tumour cells.

While singlet oxygen causes necrosis of most normal and tumour structures it does not affect supporting structures. This was well demonstrated by Barr in 1987 where the effects of PDT on the wall of the colon in rats were compared to thermal effects (Barr et al., 1987a). With both treatments full thickness necrosis of the wall of the colon occurred. With thermal treatments this resulted in weakening of the structural integrity of the colon wall as demonstrated by its bursting pressure when both ends of the segment of treated colon were ligated and the segment inflated. No such effects were seen with PDT. Furthermore histology confirmed that supporting collagen was unaffected in PDT whereas it was totally disrupted with thermal treatments. Similar effects were described by Smith et al in 1993 and are discussed below (Smith et al., 1993).

These studies were vital to understanding the effective application of PDT to hollow organs. The wall of the viscus can be treated with PDT without breakdown of the wall due to the persistence of the collagen supporting
structures. These and other studies also showed that scarring was minimal after PDT and epithelial structures could completely regenerate within periods of 1-2 months (Barr et al., 1987b). The tumouricidal effect of PDT has been shown in a variety of cancers in both animal models and humans (Dougherty, 1993).

3.3 Photosensitzers for PDT

These drugs have no pharmacological effect in the absence of light activation (Bonnett, 1995). The wavelength at which a particular photosensitizer is excited by the light is important as longer wavelengths allow greater tissue penetration by the laser light (Svaasand, 1985a). Therefore it is preferable to have drugs which absorb light further into the electromagnetic spectrum. Deeper penetration of light allows deeper excitation of the photosensitizing drug and therefore deeper effect (Stewart, 1993).

The major drawback of photodynamic therapy is the inherent capacity of photosensitizing drugs to cause photosensitizing skin reactions (Razum et al., 1987; Zalar et al., 1977). This is the only consistently reported side effect of PDT. Exposure to sunlight can cause a range of reactions from mild erythema to full thickness burns.

The ideal photosensitizing drug is one which has a high specificity of uptake into the tumour as compared to normal surrounding tissues, has an absorption peak which is at a long wavelength allowing deep tissue penetration, and which does not cause excessive skin photosensitivity. This has lead to a great deal of research into finding a photosensitizer with all of these features (Bonnett, 1995). As yet the perfect photosensitizer has not been found; however there have been great improvements since the first clinical trials using haematoporphyrin derivative.
Table 3.1 lists the most commonly used photosensitizing drugs and compares some aspects important for their clinical application. They are listed in chronological order of their discovery. All are administered intravenously except ALA which is given either orally or topically. An intravenous preparation of this drug is however being developed.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Chemical purity</th>
<th>Wavelength of excitation (nm)</th>
<th>Singlet oxygen yield</th>
<th>Tumour selectivity</th>
<th>PDT Lesion size</th>
<th>Skin photosensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPD</td>
<td>+</td>
<td>628</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>Photofrin</td>
<td>++</td>
<td>630</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>A1S2PC</td>
<td>+++</td>
<td>675</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>mTHPC</td>
<td>++++</td>
<td>652</td>
<td>++++</td>
<td>++</td>
<td>++++</td>
<td>++</td>
</tr>
<tr>
<td>ALA</td>
<td>++++</td>
<td>635</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 3.1 Characteristics of Photosensitizers for PDT.

Haematoporphyrin derivative (HpD) was initially used for tumour diagnosis due to its relative tumour selectivity. It was subsequently the first widely used drug for PDT with treatments of head and neck tumours, oesophagus, bronchus and papillary carcinoma of the bladder (Patrice et al., 1990; Wenig et al., 1990; Edell and Cortese, 1992; Jocham et al., 1989). It is a mixture of more than 10 different porphyrins and the active component is not known (Kessel and Thompson, 1987). This impurity hampered the reproducibility of early research efforts to characterise the pharmacokinetics and tissue responses of HpD (Bonnett, 1995). Although HPD had some selectivity for the tumour compared to its surrounding normal tissue, subsequent agents have shown similar or greater tumour selectivity (Stewart, 1993). Its half life is long (19 days) and this contributes to the long period (up to 6 months) during which patients can have photosensitising skin reactions (Brown et al., 1992).

Photofrin is the commercially available purified form of HpD (Byrne et al., 1990). It is still an impure mixture of porphyrins. Many patients have been effectively treated with both HpD and Photofrin however the major drawback is the skin photosensitivity.
Subsequent agents are referred to as second generation sensitizers. Disulphonated aluminium phthalocyanine ($\text{AlS}_2\text{Pc}$) and meso tetra hydroxy phenyl chlorin ($\text{mTHPC}$) were chemically synthetised after systematic searches for newer photosensitizers which absorbed light of longer wavelength, further into the red spectrum, thereby allowing deeper tissue effects.

Of the second generation agents $\text{mTHPC}$ is the most promising in terms of the magnitude of its tissue effects (Ris et al., 1991). It was found by Berenbaum and Bonnett working at Queen Mary's College in 1986 (Bonnett et al., 1989). These workers were testing a large panel of chemicals for their light absorbing and tumour necrosing properties using an animal tumour model. Of over 100 substances tested $\text{mTHPC}$ showed the highest yield of singlet oxygen. Also its absorption at a longer wavelength than Photofrin allowed deeper tissue penetration. On a mole for mole basis it is more than 100 times as active as HpD as judged by amount of tumour necrosis in animal models. Therefore treatment times can be shorter with this agent. A wide variety of tumours have now been treated with $\text{mTHPC}$ in clinical studies including squamous cell carcinomas of the head and neck, bronchus, oesophagus, and adenocarcinomas of the prostate (Dilkes et al., 1995; Grosjean et al., 1997; Chang, 1996).

Phthalocyanines have the potential to be active PDT agents because they absorb light at 675nm, a wavelength which allows deeper tissue light penetration into tissues. Sulfonation is used to make them water soluble, and the most active isomer is the disulfonated derivative $\text{AlS}_2\text{Pc}$ (Chan et al., 1990). Numerous preclinical studies have shown their activity against tumours (Chan et al., 1987; Barr et al., 1990b; Bachor et al., 1992; Chatlani et al., 1992). A significant disadvantage is the long treatment times required to achieve PDT affect. To date no clinical studies with $\text{AlS}_2\text{Pc}$ have been reported.

Studies on the effect of PDT using $\text{AlS}_2\text{Pc}$ on the normal trachea were reported by Smith et al in 1993 (Smith et al., 1993). These were performed to increase understanding of the tolerance of tracheal and bronchial structures to endobronchial PDT. Treatments were performed in rats under general anaesthetic by touching the laser fibre tip onto the surface of the tracheal mucosa after opening the trachea with a small incision. Using treatment parameters of up to 100 J (100 mW for 1000 seconds) lesions up
to 8 mm in diameter were created. In the larger lesions full thickness necrosis of the trachea occurred; however, these healed completely by 2 months and there was no reduction in the integrity of the tracheal wall as measured by its bursting pressure. In contrast thermal lesions created by a YAG laser reduced the strength of the trachea. The conclusion was that accidental irradiation of normal trachea and bronchial structures would be safer with PDT than with thermal lasers. These results allowed further development of the use of PDT on small endobronchial tumours (Lam, 1994).

It is noteworthy that these studies were performed some 12 years after PDT was first used to treat endobronchial tumours. Thus unfortunately the understanding of the normal tissue responses, a major prerequisite for the effective use of a cancer therapy, lagged a long way behind the first clinical use of endobronchial PDT.

5 aminolaevulinic acid (ALA) is a naturally occurring precursor in the heme biosynthetic pathway (Kramer et al., 1973; Charlesworth and Truscott, 1993). It does not have photosensitising properties but is converted to protoporphyrin IX (PpIX) which is the active derivative. Cells with high capacity for haem synthesis and rapid turnover accumulate protoporphyrin IX more effectively than normal cells, for example cancer cells and mucosal cells of the gastrointestinal tract. The drug has a short half life and is rapidly metabolised so that risk of skin photosensitivity is limited to 1-2 days. It can be administered topically and its major clinical application to date has been in management of skin basal cell and squamous cell carcinomas (Kennedy et al., 1996; Lui et al., 1995). It is also showing some promise in treatment of early bronchogenic carcinomas and Barretts oesophagus (Barr et al., 1996; N. Awadh and S. Lam, unpublished work, presented at 6th biennial meeting of the International Photodynamic association, Melbourne 11th March 1996). To date only thin tumours have been well treated with ALA (Fan et al., 1996; Mlkvy et al., 1995).

3.4 Light for PDT

3.4.1 Lasers for PDT

Any visible light source can be used for PDT in theory (Absten and Joffe, 1993). However laser light sources have several advantages. They can be transmitted by optical fibres and therefore can be passed down endoscopes or in the case of interstitial treatments down thin introducer needles.
Secondly they are monochromatic and therefore different lasers can be chosen for the specific wavelength of light they generate.

Lasers in PDT are not used to exert a thermal effect as this limits the diffusion of the red light into the tissues and therefore the overall size of PDT effect (Chang et al., 1996a). The highest possible power which does not cause thermal effects is used. The role of the laser light is solely to activate the photosensitizing drug in the tissues. Therefore the laser is not the cause of the tissue effects, rather it is the cytotoxic oxygen released by the interaction of the laser and the drug which causes the necrosis.

The most commonly used lasers for PDT are dye lasers (Mills, 1995). Organic dyes such as rhodamine are illuminated to produce light of various wavelengths. The dye absorbs light over a band of certain wavelengths; this causes the dye to fluoresce, emitting light of a different wavelength which is used for PDT. Various different lasers are used to provide this light which causes the dye to fluoresce. One example is the copper vapour laser, which was used for PDT in this thesis in Chapter 8 (termed a copper vapour pumped dye laser). The dye is in liquid form and it is pumped through a nozzle to form a thin jet which crosses the optical path of the light from the copper vapour laser. These lasers are tunable, that is the colour of the light produced can be varied by changing the dye and more finely tuned by changing the angle at which the light from the copper vapour laser meets the dye jet. This enables one laser to be used for PDT applications using a variety of photosensitizers, for example giving wavelengths of between 600 and 680nm.

Diode lasers have recently become available for use in PDT (Manni, 1992). They offer the same advantages as diode lasers for thermal use, namely they are portable, cheap, and simple to use. They are designed to deliver only one wavelength which is specific for one photosensitizer, for example 652nm for mTHPC. Such a laser was used in the experiments described in Chapter 11.

3.4.2 Light penetration

When light is incident upon a tissue it may either be reflected or enter the tissue and be scattered, absorbed or transmitted (Absten and Joffe, 1993). The penetration of light into tissues is limited mostly by scattering except in highly pigmented tissues such as the liver where absorption is stronger
Light distribution in the parenchyma of the lung is by scattering (Shunsuke et al., 1985). To cause a PDT effect light does not need to be uniformly distributed but present in just enough quantity to allow the necessary threshold for drug and light dose to be exceeded. The depth to which light penetrates tissues is usually expressed as the depth at which the light intensity of a given wavelength has fallen to 37% of its incident value. This ranges from 1-2 mm in brain tissue to 5 mm in muscle and some tumours including squamous cell carcinoma of the lung (Svaasand, 1984). In the lung parenchyma this is 2-3 mm and is less in heavily pigmented areas such as carbon deposits in heavy smokers. PDT effects are however seen at depths up to 1 cm where the light intensity is only 3-10% of the incident intensity (Gilson et al., 1988; Lowdell et al., 1993). This is due to the threshold effect described above. In theory treatment at 850 nm would give penetration depths of 8 mm but photosensitizers which are specific for this wavelength have not yet been developed.

Interstitial photodynamic therapy overcomes the problem of light penetration into tissues by delivering the light within the tissues and with the use of multiple laser fibres inserted simultaneously (Marijnissen et al., 1989). These can either be bare tipped fibres, as described for the ILP treatments, or diffuser fibres (Chang et al., 1996a; Lowdell, 1993 #1721). The treating section of diffuser fibres is elongated and cylindrical permitting up to 4 cm length of even illumination of tissue. These fibres are usually of a larger diameter than bare tipped fibres requiring larger introducer needles.

3.5 Clinical experience with PDT

The early clinical applications of PDT were on advanced tumours. This is often the case in the early development of a tumour treatment which was due to a desire to avoid any possible side effects in patients whose outlook was better, but also possibly an overconfidence in the ability of this treatment to treat large volumes of tumour. This was not only ineffective but hazardous. For example blood vessels at the perimeter of the tumour treatment zone in colon and bronchial tumours were occasionally left exposed (Barr et al., 1990a; Cortese and Kinsey, 1982). It is now appreciated that given the limited penetration depth of red light the role of PDT is in the management of small volume tumours. Small tumours are best removed by surgical excision; however PDT can be used in cases where the tumour involves vital structures or where a patient is unable to undergo surgery. For PDT to be effective light must be able to reach all parts of the tumour which
is best achieved in small tumours. It is a minimally invasive therapy, it is usually painless, it does not have the systemic side-effects of chemotherapy, it necroses all tumour types and does not have the problem of development of tumour resistance seen with chemotherapy (Stewart, 1993).

3.5.1 PDT and Bronchogenic Carcinoma:

PDT of endobronchial tumours was one of the first clinical applications of PDT dating from the early 1980's (Hayata et al., 1982; Vincent et al., 1984; Kato et al., 1986). Most of the work has been performed with photophrin, however recently there has been interest in treating early endobronchial carcinomas with mTHPC (Van den Bergh, 1994). Early treatments were in large obstructing tumours with a palliative intent to open obstructed airways (Balchum and Doiron, 1985, McCaughan et al., 1986). Post treatment haemorrhage was seen in some cases and was thought to be due to the treatment not being deep enough to incorporate all of the tumour vasculature. All grades of endobronchial tumours have been treated however, including carcinoma in situ (Hayata et al., 1984; Kato et al., 1989). In these early tumours PDT is used for cure of small tumours in high risk surgical patients because of co-existing cardiovascular or lung disease. The technique offers preservation of lung tissue by treating early stage lung cancers as conservatively as possible. Overall over 500 patients have been treated with PDT for endobronchial malignancy. There are consistent complete and partial remission rates ranging from 70%-100% (Marcus and Dugan, 1992). Disease free intervals following PDT of early endobronchial tumours have been observed for up to 11 years.

The largest experience has been by Kato in the Tokyo Medical School (Kato and Okunaka, 1995). They have treated over 200 patients with PDT including 59 early stage lung cancers (Stage 0), 26 Stage 1, 10 Stage 2, 80 Stage 3, and 24 Stage 4. The best results have been observed in early stage lung cancer. Of the stage 0 group (69 lesions in 59 cases) all were squamous cell carcinomas except for one adenocarcinoma. After treatment they defined complete remission as when no tumour was observed by biopsy and/or brush cytology for at least 4 weeks; partial remission was a reduction in tumour volume greater than 50% but with persisting cancer on biopsy or brushing at 4 weeks. Complete remission was obtained in 45 out of 69 lesions (65.2%). However the partial response in 24 lesions was due to the inability to visualise the entire extent of the lesion endoscopically. These cases were given additional therapy (10 surgery, 7 radiotherapy, 6
chemotherapy, and 1 Nd-YAG laser coagulation). Recurrence was recognized in a total of 5 cases which had initially been complete responders. All of these cases were then treated by surgery and radiotherapy and a 100% complete remission rate was therefore obtained. Follow-up was from 1-152 months and 41 patients were disease free (51 lesions) during this time. However 4 patients died due to lung cancer.

A multicentre study on early stage lung cancer involving 66 carcinomas showed a 77.3% complete remission after initial PDT and 28 carcinomas which were 1cm or less in length had a 100% complete remission rate after initial PDT. Others have reported similar results (Sutedja et al., 1993). The Mayo clinic has treated more than 65 patients with radiologically occult carcinoma where the surface area of the carcinoma was $3\text{cm}^2$ or less (Cortese, 1995). The complete response rate was greater than 55%. They also have a series of 13 patients who were surgical candidates at the time of treatment but elected to receive PDT as a primary therapy. In this group 13 of 14 cancers had an initial complete response (93%) but two of the 13 developed a recurrence (15%). One patient failed to have a complete response. Therefore overall 10 of the 13 patients (77%) were spared a surgical procedure. Six patients received a complete response after a single session of PDT while 4 patients achieved this after a second session of PDT. The range of follow-up was 16-49 months.

This group as well as Kato's group emphasize the importance of tumour selection in application of endobronchial PDT for cure. The usual recommendation is to treat tumours less than 1cm in their longest dimension and where all of the length of the tumour is visible endoscopically. Furthermore pre-treatment screening should demonstrate no regional lymph node involvement. Importantly in these early lesions adjacent lymph nodes are usually free of carcinoma. Hayata reported in 1993 that of the 13 cases of carcinoma in situ resected at their hospital there was no lymph node metastasis (Hayata et al., 1993). Also in 92 patients in another series of occult lung cancer no evidence of lymph node metastasis was seen in tumours less than 2 cm in diameter (Nagatomo et al., 1989). Complete remission was not obtained in lesions which were anatomically difficult to photoirradiate or where the tumour was in a submucosal location if photoirradiation from an angle of 90 degrees to the surface of the lesion was not possible. Complete remission also was not achieved where the tumour extended beyond the cartilage or in extensive lesions. Alterations in fibre design including the application of cylindrical quartz fibres with a
divergence of 360 degrees and increased laser power have meant that better light delivery to all of the tumour surface is now possible (Van den Bergh et al., 1995).

Endobronchial PDT can be used to treat synchronous or metachronous lung tumours (Cortese, 1995). Other applications include the pre-operative treatment of tumours overlapping the carina or other sites where shrinkage of tumours allows subsequent surgical resection (Kato and Okunaka, 1995). Kato has reported 24 patients receiving this form of treatment 21 of whom went on to have surgery which either had not been previously possible or was less extensive.

Recently results were reported of a series of patients treated with mTHPC for early squamous cell carcinomas of the bronchus and oesophagus (Grosjean et al., 1997). PDT was performed because of inoperability due to severe cardiopulmonary disease, or patient preference for a minimally invasive therapy as opposed to surgery. 16 bronchial tumours were treated ranging in maximum dimension from 0.3 to 2 cm (mean 0.9 cm). Of these there was a complete response in 13 with no tumour visible bronchoscopically or on biopsy and brushings with follow-up of up to 38 months, (mean 12 months). The other 3 cases had incomplete responses possibly due to positioning of the tumour at bronchial spurs where the complex geometry prevented homogenous light delivery to the mucosa. This aspect of endobronchial PDT is the subject of intense research to develop better light delivery mechanisms (Van den Bergh, 1994). The only adverse event in the bronchial tree was 1 case of bronchial stenosis. The results indicate that mTHPC is at least as effective as the first generation photosensizers in this setting. Furthermore bronchial perforation was not seen in these treatments of early carcinomas whereas it is a theoretical risk in treatment of large invasive carcinomas. Also bronchial cartilage does not take up photosensizers including mTHPC and AIS₂Pc (Smith et al., 1993).

3.5.2 PDT as adjunctive treatment in mesothelioma

Mesothelioma has also been treated with mTHPC PDT using specially developed surface illuminators applied to the pleural surface under direct vision via thoracotomy (Ris et al., 1991). This small series of 4 cases was the first reporting the clinical use of mTHPC. Depths of necrosis of 1 cm were seen with this technique and are amongst the largest reported for PDT. No damage to aorta, oesophagus or nerve ganglion cells was seen, even when
full thickness necrosis occurred adjacent to these areas. Adverse effects reported in this series included mild skin photosensitivity up to 14 days and severe chest pain and fevers, probably due to the large area of tumour and chest wall treated. Pass in 1994 reported use of intrapleural PDT with Photofrin in 42 patients as an adjunct to debulking surgery for mesothelioma (Pass et al., 1994). Most tolerated the procedure well however some complications occurred including empyema, bronchopleural fistula and oesophageal perforation. The surgery was extensive including pneumonectomy and lobectomies, and the complications may have been due to the surgery rather than the PDT itself. These authors and others are now undertaking phase 2 and 3 clinical trials (Takita et al., 1994).

3.5.3 Interstitial photodynamic therapy

A number of authors have investigated the effects on normal tissue in animal experiments (Chang et al., 1996a; Pantelides et al., 1990). Chang used mTHPC interstitial PDT with the fibres placed transperineally with the aid of a transrectal ultrasound probe. With 4 fibres necrotic lesions up to 35 x 25 x 22 mm occurred, covering more than 80% of the total prostate volume. Two fibre treatments gave smaller volumes of necrosis but demonstrated that localised necrosis of small volumes of tumour can be performed if this is all that is required. Therefore as other reports suggest that PDT of adenocarcinomas is at least as effective on tumour tissue as the surrounding normal tissue, it is likely that any part of the normal prostate which can be necrosed by PDT can also be necrosed if it is replaced by cancer (Barr et al., 1990a; Chang et al., 1996a). The lesions healed by scarring in the stromal areas however there was complete regeneration of the prostatic urethra (which had initially shown haemorrhagic necrosis) with no stricture formation. 2 of the 7 animals however had acute urinary retention after the treatment, requiring catheterisation up to 5 days before resuming normal urination. There was no effect on the fibrous prostate capsule in any case and effects on the adjacent rectum were only minor. It was likely that the white coloured capsule reflected light back into the organ reducing the transmission of light to adjacent tissues. This study demonstrates some of the interesting and clinically useful features of PDT. Firstly that full regeneration of epithelial structures occurs and secondly that necrosis only occurs where light is transmitted thereby limiting effects on adjacent vital structures.

Interstitial PDT may become a useful modality for managing localised prostate cancer and pilot studies using mTHPC are underway at the
Middlesex Hospital. Windahl reported 2 patients treated for residual prostate cancer after transurethral resection of the prostate. He used the transurethral route. Both patients had significant reductions in prostate specific antigen after PDT (Windahl et al., 1990). One subsequently died of a second primary tumour (bronchial) and had no residual prostatic disease at post mortem.

There are few other reports of the use of interstitial PDT. Lowdell and colleagues reported an exploratory study of 50 interstitial treatments of subcutaneous and cutaneous tumours in 9 patients using photofrin (Lowdell et al., 1993). Here the objective was to treat large volumes of tumour while limiting the extent of skin necrosis which occurs with surface PDT. They used cylindrical diffuser fibres positioned using 19 G needles under local anaesthetic. Up to 8 fibres were placed for larger tumours which had volumes of up to 60 cm$^3$. Complete responses at 1 month were seen in 81% of tumours treated at the highest drug and light doses. Necrosis occurred up to 16 mm from the fibres. Longer followup was not reported. With treatment of larger tumours large areas of skin necrosis occurred; however, there was complete healing of these with no scarring usually by 1 month. This was thought to be due to the preservation of subcutaneous collagen allowing healing to occur by regeneration rather than by scarring and could not have occurred if radiotherapy had been used.

Interstitial PDT of a pancreatic tumour using mTHPC has been performed on 1 patient at the Middlesex Hospital (D Whitelaw, 1997, personal communication). A percutaneous radiologically guided technique was used. Well defined radiological necrosis of 3 cm diameter was seen on CT scan 3 days after treatment.

3.6 Summary of PDT

PDT has unique features as a cancer therapy, the most important of which are the excellent tissue healing after treatment and the ability to cause necrosis in specific compartments of an organ. Its efficacy has been shown in many studies. Endobronchial PDT for early bronchogenic carcinoma has been one of the most commonly performed procedures and demonstrates the susceptibility of these tumours to PDT. Interstitial PDT can cause large volumes of necrosis deep inside tissues with minimal toxicity. Therefore this form of therapy could potentially be applied with curative intent to small peripheral lung tumours in medically inoperable patients.
Chapter 4 Rationale and objectives of the thesis
In patients who have localised lung cancer who are unable to undergo surgery cure may be possible if the localised tumour can be eradicated in situ. Up till now the main alternative to surgery in this situation has been radical radiotherapy. The previous introduction has highlighted some of the short-comings of radical radiotherapy in this situation.

There are two interstitial laser therapies which are currently being applied to tumours in other organs which could be applied to lung tumours. Lung tumours are suitable for this type of therapy as they are solid tumours surrounded by normal parenchyma. As previously discussed these two therapies are interstitial laser photocoagulation and interstitial photodynamic therapy. Both could be applied by passing the laser fibre into the centre of the tumour through an introducer needle passed percutaneously in the manner of a fine needle aspiration biopsy under local anaesthetic with CT guidance.

This thesis therefore has as its rationale the need to find an alternative therapy for patients with small tumours who are unable to undergo surgery and seeks to determine the feasibility of this new treatment approach.

The ability of interstitial laser photocoagulation and photodynamic therapy to cause tumour necrosis has been well documented. Adenocarcinomas in the liver and prostate and squamous cell carcinomas in the head and neck have been effectively treated with both interstitial laser photocoagulation and interstitial photodynamic therapy. It is also well known that endobronchial squamous cell carcinomas are susceptible to photodynamic therapy when applied superficially. There is no reason to presume that parenchymal lung tumours will differ from endobronchial tumours in their response to these therapies. What is unknown however is what the response of normal lung parenchyma will be to these two forms of treatment and whether in fact this type of treatment can even be applied in this situation. In treating tumours it is impossible to avoid some damage to normal surrounding structures. If this occurred without the normal tissue responses having been assessed beforehand then the effects of the treatment could potentially be hazardous to the patient. Furthermore it is desirable to include some normal parenchyma in the treatment field to ensure that small microscopic deposits of tumour are removed along with the main bulk of the tumour. This is a concept familiar to surgeons removing tumours in a
variety of organs. Also the size of the effects of these two types of laser treatments on normal lung parenchyma can give some idea as to the likely size of effect on tumours. The effect on tumours need only be as great as that on the normal tissue for it to be an effective treatment.

Therefore the work in this thesis concerns the effect of the two forms of laser treatment on the normal lung parenchyma. The lung is a unique organ in that it is collapsable, has a vast vascular supply and is an aerated organ. All of these factors contribute to laser tissue interactions. Furthermore the lung is an inhomogeneous organ with structures of varying size and shape and density placed side by side. Also the lung is adjacent to some vital organs including the heart, oesophagus, and great vessels. The effects on these organs also need to be assessed before the treatment can be applied to the lung itself to ensure that no adverse effects on surrounding structures occur.

The objectives of the thesis were therefore to assess the two treatment types, initially in small animals (rats) in Chapters 5 to 10, and then to assess the effects in large animals (pigs) (in Chapter 11), which have anatomy and lung structure similar to humans.

The aim with the small animal experiments was to see whether well defined lesions can be created in the lung given the variable nature of the lung structure as outlined above. Secondly the effect of changing the treatment parameters on lesion size was determined. In the case of interstitial laser photocoagulation this simply depends on changing the laser power and treatment time. With photodynamic therapy it is a more complex process requiring assessment of changes in the interval between drug administration and laser treatment and the duration of laser exposure. Furthermore the differences in size of lesion between three different photodynamic therapy drugs were assessed to determine which gave larger lesions. The macroscopic and microscopic aspects of the healing process are described; if normal lung adjacent to the tumour is treated it must be able to heal safely.

Preceding the PDT treatments fluorescence microscopy on lung sections was performed to determine the quantity and distribution of photosensitizer in the lung parenchyma after intravenous injection. This was used to find the optimum interval between drug administration and the time when light should be administered. Also by showing how the drug was distributed fluorescence microscopy gave information which assisted in interpreting the histology of the subsequent PDT effects.

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The functional impact of the treatments on the lung was also assessed; if this treatment is to be an alternative to radiotherapy it is important to know any adverse effects on the physiology of the treated lung. Also the risk of pneumothorax was assessed using fluoroscopy.

With the small animal experiments only a single fibre was used. However to treat the size of tumours seen in patients will require the use of several fibres inserted simultaneously. This is one of the other reasons that treatments were performed in large animals, as the small animal experiments could not accommodate the size of lesion required. Therefore using the large animals information could be obtained which would allow the multiple fibre treatments to be performed in patients with tumours. As described above if a spherical lesion of 3 cm diameter could be created in normal parenchyma, it would only require the tumour to be at least as sensitive to the effects of the treatment for the tumour within this volume to be ablated. Experiments in tumours of other organs have shown this to be the case. PDT lesions were also made with diffuser fibres to compare the lesion size with bare fibre tips. The macroscopic and histological features of the lesions are described both acutely, and up to 3 months after treatment to determine the healing response. Fluoroscopy at the time of treatment and subsequent Xrays were used to check for pneumothorax and any other effects of the lesions on the lung. The animals' breathing and general behaviour were also monitored to ensure no significant effects on lung physiology occurred.
SECTION B EXPERIMENTAL STUDIES

CHAPTER 5  Macroscopic effects of ILP on lung parenchyma in rats

CHAPTER 6  Microscopic appearances of ILP on lung parenchyma in rats

CHAPTER 7  Studies of photosensitizer quantity and distribution in lung parenchyma in rats

CHAPTER 8  Macroscopic effects of PDT on lung parenchyma in rats

CHAPTER 9  Microscopic effects of PDT on lung parenchyma in rats

CHAPTER 10  Effect of ILP and PDT on lung physiology and mechanical integrity.

CHAPTER 11  ILP and PDT treatments in lung parenchyma using multiple fibres in pigs.
Chapter 5 Macroscopic effects of ILP of the lung parenchyma in rats

5.1 Introduction

5.2 Methods
   5.2.1 Preliminary experiments - Ex vivo
   5.2.2 Development of the in vivo treatment method for rats.
   5.2.3 In vivo treatments
   5.2.4 Assessments on treated lungs

5.3 Results
   5.3.1 Ex vivo ILP
   5.3.2 In vivo treatments
   5.3.2.1 ILP lesion appearance
   5.3.2.2 Lung volume measurement.
   5.3.2.3 Angiograms
   5.3.3 Complications of treatment

5.4 Discussion
5.1 Introduction

This chapter describes the macroscopic effects of interstitial laser photocoagulation on normal lung parenchyma. Because this had not been done before preliminary experiments were performed on ex vivo lungs to determine the general nature of the lesions. Most of the experiments were then performed in vivo. The methods used for this were adapted from in vivo experiments of ILP in other organs but required some novel modifications due to the inherent difficulties caused by the shape of the chest wall and proximity of the lung to vital structures. Experimental treatments in other organs such as the liver and pancreas have been performed by inserting the fibre under direct vision at laparotomy into the organ. In the lung however the treatments were performed with a closed chest, inserting the laser through the chest wall into the lung. Although this initially made the experimental technique more difficult, it was preferable to perform the treatments in this way as thoracotomy would have made the procedure unnecessarily extensive for the animals, and treatments will be performed through a closed chest in patients.

5.2 Methods

5.2.1 Preliminary experiments EX VIVO

The first treatments were performed ex vivo in lungs removed from rats and pigs. Lungs were inflated with air and the laser fibre inserted into the parenchyma. In the pig lungs the fibre was inserted with the use of an introducer needle which was then withdrawn a short distance leaving the bare laser fibre tip exposed. A comparison was made between the size of lesions made with fibres which were pre-charred and with normal fibres. Charring was induced by placing a small drop of blood on the fibre tip and activating the laser at 10 W for 3 seconds (Amin et al., 1993b). These treatments were performed with laser parameters of 2 watts for 500 seconds using a diode laser, wavelength 805nm (Diomed, Cambridge.). Lungs were sectioned perpendicular to the direction of laser fibre entry into the lung. These sections were ellipsoid in shape and the lesion size was taken as the largest dimension of the section. Histological confirmation of thermal necrosis was made and is described in the next chapter.
5.2.2 Development of the in vivo treatment method for rats.

All experiments were performed under general anaesthetic. After shaving the fur on the left hemithorax a verres needle was inserted into the pleural space via a small incision. This needle has a spring loaded blunt tip which covers the sharp part of the needle tip once the pleural space is entered. It therefore prevents the introducer needle from penetrating the lung surface. Once this was in place the laser fibre was then inserted down the needle and pushed into the lung. Although this avoided puncturing the lung with a relatively large needle it was a problematic technique as some treatments only showed laser effects on the pleural surface indicating the laser fibre did not always enter the lung parenchyma. Subsequent treatments were therefore performed by inserting a 19 G introducer needle containing the laser fibre directly through the intercostal membrane into the lung. The introducer needle was then withdrawn a short distance leaving the laser fibre exposed. The rats tolerated this well.

Finding the optimal site for needle insertion was also problematic initially. The left lung was chosen as this has only one lobe allowing easier assessment of the lesion size without interference from interlobar fissures. Preliminary dissections had shown that the greatest volume of lung which would be likely to accommodate a laser lesion of up to 1 cm in diameter was to be found in the lower part of the left lung. Therefore the lower part of the left lung was chosen as the site of treatment. The first trials were performed with the rat in a supine position passing the needle through the anterior chest wall. Although this successfully caused lesions in the lung, in a number of animals the laser fibre appeared to have penetrated the diaphragm at its uppermost point causing laser effects in the liver immediately beneath it.

Subsequent treatments were therefore performed with the rat in a lateral position. Preliminary dissections allowed the use of surface landmarks to determine a site for entry. Initially rats were placed in a prefabricated mould with the aim of having each rat in an identical position to allow reproducibility of treatment site. Despite these efforts however there was still variation in the site on the lung which was actually treated. Lesions were either too far posterior affecting the spinal cord, too far anterior missing the lung or too far rostrally hitting the aorta.
Two changes were therefore made. Firstly Xray was used to show the position of the lung without the need for relying on surface landmarks. Secondly a frame was made into which the anaesthetised rat was placed in a standard lateral position. The frame held the needle and allowed accurate positioning of the needle over the chest wall. By the simultaneous use of fluoroscopy the needle site was accurately determined in most subsequent cases and the frame allowed the necessary small adjustments of needle position either in an antero-posterior or rostral-caudal direction (Figure 5.1).

5.2.3 In vivo treatments

A diode laser, wavelength 805nm (Diomed, Cambridge UK.) was used to deliver laser powers ranging between 1 and 3 Watts. The laser fibres were 400 um core diameter glass fibres with silica cladding. These were cleaved and calibrated before each treatment and 5 mm of bare fibre was left exposed after paring back the cladding. All treatments were performed with fibres which were precharred (Figure 5.2).

Normal male Wistar rats weighing between 300 and 350 g were used. Treatments were performed under inhalational general anaesthetic using halothane and oxygen. Rats were placed in the right lateral position and the fur over the left hemithorax was shaved. A longitudinal 1 cm incision was made in the left lateral chest wall. By blunt dissection the intercostal membrane of the 5th or 6th interspace was exposed. The rat was then placed in the frame as described above. An image intensifier was used to position the introducer needle over the mid point of the lower part of the left lung. Then the 19 G introducer needle containing the laser fibre was passed perpendicularly through the intercostal membrane into the lung. The needle was then withdrawn keeping the tip of the laser fibre at the original depth for the actual treatment.

After each treatment fluoroscopy was used to exclude pneumothorax. Conventional chest Xrays were performed after sacrifice before opening the chest at times from 3 days to 6 months following treatment.

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Figure 5.1 (A) Experimental set up for ILP treatments in rats. Shows the frame holding the introducer needle through which the laser fibre is passed. (B) Fluoroscopic view-lateral. (C) Fluoroscopic view- antero-posterior.
5.2.4 Assessments on treated lungs

The acute lesion size was assessed 3 days after treatment for a range of powers 1-3 W and time exposures giving a total of 500 or 1000 J for each power. For the experiments to study healing the treatment parameters were 2W for 250 seconds, (500 joules total energy). Rats were killed at 10, 21, 60, 120 and 180 days after treatment. At post mortem the lungs and mediastinum were removed en-bloc in a standard manner (Freeman et al., 1972). A cannula was placed in the trachea and tied off, after which 10% formaldehyde was run in at 25 cm pressure to inflate the lungs. The trachea was then tied off and the lungs and mediastinum placed in formaldehyde. After 3 days the left lung was sectioned at approximately 1mm intervals in a plane perpendicular to the line of the laser fibre insertion. Lesions were measured by taking the largest diameter on these sections and necrosis was confirmed by histology in representative sections as described in the next chapter. This method has been used by others to compare different laser treatment parameters (Matthewson et al., 1987).

The volume of the left lung was measured following ILP to determine if any acute or chronic shrinkage of the lung occurred as a result of the treatment. After sacrifice lungs were removed and the trachea cannulated. The lungs were then filled with formaldehyde at a pressure of 25 cm of water until the pleural surface was smooth. The left main bronchus was ligated and the lung was dissected free carefully removing any attached structures. The volume of the lung was measured by displacement in water. Measurements on rat lung volumes have been performed by others with a similar method (O'Neil and Raub, 1984). By using formaldehyde as the inflating agent the lungs were then able to be subsequently assessed by histology. The volumes were compared to the volume of left lungs from weight matched animals. Measurements were performed immediately after ILP and at 2, 4, and 6 months. The ILP parameters were 2 W for 250 seconds and 2 W for 500 seconds at the immediate time point (8 rats for each of these treatment parameters), and 2 W for 250 seconds for the remainder of the assessments (at least 4 rats at each point).

Angiograms were performed to demonstrate any effects of the ILP lesion on parenchymal vessels. Immediately after sacrifice a cannula was placed into the main pulmonary trunk and secured. Normal saline was initially injected to flush out the blood in the vessel, then 0.5 ml of a dilute mixture of barium and gelatin was injected into the pulmonary circulation via the cannula.
When the gelatin solidified it kept the barium fixed in the vessels. Xrays were then taken of the lung using mammography equipment.

5.3 Results

5.3.1 Ex vivo ILP

The effects on ex vivo lungs were firstly creation of a well circumscribed ILP lesion and secondly shrinkage of the lung at the site of the lesion. Lesions were ellipsoid in shape in the plane of the laser fibre with the widest region close to the position of the tip of the fibre. The lesions were circular on cross section. There was central charring of lung tissue surrounded by thermally coagulated tissue which appeared dark grey (Figure 5.3). Necrosis of this outer part of the lesion was confirmed by histology discussed in Chapter 5.

In the rat lungs lesions created using 2-3 W could be accommodated in the lung spreading in a radial direction from the tip of the laser fibre. In contrast when treating with 4 or 5 watts the lesions tended to enlarge in a forward direction penetrating directly out of the lung. The laser has a red aiming beam at its tip which was visible within the lung parenchyma at the start of the treatment. After approximately 1 minute this aiming beam could no longer be seen due to the commencement of tissue charring at the laser fibre tip. As the treatment proceeded it was also apparent that there was shrinkage of parenchyma with lung being drawn in towards the thermal centre of the lesion.

Figure 5.4 shows the effect of precharring on lesion diameters as measured perpendicular to the direction of treatment in the ex vivo pig lungs. There was a significant increase in lesion size (T-test, P<0.01). Furthermore there was less variation in lesion size in the pre charred fibre treatments. On the cut surface after both treatments the lesion showed a charred centre within which there was a small zone of complete ablation of lung tissue. The size of this zone within the overall lesion was slightly larger with the precharred fibres, though this was not a significant increase.
Figure 5.2 Appearance of normal and pre charred (i.e. +) bare tipped laser fibres for ILP.

Figure 5.3 Macroscopic appearance of ILP lesion in ex vivo lung. Lesion sectioned longitudinally in the plane of laser fibre insertion into the tissue.

Figure 5.4 Chart of lesion size in ex vivo experiments using charred and normal laser fibres. 6 treatments with 2W for 500 seconds with each fibre type.
Figure 5.5 Macroscopic appearance 3 days after in vivo ILP in rats, 2W for 500 seconds. (A) Lung surface, posterolateral and posterior views. (B) Cut surface, perpendicular to line of laser fibre insertion.
5.3.2 In-vivo treatments
5.3.2.1 ILP lesion appearance

As with the ex vivo experiments the lesions were ellipsoid in shape. Three days after treatment on the cut surface perpendicular to the fibre direction there was a central area of charred tissue surrounded by sequential rings of pale then hemorrhagic tissue (Figure 5.5b). There was a sharp demarcation between treated and normal tissue. These appearances were also evident on the pleural surface immediately superficial to the parenchymal ILP lesion. Here there was shrinkage of the lung surface due to the thermal effects (Figure 5.5a).

Lesions were highly reproducible in size; 3 days after lesions produced with 2W for 500 seconds in 10 rats the mean lesion diameter was 8.7 mm with a standard deviation of 1.1 mm. Figure 5.6 shows the mean lesion sizes at 3 days by different treatment parameters. The maximum diameter of necrosis seen was 12 mm using 3W for 333 seconds.

![Diagram](image.png)

Figure 5.6 Effect of changing laser parameters on lesion diameter as measured 3 days after ILP. At least 3 animals at each point (Mean, Standard deviation)
By 3 weeks there was puckering of the lung surface adjacent to the ILP lesion which persisted up to 6 months (Figure 5.7). By 2 months the lesion had shrunk and the necrotic tissue had been predominantly replaced by white scar tissue. This process appeared complete by 2 months as lesions appeared the same at 6 months (Figure 5.8). In all cases except one there was no adhesion between the parietal and visceral pleura. In the case where adhesions did occur the parietal pleura had been inadvertently treated as well. Figure 5.9 shows the size of lesions at times up to 6 months following initial ILP treatment of 2W for 250 seconds. It demonstrates that shrinkage of the lesion was complete by 2 months.

5.3.2.2 Lung volume measurement.

Figure 5.10 compares the volume by displacement of lungs treated with ILP compared to untreated controls. In the lungs measured immediately after treatment the amount of shrinkage was greater at higher delivered energies; the volume after 2W for 500 seconds being significantly less than controls (p<.01). There was persisting shrinkage of the lung at 2 and 4 months although this was not a significant difference. This shrinkage at later times was due to fibrosis of the ILP lesion (as demonstrated on histology) rather than the thermal shrinkage of the lung which occurred immediately after treatment.

5.3.2.3 Angiograms

Angiograms in 6 rats with typical ILP lesions performed immediately after treatment showed obliteration of the microvasculature in the immediate vicinity of the treatment field (Figure 5.11a). In 2 cases angiograms were performed where the ILP lesion was directly placed at a central vessel. These demonstrated occlusion of the vessel, although no leak of dye occurred indicating that the vessel was sealed (Figure 5.11b). Angiograms performed in 4 rats 2 months after ILP showed no disturbance of the vascular architecture.
Figure 5.7 Appearance of lung surface 4 months after ILP2W for 250 seconds

Figure 5.8 ILP lesion 6 months after ILP( 2W for 250 seconds).
Figure 5.9 Chart of lesion size up to 6 months after ILP. At least 4 animals at each point. All had initially been treated with 2W for 250s.

Figure 5.10 Chart of left lung volume in treated rats compared to controls at times up to 6 months. At least 4 rats at each point. Volume significantly reduced for ILP2W500 seconds immediately after treatment (p<.01). No other significant differences. The 6 month rats were larger and were compared to a different set of controls.
Figure 5.11 Angiograms of rat lungs immediately after ILP. (A) Loss of microvasculature at lesion site. (B) Occlusion of larger vessel at lesion site.
5.3.3 Complications of treatment

Of the 130 rats treated with ILP, 90 developed no complications. Table 5.1 lists the complications which did occur. These were mainly due to the small size of the animal used in these experiments, which meant that the tip of the laser fibre was inevitably close to major structures including the hilar vessels, major bronchi and the oesophagus. By necessity the lesions needed to be created near the oesophagus as most of the lung volume is located posteriorly.

<table>
<thead>
<tr>
<th>Time when complication occurred</th>
<th>Immediate</th>
<th>Up to 3 days</th>
<th>Up to 3 weeks</th>
<th>Up to 6 months</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumothorax</td>
<td>8 (3)</td>
<td></td>
<td></td>
<td></td>
<td>8 (3)</td>
</tr>
<tr>
<td>Vascular effects</td>
<td>5 (5)</td>
<td>4</td>
<td>2 (2)</td>
<td></td>
<td>11 (7)</td>
</tr>
<tr>
<td>Lung and mediastinal infection due to oesophageal perforation</td>
<td>15 (9)</td>
<td>1 (1)</td>
<td>1</td>
<td>17 (10)</td>
<td></td>
</tr>
<tr>
<td>Lung abscess without oesophageal perforation</td>
<td>2</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Lobar collapse</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 5.1 Complications of ILP. Numbers in brackets indicate the number which were sacrificed or died due to the complication. No rats had more than 1 complication.

Figure 5.12 gives an example of a pneumothorax as shown by fluoroscopy immediately after ILP. The cause of pneumothorax in 4 rats was inadvertent laser treatment of the intercostal membrane creating a hole allowing air to be drawn into the chest cavity (Figure 5.13). In 2 rats the laser lesion extended from the outer surface of the lung to the inner surface, creating an open channel. In one rat where the fibre was placed too deeply the main bronchus was perforated by the laser effect. In rats where no pneumothorax was seen on fluoroscopy at the time of treatment no rat subsequently developed a pneumothorax, as determined by conventional chest radiographs performed immediately after sacrifice at times from 3 days up to 6 months after treatment. Therefore there was no chronic airleak from the laser treated area. This shows that laser lesions which seal the lung parenchyma at the time of treatment do not subsequently break down to cause a pneumothorax.

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Figure 5.12 (A) Fluoroscopic appearance of pneumothorax in rat after ILP (B) Normal lung

Figure 5.13 Inadvertent ILP effect on chest wall which lead to pneumothorax.
Where the laser lesion was sited immediately adjacent to the oesophagus a small perforation occurred in the wall of the oesophagus (Figure 5.14a). In the short term this caused macroscopic mediastinitis which was usually fatal. Barium contrast injected into the oesophagus in these specimens escaped through the perforation and entered the mediastinum as well as the adjacent lung parenchyma (Figure 5.14b, c). This resulted in acute and chronic lung infections, the histology of which is described in the next chapter. Late lung infections were seen in 4 rats after 3 weeks. Two of these were in animals which had an oesophageal perforation while in 2 no perforation could be demonstrated. This is discussed further in the next chapter.

Acute lung congestion peripheral to the laser lesion was seen in 9 rats (Figure 5.15a). Here the lesion was close to the hilum at the site of the pulmonary veins and lymphatics draining the lower part of the lung and as a result capillaries and larger vessels distal to this showed intense engorgement. Five of these rats died during the procedure due to extensive lung oedema. The remaining 4 did not show any effects of this complication but were sacrificed at 3 days for lesion size assessments. Major haemorrhage due to laser penetration of vessels was not seen, even where the lesion was directly on a vessel (Figure 5.10b).

In 2 cases there was apparent infarction of a large segment of the lung due to ILP damage of the blood supply to that region. This caused significant breathlessness and malaise and necessitated sacrifice in both cases. In 2 rats there was collapse of a segment of the left lung distal to the ILP lesion which had obstructed the major bronchus in these cases (Figure 5.15b).
c. 
Figure 5.14 Effect of ILP on oesophagus (A) Before and (B) after injection of barium into oesophagus. (C) Lung adjacent to the oesophagus showing entry of barium into parenchyma.

Figure 5.15 (A) Acute lung congestion peripheral to an ILP lesion. (B) Collapse of lung distal to ILP lesion.
5.4 Discussion

These studies have shown that it is technically possible to treat normal lung parenchyma with ILP. In treating patients with tumours one or more laser fibres will be inserted into the tumour, but some surrounding normal tissue (1-2cm) must be treated as well to maximise the chances of complete tumour ablation (Ginsberg, 1993; McIntosh and Thatcher, 1990). While complications have occurred in these experiments the risks can be minimised in clinical use with selection of tumours away from vital structures and by careful placement of the fibre tips under CT control.

In thoracic medicine lasers have been used at high powers primarily for the endoscopic palliative debulking of endobronchial malignancies (Hetzel et al., 1983; Dumon et al., 1982). This chapter has described a means of treating specific areas of lung parenchyma with a minimally invasive technique using low laser power. ILP has been applied successfully in other organs such as the liver where there were small tumours surrounded by normal tissue (Amin et al., 1993a). The size of lesions in the lung was comparable though slightly smaller than those seen with ILP in the normal liver which may be due to the aerated nature of the lung with high vascularity. Both these factors could be important in dissipating heat away from the lesion and consequently limiting its size.

Conversely the lesions made in vivo were slightly smaller than those made ex vivo which is probably due to the effects of flowing blood and the movement of air in air passages adjacent to the ILP lesions (Bown and Hetzel, 1995). The ex vivo experiments also confirmed that precharring the fibres was important in terms of creating lesions which were generally larger and more reproducible in size. Others have documented the importance of precharring in both tumour and normal tissue (Harries et al., 1994b; Amin et al., 1993b). Harries showed that in breast tissue the size of ILP necrosis was doubled and that precharring gave a more predictable lesion diameter. Therefore as for other organs, in the lung it is important that an ILP lesion quickly develops a charred centre, becoming the point heat source from which further heat dissipates into the tissues.
The size of the lesions for a given set of laser parameters was highly reproducible and increased in diameter at higher powers. At a given power longer exposures gave larger lesions although at 3W the increase was only comparatively less than with 1 and 2 W. This was most likely because at 3W the threshold for heat to cause tissue damage was reached more quickly, so that treating for longer had comparably less effect than with 1 and 2 W. In experiments of ILP in normal liver Matthewson demonstrated a plateau effect on lesion size above a total energy of 1000 J for treatments using 1-2 W; at 1000J lesion sizes were similar for 1 and 2 W (Matthewson et al., 1987). The results in these rat lungs do not show this plateau effect at 1 and 2 W, however this may have become apparent if the total delivered energy was increased for example to 1200 or 1500 J. It may be that it takes longer in the lung than the liver for the ILP lesion to reach an equilibrium between the effects of heat at the centre of the lesion and cooling by blood flow at the periphery of the lesion. At 3 W however the lesion sizes suggested that a plateau effect was starting to occur, probably because this equilibrium was reached more quickly than at 1 and 2 W. This equilibrium effect, and the results of the ex-vivo studies showing "forward firing" of the lesion at high powers are the reasons that ILP is best performed at 2-3 W. Forward firing causes lesions which are linear and therefore poorly suited to treating tumours which are basically spherical in shape.

The lesions healed by scarring limited to the site of injury. The macroscopic changes were complete by 2 months. Lung tissue does not regenerate to replace cells lost by thermal injury, in contrast to ILP of the liver where lesions are partially replaced by regenerating normal tissue (Steger et al., 1992a). Despite this difference lung lesions healed safely and there was no residual cavity in these experiments. There was a trend for reduction in lung volumes with the healing process which is consistent with the localised fibrosis at the ILP lesion seen macroscopically on the cut surface. However there was only minimal change in the overall contour of the lungs and the animals coped with the lesion well, which is validated in chapter 10. The size of the laser lesion with 2W 250 seconds relative to the whole lung in these rats, approximately 13% of the volume, is comparable to that required in a patient to treat a 2 cm diameter tumour with a 2 cm normal lung perimeter ( volume 270 ml in a typical single lung volume of 3 litres, 9%) . Therefore although the volume effects with 2W250 seconds were less than with 2W 500 seconds, there was still no progressive shrinkage of the lung due to a lesion which was not insignificant in size compared to the size of the lung.
Most of the complications seen were due to the small size of the animal treated and were related to treatment of the medial aspect of the lung in the region of the major bronchi and vessels as well as the oesophagus which passes close to the posterior aspect of this region. Oesophageal perforation led to mediastinitis and infection of the laser treated part of the lung. Patients with tumours adjacent to the oesophagus would therefore not be suitable for ILP. In the 2 rats with lung abscess where no oesophageal lesion was seen it is possible that major bronchial wall perforation by the laser may have allowed entry of bacteria into the laser wound as some bacteria normally exist in the major bronchi. If a peripheral bronchus was penetrated it is unlikely that infection would result as these bronchi are sterile. It may be advisable, however, to place patients on prophylactic antibiotics at the time of treatment in view of the small chance of (inadvertently) penetrating a more medial bronchus. Also there is the theoretical risk of infection arising in the necrotic tissue due to bacteremia which could also be reduced by antibiotics at the time of treatment.

A small amount of haemorrhage occurred in the perimeter of the laser treated area which is typical of thermal effects observed in other organs. Major haemorrhage due to perforation of a pulmonary artery was not seen. Haemorrhage has not been a problem in clinical ILP of liver tumours, including where treatments are performed close to large vessels (Amin, 1993). This is due to the vessels acting as a heat sink. In studies on the liver, angiograms showed that the small vessels in the laser treatment area up to 1.5 mm in diameter were totally obliterated (Matthewson et al., 1987). The occlusion of 1-2 mm diameter vessels in this study where the lesion was placed immediately upon them was in keeping with this. Small vessel damage is likely to be a mechanism of ILP tumour destruction, therefore this was a positive finding. In 2 cases there was long term ischaemia of the normal lung distal to the lesion due to vessel occlusion. This complication is unlikely to occur in patients as only peripheral tumours would be treated and there is an extensive collateral circulation. In the most peripheral treatments there could possibly be some ischemia of lung which does not have collateral circulation but this would involve only a very small volume of lung. For clinical use contrast enhanced CT scanning could be performed to detect major vessels in the vicinity of the tumour so that these may be avoided in placement of the laser fibres.
Pneumothorax was surprisingly uncommon given the relative size of the laser fibre and lesion to the size of the lung itself. By keeping the laser lesion only in the outer part of the lung and by avoiding the chest wall this problem is likely to be reduced even further in patients. The lung volume studies demonstrated acute shrinkage of the lung after ILP. This raises the possibility that the shrinkage around the ILP lesion is serving to seal it off. Virtually all tissues shrink when gently heated as with ILP (Thompsen, 1991). This was demonstrated in the ex vivo experiments where the lung appeared to be drawn in towards the heat of the ILP. Retraction and shrinkage of lung parenchyma is commonly observed in contact laser treatment of pulmonary blebs (Torre et al., 1994). The macroscopic appearance of the charring at the centre of the lesion and the surrounding rim of tissue which was immediately coagulated by ILP would also serve to seal off the hole made by the introducer needle. This is discussed further in chapter 10.

In summary the effects of low energy interstitial laser treatment on normal lung parenchyma show that reproducible well defined lesions can be created and that these heal safely. The histology of the healing process is described in the next chapter. The side effects of the treatment described in this animal model mean the treatment must avoid tumours near the oesophagus. In Chapter 11 large animal experiments are described which show that the size of lesion created using multiple fibres could allow a 2-3 cm tumour to be accommodated in the lesion.
Chapter 6  Microscopic appearances of ILP of lung parenchyma in rats

6.1 Introduction

6.2 Methods

6.3 Results

6.3.1 ILP lesions

6.3.2 Complications

6.4 Discussion
6.1 Introduction

The previous chapter demonstrated the macroscopic effects of ILP on lung parenchyma. This chapter concerns the histological effects of ILP. It is essential that histology proves that the observed macroscopic changes are in fact necrotic. Histology is required to confirm that all of the ILP lesion is in fact necrotic as the more peripheral effects are more subtle macroscopically. Histology is also useful in elucidating the pathogenesis of some of the complications of ILP described in the previous chapter. In the ex vivo experiments only the immediate effects of ILP can be seen. In in vivo treatments it takes up to 72 hours for the full extent of necrosis to become apparent, even though cell death occurred immediately at the time of treatment. The macroscopic appearances at 72 hours are a combination of the immediate effects in the centre of the lesion and the effects surrounding this which come on more gradually. Therefore in the ex vivo treatments a special stain, MTT diaphorase, is required to show the full extent of necrosis. Only viable cells accumulate the stain. This stain is not needed for the in vivo specimens because after 72 hours the full extent of necrosis can be confirmed by conventional histology.

6.2 Methods

Histology was performed on the specimens used for the treatments in the previous chapters. For the analysis of ex vivo specimens tissues were frozen and cut perpendicular to the line of the laser fibre insertion. The MTT diaphorase stain differentiates between viable cells which have an intact electron transport chain and non viable cells which do not. MTT (3- (4,5-dimethyl-thiazoyl-2)-2,5-diphenyl tetrazolium bromide) is an intermediate electron carrier in the electron transport chain. Normally this compound becomes oxidised when the enzyme NADH diaphorase couples the oxidation of NADH and cytochrome C in the electron transport chain. If this occurs the MTT becomes reduced to a dark green colour. In non viable cells the electron transport chain is uncoupled and the MTT is not oxidised and does not change colour and these cells remain colourless.

For the analysis of lungs treated in vivo paraffin blocks were made from the sections taken perpendicular to the line of laser insertion into the lung. Conventional haematoxyllin and eosin staining was used. Reticulin and elastin Van Geison stains were used to demonstrate the effects of ILP on lung collagen and elastin fibres respectively.
Electron microscopy was performed on lungs treated with ILP to determine the submicroscopic effects of ILP on the elastin and collagen fibres. Comparisons were made with fibres in the ILP lesion and in parts of the lung 1-1.5 cm away from the lesion which were normal by conventional light microscopy. Specimens were fixed in formaldehyde then cut into small blocks, soaked in ethanol and propylene oxide then fixed in resin. Blocks were then embedded in beem capsules. An ultramicrotome was used to cut the blocks; the slices were placed on copper palladium grids then stained with uranyl acetate and lead citrate. They were examined using a JEOL 1200EX electron microscope.

6.3 Results

6.3.1 ILP lesions

H&E stained sections of lung treated with ILP ex vivo showed ablation in the centre of the lesion but only minimal effects in the parenchyma outside this (Figure 6.1a). However the diaphorase stained section showed that this zone was in fact necrotic, even though it did not appear so on the Haematoxylin and eosin stain (Fig 6.1b). It showed a clear margin of the lesion with normal tissue demonstrating the well marginated extent of the thermal lesion.

Figure 6.1 Histology of an ex vivo specimen of lung treated with ILP. Contiguous sections stained with Haematoxylin and eosin (left) and MTT Diaphorase stain (right).
The zone of nonstaining with the diaphorase stain corresponded to the zone of grey tissue outside the charred region seen macroscopically. Therefore all of the measured width of the lesion for the ex vivo experiments in the previous chapter was in fact necrotic.

Histology of the in vivo specimens confirmed that there was necrosis across the full width of the lesions as measured macroscopically. At 3 days histology showed a central charred zone with complete destruction of parenchyma including a small volume of lung tissue which had been vapourized (Fig 6.2a). Surrounding this were 2 zones of eosinophilic coagulative necrosis with the more central zone showing having a "condensed" appearance (Figure 6.2 b). There appeared to have been immediate coagulation of proteins in this more central zone, whereas the outer zone of coagulative necrosis had most likely been the result of lower temperatures and occurred more gradually. In these zones necrosis was evident in alveolar walls as well as bronchioles and small vessels (Figure 6.3). Surrounding this there was a perimeter of haemorrhagic tissue where leakage of red cells had occurred from sublethally injured small vessels. This was in contrast to the inner zones where the coagulating effect of the heat on the vessel had immediately sealed it. There was a distinct demarcation between the laser lesions and normal lung.

Outside this there was viable lung which contained an acute inflammatory cell infiltrate (Figure 6.4). There was type 2 pneumocyte hypertrophy indicative of sublethal injury in this normal lung adjacent to the ILP lesion (Adamson and Bowden, 1974). Collagen (Figure 6.5) and elastin stains showed complete destruction of connective tissue at the site of the lesion but no effect on the lung parenchyma surrounding it.

Electron microscopy of the ILP lesions immediately after treatment showed expected severe damage to alveolar walls, pneumocytes and endothelial cells. The elastic fibres in the centre of the lesion were damaged to variable degrees with a "motheaten" appearance, although not totally obliterated (Figure 6.6a). Collagen fibrils appeared to be fused together. In contrast elastic fibres at the periphery of the lesion and in sections of lung 1 cm distant from the lesion showed no effect on the elastic fibres (Figure 6.6b). Lesions at 3 days showed an increase in collagen close to the centre of the lesion (Figure 6.6c) presumably indicating the start of healing.
Figure 6.2 (A) Haematoxylin and eosin stain of ILP lesion at 3 days. (Magnification x 2)
High magnification of central condensed zone (3) of coagulation necrosis (x 10)
Fig 6.3 Necrotic small vessel in thermally coagulated zone of ILP. (x10)

Figure 6.4 x4 magnification of perimeter of ILP lesion. Haemorrhagic necrosis(5), inflammatory infiltrate(6) and normal lung(7).
Figure 6.5 Reticulin stain of ILP lesion at 3 days, showing complete destruction of collagen in the centre of the lesion and distortion of fibres in the remainder of the lesion. Fibres in surrounding tissue appear normal.

Figure 6.6 Transmission electron microscopy of lungs after ILP. Large arrows show collagen, small arrows show elastin. Magnification x10,000. (A) Centre of lesion immediately after treatment showing collagen destruction (B) 1 cm away from lesion immediately after treatment showing normal collagen and elastin (C) Increase in collagen fibres in lung adjacent to the centre of the lesion 3 days after treatment.
At 10 days the surrounding tissue was becoming organized into granulation tissue with an increase of fibroblasts and macrophages, and dilatation of blood vessels at the perimeter of the lesion (Figure 6.7). The central charred zone persisted. Remnants of silica laser fibre cladding from the time of treatment were evident at this time. These were surrounded by foreign body giant cells. Bronchial walls which had been breached by the laser effect were starting to heal with replacement by granulation tissue.

After this time, there was progressive ingrowth of granulation tissue into and replacing the original area of necrosis (Figure 8a). The charred perimeter of the central zone remained. There was a neat cut-off between the lesion and normal tissue. The whole lesion was contracted relative to its initial size and as a result there was some mild dilatation of airways and alveolar spaces in the immediate surrounding normal lung. The final appearance was of a granuloma containing remnants of charred material (Figure 6.8b). This was complete at 2 months as the histological appearance was the same at 2 and 6 months.

Figure 6.7 Histology of ILP at 10 days.
Figure 6.8 Healing of ILP lesion (A) 3 weeks and (B) 2 months after ILP 2 W for 250 seconds.
6.3.2 Complications

Thermal damage of major hilar arteries and associated veins was seen on histology in cases where the lesion was close to the hilum (Figure 6.9a). Two different effects occurred as a result of thermal damage to these vessels; firstly an acute congestive process and secondly a chronic infarction of the lung (Figure 6.9b and c). As described in the macroscopic findings of the acute congestive process, bleeding and alveolar oedema occurred, but only in parts of the lung peripheral to the ILP lesion. It appeared therefore that this congestion was due to obstruction to venous outflow from the lung by the coagulated hilar veins.

Major intrapulmonary haemorrhage did not occur. This was also shown in the histology of lungs described in the previous chapter which had angiograms showing cut off of the major vessel due to the ILP lesion. There was coagulative necrosis of the vessel wall however this had not resulted in a space occupying haematoma on both sides of the ILP lesion. These sections did demonstrate some alveolar haemorrhage although this was only on the side distal to the ILP lesion. The second type of vascular event was infarction of lung distal to the point of vascular occlusion due to the ILP effect. This occurred in 2 cases both of which needed to be sacrificed due to breathlessness and malaise occurring at 5 and 17 days respectively.

Oesophageal perforation was identified in all but 2 of the cases which developed mediastinal and intrapulmonary infection (Figure 6.9d). In the ILP treatments assessed at 3 days histology showed full thickness thermal necrosis of the oesophageal wall. The ILP lesion showed neutrophil infiltration into the centre of the lesion (Figure 6.9e). In the treatments assessed at 3 weeks the ILP lesion had formed into an abscess (Figure 6.9f). Oesophageal sections showed focal scarring at the site where the thermal effects had perforated the wall. As a result of the oesophageal perforation food matter was present in the lung parenchyma (Figure 6.9g). Two other rats developed a lung abscess at 3 weeks although a site of esophageal perforation could not be located. As the lung lesion was close to the main bronchus in these rats the possible cause of the abscess was perforation of the main bronchus with subsequent seeding of infection into the lesion.
Figure 6.9 Complications of ILP. (A) Vascular damage at hilum (B) Acute pulmonary congestion due to vascular occlusion (C) Infarction of lung at 2 months due to vascular occlusion (D) Acute thermal oesophageal perforation (E) Neutrophil infiltrate into centre of ILP lesion 3 days after treatment in a case of oesophageal perforation (F) Lung abscess at 3 weeks (due to oesophageal perforation) (G) Food matter in parenchyma adjacent to abscess (due to oesophageal perforation)
Figure 6.9 continued
The alternative cause of the lung infections was that the necrotic charred tissue was acting as a source of infection. Therefore to determine if prophylactic antibiotics could prevent lung and mediastinal infections a further series of 16 rats which were being used for different assessments were given prophylactic broad spectrum antibiotics (Enrofloxacin, intramuscular, 10 mg/kg) at the time of ILP and observed up to 2 months. However 4 cases of acute lung and mediastinal infection still occurred in this group and all had evidence of oesophageal perforation.

In 2 rats the laser lesion involved the proximal main bronchus to the lower part of the left lung resulting in collapse of the lung distal to the point of obstruction. Histology showed occlusion of the proximal bronchus by charred material and collapse of the distal lung. The collapsed lung did not show any evidence of infection.

6.4 Discussion

Histologically the changes were typical of ILP in other tissues (Matthewson et al., 1987; Pearce and Thomsen, 1995). At the highest temperatures, in the centre of the lesion, there was carbonisation and tissue destruction. The sequential zones of destruction outside this were due to progressively lower temperatures the further from the centre of the lesion. The outer inflammatory zone had evidence of the reversible injury seen at temperatures previously shown to be 42-46 °C in other tissues (Thompson, 1991).

The presence of charring per se did not have any adverse effects on the healing process. Even though the tissue temperatures must have temporarily been very high to create this effect it only caused adverse effects when the lesion was immediately adjacent to vital structures in these small lungs. The final histological appearances were very similar to those described by Matthewson in the normal liver 3 months after ILP (Matthewson et al., 1987). He showed a small fibrous nodule with some residual central carbonisation. In multiple fibre treatments in normal canine liver Steger et al showed progressive regeneration of normal liver to the point at 12 months where there were 4 small charred areas set in normal liver (Steger et al., 1992a). Although there is some cladding from the laser fibre left in the
Perimeter of the lesion this is not likely to cause problems as it has been effectively sterilized by the 200-300°C temperatures occurring at the tip of the fibre at the time of treatment. It is possible that with improvements in fibre delivery systems this problem may be avoidable by using diffuser fibres which do not have cladding of a similar material and which give necrosis over a longer length (Nolsoe et al., 1992).

Pearce and Thompsen have demonstrated similar electron microscopy of thermal affects on collagen, namely fusion of fibrils and loss of striations of fibres seen longitudinally (Pearce and Thomsen, 1995). Sawabata et al examined lung tissue and pleura removed at thoracotomy in lung volume reduction surgery by the use of a high energy contact laser (Sawabata et al., 1995). Macroscopically there was contracture of the lung tissue as it was cut by the laser. Scanning electron microscopy demonstrated laser thermal damage to lung collagen at the site of the lesion where it formed into a smooth gel. This is the likely cause of preventing air leak after this form of surgery and it is possible the same effect of sealing the ILP lesions is occurring in the rat lungs. Also using elastin Van Gieson stains Sawabata et al showed condensation of elastin which was probably the histological correlate of the observed shrinkage of the bulla and pleura.

In the present experiments the electron microscopy showed no damage to collagen and elastin at sites distant from the ILP lesion. This is reassuring as there was a theoretical possibility that low temperatures transmitted to parts of the lung distant from the ILP lesion may have altered the lung connective tissue and consequently affected lung physiology. The absence of physiological effects of ILP on the rest of the lung is confirmed in Chapter 10.

The vascular effects seen in the lungs were seen in ILP treatments of normal liver in rats. In those studies both the microvasculature and 1-2 mm vessels were coagulated by the effects of heat. Histology of these vessels showed coagulation necrosis of the vessel wall. There were no cases of massive bleeding as a result of this and the angiogram showed no leakage of dye from the damaged vessel. Indeed thermal laser treatment is used to cause haemostasis of small vessels in thoracoscopic lung resections (Wakabayashi, 1995).

The cause of the alveolar haemorrhage was most likely due to the combination of the arterial insult with occlusion of venous drainage. This led to overwhelming lung oedema and haemorrhage which was always fatal. In
the cases where non haemorrhagic infarction occurred the rats became breathless after a more prolonged period. In these cases there must have been only minimal alveolar oedema soon after treatment. As mentioned in the previous chapter these effects are unlikely to be significant in larger lungs and treatments in large animals are presented in Chapter 10.

Histology helped to confirm the impact of oesophageal perforation in causing lung and mediastinal infection. Oesophageal contents were seeded into the mediastinum and lung soon after the treatment which had severe results.

In summary the histology of ILP in the lung is predictable and similar to its effects in other organs where it has been used effectively to treat tumours surrounded by normal tissue. ILP causes reproducible necrotic effects in tumours which heal safely in the same way as it does in normal tissue (Matthewson et al., 1988; Harries et al., 1994b). The results from this chapter suggest that a perimeter of normal lung tissue can be safely included in the treatment field around a lung tumour. Based on previous studies the lung tumour is likely to be at least as sensitive to the effects of ILP as normal tissue. Selection of the appropriate patient will be of paramount importance to avoid treating tumours close to the oesophagus or hilar vessels.

In the following three chapters a different form of treatment is explored which causes local necrosis of the lung via a different mechanism with different tissue effects.
Chapter 7  Studies of photosensitizer quantity and distribution in lung tissue

7.1 Introduction

7.2 Methods

7.3 Results

7.4 Discussion
7.1 Introduction

The tissue effects of photodynamic therapy depend on the quantity of photosensitizer in the tissue at the time it is activated and the distribution of the photosensitizer (Dougherty, 1993). The information derived from studying the uptake of drug into the tissue can be used to firstly determine the time at which the effects of administering the laser light will be maximal, and secondly the likely effects of the laser light on the tissue as predicted by the distribution of the drug within particular structures in an organ (Barr et al., 1987b). After the injection of a photosensitizer the drug is distributed throughout the body. The time course of uptake of the drug into a particular organ depends on a variety of factors, particularly the blood supply of that organ (Whelpton et al., 1995). Therefore each organ should be tested separately before photodynamic therapy is performed on it.

The method used in this chapter is fluorescence microscopy of tissue sections taken after the animal has been injected with photosensitizer. It may be performed on normal tissues however the technique is particularly useful in animal tumour experiments to determine the time of maximum uptake of photosensitizer in tumour tissue compared to normal tissue (Bedwell et al., 1992). Different techniques are being developed for use in humans to determine the relative concentration of photosensitizer in tumour and surrounding normal tissue. These do not require biopsy and work by analysing light emitted from the tumour after excitation with specific wavelength light (Braichotte et al., 1996). The other common method used is assay of drug extracted from tissue or plasma (Ronn et al., 1996). The advantage of fluorescence microscopy compared to these methods is that it in addition to giving quantitation of the drug in tissues it shows where in the organ the drug is distributed (MacRobert and Phillips, 1990).

7.2 Methods

The method used for these studies was fluorescence microscopy (Chan et al., 1989b; Pope et al., 1991b). This technique measures the fluorescence of photosensitizers in tissue sections when they are excited by a particular wavelength of light. Low power laser light is applied to thin sections of tissue which have been taken from animals which have received an IV injection of the photosensitizer. The quantity of fluorescence obtained from these
sections is often small and short lived and therefore requires highly sensitive techniques to detect it. There is good correlation between tissue levels obtained with this method and chemical extraction methods (Chatlani et al., 1991).

The experimental set-up was as follows. An inverted microscope (Olympus IMT-2) with attachments for epifluorescence and phase contrast attachments was used (Figure 7.1). The excitation light was provided by a 1.8mW helium-neon laser with a wavelength of 543 nm. This laser light was directed through a liquid light guide via a filter centred at 540 nm to remove extraneous light, through a dichroic mirror to direct the light onto the microscopic slide. This caused the excitation of fluorescence in the microscopic slide which was detected in the range of 630-700nm using a combination of bandpass and longpass filters. Imaging of the fluorescence was performed using a charge-coupled device (CCD) camera. A personal computer with a high resolution colour monitor was used to control the operation of the camera and provided digital image processing, display, and storage. Using a software package, a false colour coded image of fluorescence intensity could be derived giving an overall picture of the distribution of the photosensitizer within the tissues. Quantification of the fluorescence signal was made by superimposing boxes on areas of interest in the false colour coded image from which the software package was able to obtain a fluorescence score.

The photosensitizers tested were ALA, ALS2PC, and mTHPC. The injected dose was 200mg/kg, 1mg/kg, and 1mg/kg respectively. After intravenous injection animals were sacrificed at serial time points. These were as follows: for ALA 2, 3, 4, 5 and 8 hours, for ALS2PC 1/2, 1, 4, 6, 24 and 72 hours, and for mTHPC 1, 3, 4, 6 and 8 days after injection. The range of times for each drug were chosen with reference to previous experimental and clinical tumour treatments performed with each drug (Messmann et al., 1995; Smith et al., 1993; Ris et al., 1993b). After sacrifice sections of the lung parenchyma were taken and immediately frozen in liquid nitrogen. Frozen sections (6 um thickness) were subsequently made and these slides were used for the CCD analysis. The amount of photosensitizer was quantitated from the CCD pictures of at least 2 large areas of each slide. Each animal had 2 slides. Therefore at each time point there were 4 separate recordings from 3 different animals giving a mean of at least 12 measurements at each time point.
Further analyses were performed on the distribution of mTHPC. There was a nodular pattern of tissue fluorescence with all drugs which was most marked with mTHPC. This suggested a concentration of the drug in specific cell types. To test the hypothesis that these cells were macrophages immunocytochemistry was performed on frozen sections using a monoclonal antibody (FA11, macrosialin) specific for rat macrophages (gift of Dr R. De Silva, William Dunn School of Pathology, Oxford.) (Smith and Koch, 1987). The antibody was counterstained with peroxidase. Fluorescence testing was performed first with the CCD camera and the images stored, then the macrophage stain was used on the same slide which allowed direct correlation.

Secondly the microvascular distribution of mTHPC was assessed using fluorescein as others have done for different photosensitizers (Bellnier et al., 1995). This was achieved by injecting fluorescein 1 mg/kg intravenously 5 minutes before sacrifice into rats which had already received mTHPC 1 and 3 days before. Frozen sections made from this tissue had CCD analysis as above. Then on the same slide the distribution of fluorescein in the tissues was imaged with the CCD camera using a halogen lamp to excite its fluorescence.

7.3 Results

Figure 7.2 shows the tissue quantification at times following intravenous injection of mTHPC, ALS2PC and Protoporphyrin IX (PpIX) from ALA, respectively. With ALS2PC and mTHPC there was an initial peak followed by a gradual decline in concentration. With ALA there was a buildup to a peak at 3-4 hours due to the time required for protoporphyrin IX to accumulate.

The intensity of tissue fluorescence was greatest with mTHPC however the pattern of uptake of photosensitizers showed a similar progression of changes with time for each of the 3 drugs. Early time points showed a diffuse uptake of the drug across the lung parenchyma (Figure 7.3a). Vessels and bronchioles did not stand out from the background general uptake. This is in contrast to figures 7.3 b and c which show the uptake of mTHPC at 3 and 6 days. Here the vessel and bronchus stand out from the background. This is most obvious at 6 days when the background contains far less drug (The H&E sections which accompany the CCD images appear less aerated than typical lung sections due to the freezing of the tissue in the slide preparation).
Figure 7.1 Diagram of laser, microscope and camera used for fluorescence quantitation. (Modified from Loh, 1996.)

Figure 7.2 A Plot of tissue quantitation of photosensitizers in untreated lung parenchyma. (A) mTHPC (B) ALS₂PC and (C) ALA (PpIX)
Figure 7.2 (Continued) Plot of tissue quantitation of photosensitizers in untreated lung parenchyma. (B) ALS2PC and (C) ALA (PpIX)
Figure 7.3 CCD images (top) and corresponding H&E sections (bottom) of lung from rats injected with mTHPC. (A) 24 hours after injection (B) 72 hours after injection (C) 144 hours after injection.
Figure 7.3 (B) CCD image (top) and corresponding H&E section (bottom) of lung from rats injected with mTHPC 0.72 hours after injection.
Figure 7.3 (C) CCD image (top) and corresponding H&E section (bottom) of lung from rats injected with mTHPC. 144 hours after injection.
There were also sequential changes in the appearance of parenchymal blood vessels with each of the 3 photosensitizers which were best shown in the sections which contained both mTHPC and fluorescein. Sections taken 24 hours after drug injection showed concentration of photosensitizer in the intimal layer (Figure 7.4a). At 72 hours however there was no direct match on the same sections between mTHPC fluorescence distribution and the fluorescein pattern at high magnifications (Fig 7.4b). Most of the mTHPC appeared to have left the microcirculation and entered the interstitial and cellular compartments. Low power views taken at later times showed photosensitizer in the muscular layer of the larger vessels (Fig 7.3 b and c). This was still present as late as 8 days after injection with mTHPC.

All drugs showed uptake of photosensitizer in the epithelium however this was most marked with PpIX (ALA) (Figure 7.5). With mTHPC and ALS2PC the fluorescence was diffusely distributed through the bronchial wall and epithelium. The strong uptake of PpIX in bronchial epithelium from 1 hour onwards, and this persisted up to 8 hours. As with the other photosensitizers there was a strong uptake in the parenchymal vessels with PpIX (ALA).

The appearance of the background changed from the initial diffuse uptake to a more nodular distribution which did not conform to any underlying lung structure. This was most obvious in the mTHPC sections and occurred from 48 hours onward and is illustrated in figure 7.3b and c. It was also seen with ALA at 4-8 hours and with ALS2PC from 4-6 hours onwards. Fluorescence scores from these nodules were typically 10-20 times as high as the adjacent background tissue. This nodular pattern was still present at 8 days but was less intense. Immunohistochemistry for macrophages demonstrated that the nodular pattern of uptake was due to uptake of the sensitizer into macrophages. Sections showed an exact match of these nodular foci of photosensitizer uptake with the immunoperoxidase stain for the macrophages (Figure 7.6). Figure 7.7 shows the contribution of fluorescence contained in macrophages to the overall tissue fluorescence. This data was obtained by comparing areas on slides containing large numbers of macrophages with areas with no macrophages (Fig 7.8). It shows that tissue containing macrophages contained up to twice the amount of mTHPC compared to tissues not containing macrophages. Also, at 6 and 8 days, although the overall quantity of sensitizer level at this time was lower than at 3 and 4 days, most of the total amount of fluorescence was contained in macrophage containing lung.
Figure 7.4 Black and white images of parenchymal vessels in rats which had received both fluorescein (left) and mTHPC (right). (A) 24 hours after mTHPC, shows intimal location of mTHPC at this time. Magnification x 10 (B) 72 hours after mTHPC injection, shows that mTHPC is not exclusively in the microvasculature at this time. There are nodular deposits which do not correspond to the fluorescein distribution; these are macrophages taking up mTHPC - see Figure 7.6
Figure 7.5 PpIX uptake in bronchial epithelium 1 hour after intravenous injection of ALA. (A) CCD image (B) Corresponding H&E section
Figure 7.6 Lung sections 72 hours after intravenous injection of mTHPC. CCD images (Top) and immunohistochemistry for macrophages on the same slide (Bottom)
Figure 7.7 Graph of mTHPC fluorescence of macrophage containing lung compared to overall lung tissue fluorescence. Each bar is the mean of 4 regions from each of 3 animals, shown with standard deviation.

Figure 7.8 Typical images used for Figure 7.8. (A) Macrophage containing lung (B) Lung without macrophages
Therefore after the initial strong diffuse uptake with all sensitizers, at later times there was a concentration in the walls of the larger blood vessels and in macrophages. ALA (PpIX) differed from mTHPC and ALS$_2$PC in having concentration of sensitizer in the bronchial epithelium.

7.4 Discussion

The lung is a highly vascular organ and consequently the pharmacokinetics of the uptake of these drugs into the tissues is determined by this high blood supply. With mTHPC and ALS$_2$PC there is a high initial peak of tissue photosensitizer concentration with a subsequent gradual decline. Whelpton has described quantitation of mTHPC in different organs in rats using high performance liquid chromatography (Whelpton et al., 1995). The lung had the highest initial concentration of all organs studied as this is the first organ to be perfused after an intravenous injection. The high tissue concentrations are due to the high plasma levels causing a concentration gradient into the tissues.

Ronn et al have studied the pharmacokinetics of mTHPC in plasma (Ronn et al., 1996). In patients and animals they have demonstrated there is a high plasma level immediately after intravenous injection as expected. There is then redistribution out of the vascular system probably into the liver with subsequent re-release of the drug into the plasma. This gives a second peak of plasma concentration between 12 and 24 hours of similar magnitude with the first. Therefore although 24 hours was the first time point measured in these experiments it is unlikely the levels of mTHPC would have been significantly higher at earlier time points. This is confirmed in a study by Peng et al (Peng et al., 1995).

The early peak levels of ALS$_2$PC around 1 hour in this study have been demonstrated in the normal stomach (Loh et al., 1992), the liver (Bown et al., 1986), and muscle and skin (Tralau et al., 1987b).

Smith described the pharmacokinetics of ALS$_2$PC uptake in rat trachea. The quantity in the trachea appeared greatest at 1-4 hours after injection (Smith et al., 1993). The fluorescence microscopy of the trachea however revealed that there was a distinct highly fluorescent area surrounding the cartilage corresponding to the perichondrium which is a highly vascular membrane.
The lung uptake was also shown by drug extraction to be highest at 10 minutes after injection when most of the drug must still have been in the vascular tree. After that there was a subsequent gradual decline in the tissue concentration. No description of smaller bronchi was given in this paper. In the current study there was some selectivity of uptake in bronchial walls however this was not as marked as in adjacent vessels. As in the study by Smith there was no affinity for the bronchial epithelium. In contrast affinity of ALA for the bronchial epithelium in large bronchi has been shown by some workers in humans (Huber et al., 1996). Here the protoporphyrin IX accumulates in the more rapidly dividing cells of the bronchus.

The pharmacokinetics of protoporphyrin IX (PpIX) differed from the other 2 drugs in showing a gradual increase to a maximum at 3-4 hours after injection. This is due to the necessary time interval for PpIX to accumulate in cells (Konig et al., 1993). Others have shown peak fluorescence of PpIX at around 3-4 hours for example in the wall of the stomach (Loh et al., 1992), tumours of the oral cavity (Grant et al., 1993), and experimental colonic tumours (Bedwell et al., 1992).

mTHPC was retained in the tissues longer than the other photosensitizers as has been reported by others (Bonnett, 1995). It is a highly lipid soluble drug and it is believed that this slow elimination is due to the slow release of drug back into the plasma from tissues which can have a half life of up to 100 hours (Peng et al., 1995, Whelpton et al., 1995). An elimination half life in mice of 10-12 days has been reported by Whelpton (Whelpton et al., 1996).

The results of these experiments allow prediction of when normal tissue damage is likely to be maximal and how long after injection there is likely to still be an effect. Some normal tissue damage around a tumour should be created to allow better chance of a curative treatment, and the unique features of PDT mean that normal tissue damage will heal with minimal disruption of surrounding tissue. Therefore it is not vital to know the relative concentration of drug in tumour and normal tissue. Rather, it is sufficient only to treat when it is predicted there will be as much effect on tumour as on normal tissue.

Based on the pharmacokinetic data alone it would appear that the maximal tissue effect would be at 1 day with mTHPC, 1 hour with ALS\textsubscript{2}PC, and 3-4 hours with ALA. However as is discussed in the next chapter tissue effects are
modified by the drug distribution in the tissues, particularly in blood vessels and also in macrophages.

These results show the importance of small blood vessels in the lung parenchyma as a site of distribution of photosensitizers. Uptake persisted at times when the general uptake in the lung had declined to low levels. Similar findings for the uptake of photosensitizers into blood vessels have been described by other workers. Anholt et al demonstrated uptake of tetra sulfonated phthalocyanine in the vasculature of experimental tumours (Anholt et al., 1994). Two hours post injection the drug was strongly localised in tumour vessel walls whereas this was much weaker at 24 and 48 hours. This study also demonstrated that although the drug levels in the tumour were highest 1 hour after injection, that ALS$_4$PC was located in the periphery of the tumour. It was not until 24-48 hours after injection that drug was present in the central part of the tumour.

Recent studies have shown similar findings for mTHPC (Andrejevic Blant et al., 1997). They also demonstrated that this different uptake caused different patterns of necrosis. Treating when there was intimal drug caused vascular occlusion and ischemic necrosis. In contrast treating when there was mural uptake caused coagulative necrosis. In a separate study this group showed no difference between histological appearance of PDT treatment of tumours with 4 and 8 day drug light intervals (Andrejevic Blant et al., 1996).

Arterial fluorescence has also been demonstrated for ALA (PPIX) in preliminary studies for arterial PDT (Nyamekye et al., 1995). One hour after injection there was strong fluorescence in the media of rat carotid arteries with weaker fluorescence in the intima and adventitia. Others have shown intimal fluorescence at this time in rat carotid arteries (Grant et al., 1994). Here there was preferential accumulation in the media of the vessel.

This is the first description of the appearance of mTHPC uptake into macrophages in tissues. Previous workers have demonstrated concentration of ALS$_2$PC in macrophages in tissue culture and also showed ALS$_2$PC concentration in liver macrophages in tissue sections (Chan et al., 1989a). Like the liver the lung is an organ with plentiful numbers of macrophages and these cells are important in the pathogenesis of many disorders in the lung. The reasons macrophages concentrate photosensitizers are not well understood. One reason is that photosensitizers interact with low density lipoprotein (LDL) and macrophages take up modified LDL (Jori et al., 1984;
Hamblin and Newman, 1994b). Nearly all cells express the normal LDL receptor. However macrophages also express at least two scavenger receptors which take up a wide range of modified and oxidised proteins. They also have a receptor which avidly phagocytoses aggregated LDL (Suits et al., 1989). Endothelial cells also take up altered LDL which may explain the avidity of many photosensitizers for the endothelium (Henderson and Dougherty, 1992).

Concentration in macrophages is an important mechanism for localization of photosensitizers in tumours. Studies using haematoporphyrin derivative have shown that tumour associated macrophages contain up to nine times the quantity of photosensitizer as the tumour cells themselves (Korbelik et al., 1991a; Korbelik et al., 1991b). More recently Korbelik et al used flow cytometry to analyse the macrophage uptake of photosensitizers in a wide variety of tumours (Korbelik et al., 1991a). They showed that photosensitizer levels in tumours were highly heterogeneous due to the relative concentration of tumour macrophages between tumours. Also certain populations of tumour macrophages concentrate the photosensitizer 10-20 times more than the average level seen in the malignant cells. Other populations aggregate levels similar to that seen in malignant cells. Lung tumours contain often greatly increased numbers of macrophages which may enhance lung tumour uptake of photosensitizers (Mantovani et al., 1992, Yasumoto et al., 1988).

Macrophage uptake of photosensitizers may be important in causing the observed tissue effects once these cells are activated by laser light. Evans et al have shown that macrophages in vitro produce TNF alpha when stimulated by photodynamic therapy (Evans et al., 1990). Importantly there was a dose response relationship between the production of TNF alpha and the dose of PDT. Therefore photodynamic therapy did not destroy the macrophages, rather it appeared to stimulate them to secrete the active cytokines. TNF alpha has important functions in the lung and therefore the potential for this interaction in the lung may be significant in the treatment experiments. In the lung TNF alpha causes neutrophil and macrophage influx, endothelial and epithelial damage and oedema. TNF alpha also is a potent inducer of thrombosis.

Macrophages are important in the cellular response to PDT. Yamamoto in 1992 demonstrated that macrophages which had taken up haematoporphyrin derivative had an enhanced phagocytic activity compared
to normal macrophages (Yamamoto et al., 1992). Korbelik in 1994 demonstrated enhanced cytotoxicity of macrophages for human lung adenocarcinoma cells treated with PDT (Korbelik and Krosl, 1994). There was enhanced macrophage mediated killing of these tumour cells which had been treated with PDT compared to tumour cells which had not been treated with PDT. It appears that PDT causes damage to tumour cell membranes which are recognized by macrophages helping to identify these affected cells as their targets. Also an important recent study has shown that immunomodulators which stimulate macrophages (Vitamin D3 binding protein derived macrophage activating factor - DBPMAF) administered immediately following PDT enhanced the PDT effect on squamous cell carcinomas in experimental animals (Korbelik et al., 1997).

The effects of activating the photosensitizer in the tissues are described in the following chapter. The same drug light intervals as were used in the pharmacokinetic studies are used for treatment to determine the relationship between tissue drug levels and PDT effects.
Chapter 8 Macroscopic effects of PDT on the lung parenchyma in rats

8.1 Introduction

8.2 Methods
   8.2.1 Photosensitizers
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8.3 Results
   8.3.1 Light distribution in the lung
   8.3.2 Macroscopic appearances of PDT
      8.3.2.1 Acute effects
      8.3.2.2 Chronic effects
   8.3.3 Adverse effects

8.4 Discussion
8.1 Introduction

Having determined the distribution of drug in the lung parenchyma in the previous chapter I will now discuss the findings when PDT is performed on rat lungs. Drug was distributed throughout the lung parenchyma and therefore PDT lesions were to be expected in a lung. The relationship between the quantity of drug in the lung at different time points after injection and the subsequent tissue effects are also analysed in this chapter.

8.2 Methods

8.2.1 Photosensitizers

The three photosensitizers used were ALA, AIS₂PC, and mTHPC. The doses of each were 200mg/kg, 1mg/kg, and 0.3mg/kg respectively. These are typical doses used in rat experiments previously. ALA was prepared as a solution with phosphate buffered saline. AIS₂PC solution was prepared by adding the powder to 0.1M NaOH and 0.01M NaH₂PO₄. mTHPC was prepared immediately prior to injection by dissolving in a solution composed of ethanol, polyethylene glycol, and distilled water. All photosensitizers were injected slowly via the tail vein. Thereafter the rats were protected from direct light to prevent skin photosensitivity.

8.2.2 Light distribution in ex vivo lungs

This was performed by inflating an ex vivo rat lung and a porcine lung, and inserting the PDT laser fibre through the lung surface. The distribution of light in the lung could therefore be directly observed.

8.2.3 Photodynamic Therapy

Rats were placed under a general anaesthetic and the left side of the chest shaved and sterilized with a topical preparation. The rat was placed in the same frame as used for the ILP experiments and again the optimum site of placement into the left lung was chosen by fluoroscopy. Once this was determined a small incision was made and the introducer needle passed into the lung parenchyma. The laser fibre within the introducer needle was then held stationary while the introducer needle itself was retracted a short distance leaving the bare laser fibre tip exposed in the lung parenchyma. The laser light source was a copper vapour pumped dye laser (Oxford
Lasers), which is tunable so that the different wavelengths for each photosensitizer could be obtained. The laser fibre was a 400 um internal diameter bare-tipped glass fibre with silastic cladding. The fibre was cleaned between each treatment and carefully recalibrated to check the power before and after each treatment. After sacrifice by CO\textsubscript{2} inhalation lungs were fixed and sectioned as described in Chapter 5. The largest diameter of necrosis (black tissue) was measured. Lung volumes were also measured as described in Chapter 5.

Four animals were treated with laser light alone without prior administration of photosensitizer to determine if any thermal effects were produced on the tissues by the laser power used. The light dose was 100 mW for 1000 seconds (100 joules) at a wavelength of 635nm. These animals were killed three days after treatment for histological examination. Further control rats were also kept up to 6 months after insertion of the laser fibre to exclude any effect on macroscopic appearance of this intervention.

The wavelengths for ALA, AIS\textsubscript{2}PC, and MTHPC were 635, 675, and 652nm respectively. All treatments were performed using a laser output of 100 mW. In the first set of experiments the drug light intervals were varied between 2 and 8 hours for ALA, 1 and 72 hours for AIS\textsubscript{2}PC, and 1 and 6 days for mTHPC. These experiments were to determine the time at which PDT damage measured 3 days after treatment was greatest with each sensitizer. In the subsequent experiments one treatment time was chosen and the light dose varied between 20 and 300 Joules. The treatment time selected was that which gave the largest lesion size in the above experiments. For mTHPC this was chosen at 3 days due to marked vascular effects of PDT at earlier times which may have confounded lesion size measurements. Also previous reports have shown that the best ratios of mTHPC between tumour and normal tissue are seen at 72 hours after sensitization and therefore information obtained at this drug light interval was more relevant than the effects at 24 hours, since the 72 hour interval would be the likely interval chosen in tumour treatments (Ris et al., 1993a).

The effects of PDT were assessed between 3 days and 6 months after initial PDT treatment. An assessment of healing after PDT was made with all photosensitizers however most of this assessment was performed with mTHPC. Histology was performed at 10 days, 3 weeks, and 2 months after ALA and AIS\textsubscript{2}PC, however mTHPC was also assessed at 6 months. Groups of
five animals were used at each time point with mTHPC at times up to 6 months.

Fluoroscopy was performed immediately after treatment to determine if pneumothorax had occurred. Conventional chest X-rays were used post-mortem to determine if late pneumothorax occurred. These were taken on groups of rats at each time point up to 6 months. Angiograms of pulmonary arteries were performed as described in Chapter 5. These were done on lungs removed 2 months after mTHPC PDT.

8.3 Results

8.3.1 Light distribution in the lung

Inserting the laser fibre into an inflated lung ex vivo resulted in uniform illumination of the lung (Figure 8.1). The light appeared to be scattered in all directions in the lung; it did not simply continue in a forward direction from the laser. The pleura appeared to internally reflect the light from the laser back into the lung. This was demonstrated by putting the laser fibre through the lung to the opposite pleural surface. The light still gave a diffuse glow over the pleural surface and inside the lung even when the tip of the fibre was touching the pleural surface. When the fibre was advanced through the pleural surface the point source of the light immediately became apparent and the light shone in a forward direction onto adjacent structures.
8.3.2 Macroscopic appearances of PDT

8.3.2.1 Acute effects:

On the cut surface perpendicular to the line of the laser fibre the PDT effect with all photosensitizers was seen as a circular uniformly dark area centred around where the tip of the fibre had been placed. There was a clear distinction between PDT lesions and the surrounding untreated parenchyma (Figure 8.3). As with ILP the PDT effect from the parenchymal lesion was also visible on the adjacent lung surface (Figure 8.2). The pleural surface had a normal contour and there was no shrinkage of the lung at the lesion site. No adhesions between visceral and parietal pleura were seen at 3 days. The PDT effect was confined to the lung and visceral pleura and there was never any PDT effect on the parietal pleura (Figure 8.4). Also, the mediastinal structures such as the great vessels or oesophagus were not damaged by the PDT occurring in the immediately adjacent lung (Fig 8.5). An exception to this was the few cases where the laser fibre had inadvertently been placed through the medial surface of the visceral pleura (see below, adverse effects).

The remainder of the lung appeared normal in virtually all cases apart from some rats treated with mTHPC with a drug-light interval of 24 hours. Here there was evidence of vascular congestion on the lung surface and on sectioning the lung appeared oedematous (Figure 8.6).

Lesion sizes were highly reproducible. The lesion diameter in 10 rats treated with mTHPC with a 3 day drug light interval and 80 J was 9.8mm, standard deviation 1 mm. The effect of changing drug light interval is shown for each of the photosensitizers in figures 8.7 a, 8.8a and 8.9a. The effect of increasing the light dose when a single drug light interval is used is shown in figures 8.7b, 8.8b and 8.9b. With mTHPC a 72 hour drug light interval was chosen as although the results with a 24 hour interval were slightly larger there was the potential for lesions to be obscured by thrombosis and oedema seen using this earlier treatment time. With each drug there was a plateau above which increasing the light dose does not increase the diameter of PDT necrosis. The largest lesion seen was with mTHPC and this was 12mm in diameter. The drug light interval for ALS₂PC was 1 hour. Only 1 animal was used for the 300 J treatment with ALS₂PC. For ALA 4 hours was chosen as there was a smaller standard error than for 3 hours even though the mean results were very similar.
Figure 8.2 Macroscopic appearance of lungs 3 days after mTHPC PDT. (A) Light dose 40 J. (B) Light dose 100 J. Both treated with a 3 day drug light interval.

Figure 8.3 Appearance of the pleural surface superficial to the interstitial PDT lesion shown in Figure 8.3 a.
Figure 8.4 Lung and chest wall post mortem 3 days after PDT demonstrating PDT effect on visceral pleura but no effect on parietal pleura.

Figure 8.5 PDT effect in lung adjacent to oesophagus which is unaffected. Treated with 80 J with 3 day drug light interval.
Figure 8.6 Vascular effects of PDT with mTHPC with 24 hour drug light interval, light dose 80 J. (A) Whole lung (B) Cut surface perpendicular to line of laser fibre insertion.
Figure 8.7 Lesion sizes (Mean,SD) at 3 days after PDT with mTHPC.(A) Effect of changing drug light interval with constant light dose(80J) 6 animals at each time point(B) Changing light dose at drug light interval of 72 hours. At least 3 animals at each time point.
Figure 8.8 Lesion sizes (Mean, SD) 3 days after PDT with ALS2PC. (A) Effect of changing drug light interval with constant light dose (100J). At least 3 animals at each time point. (B) Changing light dose at drug light interval of 1 hour. At least 3 animals at each time point, (apart from 300 J, n=1)
Figure 8.9 Lesion sizes (Mean, SD) 3 days after PDT with ALA. (A) Effect of changing drug light interval with constant light dose (100 J) At least 3 animals per time point. (B) Changing light dose at drug light interval of 4 hours. 3 animals per time point.
8.3.2.2 Chronic effects

From 3 weeks onward mild localised indrawing of the pleural surface occurred. This appeared to be maximal at 2 months after the treatment and was still evident 6 months after treatment (Figure 8.10). No adhesions between the visceral and parietal pleura were seen. On the cut surface there was a localised area of pale fibrous tissue which had replaced the initial zone of PDT necrosis (Figure 8.11). There was no cavitation. Where the lesion was adjacent to major structures there was no effect on these, including no evidence of localised bronchial stenosis in major bronchi nor occlusion of major blood vessels. Angiograms of vessels in the vicinity of PDT lesions at 2 months after treatment showed no disruption of vessel architecture (Figure 8.12a and b). Figure 8.12 b shows it was possible to demonstrate growth of new vessels in the scar of the PDT lesion.

Figure 8.13 shows the effect of time on lesion size after all had initially received mTHPC PDT with a 3 day drug light interval and light dose of 80 J. Figure 8.14 shows the volume of the left lung at various times after PDT. It demonstrates that despite the shrinkage of the PDT lesion no significant impact on the volume of the lung was observed.

Figure 8.10 Appearance of whole lung 6 months after PDT with mTHPC (Drug light interval 3 days, light dose 80 J)
Figure 8.11 Appearance of cut section at 6 months after PDT with mTHPC (Drug light interval 3 days, light dose 80 J)

Figure 8.12 Angiograms of lungs at 2 months after PDT with mTHPC (Drug light interval 3 days, light dose 80 J) a. No effect on vessels. b. Proliferation of vessels in the healed PDT lesion
Figure 8.13 Effect of time on mean lesion size up to 6 months after PDT with mTHPC (Drug light interval 3 days, light dose 80 J). At least 5 animals at each time point.

Figure 8.14 Volume of left lung after PDT with mTHPC (Drug light interval 3 days, light dose 80 J) compared to untreated controls matched by animal weight. (Different controls used for 6 month rats). At least 4 animals at each time point.
8.3.3 Adverse effects

This treatment was very well tolerated and despite the size of the lesion created no animal died in the operative or peri-operative period.

Pneumothorax was seen on fluoroscopy in 11 out of 91 cases. Tension pneumothorax was not seen and no animal died as a result of pneumothorax. Chest X-rays performed post-mortem on animals killed at 3 days and at 2 and 6 months showed no pneumothorax in any animal.

Out of 120 rats treated there were only 4 serious adverse events and these were all due to oesophageal penetration with mediastinitis. Two of these were detected at three days and two were detected at two weeks post treatment. These 4 animals were all from the same batch of treatments. This effect necessitated sacrifice of the animal in all cases. The PDT zone of necrosis in these animals was centred on the oesophagus itself with PDT effects seen on the mediastinal aspect of the pleura both on the left and the right lungs, indicating that the laser fibre had been passed too deeply into the lung, passing just out of the visceral pleura on the medial aspect of the lung, and had come into direct contact with the adventitial surface of the oesophagus. In all the other treatments the laser fibre had been correctly placed within the visceral pleural surface and no effects on the oesophagus were seen. Histology of the oesophagus showed necrosis and haemorrhage, and reticulin and elastin Van Gieson stains at the site of perforation showed marked disruption of the collagen architecture, which may have been due to thermal effects on the oesophagus due to the proximity of the tip of the laser fibre, although thermal coagulative necrosis was not seen on H&E sections.

8.4 Discussion

These results indicate that reproducible zones of PDT necrosis can be created within the lung parenchyma by the percutaneous technique. The lesions were highly reproducible and well demarcated from the surrounding parenchyma which was macroscopically normal in virtually all cases. The localised nature of the PDT insult and the preservation of normal tissue architecture surrounding the zone of PDT is important in that it suggests the potential for this therapy to treat a localised lung lesion.
The long term effects of the PDT treatment were reproducible, that is a small scarred zone at the site of initial treatment was always observed. Only minimal in-drawing of the pleural surface immediately adjacent to the area of fibrosis while the rest of the lung appeared normal in shape and size as was supported by the lung volume studies. Fibrosis appeared to be complete by 2 months. Surrounding structures were unaffected and the remaining lung parenchyma did not have any scarring or other residual effects from the initial treatment. Therefore it is possible to create a localised lesion with this therapy which heals in a safe and reproducible manner.

The appearance of the ex vivo lung illuminated by the laser is probably due to scattering of the light within the parenchyma. Others have demonstrated the scattering of light in human lungs in both theoretical models and practical experiments (Suzuki et al., 1985). This scattering is the likely explanation for the spherical shape of the lesions distributed evenly around the fibre tip. Light cannot be scattered indefinitely and is eventually absorbed. Studies on the distribution of light within the lung parenchyma have shown that 70% of the light intensity is lost within distances of 3-4mm of the point light source (Doiron et al., 1983). This is the likely explanation for the plateau effect seen in the maximum lesion sizes. Prolonging the light exposure increases the light dose however above 1000-1500 seconds there was no significant improvement in lesion size in any of the sensitizers. In the previous chapter CCD quantitation showed that with a 24 hour drug light interval the quantity of mTHPC in the tissues was up to 5 times greater than that seen at 72 hours. However the lesion size when PDT was performed at 24 hours and 72 hours was virtually the same. Therefore it is likely that the limiting effect was light penetration.

There were differences in maximum lesion sizes between the 3 photosensitizers. These differences would be even greater if the 3 dimensions of the lesion rather than just the single largest dimension were taken into account. mTHPC gave the largest lesion size. This is due to its known ability to strongly absorb red light and its high yield of singlet oxygen. Lesion sizes with A1S2PC were slightly greater than with ALA. These were also consistent with their effects in other organs for example the prostate where lesions with ALA were significantly smaller than with mTHPC (Chang, S. 1996b).

With mTHPC and ALS₂PC the lesion sizes at later drug light intervals were larger than would be predicted by the CCD quantitation in the previous
chapter. Others have demonstrated this fact, namely that the size of PDT effect is not necessarily proportional to the tissue concentration and is dependent on binding of the sensitizer to important biological targets (Star et al., 1986; Henderson and Bellnier, 1989a). Lesions at 6 days with mTHPC were still 6-7 mm in diameter whereas the low quantity of drug in tissues on CCD at this time would have predicted a much smaller lesion. This is true for the lesions at 24 hours with ALS₂PC. This is likely to be a result of the importance of distribution of drug in the tissues. CCD images showed that at these later times drug was present mostly either in the walls of small vessels or in macrophages. Clearly either or both of these sites are important in causing the PDT tissue effects. This is discussed further in the next chapter.

Peak levels of ALS₂PC in experimental tumours are normally seen 24-48 hours after injection compared to normal tissue which peaks at 1 hour (Tralau et al., 1987b). In this study of PDT on transplantable rat fibrosarcoma there was peak necrosis of 6 mm diameter at 24 hours however there was still a mean diameter of 5 mm of necrosis when treatments were performed at 1-3 hours.

In a study of experimental pancreatic tumours Regula et al showed that PPIX levels were maximal in both normal and tumour tissues at 4 hours and these tumours were treated at that time (Regula et al., 1994). Minimal necrosis was observed in the normal pancreatic tissue around the tumour as the light was applied under direct vision only onto the tumour surface. Lesions in the tumour were up to 8 mm in diameter.

These studies have shown that normal tissue in the lung parenchyma can be treated with PDT and this therefore raises the prospect of treating a zone of normal tissue surrounding a tumour. There are numerous reports of the effect of PDT on tumours in other organs which show that there is at least as much necrosis in the tumour as in the normal tissues surrounding it (Barr et al., 1990b). To achieve tumour necrosis all that is required is for the tumour to be at least as susceptible to PDT as the normal tissue in which it arose (Bown, 1990). This has been demonstrated to be the case in a variety of tumours including the pancreas (Chatlani et al., 1992; Regula et al., 1994).

The size of lesions created in this study indicate that simultaneous use of multiple fibres will be needed to treat lung tumours of up to 3cm in diameter in patients. Interstitial PDT in solid organs using mTHPC has given lesion
sizes of a similar magnitude to that observed in these lungs (Chang et al., 1996a). This along with the selective uptake of drug into tumours means that interstitial PDT of a solid tumour such as a bronchogenic carcinoma is likely to be at least as effective as on the normal surrounding lung.

In tumour treatments changing the drug light interval has a major impact on tissue responses. In experiments on human mesothelioma xenografts in nude mice Ris et al demonstrated that the optimum time to treat with mTHPC was 3 days (Ris et al., 1993a). At this time there was much greater effect on tumour than on normal tissue even though there were similar quantities of drug present in both. Also the tumour lesion sizes were the same as treatments at 24 hours where there was more drug present in the tumour. Furthermore at this early time there was proportionately more effect on normal tissue. These results indicated the importance of allowing time for the drug to distribute to regions of the tumour, probably vascular, which were important in causing the PDT effect. mTHPC is excreted unchanged via the biliary system, so the effects are not due to a metabolite of mTHPC (Ronn et al., 1996). A similar effect was described by Chang in the normal prostate (Chang et al., 1996a). Interstitial PDT of using mTHPC showed similar sized lesions at 24 and 72 hours despite higher drug levels at the earlier timepoint.

The results in this study are therefore in keeping with results in other organs and offer the prospect that a sizeable amount of normal tissue around a tumour can be necrosed at a time when there is an ideal distribution of drug in the tumour to cause the most effective tumour necrosis. Others have shown that treatment of endobronchial tumours with mTHPC PDT is best performed at least 4 days after injection of the drug. Drug excretion is more rapid in these small experimental animals than in humans. Therefore sizable lesions at 3 days in this study indicate large lesions will still be able to be made at 4 days or later in humans.

In view of the oedema and congestion seen with the 24 hour drug-light intervals in these experiments such short drug-light intervals should be avoided in treating patients. The appearance of lung oedema was more severe than would be likely to occur in patients as here the volume of lung affected in proportion to the total lung volume would be much smaller.

The absence of any deleterious effects on surrounding structures when the needles were correctly placed within the lung is a significant finding. The reason for this may be limitation of light penetration out of the lung into
these structures. This has been observed in other organs such as the prostate where the fibrous capsule of the prostate served to internally reflect light back into the tissues during interstitial photodynamic therapy (Chang et al., 1996a). The pleural surface may be working in a similar manner. The pleural fluid interface with the thin pleural membrane may accentuate this reflection and thereby help to contain light within the lung. This has two potential advantages. Firstly the absence of effect on the parietal pleura suggests that this may be an entirely painless procedure. Others have performed PDT in the pleural space with the aim of including the parietal pleura in the treatment field in the management of mesothelioma. In these cases PDT necrosis up to 1cm deep was observed on the pleural surface and in most cases was associated with significant albeit transient pain (Ris et al., 1991). In contrast these interstitial treatments within the lung parenchyma do not affect the parietal pleura and therefore as a result may be painless.

By comparison with ILP the risk of oesophageal perforation when PDT lesions are adjacent to the oesophagus is far lower. It only occurred in the small number of animals in this study where the laser fibre was incorrectly placed directly against the oesophagus wall. In large animal experiments the risk of oesophageal perforation following PDT lesions made in this way is lower still. Tochner performed surface PDT to the pleura and intrathoracic organs in dogs using Photofrin (Tochner et al., 1994). This was a preclinical normal tissue tolerance study prior to commencing human studies of treatment of mesothelioma. Organs tested by creating 2 cm diameter lesions included the chest wall, lung, heart, oesophagus and diaphragm. No functional injury to any intrathoracic organ occurred even when applying the PDT effect directly onto the organ in a worst case scenario. This was confirmed in pilot studies of mTHPC PDT of the pleura for treatment of mesothelioma; here there was no effect on oesophagus, aorta, subclavian artery and brachial plexus even in the peence of a close relationship between tumour and normal tissue (Ris et al., 1992). Therefore the perforation in these rat experiments may have been simply due either to thermal effects of the laser, or PDT effects on this very thin walled oesophagus, or a combination of the two. It is likely therefore that tumours close to the oesophagus could be more safely treated with interstitial PDT than ILP, as long as the tumour or the needles do not breach the visceral pleura.

The incidence of pneumothorax is consistent with the known incidence of pneumothorax in patients receiving fine needle aspiration biopsies (Lane and Gleeson, 1995). However considering the duration of placement of the fibre
in the lung parenchyma and the size of the needle with respect to the overall size of the lung the incidence of pneumothorax is reassuringly low. Unlike the effects with interstitial photocoagulation, there is no immediate necrosis with photodynamic therapy and no immediate shrinkage of the parenchyma. Histology showed that vascular effects occurred virtually immediately with PDT and it may be that microvascular thrombosis and local haemorrhage with photodynamic therapy is in some way assisting in sealing the site of needle placement into the lung and therefore reducing the incidence of pneumothorax. The absence of tension pneumothorax in these rats implied that no bronchial penetration occurred. This was confirmed histologically (discussed in the next chapter) in that the bronchial connective tissue was unaffected by PDT. In contrast the tension pneumothorax with interstitial laser photocoagulation was usually associated with a large broncho-pleural fistula due to bronchial penetration by the thermal effects.
Chapter 9 Microscopic effects of PDT on lung parenchyma in rats

9.1 Introduction

9.2 Methods

9.3 Results
   9.3.1 Histology
   9.3.1.1 Controls
   9.3.1.2 PDT treated lungs

9.4 Discussion
9.1 Introduction

This chapter describes the histology on the lesions created for the previous chapter. The histological appearances of PDT are unique and provide insights into the practical ways PDT can be employed most beneficially as a cancer treatment. Histology also allows comparison between the appearances of drug distribution as described in chapter 6 and the tissue effects when the drug is activated by the laser light in PDT.

9.2 Methods

Sections were taken from lungs treated as described in the previous chapter. Histology was performed on groups of animals at times from 3 days up to 2 months with ALA and ALS2PC and up to 6 months with mTHPC. Also, histology was performed on lungs removed from rats sacrificed immediately after completion of PDT using mTHPC with a 3 day drug-light interval. Haematoxylin and eosin stains were used on most slides. Reticulin staining was used to demonstrate collagen fibres. Gramm's stain was used in some sections to exclude the presence of bacteria as a cause of lung inflammation after PDT.

Due to the differences observed in the macroscopic appearance of lungs treated at different drug light intervals with mTHPC a measurement of tissue wet weight was used to compare the amount of tissue water and vascular congestion in lungs treated with mTHPC at 1, 3 and 6 day drug light intervals. 3 days after treatment rats were sacrificed. Each lung was weighed separately having been carefully dissected free of any attached structures. This ratio of the weight of the left (treated) lung to right (untreated) lung was compared to the ratio of left to right lungs in normal rats. Any increase in left lung weight would be a reflection of the amount of increased tissue water. 6 rats were analysed at each of the drug-light intervals.

Bronchoalveolar lavage fluid was removed from the lungs after mTHPC PDT in 12 rats. This was used for microscopy and bacteriological culture. This assessment was made as some sections showed a neutrophil infiltrate surrounding PDT lesions. After sacrifice the left main bronchus was cannulated and the cannula secured in place by a suture. Then 5 ml of
normal saline in 1 ml aliquots were instilled and aspirated. The first 1 ml was discarded.

9.3 Results
9.3.1 Histology

9.3.1.1 Controls

Histology on control specimens where 100 mWatts of light were administered showed no thermal effects. There was evidence of an acute mild inflammatory response typical of the response of the lung to any form of injury and this was consistent with the insertion of the laser fibre and introducer needle. However none of the typical coagulative necrosis or charring was evident in these specimens. No histological effects of insertion of the fibre alone were seen in specimens removed 2 and 6 months after insertion.

9.3.1.2 PDT treated lungs

With all of the photosensitizers histology at 3 days showed a well demarcated circular zone of haemorrhagic necrosis (Figure 9.1). This corresponded to the necrosis seen macroscopically. The necrosis had a uniform bland eosinophilic appearance. At high magnification red cells could be seen leaking from damaged alveolar capillaries and alveoli contained fibrin and fluid. Although there was uniform cell death the tissue architecture was not disturbed. There were the ghost outlines of alveolar walls and the structures such as blood vessels and bronchi were still easily identifiable. Reticulin stains at 3 days revealed that the underlying supporting collagen in the lung tissue was undisturbed with no destruction of the supporting tissue in alveolar walls, blood vessel walls, and bronchi (Figure 9.2).

Necrosis affected the epithelium and walls of the bronchi (Figure 9.3). All photosensitizers caused necrosis of the bronchial epithelium even though in the CCD images there appeared to be better uptake of PpIX in the epithelium than the other drugs. Necrosis was present in the vascular endothelium and muscular layer (Figure 9.4). Some vessels showed intraluminal thrombosis along with damage of the walls. Surrounding the PDT necrosis was a rim of acute inflammatory cells including neutrophils and lymphocytes (Figure 9.1a).
Figure 9.1 H&E sections of lungs 3 days after PDT treatment using mTHPC (Drug light interval 3 days, light dose 80 J) (A) Magnification x2 (B) The centre of the lesion, Magnification x40
Figure 9.2 Reticulin stain of PDT treated lung. Same section as Figure 9.1.

Figure 9.3 Bronchial epithelial necrosis after mTHPC PDT. Same section as Fig. 9.1.

Figure 9.4 Necrosis of the vascular wall after mTHPC PDT. Same section as fig. 9.1.
Sections taken immediately after PDT was completed did not show the PDT necrosis in the parenchyma which developed subsequently, but showed extravasation of red cells through the wall of larger vessels, both arteries and veins, along with endothelial damage and occasional thrombosis (Figure 9.5).

Figure 9.5 H&E section of lung immediately after mTHPC PDT (80 J, 3 day drug light interval) showing extravasation of red cells (A) (x 4), and endothelial damage in larger vessels (B) (x40)
Histology of lesions adjacent to the mediastinum showed that structures in this area were not affected by PDT. Some sections demonstrated typical PDT necrosis in the lung parenchyma however for example oesophagus immediately adjacent to lesions such as this was not affected histologically by the PDT (Figure 9.6). The pleura appeared to demarcate the zone of necrosis.

Histology 10 days after PDT showed resorption of red cells from the central part of the PDT necrotic area (Figure 9.7). By three weeks there was replacement of necrotic tissue by early granulation tissue (Figure 9.8). The bronchi and blood vessels within the PDT zone remained structurally intact and by this time had regrown normal epithelium and endothelium. By 2 months the initial PDT lesion had become a small scarred zone with residual bronchi and vessels within it (Fig 9.9). Despite the initial severe PDT necrosis, structures adjacent to the PDT such as large bronchi and vessels remained histologically normal. There was no residual scarring or architectural disruption of any surrounding tissue, including zones which may have included alveolar oedema and haemorrhage initially. The histology at 6 months was the same as that at 2 months indicating that the process was complete by 2 months.

At the early time points after mTHPC some sections showed intra - alveolar oedema, haemorrhage, and marked neutrophil infiltrates in viable lung surrounding the PDT zone (Figure 9.10 a and b). These changes were more marked than the typical inflammatory response seen with other PDT lesions. These changes were particularly apparent at 24 hours although they were occasionally seen at a 72 hour drug light interval. Only about a third of the 24 hour treatment histology sections had this appearance. This did not occur with the other drugs and apart from this there were no differences in the histological appearance of the PDT necrotic area between mTHPC, ALA, and ALS2PC.

These changes were most marked where the PDT lesion had been made close to the hilum of the lung. Thrombosis of the large hilar vessels was sometimes seen in these cases. There was often fluid in the interstitium immediately surrounding the larger vessels (perivascular cuffing ). The marked oedema and haemorrhage in the periphery of the lung therefore appeared to have been increased by proximal obstruction to pulmonary venous drainage. The sections which showed neutrophil infiltrate were counterstained with
Figure 9.6 H&E section demonstrating normal oesophagus immediately adjacent to PDT lesion. (mTHPC, 80J, 24 hour drug light interval).

Figure 9.7 H&E section of PDT lesion at 10 days.
Figure 9.8 H&E section of PDT lesion at 3 weeks showing regeneration of the bronchial epithelium within the lesion.

Figure 9.9 H&E section of PDT lesion at 2 months
Gramm’s stain which did not show any bacteria present. None of the rats from which these lung sections were taken showed any appearance of ill health or sepsis prior to sacrifice at 3 days.

Bronchoalveolar lavage fluid microscopy showed low to moderate numbers of polymorphs in all cases. Cultures were negative in 9 cases but showed a growth in 3 cases. The organism was Streptococcus viridans in 2 cases and proteus mirabilis in 1 case. Organisms were seen on microscopy in 2 of the 3 cases. Histological sections were made of the lungs from which the positive bronchoalveolar lavage fluid cultures were obtained. Gramm’s stain on each of these 3 slides was negative. All demonstrated neutrophil infiltrate in the vicinity of the PDT lesion. It is therefore possible that the positive BAL cultures were obtained from bacteria in the trachea or upper airway as the cannula was being inserted. Each of the bacteria cultured are normal flora in the rat airway. Furthermore all of these rats also appeared well at the time the specimens were taken.

Figure 9.11 shows the results of left lung weights (the treated lungs) compared to right lung weights (untreated). Weights were measured 3 days after treatment. This provided a measure of lung oedema and vascular congestion. The ratio of treated lungs at all drug light intervals were significantly heavier than control ratios, (T test with Bonferroni’s modification p<.001). Using Bonferroni’s modification of the T test for multiple tests there were no significant differences between the weight ratios at the 3 different drug-light intervals.
Figure 9.10 H&E sections of lung after PDT with 24 hour drug light interval (A) Haemorrhagic effects (B) Neutrophil infiltrate in surrounding lung.

Figure 9.11 Results of left lung weights with mTHPC PDT using 3 different drug light intervals. Comparison made with controls. 6 rats at each time point. All treated groups significantly heavier than control (p<.001). No significant differences between treated groups.
9.4 DISCUSSION

The necrosis was typical of photodynamic therapy in other organs in that epithelial structures were necrosed but the supporting connective tissue and the tissue architecture was not affected (Barr et al., 1987b; Bown, 1990). The ability of bronchial epithelium to regenerate is also typical of PDT (Smith et al., 1993; Pope and Bown, 1991a). The bronchus can be regarded as a hollow organ and as such its supporting structure of collagen and muscular layers remained intact following the PDT. Therefore it is likely that bronchial epithelium grew in from the unaffected parts of the bronchus outside the PDT zone. The same cannot be said however for the alveolar structures which became scarred presumably due to the lack of contact between an area of viable epithelium outside the PDT treated zone. The alveolus is a self-contained structure as compared to the hollow viscus of the bronchus. In human pathology after such severe acute damage to alveolar epithelium scarring is usually the end result (Bitterman, 1992). The regeneration of the bronchial epithelium is unlikely to have any beneficial effects from a physiological point of view as in the majority of cases of treating a peripheral lung tumour the bronchial structures will subtend only a small volume of lung parenchyma. However normal bronchial epithelial regeneration precludes the development of bronchial scarring and bronchial stenosis. This will ensure that bronchial drainage from the treated area is maintained.

There was also complete healing of blood vessel walls and at 2 and 6 months normal vessels were to be seen immediately adjacent to where the initial PDT necrosis had been. In rabbit experiments others have shown that PDT of arteries with ALA and ALS_2PC causes loss of endothelium and complete cell death throughout the media and adventitia (Grant et al., 1995). However the structural integrity of the vessels was not lost as their bursting strength was not reduced. This is typical of the effects of PDT in that the connective tissue components of the vessel wall were not disrupted.

The uniform nature of the necrosis across the zone is in keeping with uniform light distribution having occurred to all parts of the PDT zone. This is fortunate as the lung is a heterogeneous structure which potentially could have caused inhomogeneous light distribution. The histology of PDT lesions adjacent to the oesophagus supported the impression of the macroscopic
appearances that the visceral pleura is serving to limit light extension out of
the lung.

There were no thermal effect from the laser in control lung specimens so that
all necrosis was purely photochemical in origin. Others have found that
using 100mW for interstitial PDT avoids thermal effects at the laser tip
which could potentially limit the effectiveness of light distribution into the
tissues (Chang et al., 1996a). In surface treatments in experimental colon
tumours powers over 100 mW caused thermal effects (Tralau et al., 1987a).

Pelton has described the effects of photofrin PDT on thoracic organs during
surface treatments of the pleura in rats (Pelton et al., 1992). These were
studies for the development of intraoperative surface treatments of the
pleura with PDT as an adjunct to surgical resection. This included observing
the effects on lung adjacent to the pleura where the treatment was occurring.
They reported changes suggestive of diffuse alveolar damage including
epithelial and alveolar necrosis, intra -alveolar haemorrhage and fibrin
deposition. Diffuse alveolar damage, the pathological correlate of respiratory
distress syndrome, has these features in the early stages (Brigham, 1982).
The maximum depth of PDT necrosis was 3-4 mm. No evidence of acute
injury was seen at 1 month and at 6 months only mild chronic inflammation
was seen. These are similar findings to the results for interstitial PDT.
Hyaline membranes were not described in the acute lesions in Pelton's study
or this study, however as the lesions were probably primarily vascular in
origin it is reasonable to interpret these changes as localised diffuse alveolar
damage. The vascular changes seen immediately after PDT showing
endothelial injury with leakage of erythrocytes are in keeping with this.
Others have documented the sensitivity of the endothelium to PDT with in
vitro studies (Breider et al., 1993, West et al., 1990). Others have shown
slowing of flow and stasis in small vessels during photodynamic therapy
(Weiman et al., 1988). The perivascular fluid cuffs seen on some sections,
typical of increased lung lymph flow, are also features of early diffuse
alveolar damage following capillary injury (Brigham and Meyrick, 1986). In
this respect oedema and neutrophil infiltrate surrounding the lesions
treated 24 hours after mTHPC injection could be regarded as simply a more
florid manifestation of the same process. That is, a severe endothelial injury
leading to outpouring of fluid, and the accumulation of acute inflammatory
cells due to increased capillary permeability and in response to secretion of
inflammatory mediators (Krosl et al., 1995).
The results of lung weights at 3 days after PDT are supportive of this. While there was a trend for heavier lungs with a 1 day drug-light interval compared to 3 or 6 days the differences were not significant. The wide standard deviation at 1 day indicates the unpredictability of the vascular effects at this time. In contrast all PDT treatments regardless of the time of treatment were significantly heavier than control indicating inflammatory fluid accumulation in and around the lesion is an important component of the response to treatment at all drug-light intervals. It should be pointed out that the large change in ratio indicative of fluid retention was due to the large size of the lesion compared to the lung. The change in human lungs would therefore be much smaller and the accumulation of oedema fluid would only be in the vicinity of the PDT lesion. Furthermore this would be minimal if the treatment was at 3 days or longer.

The acute PDT necrosis was haemorrhagic in nature with all 3 drugs due most likely to the effects on the small vessels as was predicted by the CCD studies discussed in Chapter 6. However mTHPC demonstrated marked vascular effects in the region around the PDT necrotic zone which were not seen with ALS$_2$PC and ALA. These changes were most marked with the 24 hour drug light interval. It is surprising that the effects with ALSPC at early drug light intervals (1 hour) did not have more of a vascular nature, as this drug has also been known to cause vascular effects in other organs. There has been debate however as to whether the mechanism of action of this drug is primarily cellular or vascular.

Other authors have recognized the biphasic pattern of tissue damage with mTHPC (Anrejevic Blant, S., 1997). With short drug light intervals there is a marked vascular effect with thrombosis and oedema, however at later times there is still significant necrosis without these more florid vascular effects. In tumour treatments with mTHPC PDT there is often marked damage of tumour vessel walls as well as intraluminal thrombosis (Peng et al., 1995). This results in tumour infarction which contributes to the necrosis caused by the cellular effects of PDT. Initial clinical treatments on mesotheliomas were performed with a 24 hour drug light interval and tumour histology showed this vascular effect which included marked interstitial oedema (Ris et al., 1991). Clinical treatments now use a 96 hour drug light interval or even longer (Van den Bergh, 1994). Animal studies with ALS$_2$PC have also shown that the drug is present in high concentration in tumour vessels up to 6 hours after injection and that by 24 hours the drug is diffusely spread through the tumour, with little remaining in the vessels (Bremner et al.,
1992). With mTHPC there is also this diffuse distribution in the tumour however the vessels still show persisting drug in their walls up to 8 days after injection.

In vitro studies have shown that mTHPC does have a direct effect on cells and does not rely simply on vascular effects to cause necrosis (Ma et al., 1994). These studies on lung fibroblasts in fact showed that mTHPC was more efficient than photofrin in causing cell death. They also demonstrated that mTHPC is highly lipophilic and that maximal cellular uptake of the drug was observed 24 hours after addition of the drug to the cells. Furthermore numerous in vitro studies have demonstrated the effectiveness of PDT in causing necrosis of human lung cancer cells (Matthews et al., 1989, Perry et al., 1990).

Therefore it is likely that both vascular and cellular effects combined to cause the observed PDT necrosis.

The marked neutrophil infiltrate seen in some cases with mTHPC treatment with a 24 hour drug light interval was most likely a profound inflammatory response to cellular signals initiated by the photodynamic therapy process, rather than as a result of infection. The presence of tissue oedema may predispose to lung infections, however the absence of micro-organisms on sections counter-stained with a Gram stain and the generally negative culture results on broncho-alveolar lavage fluid mitigate against this. The broncho-alveolar lavage fluid was heavily cellular in all cases yet only had a growth on culture in three cases. Furthermore there were no adverse effects of this treatment in terms of the development of pneumonia or lung abscess and the histology at three weeks, 2 months, and 6 months showed no evidence of infection in any case. The scarring was the result of the profound initial necrosis and inflammation, with the likelihood that the fibroblastic proliferation was set in train by the initial PDT effect.

The CCD experiments discussed in the previous chapter raise the possibility that stimulation of macrophages containing photosensitizers may have an important role in causing the PDT effect. The tumoricidal capacity of macrophages for tumour cells is increased by photodynamic therapy (Yamamoto et al., 1992). Previous workers using in vitro models have shown that macrophages release cytokines such as tumour necrosis factor (TNF alpha) when stimulated with PDT (Evans et al., 1990). In the lung apart from exerting an antitumour effect TNF alpha causes a number of non specific
changes including vascular thrombosis and alveolar oedema. Whilst the observed changes in the lung sections could therefore possibly be due to the effects of macrophage stimulation these studies are unable to prove this. It is quite possible that all of these effects were mediated by photosensitizer localized in the microvasculature. However the CCD studies showed that drug was still present in both the small vessels and macrophages up to 6 -8 days after injection of the drug. Therefore either or both of these compartments could have been responsible for causing the still moderate sized lesions observed at this timepoint when the total quantity of drug in the tissues was low. It is noteworthy that the size of these lesions at 6 days, performed at a time when the quantity of drug in the tissues was at most 10% of its peak value, were equivalent to the largest lesion sizes with both ALA and ALS2PC. It may be that the PDT effects with this drug are multifaceted, that is, there is a vascular effect, seen with shorter drug light intervals but persisting at later times to a lesser extent, as well as a direct cellular effect combined with the effects of cytokines released by PDT activated macrophages. Even if macrophages only contribute partly to the PDT process this is to be welcomed as lung tumours contain large numbers of macrophages which could potentially improve the selectivity of the treatment effect on the tumour.
Chapter 10 Effect of ILP and PDT on lung physiology and mechanical integrity in rat lungs.

10.1 Introduction:

10.2 Methods
   10.2.1 Respiratory rates
   10.2.2 Lung compliance
   10.2.3 Bursting pressures

10.3 Results
   10.3.1 Respiratory rates
   10.3.2 Lung compliance
   10.3.3 Bursting pressure

10.4 Discussion:
10.1 Introduction

This chapter deals with attempts to detect any effect of ILP or PDT on lung physiology and the mechanical integrity of the lung. As these treatments will be performed on patients with poor lung function it is important to ensure that they cause no unexpected effects on lung physiology. It is unlikely that the loss of the small volume of normal tissue surrounding the tumour would have any significant impact on lung function. However these treatments could affect the lung parenchyma at sites distant from their main site of action by altering the elasticity of the lung at a submicroscopic level, particularly as the healing and scarring process occurs. Respiratory rates were measured as an indicator of the overall functioning of the respiratory system. Others have been able to match respiratory rates to the histological development of radiation pneumonitis in mice. Higher respiratory rates were matched by higher degrees of radiation pneumonitis. Also, measurement of lung compliance was performed at times from 2-6 months after ILP and PDT.

Pneumothorax is a complication of any procedure which involves placement of needles into the lung (Lane and Gleeson, 1995). Interstitial treatments for lung tumours may therefore cause pneumothorax, as was demonstrated in chapters 4 and 7. There are 2 possible causes for pneumothorax with these treatments. Firstly, air may be drawn into the pleural space via the needle insertion track through the chest wall. Secondly, air may enter the pleural space from the punctured visceral surface of the lung. The incidence of pneumothorax was low in the rat experiments when considering the size of the needle (19 G) compared to the small size of the lung, and considering that a treatment which caused tissue necrosis was being performed. In reported series of fine needle aspiration in patients the use of large needles is a risk factor for pneumothorax (Berquist et al., 1980). It is possible therefore that the effect of ILP and PDT was to seal the visceral pleural surface at the site of the needle puncture, thereby preventing pneumothorax and increasing the safety of the procedure. That is, the mechanical integrity of the pleural surface remained intact despite a large introducer needle having been introduced to perform the treatment. Others have assessed the mechanical strength of an ILP or PDT lesion in hollow viscera such as the colon or blood vessels by testing the pressure at which the organ bursts. The bursting pressure experiments described in this chapter apply the same principle to quantitate this sealing effect of each form of treatment. Lungs are inflated via the trachea and the pressure at which the ILP or PDT lesion bursts is measured.
10.2 Methods
Measurements were made on the lungs and rats described in the preceding chapters.

10.2.1 Respiratory rates
Respiratory rates were performed after the methods of Travis where rats were placed in a commercially available perspex holding chamber (Travis et al., 1979). Spontaneous breathing rates were recorded on video camera. Rats were able to walk in to this cylindrical chamber, there was no impediment to breathing by the chamber walls, and there were a number of holes to allow free entry of room air. The rats stood facing a plastic cone within the chamber which communicated with room air. Each rat was acclimatised to the chamber in three sessions of two minutes each. During these acclimatisation sessions the rats often attempted to turn around in the narrow cylindrical chamber and the breathing was erratic as a result. However in subsequent sessions they were content to stand in a forward facing direction and the breathing was more regular allowing rates to be counted. By videotaping the rats breathing, episodes of movement and sniffing could be differentiated from normal breathing and these were not recorded. Recordings based on pressure changes in the chamber do not allow for this differentiation. After allowing 30 seconds for the rats to settle in the chamber, 30 seconds to one minute of the rats' breathing was recorded. The mean of two separate recordings for each rat was taken at each timepoint. For both ILP and PDT recordings were made in 8 rats before treatment as a control and after treatment at 1 week, 2 weeks, 3 weeks, 4 weeks, 6 and 8 weeks. Assessments at 4 and 6 months were made in separate groups of at least 6 animals which did not have control measurements taken before treatment. These rats were compared to controls which had a sham operation, that is, a general anaesthetic with a chest wall incision and insertion of the laser fibre only without activation of the laser. These controls were kept out to 4 and 6 months respectively.

10.2.2 Lung compliance
A measure of lung compliance was performed after the methods of Young (Young et al., 1970). Measurements were performed on groups of 4 excised lungs at 2, 4 and 6 months after ILP and on groups of at least 5 lungs at 2 and 6 months after PDT. These were compared to untreated control lungs taken from animals in the same weight range. Up to 2 months groups of rats were either in the weight range 300-340g or 340-380 g. By 6 months there had
been some growth of the rats and therefore the controls for these were taken from heavier rats, 380-420 g.

After sacrifice and removal of the lungs the left main bronchus was cannulated and secured with a suture. Then the lung was placed in a closed chamber and was degassed by raising the chamber pressure above atmospheric and simultaneously aspirating air from the cannulated left main bronchus. This procedure was performed twice. Then from this degassed volume air was injected in 0.5ml increments into the left main bronchus. The pressure was recorded after each increment by a pressure transducer. A three-way tap was used to alternate injections of air and measurements of pressure. Pressure recordings were made after 30 seconds of equilibration after injection of each increment of air. Increments were injected until an arbitrary total lung capacity (for the left lung) of 30 mAtm was reached (Hayatdavoudi et al., 1981). Air was then withdrawn in increments of 0.5 mls down to the starting volume. A total of three inflations and deflations were performed and the second and third of these were analysed. As found by others the data from the first cycle tended to be inconsistent due to the lung reinflating from its fully collapsed state during this cycle. The second and third curves were plotted separately and a mean of the straight portions of the slopes of both the inflation and deflation limbs of the 2 curves was recorded for each animal (Hayatdavoudi et al., 1981). The pressure at an inflation volume of 3.0 ml was chosen as a way of comparing the positions of the curves, to see whether there was any shift to the right with treated lungs compared to controls. A number of recordings could not be made as the lungs occasionally burst during the inflation procedure, or a hole was inadvertently made in the left main bronchus during the cannulation or suturing procedure. This latter procedure was technically difficult and painstaking care had to be taken to ensure that the fastening suture was safely placed. The surface of the lung was periodically moistened with normal saline as drying of the lung caused stiffening and a propensity to burst.

10.2.3 Bursting pressures

After sacrifice and removal of the lungs a cannula was placed into the left main bronchus and ligated. The lung was then placed in water and kept submerged by a specially designed frame (Scherle, 1970, Figure 10.1). The lungs were slowly inflated with air via a syringe mounted on a syringe driver. Pressure was progressively increased until air escaped from the lung surface as evidenced by bubbles escaping into the water. The pressure at which this
occurred was recorded and the site where the lung surface first ruptured was carefully observed. Treated lungs were compared to normal untreated lungs and controls which had only had the laser fibre inserted with the introducer needle without activating the laser. Measurements on the treated lungs were performed immediately after treatment when the risk of pneumothorax would be highest and at 3 days when the lesion necrosis would be maximal. 6 animals were treated at each of these times for both PDT and ILP. Six and 7 animals were used to test the normal lung and laser fibre only controls respectively.

Figure 10.1. Diagram of experimental set up for bursting pressure measurements in rat lungs.
10.3 Results

10.3.1 Respiratory rates

Figure 10.2a shows the respiratory rates from 8 rats before and up to 6 months after ILP at 2w for 250 seconds. There was no significant difference in respiratory rates after ILP with all rates being within 10% of control values. Similar results were seen after the PDT treatments of 80J at a 3 day drug light interval (Figure 10.2b). No rats demonstrated changes in breathing pattern such as abdominal or flank breathing.

On dissection at 2 months it was noted that one of the ILP rats had collapse of the lower part of the lung due to the laser lesion having caused occlusion of the proximal main bronchus. In this rat all respiratory rates from 2 weeks after the treatment were significantly higher than control (mean 128 bpm). This was the only animal in which a significant increase in respiratory rate was seen and this was clearly the result of a complication of ILP due to the small size of the animal.
Figure 10.2 Respiratory rates in rats up to 6 months (A) after ILP, and (B) after PDT. Different controls used for the measurements at 6 months due to larger animals at this time.
10.3.2 Lung compliance

Figure 10.3 demonstrates typical compliance curves from both a treated lung and a control lung. They demonstrate the linear portion in the mid section of the inflation part of the curve. Measures of slopes in individual lungs were highly reproducible. In 3 specimens 4 separate inflations and deflations of the lung were performed. Standard deviations in these lungs were at most 3% of the overall slope measurement. Table 10.1 shows the slopes of inflation and deflation limbs of the curve in treated and control lungs at times up to 6 months. There were no significant reductions in compliance for either treatment although there was a slight reduction at 2 months. Table 10.2 shows that the pressure at a volume of 3 ml inflation did not differ between treated and control groups, confirming the visual impression of the graphs that there was no shift of the curve, and therefore no major difference in residual volume and total lung capacity.
Figure 10.3. Examples of compliance curves from the left lung of a control rat (A) and a PDT treated rat at 6 months (B).
### Table 10.1 Results of compliance slopes. Each cell of the table the mean and standard deviation of slopes from curves from 4 animals, except 6 months in ILP which had 1 animal.

<table>
<thead>
<tr>
<th></th>
<th>Inflation slopes</th>
<th>Deflation slopes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 months</td>
<td>4 months</td>
</tr>
<tr>
<td><strong>ILP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated lungs</td>
<td>0.2 +/- .05</td>
<td>0.23 +/- .02</td>
</tr>
<tr>
<td>Control lungs</td>
<td>.22 +/- .04</td>
<td>.22 +/- .04</td>
</tr>
<tr>
<td><strong>PDT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated lungs</td>
<td>.14 +/- .02</td>
<td>.24 +/- .03</td>
</tr>
<tr>
<td>Control lungs</td>
<td>.18 +/- .05</td>
<td>.23 +/- .04</td>
</tr>
</tbody>
</table>

### Table 10.2 Pressure at inflation volume of 3 ml in treated and control lungs. Each cell of the table is the mean (SD) from 4 animals.

<table>
<thead>
<tr>
<th></th>
<th>2 months</th>
<th>4 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ILP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated lungs</td>
<td>22.8 +/- 3.9</td>
<td>20 +/- 2.9</td>
<td>23</td>
</tr>
<tr>
<td>Control lungs</td>
<td>19.5 +/- 2.1</td>
<td>19.5 +/- 2.1</td>
<td>20</td>
</tr>
<tr>
<td><strong>PDT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated lungs</td>
<td>28.3 +/- 5.9</td>
<td>Not done</td>
<td>24.5 +/- 1.3</td>
</tr>
<tr>
<td>Control lungs</td>
<td>22 +/- 2.3</td>
<td>20 +/- 3.5</td>
<td></td>
</tr>
</tbody>
</table>
10.3.3 Bursting pressure

Table 10.3 summarises the results for bursting pressures. Untreated lungs tended to burst at the lung apex or on the lateral surface in the apical part of the lung. In the controls for insertion of the laser fibre alone the lungs burst at this insertion site in 6 out of 7 cases. In contrast the number of lungs bursting at the ILP and PDT treated sites immediately after treatment were 2 out of 6 and 3 out of 6 respectively. Using Fisher's exact test these were not significantly different from controls. At 3 days there were even fewer instances of the lungs bursting at the treatment site. In 2 of the cases of bursting at the ILP lesion, the lesion was passing from the outer to the inner surface of the lung thereby creating a channel such that at no point was there overlying lung to seal it. Furthermore the pressure at which bursting of the two ILP lesions occurred was significantly higher than the bursting pressure of the 6 where there was only needle insertion (mean 49 mAtm compared to 28.2, p< 0.01 ). The pressures at which the three PDT sites burst were also significantly higher than the fibre alone controls (67 m Atm compared to 28, p< 0.01). The pressures where the ILP and PDT lungs burst (whether at the laser site or elsewhere) were comparable to the bursting pressure of control lungs which had no intervention, although there was a wider range of bursting pressures for the ILP treated lungs.

<table>
<thead>
<tr>
<th></th>
<th>Bursting Pressure (mAtm)</th>
<th>Number bursting at site of intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONTROLS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No intervention</td>
<td>56 +/- 11</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Needle and fibre</td>
<td>36 +/- 20</td>
<td>6 out of 7</td>
</tr>
<tr>
<td>(immediately)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ILP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediately</td>
<td>43 +/- 12</td>
<td>2 out of 6</td>
</tr>
<tr>
<td>3 days</td>
<td>58 +/- 30</td>
<td>1 out of 6</td>
</tr>
<tr>
<td><strong>PDT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediately</td>
<td>66 +/- 23</td>
<td>3 out of 6</td>
</tr>
<tr>
<td>3 days</td>
<td>70 +/- 23</td>
<td>0 out of 6</td>
</tr>
</tbody>
</table>

Table 10.3 Results of bursting pressure on control and treated lungs.
Of the 6 animals which were assessed immediately after ILP treatment 2 had pneumothorax on fluoroscopy. In 1 of these rats there was a chest wall hole created by the laser and the laser site remained patent in the bursting pressure experiment. In the other the laser hole extended through the full width of the lung such that a channel was created in the lung parenchyma, and in this case the laser lesion burst at this site. Of the animals tested at 3 days 1 had a pneumothorax on fluoroscopy immediately after treatment and in this case the lesion did not burst. There was only 1 pneumothorax in the PDT group. The bursting pressure was tested immediately in this rat and the lung burst at the PDT site.

10.4 Discussion

Interest in the physiological effects of these interstitial treatments stems from the fact that radical radiotherapy, the current option for patients who are not suitable for surgery, causes effects on the lung parenchyma which range from mild to life threatening. The extent of effect depends on a number of factors including the volume of lung irradiated, the existence of prior lung disease, the fractionation schedule and concurrent use of chemotherapy (Travis, 1991; Emami et al., 1991). A treatment which could avoid these problems would therefore be preferable to radiotherapy.

Although the present experiments did not directly compare ILP and PDT with radiotherapy they suggest that physiologically these are well tolerated treatments. In patients there is a well defined dose-response relationship for radiation pneumonitis after irradiation of the whole thorax (van Dyk et al., 1981). However there is little in the literature on the clinical effects of irradiation of partial volumes of lung (Prasad, 1978). Although most experimental work has reported the effects of irradiating both lungs Liao has reported studies on the effects of irradiating partial volumes of single lungs on mortality and breathing rates in rats (Liao et al., 1995). Volumes of single lung ranging from 17 to 84% were irradiated with single doses of between 12-20 Gy. The threshold volume for mortality was greater than 50% for volumes which included the lung apex, and 40-70% for volumes which included the base. The threshold volume for increase in respiratory rates was greater than 17% for the apex and greater than 40% for the base. Breathing rates were measured twice weekly up to 32 weeks and showed a clear volume effect. Changes in breathing rate began later and peaked at lower levels as the volume of lung irradiated decreased. For mice which had 17% of one
lung irradiated, 10 % of mice had a breathing rate 20 % higher than control at 22 weeks, compared to 90 % of rats which had 40 % of the lung irradiated.

These results indicate the importance in clinical treatments of limiting the radiation port to as small a volume as possible. This requires a great deal of expertise and planning to ensure the target tumour is actually included in the treatment field. As with ILP some normal lung must be treated, but the present experiments have shown that for the small volume of lung treatment required there is no increase in respiratory rate. There is no doubt that the tumour will be in the treatment field because ILP treatment is delivered directly into the tumour using CT control.

The present studies show no significant impact on lung physiology and support the concept that ILP and PDT in the lung are truly localised treatments. The histological fibrosis around the lesion after ILP and PDT was always limited. In contrast fibrosis following radiotherapy may be unpredictable and extensive. Acute radiation pneumonitis is also unpredictable and may involve large parts of the lung. Occasionally this may include parts of the lung not treated by radiotherapy. There were no such histological findings in either the short or long term assessments after either ILP or PDT. Furthermore the size of the laser lesion relative to the whole lung in these rats, approximately 13% of the volume, would be more than that seen in treating a 2 cm diameter tumour with a 2 cm normal lung perimeter ( volume 270 ml in a typical single lung volume of 3 litres, 9%) in a patient. Therefore effects on these small rat lungs would be an overestimate of any effects which might occur in patients.

Changes in respiratory rate are observed readily in rats when there is any derangement of lung function, for example due to radiation pneumonitis and radiation fibrosis (Travis et al., 1980). Travis et al irradiated both lungs in mice and after 16 weeks measured breathing rates with the mice in a perspex airtight cylinder fitted with a pressure transducer. There was a linear dose response effect of radiation on breathing rates once the dose of radiation exceeded 11 Gy. Rates increased from controls of 300 to 450 breaths per minute at 16 weeks. The method proved a reliable non invasive means of predicting radiation pneumonitis as opposed to just recording the dose causing death of 50 % of mice (LD 50). It also allowed repeated measurements on the same mice over time. They verified the plethysmographic measurement of breathing by using high speed cine film. In the current experiments the rates were slower and it was therefore valid to
use conventional video-filming of the rats breathing as the means of recording respiratory rates.

With restrictive lung dysfunction, breathing becomes more rapid and shallow. Travis et al measured amplitude of breathing, however this did not provide additional information as the shape of the dose response curve was a mirror image of the breathing rate curve (Travis et al., 1979). There were also more fluctuations and scatter in the amplitude measurements and the authors regarded the breathing rate as the better of the 2 measurements. In further studies using this technique only breathing rate was measured (Liao et al., 1995; van Rongen et al., 1995). In the current experiments the effect on physiology of the fibrosis due to ILP or PDT would have been of a restrictive type similar to radiation effects. Therefore measuring breathing rates without measuring breathing amplitude was a valid approach. Also it would appear that the lesions were not causing any marked physiological shunt, although detailed arterial blood gas studies would have been required to confirm this.

Others have used similar measurements of lung compliance on excised rat lungs. For example it was shown to be a reproducible method in confirming increases in lung compliance in the development of an animal model of emphysema and a decrease in compliance due to oxygen toxicity (Freeman et al., 1972, Hayatdavoudi et al., 1981). Freeman showed a shift of the curve to the left due to differences in residual volume which was not seen in this study. Although due to small numbers the compliance studies cannot confirm there is no effect on lung compliance, they show no major effect, particularly considering only the treated lung was tested. If both lungs had been tested, results may have masked an effect on treated lung. Also, the results for lung compliance are in keeping with the detailed microscopic assessment of the perimeter of the laser lesion. Although the histology showed a clear margin between normal and laser affected tissue, there remained a possibility that submicroscopic damage to normal lung matrix occurred due to the effects of heat at sites distal to the lesion. Special stains for collagen and elastin did not show structural damage nor did electron microscopy outside of the lesion, and these results on compliance suggest that there is no marked effect on the function of the lung distal to the lesion.

The bursting pressure measurements showed that in most cases there was acute sealing of the hole created in the lung surface by the introducer needle. There were 2 cases, 1 with ILP and 1 with PDT, in which the
treatment site on the lung appeared to be the cause of pneumothorax as this was the site which burst first. In general however the treatments did not enlarge the needle hole even though they were causing necrosis of the underlying parenchyma. This is reassuring information with respect to the risk of pneumothorax. With ILP sealing was likely to have occurred at the coagulated zone immediately surrounding the centre of the laser lesion. Laser welding is a procedure which uses the principle that with tissue temperatures around 90-100 °C there is fusion of collagen in 2 opposing tissue surfaces (Flemming et al., 1990; Flemming et al., 1988). It provides immediate fluid-tight sealing. This has been used in surgery for example in anastomosis of segments of bowel (Cilesiz et al., 1996). It is likely that a similar effect is occurring in these ILP treated lungs such that the fusion of collagen fibres in the treated zone is serving to seal the hole created by the introducer needle.

Somewhat surprisingly there was also an acute benefit with PDT. This may have been due to the acute vascular effects of mTHPC PDT causing some local occlusion around the site of needle introduction. Necrosis of the lung parenchyma itself would be unlikely to have commenced until 24 hours after the treatment and would only be maximal by 3 days. The results indicate that if the treatment does not self seal immediately then it will seal by 3 days, particularly with PDT as none of the 7 lungs tested at 3 days burst.

Where bursting pressures have been performed before to compare thermal and PDT effects in hollow viscera, there was reduced bursting pressure with thermal treatments and maintained integrity with PDT (Barr et al., 1987a; Smith et al., 1993). The results of the current experiments are therefore in contrast. The measurement of bursting pressure in this study was to validate an impression that the incidence of pneumothorax was low considering the fragile surface of the lung had been punctured by a sharp needle. Therefore this was not a measure which had physiological implications to the functioning of the normal lung, rather it was to measure the effects of a mechanical intervention.

In the following chapter PDT and ILP are applied to the normal lungs in large animals. As will be discussed pneumothorax occurred with both ILP and PDT and the results from these bursting pressure experiments suggested that the problem was due to entry of air into the pleural space through the needle tracks in the chest wall rather than from the surface of the lung.
Chapter 11 ILP and PDT treatments of lung parenchyma using multiple fibres and diffuser fibres in pigs.

11.1 Introduction

11.2 Methods
11.2.1 Animals
11.2.2 Operative technique
11.2.3 Lasers and fibres
11.2.4 Observations

11.3 Results
11.3.1 Treatments under local anaesthetic
11.3.2 Treatments under general anaesthetic
11.3.3 Macroscopic appearances
11.3.4 Microscopic Effects
11.3.5 Adverse effects

11.4 Discussion
11.1 Introduction

Experiments in large animal lungs were performed for a number of reasons. Firstly in order to accommodate lesions made with multiple fibres inserted simultaneously a large lung was required. The use of multiple fibres in experimental and clinical work allow the creation of lesions which are large enough to cause necrosis of small tumours and some surrounding lung. In these experiments only normal lung was treated but the magnitude of lesion created would give an estimate of the number of fibres and treatment parameters required to incorporate a lung tumour of 2-3 cm in diameter and 1cm of normal surrounding parenchyma.

Secondly by using a lung the size of a human lung with a similar anatomical structure and blood supply the laser tissue interactions would be more typical of human lungs than small animal lungs. In particular the transmission of heat and light away from the centre of lesions in ILP and PDT would be similar to humans.

Thirdly in the small animal experiments a number of side-effects occurred which were due to the proximity of the laser to major structures. These included oesophageal perforation, particularly in ILP, and vascular effects when the hilar vessels were damaged by ILP and PDT lesions. These had serious effects on the outcome of the treatment in the rats. It is important therefore to show that these lesions can be created safely in larger lungs where damage to these vital structures can be avoided by positioning the fibres away from these regions. The vascular effects are likely to be similar in both rat and pig experiments, however in the larger pig lungs there is an extensive collateral circulation which would be anticipated to counteract any adverse effects on the vessels due to the laser treatments. In the rat lungs hilar vessel damage caused severe alveolar haemorrhage, oedema, and infarction. In contrast it is unlikely that such an extensive effect would occur following treatment of a peripheral portion of lung parenchyma in the pigs. Therefore while the effects in the rats were severe the treatment is likely to be much better tolerated in the larger animal even though the histological appearances may be similar at the treatment site. These effects are described in this chapter.

Fourthly in the PDT experiments the pharmacokinetics of the injected drug in large animals is almost the same as for humans (Ronn et al., 1996). This allows for information on drug light intervals in large animal experiments to
be used in treatments in humans. Only mTHPC was used for these large animal experiments as this was the most promising photosensitizer in the small animal studies. Finally with larger lesions cavitation may occur in the long term (McNicholas et al., 1993a). This is best assessed with a large animal model as with the rats it may have been that due to the small size of the lesions cavitation could not have occurred.

11.2 Methods

11.2.1 Animals

Normal large white pigs weighing approximately 100 kg were used. Pigs were used as the anatomy of the lungs including lobar structure, is similar to humans (Spencer, 1985a). There are some differences in the submacroscopic structure of the lung, including strongly developed interlobular septae, however pig lungs are more similar in this respect to humans than other large mammals such as dogs (Spencer, 1985a). One hundred kilogram pigs were chosen firstly as the size of the lung is similar to a human lung. Secondly and most importantly if smaller pigs, for example, 30 kilogram in weight had been used, then the pigs body size would have increased rapidly after the treatment by the time of the long-term assessments at 2 months. Pigs of this small size grow at a rate of 1 kilogram a day with growth in both skeletal structure and muscle and fat mass. This growth pattern continues up to about 100 kg weight (A.M. Johnstone, 1996, personal communication). Body weight gain continues after this although at a slower rate, however further skeletal growth does not occur to any significant extent. Therefore by choosing 100 kg pigs it was anticipated that no significant growth of the lung would occur (Thurlbeck, 1982; Hsia, CCW et al 1996). In other mammals including humans growth of the lung stops slightly earlier than growth of the skeletal system. If lung growth was to occur during the period after treatment of the pig lung, healing of the lesions may have been confounded by the growth of the lung which in all likelihood would have accelerated the healing response to a greater degree than would be expected in older lungs.
11.2.2 Operative technique

The first two treatments were performed under local anaesthetic but due to difficulties with this technique the remainder were performed under general anaesthetic. The local anaesthetic technique was as follows. Firstly intramuscular sedation was given as the pigs stood in a holding crate. Azaperazone was used in a dose of 5 mg/kg. The skin was prepared with topical antiseptic and local anaesthetic (1% Lignocaine) was infiltrated into the skin and down to and including the pleura. Once the pig was settled the needles were then inserted into the lung through the chest wall in a dorsal ventral direction. This was made difficult by the movement of the pig despite sedation and subsequent treatments were therefore performed under general anaesthetic which was a much more satisfactory method.

General anaesthesia was induced with Halothane after prior sedation with azaperazone. Anaesthesia was maintained via face mask with 2-3% Halothane and oxygen 7-8 l/min. No muscle relaxant was used and pigs breathed spontaneously. Pigs were placed in a lateral position and needles were passed through the lateral chest wall into the lung in a lateral to medial direction. The optimum point of insertion was determined firstly by preliminary dissection which showed that at a point on the skin 15 cm down the chest wall from the spinal cord the lung was at its thickest in a lateral to medial direction. Secondly fluoroscopy was used with the pig under anaesthetic to locate the upper border of the lower lobe and mark the skin at this point. All treatments were performed in the lower lobe, caudal to this skin mark. The 4 needles were inserted in a square pattern and imaged fluoroscopically in the lung (Figure 11.3). For ILP most treatments were performed with the needles separated by 1 cm and for PDT by 0.7 cm.

11.2.3 Lasers and fibres

For the multiple fibre treatments in ILP and PDT four 400um laser fibres (outer diameter 0.7mm) were used. 19 guage introducer needles were used to insert the fibres into the lung. Prior to insertion the laser fibres were flagged at the surface to ensure that 1 cm of the fibre would be protruding from the end of the introducer needle inside the lung. For the ILP treatments the fibres were pre-charred prior to insertion as described in Chapter 5. PDT was also performed using a single 2 cm length diffuser fibre with an outer diameter of 0.8 mm (Figure 11.2). This required a slightly larger introducer needle for insertion into the lung (17G).
Figure 11.1 Diode laser used for PDT. The laser for ILP is slightly smaller than this. The fibre splitter is seen at the left and the four fibres and introducer needles are seen in the foreground.

Figure 11.2 Bare tip fibres (left) and diffuser fibre (right) Both types of fibre are seen, extending from introducer needles. Arrows indicate the 2 cm length of the diffuser tip.

Figure 11.3 Fluoroscopic image showing anteroposterior view of pig lung with 4 needles in place for interstitial treatment.
For ILP the 25W Diode (Diomed) laser was used. The laser was connected to a production made beam splitter which divided the laser output equally into the 4 fibres. Laser parameters for ILP were 2 or 3 Watts for 500 or 333 seconds respectively. For each lesion there were 2 treatments with the second performed after pulling the four fibres back 1 cm from their original position. Each animal had up to 4 lesions made in this way, with 2 in each lung.

For PDT a diode laser (Applied Optronics, loan of Scotia pharmaceuticals) was used (Figure 11.1) This laser is designed to deliver only the necessary 652 nm wavelength for PDT with mTHPC. Like the ILP laser it is portable and works from the mains electricity supply. With the 4 fibre treatments the light dose was 100 Joules per fibre. The laser parameters for each fibre were either 100 mW for 1000 seconds or 50 mW for 2000 seconds. As for the ILP treatment each lesion was made with 2 stations each with these treatment parameters separated by a pull back of 1 cm. For the single diffuser fibre treatments the light dose was 100 J/cm of diffuser, 200J total. The laser parameters were 100 mW for 1000 seconds. No pullback was performed with the diffuser. Up to 4 lesions were made in each pig, with a maximum of 2 multiple fibre treatments in 1 lung and 2 diffuser fibre treatments in the other lung.

The dose of mTHPC was 0.15mg/kg, the same as in human treatments. The drug light interval was 4 days for the first 2 pigs but was changed to 3 days for the remainder of the treatments. Both of these drug light intervals have been used in clinical studies of mTHPC (Grosjean et al., 1997; Ris et al., 1991). Pigs were kept indoors and protected from direct light after the injection of mTHPC.

11.2.4 Observations

After each lesion was made fluoroscopy was used to check for pneumothorax. If this occurred no further lesions were made and the pneumothorax was aspirated with a cannula, 3 way tap and 50 ml syringe (Andrivet et al., 1995). Conventional chest Xrays were taken in all animals 3 days after treatment and at the time of sacrifice if this was later than 3 days.

Pigs were regularly checked for general well being and any signs of respiratory distress. A maintenance diet was used in the pigs kept for longer
periods to prevent excessive body growth which would therefore limit any further growth of the lung during this time.

Pigs were sacrificed from 3 days to 3 months after treatment. The lungs were then removed and inflated via the trachea with formaldehyde by gravity. Lungs were then placed in formaldehyde and after 24 hours each lesion was sectioned perpendicular to the line of laser fibre insertion. The size of the lesion was measured in 3 dimensions (length and breadth of the cross section of the lesion and depth of the lesion in the direction of laser fibre entry) and necrosis was confirmed on subsequent histological sections.

11.3 Results

11.3.1 Treatments under local anaesthetic

The treatments performed under local anaesthetic were difficult due to movement of the animal despite sedation. The needles were always inserted while the pig was stationary, however during the treatment the pig sometimes stood up and this shifted the position of the needles. In the first pig three lesions were made but as a result of this problem only one lesion showed uniform necrosis at 3 days. During the local anaesthetic treatments with ILP the pig did not demonstrate any signs of discomfort as a result of the ILP. In the second pig there was even more movement after insertion of the needles with the pig repeatedly standing up then returning to the lying position. Three minutes into the first treatment this pig had haemoptysis of approximately 200mls and the procedure was immediately stopped. No further bleeding occurred once the needles were removed. This pig died 24 hours after treatment. At post-mortem there was a large lung haematoma at the site of needle insertion into the lung. There was laceration of the visceral pleural surface at the site of the needle insertions into the lung. There was also a large haemothorax. On sectioning of this haematoma cavity it was not possible to distinguish any laser lesion. The haematoma had obliterated the lung parenchyma in a diameter of 6 cm. Subsequent histology was also not able to distinguish any laser effect on blood vessels. It appeared therefore that the needles had lacerated the lung parenchyma during the movement of the pig and this had caused the large haematoma and laceration of the visceral pleura with haemothorax.
11.3.2 Treatments under general anaesthetic

The treatments performed under general anaesthetic were much more satisfactory. Eleven more pigs were treated with either PDT or ILP and there were no further adverse events either during the treatments, in the peri-operative period, or during the healing phase. All animals had normal general appearance and behaviour following the treatments, indicative of good health. The maximum weight gain by any pig was 20 kg therefore during the healing phase no significant growth of the lung occurred.

11.3.3 Macroscopic Appearance

Tables 11.1 and 11.2 list the lesion sizes after ILP and PDT respectively as measured at times from 3 days to 3 months after treatment.

11.3.3.1 ILP

At three days the pleural surface at the site of the ILP lesions was slightly indrawn due to the localized thermal effects of the ILP. On sectioning the lesions appeared roughly spherical in shape ( Figure 11.4 ). The lesions showed central necrosis surrounded by lung inflammation. Within the necrotic zone four separate charred regions due to each fibre could be identified on most sections. These were placed in approximately a square pattern.

Two lesions were made with the 4 fibres separated by 1.5 cm. The laser parameters with this treatment were 3 Watts for 333 seconds. On sectioning these lesions the central ILP zone contained four separate circular zones of necrosis each with its own zone of central charring. The four lesions did not fully coalesce and macroscopically it appeared that necrosis was not present across the full extent of the ILP zone ( Figure 11.5 ). This was confirmed on histology which showed viable portions of the ILP lesion. Therefore subsequent lesions were made with the fibre separation of 1 cm. All lesions created with this fibre separation showed fully coalescing zones of ILP necrosis.

At three weeks the lesion was surrounded by fibrous tissue. There was still central necrotic tissue, which in places had cavitated. There was contracture of the pleural surface at the site of the lesion. At 2 months and 3 months this contracture was slightly more marked however the overall surface and
structure of the lung was not disturbed (Figure 11.7). On cut sections at 2 and 3 months the lesions still showed this central necrotic tissue persisting with residual charred regions within this necrotic tissue (Figure 11.5). They were surrounded by a rim of dense scar tissue. The appearances at 3 months were not different to those at 2 months macroscopically.

<table>
<thead>
<tr>
<th>Pig Number</th>
<th>Treatment per fibre</th>
<th>Time of assessment</th>
<th>Lesion size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2W 500s</td>
<td>3 days</td>
<td>2.8 x 2.5 x 2</td>
</tr>
<tr>
<td></td>
<td>3W 333s</td>
<td>3 days</td>
<td>† 3.5 x 0.5 x 1.0</td>
</tr>
<tr>
<td>2</td>
<td>2W 500s</td>
<td>3 days</td>
<td>3.0 x 1.8 x 2</td>
</tr>
<tr>
<td></td>
<td>2W 500s</td>
<td>3 days</td>
<td>3.1 x 1.7 x 1.9</td>
</tr>
<tr>
<td></td>
<td>*3 W 333s</td>
<td>3 days</td>
<td>** 4.4 x 2.8 x 2</td>
</tr>
<tr>
<td></td>
<td>*3 W 333s</td>
<td>3 days</td>
<td>** 4.2 x 3.4 x 1.8</td>
</tr>
<tr>
<td>3</td>
<td>3 W 333s</td>
<td>3 days</td>
<td>3.8 x 2.0 x 2.5</td>
</tr>
<tr>
<td></td>
<td>3 W 333s</td>
<td>3 days</td>
<td>3.2 x 2.0 x 2.5</td>
</tr>
<tr>
<td></td>
<td>3 W 333s</td>
<td>3 days</td>
<td>3.0 x 2.3 x 3.1</td>
</tr>
<tr>
<td></td>
<td>3 W 333s</td>
<td>3 days</td>
<td>3.0 x 2.8 x 2.5</td>
</tr>
<tr>
<td>4</td>
<td>3 W 333s</td>
<td>3 weeks</td>
<td>3.0 x 1.5 x 1.5</td>
</tr>
<tr>
<td>5</td>
<td>3 W 333s</td>
<td>2 months</td>
<td>1.9 x 1.1 x 1.6</td>
</tr>
<tr>
<td></td>
<td>3 W 333s</td>
<td>2 months</td>
<td>1.8 x 1.0 x 1.5</td>
</tr>
<tr>
<td>6</td>
<td>3 W 333s</td>
<td>3 months</td>
<td>2.2 x 1.1 x 1.0</td>
</tr>
<tr>
<td></td>
<td>3 W 333s</td>
<td>3 months</td>
<td>2.0 x 1.0 x 1.0</td>
</tr>
<tr>
<td></td>
<td>3 W 333s</td>
<td>3 months</td>
<td>2.2 x 1.4 x 1.1</td>
</tr>
</tbody>
</table>

Table 11.1 Lesion sizes after ILP. Lesions were ellipsoid in shape and measured in 3 dimensions. All performed with 4 fibres with 1 pullback of 1 cm.

* = fibres separated by 1.5 cm. Remainder of treatments performed with 1 cm separation. ** = some viable areas in the lesion.
† = performed under local anaesthetic and lesion affected by movement of the pig.
Figure 11.4 (A) Macroscopic appearance of ILP lesion 3 days after treatment. (4 fibres separated by 1cm, 3W for 333 seconds per fibre)
(B) Microscopic appearance of this section (x1)
Figure 11.5 (A) Appearance of lesion at 2 months. (B) Microscopic appearance of this section (x1).
Figure 11.6 Non coalescing areas of necrosis in ILP lesion with fibre separation of 1.5 cm (3W for 333 seconds per fibre)

Figure 11.7 (A) Surface appearance of lungs 3 months after ILP. (B) Cut surface of lung showing 2 separate lesions with intervening normal parenchyma.
11.3.3.2 PDT Macroscopic appearances

The first three pigs treated with PDT had unsatisfactory lesions. The first two had lesions which only had inflamed hyperaemic lung tissue at the site of needle placement. This treatment was performed with a four day drug light interval. Furthermore when the laser fibres were withdrawn from the lung after these treatments the tips of two of the fibres were charred. On sectioning the lung apart from the hyperaemia there were isolated sites of small areas of tissue charring within the PDT treated zone. Therefore the third pig was treated with different parameters. Firstly the drug light interval was shortened to 3 days so that at the time of treatment more drug would be present in the lung tissue. Also to reduce the risk of charring the laser fibre power was reduced from 100 mWatts to 50 mWatts and the treatment time doubled from 1000 to 2000 seconds. With these changes the lesion at 3 days appeared typical of PDT necrosis in that it was dark and well delineated from surrounding tissue. It had a similar appearance to the effect of PDT in the rat lung. No charring of the lesion occurred. However the lesion size was only 2 cm in maximum dimension.

Therefore new laser fibres were obtained as it was suspected that the original laser fibres were possibly the cause of these smaller lesions. These fibres had been used for multiple ILP treatments prior to the PDT treatments. The same parameters of a 3 day drug light interval, 50 mW per fibre, and 2000 seconds per treatment site were chosen. With these parameters and the new fibres there was a marked difference in the lesion size which was 3.8 x 2.1 x 2.1 cm (Figure 11.8). Therefore these parameters were used for all subsequent PDT treatments and the results of these are shown in Table 11.2.

The PDT lesions were well delineated from normal surrounding tissue and had a dark homogenous appearance across the full area of necrosis. There was no haemorrhage or oedema in surrounding tissue. At 3 days the pleura overlying the PDT site was normal apart from mild hyperemia. At 10 days the lesion still showed a necrotic black centre, however there was a pronounced rim of inflammation in the normal tissue surrounding this. At 2 months there was localised contracture of the surface of the lung which was similar to ILP, however again there was no disruption of the overall shape of the lung. On the cut surface the lesion appeared as a dense collagenous scar which contained patent bronchi within it (Figure 11.9). There was no central cavitation.
<table>
<thead>
<tr>
<th>Pig Number</th>
<th>Drug light interval</th>
<th>Fibre type</th>
<th>Laser power</th>
<th>Time of assessment</th>
<th>Lesion size (cm)</th>
</tr>
</thead>
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<tr>
<td>7</td>
<td>4 days</td>
<td>MF</td>
<td>100 mW</td>
<td>3 days</td>
<td>* 2.5x2.5x1.0</td>
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</tr>
<tr>
<td>8</td>
<td>4 days</td>
<td>MF</td>
<td>100 mW</td>
<td>3 days</td>
<td>* 2.5x1.8 x1.0</td>
</tr>
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<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>9</td>
<td>3 days</td>
<td>MF</td>
<td>50 mW</td>
<td>3 days</td>
<td>2.0 x 1.5 x 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MF</td>
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</tr>
<tr>
<td>10</td>
<td>3 days</td>
<td>MF</td>
<td>50 mW</td>
<td>3 days</td>
<td>3.8 x 2.0 x 2.2</td>
</tr>
<tr>
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<td>Diff</td>
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<td>2.2 x 1.4 x 2.0</td>
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<td>Diff</td>
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<td></td>
<td>2.0 x 1.3 x 2.0</td>
</tr>
<tr>
<td>11</td>
<td>3 days</td>
<td>MF</td>
<td>50 mW</td>
<td>10 days</td>
<td>3.5 x 2.0 x 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diff</td>
<td>100 mW/cm</td>
<td></td>
<td>1.5 x 1.0 x 1.5</td>
</tr>
<tr>
<td>12</td>
<td>3 days</td>
<td>MF</td>
<td>50 mW</td>
<td>2 months</td>
<td>2.3 x 1.8 x 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diff</td>
<td>100 mW/cm</td>
<td></td>
<td>2.2 x 1.1 x 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diff</td>
<td>100 mW/cm</td>
<td></td>
<td>1.6 x 1.0 x 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diff</td>
<td>100 mW/cm</td>
<td></td>
<td>1.5 x 1.0 x 0.9</td>
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</tbody>
</table>

Table 11.2. Lesion sizes after PDT using mTHPC (0.15 mg/kg).

MF = Multiple fibres. Total energy per fibre was 100 J. Each treatment performed with 1 pullback of 1 cm.

Diff = 2cm Diffuser fibre. Light dose was 100 J/cm.

* = No PDT necrosis on histology. The remainder of the lesions showed PDT necrosis.
Figure 11.8 (A) Appearance of PDT lesion at 3 days. Multiple fibre treatment, 3 day drug light interval 100 J per fibre (50mW for 2000 seconds) (B) Microscopic appearance of this lesion (x1)
Figure 11.9 (A) Macroscopic appearance of PDT lesion and at 2 months (B) Microscopic appearance of this section
11.3.4 Microscopic Effects

11.3.4.1 ILP

Thermal coagulative necrosis was well demonstrated in the centre of the ILP lesions (Figure 11.10). Also sections showed small zones of charring. However compared to the rat lungs the volume of charring in these lesions was comparatively small with respect to the size of the lesion overall. The coagulated tissue was eosinophilic and there was destruction of all tissue elements. Surrounding this there was a rim of inflammatory cells in response to this injury. At three weeks this necrotic coagulated tissue was still evident in the centre of the ILP lesion however surrounding this there was marked granulation tissue proliferation (Figure 11.11). Fibroblasts, macrophages and new blood vessels were present in this region surrounding the central necrotic area. In some sections cavitation of this central necrotic zone was occurring. At two months the central necrotic zone persisted however the periphery had become organized into a dense collagen rim. There was a chronic inflammatory cell infiltrate within this rim of collagen and surrounding the persisting necrotic material. There were occasional foreign body giant cells associated with persisting charred material. The appearances were the same on histology at 3 months.

11.3.4.2 PDT

The appearances were the same as for the rat lungs. There was uniform haemorrhagic necrosis across the lesion. There was necrosis of alveolar epithelial cells, vascular endothelium and smooth muscle, and bronchiolar epithelium. Epithelium on cartilaginous bronchi was necrotic although in parts it remained viable. At high magnification alveolar capillaries showed leakage of red cells. There was no distortion of the tissue architecture at three days with persistance of the collagen supporting structures of the lung parenchyma. There was no effect on bronchial cartilage. Some vessels demonstrated thrombosis and coagulative necrosis of the vessel wall. There was no significant oedema or neutrophil infiltrate in the surrounding normal tissue, however there was an inflammatory cell infiltrate at the periphery of the PDT lesion. At ten days the appearances were essentially the same (Figure 11.12a). However, although the epithelium in bronchioles was necrotic in all parts of the lesion, the epithelium of larger cartilaginous bronchi remained viable (Figure 11.12b). There was granulation tissue ingrowth starting at the periphery of the lesion. At two months the whole
Figure 11.10 Thermal coagulative necrosis in centre of ILP lesion at 3 days after multiple fibre ILP

Figure 11.11 Granulation tissue developing at perimeter of ILP lesion at 3 weeks after multiple fibre ILP
Figure 11.12 (A) Necrosis of bronchiolar epithelium at 10 days after mTHPC PDT (B) Persistence of viable epithelium in cartilaginous bronchi on the same section. (C) High magnification view of PDT scar at 2 months post treatment.
lesion had been replaced by dense fibrous tissue (Figure 11.9b). Within this fibrous tissue there were residual normal bronchi and vessels, and there was no residual necrotic tissue (Figure 11.12c). Some parts of the lesion showed haemosiderin laden macrophages which presumably had been involved in clearing the initial haemorrhagic necrosis.

11.3.5 Adverse effects

Seventeen ILP lesions were made (including the treatment which caused the haemotoma) and pneumothorax occurred after 3 of them (18%). Pneumothorax occurred after 2 of 17 lesions with PDT (11%). In 2 of the 5 cases pneumothorax only occurred as the needles were being removed; therefore the treatment had been completed. No pig developed any respiratory distress as a result of pneumothorax and no tension pneumothorax was seen. The pneumothorax was successfully aspirated in one case. On serial chest radiographs after the treatment the pneumothorax resolved spontaneously by between 3 days and 3 weeks. For example in pig 7 the pneumothorax had virtually fully resolved by 3 weeks after ILP whereas there had been a 30% pneumothorax initially.

The histology in these cases did not reveal any large bronchial perforations which may have caused a broncho-pleural fistula. It is possible that small bronchial perforations occurred which healed with scarring and were not detectable on histology. However it is also possible that the pneumothorax was due to air being drawn into the pleural space through the needle puncture holes in the chest wall. Air could also have been indrawn into the pleural cavity through the small space surrounding the fibre within the introducer needle. Therefore in subsequent treatments adhesive was used to close this potential space around the laser fibre at the tip of the introducer needle, once the fibre was positioned in the lung. Adhesive was also used to occlude the needle puncture sites at the chest wall. With these changes no subsequent pneumothoraces occurred in the ILP treatments.

A similar rate of pneumothorax was seen with PDT. Again by changing the technique and stoppering the potential sources of air entering into the pleural space no further pneumothorax occurred. With the larger introducer needle for the diffuser fibre pneumothorax occurred in 1 out of 5 treatments. X-rays on the pigs which developed pneumothorax after PDT also showed that the pneumothorax resolved spontaneously.
Two ILP lesions were associated with a small haematoma in the parenchyma immediately adjacent to the lesion (Figure 11.13 a). These pigs had not demonstrated any respiratory distress as a result of the treatment. Histology on these lesions showed that the laser effect had caused penetration of a vessel in the vicinity of the ILP lesion. There was no haematoma in the lung parenchyma at any time after PDT.

In 1 case at 3 months after ILP there was bronchiectasis distal to a lesion which had been sited directly on a bronchus (Fig 11.13 b). Some localised bronchiectasis was also evident microscopically on another of the 3 month specimens. This did not occur after PDT where lesions had been sited in similar locations. Neither treatment caused lung collapse or infarction.

The pleura showed marked adhesions in the lungs removed at three weeks after ILP. This pig had developed a pneumothorax which was detected after the second ILP treatment. It is therefore likely that the lung was collapsing during the second treatment and that the laser was therefore firing directly onto the visceral and parietal pleural surface. This therefore was a potent source of adhesion development. No other pigs showed significant adhesions. No adhesions were seen in any pig treated with PDT. No pig developed a pleural effusion as a result either of the PDT or ILP treatments. This was demonstrated both by macroscopic findings at post-mortem and on chest radiographs immediately prior to sacrifice.
Figure 11.13 Complications of multiple fibre ILP (A) Local haematoma at 3 days (B) Local bronchiectasis after 3 months
Table 11.3 summarises the differences between ILP and PDT as observed in these treatments of normal pig lungs.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>ILP</td>
<td></td>
</tr>
<tr>
<td>Simple</td>
<td>Localised Haematomas</td>
</tr>
<tr>
<td>Possible real time monitoring</td>
<td>Localised bronchial damage</td>
</tr>
<tr>
<td></td>
<td>Cavitation</td>
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<tr>
<td>PDT</td>
<td></td>
</tr>
<tr>
<td>No vascular penetration</td>
<td>Sun exposure</td>
</tr>
<tr>
<td>No cavitation</td>
<td>Numerous Rx variables</td>
</tr>
<tr>
<td></td>
<td>? Effect on large bronchi epithelium</td>
</tr>
</tbody>
</table>

Table 11.3 Summary of the differences between ILP and PDT in their effects on lung parenchyma.

11.4 Discussion

These results indicate that multiple fibre treatments can be performed successfully in the lung parenchyma using both ILP and PDT. The lesion sizes were approximately equivalent with both treatments. Similar sized ILP lesions have been created with multiple fibres in the canine liver and with PDT in the prostate (Steger et al., 1992a; Chang et al., 1996a).

The lesion size of approximately 3.5 x 2 cm is slightly less than would be needed to treat a tumour of of 2-3cm diameter and include a perimeter of 1-2 cm of normal tissue surrounding the tumour. Such treatments will therefore require more than 4 fibres. Typically in the liver ILP treatments the current practice is to use up to 8 fibres simultaneously and it is entirely feasible to perform this with the currently available technology (Amin, 1995). This requires the simultaneous use of two fibre splitters rather than the one used in this study. However for tumours of 1-2 cm in diameter it is likely that 4 fibres will be adequate.
The lesions with the diffuser fibre were reproducible and large and could be created more quickly without the need for pullbacks which were required with the multiple fibre treatments. It is likely that 3, perhaps 4 diffuser fibres could create the same size lesion as a 6 to 8 bare fibre treatment. Smaller diameter diffusers with lengths up to 4 cm or greater are available and future treatments could explore the potential benefits of these fibres. The bare fibres none the less are effective in causing large lesions when used with other fibres simultaneously and are cheaper. The diffuser did not offer any advantages over the multiple bare fibres in terms of the uniformity of the necrosis. This may have been due to light scattering in the parenchyma effectively improving the light distribution from the bare fibres.

These results in normal tissue are useful in consideration of treatment of tumours. Both ILP and PDT have always been at least as effective in causing the necrosis in tumours as in the surrounding normal tissue in a variety of different organs (Barr et al., 1990a; Bown, 1990; Masters et al., 1991). With respect to ILP it is possible that the tumour may be even more susceptible to the effects of heat as it will not have the cooling effect of the movement of air and the normal blood supply in the normal lung. PDT also is likely to be at least as effective in the tumour, particularly as the uptake of mTHPC into tumours is normally slightly greater in tumour tissue than in surrounding normal tissue.

Whilst PDT offers the chance of some selectivity of effect on tumours compared to background normal tissue, its best feature is the unique acute tissue effects it causes and the healing process which follows (Bown, 1990). PDT treatments did not cause any damage to large vessels in these experiments. With ILP it was possible for the vessels to be penetrated by the thermal effect which lead to haematomas into the lung tissue. This did not have serious consequences in these cases. However if large vessels were present in the vicinity of a tumour it would probably be preferable to use PDT. Secondly, the long term healing effects with ILP demonstrated that the central part of the lesion remained necrotic and in one case showed cavitation. McNicholas showed similarly that ILP of the prostate lead to cystic degeneration of the lesion at 2-3 months (McNicholas et al., 1993). Whilst having a cavity of necrotic tissue is preferable to having a tumour the cavity may potentially become infected which in turn would have a small chance of causing haemoptysis (Varkey and Rose, 1976). PDT did not result in cavitation of the lesion. It is likely that the persistence of interstitial collagen...
and elastin in the lesion immediately after treatment formed a scaffold on which fibrous tissue was subsequently able to completely replace the necrotic area. The tumour itself would be unlikely to cavitate as tumour connective tissue is also not damaged by PDT (K.Fan, 1997, personal communication). In contrast after ILP this connective tissue structure is completely destroyed in the centre of the lesion. Clinical studies with mTHPC in the pleura showed that vessels, such as the subclavian artery, and the brachial plexus in the vicinity of treatment of a mesothelioma were unaffected by the necrosis around them.

Thirdly there was no bronchial obstruction with PDT. In contrast the damage to the bronchial cartilage and scarring of the bronchial epithelium after ILP lead in 1 case to localised bronchiectasis. The ability of PDT treated epithelium to regenerate on the undamaged cartilage of the bronchus is another example of the attractive healing features of PDT.

The persistence of some viable epithelium on the cartilaginous bronchi after PDT was of some concern if this is to be a treatment for bronchogenic tumours. It is possible this was due to differences in bronchial structure compared to humans, namely more prominent cartilage plates (Spencer, 1985a) and well developed fibrous connective tissue around bronchi which were seen in these sections. Others have noted thicker fibrous tissue septae in histology of pig lungs (McLaughlin et al., 1961) compared to humans. The thicker bronchial wall may have reduced light penetration through the bronchial wall into the mucosa. Cartilage itself does absorb light, but only weakly compared to the mucosa (Murrer et al., 1995), therefore it is unlikely that it was the cartilage which prevented light penetration. The epithelium on smaller bronchial structures (bronchioles) was uniformly necrotic, so the absence of PDT necrosis was not due to the photosensitizing drug being absent from bronchial epithelium. Uptake of mTHPC in large bronchial mucosa has been well described in humans (Van den Bergh, 1994) and indeed endobronchial PDT with mTHPC is extremely effective, causing necrosis of both tumour and some surrounding normal tissue (Grosjean et al., 1997). Peripheral lung tumours contain very little cartilage (M.Griffiths, unpublished observations, 1997) and any residual cartilage would be disrupted. Therefore tumour epithelium does not conform to an endobronchial pattern in most cases. Rather the tumour cells are poorly organised, often extending directly into the lung parenchyma (Spencer, 1985b), where they would be susceptible to PDT. Also, selectivity of PDT would mean that tumour epithelium was more likely to be necrosed than
normal epithelium due to the way the abnormal tumour vasculature determines PDT effects (Ris et al., 1993a). It is not so much the quantity of photosensitizing drug as the way it is distributed in the tissues which gives increased tumour necrosis compared to normal tissues. It appears that after a 3 day drug light interval with mTHPC, there has been a favourable combination of drug with tumour vasculature and stroma that enables optimum PDT necrosis to occur (Ris et al., 1993a). As discussed in Chapter 12, studies on parenchymal lung tumours will be required to confirm this.

The pigs tolerated the effects of ILP and PDT well, with up to four lesions being created in each pig. Respiratory rates were not elevated at any time during the procedure and their general behaviour was normal. The appearance of the lungs at post-mortem also suggested only minor disturbance of the tissue architecture of the whole treated lung.

Neither treatment showed an advantage from the point of view of the incidence of pneumothorax. When it did occur in either ILP or PDT it was not associated with any severe consequences and resolved spontaneously in all cases. In the rat experiments it was demonstrated that the treatment has some sealing properties on the lung parenchyma, however this was not universal. It was not possible with these experiments to determine which was the main cause of pneumothorax - air leak from the visceral pleural surface or indrawing of air through the chest wall holes into the pleural space. However after occluding the narrow space around the fibre at the introducer needle head only 1 further pneumothorax occurred. Therefore by carefully limiting any possible entry site of air into the pleural space pneumothorax appeared to be reduced.

Although there was no physiological impact of pneumothorax in these pigs the treatments demonstrated that if pneumothorax occurs then the needles will be shifted out of their original site as the lung collapses. This was particularly true in the cases where the laser fibres appeared to have been firing on the pleural surface after the lung collapsed in ILP. Therefore clinically it will be important to check during treatments that pneumothorax is not occurring. This effect lead to pleural adhesions. The fact that pleural adhesions occurred is not surprising as the thermal properties of lasers have been used to cause pleurodesis in thoracoscopic surgical techniques (Wakabayashi, 1995). Here high power laser light is applied across the visceral and parietal pleura to abrade and irritate the surface and cause subsequent pleural adhesions for a therapeutic benefit.
If pneumothorax occurred with interstitial treatments of the lung in patients the treatment would have to be stopped or if the pneumothorax could be aspirated, the needles could be repositioned and the treatment continued. Alternatively with ILP if pneumothorax occurred the treatment could be attempted again after 2 to 3 days. This would need to be explained to patients prior to treatment. If the treatment had not been completed prior to the pneumothorax occurring PDT would have a major disadvantage in this situation as the patient would have received a photosensitizing drug. This would mean the patient would remain photosensitive to light for up to two weeks without having received the therapeutic benefit of the treatment.

It would be important that patients remained stationary during interstitial treatments of the lung as demonstrated by the severe laceration of the parenchyma which occurred when there was excessive movement of the pig in the second case. Obviously patients can be instructed to lie still once the needles are in place, however it would be preferable to use sedation or even a neurolept anaesthesia to ensure that the patients remained stationary when the needles were in place in the lung. The important information from the first two treatments which were performed with the pig awake was that the thermal effects of ILP did not cause pain to be experienced during the treatment. Therefore the ILP treatment should not be painful in patients.

The initial PDT treatments which gave unsatisfactory results demonstrate the relative complexity of PDT compared to ILP. Numerous factors had to be considered as a possible cause of the failure of the treatment before successful reproducible lesions could be made. The two factors which limited the size of the lesion were the presence of charring in the tissues and the probable poor quality of the emitted laser light from the older laser fibres. Indeed the charring of the fibres at these low powers probably also resulted from the fibres being older and less resistant to the effects of the low powers emitted from the fibres. This was evident as treatments subsequently performed at 100 mWatts per fibre with the new fibres did not show any evidence of charring. It is likely therefore that in patient treatments 100 mWatts per fibre can be used which would significantly reduce the treatment time. Furthermore the experiments in small animals with control fibres did not show any evidence of thermal effects in over 100 cases treated at this power.
It is more difficult to substantiate the reasons why changing the laser fibres resulted in larger lesions due to a better quality of emitted beam. The laser beam was calibrated with the old set of fibres and the laser output was shown to be the same for both the old and the new fibres. However by making this change the new fibres gave significantly larger lesions for the same power settings with no other parameters changed. Others have found that unexplained differences in lesion size occurred when different sets of laser fibres were used in experimental work (G. Buonaccorsi, 1996, personal communication). This has been seen only in ILP, however the effects may also be evident in these PDT treated cases. This effect has not been reported previously and has not been proven however the evidence based on these experimental studies does suggest that all due care should be taken in choosing as new fibres as possible for bare fibre treatments with PDT.

In contrast the ILP method of treatment is more straightforward. Furthermore with future developments in ILP monitoring with MRI scanning it will be possible to monitor the tissue effects of ILP in real time and stop the treatment when the full amount of desired necrosis has occurred (Fried et al., 1996). MRI detects changes in tissue temperature, therefore this will not be possible with PDT.

No features of alveolar haemorrhage or ARDS occurred in the tissues surrounding the PDT lesion. This validates the assumption that most of these effects in the rats were due to either the use of short drug light intervals or effects on vessels at the lung hilum, or a combination of both. Whilst hilar lesions were not created in these pigs it is unlikely that such lesions will be treated in this location in patients in any case.

The effects of ILP and PDT on bullous lung were not assessed by these experiments as the lung was entirely normal. Elderly patients with small peripheral tumours are likely to have coexistent bullous lung disease. It is known however that bullae respond well to the effects of thermal laser treatment, and this is being used in lung volume reduction surgery. The thermal sealing effects on bullae are well known and air leak is very uncommon in this situation. The effects of PDT on the normal tissue in this situation may differ slightly from those seen in the normal lung treated in this experiment. However the penetration of light is likely to be at least as great if not greater in the presence of bullous disease, as there would be less scattering due to multiple alveolar walls being absent in bullae. There may
be a slightly increased risk of pneumothorax with PDT compared to ILP in this situation.

With ILP separating the fibres by 1 cm ensured that all of the lesion was necrotic whereas some lesions made with fibre separation of 1.5 cm still showed viable regions. The problem of fibre separation has been investigated by other workers. Steger performed ILP with 4 fibres in normal canine liver with a total energy of 4000 J (Steger et al., 1992a). In 12 out of 16 treatments at 1.5W the 4 lesions overlapped. In a separate paper these authors described the evolution of multiple fibre ILP lesions in real time using ultrasound (Steger et al., 1992b). Using 1.5W into each of 4 fibres there was a progressive coalescence of the 4 separate lesions into 1 large oval shaped lesion. This took between 400 and 600 seconds. Before this each lesion was surrounded by normal parenchyma. This is the likely explanation for the persistence of normal parenchyma in the lesions created with a fibre separation of 1.5 cm. Bringing the fibres closer together on subsequent treatments ensured that all of the lesion was necrotic.

In conclusion these encouraging results demonstrate the possibility of applying these treatments to peripheral lung tumours. The unique features of PDT tissue effects give preferable acute results and better long term healing with no cavitation. It will be up to the doctor and patient to decide if they consider these beneficial effects outweigh the inconvenience of having to avoid sun exposure. This inconvenience would be much greater if pneumothorax occurred particularly if this happened during the procedure and the treatment could not be completed. However with PDT in these experiments pneumothorax seemed to occur after the treatment was finished, as the needles were being removed. This would offer the reassurance that in most cases even if pneumothorax occurred, that the treatment had been completed. Also, PDT would be the preferred treatment in lesions immediately adjacent to large vessels or in tumours close to the mediastinum. The advantages of ILP are its comparative simplicity and the long term potential for real time monitoring with MRI.
SECTION C: CONCLUSION

CHAPTER 12: Summary of findings of the thesis and future prospects

12.1 Summary of findings of the thesis

12.2 Further research resulting from this thesis.

12.2.1 Clinical studies
   12.2.2 Confirmation of tumour necrosis after interstitial treatments
       clinical studies
   12.2.3 Neoadjuvant Chemotherapy
   12.2.4 Practical Aspects of pilot studies
   12.2.5 Areas of current research which could be applied to interstitial treatments
   12.2.6 Application of interstitial treatments via the fibreoptic bronchoscope

12.2.7 Other studies

12.3 Conclusion
12.1 Summary of findings of the thesis

The overall objective of this thesis was to investigate the effect on the lung parenchyma of 2 interstitial laser treatments ILP and PDT. It was to assess the macroscopic and histological appearances and the practicality of performing this new treatment.

In Chapters 5 and 6 ILP was shown to be a reproducible technique for causing thermal necrosis, with lesion diameters for single fibre treatments up to 12mm. Histology and electron microscopy demonstrated that the connective tissue and elastic tissue of the lung was not affected outside the ILP zone. This, combined with the physiological results up to 6 months presented in Chapter 10, demonstrates that this ILP effect is localised. In Chapters 8 and 9 it was demonstrated that interstitial PDT was also a reproducible technique giving a well localised zone of necrosis in the parenchyma. At shorter drug light intervals there were more marked vascular effects after PDT. In Chapter 7 fluorescence microscopy experiments preceding PDT treatments demonstrated the intravascular distribution of photosensitizers at early time points and the subsequent more even distribution of the drug throughout the lung parenchyma at later time points. All three drugs showed uptake in bronchial epithelium. The uptake of mTHPC by macrophages in the lung parenchyma raises the possibility that macrophages were involved in causing the tissue effects of PDT. As for ILP, there were no significant effects on the physiological function of lungs treated with PDT. The adverse effects of ILP and PDT in the rats predominantly related to the small size of the animal lung. This was particularly true in the ILP treatments where the proximity of the ILP lesion to the oesophagus caused oesophageal perforation.

In the large animal experiments in Chapter 11 multiple fibres caused large lesions which were well tolerated. There was central cavitation at 2 and 3 months after ILP whereas this was absent with PDT due to the unique healing features of PDT. Furthermore because bronchial cartilage and vascular smooth muscle was not damaged by PDT these structures were able to re-epithelialize. As a result there were no long term effects on the bronchi or vessels due to PDT. The significance of the viable epithelium in larger
bronchi after PDT remains to be determined in tumour treatments, as discussed below.

The adverse effects seen in the rat lung tissues were not observed in the large animal experiments. Here the ILP and PDT lesions did not cause widespread vascular congestion or oedema. Although the lesions in pigs were not placed adjacent to the hilum they were close to large vessels and no significant congestive phenomena were observed. The importance of keeping the subject stationary while the needles were in place was demonstrated by the case of the large lung haematoma in the pig treated under local anaesthetic. Other than this there were only minor side-effects in the lung parenchyma and these were only observed with ILP. These included local haematoma and local bronchiectasis due to thermal damage to adjacent vessels and bronchi respectively.

12.2 Further research resulting from this thesis.

12.2.1 Clinical studies

The results have shown that interstitial laser photocoagulation and interstitial photodynamic therapy are potential new treatments for selected patients with lung cancer which heal safely. The size of the lesions created with both of these treatments was of a magnitude which will allow pilot clinical studies to be performed. Pilot studies would be the best way to confirm that not only were these practical treatments for patients but also that tumour necrosis could be achieved. In performing these pilot studies the selection of patients would be paramount. The main consideration would be choosing patients with tumours no greater than 2-3cm in diameter with no evidence of lymphadenopathy at the hilum or mediastinum on CT scanning and no evidence of distant metastases. Between 4 and 8 bare fibres would be needed to incorporate a 3 cm diameter tumour based on the experiments in these normal lungs. To attempt cure of larger lesions would firstly reduce the likelihood that the treatment would encompass all of the tumour and secondly raise the likelihood that the patient already had metastatic disease. In such cases a local curative treatment would not be in the patient's interest. Attempts to treat tumours larger than 3cm in diameter with liver ILP were not successful, since contrast CT showed some persisting viable tumour. This was due to the necessity for much more accurate targeting of the tumour with the needles with larger tumours and the inability to treat a 1-2 cm rim of normal tissue around the tumour (Amin et al., 1993c). Consequently the
tumour size selected is now less than 3cm, preferably 2cm in diameter. Furthermore the greater amount of normal tissue surrounding the tumour which can be treated raises the possibility of local cure. In the lung, tumours which were surrounded by 1 - 2 cm of normal parenchyma would be ideal for these treatments.

Presently patients with small tumours who are medically inoperable comprise only a small proportion of all patients with lung cancer. There is however renewed interest in lung cancer screening using new techniques to improve sputum analysis. These include molecular markers, surface antigens, and malignancy associated changes in cell nuclei (Mao et al., 1994; Tockman et al., 1988; Palcic and MacAulay, 1994; Lam et al., 1993; Rabbitts, 1991). These techniques have been applied to series of archival sputum samples which had normal conventional cytology and predicted the subsequent development of malignancy in up to 80 % of cases (Palcic and MacAulay, 1994; Tockman et al., 1988). If over the next 5-10 years these techniques become widely used in clinical medicine, increased numbers of patients with early carcinomas will be found, including patients who would be suitable for these percutaneous interstitial treatments.

Patients could be considered for these treatments either as a primary treatment modality or in the situation of recurrence following surgery or radiotherapy with curative intent. In the latter situation the patient would have no alternative option and these treatments could then be offered.

The interstitial treatments examined in this thesis would also be suitable for the management of isolated lung metastases in cases where resection would be considered for improvement in the patient's prognosis (Rusch, 1995; Mountain et al., 1984). Such tumours include osteogenic sarcomas, soft tissue sarcoma and renal cell carcinomas. Often the decision to perform an open operation in these cases is even more difficult than in the patient with a primary carcinoma who has borderline lung function or has some other relative contraindication for surgery. Therefore an ablative technique which was minimally invasive and did not inconvenience the patient with secondary carcinoma would be a preferable and highly desirable treatment option. Furthermore in the event of further secondaries becoming evident repeat ILP or PDT treatments could be performed at subsequent times. Improvement in survival after a second surgical metastasectomy is the same as after the first metastasectomy (Rusch, 1995). It is noteworthy that although surgical resection is considered in these patients, radiotherapy is
not usually considered an alternative treatment due to its lack of efficacy and likelihood of impairing lung function.

12.2.2 Confirmation of tumour necrosis after interstitial treatments in pilot clinical studies

To confirm necrosis of lung tumours treated by ILP or PDT it would be necessary to use either contrast CT or histology on tumour biopsies following treatment, or a combination of both.

In pilot studies of ILP for metastatic liver cancers Amin et al used avascularity of the lesion on contrast CT as the index of tumour necrosis (Amin et al., 1993c). In selected cases they also used core needle biopsies of regions of avascularity to confirm the necrosis. Other reasons for the validity of the CT index were firstly that experimental ILP in normal canine liver (using the same parameters as the CT guided treatments in patients) caused well defined areas of coagulative necrosis in which there was angiographic evidence of obliteration of small vessels and occlusion of some larger vessels (Matthewson et al., 1987; Steger et al., 1992a). Secondly, temperature measurements had shown that at the centre of the lesion the temperature was 100 °C and that 8 mm away it was still 50 °C making it very unlikely that tumour cells could survive these temperatures for the 500 second duration of the treatment (Matthewson et al., 1987). Others have found good correlation between non enhancement of therapeutically embolized tumours on CT and histological necrosis on the surgically resected specimens (Takayasu et al., 1984).

The same approach would be used in lung tumour ILP, performing contrast CT at 24 hours and in selected cases performing needle aspiration biopsies of areas of non contrast. It may be possible to perform tru-cut biopsies in some cases where the tumour is close to the pleura and therefore easily accessible. This would however carry a greater risk of pneumothorax. As was done in the liver ILP study, follow up contrast CT scans could be performed at 6 months then at 12 months with additional scans if chest radiographs suggested any change in the intervening period. Change in contrast CT appearance of lung tumours is a reported method of differentiating benign from malignant parenchymal lung tumours (Swenson et al., 1992). In the only ILP treatment on a lung tumour performed to date the post treatment
CT showed loss of contrast in the treated field in the tumour (Brookes et al., 1996).

A similar approach could be taken following PDT treatments. This would address the question raised in Chapter 10 of the viability of the normal epithelium in larger bronchi. As previously discussed, it is anticipated that PDT on any tumour tissue in a similar location would result in necrosis; however, this needs confirmation in pilot studies. Interstitial PDT is known to cause necrosis of bulky endobronchial tumours. Kato and others have performed this with Photofrin using bare fibres inserted into tumours bronchoscopically (Kato and Okunaka, 1995). Furthermore recent data confirms the effectiveness of PDT using mTHPC on small endobronchial tumours with up to 5 year disease free follow-up (Grosjean et al., 1997). It is therefore very likely that interstitial PDT will cause necrosis of peripheral lung tumours. Recent interstitial PDT treatments with mTHPC in tumours of the pancreas and prostate using near identical treatment parameters to those used in the large animal experiments in this thesis have confirmed the ability of this technique to cause tumour necrosis with lesions of up to 3 cm diameter using between 2-4 fibres (D. Whitelaw, 1997, personal communication). Lung tumours are more solid than the surrounding lung parenchyma, therefore the ability to create a lesion of 3-4 cm diameter in normal parenchyma, along with this recent clinical information regarding the efficacy in causing necrosis in solid tumours, implies that the interstitial treatment of a peripheral lung tumour is feasible. In the event that necrosis was incomplete a repeat treatment could be performed. An interval of 1 month is usually taken between repeat treatments with mTHPC. Alternatively, ILP could then be used.

Performing ILP and PDT treatments in a large animal lung tumour model would pose a number of difficult problems. The most important would be the differences in submacroscopic structure of animal lungs referred to in the previous chapter (McLaughlin et al., 1961). These may affect light transmission in tissues, particularly in PDT experiments. Secondly chemically induced or metastatic tumours would have different structures to slow growing primary tumours in patients (Fidler, 1990). Tumour models often require animals to be immunodeficient (Wang et al., 1992) which could affect the inflammatory response which is undoubtedly an important part of the mechanism of action and healing following these treatments. Finally CT imaging would be required to ensure that the fibres were correctly placed in the tumour; it would be difficult to have access to this in animals. For these
reasons, along with the promising findings in normal tissue, it is preferable
to procede to patient studies.

12.2.3 Neoadjuvant Chemotherapy

Despite a number of non randomised and 2 randomised trials demonstrating
the benefits of neo adjuvant chemotherapy in non small cell lung cancer, its
role is not yet established (Masters and Vokes, 1995). There are however at
least 3 large studies under way in the UK and Europe to address the issue of
survival benefit with neo adjuvant and adjuvant chemotherapy (Depierre et
al., 1995; Non small cell lung cancer collaborative group 1995). If these
studies confirm a benefit then future interstitial laser treatments for lung
cancer could be preceded by short course chemotherapy. Apart from effects
on occult metastatic disease, chemotherapy could reduce the size of the
primary tumour, making the interstitial laser treatment easier. Significant
initial reduction in primary tumour size occurs in up to 20-50% of cases
following commonly used Cisplatin-based regimens for non-small cell
carcinoma, including MIC, or MVP (Ginsberg, 1997). A recent meta
analysis demonstrated the benefits of chemotherapy for non-small cell
carcinoma in all treatment approaches including surgery, radiotherapy, and
best supportive care (Non small cell lung cancer collaborative group 1995).
In the surgery and radiotherapy groups most patients had relatively
advanced disease. Neoadjuvant surgical studies have also been performed in
stage 3A disease (Rosell et al., 1994; Roth et al., 1994). However the above
studies will address the issue of chemotherapy in those with small tumours,
T1 or T2. As tumours grow they accumulate increasing numbers of somatic
genetic mutations which confer chemotherapy-resistance on the tumour. If
chemotherapy was given at an earlier time the tumour would have fewer
such mutations (Goldie and Coldman, 1979). A good long term prognosis
with T1 and T2 could potentially be made even better by chemotherapy as
these tumours would also have a smaller occult metastatic tumour load than
more advanced tumours (Pastorino, 1996).

12.2.4 Practical Aspects of pilot studies

Patients would need to be advised on the risks of the interstitial treatments
which have been elucidated in this thesis. Pneumothorax poses two
problems with respect to these treatments. Firstly in these patients with

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poor lung function there is the risk of respiratory embarrassment due to pneumothorax. However needle aspiration of the chest and intercostal catheter (ICC) placement are well established techniques which in virtually all cases adequately deal with pneumothorax when it occurs following fine needle aspiration (FNA) biopsy of the chest. Furthermore the duration of placement of the intercostal catheter when it is required is seldom more than 3 days. Not all patients who develop pneumothorax require ICC placement. Of the 10-20% of patients who develop pneumothorax following FNA only 15-20% will require ICC placement. Patient selection is important here also. Patients with severe breathlessness prior to the treatment would be considered too great a risk in the event that pneumothorax occurred due to the treatment. In the study of fine needle brachytherapy referred to in Chapter 1, 9 patients had up to 3 needles inserted percutaneously, with dwell times of up to 2 hours (Hayman et al., 1995). A chest physician was always on hand for the insertion of an ICC if necessary. This was required in 1 of the 9 cases and there were no complications from this. All of these patients had an FEV1 of < 600 ml and had been judged by a chest physician as able to cope with a pneumothorax if it occurred.

Secondly the development of pneumothorax would make it difficult to continue the treatment. The options in this case would be to either cease the treatment or aspirate the pneumothorax and continue with the treatment once the needles had been repositioned using the CT scan imaging. To reduce the risk of pneumothorax patients would be given oxygen prior to and during the treatment, be asked to breathe in as the needles were being inserted through the pleura, and to lie on the treated side following the treatment. These techniques have been shown to reduce the risk of pneumothorax in various series of FNA (Cormier et al., 1980; Moore et al., 1990; St Louis et al., 1984). The pig experiments showed that it was important to occlude any potential apperture for air entry into the pleural space. This may be addressed by custom building an introducer needle which has a self-sealing port through which the laser fibre could be introduced. Despite there being a risk of pneumothorax this thesis has demonstrated the surprisingly low incidence of this complication considering the number of needles being placed in the lung and the fact that necrosis of the underlying lung is being created. The bursting pressure experiments showed a trend for a protective effect for the development of pneumothorax due to these treatments. This protective effect however is only relevant to the escape of air from the visceral pleural surface and cannot prevent
pneumothorax from occurring due to air being entrained into the pleural space through the chest wall.

There were no other complications in the lung parenchyma with PDT. In contrast, in two cases with ILP there were localised haematomas around the ILP lesion due to thermal effects on the adjacent blood vessels. Vascular complications are only infrequently seen in the liver treatments with ILP. This includes cases where the ILP lesion is made next to large vessels. It has been considered this was due to a heat sink effect by the flowing blood in these larger vessels. In the initial pilot study of 93 ILP treatments for liver metastases in 31 patients there were 6 small subcapsular haematomas and in 1 case there was a drop in haemoglobin by 2 g/dl after 2 treatments 1 week apart (Amin et al., 1993c). Haemoptysis and lung haemorrhage are recognised complications of fine needle aspiration biopsy. Usually these are self limited although in the 1970's a number of case reports presented the risk of fatal haemoptysis following cutting needle biopsy of the lung with large needles (Pearce and Patt, 1974; Norenberg et al., 1974). By using CT scan images prior to the treatment it would be possible to identify any large vessels in the vicinity of the tumour to be treated. These could be avoided in the placement of the needles such that the thermal effects were not being directly placed onto the vessel. This would reduce the risk of bleeding due to the treatment. It would be important to avoid treating tumours in the vicinity of major pulmonary vessels. This is unlikely to occur as ILP treatments would only be applied in the periphery of the lung away from the hilum and mediastinum, due to the potential risk of oesophageal perforation in treatments applied in this region.

In contrast to PDT which healed with scarring of the whole lesion, following ILP there was a cavity within the scarred perimeter. This is unlikely to cause significant problems although there is a potential long term risk with superinfection of a cavity. Despite this tumours can cavitate and this includes small tumours. Therefore treating a tumour with curative intent and leaving a cavity as a result may not be particularly disadvantageous to a patient. The other problem with ILP was local bronchial obstruction leading in one case to a localised region of bronchiectasis. Again if the curative treatment was successful this may not be a significant complication.

PDT had neither of these complications and therefore patients could be advised that the long term healing effects were better with this treatment. The only disadvantage clinically with PDT is the necessity for patients to
avoid direct sun exposure for up to 2 weeks with mTHPC. As more clinical experience is being gained with this drug the regime for graduated re-exposure to sunlight after the initial injection of the drug is now well established. There have never been any severe reactions to sunlight following mTHPC later than 2 weeks after the injection, whereas this has occurred up to 6 months after photophrin and HPD. Patients are now given hand held light meters to use both in the hospital ward and at home following the administration of a photosensitizer. Using a graded scale of exposure to ambient light they can check that they are within this scale by using their light meter. For example, up to 24 hours the patient must stay indoors in a darkened room (Maximum light exposure on light meter 100 Lux). On days 2 to 7 the patient can have normal indoor lighting, with safe lux levels increasing from 200 at day 2 to 700 at day 7. After one week it is possible for patients to go outside, so long as their light meter registers less than 800 on day 8 and less than 1400 on day 14. Thereafter there is a gradual re-exposure to normal outdoor light. The use of these light meters is very reassuring to the patient and provides an objective scale for patients to adhere to which improves the safety of the treatment.

12.2.5 Areas of current research which could be applied to interstitial lung treatments

Future improvements in ILP include the development of MRI scanning to monitor the treatment of ILP. Images performed prior to the commencement of the treatment can be compared to images collected based on thermal changes in the tissue as the treatment progresses. ILP treatments to tumours of the same size may result in different lesion size for the same parameters due to differences in the vascularity and the heterogeneity of the tumour compared to the normal surrounding tissue (Amin et al., 1993c). The aim is to be able to stop the treatment when the extent of heat effects on the tumour have exceeded the limits of tumour on the pre-treatment scan and incorporate a rim of surrounding normal tissue. Therefore it would be possible to tailor the treatment to the tumour. In the meantime contrast CT performed 24 hours after treatment provides an accurate assessment of the extent of ILP necrosis (Amin et al., 1993c). If imaging of the lesion suggests that complete tumour necrosis has not occurred these treatments can be repeated. This is one of the main advantages of using a minimally invasive technique.
MRI scanning of the lung parenchyma does not have any advantages over CT scanning (Hansell, 1995). However if the purpose of the MRI is to image the solid tumour alone then high repetition sequences focussed on this solid tissue should be extremely effective in the monitoring of the treatment. Monitoring of the effects on the aerated lung parenchyma around the tumour would potentially be more difficult however the treatment could be continued for a period estimated by the rate at which necrosis occurred in the tumour itself.

Advances in diffuser fibre technology may assist the development of interstitial lung treatments, particularly PDT. With thinner fibres the treatment could be performed with only the same risk of pneumothorax as for insertion of the bare fibre tips. This would allow shorter treatments.

12.2.6 Application of interstitial treatments via the fibreoptic bronchoscope

Lung tumours are most commonly situated in the large airways, therefore it would be useful to be able to apply the results of these parenchymal studies to endobronchial treatments. With advances in imaging of bronchial tumours such as endobronchial ultrasound and MRI it may be possible to insert a laser fibre through the bronchial wall in the manner of a transbronchial needle aspiration (Wang and Terry, 1983) into a tumour immediately outside the bronchus. This would only be of use if staging investigations revealed no lymphatic spread and the patient was medically inoperable. PDT would probably have an advantage in this situation as there would be no effect on bronchial cartilage or large vessels however ILP could also possibly be performed with careful real time monitoring.

12.2.7 Other studies

There are a number of recent developments in PDT which warrant investigation and further development in the clinical setting. The first is fractionation of the light. Messman et al demonstrated in a paper on the effects of ALA on normal colon that the area of necrosis could be improved by a factor of up to three fold by dividing the light dose into two or more separate periods with light free intervals in between (Messmann et al., 1995). These authors felt the most likely reason for the effect of this fractionation related to vascular shut down and tissue oxygenation. The vasoconstriction occurring during the first fraction of treatment possibly relaxes during the interval in the light administration. This may permit re-oxygenation of the
target area, making it more susceptible to PDT when the next light dose is given. Fractionation therefore may allow recovery of normal oxygen levels in the tissues. Oxygen is an essential component of the PDT effect (Star et al., 1986). Alternatively it may be that the increased tissue effect is related to a reperfusion injury as there is a release of oxygen radicals upon reperfusion of damaged tissue (Klausener et al., 1989). Although this study was performed with ALA there is potential for fractionation to be used with other photosensitizers including mTHPC.

ALA PDT gives relatively small lesions with interstitial PDT and methods of amplifying this effect have been pursued in other organs. The second potential mechanism of amplifying the effect of ALA is the use of iron chelators (Chang et al., 1995). These compounds such as CP 94 (1, 2-diethyl-3-hydroxypyridine-4-1) cause inhibition of conversion of PpIX to haem and thus temporarily raise the tissue levels of PpIX. Chang demonstrated that this caused increased levels of PpIX in the rat bladder by simultaneously administering CP 94. This technique has not yet been tested clinically.

The studies by Korbellik et al raise the possibility that immunotherapy will be used to amplify the tissue effects of photodynamic therapy (Korbelik et al., 1997). In this way the cytotoxic effects of macrophages can be used to beneficial effects. Clinical and experimental treatment of tumours using macrophage related cytokines such as TNFalpha have showed promise, however the difficulty was achieving tissue levels of the cytokine at high enough levels in the vicinity of the tumour without causing adverse systemic effects (Pogrebniak et al., 1994). PDT may therefore be a de facto way of producing large quantities of TNF alpha immediately in the vicinity of the tumour. Further laboratory work could be done to determine if there is an immediate liberation of TNF alpha from mTHPC containing macrophages immediately after light activation or whether the macrophages involved in the tissue effects of PDT are recruited to the site as they would be in other forms of lung injury.

Other workers in respiratory pathology could potentially use the PDT treatment in rats as a model for endothelial injury, for example in the investigation of new treatments to reduce vascular injury in the lung. The rate at which bronchial epithelium regenerates could also be assessed using PDT as the initial injury. Agents which either reduce the extent of the initial injury or accelerate healing afterwards could potentially be tested.
12.4 Conclusion

In conclusion ILP and interstitial PDT are potential new treatments for patients with peripheral lung tumours who are medically inoperable. They have the advantage over radical radiotherapy as fibres can be placed accurately into the tumour without the requirement for time-consuming planning, and treatment can be performed in a single session. Pilot clinical studies can now be performed using the information from this thesis, as well as information from treatments using ILP and interstitial PDT in tumours of other organs. In the long term it may be that ILP and interstitial PDT have complimentary roles, given the differences of their acute and long term effects on the lung parenchyma which have been observed in this thesis.
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LIST OF PUBLICATIONS


PRESENTATIONS TO INTERNATIONAL SCIENTIFIC MEETINGS

1. Preclinical studies on the use of Interstitial Laser photocoagulation in lung cancer. European Laser Association, Biomedical Optics Society annual joint meeting, Vienna September 1996 (Oral presentation)


OTHER PRESENTATIONS


Preclinical studies on the use of Interstitial Laser Photocoagulation in the lung parenchyma.


University College Medical School, and Imperial Cancer Research Fund, London UK.

ABSTRACT

A preliminary assessment was made of the effect of interstitial laser photocoagulation on normal lung parenchyma. Using rats lesions were created by passing the laser fibre percutaneously into the normal lung under general anaesthetic. The lungs were removed post mortem at 3 days. The lesions were ellipsoid in shape and well circumscribed. Histology showed central charring surrounded by zones of coagulative and haemorrhagic necrosis. There was a clear margin between the treated and normal tissue. These results indicate that further examination is warranted of the use of ILP for treatment of small primary lung tumours in patients unsuitable for surgery.

Keywords: Lasers, Interstitial therapy. Lung neoplasms, therapy.

1. INTRODUCTION

Interstitial laser photocoagulation (ILP) has been used effectively in the treatment of liver tumours as well as tumours of the prostate and pancreas. \(^1,2\) In ILP bare tipped or diffuser fibres cause hyperthermic necrosis of tumour as well as a small perimeter of surrounding normal tissue. \(^3,4\) Up to now thermal applications of laser in the lung have been on tumours of the bronchus using high power YAG laser to palliate obstructing tumours. \(^5,6\) ILP using low laser powers has been attempted on endobronchial tumours, however the effects of ILP on the peripheral lung tumours have not been studied. \(^7\)

Small lung tumours arising beyond the limit of reach of a bronchoscope (peripheral tumours) are best treated by surgical resection and may be cured in up to 70% of cases with best results for tumours less than 3 cm in diameter with no lymph node metastases. \(^8\) However some patients are unfit for surgery due to poor respiratory reserve or other concurrent medical condition. The main treatment for these patients is radical radiotherapy however long term results with this are poor (6-32% 5 year survival) and side effects may occasionally be severe. \(^9\) Therefore ILP may provide an alternative treatment in these patients. Furthermore many patients with lung cancer are aged over 70 years and for a number of reasons a minimally invasive therapy may be applicable in this group if surgery is not possible. The aim would be to pass laser fibres through the chest wall under local anaesthetic using introducer needles into the centre of the lung parenchymal tumour.

In this paper we assessed normal lung parenchyma in a rat model to see the acute effects of ILP as a preliminary step to treating patients with lung tumours. Some normal lung will need to be included in the treatment field and the effects of ILP on the parenchyma should therefore be determined. In particular the risk of pneumothorax needs to be known since most patients selected for this treatment will have poor lung function and a pneumothorax could be severely compromising with severe breathlessness.
2. METHODS

Preliminary assessment of the effects of ILP was made on ex vivo porcine lungs. This was to determine the likely overall shape of lesions. These lungs were inflated with air via the trachea which was then tied off. The laser fibre was inserted into the lung parenchyma with the use of a 19 gauge introducer needle. The needle was then withdrawn a short distance to expose the fibre tip. Laser treatment was then performed using powers of up to 3 watts.

Subsequently male Wistar rats, weighing between 350 - 400 g, with normal lungs, were treated under inhalational general anaesthesia using halothane and oxygen via a face mask. ILP was performed using a 25 W semiconductor laser (805 nm). The silastic cladding at the tip of the laser fibre was pared back 5 mm and the distal 1-2 mm of fibre was pre charred according to the method described by Amin et al. Preliminary experiments at 2 W had shown that at most 1-2 mm of back-burning of the silica cladding occurred with this fibre preparation. Rats were placed in the right lateral position in a specially designed frame which allowed insertion of the introducer needle reproducibly into the lower part of the left lung. The site for correct fibre placement was checked by fluoroscopy using a Siemens image intensifier (Siremobil 2000). (Fig. 1). A small incision was made in the lateral chest wall and blunt dissection down to the intercostal membrane was performed. The 19 Gauge introducer needle containing a 400 um core diameter bare tipped laser fibre was inserted 5 mm through the intercostal membrane into the lung. The introducer needle was then withdrawn a short distance leaving the tip of the laser fibre exposed in the lung parenchyma. After completion of the laser treatment the fibre was withdrawn and the rat allowed to recover. An assessment of a range of powers was performed with parameters of 1-3 W and treatment times up to 1000 seconds.

Fig. 1 Fluoroscopic view of thorax of rat in treatment position. The laser fibre was passed into the lung via an introducer needle passed through the centre of the cross bar of the frame using this lateral view.
Rats were sacrificed at 3-4 days. After sacrifice lungs were removed and inflated with formaldehyde via the trachea at 25 cm water pressure until all pleural surfaces were smooth, after which the trachea was tied off. (Fig.2) After fixing for 3 days the left lung was sectioned perpendicular to the line of laser treatment.

All rats had fluoroscopic screening immediately after treatment to detect if pneumothorax had occurred. Rats sacrificed immediately after treatment also had standard chest X-Ray to confirm the findings on fluoroscopy.

Fig 2. Posterolateral view of lungs after filling with fixative. The left lung has been treated with ILP. Note there is no leak of fixative from the site of laser treatment.
3. RESULTS

The ex vivo experiments showed that the laser lesions were ellipsoid in shape with the greatest diameter of effect being close to the laser tip. In the treatments at higher powers the effect was more elongated indicating forward firing of the laser. Lesions created at lower power were more spherical, being centred more closely around the tip of the fibre.

Similar findings occurred in the rats treated under general anaesthetic. Macroscopically on the cut surface, lesions showed a central zone of charring surrounded by sequential zones of white coagulated tissue, haemorrhagic necrosis and pale tissue outside which the lung appeared normal. (fig 3).

Prior to sectioning the lung where there was laser effect on the lung surface this appeared to be sealed as no formaldehyde leaked out from this site during preparation of the lung specimens. Furthermore where there was no laser effect on the surface there was no leak from the site of fibre insertion into the lung.

Histology of the laser lesion showed a central zone of charring with complete destruction of lung tissue. Surrounding this was a zone of coagulative necrosis with no charring which also had loss of the normal tissue architecture. Outside this was a rim of haemorrhagic necrosis where capillaries

Fig.3 Sections of one lung after ILP. The centre of the lesion shows charring surrounded by sequential zones of pale then haemorrhagic tissue then normal tissue.
and small vessels had been injured but not coagulated, leading to leakage of red cells into the tissue although the overall structure of the tissue was not significantly affected. (Fig. 4). Outside this was viable tissue which contained an acute inflammatory cell infiltrate in the tissue immediately adjacent to the laser lesion, typical of the response of the lung to any type of acute injury. (Fig. 5).

Laser lesions were reproducibly localised to the lower part of the left lung and were consistent in size and shape with lesions up to 12 mm in diameter when using higher powers.

Fig. 4 Histological section of lung showing effects of ILP at 3 days. The centre shows charring and an apparent hole where charred tissue has been removed in specimen preparation. There are surrounding zones of coagulative and haemorrhagic necrosis outside which there is an acute inflammatory infiltrate. (Haematoxyllin and eosin. Magnification x10)

Pneumothorax was detected on fluoroscopy immediately after laser treatment in a small number of rats. The usual cause of this was inadvertent laser treatment of the intercostal membrane creating a hole which allowed air to be drawn into the chest cavity during treatment. If necrosis of a large bronchus occurred and there was no overlying lung tissue to seal the hole then a pneumothorax also occurred. However this occurred uncommonly and in general the rats tolerated the treatment process well. In rats which had conventional Xray immediately after sacrifice no pneumothoraces were seen which corresponded to the information obtained from fluoroscopy immediately after treatment.
Due to the small size of the animal used and the proximity of the mediastinum to the treatment area in a number of cases a laser lesion was inadvertently made in the oesophagus which resulted in infection in the mediastinum and adjacent lung.

Fig 5. Histological appearance of normal lung (left) immediately adjacent to the rim of inflammatory infiltrate at the outer part of the ILP lesion (right).

Fig 6. Left pneumothorax on fluoroscopy in a rat after ILP.
4. DISCUSSION

As in other organs these experiments in the normal lung have shown that ILP can create zones of necrosis of roughly predictable size and in a specified location. This opens the prospect of treatment of a perimeter of normal lung parenchyma around a peripheral lung tumour. In ILP the fibres would be placed in the centre of the tumour as well as on the periphery to include some of the normal surrounding lung and these results will help in planning such treatments. The sharp demarcation between normal and necrotic tissue on light microscopy is important in this respect, as too great an injury to the surrounding normal lung would be detrimental in patients with poor lung function.

The lesion size is comparable to that seen in the liver at similar power settings and the lesion shape is also ellipsoid, becoming more elongated at higher powers due to more forward firing. As in treatments in the liver and prostate the centre of the lesion was charred and therefore probably acted as the source of heat propagation once it reached a critical temperature. Compared to the liver the lung is structurally less dense and the movement of air in and out of the lung in the vicinity of the treatment area may reduce the size of thermal lesion created. The lung is a highly vascular organ and as in other organs the flow of blood may reduce the transmission of heat further into the tissues. Histology was similar to that seen when ILP is used in other organs, with a localised area of thermal necrosis and a sharp demarcation from normal surrounding tissue. Unlike the liver however the lung does not regenerate after necrosis has occurred and this will impact on long term healing which is currently being assessed in further experiments, however given the early tissue reaction is likely to lead to replacement of the necrosed area with fibrous tissue.

An assessment of pneumothorax with this technique is important as patients who are likely to receive this type of treatment will have little pulmonary reserve. Although pneumothorax can usually be easily treated with intercostal catheter the acute episode may cause severe respiratory difficulty in such patients. Pneumothorax is a recognised complication of percutaneous fine needle aspiration biopsy of pulmonary masses with the incidence at around 28% varying between 8% and 56% depending on the technique used. Because pneumothoraces may spontaneously resolve with time it is important to detect them immediately after treatment, hence the value of fluoroscopic screening in these experiments. The additional conventional X-rays showed that the fluoroscopic findings were valid.

In this experimental technique lesions were created in the same way as is proposed with ILP treatment of lung tumours. Therefore pneumothorax may occur with this treatment, however the small size of the experimental animal must be taken into consideration, in that laser lesions were likely to damage the chest wall or extend from one pleural surface to the other which would not occur when applied in humans since fibres could be positioned much more easily to avoid these complications. The inadvertent treatment of the chest wall is not likely to occur in patients because the parietal pleura can be avoided by treating lesions more than 1 cm inside the visceral pleura. Future experiments will need to quantitate the rate of pneumothorax to ensure it is likely to be comparable to that seen in diagnostic lung procedures. The laser effect seems to seal the hole created by the introducer needle possibly by a laser welding mechanism.
The complication of oesophageal perforation and mediastinitis was a consequence of the small size of the animal model used as it was difficult to keep the fibre further out in the lung parenchyma without affecting the parietal pleura and chest wall. This indicates that the laser fibre should not be placed near the mediastinum and that central tumours would probably be unsuitable for this technique.

Further experiments will need to be performed before applying this treatment in humans, including the simultaneous use of multiple fibres to create lesions large enough to destroy small tumours. The healing process of the ILP lesions needs to be documented, as well as any effect on overall pulmonary function.

5. REFERENCES