Photodynamic therapy for the treatment of cancer of the pancreas

A thesis submitted in fulfilment of the degree of doctor of medicine (MD)

By
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Abstract of thesis

Title. Photodynamic therapy for the treatment of cancer of the pancreas

Cancer of the pancreas is the fifth commonest cause of cancer death and even with treatment, 80-90% of patients die within a year of diagnosis.

Photodynamic therapy (PDT) is a non-thermal technique for inducing tissue necrosis. Cytotoxic oxygen species are produced from the interaction between light of a specific wavelength and a photosensitising agent in an oxygenated tissue. Experimental studies using the photosensitisers mTHPC and ALA have shown PDT to have a substantial therapeutic potential for the treatment of pancreatic cancer. mTHPC gave the largest volume of necrosis around treatment sites but clinically causes skin photosensitisation for up to a month. ALA photosensitivity only lasts 1-2 days, but clinical studies show that at the maximum tolerated dose (60mg/kg) the effect is too superficial.

The aims of this thesis were: 1. To assess the safety and feasibility of interstitial PDT using mTHPC for the treatment of inoperable pancreatic cancer. To undertake animal studies to investigate a method of enhancing ALA PDT by the addition of the hydroxypyridinone iron chelator, CP94, to slow down the conversion of PPIX (the photoactive derivative of ALA) to haem.

The pilot clinical study on 16 patients with localised but inoperable pancreatic cancers (2-6cm in diameter) showed that PDT could produce tumour necrosis with no treatment related mortality and a median survival of 12.5 months. Major complications included bleeding and duodenal stenosis but these were manageable.

Experiments on hamsters with cancer transplanted into their pancreas showed significantly higher tissue levels of PPIX and significantly larger volumes of PDT produced tumour necrosis in those given ALA and CP94 compared with ALA alone.

This thesis has shown that clinical, interstitial PDT with mTHPC for pancreatic cancer is technically feasible and could produce some survival benefit. This should now be tested in a randomised, controlled trial. The animal experiments showed that adding an iron chelator may enhance ALA PDT. This could now be tested clinically. These encouraging results justify further studies of the possible role of PDT in the management of cancer of the pancreas.
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For my Parents
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Abbreviations

AGA American Gastroenterological Association
AJCC American Joint Committee on Cancer
ALA 5-Aminolaevulinic Acid
AlPc Aluminium Phthalocyanine
AlSPc Aluminium Sulphonated Phthalocyanine
ATP Adenosine Triphosphate
a.u. Arbitrary Units
BOP N-nitroso-bis-(2-oxypropyl) amine
BPD Benzoporphyrin Derivative Monoacid
CCD Charge Coupled Device
CP20 1,2-dimethyl-3-hydroxypyridin-4-one
CP94 1,2-diethyl-3-hydroxypyridin-4-one
CT Computerised Tomography
DHE Dihaematoporphyrin Ether/Ester
DFO Desferoxamine
DMSO Dimethyl Sulphoxide
DNA Deoxyribonucleic Acid
ECOG Eastern Cooperative Oncology Group
EDTA Ethylene Diamine Tetra-acetic Acid
ERCP Endoscopic Retrograde Cholangiopancreatography
EUS Endoscopic Ultrasound
FAM 5-fluorouracil Adriamicin Mitomycin (regime)
FAMMM Familiar Multiple Mole Melanoma (Syndrome)
Fc Ferrochelatase
Fe $^{2+}$ Ferrous Iron
Fe $^{3+}$ Ferric Iron
FNA Fine Needle Aspiration
GITSG Gastrointestal Tumour Study Group
GTT Glucose Tolerance Test
H&E Haemotoxylin and Eosin
$\text{H}_2\text{O}_2$ Hydrogen Peroxide
HpD Haematoporphyrin Derivative
HPO Hydroxypyridinone
IGT Impaired Glucose Tolerance
MRCP Magnetic Resonance Cholangiopancreatography
mTHPC meta-terahydroxyphenylchlorin
MRI Magnetic Resonance Image
Nd: YAG Neodymium: yttrium aluminium garnet
NPe6 Mono-Aspartyl Chlorin e6
$^{1}O_2$ Singlet Oxygen
$O_2^{-}$ Superoxide
OH Hydroxyl Radical
PBS Phosphate Buffered Saline
PD Pancreaticoduodenectomy
PDT Photodynamic Therapy
PET Positron Emission Tomography
PPPD Pylorus Preserving Pancreaticoduodenectomy
PPIX Protoporphyrin IX
PTD Percutaneous Transhepatic Drainage
PTE Percutaneous Transhepatic Endoprosthesis
PUVA Psoralen Ultraviolet A
RNA Ribonucleic Acid
SCC Squamous Cell Carcinoma
SEER Surveillance Epidemiology and End Results (programme)
SMF Streptozotocin Mitomycin 5-fluorouracil (regime)
SMPV Superior Mesoportal Venous System
SMV Superior Mesenteric Vessel
SnET$_2$ Tin Etiopurpurin
Suc-CoA Succinyl CoA
TK Thymidine Kinase
TNF-alpha Tumour Necrosis Factor-alpha
TPPS$_4$ meso-Tetraphenylporphine tetrasulphonate
US ultrasonography
UICC Union Internationale Contre le Cancer
ZnPc Zinc phthalocyanine
5-FU 5-fluorouracil
Chapter 1. Pancreatic cancer

1.1 Epidemiology and pathology

Epidemiology

The incidence of pancreatic cancer is estimated at approximately 10 cases per 100,000 per year in Western Europe and North America. This ranks it twelfth in cancer incidence and due to its extremely poor prognosis, pancreatic cancer is the fifth leading cause of cancer death in the developed countries, exceeded only by cancers of the lung, colon and rectum, breast, and prostate (Landis et al, 1999).

Worldwide, pancreatic cancer is 13th in cancer incidence and the ninth most common cause of cancer related death (Parkin et al, 1999). Pancreatic cancer is more frequent in the industrialised regions, where approximately 66% of cases occur. However, a similar rate of incidence as in the developed countries has been reported in Eastern Asia, Central and temperate South America and the Caribbean (Parkin et al, 1999). The lowest incidence (approximately 2 per 100,000) is observed in India. The reason for these variations is not clear, however misdiagnosis leading to underestimation of pancreatic cancer is one probable factor. Genetic and envoirmental factors may also play an important role.

In North America, the incidence of pancreatic cancer increased threefold from 1930 to 1973. After 1974 the rate of pancreatic cancer started to decline slightly due to a decreasing incidence in men. However, the rate of pancreatic cancer continued to grow among woman and since the mid 80’s slightly more women die of pancreatic cancer than combined uterine cervix and corpus cancers (Landis et al, 1999). For year 2000, the estimated number of cases of pancreatic cancer in women is expected to exceed the rate for men (male-13,700, female-14,600) (Greenlee, 2000).

Similar time and gender trends in the incidence of the disease can be observed in the UK. Although full epidemiological data is not available, analysis of all cases of the pancreatic cancer from 1957 to 1986 in the West Midlands performed by Bramhall et al showed a similar development to the America pattern (Bramhall et al, 1995).

The occurrence of pancreatic cancer rises steadily with age to peak in the seventh and eight decade. Pancreatic cancer is 30-40% more frequent in blacks than in whites (Ahlgren, 1996a).
The prognosis of pancreatic cancer is extremely poor. Official epidemiological statistics from Europe and North America reveal that only up to 15% of diagnosed patients survive one year and approximately 4% five years (Faivre et al, 1998, Landis et al, 1999). Despite the substantial progress of medicine and introduction of new imaging techniques, statistically survival of pancreatic cancer remained unchanged over the past two decades and at present is the worst of any type of cancer (Landis et al, 1999).

However, even this miserable prognosis may be in fact artificially inflated by misdiagnosis of benign diseases or other neoplasms (e.g. ampullary) as pancreatic cancer, which are associated with a better outcome (Ahlgren, 1996a). In the epidemiological study from the West Midlands, there were more 5 years survivors following only palliative treatment than after potentially curative surgery (40 versus 20, respectively), presumably owing to false positive diagnosis (Bramhall, 1996-author’s reply). Also some rare types of pancreatic neoplasms have a much more favourable biological behaviour than pancreatic adenocarcinoma and may substantially prolong the average survival of the whole group.

The reported 5 years survivals of patients with histologically confirmed ductal adenocarcinoma were 0% and 1.3% in two big epidemiological American studies (Carriaga and Henson, 1995, Riela et al, 1992). This data confirm that pancreatic adenocarcinoma is lethal and virtually incurable.

**Aetiology and risk factors**

The aetiology of pancreatic cancer is unknown, however, several risk factors have been identified.

Tobacco smoking is the major well established risk factor, which has been shown to increase approximately 2.5 fold the risk of pancreatic cancer in both a number of prospective and case-control studies (Boyle et al, 1996). Based on the results of the large, prospective American study, it was calculated that 25% of all pancreatic cancers could be attributed to cigarette smoking (Fuchs et al, 1996).

Smoking is also believed to be responsible for the increased incidence of pancreatic cancer among women in the last three decades.

Most studies found a positive correlation between the number of cigarettes smoked (and in some surveys the duration of habitual smoking) and pancreatic cancer.
A reduction of risk by 48% within 2 years of quitting smoking has been noted too, however, 10 to 15 years must elapse following smoking cessation until the risk falls to the level of never-smokers (Fuchs et al, 1996, Boyle et al, 1996). Epidemiological studies demonstrating a casual relationship between smoking and pancreatic cancer have been supported by histopathological evidence. Precarcinogenous cells with nuclei atypia were detected in the pancreatic duct of the majority of smokers at autopsy and rarely in non-smokers (Auerbach and Garfinkel, 1986).

There was a strong positive relationship between the degree of these alterations and the amount of cigarettes smoked. Similar abnormalities associated with cigarette smoking were observed also in the trachea, bronchi and larynx, where cancers are well established tobacco-related malignancies.

Furthermore, K-ras point mutations, which are present in more than 90% cases of pancreatic cancer, were detected in normal pancreas from 39% of heavy smokers but in no non-smokers (Berger et al, 1999).

In an experimental study, tobacco specific nitrosamines induced pancreatic and lung cancers in rats, when administered with drinking water (Rivenson et al, 1988).

A number of dietary factors have been linked with pancreatic cancer. Several studies have suggested that high protein (mainly meat) consumption elevate the risk of pancreatic cancer (Gold, 1995). Increased saturated fat and cholesterol intake also seemed to promote development of pancreatic cancer but polyunsaturated fat may be in fact protective and significantly reduce the risk (Zatonski et al, 1991).

In an experimental study, a diet rich in fat enhanced the incidence of the nitrosamine induced pancreatic cancer in hamsters (Birt et al, 1989).

Several studies found that fibre, raw vegetables, fruits, vitamin C may have a significant protective effect against pancreatic cancer (Jain et al, 1991, Gold, 1995). Evidence regarding the influence of coffee and alcohol consumption on the tumorigenesis of pancreatic neoplasms are inconsistent and often contradictory.

Frequent alcohol consumption has been shown to increase 5-fold the risk of pancreatic cancer in a large, prospective Norwegian survey (Heuch et al, 1983), however subsequent case-pooled studies did not show any excess risk related to the alcohol consumption (Ghadirian et al, 1990, Zatonski et al, 1993).

Additionally, some studies found a negative correlation between pancreatic cancerogenesis and alcohol intake (Clavel et al, 1989).
A dose-response effect of coffee consumption on the risk of pancreatic cancer has been observed in several case-controlled studies (MacMahon et al, 1981), especially in women (Clavel et al, 1989). However, the reported increase of risk was usually low and all prospective studies failed to demonstrate a significant relationship between coffee consumption and pancreatic cancer (Heuch et al, 1983, Gold, 1995). Therefore, if any associated with coffee risk exists it cannot be strong.

Exposure to a number of chemicals may be an important factor in the carcinogenesis of pancreatic cancer.

An increased incidence of pancreatic cancer has been reported among workers employed in metal, building, printing, publishing and chemical industries. The mortality rate for pancreatic cancer was five times higher in workers exposed to beta-naphthylamine and benzidine compared to the general population. Other identified carcinogenic chemicals include ethylene and propylene chlorohydrin, ethylene dichloride and DDT (Gold, 1995). Recently, it has been discovered that patients with pancreatic cancer (especially cases whose tumour carries the K-ras mutation) have significantly higher plasma concentrations of organochlorides than a control group (Porta et al, 1999). Organochloride pesticides have extremely long half-lives of 10-30 years and can accumulate in adipose tissue. Although their usage has been prohibited in most European countries in the past few decades, pesticides are still present in food, especially meat.

Recently it has been suggested that most of the environmental risk factors of pancreatic cancer can be related to the exposure to aromatic amines (DiMagno et al, 1999).

Aromatic amines are present in tobacco smoke, cooked meat and a number of industrial chemicals. However, it seems to be clear that a very strong mutagen for pancreatic cancer, which could have the same impact on the development of pancreatic cancer as for example tobacco smoke on lung cancer, does not exist or has just not yet been identified.

Other than environmental factors, a number of genetic alterations may play a significant role in the aetiology of pancreatic cancer. It is estimated that approximately 10% of patients with pancreatic cancer have an inherited genetic defect predisposing to this (and often also to other) cancers (Lowenfelds et al, 1999).

In a case-control study conducted among French Canadians, 7.8% of patients with pancreatic cancer had a relative with the same disease compared to 0.6% in the control population.
This gave a 13-fold higher incidence in the occurrence of a family history of pancreatic cancer in the individuals suffering from pancreatic cancer than in healthy controls (Ghadirian et al, 1991).

Another case-control study, which investigated a familial association between pancreatic and other types of cancer, revealed a 5-fold increased risk associated with a family history of pancreatic neoplasm and a 2-fold increased risk of pancreatic cancer related to a family history of any malignancy (Falk et al, 1988). Finally, Silverman et al reported a 3-fold increased risk of pancreatic neoplasm allied with the occurrence of pancreatic cancer in first-degree relatives and a lower but still significant risk associated with a family history of cancers of colon, ovary, breast and endometrium. The increased familial incidence of colorectal, ovary, endometrium, pancreas and breast cancers is typical for hereditary non-polyposis colorectal cancer (Lynch II variant). However, the presence of the element of the syndrome in the relatively small, population-based study suggests a much higher than anticipated occurrence of the syndrome in the general population.

Moreover, cigarette smoking additionally aggregated the risk of pancreatic cancer associated with a family history of malignancy (Silverman et al, 1999). This finding shows clearly the correlation between environmental factors and genetic predisposition to cancer.

Other known genetic diseases with an increased risk of pancreatic cancer include ataxia-teleangiectasia, cystic fibrosis, familial adenomatous polyposis, Li-Fraumeni syndrome, Peutz-Jeghers syndrome, and familial atypical multiple mole melanoma syndrome (FAMMM) (Lowenfelds et al, 1999).

The BrCa2 gene, which is responsible for some hereditary breast cancers, has been also linked with higher susceptibility to pancreatic cancer and found in 10% of patients with pancreatic malignancy. In fact the BrCa2 gene may be one of the most frequent genetic disorders leading to the development of pancreatic cancer (Lowenfelds et al, 1999).
Probably the highest risk of pancreatic cancer associated with an inherited germline defect has been found in the patients with hereditary pancreatitis, which have a 40% cumulative risk of developing pancreatic cancer by 70 years of age (Lowenfelds et al, 1999).

The mutation responsible for this disease, which has been traced back to the gene coding for cationic trypsinogen, leads to the disturbances in the inactivation of trypsin in acinar cells and as a consequence to attacks of acute pancreatitis (Gates et al, 1999). Subsequently, typical chronic pancreatitis develops with pancreas calcification and endocrine and exocrine insufficiency. The link between hereditary pancreatitis and pancreatic cancer remains unclear, however, as cancer usually develops approximately 40 years after the onset of disease. There seems to be a correlation between long-standing inflammation of the pancreas and cancer.

Tropical pancreatitis, which is another form of chronic pancreatitis, is also associated with an excess incidence of pancreatic cancer (Lowenfelds et al, 1999).

It has still not been established, however, if there is a causal relationship between chronic alcoholic pancreatitis, which is the most common type of chronic inflammation of the pancreas, and pancreatic cancer.

Several case-control studies supplied conflicting and not convincing data, mainly due to the small number of patients with chronic pancreatitis and recall bias (Jain et al, 1991, Bueno de Mesquita et al, 1992). One large, historical cohort study revealed a 16-fold elevated relative risk of pancreatic cancer in patients with chronic pancreatitis (Lowenfelds et al, 1993). The risk decreased slightly after excluding all patients with pancreatic cancer diagnosed during the first year of follow-up, however, the cumulative risk of pancreatic cancer increased with longer duration of the disease and was 4% twenty years after diagnosis of chronic pancreatitis. However, another large, record linkage study from Sweden demonstrated a much lower, 7-fold increased risk for pancreatic cancer in chronic alcoholic pancreatitis, which declined to only 2-fold after ten years of follow-up (Karlson et al, 1997). This indicates that possible misclassification of the early pancreatic cancer as chronic pancreatitis could lead to substantial overestimation of the role of chronic pancreatitis in the development of pancreatic cancer (Gold and Cameron, 1993).
However, as both studies showed some relationship between chronic pancreatitis and subsequent development of pancreatic cancer, there is probably a moderately elevated risk of pancreatic neoplasm associated with chronic pancreatitis, which could be the cause of up to 4% of all cases of pancreatic cancer (Lowenfelds et al, 1999). Diabetes and pancreatic cancer are closely associated. 20-70% of patients with pancreatic cancer are diabetic or have impaired glucose tolerance.

It is well documented that pancreatic cancer itself induces diabetes (Ding et al, 1998). However, whether the diabetes is a consequence of the diabetogenic action of tumour in all cases or maybe sometimes a truly etiological factor remains unclear. The largest case control study showed that the increased risk of pancreatic cancer was limited to the patients with the onset of diabetes two years before the diagnosis of cancer (Gullo et al, 1994).

Other studies, however, have demonstrated an elevated risk of pancreatic cancer associated with diabetes of more than 5 years standing (Cuzik and Babiker, 1989) or even a slight positive relationship between duration of diabetes and the occurrence of pancreatic cancer (Silverman et al, 1999). At present, it is thought, that a group of patients with newly diagnosed diabetes, more than 60 years old, not obese and with no family history of diabetes may have an increased risk of pancreatic carcinoma (DiMagno et al, 1999).

Gastrectomy is another medical condition, which is though to predispose to the pancreatic tumorigenesis. It has been established that the relative risk of cancer is increased two to five times, twenty years after surgery (Warshaw and Fernandez-del Castillo, 1992).

Allergic disorders like hay fever, asthma or eczema on the other hand, were shown to reduce the risk of pancreatic cancer (Silverman et al, 1999, Jain et al, 1991).

**Pathology of pancreatic neoplasms**

Primary malignant neoplasms of the pancreas can originate either from the exocrine parenchyma of the pancreas or from the endocrine component of the organ, which consists of the islets of Langerhans.

However, the overwhelming majority of pancreatic tumours are exocrine neoplasms; endocrine tumours are rare and make up less than 2% of all pancreatic tumours.
More than 90% of the malignant exocrine tumours are adenocarcinomas of epithelium cell origin. Approximately 90% of them are ductal adenocarcinoma, which is the most common type of pancreatic neoplasm, accounted for 75%-92% of all pancreatic tumours.

The predominant site of the ductal adenocarcinomas is the head of the gland (78%); the remaining 22% of cancers are equally located in the body or the tail of the pancreas (Sener et al, 1999).

Ductal adenocarcinoma is an extremely aggressive cancer, infiltrating all adjacent organs and metastasising early to adjacent and distant lymph nodes, peritoneum and liver. It has also proclivity for perineural invasion.

Due to its highly malignant behaviour and late clinical presentation pancreatic ductal adenocarcinoma carries a very poor prognosis. Median survival does not exceed 4 months and the five years survival was found to be 0%, 0.04% and 1.3% in the three big analyses (Riela et al, 1992, Gudjonnson, 1987, Carriaga and Henson, 1995).

Mucinous and mucin producing adenocarcinomas comprise 6% of all pancreatic neoplasms and their outlook is similarly poor to ductal adenocarcinoma. However, other mucin producing carcinomas, which form cystic lesions are known to have a more favourable biologic behaviour and better prognosis. Mucinous cystadenocarcinoma has the best outcome of all pancreatic cancers. Median survival is longer than 12 months and approximately half of patients remain alive after 5 years from the diagnosis. Mucinous cystadenocarcinoma probably arises from the relatively benign lesion, described in the past as cystadenoma.

However, it is believed now, that all mucinous cystic tumours have malignant potential and should be treated as cancerous lesions.

Unfortunately, this highly curable cancer is very rare and accounts for less then 0.5% of all pancreatic tumours.

Cystadenocarcinoma, like its mucinous type, is more frequent in women. Also this form of cancer carries better prognosis then ductal adenocarcinoma.

Intraductal papillary neoplasm (described also as intraductal papilloma, intraductal papillary mucinous tumour, mucin-producing cancer, mucin-hypersecreting neoplasm, ductetatic cystadenoma) is sometimes also classified as a subtype of mucinous cystadenocarcinoma. Intraductal papillary neoplasm is relatively highly curable by surgery, thanks to its favourable biologic nature and early production of clinical symptoms (Compton and Mulvihill, 1997).
This tumour is characterised by slow, localised growth and initially may be benign. However, it has considerable malignant potential and invasive cancer is present at surgery in up to 50% of cases.

At ERCP, cystic dilatation of the main pancreatic duct or its branches owing to cancer production of mucous material is the typical finding. A dilated papilla filled with and exuding mucous is also commonly observed. Sometimes only cystic lesions, separate from pancreatic ducts, are seen within the pancreas (DiMagno et al, 1999).

Papillary adenocarcinoma and papillary cancer, are rare tumours, more common in females and have 40% 1 year and 8% 5 year survival rates.

Approximately 1% of all pancreatic tumours are adenosquamous and squamous cell carcinoma in origin, which exhibit similarly dismal outcome to ductal adenocarcinomas.

Only 0.2% of all pancreatic neoplasms are made up by acinar cell carcinomas. This rare cancer arises from the acinar cells of the pancreas and is more frequent in men. Like the majority of pancreatic tumours it is associated with a short, 5 months, median survival.

Other types of cancer are extremely uncommon and include undifferentiated, anaplastic, large cell and pleomorphic (giant cell) cancers. These rare neoplasms usually have a very poor outcome, even worse than ductal adenocarcinoma.

Islet cell carcinomas are a heterogeneous group of neoplasms, accounting for 1.7% of all pancreatic tumours. They arise from the endocrine part of the pancreas and about 50% of tumours secret hormones normally produced by the cells of their origin. Depending on the secreting substance and resulting clinical syndrome, five main types of tumours have been described: gastrinoma, glucagonoma, insulinoma, VIPoma and somatostatinoma.

The biology of these neoplasms is unpredictable and as histological examination cannot distinguish malignant tumours from benign, the diagnosis of malignancy can be made only in the presence of liver or lymph node metastases. Many of these tumours are detected at an advanced state, however, due to their usually more favourable behaviour and often positive response to surgery and/or chemotherapy, they have a variable but generally better prognosis than exocrine tumours (Bieligk and Jaffe, 1995).
Tumours of uncertain origin comprise clear cell carcinoma, small cell carcinoma, pancreatic giant cell tumour with osteoclast-like giant cells and papillary cystic neoplasms. All of these are exceptional and all but papillary cystic tumours are associated with a bad prognosis.

Other rare primary neoplasms found in the pancreas include fibrosarcoma, leiomyosarcoma, hemangiopericytoma, histiocytoma and lymphoma.

Most data in this section was obtained from the Surveillance, Epidemiology, and End Results (SEER) programme of the National Cancer Institute, which recorded all cases of pancreatic neoplasms from 1973 to 1987 in the United States (Carriga and Henson, 1995).

**Molecular biology of pancreatic cancer**

The molecular events involved in the carcinogenesis of pancreatic tumours are still poorly understood. However, it seems likely that accumulation of genetic abnormalities is necessary to trigger the development of cancer.

Point mutations of the K-ras oncogene at codon 12 are the most common genetic alterations associated with pancreatic cancer, which were found in more than 95% of pancreatic cancer tissue specimens (Almoguera et al, 1988).

K-ras mutations were also detected in 77% of cases in the pancreatic juice (Tenner et al, 1996) and in 63% of cases in the duodenal juice (Iguchi et al, 1996) obtained during ERCP from patients with pancreatic cancer, and in stool from 6 of 11 patients suffering from pancreatic carcinoma (Caldas et al, 1994). In these cases mutations were found in the cancer cells shed to the gastrointestinal tract.

Mutated K-ras from the cancer cells circulating in the peripheral blood was observed only in 2 cases of 6, in which one had evidence of liver metastases (Tada et al, 1993). However, much higher sensitivity (81%) was obtained when K-ras mutations were identified in cancer DNA present in the plasma of pancreatic cancer patients (Malucahy et al, 1998).

Due to the strong correlation of K-ras mutations with pancreatic cancer, genetic analysis could be of great value for diagnosis and early detection of pancreatic cancer. Unfortunately, K-ras mutations are not specific for pancreatic cancer.
They have been found commonly in the normal pancreatic ducts, hyperplastic ducts (Tada et al, 1996), squamous metaplastic ducts and periductal fibrosis present both in the normal pancreas and the pancreas from patients with chronic pancreatitis or cancer (Luttges et al, 1999, Berger et al, 1999, Yanagisawa et al, 1993).

K-ras mutations probably also do not precede (or at least not in high frequency) the development of pancreatic cancer in humans.

In the study by Furuya et al none of 20 patients with chronic pancreatitis and K-ras mutations detected in the duodenal juice developed pancreatic cancer during a mean follow-up period of 78 months (Furuya et al, 1997).

Also K-ras mutations were detected in up to 32% of the pancreas taken at autopsy from patients who died of non-pancreatic disease and did not develop cancer (or other pancreatic diseases) during their lifetime (Tada et al, 1996, Berger et al, 1998).

These findings indicate that K-ras mutations are very common events in the pancreas, and may not have relevance to carcinogenesis. Also the negative predictive value of the K-ras mutations for pancreatic cancer is not high as the mutations may be present in only 64% of cancer specimens (Porta et al, 1999).

Therefore, at the present, detection of K-ras mutations has very limited (if any) value for diagnosis, screening or follow-up.

Pancreatic cancer has a 60% prevalence of p53 apoptosis gene mutation, which is similar to other human neoplasms (Barton et al, 1991). However, also this mutation is not specific for cancer as it occurs in 10% of chronic pancreatitis (Gansauge et al, 1998). A serological test based on the detection of p53 protein antibodies had a very low sensitivity (21%) and failed to be useful in tumour detection (Gessner and Baillie, 1996). Other identified genetic alterations in pancreatic cancer are numerous, however at the moment they have no clinical utility.

1.2 Diagnosis of pancreatic cancer

Clinical features of pancreatic cancer

The initial clinical symptoms of pancreatic cancer are not specific and are usually described as generalised dyspepsia or abdominal discomfort. Obstructive jaundice, abdominal pain and weight loss are the classical presenting features of pancreatic carcinoma but are usually associated with more advanced disease (Barkin and Goldstein, 1999).
Jaundice at presentation owing to the obstruction of the common bile duct occurs in at least 50% of patients with pancreatic cancer (Bakkevold et al, 1992). Jaundice is a typical, and sometimes the first, manifestation of cancer localised in the head of the pancreas.

However, it is rare for cancers arising from the body or the tail of the gland, and is caused in these cases by liver metastases or by compression of the biliary tree by metastatic lymph nodes in the porta hepatis (Hawes et al, 2000).

Painless jaundice, believed to be a classical symptom of pancreatic cancer is observed only in the minority (18%) of patients; in most cases jaundice is associated with pain (Bakkevold et al, 1992). A painless palpable gall bladder (Courvoiser’s sign) is found sometimes at examination (Kumar and Clark, 1998).

Abdominal pain is the most common symptom of pancreatic cancer, occurring in at least 80% of patients at some time during the course of the disease (Hawes et al, 2000).

The characteristic pain is a dull ache localised in the midepigastrum, which may radiate through to the back, especially in cases of cancer of the body or the tail of the pancreas. Pain is usually more severe at night and in the supine position and may be relieved by sitting forward (Kumar, 1998). Pain is caused by cancer infiltration or compression of the neural plexus around the superior mesenteric artery and celiac axis or by obstruction of the adjacent structures like the pancreatic duct (Hawes et al, 1999, Barkin and Goldstein, 1999).

Weight loss is the third main presenting feature of pancreatic cancer. It may result from anorexia, pancreatic exocrine insufficiency or gastric outlet obstruction. Duodenal stenosis, which manifests clinically as vomiting, is a late symptom of advanced cancer, which occurs in approximately 20% of patients with pancreatic cancer. However, vomiting often is not associated with duodenal obstruction and reflects stomach dysmotility owing to cancer neuropathy. Commonly only symptoms of delayed gastric emptying are present, which include early satiety, nausea and bloating (Barkin and Goldstein, 1999).

Diarrhoea/steatorrhoea resulted from exocrine insufficiency was reported in 12% of patients in a large study (Bakkevold et al, 1992).

Approximately 70% of patients with pancreatic cancer suffer from diabetes or impaired glucose tolerance (Permet et al, 1994).
Diabetes may be in fact a very early sign of the disease and precede even by two years the diagnosis of cancer (Gullo et al, 1994).

The pathology of diabetes associated with pancreatic cancer is complex. Currently, it is though that diabetes is caused by a factor released by cancer cells, which selectively stimulates the production and secretion of amylin (islet amyloid polypeptide) by the beta cells of the island of Langerhans (Ding et al, 1998). Amylin is a diabetogenic factor, which induces profound peripheral insulin resistance and impairs synthesis of glucagon. Significant improvement of glucose tolerance is observed after resection of the tumour (Permet et al, 1994).

Other non-specific symptoms of pancreatic cancer include weakness and generalised malaise. Migratory thrombophlebitis, gastrointestinal haemorrhage, acute pancreatitis and splenomegaly are infrequent signs and symptoms of pancreatic cancer (Hawes et al, 2000).

**Staging of pancreatic cancer**

The aim of the work-up of pancreatic cancer is to establish the diagnosis and to ensure the most appropriate treatment for each patient. In practice this means mainly identifying the small group of patients with potentially resectable disease. Pancreatic cancer is usually deemed to be resectable if there is no extrapancreatic disease present and the tumour does not invade the great peripancreatic vessels (hepatic artery, superior mesenteric artery and superior mesoportal venous system (SMPV)).

However, some of the criteria of operability are consider to be relative (e.g. involvement of the SMV or SMPV) and differ between surgical centres depending on the extent of the resection normally performed.

The TNM staging system of pancreatic cancer approved by the Union Internationale Contre Le cancer (UICC) and the American Joint Committee on Cancer (AJCC) (Table 1) although reproducible, simple and associated with considerable prognostic value, does not always reflects surgical resectability of the disease.

Usually stage I (localised disease) and sometimes stage II (regional disease) may be suitable for curative surgery, however, resections are in fact sometimes performed in all stages of pancreatic cancer.
**Table 1.** Union Internationale Contre Le Cancer and American Joint Committee on Cancer TNM staging system for pancreatic cancer.

<table>
<thead>
<tr>
<th>Primary tumour (pT)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TX</td>
<td>Primary tumour cannot be assessed</td>
</tr>
<tr>
<td>T0</td>
<td>No evidence of primary tumour</td>
</tr>
<tr>
<td>Tis</td>
<td>Carcinoma in situ</td>
</tr>
<tr>
<td>T1</td>
<td>Tumour limited to the pancreas and 2 cm or less in greatest dimension</td>
</tr>
<tr>
<td>T2</td>
<td>Tumour limited to the pancreas and more than 2 cm in greatest dimension</td>
</tr>
<tr>
<td>T3</td>
<td>Tumour extends directly to any of the following: duodenum, bile duct, peripancreatic soft tissues*</td>
</tr>
<tr>
<td>T4</td>
<td>Tumour extends directly to any of the following: stomach, spleen, colon, adjacent large vessels**</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Regional lymph nodes (pN)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NX</td>
<td>Regional lymph nodes cannot be assessed</td>
</tr>
<tr>
<td>N0</td>
<td>No regional lymph nodes metastases</td>
</tr>
<tr>
<td>N1</td>
<td>Regional lymph node metastases</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Distant metastases (pM)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MX</td>
<td>Presence of distant metastases cannot be assessed</td>
</tr>
<tr>
<td>M0</td>
<td>No distant metastases</td>
</tr>
<tr>
<td>M1</td>
<td>Distant metastases</td>
</tr>
</tbody>
</table>

**Stage groupings**

- **Stage 0**: Tis, N0, M0
- **Stage I**: T1-2, N0, M0
- **Stage II**: T3, N0, M0
- **Stage III**: T1-3, N1, M0
- **Stage IVA**: T4, any N, M1
- **Stage IVB**: any T, any N, M1

* For T3, peripancreatic soft tissues include soft tissues adjacent to the pancreas in addition to the common bile duct and duodenum (including ampulla of Vater). Specifically, peripancreatic soft tissues include the surrounding retroperitoneal fat (retroperitoneal soft tissue), mesentery (mesenteric fat), mesocolon, and greater and lesser omentum.

** For the T4 classification, the adjacent large vessels are the portal vein, the celiac artery, the superior mesenteric, and common hepatic arteries and veins (not the splenic vessels). Direct extension to an organ or stricture not listed in T1-T4 is M1. Peritoneal seeding is also considered M1.

*Source: Compton and Mulvihill, 1995.*
**Imaging**

Abdominal ultrasonography (US) is currently much less frequently used for the diagnosis of pancreatic cancer than in the past, being replaced by the other more accurate examinations. The latest American Gastroenterological Association (AGA) recommendation regarding pancreatic cancer does not include abdominal US as a useful diagnostic test (DiMagno et al, 1999).

However, ultrasonography seems to be still suitable for initial evaluation of patients with jaundice or abdominal pain. It is cheap, available, quick and not associated with exposure to ionising radiation. Ultrasonography accurately distinguishes obstructive from intrahepatic jaundice, easily demonstrating dilatation of the biliary tree. Ultrasonographic examination may also show dilatation of the pancreatic duct, which is the other important secondary sign of pancreatic cancer, or/and ascites and liver metastases. Sometimes a pancreatic mass, lymphadenopathy and even detail of the local cancer extension can be seen on US too. Furthermore, biopsy of any suspected lesions can be often made under US control.

Up to 20% of examinations, however, are technically unsatisfactory owing to the bowel gas interference, obesity, and previous surgery. It is estimated that the whole pancreas may be adequately visualised only in up to 67% of cases (Moossa and Gamagami, 1995). Despite these limitations the sensitivity of US for the detection of pancreatic tumours could be as high as 73% in the latest series.

US performs very poorly, however, at assessing lesion resectability, especially compared to other techniques (Rosch et al, 1992a). Therefore, US can only be used as an initial screening examination, to select the group of patients with suspected pancreatic cancer for further evaluation but not to establish the diagnosis or stage of the disease.

Computed tomography (CT) is the well recognised modality for both detection and staging of pancreatic cancer. CT can reveal an abnormal mass in the pancreas or show indirect signs of the cancer like bile and/or pancreatic duct dilatation (the double duct sign), ‘postobstructive pseudocyst’ and/or pancreatic atrophy (Moossa and Gamagami, 1995). CT also provides information about resectability of the cancer, showing the very fine anatomical details regarding local tumour extension to the peripancreatic tissues, contiguous organs or great vessels and demonstrating lymphadenopathy, ascites and liver metastases associated with the disease.
The overall sensitivity of conventional dynamic CT for the detection of pancreatic cancer is approximately 69%, which diminishes substantially to only 40% for lesions smaller than 2 cm (Muller et al, 1994). Dynamic CT is an excellent modality for predicting tumour unresectability for which its positive predictive value was 89% and 100% in two series (Megibow et al, 1995, Freeny et al, 1988). However, CT usually understages pancreatic cancers and has poor ability to predict if tumour is truly amenable for resection.

In a large retrospective study, only approximately 21% of the patients deemed to have resectable disease on the basis of CT appeared to have truly operable tumour at surgery. This result mainly reflects the poor ability of CT to spot small peritoneal and liver metastases and to distinguish metastatic from hyperplastic lymph nodes (Freeny, 1999).

Also the extend of local cancer spread and the involvement of great vessels by the tumour is often underestimated by conventional CT (47% sensitivity and 69% specificity for assessment of vascular invasion in the large study by Megibow (Megibow et al, 1995).

Major improvement in both detection and preoperative assessment of pancreatic cancer has been achieved by the introduction of spiral, dual-phase CT. This modality gives high quality, free of respiratory misregistration, thin section images during phases of the optimal contrast medium enhancement. The arterial phase represents the time of the maximal pancreatic enhancement and provides the best tumour-pancreas contrast. The portal phase, which is obtained during the peak of the vessel and the liver opacification following injection of the contrast medium, enables improved detection of liver metastases and superior visualisation of the vessels’ anatomy.

Sensitivity and specificity of dual-phase spiral CT for tumour detection were between 92-97% and 92-100% respectively in recent studies (Diehl et al, 1998, Sheridan et al, 1999, Legmann et al, 1998), significantly exceeding values achieved by dynamic CT. However, biphase spiral CT may still miss cancers smaller than 15-20mm (Legmann et al, 1998, Diehl et al, 1998) as the sensitivity for the detection of lesions smaller than 15mm was only 67% (Legmann et al, 1998).

Spiral dual-phase CT substantially improves the preoperative evaluation of pancreatic cancers.
In the studies comparing CT assessments with the surgical findings, the overall accuracy for predicting resectability was 91% for dual-phase and 70% for uniphase spiral CT. Uniphase CT correctly identified only 21 patients among 40 with unresectable disease (sensitivity 53%, 100% specificity), whereas dual phase spiral CT showed 50 among 55 patients with inoperable disease (91% sensitivity, 79% specificity) (Bluemke et al, 1995, Diehl et al, 1998). Improvement in tumour staging is achieved by better visualisation of local tumour invasion, especially by the improved assessment of the peripancreatic vessel involvement.

Compared with results at operation, vessel encasements were evaluated by the spiral dual-phase CT with 84%, 88% sensitivities and 97%, 98% specificities in two recent studies (Lu et al, 1996, Diehl et al, 1998).

Spiral CT is still inadequate, however, in the detection of liver (sensitivity 75%, Diehl et al, 1998) and lymph nodes metastases. This reflects the inability of CT to determine metastases in normal size lymph nodes and to identify small, 2-3mm secondary lesions in the liver. However, patients with disseminated disease often have other signs of inoperability (like extensive retroperitoneal cancer spread), which assist in precluding unnecessary surgery (Diehl et al, 1998). Despite these limitations, spiral dual-phase CT is currently recommended as the best and normally the only one required test to detect and stage pancreatic tumours (DiMagno et al, 1999).

Probably the highest accuracy for the detection of pancreatic tumours can be achieved by using endoscopic ultrasound (EUS). Sensitivity and specificity of EUS for the detection of pancreatic cancer were close to 100%, in several studies. EUS was significantly superior for tumour diagnosis to conventional CT, angiography and abdominal ultrasound (98%, 85% and 73% sensitivities for EUS, CT and US respectively) (Rosch et al, 1992b).

In a direct comparison with spiral dual-phase CT in two studies, which correlated results of imaging examinations with operative findings, EUS had a slightly higher sensitivity for tumour detection than CT (Midwinter et al, 1999, Legmann et al, 1998). Differences were not statistically significant and both modalities performed very well, however, CT missed several small lesions, detected by EUS, in both series.
Therefore, EUS seems to be especially useful for the localisation cancers smaller than 2 cm, which are often not visualised by other tests (Rosch et al, 1992b, DiMagno et al 1999).

EUS, due to limited penetration of the ultrasound wave, is not suitable for evaluation of the liver metastases; however, it is highly accurate for local and nodal staging of pancreatic cancer.

In a large study, consisting of 81 patients with pancreatic cancer who underwent surgical exploration, EUS was 93% accurate for predicting local resectability versus 60% for conventional CT. EUS was superior in assessing T stage (85% versus 30%), N stage (72% versus 55%) vascular invasion (93% to 62%) to CT (Greco et al, 1999). Other studies brought similar results (Hawes et al, 2000).

However, EUS was found to have similar sensitivity to state-of-the-art dual-phase spiral CT sensitivities for evaluation of the venous system involvement and nodal metastases (Midwinter et al, 1999), and the same as dual-phase spiral CT overall (92%) accuracy for the prediction of tumour resectability (Legmann et al, 1998). Therefore, it seems that EUS offers diagnostic value equivalent to that of non-invasive spiral dual-phase CT for staging pancreatic cancer and should rather be limited to searching for small tumours, not seen by CT (diMagno et al, 1999).

EUS however, may allow tissue diagnosis to be obtained either from the primary lesion and/or lymph nodes.

EUS with guided fine-needle aspiration (FNA) biopsy seems to overcome the main drawbacks of EUS - poor ability to distinguish pancreatic cancer from focal pancreatitis (Lengmann et al, 1998) and limited accuracy to differentiate metastatic from inflammatory lymph nodes.

EUS- FNA biopsy had sensitivities of 86%, 92% and specificities of 94%, 100% for pancreatic masses and sensitivities of 92%, 83% and specificities of 93%, 100% for lymph nodes in two large studies (Wiersema et al, 1997, Chang et al, 1997). EUS with guided biopsy upstaged and precluded surgery in up 40% of patients with pancreatic cancer in another study by demonstrating portal vein invasion or metastases to coeliac lymph nodes (Chang et al, 1997). EUS-guided FNA is a safe procedure, with a low rate of complications, not exceeding 2%. Thus EUS coupled with EUS-guided biopsy may be a useful, accurate test, especially for patients, who have undefined, equivocal findings on CT.
Magnetic resonance (MR) is a relatively new imaging technique that can be employed for the detection and assessment of resectability of pancreatic cancer. Conventional spin echo techniques of MR provided poor quality images owing to motion artefacts and poor spatial and axial resolution.

However, the new type of dynamic dual-phase gadopentate enhanced MR seems to overcome these limitations. Dynamic MR was significantly superior to dynamic CT for the detection of pancreatic (especially small) lesions, but not for preoperative staging (Muller et al, 1994). Compared with uniphasic spiral CT, MR had slightly better or equal accuracy in the identification and staging of pancreatic tumours but the differences were not statistically significant (Ichikawa et al, 1997).

The latest studies showed that MR did not offer any definitive advantages (or was even statistically worse) in the diagnostic evaluation of pancreatic cancer over technically optimal dual-phase spiral CT (Sheridan et al, 1999, Nishiharu et al, 1999). In addition, MR is much more time-consuming and difficult to interpret than CT. MR may be however, valuable in differentiating between chronic pancreatitis and cancer in the patients with isoenhancing enlargement of the pancreas (Semelka and Ascher, 1993) and is useful in case of iodine allergy. MR has been postulated to be an alternative when CT has not solved queries regarding detecting or preoperative assessment of a pancreatic neoplasm, however no study has proved that these examinations are truly complementary rather than do no more than duplicate the findings.

Magnetic resonance cholangiopancreatography (MRCP) is a relatively recently developed application of MR technology for imaging the biliary and pancreatic ducts. MRCP is a fast, non-invasive modality, which visualises fluid-filled structures without the need for administration of a contrast medium. MRCP provides similar (or better) than endoscopic retrograde cholangiopancreatography (ERCP) description of the biliary anatomy and in virtually 100% of cases shows the site of biliary obstruction (Varghese et al, 1999a, Lee et al, 1997, Macaulay et al, 1995). The overall sensitivity, specificity and diagnostic accuracy of MRCP for the various biliary tree diseases were 97%-100% including patients in whom ERCP failed or was inadequate (Varghese et al, 1999b). MRCP has a diagnostic value equal to ERCP (Lee et al, 1997), close to 100% success rate for performing technically adequate examination and carries no complications.
These features favourably judge MRCP over ERCP and suggest that in the future MRCP may replace ERCP for the diagnostic evaluation of patients with suspected pancreatobiliary diseases. However, further studies especially concerned with pancreatic malignancies are warranted.

ERCP is used as the initial diagnostic and therapeutic procedure for most patients with pancreatic cancer, who present with obstructive jaundice. However, in fact the role of ERCP for diagnosis and staging of pancreatic cancer has diminished considerably. ERCP usually accurately distinguishes malignant from benign pancreatobiliary obstruction, shows the site of the stricture and sometimes facilitates obtaining a tissue diagnosis. However, ERCP can not provide any additional information when the pancreatic tumour has been properly evaluated by spiral CT.

In particular, patients who are candidates for curative surgery should not undergo ERCP due to the risk of cholangitis and pancreatitis associated with the procedure, which may even exclude further operation and increases the risk of perioperative infection (Gloor et al, 1997). In summary, ERCP is advocated for patients with cholangitis to relieve biliary obstruction, with no mass or equivocal findings on CT or jaundiced patients with inoperable disease for palliative placement of a biliary stent. The icteric patients, who qualify for curative resection should not have preoperative biliary drainage unless the surgery is delayed for more than several weeks, in which case endoscopic placement of a plastic stent is recommended (DiMagno et al, 1999).

Positron emission tomography (PET) is a new, non-invasive imaging technique, that evaluates tissues on the basis of their intracellular (most commonly glucose) metabolism.

PET has considerable potential for the differentiation of benign from malignant diseases. In a very recent study based on 48 patients with pancreatic cancer or pancreatitis, PET diagnosed pancreatic cancer with 100% sensitivity and 97% specificity and chronic pancreatitis with 96% specificity and 100% sensitivity as correlated with the histopathological diagnosis. PET also correctly assessed control cases with the exception of patients with acute pancreatitis (Imdahl et al, 1999). In another study liver metastases were detected by PET with 90% accuracy with two false positives and one false negative (Nakamoto et al, 1999). In future PET may be especially useful for the final evaluation of lesions the undetermined by other tests and detection of distant metastases.
**Biopsy**

Tissue diagnosis of pancreatic cancer can be obtained by percutaneous fine-needle cytology or core needle biopsy under ultrasound, CT or EUS guidance. Percutaneous FNA cytology has 57-96% sensitivity and close to 100% specificity (Warshaw and Fernandez-del Castillo, 1992). Complications are rare (1%), however, pancreatitis, pseudocyst, pancreatic cysts, abscesses and even procedure-related deaths have been reported. (Moossa and Gamagami, 1995). Also cancer cells seeding along the needle tract is probably very rare but is a possible adverse event (Ferruci et al, 1979).

Histopathological confirmation is vital for patients with inoperable disease. Before any palliative treatment may be applied, it is necessary to exclude curable diseases like tuberculosis, sarcoidosis, lymphoma and rare pancreatic neoplasms like islet cell carcinoma, often associated with much better prognosis and requiring a different therapeutic approach (Warshaw and Fernandez-del Castillo, 1992).

However, tissue diagnosis is not necessary before planned potentially curative surgery (Gloor et al, 1997). The result of the biopsy will not usually affect the decision about surgery as only a positive biopsy has diagnostic value. Otherwise the sampling error, which could be especially high for small operable cancers, can not be excluded. On the other hand, complications of the biopsy that include the possibility of peritoneal seeding along the needle tract and sever pancreatitis, however uncommon if it occurs may reduce the patient’s chances for cure. Therefore, pancreateoduodenectomy is normally performed only on the basis of a typical patient medical history and results of imaging tests, in all centres, which have low operative mortality rate.

**Surgical exploration**

Despite careful diagnostic and staging evaluation, some patients deemed to be operable on the basis of the imaging examinations have small peritoneal and/or liver metastases, which could not be detected other than by direct inspection. Therefore, surgical exploration, preferably by laparoscopy was advocated as the last preoperative test (Warshaw and Fernandez-del Castillo, 1992). Such practice seemed to be fully reasonable in the past, when owing to limited accuracy of the staging techniques, up to 40% of patients assessed to be resectable had in fact small metastases precluding curative surgery.
However, at the present, thanks to significant advances in imaging modalities, this group of patients has shrunk to only approximately 12%. It is so believed that it is not justified to perform laparotomy or even less invasive laparoscopy in all patients to spare further surgery only in a small fraction of patients. Surgical exploration is however, recommended as the last proof of unresectability in patients with advanced disease without the final histological confirmation (Gloor et al, 1997, DiMagno et al, 1999).

**Tumour markers**

Several substances: hormones, tumour antigens and enzymes have been proposed as a tumour marker of pancreatic cancer. Unfortunately all of them suffer from low sensitivity and specificity for pancreatic malignancy (Warshaw and Frenandez-del Castillo, 1992). CA19-9, a tumour antigen associated with the Lewis blood group, is the most clinically useful marker. However, owing to poor sensitivity, which does not exceed 50% for small, early tumours (Birk et al, 1998) it is not suitable for screening purposes. CA19-9 is also not specific for pancreatic cancer as an elevated level of CA19-9 has been found in other malignancies (mainly biliary) and also benign pancreatobiliary diseases, especially associated with jaundice. Probably the best correlation is between CA19-9 level and treatment response and therefore CA19-9 has may be useful as a follow-up test such as predictor of recurrence following surgery (Barkin and Goldstein, 1999) or to monitor the effect of chemo and/or radiotherapy (Willett et al, 1996). Its role however, is still not widely accepted.

**Summary**

Dual –phase spiral CT is recommended as the test of choice to detect and assess the stage of pancreatic cancer. Other examinations are usually not required. EUS is advocated to be used to search for small lesions, not seen by CT and coupled with FNA to obtain a tissue diagnosis in inoperable cases. ERCP has a more therapeutic than diagnostic role for pancreatic cancer as the less invasive examinations (CT, EUS, and MRCP) have equal or much higher diagnostic value. Laparoscopy is limited to providing the definitive proof of unresectability for patients with probably inoperable but histologically not confirmed cancer.

Tissue diagnosis is not required before planned curative surgery but is obligatory for patients with inoperable disease.
Serum CA19-9 is the most useful tumour marker, however the unsatisfactory sensitivity and specificity grossly limit its clinical value. CA19-9 is usually used to monitor treatment response.

1.3 Treatment

1.3.1 Surgical resection

Resectability

Surgical resection is the only chance for cure or substantial prolongation of the disease-free survival for patients with pancreatic cancer. Unfortunately, only a minority is amenable for potentially curative operation. The resectability rates were from 11% to 23% in the seven large (each included more than 500 patients) hospital series. Similar resections rates; 17.5% and 20%, were calculated from the data collected from 37 studies published from 1951 to 1989 (Michelassi et al, 1989) and 12 studies published between 1990 to 1994. Some discrepancies observed between studies may have resulted from the different resection criteria or patient selection. Nevertheless, the number of procedures performed in the specialist pancreatic units does not seem to represent a real resection rate of pancreatic cancer for an entirely unselected population. In a large epidemiological study from the West Midlands of nearly 14000 cases of pancreatic cancer, only 2.6% of patients underwent resection with curative intent (Bramhall et al, 1995).

A similarly low number (4.6%) of attempted curative surgeries was recently reported in the Swedish population study, which reviewed all cases of pancreatic cancer recorded from 1977 to 1991 in the local tumour registry (Hedberg et al, 1998). Differences between resection rates in the institutional and epidemiological studies are mainly due to patient selection in the specialist centres and (probably to lesser degree) owing to a possibly more conservative approach to pancreatic resection in the local hospitals.

The resection rate increased from 1.9% in the 1977-1984 (5 of 261) to 6.7% (19 of 314) in 1985-1991 in the Swedish study but remained unchanged over forty years (1952-1987) in the British series.
The slight increase of the operability rate in the Swedish survey could be obtained by the introduction of new imaging techniques and a more aggressive attitude to resection surgery in the last decade. However, it is important to emphasize that the low resection rate reflects the late clinical presentation of pancreatic cancer when the overwhelming majority of patients have inoperable, metastatic disease. Unless efficient screening tests are available, the resection rate for pancreatic cancer will remain under 10%.

**Types of pancreatic resection**

The classical, standard operation for pancreatic cancer is the Kausch-Whipple pancreaticoduodenectomy (PD). It consists of resection of the antrum, gall bladder with distal common bile duct, duodenum with proximal jejunum, head of the pancreas and lymph nodes situated intimately around the pancreatic head and along the right side of the distal common bile duct (Pitt, 1995).

Reconstructive pancreatico-jejunostomy or -gastrostomy, hepaticojejunoanostomy and gastrojejunostomy may be performed using several different anastomosis techniques (Pitt, 1995).

The modification of the standard Whipple procedure is the pylorus-preserving pancreaticoduodenectomy (PPPD), which leaves the whole stomach, pylorus and proximal part of the duodenum, restoring the gastrointestinal tract by duodenomejunostomy. This technique is considered to be more physiological and was introduced in an attempt to reduce postoperative malnutrition and dumping syndrome. However, whether postoperative nutritional status is really improved following PPPD comparing with standard PD remains unclear (DiMagno et al, 1999). Moreover, postoperative delayed gastric emptying seems to be more frequent after a pylorus-preserving resection. Mortality rate, operative time and blood loss are equal for both procedures (Lin and Lin, 1999, Roder et al, 1992), but it is claimed that PPPD is associated with lower incidence of marginal ulcers and enterogastric reflux (Pitt, 1995).

PPPD is a less extensive operation and obviously not suitable for tumours infiltrating the prepyloric region.

In addition, perigastric and retropyloric lymph nodes are not removed, so doubts concerning the oncological radicality of PPPD can be raised.
Although there are no large prospective randomised studies comparing long term outcome following PPPD with standard PD for pancreatic cancer, in all surgical series but one (Roder et al, 1992) survival time was comparable and definitely not adversely affected by the PPPD resection (Yeo et al, 1995, Trede et al, 1990).

PPPD is currently thought to be the resection of choice if it is technically possible and ensures adequate resection margins.

To improve the survival rates some surgeons have advocated more extensive operations with wide excision of the peripancreatic, retroperitoneal tissue and extended dissection of the lymph nodes. Also resection of the segment of the portal vein or superior mesenteric vein with subsequent venous reconstruction is required usually for the en-block radical pancreaticoduodenectomy. Some, mainly retrospective, analysis showed a survival advantage following radical surgery, but most studies did not demonstrate any significant differences in the survival time between patients undergoing radical or standard type of resections (McGarth et al, 1996).

Pancreaticoduodenectomy with venous resection and segmental reconstruction however, is the only option for potentially curative surgery for patients with short encasement of the superior mesenteric-portal vein confluence. This procedure has been shown recently, to be associated with similar survival time to standard pancreatectomy and not to increase the operative mortality rate. In the study by Leach et al, the median survival of patients who required venous reconstruction was 22 months comparing to 20 months for patients who underwent standard PD. There were no perioperative deaths in either group, however two patients died later than 6 months following surgery due to occlusion of the reconstructed vessel (Leach et al, 1998). Nevertheless, PD with vessel reconstruction offers a relatively safe tumour resection and substantial prolongation of the survival for this group of patients, normally deemed as inoperable.

Total pancreatectomy carries a higher risk of postoperative complications than partial PD and causes very brittle and difficult to manage diabetes (Pitt, 1995).

Like the other radical resections, total pancreatectomy also does not bring any survival benefit and is rather reserved for the removal of tumours arising from the body of the pancreas or cancers, which extensively involve the whole gland.
Mortality and morbidity

Mortality following potentially curative surgery for pancreatic cancer used to be high. A review of the 37 surgical studies published from 1951 to 1987, in which a total of 930 resections were performed, revealed 20% overall postoperative death rate (range, 0% to 54%) (Michelassi et al, 1987). Similarly, a 27.6% rate of 30-day mortality associated with pancreatic cancer operations was found in the large epidemiological survey, which included all cases of pancreatic cancer treated from 1977 to 1986 in the West Midlands (Bramhall et al, 1995). However during the past two decades the in-hospital mortality following pancreatic cancer surgery has been dramatically reduced. Large analyses of the outcome of pancreatic resection in the two different centres over 40 (Michelassi et al, 1987) and 20 years (Sperti et al, 1996) showed a substantial decrease of the mortality rate from over 20% before 1981 to 5% after that date. In four other large multi-institutional surveys the overall or 30-day postoperative death rates were between 7.8% to 10.7% for pancreatitectomies performed in the 80's and the first half of the 90's. Even lower fatality rates; usually less than 5% were registered in the series from the specialist pancreatic centres during the same time period (Yeo et al, 1995). Observation that the best surgical outcome is achieved in the specialist units has been confirmed by the epidemiological surveys.

In the Canadian, population based retrospective study, which examined mortality rate following pancreatic resection for neoplasm from 1988 to 1995, in-hospital death rate was only 3.4% in the high-volume centres, which performed at least 6 procedures per year and 11.3% for units with a lower caseload (Simunovic et al, 1999). A similar pattern was found in the British analysis comparing mortality rate reported from specialist units with results published in general surveys (4.8% versus 9.8% mortality rate) (Neoptolemos et al, 1997). Currently, it is estimated that pancreatic resections mortality rates are less than 2% in experienced hands (Yeo et al, 1995, DiMagno et al, 1999).

Morbidity after resection for pancreatic cancer remains significant even in the world-leading institutions. 33% - 52% complication rates have been reported from the Mayo Clinic (Nitecki et al, 1995) and John Hopkins Hospital (Cameron et al, 1993).

**Survival**

The comprehensive review of 37000 cases of pancreatic adenocarcinoma reported in the literature over the 50 years up to 1986, revealed only 3.5% rate of 5-years survival following pancreatic resection and 0.4% for overall 5-years survival rate (Gudjonsson, 1987). More recent series, however, reported 5-years post resection survival rates approaching 25% (Geer et al, 1993, Trede et al, 1990), suggesting a significantly better outcome of pancreatic cancer surgery. In many studies however, high values of the 5-years survival seem to be more the result of confusion than a real improvement.

Currently, many authors calculate the actuarial 5-years survival, which does not reflect the real number of 5-years survivors and should not be compared with the previously reported actual survival rates. Yeo and associates stated 21% actuarial 5 years survival but in fact only 11 of 201 (5.47%) patients who underwent resection lived longer than 5 years (Yeo et al, 1995). In a review of 15 studies published from 1990 to 1994, only 77 of 2055 operated on patients survived 5 years, which gave an actual survival rate of 4%, similar as reported by Gudjonsson. However, actuarial survival in these separate studies ranged from 0 to up 24% (Sperti et al, 1996).

Long-term survivors with falsely positive diagnosis of ductal adenocarcinoma can also significantly inflate survival time (Nitecki et al, 1995). Histopathological revision lead to reclassification and exclusion from the further analysis approximately of 50% of the patients who lived longer than 5-years in the three surgical series from the referral centres including the Mayo Clinic (Nitecki et al, 1995, Conlon et al, 1996, Connoly et al, 1987). Therefore, pathologic confirmation of the tumour type in all long-term survivors should be obligatory practice before reporting survival rates following resection for pancreatic adenocarcinoma.

Methodological incorrectness like excluding “palliative” resections (Klinkebijl et al, 1993) and patients who died postoperatively from the survival statistic (Fortner et al, 1996, Trede et al, 1990) can be also misleading.
Finally, patient selection and resection of only the “favourable” cases may strongly affect the survival rates.

Median survival following pancreatic resection in the studies published in the 1990’s, which had pathological re-assessment of the long-term survivors was 14.3, 15.5, 17.5 and 18 months respectively (Nitecki et al, 1995, Yeo et al, 1995, Conlon et al, 1996, Geer et al, 1993) and actual 5 years survival approached 10% (Conlon et al, 1996, Sperti et al, 1996). Therefore, some limited progress could be made, especially as the improvement in the survival from decade to decade was reported both in the single-institution study (Yeo et al, 1995) and in the large epidemiological survey (Bramhall, et al, 1995).

It is important, however, to emphasise that as for many other malignancies, pancreatic cancer 5-years survival does not mean a cure. Probably more than 50% of the patients who pass the 5 years mark die eventually of cancer recurrence (Trede et al, 1990, Conlon et al, 1996, Connoly et al, 1987). Therefore, at the moment surgery offers so slim a chance for cure that it should be considered more as palliative than radical treatment, and furthermore, is available only for small fraction of patients suffering from pancreatic cancer.

**Prognostic factors**

Although the overall results of potentially curative surgery are poor, a number of variables have been evaluated to identify factors predicting a better outcome of pancreatic resection.

Perioperative factors such as type of resection, estimated blood loss, volume of transfused blood and operative time were found not to have a meaningful impact on survival (Yeo et al, 1995). Instead, stage of the disease and biology of the tumour are the most important determinants of the prognosis.

In the multivariate analysis of 201 pancreatic resections, tumour diameter less than 3cm, no lymph node metastases, negative resection margins and diploid tumour DNA content were the strongest, independent factors associated with long-term survival (Yeo et al, 1995). Patients who underwent pathologically radical resections had significantly longer median survival and higher 5-years survival rates than patients, with positive resection margins (18 months versus 10 months and 26% versus 8%, respectively).
Lymph nodes and resection margins free of cancer both ensured the best outcome with median survival of 32 months and 40% likelihood of 5 years survival. Other studies have also demonstrated the worsened prognosis associated with incomplete resection, lymph node metastasis and extensive local cancer invasion (Allema et al, 1995, Nitecki et al, 1995, Trede et al, 1990, Wade et al, 1995). Tumour size has a high predictive value (Geer et al, 1993, Fortner et al, 1996) but is a significantly less important indicator of the outcome than the aforementioned factors (Birk et al, 1998).

Thus, 50% of tumours smaller than 2cm are estimated to invade retroperitoneum or lymph nodes at the time of surgery (Tsuchiya et al, 1986) and seem not to have a better prognosis than larger lesions of the same stage.

As in fact, all of the previously discussed factors reflect the stage of the disease, there is a positive correlation between TNM stage of the tumour and prognosis following surgery. The mean survival is 20 months for stage I-II cancers and 14 months for stage III disease after attempted curative resection. Interestingly, differences in the survival between stages are not large for the unresected tumours; the mean survival was found to be 8 months for stage I-II cancers and 6 months for stage III malignancies (Kokoska et al, 1998).

However, long-term survival, probably depends most strongly on biology of the tumour, which is currently poorly understood. Pathological review of the 5-years survivors demonstrated that surprisingly many of them had advanced disease (lymph node metastases, large size of the tumours, extrapancreatic, perineural and lymphatic invasion) (Connolly et al, 1987, Conlon et al, 1996, Fortner et al, 1996). DNA content but rather not histological differentiation of the cancer, may to some degree reflect the biological nature of the tumour and therefore, was shown to have the highest predictive value in multivariate analysis (Yeo et al, 1995, Bottger et al, 1994).

In summary, the classic oncological rule that cancer prognosis is associated with the stage of the disease and the radicality of the treatment is true also for pancreatic cancer. However, unpredictable biologic behaviour of individual tumours may be one of the most significant factors influencing long-term survival.
1.3.2. Chemotherapy for inoperable pancreatic cancer

Pancreatic cancer is considered to be highly resistant to chemotherapy and therefore all chemotherapy treatments have only very moderate palliative potential at their best. The maximum objective response rate to chemotherapy does not exceed 20% and no single agent or combination regimens provide a meaningful impact on the median survival. Chemotherapy, however, may in a moderate and usually brief way palliate symptoms of the cancer (e.g. pain) or delay their onset, thus significantly improving quality of life. Importantly, symptomatic relief may be achieved even in patients who do not fulfil the criteria for an objective response.

5-fluorouracil (5-FU) is the most extensively studied agent, whose moderate antitumour activity for gastrointestinal malignancies is well documented. However, 5-FU was found to be less effective for pancreatic cancer than for other solid tumours of the gastrointestinal tract. An overall response rate of 28% to a bolus of 5-FU was calculated following analysis of over 200 patients treated in several trials in the 1960’s and 1970’s. This relatively high figure, however, resulted from substantial over estimation owing to the evaluation of response by clinical criteria. The response rate based on the more precise (but still not ideal method, due to difficulties in measuring accurate tumour dimensions without surrounding desmoplastic tissue) assessment of lesions size by imaging techniques was found to be much lower (Cullinan et al, 1990). Currently, it is thought, that the real objective response rate to 5-FU is probably below 10% (Ahlgren, 1996b).

Median survival also does not seem to be significantly (if at all) prolonged by 5-FU chemotherapy, being disappointingly no longer than 5 months (Cullinan et al, 1990). Addition of biochemical modulators like leucovorin, alpha-interferon and PALA (N-(phosphonacetyl)-L- aspartic acid) did not result in the increase of the response rate and effectiveness of 5-FU, opposite to that observed in colorectal cancer (Schnall and Macdonald, 1996).

Several combination regimes based mainly on 5-FU and mitomycin were evaluated for the treatment of pancreatic carcinoma. The most widely used include FAM (5-FU, adriamycin, mitomycin), SMF (streptozotocin, mitomycin, 5-FU) and the Mallinson regimen (5-FU, mitomycin, methotrexate, vincristine, cyclophosphamide).
Early single institution studies sometimes demonstrated encouraging results for combination therapy with the response rate as high as 40% (Ahlgren, 1996b), however all regimens failed to reproduce a similar outcome in larger, randomised studies.

A comprehensive review of all the single-agent and combination regimen randomised studies from 1978 by DiMagno clearly shows that no combination treatment is superior to monotherapy with 5-FU and all of them (including 5-FU) have probably the same minimal impact on survival (DiMagno et al, 1999).

As the efficacy of all the studied agents and chemotherapy regimes with respect to survival benefit is marginal if not doubtful, an improvement of the quality of life may be a more realistic and noteworthy goal for the chemotherapy treatment of pancreatic carcinoma.

Gemcitabine was the first drug approved by the FDA for the treatment of locally advanced and metastatic pancreatic carcinoma, based only on its moderate ability to relieve cancer related symptoms.

Antitumour activity of gemcitabine for pancreatic cancer was equivalent to that exhibited by 5-FU with objective response rates of 6.3%, 11%, respectively, and median survival time of 6.3, 5.6 months as evaluated in two phase II trials (Casper et al, 1994, Carmichael et al, 1996). However, significantly more patients (approximately 25% of total) than those fulfilling the objective response criteria, experienced detectable improvement in the disease–related symptoms. In addition, gemcitabine was well tolerated; dose dependent neutropenia, thrombocytopenia, flu-like symptoms, transient nausea and vomiting were rare and usually mild.

In further studies a clinical benefit response expressed as the marked, sustained improvement in at least one of the following: pain intensity, performance status, analgesic consumption or weight gain was established as a novel endpoint. In a study including 63 patients in whom 5-FU had previously failed, 27% of them experienced clinical benefit (Moore, 1996).

Compared with 5-FU in a randomised study, gemcitabine was significantly superior to 5-FU with regard to symptoms alleviation (23.8% clinical benefit response in the gemcitabine group versus 4.8% in 5-FU arm) and produced also a slight but statistically significant increase of the median survival time (5.7 versus 4.4 months) (Burris et al, 1997). An investigational new drug treatment program finally confirmed the efficacy of gemcitabine to palliate symptoms of pancreatic cancer.
In this large study, 18.4% of 3023 patients with locally advanced or metastatic pancreatic cancer experienced improvement in the disease-related symptoms (Storniolo et al, 1999). However, the objective response rate was still only 12% and the median survival time remained short (4.8 months, 15% of 12-month survival) and not really better than for any other chemotherapy regimes. These findings clearly indicate that gemcitabine has significant potential for symptomatic relief but rather does not produce any meaningful survival advantage for the majority of patients. At the moment however, gemcitabine is one of the few noteworthy options for the treatment of patients with unresectable pancreatic cancer and even for patients with poor performance status and/or pain. Chemotherapy with gemcitabine may be also a standard, control arm for future investigational studies (DiMagno et al, 1999).

1.3.3 Radiotherapy and combination therapy for inoperable pancreatic cancer

Pancreatic cancer was believed to be highly unresponsive to radiotherapy. However, a small, early study from Duke University showed some survival advantage achieved by radiotherapy compared with untreated patients. The next randomised study from the Mayo Clinic demonstrated promising results obtained by the use of radiotherapy with concomitant administration of 5-FU. The median survival of patients who received radiation and chemotherapy with 5-FU was substantially longer than in the radiotherapy and placebo group (10.4 versus 6.3 months respectively) (Thomas, 1996). Subsequent trials conducted by the Gastrointestinal Tumor Study Group (GITSG) tried to determine the efficacy of radiotherapy and the optimal treatment protocol for the management of pancreatic cancer. The first randomised study of GITSG showed the superiority of the combined modality therapy (radiotherapy plus 5-FU) to radiotherapy alone. Median survival was nearly double in the chemoradiation group compared to that produced by radiation only (42 versus 23 weeks) (Moertel et al, 1981). However, toxicity of the treatment was also increased in the combined modality arm. Other regimes, which included chemoradiation with doxorubicin (GITSG, 1985) or hyperfractionation radiation along with administration of SMF chemotherapy (Seydel et al, 1990), failed to improve results over chemoradiation with 5-FU and carried significantly higher morbidity.
Surprisingly, the Eastern Cooperative Oncology Group (ECOG) randomised study demonstrated no differences in the median survival produced by 5-FU alone and in combination with radiotherapy (8.3 months for chemoradiation treated patients and 8.2 for 5-FU only treated group), with double the toxicity rate in the combined modality group (Klaassen et al, 1985). The next GITSG trial, however, showed that statistically more patients receiving radiotherapy and SMF based chemotherapy remained alive at 1 year compared with patients receiving only chemotherapy (41% versus 19%, respectively) and clearly proved the advantage of the combined radio and chemotherapy over chemotherapy alone (GITSG, 1988). At the moment it is thought that chemoradiation with 5-FU is a therapeutic option for patients with inoperable pancreatic cancer.

The overall benefit of the treatment is however, questionable, as it is uncertain if the survival benefit really outweighs toxicity of the treatment and the burden of the frequent hospital visits.

### 1.3.4 Adjuvant therapy

Adjuvant therapy applied before or after potentially curative surgery improves survival and cure rates for many malignancies and is often a standard practice. However, the value of adjuvant treatment for pancreatic cancer is unproven.

A small GITSG randomised study showed encouraging results of adjuvant chemoradiotherapy with 5-FU. The median survival of patients who additionally received chemoradiation following attempted curative resection was 20 months compared with 11 months for the surgery only patients. Also experience from the John Hopkins Hospital suggested statistically improved long-term survival for patients who were chemoradiated after resection of pancreatic cancer (Yeo et al, 1995). However, results of a recently completed randomised trial, which evaluated effectiveness of adjuvant radio and chemotherapy were disappointing. Post operative irradiation with chemotherapy produced only small, statistically not significant survival benefit compared with resection only without any impact on the 2-years survival and locoregional recurrence rates (24.5 months median survival of the combined modality group versus 19 months for the resected only patients, p=0.09) (Klinkenbijl et al, 1999).
The results of the ongoing large European randomised study will probably finally establish the role of the adjuvant radiotherapy plus chemotherapy for the management of pancreatic cancer.

Neoadjuvant chemoradiation carries equal survival and morbidity rate to postoperative adjuvant treatment.

However, as 20% of patients do not receive post operative adjuvant therapy due to complications or delayed recovery from the surgery, delivery of the adjuvant treatment before surgery may ensure completing adjuvant treatment in all cases. (Spitz et al, 1997). On the other hand up to 18% of deemed to be resectable patients may continue local tumour development or progress with metastases during preoperative chemoradiation and thus, lose a chance for potentially curative surgery (Hoffman et al, 1998, Evans et al, 1992, Wanebo et al, 2000). Some reports have suggested, however, that chemoradiation pathologically downstaged (Wanebo et al, 2000) and even enabled resection of 12% (2 of 16) of patients with locally advanced inoperable disease (Jessup et al, 1993). The survival benefit of this practice is uncertain and a high rate of fatal complications was observed following resection of the advanced, preirradiated cancers (Wanebo et al, 2000, Hoffman et al, 1998).

The results of IORT, interstitial irradiation and radiotherapy with a number of radiosensitisers including misonidazol and hycanthone for the treatment of pancreatic cancer were not encouraging (Thomas, 1996).

Preoperative or postoperative adjuvant therapy was carefully recommended by the AGA (DiMagno et al, 1999) but the survival benefit produced by such an approach is still questionable. Clearly, more studies are necessary to evaluate the real potential of adjuvant therapy for pancreatic cancer and draw a final conclusion.

1.3. 5 Palliative approach to pancreatic cancer

As pancreatic cancer is rarely suitable for potentially curative resection, adequate palliative treatment, which alleviates symptoms of the disease and improves the quality of life, is the primary goal of medical care for the vast majority of patients with pancreatic neoplasms.

Obstructive jaundice, which is present in at least 50% of patients with pancreatic carcinoma, remains the main symptom requiring treatment.
Palliation of obstructive jaundice prevents progressive cholestatic injury of the liver, cholangitis, malabsorption and treats unbearable pruritus and therefore precludes premature death and brings often dramatic albeit short improvement of the quality of life. Decompression of the biliary system can be obtained by three ways: percutaneous transhepatic drainage (PTD) or endoprosthesis (PTE), endoscopic biliary stenting and surgical bypass.

Endoscopic insertion of a biliary stent was found to be a more successful and safer approach compared to percutaneous transhepatic stent placement in a prospective, randomised trial (Speer et al, 1987). In this study, adequate biliary drainage was achieved in 81% of patients following endoscopic treatment and in only 61% in the PTE group (p=0.017). PTE treatment was also associated with significantly higher 30-day mortality (33% for PTE versus 15% for endoscopic stenting) and complications rate (67% versus 19%, respectively). Therefore, as the percutaneous method for relieving malignant biliary obstruction is less effective and carries a substantial risk of fatal complications it should be performed only if endoscopic stent placement failed or is not possible due to altered anatomy of the duodenum.

Decompression of the biliary system can be also obtained by performing a surgical bypass (preferably by choledochojejunostomy), which ensures adequate biliary drainage in over 90% of cases (Watanapa and Williamson, 1992). In addition, biliary bypass can be combined with gastroenetrostomy to prevent (or to relieve) gastric outlet obstruction and with chemical splanchincectomy, which provides substantial pain relief (Lillemoe and Pitt, 1996).

However, many patients are too frail to undergo surgical management and the overall 12% (range, 0-31%) perioperative mortality of surgical biliary bypass is considerable. Also a long hospital stay (median 17 days) and nearly 50% complication rate are the other serious drawbacks of surgery (Watanapa and Williamson, 1992). Therefore, non-operative, endoscopic techniques are a valuable alternative. In a large randomised prospective study comparing endoscopic stenting with surgical bypass for the treatment of malignant biliary obstruction, the initial success rate for the relief of jaundice was equally high in both groups (92%) (Smith et al, 1994).
However, endoscopic biliary decompression carried a significantly lower procedure related mortality rate (3% versus 10%), caused fewer complications (11% versus 29%) and required shorter hospitalisation.

The major disadvantage of the biliary prosthesis appeared to be stent clogging, which was the reason for recurrent jaundice in 36% of patients in the endoscopic treatment group. To compare, recurrent extrahepatic jaundice caused by peritoneal metastases was present only in 2% of patients, who underwent surgical bypass. Also in the stenting group, 19% of patients developed late symptomatic duodenal stenosis, which was largely avoided in the surgical group by performing a prophylactic gastric bypass. In conclusion, the study of Smith et al clearly shows that none of the techniques have a significant superiority. Both methods are excellent for the initial relief of jaundice but endoscopic treatment is associated with lower procedure-related mortality, morbidity and requires shorter hospitalisation; surgical bypass however, provides more permanent palliation. In practice, therefore fitter, younger patients with longer anticipated survival are often referred for double bypass surgery to avoid further stent changes, duodenal obstruction and repeated hospital admissions.

For elderly, with significant comorbidity or/and shorter life expectancy, the endoscopic method is the procedure of choice (Crinnon and Williamson, 1997). It seems, however, that non-operative methods of palliation are gaining increasing popularity, especially as self expanding metal biliary stents may substantially reduce the incidence of recurrent jaundice and decrease the number of further endoscopic procedures (Davids et al, 1992). Expandable metal stents, owing to their large diameter, have considerably longer median patency than plastic prostheses (8.2-9 months versus 4 months) (Davids et al, 1992, O'Brien et al, 1995) and therefore stent dysfunction occurs less quickly. However, due to the much higher cost of the metal stent comparing to plastic alternative (2000£ versus 45£ respectively) insertion of the metal stent is the most economic option only for patients who are expected to live at least 6 months.

Development of minimally invasive, laparoscopic techniques for performing biliary and gastric bypass promises further progress in the surgical palliation of pancreatic cancer. In a small study, laparoscopic hepaticojejunostomy or/and gastroenterostomy was extremely well tolerated and associated with low mortality, complications rate and hospitalisation period (Rhodes et al, 1995).
A prospective, randomised assessment of laparoscopic surgery is warranted to establish its role in the palliation of pancreatic cancer.

Obstruction of the duodenum due to tumour invasion is rare at presentation and initially affects only 5% of patients with pancreatic cancer.

As the disease progresses however, up to 17% (range, 4 - 44%) of patients who undergo biliary bypass alone, develop duodenal stenosis, which requires medical treatment (Watanapa and Williamson, 1992). The incidence of gastric outflow obstruction is even higher (50%) for patients who survive one year (Gudjonsson, 1987).

Mortality associated with a second bypass operation is substantial and approaches 25% (Watanapa and Williamson, 1992) but is not increased if gastroentenrostomy is added to the initial biliary bypass and remains not higher than 15% (Watanapa and Williamson, 1992). Therefore many surgeons advocate combining biliary bypass with a prophylactic gastrojejunostomy (Crinnion and Williamson, 1997).

Some authors however, do not favour double bypass procedure as delayed gastric emptying (usually transient) was observed in up to 20%of patients following surgery. Gastrointestinal bleeding due to anastomosis ulceration was also reported (Lillemoe and Pitt, 1996).

Endoscopic techniques for relieving malignant duodenal obstruction are in the early stage of evaluation. Preliminary reports showed that insertion of a duodenal self-expanding metal stent was a safe and reasonably effective approach. In small series, most of the endoscopically treated patients were able to commence a normal or pureed diet with no mortality and minimal morbidity associated with the procedure (Nevitt et al, 1998, Soitenko et al, 1998). However, in the opinion of some authors, metal stents are still highly imperfect, being too short and rigid to pass natural or stenotic curvatures (Nevitt et al, 1998).

It is important to mention that as many as 60% of patients with pancreatic cancer have delayed gastric emptying and one third of them experiences nausea and vomiting without evidence of any mechanical obstruction in the gastrointestinal tract (Barkin et al, 1986). Gastric dysmotility due to cancer neuropathy may explain this phenomena. It is vital, however, to identify this group of patients before any invasive treatment is planned because surgery or endoscopic therapy will not improve the symptoms of gastric outlet obstruction. Prokinetic drugs may be beneficial, but vomiting is usually unresponsive to any treatment (DiMagno et al, 1999).
Progressive and often unbearable pancreatic pain is experienced by the vast majority of patients and its relief has a major impact on improving their quality of life. Typically, the pain is managed by high doses of opiates given on a regular basis. Significant reduction of the visceral pain in particular is also achieved by chemical ablation of the celiac ganglia, which can be performed during surgery (Lillemoe and Pitt, 1996), percutaneously under fluoroscopic or CT guidance (Polati et al, 1998) or using EUS (Hawes et al, 2000). Radiotherapy can also help to control pancreatic pain and this option is recommended for patients who experience recurrent, intractable pain following chemical coeliac plexus block (Crinnion and Williamson, 1997). Pancreatic exocrine insufficiency, which typically manifests as diarrhoea/steatorrhoea and may contribute to the weight loss and malnutrition, should be treated with high doses of the pancreatic enzymes. To correct steatorrhoea and prevent malabsorption, tablets containing a total of at best 30,000 IU of lipolytic activity should be taken during each meal (DiMagno et al, 1999). In summary, palliative therapy of pancreatic cancer requires a multidisciplinary approach, mainly due to the variety of disease-related symptoms. A wide range of palliative techniques is available but most of the methods are associated with considerable drawbacks and limited effectiveness. Also, no palliative treatment has any impact on the course of the disease and the survival time.
1.4 Summary

Pancreatic cancer is the fifth leading cause of cancer death in Western Europe and North America and kills 90% of patients within one year from the diagnosis. Despite development of the new imaging techniques especially spiral dual contrast CT, EUS, and MRI/ MRCP, which significantly facilitate diagnosis and reduce the need for use of the invasive examinations the resection rate remains unchanged. At the presentation, majority of patients have locally advanced or metastatic disease and only approximately 10% of patients are suitable for potentially curative surgery. However, even in this highly selected group results of the treatment are unsatisfactory. After resection, the median survival time is 13-18 months and only 10% of the resected patients survive 5 years. There is a significant progress in the reduction of the perioperative mortality, which should not exceed 2-3% in the specialist centres, however, numbers of the 5-years survivors remain very low.

Options available for inoperable patients include chemotherapy, radiotherapy and combined treatments; however, responses to these regiments are very poor. Gemcitabine, the only chemotherapy agent has been shown to produce some symptomatic relief but probably no chemotherapy drug induces any meaningful impact on the survival and course of disease. Radiotherapy alone or in combination chemotherapy produces some modest survival advantage but side effects of the treatment could be substantial.

Overall, the long-term prognosis of the disease is extremely poor and the dismal fate of the patients cannot be improved by the existing therapeutic modalities. Therefore, there is an urgent need for the development of the new minimally invasive treatments, which could have a significant impact on survival time and/or quality of life.
Chapter II. Principles of photodynamic therapy

2.1 Historical review

Therapeutic properties of light were recognised by ancient civilisations. 3-4000 years BC in India, light in combination with the plant *Psoralea coryforia*, (containing furocoumarins, the photoactive agent) was used for the treatment of vitiligo (the similar technique known as PUVA (psoralen ultraviolet A) is employed in modern dermatology). At the same time Hippocrates recommended sunlight for building up wasted muscles and the houses of Greeks and Romans, who appreciated the benefits of the sun, often contained a *solarium*, a special room just for sunbathing (Bonnett, 1999).

Phototherapy has been used in many forms since antiquity but modern photodynamic therapy, understood as a process, which requires photosensitiser, light and oxygen began in the XX century. In 1900, a medical student, Oscar Raab discovered that acridine dye effectively killed *Paramecium* only in the presence of light. He found that dye in the dark and light alone were not toxic and observed that the photo killing effect occurred only at certain light wavelengths (Bonnett, 1999). Raab’s supervisor Professor Hermann von Tappeiner and his co-workers further investigated this phenomenon and in 1904 Tappeiner with Jodlbauer discovered that oxygen was essential for this process. They introduced a term “Photodynamische Wirkung” (photodynamic action) to describe a new oxygen-dependent photosensitised reaction in biological systems (Spikes, 1997). Over the next 30 years, several mechanisms of the photodynamic effect were proposed. In the early 1930s, Kautsky and de Bruijn suggested that photoinactivation was mediated by a reactive oxygen molecule, which resulted from the energy transfer from the light excited photosensitiser to ground state oxygen.

30 years later, Foote and others confirmed this hypothesis, demonstrating that singlet oxygen was the active product of the sensitised photoreactions (Spikes, 1997). This was further supported by the experiments performed by Weishaupt in 1976 (Weishaupt et al, 1976).
At the present, it is generally accepted that the type II reaction, which involves production of the highly reactive singlet oxygen species by the photoactivated photosensitiser, is the major mechanism of PDT (Oleinick and Evans, 1998). Clinically, PDT was used for the first time in 1903 by von Tappeiner and Jesionek, who used photodynamic technique for the treatment of several skin diseases, including cancer. They used topical eosin as a photosensitiser but the development of therapeutic PDT is closely correlated with the history of haemotoporphyrins, another group of photosensitising agents.

Haematoporphyrins were isolated by Scherer in 1884 and first observations on their photosensitising properties were made by Hausmann in 1908, who reported hematoporphyrin mediated cell photokilling and cutaneous photoreactions in animals (Daniell and Hill, 1991). Later, several investigators (Policard in 1924, Figge in 1948) observed porphyrin fluorescence in cancer tissue or even PDT induced tumour necrosis like Auler and Banzer in 1942; however, these findings did not generate much interest (Bonnett, 1999). This changed dramatically in the early 1960s, when Lipson found that a new, acetic-sulphuric acid derived porphyrin (haematoporphyrin derivative-HpD) produced by Schwartz, gave excellent fluorescence in human tumours. Lipson and his colleagues saw the application of haematoporphyrin derivative mainly for tumour detection and diagnosis, however in 1966 they reported the use of this compound for the treatment of a patient with a recurrent, ulcerating breast cancer (Daniell and Hill, 1991).

The therapeutic potential of PDT was clearly demonstrated 8 years later by Diamond, who showed tumour necrosis in an experimental glioma in rats, photosensitised with haematoporphyrin and subsequently illuminated with visible light (Diamond et al, 1972).

The real breakthrough in the PDT field was, however; produced by the work of Dougherty and his colleagues who reported a cure of 48% of rats and mice with subcutaneously implanted tumours treated with HpD mediated PDT (Dougherty et al, 1975). The same group announced 3 years later, highly encouraging results of the first clinical trial on 25 patients, where various types, mainly metastatic, cutaneous or subcutaneous tumours were found to be responsive to photoinactivation (Dougherty et al, 1978).

During the next decades the initial enthusiasm evolved into a more scientific approach and much needed basic and clinical research was carried out.
Much was discovered about the mechanism of PDT, the next generation of the photosensitising agents was developed and several thousand patients were treated with PDT for a wide range of diseases. Now PDT is a recognised therapeutic modality, which has regulatory approval (using Photofrin, a more purified preparation of HpD, as a photosensitiser), for the treatment of bladder cancer in Canada and France, oesophageal cancer in Japan, USA, the Netherlands and France, lung cancer in the Netherlands, France, Japan, USA, and stomach and cervix cancers in Japan (Dougherty et al, 1998).

2.2 Mechanisms of photodynamic therapy

2.2.1 Introduction

Photodynamic therapy (PDT) is a photochemical process for producing localised tissue necrosis, which involves interaction of a photoactive agent (photosensitiser) accumulated in a targeted tissue with light of a specific wavelength corresponding to the absorption characteristics of the photosensitising drug in the presence of molecular oxygen. This combination is thought to generate a highly reactive, cytotoxic oxygen species, which destroys vital cell structures leading to cell death, whereas there is no biological effect from either light or photosensitiser applied alone. Although the principle of PDT is well established and seems to be straightforward, PDT induced tumour toxicity is a highly complicated reaction, for which the exact mechanism is still not entirely understood and defined.

The current concepts on the basics of PDT will be briefly discussed in this section of the thesis.
2.2.2 Photochemistry of photodynamic therapy

Photodynamic therapy requires a photoactive substance (photosensitiser) to absorb light (photon) of a specific wavelength matching the absorption characteristics of the photosensitising agent. On illumination, the photosensitiser is activated from a stable electronic ground state \( (S_0) \) to the lowest excited singlet state \( (S_1^*) \) (equation 1).

\[
S_0 + h\nu \rightarrow S_1^* \quad \text{absorption} \quad (1)
\]

\( S_1^* \) is the short-lived (usually less than 1µs) highly unstable structure, which undergoes intersystem crossing to form the lowest, metastable triplet state \( (^3S) \) (equation 2).

\[
S_1^* \rightarrow ^3S \quad \text{intersystem crossing} \quad (2)
\]

The efficiency of this reaction, defined as a probability of triplet state formation per photon absorbed (\( \Phi_T \) triplet state quantum yield) is 0.2-0.7 for most photosensitisers (Moan et al, 1998). Ideally for PDT, this parameter should be one, however other processes transforming the excited singlet state of the photosensitiser, like fluorescence (quantum yield 0.01-0.2), internal conversion and fluorescence quenching may also occur (equations 3-5) (all equations adapted from MacRobert et al, 1992).

\[
S_1^* \rightarrow S_0 + h\nu \quad \text{fluorescence} \quad (3)
\]

\[
S_1^* \rightarrow S_0 + \text{heat} \quad \text{internal conversion} \quad (4)
\]

\[
S_1^* + M \rightarrow S_0 + M \quad \text{fluorescence quenching} \quad (5)
\]

The triplet state of the photosensitiser has a relatively long lifetime and can undergo two types of reaction. It can transfer an electron or hydrogen atom to a biomolecule and go through a type I reaction to form free radicals or (preferably) transfer energy with molecular oxygen to generate singlet oxygen via a type II pathway (Fig. 1) (Henderson and Dougherty, 1992). Both processes lead to tissue necrosis and will be discussed below.
Photosensitiser

\[ + \]

\[ photon \]

\[ ^3S \]

Type I reaction (electron or hydrogen atom transfer)

Type II reaction (energy transfer)

free radicals

singlet oxygen

Fig. 1. Photochemistry of PDT (\(^3S\)-triplet state of photosensitiser).

**Type I reaction (free radicals hypothesis)**

The type I process of photooxygenation involves direct oxidation-reduction reactions based on hydrogen atom and/or electron transfer between the photosensitiser triplet state and a nearby suitable molecule (e.g. membrane lipid). The majority of the resultant radical or radical ions (semioxidized substrate and semireduced sensitizer) react with oxygen and generate highly reactive oxygen intermediates (\(H_2O_2\) and \(OH\)), which are powerful oxidisers of most cell biomolecules (Ochsner, 1997).

Superoxide radical anions can be also produced directly by the transfer of an electron from a photosensitiser triplet state to ground state oxygen. (Fig. 2)
Degeneration of the cell structure caused by free radicals is extensive. Superoxide radicals directly damage critical cell organelles, enzymes and proteins or alternatively promote generation of the hydroxyl radicals, which are the most toxic oxygen metabolite known. OH· has been found to initiate chain reaction causing breakdown of the cell membranes by producing the lipid radicals, lipid aloxyl radicals, lipid peroxides and lipid hydroperoxides from polyunsaturated fatty acids.

The contribution of the type I mechanism to biological damage induced by PDT remains, however, poorly established. Several experiments, which used specific radical scavengers, indicated that free radicals are produced upon photodynamic action and can play some role in PDT photokilling. Especially under anaerobic conditions, generation of free-radicals appears to be a favoured process (Ferraudi et al, 1988, Ochsner, 1997) and type I reactions can be the major mechanism of PDT induced phototoxicity. It is an important observation as hypoxia is common in rapidly growing neoplastic tissues due to inefficiently developed capillary networks and can also occur during PDT action owing to vascular shutdown and/or PDT induced oxygen consumption. The type I mechanism of phototoxicity does not seem however to be sufficient for producing satisfactory cytocidal effects on its own as it is well known that oxygen depletion significantly decreases the PDT response.

The type I mechanism seems to be also promoted when the photosensitiser is bound or in the close proximity to especially reactive macromolecular substrates (e.g. cell membrane), which increases the probability of electron transfer and thus formation of free radicals. It has been also suggested that radical type reactions may dominate over singlet oxygen species production in a hydrophilic, polar environment, although experimental data does not support these theoretical considerations (Ochsner, 1997).
In fact, most experiments showed the predominant role of the type II pathway for producing PDT necrosis (as discussed below), although the real involvement of the type I reaction in PDT remains uncertain and needs further investigation.

**Type II reaction (singlet oxygen hypothesis)**

The type II mechanism of photodynamic action involves energy transfer from the excited photosensitiser in the triplet state to molecular oxygen leading to generation of the non radical, highly reactive singlet oxygen species. Singlet oxygen has very strong oxidative properties. It reacts rapidly with electron-rich regions of many biocompounds such as unsaturated fatty acids, cholesterol and the indole moiety of aminoacids (Stewart, 1993) and thus destroys the structures of all cell organelles.

\[
\begin{align*}
^{3}S + O_{2} & \rightarrow S_{0} + ^{1}O_{2} \\
^{1}O_{2} + R & \rightarrow \text{peroxidation of lipid, guanine, histidine, tryptophan}
\end{align*}
\]

**Fig.3.** Scheme of the type II mechanism of photooxygenation reaction (\(^{1}O_{2}\) – singlet oxygen) (adapted from MacRobert et al, 1993 and Stewart, 1993)

Singlet oxygen species, due to their avid reactivity and short intracellular lifetime (0.1microsec) (Moan and Berg, 1991) have however, a very short radius of action, which is of the order of 0.01μm. When compared with the diameter of human cells, which ranges from 10 to 100μm, it is obvious that, only subcellular sites of the primary singlet oxygen formation, which correspond to the intercellular photosensitiser localization, can be accessed and attacked (as discussed in the next section) (Henderson and Dougherty, 1992).

The efficiency of generating singlet oxygen by photosensitiser is described by the quantum yield of singlet oxygen (\(\phi_{\Delta}\)). It depends on the photosensitiser triplet yield (ideally close to one), triplet state lifetimes, which should be greater than 10μs, and energy of the triplet (Bonnett, 1995, MacRobert et al, 1992).

The latter needs to be > 94kJ/mol as such an amount of energy is required for raising oxygen from the triplet ground state into the excited singlet state and is achieved only by agents which absorb light of wavelength 850nm and below (Stewart, 1993).
Singlet oxygen has not been found directly in tissues owing to its high reactivity and thus short lifetime in cellular systems. Available techniques, which allow detection of singlet oxygen generation during PDT are based mainly on the inhibition of the PDT effect by singlet oxygen scavengers or/and assessment of product formation resulting from the interaction of singlet oxygen with its quencher. Alternatively D$_2$O, which prolongs the lifetime of oxygen species and thus enhances PDT photodamage (Moan et al, 1979, Weishaupt et al, 1976) can be used. These methods suffer, however, from impaired specificity (Rosenthal and Ben-Hur, 1994). Accepting these limitations, results of most experiments indicate that singlet oxygen is the major cytotoxic intermediate of PDT for all widely used photosensitisers: haematoporphyrins, phthalocyanines, protoporphyrin IX and mTHPC (Weishaupt et al, 1976, Moan et al, 1979, Ravanat et al, 1992, Ito, 1978, Ma et al, 1994). Despite some observations, which suggest that in vivo, free radicals could significantly contribute to PDT photokilling (Ochsner, 1997, Rosenthal and Ben-Hur, 1994) it is generally accepted that the type II photoreaction is probably the main mechanism of PDT induced cell photoinactivation (Henderson and Dougherty, 1992, Oleinick and Evans, 1998).

**Oxygen effect**

It has been shown that oxygen is essential for photodynamic processes (Moan and Sommer, 1985, Bown et al, 1986) with the exception of a cyanine dye, which seems to have oxygen –independent mode of action. (Henderson and Doughtery, 1992). Nevertheless, for the majority of photosensitising drugs, oxygen depletion strongly limits PDT induced photoinactivation. Unfortunately, hypoxia, which is often present in malignant tumours due to their inefficient capillary network, could be further increased (or occur primarily) during treatment as a result of the PDT induced vessel damage and/or vascular shutdown and/or oxygen consumption by photooxidation reactions. The latter process could be so substantial that it can not be compensated by blood circulation and may lead to cell anoxia within a few tens of seconds, thus preventing further PDT action (Foster et al, 1991).

This problem probably could be partially overcome by illumination with light of a low fluence rate for which, tissue deoxygenation during treatment is decreased (Sitnik et al, 1998).
Several experiments demonstrated significant enhancement of ALA, mTHPC or porfimer sodium based PDT for treatment with a low fluence rate that could have resulted from the better maintenance of tissue oxygenation during illumination with light of a reduced fluence rate (Andrejevic-Blant et al, 1996, Sitnik et al, 1998, van Geel et al, 1996).

Augmentation of the PDT effect obtained by introducing dark intervals during light illumination (fractionation) could be attributed to minimizing the development of hypoxia during treatment (Messman et al, 1995, Curnow et al, 1998).

It has been suggested that interruption of photochemical reactions and/or reversing PDT induced microvascular constriction during the dark period allows for tissue reoxygenation sufficient for PDT. However, other processes, namely reperfusion injury and relocation of the photosensitiser could be involved in the enhancement of PDT by light dose fractionation (Curnow et al, 1999). It is also likely that the biological effect of fractionation is dependent on the fractionation schedule used (e.g. on the length of the dark period) and also varies between different photosensitising agents.

Photobleaching, defined as photochemical degradation of photosensitiser during light exposure, is another reaction, which could significantly decrease the rate of photodynamic oxygen consumption during treatment and thus sustain sufficient tissue oxygenation for PDT. Photobleaching may however lead to reduction of the active photosensitiser concentration to below the threshold level which produces PDT tissue necrosis and therefore may inhibit the PDT effect (Potter et al, 1987). Photobleaching can be, however, used to enhance PDT therapeutic selectivity between normal and tumour tissue. In a colonic tumour model, the cancer to normal tissue ratio of only 2:1 for photosensitiser concentration was exploited to obtain tumour selective necrosis by keeping the photosensitiser tissue concentration at a low level, close to the photobleaching threshold level (Barr et al, 1990).
2.2.3 Biological effects of PDT

Introduction
The biological effect of PDT is very complex and despite more than 20 years of extensive research, still poorly understood. Moreover, data from experimental studies is confusing and often apparently contradictory.
These discrepancies can be partially attributed to the differences in the experimental models used, which were more or less relevant to real situations and to human disease. The mode of PDT produced photodamage seems to be also dissimilar for each photosensitising agents being dependent on its physicochemical and biological properties and appears to be greatly modulated by the therapeutic conditions such as the light and photosensitising drug dose.
It is clear however, that at least three different mechanisms: direct killing of malignant cells, vascular effects and immunological responses are involved in PDT induced tumour damage (Oleinick and Evans, 1998) and will be discussed below. Studies on animal models suggest that all of them are required for long-term tumour control (or cure), however, their contribution in tumour eradication remains unclear. The effect of PDT on normal tissue cannot be also overlooked as for the safe eradication of cancer, it is essential to understand how adjacent normal tissues respond to PDT action. PDT effects on the normal pancreas and surrounding organs will be discussed in the relevant chapter of this thesis, which summarises results of studies on PDT for the treatment of pancreatic cancer.

Cellular targets
Sites of singlet oxygen mediated cellular damage are closely related to the localization of photosensitiser during illumination, owing to the minimal diffusion distance from the place of origin and thus short radius of action of the active oxygen species. Intracellular distribution of photosensitiser depends on its structure and chemical properties namely lipophilicity, charge of the molecule, type and number of ring and core substitutions (Oleinick and Evans, 1998) and also is determined by the intracellular transport of a photosensitising drug.
Aggregated and hydrophilic dyes are usually taken up into cells by endocytosis or pinocytosis and then build up in lysosomes or endosomes from which they can be further released into the cytoplasm and/or nucleus upon illumination, due to photodynamically increased permeability of the lysosomal membrane (Dougherty et al, 1998, Boyle and Dolphin, 1996). Lyophilic agents like HpD or zinc-phthalocyanine directly penetrate the plasma membrane (Berg and Moan, 1997), and usually bind to and then subsequently damage all cell membranes (Henderson and Dougherty, 1992), which is probably the most efficient mechanism for producing cell photolethality. Hydrophilic photosensitising agents, which lead to cell destruction by releasing lysosomal hydrolytic enzymes seem to generate a much poorer PDT effect as the quantum yield of cell inactivation, defined as 1/N of the number N of photons per unit area, which causes photodamage of 50% of the cells, can be as much as 10 times larger for the lyophilic agents as for hydrophilic drugs of similar singlet oxygen yield (Moan and Berg, 1992).

The intracellular photosensitiser accumulation (and thus mode of PDT inactivation) is also dependent on the cell type as remarkable differences in the drug location were reported for the same photosensitising agent in the various cell lines (Moan and Berg, 1992).

Early work on HpD, which investigated drug position in cells, is difficult to interpret, as this agent is a mixture of poorly defined compounds of different activity, so localization cannot automatically identify the real site of action. New generations of photosensitisers include chemically pure and usually lyophilic, agents, which have an affinity to cell membranes and for which structural (mainly lipid peroxidation and protein cross-linking) and functional (e.g. impaired action of pump ions, Ochsner, 1997) disruption of the cell membranes appear to be the initial mechanism of action. Specific intracellular targets of PDT damage are briefly discussed below.

**Lysosomes and microtubules**

Lysosomes seem to be the primary target for the photodynamic action mediated by photosensitisers localised preferentially in this cell structure, however the overall role of their photodestruction for cell photoinactivation is unclear.

Extensive photodamage of lysosomes was reported following PDT with Nile blue derivatives, (photosensitising dyes highly selective for lysosomes), but there was no correlation between the degree of lysosomal destruction and the extent of
phototoxicity produced (Lin et al, 1993). This could be partially explained by inactivation of hydrolytic enzymes, released from photodynamically ruptured lysosomes during PDT, that was observed with meso-tetraphenylporphine tetrasulphonate (TPPS₄). Photochemical inactivation of cells with lysosomally located sensitisers could therefore be dependent on the relocation of photosensitiser from lysosome into cytoplasm or/and nucleus during light exposure and subsequent photoattack of the cytosomal organelles or alternatively, could be mediated by the fraction of the originally extralysosomally situated photosensitiser (Berg and Moan, 1997).

Microtubules, cytoskeletal elements involved in cell mitosis, which constitute the target of action for some conventional oncological drugs (e.g. vincristine), were found to be the object of the photoinactivation produced by several hydrophilic agents (Berg and Moan, 1997). Polymerisation of the free form of tubulin (the main component of microtubules) was inhibited following photodynamic therapy with tetraphenylporphine tetrasulphonate, a water-soluble photosensitiser with affinity to tubulin - (Winkelman et al, 1993), that resulted in the accumulation of cells in mitosis and subsequent cell death (Berg et al, 1992). Other effects of the inhibition of microtubules could include formation of micronuclei and giant cells, which were observed following PDT with HpD, AlPcS and but interestingly, also in cells incubated with 5-ALA but not exposed to light.

Mitochondria

Mitochondria are recognized as one of the most important subcellular targets of PDT mediated phototoxicity. Morphological and/or functional mitochondrial changes, affecting the porphyrin ability to bind to mitochondrial membranes found in Photofrin-PDT resistant (RIF-8) sarcoma cells, imply that mitochondria constitute the primary target for the Photofrin photosensitisation (Sharkey et al, 1993, Wilson et al, 1997).

Mitochondria appear also to be a notably vulnerable site for inducing cell photodamage.

In a study by Wilson, a higher dose of light was necessary to produce the same level of cell kill when PPIX was distributed in the whole cell after long incubation with ALA compared with a short time of incubation when PPIX was localised predominantly in mitochondria (Wilson et al, 1997).
Other studies demonstrated the development of mitochondrial functional alterations such as inhibition of the activity of the oxidative phosphorylation pathway enzymes: cytochrome c oxidase, succinic dehydrogenase, and ADP/ATP translocator, promptly following PDT action, even before the viability of cells assessed by trypan blue infiltration was impaired (Singh et al, 1987).

An increased mitochondrial potential or dissipation of the electrochemical gradient over the mitochondrial membrane was one of the other earliest detected signs of PDT induced cytotoxicity (Moan and Berg, 1992, Singh et al, 1987).

In support of the functional changes observed after PDT, morphological transformations suggesting mitochondrial damage were shown minutes after illumination using electron microscopy (Linuma et al, 1994).

Reduced activity of mitochondria due to PDT, leads to depletion of the intracellular ATP, which in turn severely affects all energy dependent processes, vital for cell function (Ochsner, 1997). Lethality of the mitochondrial photodamage could also be mediated by releasing cytochrome c and triggering apoptosis, which is implicated as one of the highly effective mechanisms of PDT induced cytotoxicity (Oleinick and Evans, 1998).

**Nucleus**

The most frequently used photosensitisers do not accumulate inside cell nuclei, although they may bind to the nuclear membrane and affect DNA located in that vicinity (Oleinick and Evans, 1998). This is in agreement with the result of the study by Evenson and Moan, which showed high susceptibility of chromosomal telomere and centromere regions, located in the immediate proximity of the nucleus membrane, to PDT photodestruction (Evensen and Moan, 1982).

The cellular DNA damage produced by PDT in various types of cell lines (malignant or normal, human and animal) included single-strand breaks, alkali-labile sites, DNA-protein cross-links and probably as a result of these changes sister chromatid exchanges and chromosome aberrations were also observed (Moan et al, 1980, Gomer et al, 1983, Ramakrishnan et al, 1989, Evenson and Moan, 1982), in frequencies lower or comparable to those generated by X-rays or UV radiation (Gomer et al, 1983, Moan and Berg, 1992).

The incidence of photosensitisation induced mutagenicity appears to be lower in comparison with alterations produced by ionising radiation and UV exposure.
However, true assessment of the mutagenic potential of PDT is difficult as it varies significantly between different type of cells and the targeted, examined gene. Photofrin photosensitisation did not induce any mutagenic activity above background level at the hypoxanthine-guanine phosphoribosyltransferase (hppt) locus in Chinese hamster ovary cells in distinction to treatments with X-rays or UV, which effectively produced mutant types of this gene (Gomer et al, 1983). A similar conclusion was reached in work, which investigated the mutagenicity of the Na/K- ATPase gene in a hamster cell line produced by phthalocyanine –PDT (Ben-Hur et al, 1987). Studies on PDT induced mutagenicity of the heterozygous autosomal thymidine kinase (TK) gene in a murine and human lymphoblastic cell line, reviewed by Oleinick and Evans, brought however, different data, maybe due to a higher frequency of recoverable mutants in the tested system (Oleinick and Evans, 1998). In these experiments, mutations at TK loci were observed following PDT with phthalocyanine or PF, although human cells exhibited a significantly lower mutability rate than an animal cell line (Evans et al, 1997, Oleinick and Evans, 1998). These results could imply that human cells are relatively resistant to the mutagenetic action of PDT, although considering the extensive variation of the human genome, more work is necessary to determine the real mutagenic potential of PDT in humans.

Also involvement of PDT induced DNA photodamage in the overall treatment photocytotoxicity is not well established. Most of the available data suggest that nucleus photoinjury is not the main mechanism of PDT photoinactivation (Moan and Berg, 1992). Other experiments showed, however, that nucleus damage could significantly contribute to PDT induced cell kill (Ramakrishan et al, 1988).

**Apoptosis in PDT**

Apoptosis is an active, biochemical process for the self-elimination ("suicide") of individual cells, and has been found to be a common mechanism of cell death following PDT. In vitro, rapid cell death by apoptosis in response to PDT photosensitisation with various agents has been reported in several human and animal cell lines (Agarwal et al, 1991, He et al, 1994, Noodt et al, 1996). In vivo, apoptotic cells were visualised in biopsies of PDT treated murine skin papillomas and human skin tumours (Oleinick, 1998). Apoptosis, however, is not a universal mode of cell death following PDT.
Some of the investigated line cells, sensitive to PDT action, which include a human non-small cell lung carcinoma cell line exposed to Photofrin-PDT and a WiDr human adenocarcinoma cell line sensitised by ALA were found not to undergo apoptosis and died via necrosis after PDT photoinactivation (He et al, 1994, Noodt et al, 1996). The factors, which determine the PDT induced pathway of cell death and are responsible for the observed variations are not yet fully understood.

Subcellular localization of the photosensitiser is one of the factors influencing the mode of PDT produced cell death. Results of a study, which examined the cellular response to PDT with two purpurin analogues of different subcellular localization, suggest that accumulation of photosensitiser in the mitochondria favour apoptosis, whereas photosensitisers which bind preferentially to the plasma membrane induce cell death by necrosis (Kessel et al, 1997).

Further, the “level” of PDT cell death was found to modulate the mechanism of cell death, as for other cytotoxic treatments. Lower light doses with chloroaluminium phthalocyanine caused lysosomal and mitochondrial photodamage of murine leukaemia cells and initiated apoptosis in distinction to PDT doses $\geq$ LD$_{99}$, which produced membrane destruction and inhibited the apoptotic response in favour of direct necrosis (Luo and Kessel, 1997).

Finally, the induction of apoptosis in response to PDT varies in different cell types, being probably dependant on the expression of the specific genes controlling the apoptosis process. p-53, the best known gene of apoptosis seems not to be involved in the regulation of the form of PDT induced cell kill (Oleinick, 1998).

Overexpression of the \textit{BCL-2} gene, (the product of which is the protein in the mitochondrial membrane which prevents of the release of the factors triggering apoptosis), was shown to partially suppress apoptosis and induce resistance to phthalocyanine photosensitisation of Chinese hamster ovary cells (He et al, 1994).

This observation also indicates the possible important function of mitochondria in the initiation of an apoptotic response to PDT photodamage, which could be mediated by releasing cytochrome c into cytoplasm and activation of caspases, apoptotic enzymes.
Other mechanisms leading to the induction of apoptosis following PDT action, include release of lipid second messengers, intracellular efflux of calcium ions, and activation of stress kinase pathways (Oleinick, 1998); however, details of this process are still poorly understood. Similarly, the relevance of apoptosis for the overall PDT response and tumour eradication needs to be established.

**Vascular effects**

PDT action induces a profound impact on the blood vessels of the treated tissue, which could significantly contribute to PDT produced tumour damage. The vascular response to PDT is dependent on the type of photosensitiser employed and the treatment protocol (mainly, drug dose and time interval between drug administration and light exposure), as the plasma concentration of photosensitising agent at the time of illumination is one of the major factors determining vascular photosensitivity (Henderson and Daugherty, 1992).

Vascular changes resulting from PDT action occur during or very shortly after light exposure. In Photofrin-PDT, vessel constriction leading to tissue oxygen depletion was observed within the first minutes of illumination. Other early signs of the microvasculature effects included increased permeability of the vessel wall with leucocyte adhesions and platelet aggregation, which further impaired blood flow and finally caused vascular stasis (Fingar et al, 1992), haemorrhage and oedema (Henderson and Daugherty, 1992).

Events, summarised above, which lead to the severe disruption of vascular perfusion following PDT, may be related to photodamage of the vascular wall endothelium (Dougherty et al, 1998) and are probably mediated by PDT induced release of tromboxane and/or other vasoactive products of the cyclooxygenase pathway. This hypothesis is supported by the results of the study on the experimental chondrosarcoma model in rats, where pre-treatment administration of indomethacin inhibited PDT induced intravascular release of tromboxane and concomitantly suppressed PDT-dependent vascular occlusion (Fingar et al, 1990).

Mechanisms of PDT induced vascular damage vary with different photosensitisers.
In distinction to the effect observed with Photofrin, no vessel leakage or changes of the vessel lumen implicating vasoconstriction were detected in the cremaster muscle during PDT treatment of the experimental chondrosarcoma tumour with mono-L-aspartyl chlorin e$_6$ (Npe6) and significant blood flow stasis, which occurred after Npe6 photoinactivation, resulted from platelet aggregation and thrombus formation (McMahon et al, 1994).

PDT induced microvascular destruction causes substantial changes in tissue physiology, mainly leading to tissue oxygen deprivation as demonstrated by measurements of oxygen pressure using an interstitial oxygen probe. Consequences of tumour hypoxia and/or anoxia resulting from vascular collapse may substantially contribute to the overall PDT produced tumour damage and enhance cancer eradication. Several experiments have suggested that PDT dependent-vascular breakdown is necessary for cancer cure or showed the positive correlation between the degree of vascular damage and efficacy of PDT treatment (Brasseur et al, 1996, Henderson et al, 1985).

These experiments failed however to demonstrate that the PDT response could solely rely on the vascular photodamage and indicated that direct cytotoxicity and inflammatory/immunologic effect of PDT are necessary for therapeutic success.

**Inflammatory/immunological effects**

In recent years there have been substantial advances in the understanding of the inflammatory/immunological effect of PDT, partly owing to employment of molecular biology techniques. There is increasing evidence that post-PDT inflammation and tumour-sensitised immune reactions significantly enhance antitumour activity of PDT and play an important role in long-term cancer control (Dougherty et al, 1998).

Detailed description of these very complex processes exceeds this review, but in brief they are initiated by PDT-induced cell membranes alterations, which activate a number of the signal transduction pathways, namely synthesis and release of second messengers (e.g. arachidonic acid and calcium ion), activation of protein kinase cascades (e.g. stress kinase activation) and induction of stress gene and cytokine expression (e.g. TNF-alpha) (Oleinick and Evans, 1998).
Products of these reactions are powerful mediators, which stimulate the wide variety of inflammatory/immunological responses, from induction of apoptosis, modulation of cell adhesion and antigen presentation, to production of enzymes, vasoactive substances, free radicals, interleucines, leukocyte chemoattractants, and other immunoregulators (Dougherty et al, 1998).

Most of these reactions and/or substances increase PDT produced tissue damage and further enhances PDT tumour cytotoxicity. Induction of infiltration of a PDT treated lesion by non lymphoid inflammatory cells is an example of the anticancer action associated with PDT generated inflammation, which in vivo has been shown to increase the PDT tumour response and potentate the rate of cancer cures following PDT in animal tumour models (de Vree et al, 1996, Yamamoto et al, 1992, Krosl et al, 1996).

Systemic antitumour activity attributed to the induction of immunity against malignant cells is another interesting feature of PDT. It has been shown that the immune system (especially lymphoid cells) is essential for preventing tumour recurrence following potentially curative PDT treatment of experimental tumour models in the normal, nude or severe combined immune deficiency (scid) mice (Hendrzak-Henion et al, 1997, Korbelik et al, 1996).

Other studies, which further investigated this phenomena, defined accurately that long-term treatment success following curative dose of Photofrin-PDT was dependent on the immune memory cells sensitised to the PDT treated tumour, which were produced as a result of the immune reactions induced by photoinactivation.

It was found, however, that PDT related biologic processes, do not solely activate the cell death promoting pathways, which augment PDT efficacy but initiate also cytoprotective mechanisms.

PDT induced immunosuppression appeared to diminish its tumour toxicity (Lynch et al, 1989) but in the other experiment allowed for a prolonged skin transplant survival in mice (Obochi et al, 1997) and may have a positive impact on the treatment of non-malignant immunological diseases such as arthritis (Okunaka and Kato, 1999).
2.3 Photosensitisers for Photodynamic therapy

2.3.1 Introduction

The ideal photosensitiser for PDT should have no dark toxicity, be a pure and stable chemical compound (simple synthesis is an additional advantage), have a strong absorption in the red part of the visible spectrum, which ensures the best tissue penetration of light (as shown on Fig. 4), be an efficient generator of singlet oxygen (have a high triplet quantum yield), and should produce minimum skin photosensitivity (Bonnett, 1999).

![Diagram of photosensitiser absorbance in relation to tissue transmittance](image)

**Fig. 4.** Photosensitiser absorbance in relation to tissue transmittance. The absorption spectra are schematic only, and only Band I (the lowest energy absorption band) is shown, except for the typical absorption spectrum at the left. The transmittance curve refers to a sample of the 7mm thick, human scrotal sac. From Bonnett, 1999.

Preferential accumulation of photosensitiser in malignant tissues, which attracted a lot of interest in the past and was considered as the other important feature of the perfect photosensitising agent is probably grossly overestimated.
Most of the photosensitisers are taken up with some degree of selectivity by malignant tumours, which varies between different photosensitisers and cancer type (Moore et al, 1998), however, generally there was inadequate selectivity to produce cancer eradication without any damage to the surrounding normal tissues and organs (Bown, 1990).

2.3.2 First generation photosensitisers: haematoporphyrin derivative (HpD)

Haematoporphyrin derivative and its partly purified, commercialised variant Photofrin represent the first generation of photosensitising agents, which were until recently the most widely used photosensitisers for either basic research or clinical studies. Photofrin is the only photosensitiser, which has yet received regulatory approval in any country. In gastroenterology, regulatory authorizations were obtained for its use in the palliation of advanced oesophageal cancer when there are no other therapeutic options in USA, the Netherlands and France. In Japan this indication has been broadened to the treatment of early oesophageal cancer.

Chemically, HpD is a complex mixture of a large number of compounds derived from haematoporphyrin, which are characterised by the presence of the porphyrin tetrapyrole ring structure. Photofrin and other commercial preparations of HpD (e.g. Photosan) consist of the most active biologically, oligomeric fraction (so called Fraction D) of HpD, which is a somewhat incompletely defined combination of porphyrin oligomers with peripheral-hydroxyl and vinyl substituents (Bonnett, 1999). Photofrin absorbs light maximally in the Soret band (400 nm); its therapeutic absorption band in the red at about 630 nm, used for treatment, is relatively weak. Owing to the complex, unstable and difficult to reproduce composition of Photofrin, any pharmacokinetics study concerning this compound are difficult to interpret. It is clear however, that it is retained in the skin for several weeks (Kessel and Dougherty, 1999) when the risk of the cutaneous photosensitivity is substantial.

Clinically, porphyrin based PDT was used for the treatment of nearly every type of cancer as HpD and Photofrin have been until recently the only photosensitising agents available for clinical trials.

In gastroenterology, substantial clinical experience with Photofrin-PDT is confined to the treatment of oesophageal cancer and Barrett’s oesophagus.
A prospective randomised trial on 236 patients, which compared Photofrin-PDT with laser ablation for palliation of advanced, partially obstructed oesophageal cancers showed similar relief of dysphagia, median survival times and time to palliation failure in both groups. Also the rate of serious complications was comparable, with the exception of the oesophageal perforations, which occurred more frequently following Nd-Yag laser therapy (1% in the PDT-treated patients versus 7% for the patients, which underwent Nd-YAG ablation (Lightdale et al, 1995). Skin sunburn reactions were the major complication of PDT, which were experienced by most of the photosensitised patients.

Photofrin based PDT for the treatment of early oesophageal cancer produced promising results in a group of 123 patients, where the complete response rate at six months was 87% and the five years disease specific survival 74%. Oesophageal stenosis was the main complication of the treatment, which occurred in 35% patients, but responded to dilatation in every case (Sibille et al, 1995).

Several groups tried PDT using Photofrin for the treatment of Barrett’s oesophagus with dysplasia. Results of the largest published series on 100 patients treated with Photofrin based PDT and followed by Nd-YAG laser thermal ablation showed conversion of up to 80% of treated Barrett’s mucosa into normal squamous epithelium with the elimination of dysplasia in 78% of partients and complete elimination of Barrett’s mucosa in 43% of patients. Unfortunately there was high (34%) incidence of post-treatment strictures, which resulted probably from PDT induced muscle scarring and therefore, these were found to be more difficult to dilate than strictures arising from oesophagitis (Overholt et al, 1999, Wang et al, 1998).

A pilot study from Germany demonstrated the efficacy of endoscopic Photofrin–PDT for the recanalisation of malignant biliary obstruction, where not only satisfactory biliary drainage was restored but also patients performance status was significantly improved following treatment (Ortnet et al, 1998).

Although Photofrin is indisputably effective as a photosensitiser, results of basic and clinical studies clearly indicate that it does not fulfil the requirements for an “ideal” photosensitising drug and that it suffers from a variety of drawbacks. These include its poorly defined and unstable chemical composition, prolonged skin photosensitivity, which may last up to 6 weeks, and very modest photoactivity owing to the poor photochemical properties, which have to been compensated for by using high drug doses and long treatment times (Bonnett, 1999).
Limitations associated with Photofrin stimulated extensive research on the development of new photosensitising agents, so called second generation agents. The wide range of photoactive compounds studied includes metallo-phthalocyanines, chlorins, purpurins, cationic dyes, porphycines, texaphyrins and other porphyrin derivatives such as benzoporphyrin derivative monoacetate. Two, second generation photosensitisers that have attracted a lot of interest in gastroenterology and were investigated in this thesis, namely meta tetrahydroxyphenyl chlorin (mTHPC) and ALA (5-aminolaevulinic acid) will be reviewed in the next section.

### 2.3.3 Second generation photosensitisers

**Meta tetrahydroxyphenyl chlorin (mTHPC)**

Meta tetrahydroxyphenyl chlorin (mTHPC), developed at Queen Mary College, London by Bonnett (Bonnett et al, 1989, Bonnett, 1995) is one of the most potent photosensitising agents used for photodynamic therapy. It is a single, chemically pure and stable chlorin class photosensitiser, which consists of a porphyrin skeleton with one reduced double bond (Fig.5)

![Chemical structure of mTHPC](image)

**Fig. 5.** Chemical structure of mTHPC.

mTHPC has a strong absorption peak in the red part of the spectrum at 652nm, which gives better tissue penetration than at the 630 nm required for Photofrin. This means that a deeper effect can be obtained (Ma et al, 1994). It is also a much more effective generator of singlet oxygen species than haematoporphyrin derivative.
Thanks to such high phototoxicity, mTHPC requires low light doses (usually 10-20 J, compared to 100J for Photofrin) so treatment times can be shortened to a few minutes. Care must be taken about skin photosensitivity to bright light. This usually lasts up to a maximum of about a month and is shorter than that associated with administration of Photofrin (van Geel et al, 1995).

These characteristics make PDT with m-THPC not only technically easier and “clinically more friendly” but also much more efficient for producing PDT necrosis than an optimal therapeutic dose of Photofrin based PDT. This was confirmed in the in vitro study on Chinese hamster lung fibroblasts and in vivo on experiments on the mouse mammary carcinoma (Peng et al, 1995, Ma et al, 1994). Nevertheless, the pharmacokinetics and biodistribution of mTHPC appear to be complex and significant discrepancies were observed between several animal and human pharmacokinetics studies. These could result from interspecies and inter patient variations as well as from methodological differences.

In the murine colorectal tumour model, the blood concentration of radiolabelled mTHPC fell rapidly as the drug was taken up by tissues (especially the liver). Subsequently it diffused relatively slowly from the tissues with a half life of about 10 days (Whelpton et al, 1996). A similar pattern, typical for lipophilic, water insoluble agents, was seen with mTHPC in 20 patients, where the photosensitiser was found to be quickly eliminated from the plasma with a half life of 30 hours in comparison with a long residence time in tissues. However, in contrast to the animal study, after an initial rapid decrease of plasma mTHPC with the minimum at 45 minutes, mTHPC concentration increased again to reach a maximum 10 hours after injection (Glanzmann et al, 1998). The other human pharmacokinetic study also showed a delayed peak of plasma drug level but at 24 hours after drug administration with virtual total disappearance of mTHPC from the blood by 8-10 days corresponding to a half life of 45.4 hours (Ronn et al, 1996).

Despite such unusual and still poorly understood plasma pharmacokinetics, the other aspects of the photosensitiser metabolism are relatively well established. Most studies indicate that large amounts of the injected drug are quickly taken up by the liver, which appears also to be the major elimination pathway (via bile and than faeces) of the unchanged photosensitiser (Whelpton et al, 1996, Ronn et al, 1996).

Cellular uptake of mTHPC is greatly faciltitated by its high lipid solubility.
mTHPC easily penetrates across the cell plasma membrane and distributes freely in the cytoplasm as was demonstrated by fluorescence microscopy in the normal cell line and in the murine mammary carcinoma model (Peng et al, 1995, Ma et al, 1994). The nuclear fluorescence of mTHPC was minimal which may indicate that the risk of photoinduced mutagenicity is low.

The tumouricidal effect of mTHPC-PDT is related to the drug and light dose but also to the time interval between sensitisation and light exposure, which determines the intratumoural localisation of the photosensitiser. At the early time points following drug injection, mTHPC accumulates mainly in the tumour vascular network in distinction to the malignant cells where only a little drug fluorescence was detected in the mammary and squamous cell carcinoma (SCC) tumour animal models (Peng et al, 1995, Andrejevic-Blant et al, 1997). Gradually more fluorescence was observed in the tumour cells whereas the vascular mTHPC concentration progressively decreased and disappeared almost completely by the 4th day after injection in the chemically induced SCC in hamsters (Andrejevic-Blant et al, 1997). In the same experimental tumour model, changes in the biodistribution of mTHPC were accompanied by differences in the type of PDT induced photodamage. Severe, non-specific and non-selective ischaemic vascular necrosis of the normal tissues and tumour was observed following light illumination performed shortly after drug administration, whereas PDT carried out more than 3 days after mTHPC injection produced tissue-specific, coagulation necrosis of the tumour (Andrejevic-Blant et al, 1997). The importance of the mTHPC mediated vascular photodamage was brought out by studies which showed a positive correlation between PDT efficacy and mTHPC plasma level rather than with tumour concentration of photosensitiser in the animal colon and sarcoma tumour models (Veenhuizen et al, 1997a, Veenhuizen et al, 1997b). Other experiments revealed greater PDT induced tissue necrosis but also much higher treatment toxicity and higher risk of complications associated with treatment performed shortly after mTHPC administration (Ris et al, 1997).

The best therapeutic selectivity between tumour and normal tissues was seen 3-5 days following drug administration in the nude mice bearing human mesothelioma xenografts (Ris et al, 1993). In clinical protocols, most treatments are conducted 3-4 days after mTHPC injection.
Clinically, mTHPC-PDT has been used for a wide variety of tumours, with especially promising results achieved for the treatment of head and neck malignancies. In gastroenterology, there is substantial experience in the treatment of early oesophageal cancers from the Swiss group in Lausanne. Twenty-eight patients with early bronchial or oesophageal cancers were treated with mTHPC mediated PDT and this eradicated 77% of the tumours. Skin photosensitivity was only seen during the first week after injection, although one oesophageal fistula, one bronchial stenosis and two possible sealed oesophageal perforations were seen. These led to the subsequent use of green light for such lesions. This is absorbed by mTHPC (although not as strongly as the red light at 652nm) and penetrates less into tissue so minimising the risk of muscle damage and perforations (Grosjean et al, 1996). The main causes of treatment failure in this series were tumour under staging, inadequate light delivery to all relevant areas and low tumour concentrations of mTHPC (Grosjean et al, 1998). Substantial inter patient variations in the plasma levels of mTHPC and tissue drug fluorescence were reported that indicate that in vivo fluorescence measurements of drug levels might substantially improve treatment outcome.

PDT with mTHPC was also reported to successfully eradicate early gastric cancers in a small group of inoperable patients without any serious complications (Ell et al, 1998).

5-aminolaevulinic acid (ALA)

5-aminolaevulinic acid (ALA), in contrast to other photosensitising agents, is not an active compound developed in the laboratory but is a naturally occurring photosensitising “prodrug”, which is metabolised to a photoactive endogenous intermediate substance. The synthesis of ALA from glycine and succinyl Co-A, which is located in the matrix mitochondria of every nucleated cell represents the first committed step in the haem biosynthesis pathway and is normally controlled by a negative feedback inhibition depending on the local concentration of haem, its end product. However, following administration of an excess of exogenous ALA, the natural regulatory mechanism is bypassed and the haem biosynthesis pathway is overloaded.
The rate limiting step then becomes incorporation of iron into the tetapyrole structure of PPIX, catalysed by the enzyme ferrochelatase, which constitutes the final conversion of protoporphyrin IX (PPIX) to haem (Peng et al, 1997). As a result PPIX, which is potent photosensitiser in its own right, accumulates in the cells (Malik and Lugaci, 1987, Kennedy et al, 1996).

Also, other porphyrin intermediates like coproporphyrins and uroporphyrins are retained to some degree in the tissues following ALA induced saturation of the haem pathway, but they are rapidly eliminated from cells and do not have significant photosensitising potential.

Simplified mechanism of the PPIX accumulation for ALA PDT showing the key control points is illustrated in the Fig.6.

Fig. 6. Mechanism of PPIX accumulation for ALA PDT. Suc CoA- succinyl Co-A, Fe- ferrochelatase. From Marcus, 1996).
ALA can be given topically, orally or intravenously; however, in humans, the latter route of delivery is in an early phase of development. Following oral ingestion, ALA is rapidly absorbed from the gastrointestinal tract (with a peak plasma concentration at 60 minutes, Mustajoki et al, 1992) and probably extensively metabolised in the liver.

Due to first-pass hepatic metabolism, the systemic bio-availability of ALA administered orally is markedly reduced, hence a double oral dose of ALA was required to produce the same level of tissue photosensitisation as intravenous ALA in rats (Loh et al, 1993a).

ALA and its metabolites are readily cleared. In humans, the ALA plasma concentration fell with a half time of about 50 minutes, being excreted via bile and through the kidney (Mustajoki et al, 1992).

Similarly to ALA, the pharmacokinetics for PPIX show extremely fast metabolism. The peak of PPIX tissue concentration occurs within a few hours following ALA administration, the exact time being dependent on the tissue histology and also the ALA dose as there was observed a linear relationship between PPIX maximum concentration (expressed as PPIX tissue fluorescence) and the log of ALA dose (Kennedy et al, 1996). The excess of PPIX is however, metabolised to haem in a very short period of time so once the maximum PPIX level is achieved there is a rapid reduction of PPIX tissue content, with no specific PPIX fluorescence above background level observed by 24 hours after ALA administration. Quick elimination of PPIX from the body is one of the major advantages of ALA as skin photosensitivity lasts only 1-2 days, in contrast to weeks or even months with other drugs.

Haem biosynthesis takes place in every nucleated cell being a vital process for the production of the haem containing proteins, which include haemoglobin, myoglobin and cytochromes involved in oxidative phosphorylation; however, there are great variations in the amount of PPIX accumulated following ALA administration in different organs and tissues. A notably higher level of ALA induced PPIX was found in the epithelial lining tissues- urothelium, endometrium and mucosa of the hollow organs of the gastrointestinal tract (Chang et al, 1996, Bedwell et al, 1992, Loh et al, 1993b, Kennedy et al, 1996) than in the underlying submucosa and muscle layers.
ALA administration resulted also in the preferential PPIX built-up in the various types of cancers over the normal tissues of the same origin, with the PPIX concentration ratio between tumour and normal as high as 8:1 in an experimental pancreatic cancer in hamsters (Regula et al, 1994). The mechanism for selective PPIX accumulation is not entirely defined, however alterations of the enzyme activities in the haem biosynthesis pathway, especially ferrochelatase deficiency, was observed in malignant tumours, which retained higher concentrations of the endogenously produced PPIX than adjacent normal tissues (van Hillersgeberg et al, 1992). Also it has been postulated that more metabolically active cells such as cells forming the lining of hollow organs have a higher requirement for haem synthesis and therefore express increased capacity for PPIX production.

Others studies failed, however, to demonstrate any significant correlation between cell doubling time or activity of the enzymes involved in haem synthesis and PPIX accumulation (Inuma et al, 1994, Hua et al, 1995).

Preferential distribution of PPIX in the mucosal lining of the hollow organs has however, a significant practical implication as this makes it possible to achieve a selective mucosal necrosis without any damage to the underlying muscle (Loh et al, 1992).

Thanks to these unique properties, ALA induced PDT damage does not affect the muscularis propria of hollow organs (at the drug and light doses studied) and heals remarkably well by regeneration with little if any scarring. Also, the mechanical integrity of blood vessels was not decreased following ALA-PDT probably thanks to the preservation of the elastic and collagen fibres, and no perforation or thrombosis occurred in PDT treated arteries (Grant et al, 1995), despite the fact that full thickness loss of cellularity was observed soon after PDT. This makes PDT with ALA relatively safe and a particularly attractive technique for treating areas of circumferential, superficial disease, such as Barrett’s oesophagus. Several clinical studies reported the effectiveness of ALA-PDT for reduction of the length of the segment of columnar epithelium and eradication of dysplasia in Barrett’s mucosa (Barr et al, 1996, Gossner et al, 1998). The treated areas healed remarkably well with squamous re-epithelisation and no local side-effects were observed, in contrast to studies using Photofrin, in which there was a very high incidence of the post treatment oesophageal strictures. However, persistent columnar epithelium was found underneath regenerated squamous mucosa.
The implications of this are not yet clear but it raises the question of whether the treatment is adequate. PDT with ALA can eradicate some early, oesophageal cancers but only those that are not deeper than 2mm. PDT has been successfully employed for the treatment of superficial skin diseases and recently extremely promising results were reported in a study, which used ALA induced photosensitisation for preventing vascular restenosis after balloon angioplasty (Jenkins et al, 1999).

Attempts to use ALA-PDT for the treatment of more bulky lesions (polyps and cancers) have been, however, unsuccessful as the necrosis achieved was very superficial and did not exceed 2mm in depth (Gossner et al, 1998, Regula et al, 1995). Probably the maximum oral dose of ALA in humans is limited to 60mg/kg by drug induced nausea and hepatotoxicity (Ackroyd et al, 1999, Regula et al, 1995). This is insufficient to produce an adequate level of PPIX tissue photosensitisation for anything more than superficial PDT.

Enhancement of ALA-PDT without increasing the administered ALA dose may be achievable by treatment with light fractionations and/or illumination with light of a reduced fluence rate. Another way is to increase PPIX tissue accumulation by administration of an iron chelating agent, which removes iron essential for the conversion of PPIX to haem, using desferoxamine (Inuma et al, 1994), EDTA (Smetana et al, 1997) and oral iron chelators, as has been investigated in this thesis.

### 2.4 Light sources for photodynamic therapy

Any type of low power (to avoid thermal effects) visible, light source can be used for PDT. In the early years of PDT, a Kodak projector lamp appeared to be perfectly adequate for surface illumination of skin basal cell carcinomas. At that time, other investigators used xenon arc lamps with appropriate filters system. Currently however, lasers (especially tunable dye lasers and less expensive, more reliable portable diode lasers) are generally employed for PDT, as they provide the most convenient and valuable source of the therapeutic light.

The word laser is an acronym for Light Amplification by the Stimulated Emission of Radiation, which describes the basic mechanism of laser action.
In the process of stimulated emission, a laser medium (e.g. helium, argon, neon, \( \text{CO}_2 \), ruby crystal) emits an intense beam of photons of the same frequency, phase, direction and polarization creating the unique laser beam characteristics of coherence, collimation and monochromaticity. Monochromaticity, which refers to the fact that the light produced has a single wavelength and photon energy, is the main advantage of laser light for PDT. Another valuable feature of lasers for PDT is the possibility of light transmission via thin flexible fibres, which can be precisely positioned in the targeted tissue in almost every part of the body using direct vision, flexible endoscopes or even hollow needles.

Many different optical delivery fibres are used depending on the clinical application. The available systems include bare fibres and fibres with various lenses on the end, which produce a spot of homogeneous intensity light suitable, for example, for illumination of cutaneous tumours and many types of fibres with diffusers of various lengths, which give a cylindrical pattern of light distribution employed for the treatment of hollow organs or for interstitial PDT.

2.5 Light dosimetry

Light energy (fluence) delivered to the targeted tissue is one of the major parameters determining the volume of tumour necrosis produced and thus of the therapeutic outcome of PDT. It decreases exponentially with the distance from the light source, being mainly determined by the depth of light penetration into tissue. The penetration depth (\( \delta \)), defined as the depth at which the incident intensity falls off from 1 to \( 1/e \), is inversely proportional to the effective attenuation coefficient, which in turn is dependent on optical scattering within the tissue and on the optical absorption by the tissue endogenous chromophores, mainly haemoglobin and melanin.

Both of these parameters characterise the optical property of the tissue, which does not only vary significantly between different organ/tumour types but also between individual samples of the same histology and moreover can change remarkably during PDT treatment due to changes in local blood perfusion (Chen et al, 1997).

The depth of light transition through tissue is also greatly influenced by the wavelength of the light used. Generally, longer wavelengths penetrate tissue much more efficiently than shorter ones.
As shown in the Fig.4, there is a strong attenuation up to 580 nm, mostly due to absorption bands of haemoglobin, which is followed by a sharp increase of tissue penetration in the red region of the visible spectrum so that even a small change of the light wavelength in this region can significantly enhance the volume of necrosis produced (Moore et al., 1997). Therefore photosensitisers, which absorb strongly in the longer part of the red spectrum produce a generally deeper effect than for example haematoporphyrin with its absorption peak at 630nm.

The object of theoretical models of light dosimetry for PDT is to accurately describe the light dose delivered to the targeted tissue so enabling precise treatment planning and response assessment.

They are based on the generally accepted concept of a threshold dose for photodynamic therapy defined as a minimum number of photons absorbed by the photosensitiser per unit tissue volume required to induce a specific biological response, such as necrosis. Unfortunately due to complexity of the dynamic interactions between photosensitiser, light, oxygen and tissue and substantial inter patient variations, probably only systems for direct individual, in vivo light measurements will permit precise control of the light dose for PDT. Nevertheless, several systems for real time online measurements have been developed although they are still too far from ideal too complicated to use in everyday clinical practice. There is much need for development of adequate systems for in situ light monitoring.
Chapter III. Previous experimental studies on PDT for cancer of the pancreas.

The dismal prognosis of pancreatic cancer despite multimodality treatment protocols, which include surgery, chemotherapy and radiation, generates an ongoing search for alternative therapies. From the data available so far, PDT appears to have some promise as a new treatment for this virtually incurable disease. Experimental studies have been carried out on pancreatic cancer cell lines and on pancreatic tumour models in hamsters and rats using several photosensitising agents. The results are broadly similar with all the agents and experimental models studied as all pancreatic cancer cell lines and tumours were found to be responsive to PDT photoinactivation. Significant decrease of human pancreatic cancer cell growth in vitro was reported following PDT with pheophorbide A, hypericin and Photofrin (Hajri et al, 1999, Liu et al, 2000, Moesta et al, 1992). The latter study suggested a negative correlation between cell line sensitivity to PDT and degree of cell differentiation, which in turn determines the presence of the tight intracellular junction and thus influences Photofrin uptake (Moesta et al, 1992). Studies on experimental pancreatic cancer models in rats and hamsters demonstrated that PDT effectively necrosed the cancer tissue and could produce a significant survival advantage (Regula et al 1994). The depth of PDT damage in the experimental pancreatic cancer produced by a single fibre ranged from 5-8mm following aluminium sulphonated phthalocyanine (AlSPc) photosensitisation to up to 12mm when mTHPC was used as a photosensitiser (no necrosis diameter was given after DHE-PDT of BOP induced cancers in hamsters) (Chatlani et al, 1992, Milky et al, 1997, Schroder et al, 1988). PDT with a gallium porphyrin complex caused necrosis of tumours up to 6-10mm in diameter, whereas cancers larger than 12mm showed viable cancer cells at the edges. Thermal effects could have contributed to the tumour damage achieved (Tajri et al, 1994). Interestingly, using ALA, which in most organs only produces superficial PDT damage, necrosis up to 8mm in the depth was achieved in implanted pancreatic tumours in hamsters, following surface illumination with a cylindrical applicator (Regula et al, 1994).
Unfortunately, in our study, the only patient who underwent ALA-PDT for the treatment of an inoperable pancreatic cancer exhibited virtually no tumour necrosis and the only benefit was a good analgesic effect, which lasted for approximately 3 weeks after PDT.

Results of a very recent study on an orthotopically grown human pancreatic cancer in nude mice demonstrated that PDT not only destroys cancer but also significantly suppresses tumour growth. 4 weeks after interstitial PDT with hypericin (a new photosensitiser derived from St. John’s Wort), the size of the tumours was reduced by approximately 40% as compared with the pre-treatment cancer diameter. Untreated controls died of the disease at the same time with ascites and metastatic tumours with masses up to 600% larger than in PDT-treated animals (Liu et al, 2000). Furthermore, in the other randomised study, ALA-PDT was shown to significantly improve survival of treated, tumour bearing animals compared with untreated controls, although all animals eventually died of cancer (Regula et al, 1994). In the other non-randomised survival study with pheophorbide A as the photosensitising drug, 6 of 9 treated rats with implanted asarazine-induced intrapancreatic carcinomas were rendered disease-free 120 days after PDT (Evrard et al, 1994).

The results of these experimental works suggest that photodynamic therapy could be the first palliative treatment for pancreatic carcinoma showing a meaningful impact on the survival; however before contemplating clinical studies it was essential to assess how adjacent, normal tissues respond to PDT. Data on the treatment tolerability available from several studies on normal and tumour bearing rodents, which used various photosensitising agents and different methods of light delivery, is encouraging. Normal pancreas appeared to be relatively resistant to PDT damage as there was little PDT effect on the normal pancreas when light and photosensitiser doses were applied which were able to produce extensive damage to the cancer. For AlSPc, the threshold photodynamic dose required to produce PDT necrosis of the normal pancreas (calculated by multiplying light dose by tissue concentration of photosensitiser) was about seven time as high as for damage to pancreatic cancer (Chatlani et al, 1992). In the literature, only one animal had necrotising pancreatitis after PDT, which could be secondary to the extensive damage in the biliary/periampullary area caused by illumination of the whole pancreatobiliary region (Schroder et al, 1988).
In other studies, no evidence of pancreatitis or cyst formation was seen following treatment of pancreatic tumours using several photosensitising agents (Regula et al, 1994, Mlkvy et al, 1996, Chatlani et al, 1992). The mechanism of the increased therapeutic selectivity of PDT for pancreatic cancer remains however, uncertain. Much has been written about selective uptake of the photosensitisers in malignant tissues but generally that was found to be insufficient to induce a truly selective PDT effect limited to neoplastic tissue (Bown, 1990). For the pancreas, the ratio of photosensitiser concentration in pancreatic cancer to normal pancreas varied from 13.5:1 for pheophorbide A to only 1-3 :1 for most studied agents: AlSPc, mTHPC and DHE. Moreover, when the same light dose was applied to the tumour and normal pancreas, which contained identical DHE concentrations, PDT damage was produced in the tumour only (Mang and Wieman, 1987). Therefore, a mechanism other than preferential photosensitiser retention in the pancreatic neoplasm is responsible for this enhanced PDT selectivity. In one study, the resistance of the normal pancreas to photoinactivation was accompanied by a lack of photosensitiser photobleaching in the normal pancreas, whereas photobleaching was extensive in the neoplastic tissue (Mang and Wieman, 1987). As photobleaching is believed to be mediated by singlet oxygen, the cytotoxic product of PDT, it has been postulated that normal pancreas contains singlet oxygen scavengers (perhaps glutathione or other intracellular thiols) that are not present in the malignant tissue, which protect photosensitiser molecules from photodegradation and also inhibit PDT action in the normal pancreas (Chatlani et al, 1992).

Other studies demonstrated considerable biochemical differences between normal pancreatic acinar cells and an exocrine pancreatic carcinoma cell line (AR4-2J) following phthalocyanine based photoinactivation. In the pancreatic cancer cell line, PDT lead to the dose-dependent inhibition of amylase release, whereas in the normal cell line PDT stimulated release of this enzyme (Matthews and Cui, 1990a), however this was not associated with cell structural damage seen on electron microscopy (Matthews and Cui, 1990b). The implications of these findings are so far unknown. Unfortunately, in contrast to normal pancreas, other organs adjacent to the pancreas were more vulnerable to PDT damage.
In all experimental studies, the duodenum was the most sensitive structure to PDT action as extensive duodenal damage with sealed and free duodenal perforations associated with peritonitis were observed in animals following Photofrin, AlSPc, mTHPC and to a lesser degree, ALA photoinactivation of the pancreato-biliary region (Schroder et al, 1988, Nuutinen et al, 1991, Chatlani et al, 1992, Mlkvy et al, 1996, Ravi et al, 1996). The extent of the damage seemed to correlate with the surface area of the tissue exposed to illumination and duodenal necrosis could be only avoided by shielding the duodenum from the therapeutic light (Ravi et al, 1996, Mlkvy et al, 1997). It has been suggested however, that the extremely thin duodenum in rodents may not be the best model as the much thicker human duodenum may be more resistant to PDT damage (Nuutinen et al, 1991). Also, early clinical studies using PDT with HpD to treat lesions localised in the duodenum showed no duodenal perforations (Abulafi et al, 1995, Mlkvy et al, 1995).

PDT for the pancreatic tumours in these animals also produced damage in the stomach, small bowel and biliary tree; however, necrosis of these organs did not cause perforations, healed safely and did not lead to serious complications. In one study, dilatation of the biliary system, caused probably by the post-treatment periampullary oedema, was observed after PDT (Mlkvy et al, 1996); however, clinically, bile outflow obstruction could be prevented easily the insertion of a biliary stent prior to PDT.

Large vessels do not seem to be at risk of unacceptable damage following PDT. As shown in experimental studies using phthalocyanine and ALA as photosensitisers, PDT does not reduce the mechanical integrity of large vessels, probably thanks to the preservation of the elastic and collagen fibres, and no perforation or thrombosis occurred in the PDT treated arteries (Grant et al, 1995). In all studies addressing PDT for the treatment of pancreato-biliary region no damage to adjacent large vessels (aorta, vena cava, portal vein, hepatic artery) was reported (Schroder et al, 1988, Nuutinen et al, 1991, Chatlani et al, 1992, Mlkvy et al, 1996, Mlkvy et al, 1997).

In conclusion, experimental studies confirmed that PDT could necrose pancreatic cancers without unacceptable damage to the normal pancreas and adjacent organs.
The only exception to this was the much thinner than human duodenum; however, early clinical studies showed no serious duodenal damage following endoluminal PDT of duodenal lesions. Moreover, a significant survival benefit has been demonstrated in animals with transplanted pancreatic cancers treated with PDT that suggest that PDT could be a palliative treatment for pancreatic cancer, which might have a positive impact on the survival time. Results of the experimental studies applying PDT to the pancreatic cancer are summarised in the Table 2.
Table 2. Results of the PDT experimental studies on the pancreatic tumour model (BOP- N-nitroso—bis-(2-oxypropyl) amine).

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<td>ALA</td>
<td>Transplanted in hamster pancreas</td>
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</tr>
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<td>mTHPC</td>
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<td>Tumour necrosis up to 12 mm (using fractionation schedule)</td>
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<td>Liu et al, 2000</td>
<td>Hypericin</td>
<td>Human, transplanted in nude mice</td>
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</table>
Chapter IV. Aims of thesis

The ultimate objective of this thesis was to develop PDT for the treatment of pancreatic cancer. Previous work on the experimental pancreatic tumour model has established that PDT causes necrosis in pancreatic cancer without unacceptable complications and could produce a statistically significant survival benefit. With these encouraging experimental results, it was felt justified to undertake a pilot clinical study on a small group of patients with inoperable, localised pancreatic cancer, which was the first attempt to use PDT for the treatment of pancreatic cancer in humans.

It was decided to use the photosensitiser mTHPC as this gave the largest zone of necrosis around a single treatment fibre in the animal cancers and also because this drug requires the lowest light doses, which would mean a shorter treatment time. Therapeutic light was delivered via bare optical fibres placed through percutaneously inserted hollow needles, which technique was felt to be minimally invasive and feasible.

The aim of the clinical work was to assess technical feasibility, efficacy and safety of interstitial PDT for the treatment of inoperable pancreatic cancer.

We were however aware of the drawbacks of mTHPC, namely a long period of skin photosensitivity and no therapeutic selectivity between pancreatic cancer and adjacent normal organs. ALA is another photosensitising agent, which was shown to have a considerable potential for the treatment of pancreatic cancer and the use of which could overcome limitations associated with mTHPC. ALA photosensitivity only lasts 1-2 days and ALA produced less damage to normal tissues surrounding the pancreas than other photosensitising drugs. Clinical studies (including our own experience in the treatment of pancreatic cancer) showed however, that at the maximum tolerated dose (60mg/kg) the PDT effect is too superficial. Therefore, was conducted experiments in parallel to the clinical study, to investigate a method of enhancing ALA PDT for pancreatic cancer by addition of the new orally active hydroxypyridinone iron chelator, CP94, to slow down the conversion of PPIX (the photoactive derivative of ALA) to haem.
Earlier studies demonstrated that CP94 could significantly augment ALA PDT, however no complete study (including complete iron chelator pharmacokinetics and assessment of PDT effect) was performed in the pancreatic or any tumour model. An implanted pancreatic cancer in hamsters was chosen as the animal experimental model, because it shares a close similarity to human disease and was used successfully in many of the earlier PDT studies. The first series of experiments were designed to establish how PPIX fluorescence in the pancreatic cancer and normal pancreas could be influenced by the addition of the iron chelator CP94. Based on the timing of the peak PPIX fluorescence, the next series of experiments were performed to determine whether addition of the iron chelator could increase the volume of PDT induced tumour necrosis. Safety and tolerability of the treatment were also assessed. The results of these studies could help to establish the possible role of PDT in the management of cancer of the pancreas and may allow for optimisation of the treatment, which could be of clinical value.
Chapter V. PDT for the treatment of inoperable pancreatic cancer- a pilot study

5.1 Introduction

Experimental studies (discussed in chapter 3) confirmed that PDT could necrose pancreatic cancer and produce a significant survival advantage without unacceptable side effects. Published results have shown that normal pancreas tolerates treatment very well and that pancreatitis is unlikely following PDT. Also anatomical structures adjacent to the pancreas like large vessels seemed to be resistant to PDT damage or heal safely following treatment. The only exception to this was the duodenum, which is much thinner in rodents than in humans, where perforations were observed (Nuutinen et al, 1991, Mrkvý et al, 1997).

Most animals, however, seemed to tolerate the treatment well and significant survival benefit has been demonstrated in the animals with transplanted pancreatic cancer treated with PDT (Regula et al, 1994). Results of these experimental works suggest that photodynamic therapy could be the first palliative treatment for pancreatic carcinoma showing meaningful impact on the survival.

Therefore, considering the possible benefit of PDT, it was found to be justifiable to perform a clinical pilot study to assess the technical feasibility, efficacy and safety of PDT for the treatment of inoperable pancreatic cancer.

It was decided to use the photosensitiser mTHPC as this gave the largest area of necrosis around a single treatment site in the animal cancers and required the shortest treatment time.
5.2 Methods

5.2.1. Patients
Patients diagnosed with pancreatic cancer, who were referred to the pancreatic and biliary service in the Middlesex Hospital, London provided the selection population for the study. Patients were assessed on the basis of dual-phase contrast enhanced spiral CT, ERCP, and in some cases MR and reviewed by a pancreatobiliary surgeon. Only patients denied potentially curative surgery owing to tumour unresectability or high risk of co-morbidity were considered for PDT. All eligible patients had to have histological or cytological confirmation of the pancreatic adenocarcinoma. Patients with evidence of liver or more distant (e.g. pulmonary) metastases were not accepted, but patients with locally advanced disease and with enlarged abdominal lymph nodes were considered suitable. However, the main bulk of the tumour had to be localised to the pancreas and extensive extrapancreatic (especially retroperitoneal) spread of the tumour was an exclusion criteria. If the patient presented with obstructive jaundice, this had to be relieved by an endoprosthesis prior to inclusion into the study.
Patients were required to have Karnofsky Performance Status of over 60% and an estimated life expectancy of at least 3 months.
Eligible patients had to be over 18 years old, not pregnant or breast-feeding, had not received any previous specific treatment for cancer and were able to attend for follow-up assessment. The presence of any disease, which could be exacerbated by light (e.g. porphyria), was an exclusion criteria for PDT.
The study was approved by the local ethical committee and all treated patients gave written informed consent.

5.2.2. Photodynamic therapy

Photosensitiser
The photosensitiser used was meta-tetrahydroxyphenyl chlorin (mTHPC, Foscan, Scotia Pharmaceuticals, Stirling, UK), supplied as dark crystals which are reconstituted in a dedicated solvent containing polyethylene glycol, ethanol and water 1 hour prior to use. Drug was administered by slow intravenous injection of a single dose of 0.15mg/kg body weight 3 days prior to light delivery.
Administration of drug usually caused slight pain at the site of injection at the time of drug injection. This pain did not persist or cause anxiety if the patient was forewarned.

Following injection of the m-THPC, patients stayed in a side-room with subdued light to avoid skin photosensitivity reactions. For the first 24 hours the level of room light was kept under 100lux (equivalent to a single 60W bulb). On each subsequent day the light exposure could be increased by 100lux so by day 3 low level of indoor lighting was acceptable and by day 7 patient could tolerate normal indoor lighting. During this period, however, any exposure to direct sunlight was not safe and patients remained indoors or went outside after dusk.

If light exposure more intense than recommended was unavoidable; all areas of skin and eyes had to be shielded.

After one week patients were able to go out of doors on dull days but not on bright sunny days. All patients were advised strongly to avoid intense light sources (sunbeds, eye examination, and strong direct sunlight) for at least one month and preferably up to three months. Patients received detailed instruction about light exposure and were provided with a light meter.

**Light delivery**

The treatment was undertaken 3 days following the injection of m-THPC. Patients were kept nil by mouth for at least 6 hours and given IV fluids before the treatment. Routine blood tests, which included full blood count (FBC), clotting screen, liver function tests (LFT’s), serum amylase, urea and electrolytes (U&E) were checked before commencing the light delivery.

Due to the risk of the skin photosensitivity reactions the treatment was carried out under subdued light conditions.

Patients were sedated with midazolam or diazepam plus pethidine and prophylactic antibiotics (azlocillin or ciprofloxacin) were administered prior to the procedure. Metoclopramide as an antiemetic drug was also routinely given.

Following anaesthesia of the abdominal wall by the injection of 1% lignocaine, 4 or 6 19G needles (William Cook A/E Europe) were inserted into the deepest part of the tumour under ultrasound and CT guidance by a radiologist, using aseptic technique. Needles were placed within the tumour in a box configuration with their tips separated by 1-2cm (Photo 1).
**Fig. 7.** Needles placement. CT scan taken during needle insertion. Arrows show needles positioned in the tumour (Photo A). Photo B, corresponding topogram. Green arrow shows plastic biliary stent.
When the needles were satisfactorily positioned as determined by CT, a bare optical fibre, 0.4-0.6mm in diameter (Diomed Ltd, Cambridge, UK) was passed down to the tip of the each needle (Fig.8) such that approximately 3mm of the fibre protruded from the needle and had direct contact with the tumour.

Fig.8. PDT treatment. Bare optical fibres passed down the needles to the tumour.

The therapeutic light at a wavelength of 652+/-2 nm was produced by a diode laser (Applied Optronics Corporation, New Jersey, USA). Light was divided equally between up to 4 fibres using the beam splitter (Diomed Ltd, Cambridge, UK). The laser power output was calibrated to give 100mW at the tip of each fibre and light, (for which the energy dose varied from 20 to 40J between patients), was delivered via each fibre to the tumour (Fig. 9).

The treatment was fractionated, with a 150s break following administration of 20% of the calculated light dose. After the initial sites were illuminated, the needles with fibres could be pulled back approximately 1 cm under CT control as required to irradiate the entire tumour and the treatment was repeated with the same light doses. The number of needles and treatment sites depended on the tumour size and its position.
Fig. 9. PDT treatment. Red low power light delivered to the tumour via thin optical fibres.

During the procedure the patient’s heart rate, blood pressure and blood saturation were closely monitored and repeated doses of the sedative and analgesic drugs were administered as required. Oxygen via a facemask was routinely provided. All laser procedures were performed in accordance with the local laser safety rules as laid down by the hospital laser protection advisor.

**Follow-up**

Following the light delivery patients were carefully monitored on the ward. Heart rate, blood pressure, temperature, saturation and urine output were checked every few hours. If required, patients had a urine catheter placed.

Post-procedure pain was controlled by opiates. Patients were kept nil by mouth on intravenous fluids until the bowel sounds returned. Antibiotics were administered prophylactically for about 5 days.

Blood tests including FBC, LFT’s, amylase, U&E were repeated every few days or as necessary.
An early follow-up dual-phase contrast enhanced spiral CT scan was performed 2-5 days after the treatment and flexible duodenoscopy or ERCP, were done about a week after light delivery. Subsequent CT scans and endoscopies were scheduled for 1 month after the treatment and then as clinically indicated.

Glucose tolerance test and pancreolauryl tests were performed in several patients before and 1 month after PDT routinely, in the others only if this was clinically required.

Karnofsky Performance Status (KPS, appendix 1) was assessed before the treatment and 1 and 3 months after PDT. Analgesics requirement was recorded retrospectively from patients’ medical notes. Days of hospitalisation were calculated from the medical records.

Survival following the treatment was calculated from the date of PDT. The date of first test giving the diagnosis of pancreatic cancer was used as a proxy for the date of diagnosis in calculating survival from diagnosis.

All patients were closely followed for the remainder of their life, by subsequent visits in the hospital depending on their clinical condition or by telephone or written contact with patients or their local medical team. Subsequent interventions, which were necessary due to complications related to the treatment or progression of the disease, were undertaken in the Middlesex or referring hospitals.

Several patients, who wished were considered for chemotherapy especially if signs of the disease progression were found on the follow-up examinations.
5.3. Results

5.3.1 Patients selection

16 patients, 10 men and 6 women, aged 46 to 77 (mean 66) with pancreatic cancer met the criteria for PDT and agreed to take part in the study. All presented with obstructive jaundice, which was relieved by the placement of a biliary endoprosthesis before inclusion into the study. Associated symptoms were weight loss (11), abdominal pain (7), diabetes (6), diarrhoea (1) and anorexia (1). In all cases tumours were confirmed on biopsy or cytology as adenocarcinoma.

15 patients had carcinoma predominantly in the head of the pancreas, which in 2 cases arose from the uncinate process and in 2 others from the neck of the gland. 1 patient probably presented with ampullary cancer as the substantial bulk of the tumour was initially localised in the ampulla of Vater.

All patients were inoperable. At the time of diagnosis, in 13 cases, tumours were unresectable due to cancer involvement of the adjacent great vessels (superior mesenteric vein, superior mesenteric artery or portal vein). 3 patients had smaller lesions, which seemed to be amenable for potentially curative resection at the time of initial assessment but were unfit for major surgery.

Demographic data on patients and presenting symptoms are summarised in Table 3, biopsy results are given in Table 4.

The time from diagnosis to first PDT varied from 1 to 5 months, median 2.5 months and most patients showed some degree of disease progression since their initial evaluation.

On the basis of the most recent imaging tests before PDT, 3 patients had stage I, 7 stage II and 5 stage III disease, (data not available on one patient) at the time of PDT. Tumours were from 2.5 to 6 cm (mean 4.2 cm) in the maximum diameter as measured on CT scan.
Table 3. Demographic data and presenting symptoms.

<table>
<thead>
<tr>
<th>Age and sex</th>
<th>Presenting symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>70/M</td>
<td>Obstructive jaundice</td>
</tr>
<tr>
<td>66/M</td>
<td>Obstructive jaundice</td>
</tr>
<tr>
<td>62/F</td>
<td>Obstructive jaundice, weight loss, abdominal pain, diabetes</td>
</tr>
<tr>
<td>77/F</td>
<td>Obstructive jaundice, weight loss, diabetes</td>
</tr>
<tr>
<td>67/F</td>
<td>Obstructive jaundice, diabetes</td>
</tr>
<tr>
<td>65/M</td>
<td>Obstructive jaundice, weight loss, abdominal pain, lethargy</td>
</tr>
<tr>
<td>77/F</td>
<td>Obstructive jaundice</td>
</tr>
<tr>
<td>75/M</td>
<td>Obstructive jaundice, diabetes</td>
</tr>
<tr>
<td>59/M</td>
<td>Obstructive jaundice, weight loss, abdominal pain, diabetes</td>
</tr>
<tr>
<td>74/M</td>
<td>Obstructive jaundice, weight loss</td>
</tr>
<tr>
<td>55/M</td>
<td>Obstructive jaundice, weight loss</td>
</tr>
<tr>
<td>75/M</td>
<td>Obstructive jaundice</td>
</tr>
<tr>
<td>46/F</td>
<td>Obstructive jaundice, weight loss, abdominal pain, steathorrea</td>
</tr>
<tr>
<td>61/M</td>
<td>Obstructive jaundice, weight loss, abdominal pain</td>
</tr>
<tr>
<td>46/F</td>
<td>Obstructive jaundice, weight loss, abdominal pain, diabetes, anorexia</td>
</tr>
<tr>
<td>74/M</td>
<td>Obstructive jaundice, weight loss, abdominal pain</td>
</tr>
</tbody>
</table>

Most patients had visible regional lymph nodes on pre-treatment CT scans, which could be inflammatory related to the insertion of the biliary stent, however, lymph nodes larger than 1 cm in maximum diameter were considered as metastatic and were present in 5 patients. 12 patients had involvement of the duodenum on CT and 6 of them were found to have abnormalities in the duodenum on endoscopy before PDT. Duodenal stenosis or distortion due to cancer invasion was found in two patients. In one of them placement of a duodenal metal stent prior to PDT was necessary to relieve symptoms of gastric outlet obstruction. 4 others had an ulcerated or abnormal ampulla.

14 patients had obvious involvement of the adjacent great vessels at the time of PDT and 5 also showed signs of invasion of the soft peripancreatic tissues by cancer on the spiral CT. Malignant stenosis of the common bile duct was visible in all patients on ERCP. Pre-treatment maximum diameter of the tumour and TNM stage of disease is given in Table 4.

Clinically at the time of PDT patients were relatively fit and all patients had Karnofsky performance status of 80%, or better showing only minor signs and symptoms of the disease.
Table 4. Pre-treatment stage of disease and biopsy/cytology results. Note-tumour size was measured on the pre-treatment CT scan and the maximum diameter is expressed in millimetres. Stage of disease was assessed using UICC TNM classification from 1987 (appendix 2). N/A-data not available.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Maximum tumour diameter (mm)</th>
<th>TNM classification</th>
<th>Stage of disease</th>
<th>Histological diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>25</td>
<td>T2N1M0</td>
<td>III</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>2.</td>
<td>50</td>
<td>T3N0M0</td>
<td>II</td>
<td>Adenocarcinoma (brush cytology)</td>
</tr>
<tr>
<td>3.</td>
<td>45</td>
<td>T3N0M0</td>
<td>II</td>
<td>Adenocarcinoma-moderately differentiated</td>
</tr>
<tr>
<td>4.</td>
<td>52</td>
<td>T3N1M0</td>
<td>III</td>
<td>Adenocarcinoma-moderately to poorly differentiated</td>
</tr>
<tr>
<td>5.</td>
<td>39</td>
<td>T3N0M0</td>
<td>II</td>
<td>Adenocarcinoma-poorly differentiated</td>
</tr>
<tr>
<td>6.</td>
<td>49</td>
<td>T3N1M0</td>
<td>III</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>7.</td>
<td>28</td>
<td>T2N0M0</td>
<td>I</td>
<td>Adenocarcinoma- with perineural invasion</td>
</tr>
<tr>
<td>8.</td>
<td>60</td>
<td>T3N1M0</td>
<td>III</td>
<td>Adenocarcinoma-poorly differentiated</td>
</tr>
<tr>
<td>9.</td>
<td>40</td>
<td>T3N0M0</td>
<td>II</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>10.</td>
<td>45</td>
<td>T2N0M0</td>
<td>I</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>11.</td>
<td>30</td>
<td>T3N0M0</td>
<td>II</td>
<td>Adenocarcinoma cells-highly suspicious on brush cytology</td>
</tr>
<tr>
<td>12.</td>
<td>40</td>
<td>T3N1M0</td>
<td>III</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>13.</td>
<td>57</td>
<td>T3N1M0</td>
<td>III</td>
<td>Adenocarcinoma-moderately differentiated with focal perineural invasion</td>
</tr>
<tr>
<td>14.</td>
<td>30</td>
<td>T3N0M0</td>
<td>II</td>
<td>Adenocarcinoma- well differentiated, infiltration of the small intestine</td>
</tr>
<tr>
<td>15.</td>
<td>49</td>
<td>T3N0M0</td>
<td>II</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>16.</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Adenocarcinoma- moderately differentiated</td>
</tr>
</tbody>
</table>
5.3.2 Treatment conditions

15 patients had initial PDT treatment using m-THPC as a photosensitiser and only one using ALA. As the ALA PDT failed to induce any significant tumour necrosis, PDT was repeated 5 weeks later in this patient using m-THPC. 2 other patients had PDT performed twice and one patient three times. Secondary treatments were undertaken to destroy tumours remaining after the first PDT (2 cases) or for recurrent tumour (2 cases) in those amenable for laser treatment. All treatments but one were done percutaneously using bare fibres under ultrasound and CT guidance. The exception to this was the patient with a periampullary tumour. His first percutaneous PDT missed the main bulk of tumour as the needles slipped back after insertion and the treated area was predominately normal pancreas at the side of the cancer.

In a view of the location of this cancer, PDT was repeated several weeks later endoscopically by inserting a diffuser fibre into the distal common bile duct.

The number of treatment sites varied from 4 to 16 depending on the tumour size and position. Delivered light energy per fibre was 20 J in 13 patients and 25, 30 and 40J in the 3 others. The total light energy ranged from 80 to 480 J.

5.3.3 General response to PDT

Treatment was reasonably well tolerated and there were no treatment-related deaths or clinical or biochemical evidence of acute pancreatitis. All patients had abdominal pain, which usually appeared during light delivery, requiring administration of opiate analgesia after PDT.

Other early side effects of the treatment included mild fever, nausea and vomiting and paralytic ileus; however patients’ condition improved rapidly and usually all these symptoms settled within 48 hours. Most patients were able to resume oral intake after about 48 hours and all except two commenced a virtually normal diet by about one week after the treatment.

Biochemical tests usually showed only minor alterations after the treatment. The pre-treatment and maximum post treatment serum amylase values are shown in Table 5.
Table 5. Serum amylase following percutaneous PDT. Note. Asterix marks patients who had more than one percutaneous PDT with mTHPC.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre-PDT amylase</th>
<th>Maximum post-PDT amylase</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>140</td>
<td>90</td>
<td>70-300</td>
</tr>
<tr>
<td>2.</td>
<td>49</td>
<td>61</td>
<td>70-300</td>
</tr>
<tr>
<td>3.</td>
<td>263</td>
<td>582</td>
<td>70-300</td>
</tr>
<tr>
<td>4.</td>
<td>84</td>
<td>77</td>
<td>70-300</td>
</tr>
<tr>
<td>5.</td>
<td>262</td>
<td>119</td>
<td>70-300</td>
</tr>
<tr>
<td>6.</td>
<td>135</td>
<td>544</td>
<td>70-300</td>
</tr>
<tr>
<td>7.</td>
<td>78</td>
<td>75</td>
<td>70-300</td>
</tr>
<tr>
<td>8.</td>
<td>419</td>
<td>828</td>
<td>70-300</td>
</tr>
<tr>
<td>9.</td>
<td>53</td>
<td>160</td>
<td>0-100</td>
</tr>
<tr>
<td>10*.</td>
<td>43</td>
<td>31</td>
<td>0-100</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>8</td>
<td>7</td>
<td>0-100</td>
</tr>
<tr>
<td>12.</td>
<td>53</td>
<td>85</td>
<td>0-100</td>
</tr>
<tr>
<td>13.</td>
<td>32</td>
<td>33</td>
<td>0-100</td>
</tr>
<tr>
<td>14.</td>
<td>29</td>
<td>29</td>
<td>0-100</td>
</tr>
<tr>
<td>15.</td>
<td>47</td>
<td>292</td>
<td>0-100</td>
</tr>
<tr>
<td>16*.</td>
<td>33</td>
<td>41</td>
<td>0-100</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

The highest increase in amylase was less than 3 times above the normal limit and only occurred in two patients. One of them was the patient in whom the fiber slipped and predominantly the normal pancreas was treated. The other had an ERCP on the day of PDT. However, even these patients tolerated PDT reasonably well and did not develop clinical signs of pancreatitis. In every case elevated amylase values came back to normal within 7 days.
Following the treatment there was a mild and transient rise of the liver function tests (usually alkaline phosphatase) in 7 cases, observed in patients with a metal biliary stent. This may be explained by the temporary, post treatment oedema of the tissues in the common bile duct, which could slightly impair bile outflow. However, this has never required any specific therapy and all tests came back to pre-treatment values within the first week. Mild post treatment leukocytosis also returned to normal during the first post treatment week.

One patient developed acute renal failure after PDT. Inadequate fluid therapy in this cachexic (30 kg), severely diabetic patient probably led to this complication, which responded to conventional treatment.

All patients except two were discharged from hospital one week following light delivery. For those 14 patients the median hospital stay was 7 days after PDT (range; 5-9 days). Two patients who developed major complications (bleeding and acute renal failure) remained in the hospital for 26 and 30 days respectively.

5.3.4. Pancreatic exocrine and endocrine function following PDT

14 patients were diabetic before PDT, although only 4 required oral hyypoglycaemics and none needed insulin. 6 had significantly elevated fasting and/or random blood glucose levels and in 8 cases diabetes was diagnosed on the basis of the glucose tolerance test (GTT) performed prior to treatment. Only one had definitely normal pancreatic endocrine function before PDT (one patient was not fully assessed). Based on the results of the post-treatment GTT, 4 of 8 diabetic patients had only impaired glucose tolerance. One patient remained diabetic, however her blood glucose values were reduced (she never required other than diet treatment). 3 others had not GTT performed during follow-up, two of them were diabetic on the basis of the fasting or random glucose values but one seemed to maintain normoglycaemia during his lifetime (random blood glucose were never higher than 10 mmol/L). None of these patients required other than diet, diabetic specific treatment nor developed any problems, which could be related to diabetes during their lifetimes.
Patients who were obviously diabetic before treatment had more varied response to PDT. It was possible to withdraw medication for one patient (however, significantly reduced oral intake due to duodenal obstruction may have also played an important role), substantially reduce the drug dose in the other or maintain normoglycemia on oral antidiabetic agent in one other. Another patient who was diabetic but not on medication prior PDT seemed to have his diabetes exacerbated by PDT, however this patient inadvertently received treatment of mainly normal pancreas. After a second more successful PDT his diabetes was reasonably well controlled on the oral antidiabetic drugs. Two more patients required more aggressive antidiabetic treatment after PDT, however this seemed to be poorly related to PDT. One patient needed insulin, but partly in response to her feeding regime and despite that her diabetes was extremely difficult to control; the other patient started taking an oral hypoglycaemic drug a year after PDT. Patients' pancreatic endocrine functions before and after PDT are summarised in the Table 6.

Before PDT 4 patients had mild symptoms (transient diarrhoea) of pancreatic exocrine insufficiency. However, 9 of 10 patients had significantly reduced pancreatic exocrine function, assessed by the pancreolauryl test prior to PDT. After PDT 2 patients developed diarrhoea due to pancreatic insufficiency. 3 patients, who were symptomatic before PDT, had more severe diarrhoea after treatment. In 5 patients who had a pancreolauryl test performed before and after PDT, 2 had lower pancreolauryl ratio after PDT, while in the other 3 it remained stable. Pancreatic enzyme supplementation was given to symptomatic patients and to 6 others as they had reduced function on a pancreolauryl test although did not complain of diarrhoea. Data on patients' pancreatic exocrine function and applied treatment is given in Table 7.
Table 6. Pancreatic endocrine function before and after PDT.
Diabetes is present when fasting glucose > 6.7mmol/l and/or post prandial glucose >10mmol/l. Impaired glucose tolerance is present when fasting glucose <6.7 and post prandial is from 6.7 to 10mmol/l. Glucose tolerance test was performed approx. 1 month after PDT. (IGT-impaired glucose tolerance, F-fasting glucose, PP-post prandial glucose).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre-PDT glucose tolerance (glucose tolerance test- mmol/l)</th>
<th>Pre-PDT pharmacological treatment</th>
<th>Post-PDT glucose tolerance (glucose tolerance test-mmol/l)</th>
<th>Post-PDT pharmacological treatment/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Diabetes</td>
<td>Oral</td>
<td>Unstable diabetes</td>
<td>Insulin- partly due to feeding regime, poor pt complice</td>
</tr>
<tr>
<td>2.</td>
<td>Diabetes</td>
<td>hypoglycaemics</td>
<td>Diabetes</td>
<td>Oral hypoglycaemics after second PDT</td>
</tr>
<tr>
<td>3.</td>
<td>Diabetes (F-5.9,PP-13.5)</td>
<td>Nil</td>
<td>IGT (F-5.1, PP-9.3)</td>
<td>Nil</td>
</tr>
<tr>
<td>4.</td>
<td>Diabetes</td>
<td>Nil</td>
<td>Normal (but no GTT)</td>
<td>Nil</td>
</tr>
<tr>
<td>5.</td>
<td>Diabetes (F-6.7, PP-10.8)</td>
<td>Oral</td>
<td>IGT (F-5.9, PP-9.9)</td>
<td>Nil</td>
</tr>
<tr>
<td>6.</td>
<td>Diabetes</td>
<td>hypoglycaemics</td>
<td>Diabetes</td>
<td>Oral hypoglycaemics- started 1 year after PDT</td>
</tr>
<tr>
<td>7.</td>
<td>Diabetes (F-6.7, PP-13.1)</td>
<td>Nil</td>
<td>IGT (F-6.6, PP-9.7)</td>
<td>Nil</td>
</tr>
<tr>
<td>8.</td>
<td>Diabetes (F-5.5, PP-15.2)</td>
<td>Nil</td>
<td>IGT (F-5.4, PP-8.5)</td>
<td>Nil</td>
</tr>
<tr>
<td>9.</td>
<td>Normal (F-6.8, PP-4.5)</td>
<td>Nil</td>
<td>Normal (F-4.6, PP-5.8)</td>
<td>Nil</td>
</tr>
<tr>
<td>10.</td>
<td>Diabetes</td>
<td>Nil</td>
<td>Diabetes</td>
<td>Oral hypoglycaemics- well controlled</td>
</tr>
<tr>
<td>11.</td>
<td>Diabetes</td>
<td>Nil</td>
<td>Diabetes</td>
<td>Oral hypoglycaemics, one drug only, reduced dose</td>
</tr>
<tr>
<td>12.</td>
<td>Diabetes (F-6.2, PP-12.5)</td>
<td>Oral</td>
<td>Diabetes (F-7)</td>
<td>Nil- random glucose never higher than 10</td>
</tr>
<tr>
<td>13.</td>
<td>Diabetes (F-7.3, PP-14.3)</td>
<td>hypoglycaemics</td>
<td>Diabetes</td>
<td>Nil</td>
</tr>
<tr>
<td>14.</td>
<td>NK (F-5.1)</td>
<td>Oral</td>
<td>Diabetes (F-5.6, PP-14.1)</td>
<td>Nil</td>
</tr>
<tr>
<td>15.</td>
<td>Diabetes (F-6.3, PP-18.1)</td>
<td>hypoglycaemics</td>
<td>Diabetes (F-5.6, PP-14.1)</td>
<td>Nil</td>
</tr>
<tr>
<td>16.</td>
<td>Diabetes (F-5.6, PP-13.1)</td>
<td>Nil</td>
<td>Normal (but no GTT)</td>
<td>Nil, random glucose never higher than 10mmol/l, reduced oral intake</td>
</tr>
</tbody>
</table>
Table 7. Pancreatic exocrine function before and after PDT.
Pancreolauryl test was performed before and aprox. 1 month after PDT. Pancreatic insufficiency is present where pancreolauryl ratio is < 20%.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Before PDT</th>
<th>After PDT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symptoms</td>
<td>Pancreolauryl ratio</td>
</tr>
<tr>
<td>1.</td>
<td>No</td>
<td>---</td>
</tr>
<tr>
<td>2.</td>
<td>No</td>
<td>---</td>
</tr>
<tr>
<td>3.</td>
<td>Intermittent diarrhoea</td>
<td>2.4%</td>
</tr>
<tr>
<td>4.</td>
<td>No</td>
<td>14%</td>
</tr>
<tr>
<td>5.</td>
<td>No</td>
<td>---</td>
</tr>
<tr>
<td>6.</td>
<td>No</td>
<td>8%</td>
</tr>
<tr>
<td>7.</td>
<td>No</td>
<td>31.2%</td>
</tr>
<tr>
<td>8.</td>
<td>Intermittent diarrhoea</td>
<td>14.8%</td>
</tr>
<tr>
<td>9.</td>
<td>No</td>
<td>9.5%</td>
</tr>
<tr>
<td>10.</td>
<td>No</td>
<td>---</td>
</tr>
<tr>
<td>11.</td>
<td>No</td>
<td>11.8%</td>
</tr>
<tr>
<td>12.</td>
<td>No</td>
<td>3.1%</td>
</tr>
<tr>
<td>13.</td>
<td>No</td>
<td>7%</td>
</tr>
<tr>
<td>15.</td>
<td>Intermittent diarrhoea</td>
<td>7.9%</td>
</tr>
<tr>
<td>16.</td>
<td>No</td>
<td>---</td>
</tr>
</tbody>
</table>
5.3.5 Tumour response

In all patients spiral dual-phase CT scans taken a few days after PDT showed a new non enhancing zone in the pancreas corresponding to the treated area. (Fig 10). A biopsy obtained from this field in the first patient showed necrotic cancer (Fig. 11).

**Fig. 10. Tumour response- CT.** CT before treatment (Photo A). Tumour showed by arrows. CT scan of the same patient 6 days after PDT (Photo B). New non-enhancing zone in the treated area showed by arrows. Biliary stent in situ.

**Fig. 11. Tumour response- histology.** Pre-treatment biopsy showing pancreatic adenocarcinoma (Photo A). Biopsy performed 4 days after PDT (Photo B) showing pancreatic tissue with the extensive necrosis and residual glandular structures lined by neoplastic epithelium and prominent fibroblast cells. This picture may correspond to the necrotic tumour and would be consistent with PDT damage.
The biopsy however was uncomfortable and caused a small haematoma. This resolved spontaneously but considering the risk of the procedure it was not justifiable to undertake post PDT biopsies in other patients. The new non-enhancing areas in duodenum were also consistent with tissue necrosis as confirmed on endoscopy. Therefore, new non-enhancing areas in the pancreas were interpreted as zones of PDT induced necrosis, despite the lack of the histological proof in the remaining patients.

Assuming roughly spherical geometry of the PDT induced necrosis, the volume of the necrosis was calculated using the formula of ellipsoid (\(\pi abc/6\), where a, b, c were the width, length and depth of the non-enhancing area measured on the post treatment CT scan) and ranged from 9.0 to 60.0 cm\(^3\) (median 36 cm\(^3\)).

The volume of necrosis produced by separate fibre sites (averaged for individual patients) varied from 1.4 to 5.1 cm\(^3\) (median 2.9 cm\(^3\)) and the median radius of PDT necrosis around individual fibre tip was 9mm (range; 7-11mm). Spherical areas of necrosis, which were produced by the individual fibres and visible on CT in one patient, had a radius of 8-9mm, which was the same as that calculated above.

Pre-treatment tumours volumes, treatment conditions (total light energy and number of the treated sites) and volumes of the total and per fibre PDT volume of necrosis are shown in Table 8.

The estimated volumes of the PDT induced necrosis were larger than calculated tumour volume in 8 cases. However, no definitive cancer could be seen in the head of pancreas only in 3 patients on the early post PDT CT and in 3 others only tiny areas of probably viable cancer were found. The other patients had probably some cancer tissue left untreated, especially at the edges of the tumour e.g. around large vessels that are difficult to access.

PDT produced necrosis in the pancreatic (mainly tumour) tissue seemed to heal safely in all patients. There was no CT or ERCP evidence of a pseudocyst, abscess or pancreatic duct leak in any patient at any time after PDT. The main pancreatic duct was dilated in 11 patients prior to PDT and this was associated with atrophy of the body and tail in 7 cases. There is no evidence that PDT produced necrosis caused pancreatic duct obstruction or made this worse in any case. Also normal pancreas, which was accidentally treated in one patient, tolerated treatment extremely well, and was enhancing nearly normally 6 weeks after the PDT.
In 3 cases the overall size of the head of the pancreas shrunk as it healed presumably due to absorption of necrotic material. In other patients, the diameter of the pancreas did not change or slightly increased owing to post treatment oedema or tumour regrowth. Some inflammatory oedema around the head of the pancreas was visible on the early post-treatment scan in 10 patients and resolved spontaneously without any treatment usually within 1 month. Overall on the CT scan performed 3 moths after the treatment there was no visible necrotic, non-enhancing tissue in the pancreas nor any other related to the treatment inflammatory changes.

Over the course of the disease all patients had local recurrence of the disease with progression of cancer infiltration around the duodenum, large blood vessels, hilum, mesentery, retroperitoneum and with prominent lymphadenopathy. Tumour did not regrow from the site of the PDT necrosis but recurrence usually originated from the edges of the treated areas.

5 patients were found to have liver metastases, which in two cases were detected within 1 month after PDT. One patient was noted to develop two 1.5cm subcutaneous nodules in the anterior abdominal wall at the site of the previous needle placements for PDT, 4 months after treatment. Pathological examination revealed adenocarcinoma in the nodules, which probably represented needle tract cancer cell seeding. This was treated by local excision and subsequent chemoradiation and the patient died 8 months later (one year after PDT) having disseminated abdominal cancer.

One other patient developed painful supraclavicular, lymph node metastases approximately 14 months after PDT, which were treated palliatively with single course of radiotherapy. No other patient had documented metastases outside the abdomen.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre PDT tumour volume (cm$^3$)</th>
<th>No. of sites (No. of fibres)</th>
<th>Total energy (energy per site) (J)</th>
<th>Volume of necrosis (cm$^3$)</th>
<th>Volume of necrosis per site (cm$^3$)</th>
<th>Ratio: volume of necrosis to volume of tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>25</td>
<td>12 (4)</td>
<td>240 (20)</td>
<td>36</td>
<td>3.0</td>
<td>1.5</td>
</tr>
<tr>
<td>2.</td>
<td>63</td>
<td>16 (6)</td>
<td>320 (20)</td>
<td>51</td>
<td>3.2</td>
<td>0.8</td>
</tr>
<tr>
<td>3.</td>
<td>24</td>
<td>12 (4)</td>
<td>480 (40)</td>
<td>30</td>
<td>2.5</td>
<td>1.2</td>
</tr>
<tr>
<td>4.</td>
<td>a. 49</td>
<td>8 (4)</td>
<td>160 (20)</td>
<td>19</td>
<td>2.4</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>b. N/A</td>
<td>9 (4)</td>
<td>200 (25)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>5.</td>
<td>56</td>
<td>12 (4)</td>
<td>240 (20)</td>
<td>33</td>
<td>2.8</td>
<td>0.6</td>
</tr>
<tr>
<td>6.</td>
<td>43</td>
<td>8 (4)</td>
<td>160 (20)</td>
<td>52</td>
<td>6.5</td>
<td>1.2</td>
</tr>
<tr>
<td>7.</td>
<td>8.8</td>
<td>4 (4)</td>
<td>80 (20)</td>
<td>9.0</td>
<td>2.3</td>
<td>1.0</td>
</tr>
<tr>
<td>8.</td>
<td>a. 3.0</td>
<td>4 (4)</td>
<td>80 (20)</td>
<td>21</td>
<td>5.1</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td>b. 4.2 diff.</td>
<td></td>
<td>40 (20/cm)</td>
<td>14</td>
<td>N/A</td>
<td>3.4</td>
</tr>
<tr>
<td>9.</td>
<td>47</td>
<td>16 (6)</td>
<td>320 (20)</td>
<td>55</td>
<td>3.4</td>
<td>1.1</td>
</tr>
<tr>
<td>10.</td>
<td>29</td>
<td>12 (4)</td>
<td>240 (20)</td>
<td>60</td>
<td>5.0</td>
<td>2.1</td>
</tr>
<tr>
<td>11.</td>
<td>44</td>
<td>16 (4)</td>
<td>320 (20)</td>
<td>23</td>
<td>1.4</td>
<td>0.5</td>
</tr>
<tr>
<td>12.</td>
<td>13</td>
<td>16 (4)</td>
<td>320 (20)</td>
<td>39</td>
<td>2.4</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>12 (6)</td>
<td>240 (20)</td>
<td>35</td>
<td>2.9</td>
<td>2.5</td>
</tr>
<tr>
<td>13.</td>
<td>a. N/A</td>
<td>12 (4)</td>
<td>360 (30)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>b. 21</td>
<td>16 (6)</td>
<td>320 (20)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>c. 49</td>
<td>16 (4)</td>
<td>320 (20)</td>
<td>55</td>
<td>3.4</td>
<td>1.1</td>
</tr>
<tr>
<td>14.</td>
<td>14</td>
<td>8 (4)</td>
<td>160 (20)</td>
<td>36</td>
<td>4.5</td>
<td>2.6</td>
</tr>
<tr>
<td>15.</td>
<td>60</td>
<td>14 (4)</td>
<td>280 (20)</td>
<td>54</td>
<td>3.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>
5.3.6 Survival

The median survival time from diagnosis is 12.5 months (range; 6 to 34 months) and the survival time from PDT ranged from 4 to 30 months with a median of 10 months. 9 of 16 (56%) patients were alive a year after diagnosis and 7 of 16 (44%) survived more than a year after PDT. One patient is still alive at 19 months from diagnosis, 16 months from his first laser treatment.

3 patients received chemotherapy and one chemoradiotherapy after PDT. One patient responded very well and survived 8 months after starting chemotherapy. 3 others, however, seemed not to benefit significantly from chemotherapy and lived for less than 6 months following commencing adjuvant treatment. The patient, who had the shortest survival time, did not complete chemotherapy due to intolerable side effects. The median survival time of this group from PDT was 11.5 month (range; 7-14 months).

3 patients who had no visible cancer on the early scans following PDT, survived 16, 20 and 30 months (mean 22), none of them received chemotherapy following PDT. Two patients with the shortest survival time after treatment (4 and 4.5 months) had multiple liver metastases detected within first month after PDT.

The mean survival time after diagnosis and following PDT for patients with stage I cancers was 20 and 17.5 months respectively. Patients with stage II cancers had mean survival 13.5 months from diagnosis and 10.5 from PDT, mean survival for patients with stage III disease from diagnosis and time of laser therapy was 11 and 9 months respectively. Demographic data on patients, pre-treatment stage of the disease and survival time from diagnosis and PDT is summarised in Table 9.
Table 9. Survival after PDT. Note. Survival time is measured in months. Asterix marks patients who received adjuvant treatment following PDT. N/A-data not available.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Stage of disease</th>
<th>Survival time from diagnosis (months)</th>
<th>Survival time from first PDT (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>I</td>
<td>34</td>
<td>30</td>
</tr>
<tr>
<td>2.</td>
<td>II</td>
<td>8</td>
<td>4.5</td>
</tr>
<tr>
<td>3.</td>
<td>II</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>4.</td>
<td>III</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>II</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>6*</td>
<td>III</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>7.</td>
<td>I</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>8*</td>
<td>III</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>9.</td>
<td>II</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>10.</td>
<td>I</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>11.</td>
<td>II</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>12.</td>
<td>III</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>13.</td>
<td>III</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>14*</td>
<td>II</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>15*</td>
<td>II</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>16.</td>
<td>N/A</td>
<td>alive</td>
<td>alive</td>
</tr>
</tbody>
</table>

5.3. 7 Complications of PDT

**Bleeding**

5 patients developed mesenteric haematoma caused by the needle insertion immediately after laser treatment. All resolved spontaneously, however blood transfusion was required in two cases and in one of them blood loss was haemodynamically significant. 2 weeks later the same patient had life-threatening gastro-intestinal haemorrhage, which was successfully controlled by radiological embolisation of the gastroduodenal artery. The other serious gastro-intestinal bleed occurred 3 weeks after PDT from duodenal ulceration produced by the treatment. This was managed by endoscopic injection of epinephrine into the oozing site. In both these cases the gastroduodenal artery was clearly encased by the tumour, which also seemed to infiltrate the medial wall of the duodenum on the pre-treatment CT scan.
Both of these patients developed also an immediate haematoma after PDT and one of them received the highest energy dose (40 J per site). Both fully recovered. However, the patient who required artery embolisation developed duodenal obstruction a few weeks later, which was difficult to relieve (as discussed later).

There were 2 other gastrointestinal bleeds, which in one case required blood transfusion. That was caused by the tumour infiltrating the duodenum as identified on endoscopy and confirmed on biopsy. The other resulted from severe oesophagitis, which could be related to adjuvant radiochemotherapy. Both these events occurred in the late state of the disease (13 months after first and 14 weeks after third PDT in one patient and 10 months after PDT in the other), stopped spontaneously and did not return.

**Duodenal complications**

Prior to PDT, 12 patients had duodenum involved with cancer (mainly ampulla and the periampullary region) as assessed on the pre-treatment CT scans. 6 of them had abnormalities in the duodenal wall on the endoscopy before PDT. 4 patients had tumour or/and ulceration seen in the ampulla or periampullary region, in one case a haemorrhagic and distorted duodenum was found. One other patient had duodenal stenosis due to cancer infiltration and required a duodenal stent to relieve gastric outlet obstruction prior to PDT. 10 patients had a normal duodenum as assessed endoscopically prior to PDT, however CT scan on 6 of these individuals showed some degree of cancer infiltration into the duodenum, even though the mucosa appeared normal. Only 4 patients had duodenum free of cancer on CT and on the endoscopy prior to PDT.

On the early post treatment endoscopy performed within the first 4 weeks after PDT 7 patients (3 not assessed) had new areas of necrosis and in 4 changes were worse than before PDT (oedema and discoloration in 3 cases, frank ulcerations in 8) on the medial wall of duodenum, mainly in the periampullary region, only 2 has normal endoscopy.
In 3 patients this healed with a breakdown of the wall between the duodenum and common bile duct but there were no free duodenal perforations and none of these patients had symptoms from their fistula. In another patient a duodenal cavity (pseudodiverticulum) with a low CBD stricture appeared in place of the ampulla destroyed by the endoluminal PDT, which led to substantial technical difficulties in biliary stenting but did not produce any clinical symptoms. The other patient who had the ampulla destroyed by PDT, developed a periampullary stricture, which caused biliary obstruction.

2 patients had gastro-intestinal bleeding from the gastro-duodenal artery approx. 3 weeks after PDT (as discussed previously). One of them whose bleeding was controlled by embolisation of the artery developed a duodenal stricture (see below). The other had a virtually normal duodenum with only slight periampullary ulceration 3 months after PDT.

After PDT, 8 patients showed new endoscopically visible duodenal stenosis during their lifetimes. 4 cases were found during the first 6 weeks after PDT, 4 other strictures occurred up to 16 months after treatment.
5 of them were clinically symptomatic and required intervention. All were initially managed by the insertion of a self-expanding metal duodenal stent, which functioned satisfactorily in 3 cases. One patient who developed a tight stricture between the first and second part of the duodenum after bleeding and subsequent embolisation of gastroduodenal artery had to have two duodenal metal stents inserted to relieve gastric outlet obstruction. However, that functioned poorly and this patient was able to manage mainly fluids during her lifetime. She died 5 months later with symptoms of duodenal obstruction.

A problematic case was the patient with impending duodenal obstruction due to cancer infiltration prior to PDT. He required insertion of a duodenal stent 3 months after PDT but this failed to work satisfactorily for a longer time even though it was patent and correctly positioned. Even surgical gastrojejunostomy, which was subsequently performed, did not relieve his symptoms of obstruction nor did a percutaneous feeding jejunostomy tube.

In this group of 5 patients with symptomatic gastric obstruction, 3 cases seemed to be caused by cancer infiltration of the duodenum as confirmed on endoscopy, biopsy or CT scan.

In 2 of these, the obstruction occurred in the pre-terminal phase of their disease, 5 and 14 months after PDT and approximately 1 month before patient death.

In two other patients, however, no evidence of tumour progression into the gastrointestinal tract was visible on endoscopy and/or imaging examination. One of them was the patient with a periampullary tumour who had percutaneous and endoluminal PDT and presented with duodenal stenosis 10 months after PDT. That was successfully treated by placement of a duodenal metal stent. 16 months later he developed a second episode of obstruction, due to tumour ingrowth in his stent and angling of the stent into the wall of duodenum, which was relieved by insertion of an overlapping stent, which functioned satisfactorily until his death at 30 months.

The other was the patient who bled from the gastroduodenal artery. She developed a stricture at the site, where tumour invaded into the duodenum before PDT, however there was no proof that tumour progression or embolisation of the gastroduodenal artery led to this complication.
Two more patients had symptoms of delayed gastric emptying, which appeared shortly before their death. No endoscopy or treatment other than pharmacological treatment was applied.

One more patient had a malignant stricture of the duodenum, which required insertion of a duodenal stent before PDT. He developed a second episode of obstruction due to tumour ingrowth into the stent and stent angling 2 months after his first PDT, which was successfully treated by the insertion of an overlapping stent.

Appearance of the duodenum before PDT and the duodenal effect of PDT as assessed on CT and endoscopy, clinical symptoms and subsequent interventions are summarised in Table 10.
Table 10. Duodenal effect of PDT. D1, D2, D3- first, second and third part of duodenum respectively, G-D- gastro-duodenal artery, CBD- common bile duct. Asterix marks patient who had percutaneous and endoscopic PDT of periampullary cancer.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre-PDT</th>
<th>Early post-PDT effects (up to 4 weeks)</th>
<th>Late post-PDT effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endoscopy</td>
<td>CT</td>
<td>Endoscopy</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>Tumour approach with 7mm of mucosa above ampulla</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>Normal</td>
<td>Necrosis in D2 (no ulcer)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>Involvement of D1/D2</td>
<td>Necrosis in D2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>Normal</td>
<td>N/A</td>
</tr>
<tr>
<td>5</td>
<td>Ampullary tumour</td>
<td>Infiltration of ampulla</td>
<td>Inflammation around stent</td>
</tr>
<tr>
<td>Patient</td>
<td>Pre-PDT</td>
<td>Early post-PDT effects (up to 4 weeks)</td>
<td>Late post-PDT effects</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>---------------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td></td>
<td>Endoscopy</td>
<td>CT</td>
<td>Endoscopy</td>
</tr>
<tr>
<td>6.</td>
<td>Stenosed D2 duodenal stent in situ</td>
<td>Infiltration of D2 and D3</td>
<td>N/A</td>
</tr>
<tr>
<td>7.</td>
<td>Ulcerated ampulla</td>
<td>Infiltration of periampullary region, stricture D1-D2 on PTD</td>
<td>Necrosis in D2, duodenal stenosis in ?</td>
</tr>
<tr>
<td>8.</td>
<td>Normal</td>
<td>Identification into D2</td>
<td>Ulceration in D2?</td>
</tr>
<tr>
<td>9.*</td>
<td>Ulcerated ampullary tumour</td>
<td>Involvement of ampulla</td>
<td>N/A</td>
</tr>
<tr>
<td>10.</td>
<td>Normal</td>
<td>Infiltration of the periampullary region and D3</td>
<td>Oedema from duodenal cap to ampulla</td>
</tr>
<tr>
<td>Patient</td>
<td>Pre-PDT</td>
<td>Early post-PDT effects (up to 4 weeks)</td>
<td>Late post-PDT effects</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>----------------------------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td></td>
<td>Endoscopy</td>
<td>CT</td>
<td>Endoscopy</td>
</tr>
<tr>
<td>11.</td>
<td>Ulcerated duodenum and ampulla</td>
<td>Infiltration of ampulla</td>
<td>Ulceration of D1 to ampulla</td>
</tr>
<tr>
<td></td>
<td>4 months</td>
<td>Stenosis D1/D2</td>
<td>1 month</td>
</tr>
<tr>
<td>12.</td>
<td>Normal</td>
<td>Normal</td>
<td>Periampullary Necrosis</td>
</tr>
<tr>
<td>13.</td>
<td>Normal</td>
<td>Involvement of ampulla</td>
<td>Necrosis of D2</td>
</tr>
<tr>
<td>14.</td>
<td>Normal</td>
<td>Involvement of ventral part of duodenum</td>
<td>Duodenal necrosis</td>
</tr>
<tr>
<td>15.</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>16.</td>
<td>Hemorrhagic distorted duodenum</td>
<td>Involvement of pylorus, D1 and D2</td>
<td>Duodenal necrosis in D2</td>
</tr>
</tbody>
</table>
Biliary tract interventions after PDT

All patients presented initially with obstructive jaundice, which was caused by the malignant stenosis of the common bile duct. Jaundice was relieved prior to inclusion into the study by the placement of a biliary endoprosthesis. In 14 cases, a stent was inserted endoscopically, in 3 patients a combined endoscopic and percutaneous procedure was necessary. To avoid any problems with stent replacement after PDT, which might result from the post PDT scarring in the common bile duct and/or periampullary region, whenever practical plastic stents were replaced by self expanding metal stents before treatment. 9 patients had metal self-expanding biliary endoprosthesis and 7 plastic stent in situ at the time of PDT. The type of stent at the time of PDT and time from the stent placement to treatment is shown in table 10 as well as biliary interventions carried out after PDT.

10 patients had no symptoms of impaired biliary outflow or other biliary problems after PDT. Four of them (3 with a metal stent) required no further biliary interventions and maintained adequate biliary drainage during their lifetimes. 6 more patients showed no clinical signs of biliary obstruction and had a single, prophylactic procedure only. Two of them were noted to have some debris in their metal biliary stent at the time of the follow-up endoscopy and had it cleared by balloon trawling. One had a routine plastic stent change 11 months after PDT and 12 months after stent insertion. Two patients were found to develop a choledocho-duodenal fistula (Photo) that was clinically completely asymptomatic during their lifetimes: however, both these patients had their plastic stent replaced by a metal one prophylactically.

The last patient who had performed only a prophylactic biliary intervention was the patient who had his second PDT endoscopically with a diffuser fibre inserted through the ampulla. After PDT it was not possible to stent this patient due to oedema in the treated area and later he developed a duodenal pseudodiverticulum in place of the destroyed ampulla with a low stricture in the common bile duct, which was difficult to pass. Despite that he maintained adequate biliary drainage and was prophylactically stented 10 months later prior to insertion of an enteral stent for duodenal stenosis.
Fig. 13. Biliary complications after PDT. Clinically asymptomatic fistula between common bile duct and duodenum (arrow) seen at ERCP performed one month after PDT. At this procedure plastic biliary stent was replaced prophylactically by metal one.

Five patients (two with plastic and four with metal stent) developed clinically symptomatic biliary obstruction with jaundice and/or cholangitis after PDT. Metal stents had a mean patency time of 7 months (range; 3.5-12 months). Obstruction was caused by the tissue overgrowth into the stent (2 cases) or by tumour infiltration above the stented site (2 cases including one that obstructed shortly after PDT). Two of these patients had debris removed from the stent several times before it blocked and one patient also had also a low CBD-duodenal fistula.
Biliary blockage was relieved by endoscopic insertion of a plastic stent into the metal one (3 cases), which was followed in one patient by the placement of an overlapping metal stent. One patient with no endoscopic access due to duodenal stenosis required a percutaneous procedure. Subsequent plastic stent replacements (2 and 3 times) were necessary in two patients.

2 patients with plastic stents developed biliary obstruction 16 months and 6 weeks after PDT (16.5 and 3 months after stent placement, respectively). Cholangitis, in a patient who obstructed shortly after PDT may have been attributable to the post-PDT periampullary stricture.

This episode of blockage of bile outflow was relieved by replacing the plastic stent by a metal one, but further cholangitis 8 weeks later necessitated insertion of another plastic stent through the mesh of the metal one. This provided satisfactory drainage. In the second case a single change of the plastic stent ensured adequate drainage for the remainder of the patients' life. However, in both cases endoscopic stent replacement was technically difficult due to coexisting duodenal stenosis.

Biliary duct interventions are summarised in Table 11.
<table>
<thead>
<tr>
<th>No biliary intervention after PDT.</th>
<th>Patient</th>
<th>Stent type At PDT</th>
<th>Time from insertion of the latest stent to Intervention</th>
<th>Time from 1st PDT to first intervention</th>
<th>Biliary procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Plastic</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>None</td>
</tr>
<tr>
<td>2.</td>
<td>Metal</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>None</td>
</tr>
<tr>
<td>3.</td>
<td>Metal</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>None</td>
</tr>
<tr>
<td>4.</td>
<td>Metal</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>None</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Single prophylactic intervention</th>
<th>Patient</th>
<th>Stent type At PDT</th>
<th>Time from insertion of the latest stent to Intervention</th>
<th>Time from 1st PDT to first intervention</th>
<th>Biliary procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.</td>
<td>Metal</td>
<td>6 weeks</td>
<td>1 week</td>
<td>Stent trawled during f-up examination</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Metal</td>
<td>4 months</td>
<td>4 months</td>
<td>Stent trawled during f-up examination</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Plastic</td>
<td>12 months</td>
<td>11 months</td>
<td>Routine change of plastic stent</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Plastic</td>
<td>6.5 months</td>
<td>10 weeks</td>
<td>2nd PDT (endoluminal), no stent in situ left</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Plastic</td>
<td>3 months</td>
<td>6 weeks</td>
<td>10 months after 1st PDT metal biliary stent inserted prior to duodenal stent</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Plastic</td>
<td>2.5 months</td>
<td>6 weeks</td>
<td>CBD-duod. fistula, metal stent to replace plastic</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Metal</td>
<td>3.5 months</td>
<td>6 weeks</td>
<td>Plastic stent placed through metal</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Plastic</td>
<td>1.5 months</td>
<td>16 months</td>
<td>Plastic stent changed</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Plastic</td>
<td>3 months</td>
<td>6 weeks</td>
<td>Plastic stent replaced with metal</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Metal</td>
<td>10 months</td>
<td>5 months</td>
<td>Plastic stent placed through mesh of metal</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Metal</td>
<td>12 months</td>
<td>6 months</td>
<td>Plastic stent placed through metal</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>Metal</td>
<td>3.5 months</td>
<td>3.5 months</td>
<td>Plastic stent changed</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Intervention for jaundice and/or cholangitis.</th>
<th>Patient</th>
<th>Stent type At PDT</th>
<th>Time from insertion of the latest stent to Intervention</th>
<th>Time from 1st PDT to first intervention</th>
<th>Biliary procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.</td>
<td>Metal</td>
<td>3.5 months</td>
<td>6 weeks</td>
<td>Plastic stent placed through metal</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Plastic</td>
<td>1.5 months</td>
<td>16 months</td>
<td>Plastic stent changed</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Plastic</td>
<td>3 months</td>
<td>6 weeks</td>
<td>Plastic stent replaced with metal</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Metal</td>
<td>10 months</td>
<td>5 months</td>
<td>Plastic stent placed through metal</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Metal</td>
<td>12 months</td>
<td>6 months</td>
<td>Plastic stent changed</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>Metal</td>
<td>3.5 months</td>
<td>3.5 months</td>
<td>Plastic stent changed after 3rd PDT</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percutaneous plastic stent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percutaneous plastic stent</td>
</tr>
</tbody>
</table>
**Skin photosensitivity reactions**

Skin photosensitivity reactions occurred in 8 patients and were mainly located on the hands and in only one case on the face and neck. All of them developed during the first month following injection of the photosensitiser and were caused by exposure to sunlight in 6 cases. In three patients a small sunburn on the index finger resulted from prolonged pulse-oximeter monitoring during treatment and one patient developed approx. 1 cm blister on the abdomen (Fig. 14) from the laser fibre, which inadvertently lay on a small area of the uncovered skin during treatment. Cutaneous photoreactions were mild in 6 cases when only reddening of the skin was observed and moderate with blister development in two patients. They all healed well, usually with some skin peeling and in the most severe case only a tiny scar and decolourisation was visible afterwards. Usually no specific treatment was applied but in two cases ointment was used.

![Fig. 14. Skin photosensitivity reaction. A small blister (arrow) on the skin caused by the laser fibre, which laid accidentally on the abdomen during treatment](image-url)
5.3. Quality of life

All patients experienced clinical improvement following relief of the obstructive jaundice, which was the main presenting symptom of the disease. However, 7 patients had abdominal pain at the time of PDT and 5 of them were taking analgesics of appropriate strength (from morphine to acetaminophen). One more patient did not complain of abdominal pain but was taking non-steroidal anti-inflammatory drug regularly due to arthritis. 5 despite restoring satisfactory biliary drainage continued to lose weight. Other cancer related symptoms were diarrhea, diabetes and vomiting as discussed in the other sections. All symptoms of the disease were however, relatively minor and did not have a major negative influence on the patients’ performance status. Prior to PDT, all patients led a virtually normal life and had Karnofsky performance status of 80-100%.

PDT was well tolerated and all except 2 patients were discharged from hospital less than 10 days after PDT with a satisfactory oral intake and feeling comfortable. 1 month after PDT, all except 3 patients were back to their pre-treatment KPS of 80-100%.

The 2 patients whose KPS decreased significantly were one of the 2 who had life-threatening bleeding shortly after PDT who subsequently developed symptomatic, difficult to relieve duodenal obstruction, which had a major negative influence on her quality of life at that time, and the one who developed cancer-related gastric outlet obstruction. One of the two patients who experienced the major haemorrhage within 1 month following PDT fully recovered (KPS 100% 3 month after PDT).

3 months after PDT 10 patients had a KPS of 80-100%, two with the poorest performance developed symptomatic gastric outlet obstruction and the other 4, whose conditions deteriorated at that time, experienced pain or other cancer related symptoms.

The pre-treatment and post PDT KPS are summarised in Table 12.
Table 12. Karnofsky performance status (KPS %) before and after PDT.
(N/A-data not available).

<table>
<thead>
<tr>
<th>Patient</th>
<th>KPS (%) before PDT</th>
<th>KPS (%) 1 month after PDT</th>
<th>KPS (%) 3 months after PDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>100</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>2.</td>
<td>80</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>3.</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4.</td>
<td>100</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>5.</td>
<td>90</td>
<td>100</td>
<td>70</td>
</tr>
<tr>
<td>6.</td>
<td>90</td>
<td>90</td>
<td>50</td>
</tr>
<tr>
<td>7.</td>
<td>100</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>8.</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>9.</td>
<td>100</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td>10.</td>
<td>100</td>
<td>90</td>
<td>N/A</td>
</tr>
<tr>
<td>11.</td>
<td>90</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>12.</td>
<td>100</td>
<td>N/A</td>
<td>90</td>
</tr>
<tr>
<td>13.</td>
<td>100</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>14.</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>15.</td>
<td>90</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>16.</td>
<td>100</td>
<td>100</td>
<td>90</td>
</tr>
</tbody>
</table>

Days of hospitalisation at this hospital (including the admission for PDT and for other subsequent procedures due to complications of treatment or progression of the disease but excluding admissions for chemotherapy or for terminal care) amounted to 5% to 38% of patients survival time (mean 45 days). The median was 11% and only 3 patients spent more than 20% of their survival time in hospital. All patients required at least one more hospitalisation for other than follow-up purpose during their lifetimes.

However, they usually felt relatively comfortable at home until the problem arose which was most frequently jaundice or less frequently duodenal obstruction, which was typically managed during a short hospital stay.
During the terminal phase of the disease patients were under the care of the palliative care team and spent more time in hospices; two who had also the long hospitalisation time had repeated, longer admissions to hospices for respite care during their lifetimes.

PDT did not reduce analgesic consumption. 1 month after PDT all patients who were taking analgesics before treatment continued to use them, 3 requiring drug of the strongest analgesic potency. 3 more patients who did not receive analgesics before PDT, had increased pain and were administered dihydrocodeine and paracetamol. 3-4 months after PDT 10 patients were taking analgesics (data not available on 1 patient), 8 of them opiates. Later in the course of the disease most patients had pain, which could be controlled only by opiates. Analgesics required before and after PDT (1 and 3-4 months following treatment) are given in Table 13.

Table 13. Analgesics requirement before and after PDT. Coproxamol consists of Dextropropoxyphene hydrochloride and paracetamol, Co-dydramol consists of dihydrocodeine tartrate and paracetamol, DF118- dihydrocodeine tartrate, dicl.- diclofenak

<table>
<thead>
<tr>
<th>Patient</th>
<th>Analgesia Pre-PDT</th>
<th>Analgesia 1 month after PDT</th>
<th>Analgesia 3-4 months after PDT</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil at 10 months</td>
</tr>
<tr>
<td>2.</td>
<td>Nil</td>
<td>Coproxamol</td>
<td>Coproxamol</td>
<td>Morphine at 6/12</td>
</tr>
<tr>
<td>3.</td>
<td>Nil</td>
<td>Paracetamol</td>
<td>Fentanyl</td>
<td>Coproxamol at 1 year</td>
</tr>
<tr>
<td>4.</td>
<td>DF118</td>
<td>DF118</td>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Nil</td>
<td>Coproxamol</td>
<td>Paracetamol</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Co-dydramol</td>
<td>Dihydrocodeine</td>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>NSAID</td>
<td>Morphone</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Co-dydramol</td>
<td>Coproxamol/dicl</td>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Nil</td>
<td>Coproxamol</td>
<td>Morphine</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Nil</td>
<td>Nil</td>
<td>Coproxamol</td>
<td>Paracetamol</td>
</tr>
<tr>
<td>11.</td>
<td>Nil</td>
<td>Coproxamol</td>
<td>Morphone</td>
<td>Paracetamol at 6/12</td>
</tr>
<tr>
<td>12.</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil at 1 year</td>
</tr>
<tr>
<td>13.</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Nil</td>
<td>Nil</td>
<td>Morphone</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Morphine</td>
<td>Morphone</td>
<td>Fentanyl</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Co-dydramol at 2 years</td>
</tr>
</tbody>
</table>
4.4 Discussion

This is a first study to assess the feasibility and safety of interstitial m-THPC PDT for treatment of pancreatic cancer in humans. Our results confirm the results of the previous experimental work that showed that PDT could necrose pancreatic cancer (Mlkvy et al, 1997, Regula et al, 1994).

All patients had new non-enhancing areas in the treated sites in the pancreas on the early post treatment CT scans, which were consistent with cancer necrosis as confirmed on the biopsy from the first patient. It is not possible to assess if there were no viable cancer cells in the necrotic zones without pathological examination of the whole tumour but probably the local destruction was complete as it seems that there was no cancer recurrence from the treated sites. The tumour destruction volumes produced around each treatment site were considerable (mean 2.9 cm$^3$) and the mean diameter of necrosis (18 mm) was much larger than that observed in the animal pancreatic cancer model using double the dose of photosensitisier and much higher light energy (Mlkvy et al, 1997). However, there were some variations in the volume of necrosis produced around treatment sites between patients, an effect that was observed also in the previous clinical studies with endoluminal m-THPC PDT (Grosjean et al, 1996). This may be explained by large interpatient fluctuations of the tissue concentration of photosensitisier (Glanzman et al, 1998), a small amount of blood around the fibre tip, which could substantially reduce light transmission as has been shown to occur in other organs or variations in light penetration in different tumours (Chen et al, 1997). It has been suggested that adjusting the light dose to the tissue drug level can overcome some of these variations and result in the production of the a uniform, predictable area of necrosis in every patient (Braichotte et al, 1996). Tissue concentration of m-THPC seems to correlate well with the light induced fluorescence (Wang et al, 1995) and measurement of the fluorescence signal from bronchi, oesophagus and oral cavity (Zellweger et al, 2000) were undertaken to evaluate the tissue level of m-THPC before PDT. It may be interesting to assess whether there is a correlation between PDT induced damage of pancreatic tumour and fluorescence signal from more easily assessable (e.g. oral cavity) sites than the pancreas. However, it may happen that future studies will be necessary to develop ways to monitor drug level and light intensity in the pancreas, possibly by inserting thin fiberoptic sensor into the treated areas.
Clearly more work is needed to solve the accurate light and drug dosing problems; however it has to be pointed out that all tumours seem to be sensitive to PDT in terms of the ability of PDT to induce tumour necrosis. In most cases, however, some cancer tissue was left untreated by PDT and in the later stage of the disease tumour regrew from the edges of the PDT treated area.

Despite that PDT is a localised treatment and in most cases of the advanced pancreatic cancer only palliative debulking of the major tumour mass has been achieved, the survival figures look promising. The median survival time from diagnosis was 12.5 months with 56% (9 of 16) patients alive a year after diagnosis and the survival time from PDT ranged from 4 to 30 months with a median of 10 months and 44% (7 of 16) alive a year after the treatment. 1 patient is still alive at 16 months after PDT.

These encouraging results did not seem to result from patients preselection. The treated cancers were not “small early” tumours. The mean diameter was 4.2 cm and most patients had at least (considering the well-known understaging by CT) inoperable stage II or III disease. PDT patients with stage I-II disease had longer mean survival from PDT compared with inoperable patients with the same stage of disease as reported in the recent large American survey (12.5 versus 8 months) but shorter than patients who underwent potentially curative resection (20 months) (Kokoska et al, 1998). Some survival advantage also seems to be achieved by the PDT treated patients with stage III tumours (9 months mean survival from PDT compared with 6 months for untreated patients in the same study). The clinical survival data seems to agree well with results of an experimental study demonstrating significantly prolonged survival following PDT of animals with a transplanted pancreatic cancer (Regula et al, 1994). The median survival following PDT is probably longer than for untreated patients; however a similar survival time was reported following “palliative” pancreatectomy with positive resection margins (Yeo et al, 1995) and after palliative radiochemotherapy of inoperable pancreatic cancer in one of the GITSG studies (Moertel et al, 1980). Lack of an untreated control group and the relatively small number of patients are significant limitations of this study. Several patients had adjuvant chemotherapy too, which could have had an impact on their survival. Therefore, it is difficult to comment on whether PDT really had a positive influence on the course of these patients’ disease; however it seems to have had some benefit for a few patients.
In particular, those who had no visible cancer on the early post-treatment CT scan (mean survival- 22 months) could have benefited from the treatment. On the other hand, two patients with multiple liver metastases detected within the first month after PDT did poorly, and had the shortest survival time (4 and 4.5 months). It is not likely that PDT could influence their survival. Therefore, it looks as though PDT may be a suitable treatment for patients without distant metastases but including patients with lymph node metastases (stage III disease), who may achieve some improvement of their survival time following PDT.

It is not clear if any adjuvant treatment (chemotherapy or radiotherapy) may be beneficial in conjunction with PDT. 4 patients who received adjuvant chemotherapy some time after PDT seemed to do slightly better than PDT only patients (11.5 versus 8 months median survival), however this difference and the number of patients are too small to draw any definitive conclusions. The answers to this question and the true impact of PDT on survival can be only established in a randomised, controlled study preferably in comparison with other existing therapeutic options.

The benefit of any treatment must be balanced against risk. Treatment of pancreatic cancer seems to be especially difficult as many vital structures (duodenum, bile ducts, stomach, large vessels) are in the vicinity of the pancreas and in addition, they are often infiltrated by the tumour. It is essential to understand whether PDT can destroy pancreatic tumour without unacceptable damage to the surrounding normal tissues and how other organs, which could be involved by the tumour, tolerate the treatment.

Results of the experimental animal studies suggested that normal pancreas is relatively resistant to PDT damage and pancreatitis is unlikely following PDT (Chatlani et al, 1992, Nuutinen et al, 1991, Mlkvy et al, 1997). Even extensive necrosis of the implanted pancreatic tumour did not cause an inflammatory response in the pancreas. Results of this work confirm these findings. No patients had clinical or biochemical evidence of acute pancreatitis. The highest serum amylase was less than 3 times the normal upper limit even in the patient in whom the normal pancreas was inadvertently treated and always returned to pre-treatment values within a few days.
Other abnormalities in the blood tests were mild and all returned to normal within the first week after PDT. Although all patients initially experienced pain, which had to be controlled by opiates this usually settled within 48 hours and 1 week after the treatment all patients except two, whom developed complications other then acute pancreatitis could be discharged from hospital. There was some peripancreatic oedema on the CT scan performed a few days after PDT in most cases but the treated area in the pancreas always healed uneventfully, without any sign of the pseudocyst, pancreatic leakage, abscesses, pancreatic infection or aggravation of any pre-existing obstruction of the pancreatic duct. Treated normal pancreas also healed extremely well and was enhancing normally 6 weeks after PDT. Destruction of the pancreatic parenchyma, however, caused or exacerbated pancreatic exocrine insufficiency. 2 patients developed diarrhoea attributed to pancreatic insufficiency within the first month after PDT and 3 more had these symptoms aggravated following PDT. 2 of the 5 patients who had a pancreolauryl test performed before and month after PDT showed reduction of the pancreolauryl ratio following PDT. Interestingly, asymptomatic pancreatic exocrine insufficiency seems to be more common among patients with pancreatic cancer than expected, as 9 of the 10 patients tested had a significantly reduced pancreolauryl ratio before PDT. Pancreatic exocrine insufficiency, however, responds, well to enzyme supplementation if the appropriate high dose of lipase is administered (DiMagno et al, 1999).

Unlike the pancreatic exocrine function, the endocrine function could even improve following PDT. Most of the patients (14 of 16) were diabetic before PDT. Following PDT, 8 improved their glucose tolerance and none, except the patient who had mainly normal pancreas treated by PDT, seemed to have worse endocrine function. Improvement of the glucose tolerance following PDT is not surprising as pancreatic adenocarcinomas are known to produce a diabetogenic factor causing profound peripheral insulin resistance (Ding et al, 1998) and normoglycemia can be restored following tumour resection (Permert et al, 1994). A similar result seems to have been achieved by destroying the main bulk of cancer using PDT.

The duodenum was the most vulnerable organ to PDT damage in all the experimental studies where extensive duodenal necrosis with duodenal perforations was observed (Mlkvy et al, 1997, Nuutinen et al, 1991).
Human duodenum, although much thicker than rodent gut, seems to be equally sensitive to PDT effects, but no free perforation was seen. Most of the patients however, had duodenal necrosis, often with extensive ulceration of the medial (adjacent to the pancreas) wall following PDT. Smaller areas of ulceration and necrosis heal uneventfully but larger areas of damage could lead to the development of a duodenal pseudodiverticulum, low CBD stricture or fistula between common bile duct and duodenum.

In all 3 patients in whom the wall between the duodenum and the common bile duct broke down, there was CT evidence of involvement of the duodenal wall in that area with tumour and these fistulae were asymptomatic, needle position for light delivery in close proximity to that area may play also an important role. Scarring following extensive duodenal necrosis could have also contributed to the development of duodenal stenosis and explain the relatively high rate of duodenal obstruction in the PDT treated patients. 6 (37%) patients developed clinically symptomatic gastric outlet obstruction compared with the average of 17% reported in previous studies on pancreatic cancer patients (Watanapa and Williamson, 1992) and 19% in the series from this hospital during a median survival period of 5 months (Smith et al, 1994). 4 of them (25%) seemed to result from cancer progression as confirmed on endoscopy, CT and/or biopsy. In the two other cases however, post PDT scarring (and in one of these patients also embolisation of the gastro-duodenal artery) may be the true causative factor of duodenal stenosis. It is also not clear if the post-PDT scarring could in any way exacerbate the impending duodenal occlusion due to cancer invasion of the duodenum. Longer patients survival time may also contributed to the higher incidence of duodenal stenosis as another study, 50% incidence of duodenal obstructions were reported for patients who lived longer than 1 year from the diagnosis (Gudjonsson, 1987).

It is unknown why the duodenum is so sensitive to PDT. A much higher concentration of photosensitiser in the duodenum than in the pancreas (as was observed following administration of phthalocyanine, ALA and mTHPC) could be an important factor (Nuutinen et al, 1991, Ravi et al, 1996, Mlkvy et al, 1996). Most patients had also showed duodenal wall involvement with the tumour at the site of the subsequent duodenal necrosis as assessed on the pre treatment endoscopy and/or CT.
In the animal studies, duodenal necrosis could be avoided only by shielding the duodenum from the therapeutic light (Mlkvy et al, 1997). This does not seem to be possible in humans using the percutaneous route of light delivery, however reducing the photosensitiser dose and optimising the drug – light interval may decrease the risk of duodenal necrosis. There is also the possibility to optimise the positioning of the fibres. As the median radius of necrosis in the pancreas around a single fibre tip is approximately 9 mm, positioning the fibre tip that distance from the duodenum should help to avoid duodenal necrosis.

Studies on light dosimetry and drug level in pancreatic and duodenal tissues could help to understand better the mechanism of PDT in the pancreatobiliary region and reduce complications. There is also a question whether patients with duodenum involved with tumour should be treated by PDT and to what extent. Clearly more work is necessary to establish the best treatment conditions for pancreatic PDT.

Considering the anatomical position of the pancreas and its close proximity to major blood vessels, ablative treatment of advanced pancreatic cancer could be associated with the risk of haemorrhage. Pre-clinical studies suggested however, that vessels exposed to PDT are not at risk of perforation or occlusion. In experimental studies using phthalocyanine and ALA as photosensitisers, PDT caused cell death of the endothelium and smooth muscle of the large arteries (Grant et al, 1994). The mechanical integrity of vessels was, however, not compromised following PDT injury probably thanks to the preservation of the elastic and collagen fibres, and no perforation or thrombosis occurred in the PDT treated arteries (Grant et al, 1995). Similar work was not performed using mTHPC but no evidence of haemorrhage or occlusion of the large vessels (portal vein, vena cava, and aorta) was seen following m-THPC PDT of the pancreatobiliary region or transplanted pancreatic tumours (Mlkvy et al, 1996, Mlkvy et al, 1997). It seems that PDT may not have an adverse effect on the normal vessels that run in the proximity of the tumour but there is likely to be more risk if the tumour actually invades the wall of the vessel. In this study two clinically significant bleeds associated with PDT induced tumour necrosis were from the gastro-duodenal artery, which was encased by tumour on the pre-treatment CT scan and involved in the PDT induced necrosis. However, other vessels particularly the portal vein and mesenteric vessels were usually encased, compressed or distorted by the tumour before PDT too and none of them perforated or thrombosed even in the late stage of disease. Therefore, other factors may contribute to the haemorrhage.
Of the two patients who had the most severe bleeds, one was treated with the highest light dose per fibre site. The other might have had an unexpectedly high tissue photosensitiser level as the interpatient variation of the drug concentration is considerable as has been reported before (Glanzmann et al, 1998).

As discussed above, a system that allows drug level to be monitored and to adjust light dose may prevent “overtreatments” and decrease the risk of complications.

All patients with a stent in the common bile duct may develop biliary obstruction due to clogging of the plastic stent or tissue overgrowth into the metal biliary prostheses. 36% of patients with a low malignant biliary stricture relieved by insertion of a plastic stent developed recurrent obstructive jaundice during their lifetimes in the prospective study by Smith et al, 1994. Self expanding metal biliary stents had a much longer patency time (8 months versus 4 months for the plastic stents) and therefore stent dysfunction occurred less quickly (Davids et al, 1992, O'Brien et al, 1995). In the present study, metal stents were inserted prior to PDT or shortly after treatment, wherever practical, to minimise the need for subsequent interventions. At the time of PDT 9 patients had a metal stent in situ and 2 more had a plastic stent replaced prophylactically by a metal one within 6 weeks following PDT. Stent blockage occurred in 36% (6 of 16) of patients during follow-up and the mean patency time for the plastic and metal stents were 3.5 and 7 months respectively, which is comparable to that in other patients with malignant biliary stenosis not receiving PDT.

There is no evidence that treatment damaged either type of stent in any way. Occlusion of metal stents seemed to be caused mainly by tumour progression and plastic stents blocked due to accumulation of debris. All seemed to be unrelated to PDT with the exception of one patient in whom post-PDT scarring of the periampullary region impaired stent patency. As the periampullary region is often involved in PDT induced necrosis and subsequent healing with scarring, fistulisation or development of a diverticulum may occur, insertion of a metal stent prophylactically prior to PDT seems to be the right procedure. Also placement of a metal stent is recommended as the most economic option for all patients who are expected to live longer than 6 months, which seems to be achievable for the majority of the PDT treated patients.
Endoscopic restoration of biliary tree patency was difficult in 3 cases due to coexisting duodenal stenosis and 1 patient required a percutaneous procedure as he already had a duodenal stent. All these patients seemed however, to have a local tumour progression into the duodenum so post-PDT scarring probably did not contribute to their duodenal stenosis and difficulties in stent replacement.

There is concern that biopsy of pancreatic carcinoma can cause dissemination of cancer cells. Malignant seeding along the needle tract seems to be a possible (Ferrucci et al, 1979) but rare event.

Pancreatic biopsy was not associated with the shortest survival time in the group of patients with inoperable cancer (Balun et al, 1994) nor did it cause statistically significant differences in the number of positive peritoneal washings, the incidence of peritoneal recurrence or the survival time for patients who had a pancreatic resection with curative intent (Johnson et al, 1997). In the present study one patient developed subcutaneous cancer nodules at a site of needle insertion, which probably represented needle-tract dissemination. It is difficult to determine if this event had a major impact on the course of her disease and survival and if needle placements for PDT are likely to be associated with clinically significant spread of malignant cells. The answer is probably not, considering promising survival figures in the present study: however, reducing the number of needle passes seems to be desirable.

Skin photosensitivity reactions occurred in 8 patients and with one exception were really very mild, healed well and did not pose a significant problem. Avoidance of bright sunlight during the first month following photosensitiser administration should eliminate any cutaneous phototoxicity reactions and patient education and compliance is the most important factor.

Assessment of quality of life following PDT was not the main aim of this study and only limited observations was made. The treatment was however, well tolerated and most patients could be discharged home feeling comfortable within a week following the treatment and recovered fully in less than a month.

It is encouraging to see that only 3 patients had a significantly lower Karnofsky performance status one month after PDT than they did prior to PDT and that at this time point, 10 patients sustained a status of 90-100% in this highly progressive disease.
As our patients were fairly asymptomatic at the time of PDT and there was no control group it is difficult however, to evaluate if PDT could palliate or delay the onset of the cancer related symptoms. This question could be answered in the prospective randomised study with a control group.

This is the first clinical study to evaluate the use of PDT for treatment of pancreatic cancer in humans. It has shown efficacy without any treatment related mortality and acceptable morbidity. Generally, the treatment was well tolerated, however more work is necessary to decrease the risk of post-PDT bleeding and duodenal obstructions, which seem to be the most serious complications of PDT.

The impact of PDT on survival looks encouraging, however only a controlled randomised study, to compare PDT with other therapeutic options will show the real potential and value of PDT for the management of pancreatic cancer.
Chapter IV. Pharmacokinetics of the iron chelator, CP94 in the experimental pancreatic tumour model

6.1 Introduction

Benefits of ALA as a photosensitiser for PDT include short-term skin photosensitivity (1-2 days compared with 6 weeks with HpD), oral, convenient route of administration and possibility of the retreatment within days. Also promising selectivity of the photosensitiser accumulation between diseased and normal tissue has been reported (Kennedy et al, 1996).

In animal studies ALA PDT showed great potential for the treatment of pancreatic cancer. In intrapancreatically transplanted cancers in hamsters, tumour necrosis up to 8mm in depth was obtained following administration of 400mg/kg of ALA. Also the survival of the treated, tumour bearing animals was statistically prolonged (Regula et al, 1994).

Unfortunately, in human, the result of the ALA PDT was poor; as only tiny, patchy areas of necrosis were produced in the pancreatic tumour of the only one patient who received PDT with ALA (personal data).

This finding, confirmed results from the previous clinical ALA PDT studies, which showed that the maximum ALA dose (60mg/kg) tolerated by humans induces only superficial (maximum 2mm in depth) necrosis (Regula et al, 1995, Gossner et al, 1998).

It seems to be likely, that ALA induced hepatoxicity, which considerably limits its safe dose after oral administration (Regula et al, 1995, Lucroy et al, 1999), is caused by ALA overload of the liver, not by produced PPIX due to high first-pass liver metabolism of ALA (Loh et al, 1993a, Dowdle et al, 1968).

In this situation, any technique, which enhances the PPIX level and thus PDT effect without increasing the administered ALA dose may be of clinical value.

One of such method is based on administration of iron chelator and has been investigated in this thesis.
Iron chelator agents enhance PPIX accumulation in the tissues by reducing its conversion into haem. They do this, mainly by removing ferrous ions, which are a substrate for this reaction and (at least the most active chelators) by inhibiting the activity of the catalysing enzyme-ferrochelatase (Smith et al, 1997).

Ethylenediaminetetraacetic acid (EDTA) was the first iron chelator tested showing the ability to increase PPIX concentration and to enhance PDT. It has been shown that EDTA multiplied the PPIX level in the human leukemic cells K562 by a factor of two and triple photodynamic inactivation of these cells (Hanania et al., 1992). In another study, EDTA mixed with ALA in a cream formulation was successfully used for the PDT treatment of a skin viral infection (Smetana et al, 1997). However, EDTA activity as a chelator seems to be limited by cell membrane impermeability (Richardson et al, 1994) and the lack iron specificity (Keberle et al, 1964), which can lead to clinical problems if given systemically.

Desferoxamine, has more favourable chemical properties then EDTA, in particular very high affinity to iron (Keberle et al, 1964) and good penetration into cells. It was found to be more effective than EDTA both in inducing PPIX accumulation and PDT effect in two cell lines (Berg et al, 1996). Desferoxamine however, suffers from the several drawbacks; it's expensive and must be given as a very long (9-12hr) parenteral infusion since it is poorly absorbed orally and rapidly eliminated. Serious neurotoxicity may occur following prolonged usage (Brittenham, 1992).

Hydroxypyridinones are the new group of orally active, iron chelators, which were initially developed as an alternative to desferoxamine for the patients with transfusional iron overload.

They are rapidly absorbed from the gut and easily cross biological membranes thanks to favourable chemico-physical properties, namely: low molecular weight, neutral charge (both in the iron-free and iron-complex form and high lipid solubility (Porter et al, 1989). Two hydroxypyridinones had been extensively investigated: 1,2-dimethyl-3-hydroxypyridin-4-one (CP20) and 1,2-ethyl-3-hydroxypyridin-4-one (CP94). They both remove intracellular iron rapidly, however CP94 seems to be more effective probably due to its increased lipophylicity (Porter et al, 1990) and lack of inactivation by glucuronisation in most species (Porter et al, 1993).

CP94 proved better than CP20 at significantly enhancing the production of PPIX from endogenous porphyrin inducing clinical porphyria, following its prolongate administration to mice (Smith et al, 1997).
Administered with ALA, CP94 was found to double the PPIX level and photosensitivity in vitro in all studied cell lines (including pancreatic cell line PC-1, which was used in this thesis for the tumour implantation) (Bech et al, 1997) and in human skin explants (Casas et al, 1999).

In vivo, CP94 administered intraperitoneally doubled the PPIX fluorescence in the bladder mucosa produced by the intravesical instillation of 10% ALA and increased the PPIX ratio between urothelium and underlying muscle in rats (Chang et al, 1997). In another study, CP94 was found to produce greater enhancement of the PPIX fluorescence in the rat colonic mucosa than CP20. The same study also showed that simulations iv administration of ALA and CP94 was the most effective regime to enhance PPIX level and produced three times larger area of the PDT necrosis compared to ALA alone (Curnow et al, 1998).

Based on these results, CP94 as an iron chelating agent was selected for this study. ALA and CP94 was given orally, as both CP94 and ALA are normally administered orally to patients and none of the aforementioned studies explored this route of administration of CP94 for the PPIX enhancement. In addition, effectiveness of iron chelator to increase PPIX concentration and iron chelator pharmacokinetics has not been fully investigated in any animal tumour model.

The aim of this experiment was to determine the efficacy of CP94 for the enhancement of the PPIX fluorescence in the pancreatic tumour model in vivo and the dose response of both ALA and CP94 as well as the time course of PPIX fluorescence were had to establish the optimal treatment parameters for the photodynamic studies.
6.2 Material and methods

**Chemicals**

ALA (ALA.HCl, 99% purity powder, DUSA, Pharmaceuticals, Inc., New York, USA) was dissolved in physiological strength phosphate-buffer saline (PBS) to a concentration of 40mg/ml alone or with the iron chelator, CP94 (95% purity powder, Department of Pharmacy, Kings College London), which was added at a concentration 20, 40, 80 or 160mg/ml.

Chemicals were mixed in one solution as the preceding study (Curnow, thesis, 1998) showed no differences in PDT effect when ALA and CP94 were administered either in separate or mixed solutions.

Drugs were prepared shortly before administration and given as oral gavages using a long bulb-tip feeding needle (maximum volume < 1ml). Oral route of administration was chosen because both ALA and CP94 are given normally by this means to patients.

**Tumour model**

Normal, female, 100-120g Syrian Golden hamsters were used in all experiments. Laparotomies were performed under general anaesthesia, which was achieved by the intraperitoneal injection of the 1:2:1 mixture of midazolam (Roche Pharmaceuticals), water for injection and Hypnovel (fentanyl and fluanisone, Jansen Pharmaceuticals Ltd) (6-12ml/kg). For longer procedures anaesthesia was maintained by the repeated injection of these drugs or/and inhaled halotane (ICI Pharmaceuticals, Cheshire, UK). Buprenorfine (Buprenorphine hydrochloride, Reckitt & Colman Products Ltd, Hull, UK) was given subcutaneously as post laparotomy analgesia.

Pancreatic tumour model was obtained by injection 10-15 million PC-1 cells (kindly donated by The Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Centre, Omaha, USA) into the gastric lobe of the hamster pancreas during laparotomy (Fig. 15).
PC-1 cell line was derived from the N-nitroso-bis (2-oxypropyl) amine (BOP) induced hamster pancreatic ductal adenocarcinoma (Egami et al., 1989). This model shares histological, antigenic and biological (including ability to produce liver metastases) similarities to human cancer (Takiyama et al., 1990) and was successfully employed in previous PDT studies (Regula et al., 1994, Mlkvy et al., 1997).

PC-1 cells were cultured in RPMI-1640 medium supplemented with 10% foetal calf serum, 5,000 IU/ml Penicillin G, 5,000 µg/ml streptomycin, 200mM L-Glutamine (all Imperial Laboratories, UK) in a 5% CO₂ at 37°C. Before animals inoculation, cells were incubated with 0.1% trypsin (Sigma-Aldrich, UK) in 0.02% EDTA for 7-10 minutes, then trypsin was neutralised by the culture medium and following several washes, cells were finally suspended in normal saline. Cells were injected using small syringe and 25G needle (total volume 0.1-0.3ml).

2-3 weeks following inoculation approximately 80% animals developed pancreatic tumour, although liver metastases or disseminated disease in the abdomen were rarely present at that time.
Fluorescence microscopy studies

Normal and tumours bearing animals were used through this experiment. At every time point and drug dose from 3 to 9 animals were analysed. 200mg/kg ALA was administered alone or with 100, 200, 400 or 800mg/kg CP94 as an oral gavage. Animals in CP94 only group received 100 or 200mg/kg CP94 alone. Non-sensitised animals were used as controls.

Following sensitisation animals were serially killed at various times (from 30 minutes to 6hrs), and specimens from the pancreatic tumour or/and normal pancreas were removed and snap frozen in a bath of isopenthane (BDH, UK) precooled in liquid nitrogen and then stored in liquid nitrogen.

When the peak of PPIX fluorescence in the normal pancreas and pancreatic tumour following administration of 200mg/kg ALA alone or with 100mg/kg CP94 was established, further studies were conducted only at that time point (2 hours).

For fluorescence microscopy studies 10 μm cryosections were cut with adjacent matching sections stained with haematoxylin eosin for histological comparison.

An inverted microscope (IMT-2, Olympus) with a slow-scan liquid nitrogen cooled, charged-coupled (CCD) camera (Wright Instruments) was used to obtain fluorescence images. Fluorescence was excited by 8mW helium neon laser (632.8nm) and detected in the optimal for PPIX wavelength range 660-710nm using a combination of bandpass and longpass filters as described elsewhere (Bedwell et al, 1992).

A 10x objective was used and the image integration time was 50 s, as the PPIX fluorescence signal was low.

Special software was used to process a fluorescence signal into false colour coded images and fluorescence was measured digitally in arbitrary units of counts per pixel. The system described has proven to be accurate and sufficient for PPIX pharmacokinetics studies as fluorescence measurements of PPIX, (which was found to be a predominant porphyrin synthesised following ALA administration) were shown to correlate highly with PPIX chemical extraction values (Loh et al, 1993b).

Fluorescence was measured in at least two sites per section and the mean values were corrected for the tissue autofluorescence (calculated from the measurements of non-sensitised animals).
**Statistical analysis**

Data were analysed using Student unpaired t-test. Changes were considered significant if $P < 0.05$. Data are reported as mean and the error bars represented the standard error of the mean.

**Emission spectroscopy**

Fluorescence emission spectra were measured from separate representative tumour and normal pancreas specimens. Fluorescence was excited using 543nm light produced by the 1mW HeNe laser (1676, Uniphase, Meteca, Ca, USA), which was passed via a bifurcated fibre bundle (luminescence probe, PDT Inc, Santa Barbara, USA) to the tissue. Emitted light was transmitted to the spectrograph (Multispec 1/8m, Oriel Instruments, Connecticut, USA) connected to a slow-scan cooled CCD camera (Wright Instruments Ltd, Enfield, London, UK). The spectrograph was fitted with a Schott glass filter (RG 590, Image Optics) to eliminate scattered laser light and grating blazed at 540nm to provide uniform detection over 615-735nm.

The recorded signal was processed using Mogina software (Dept of Medical Physics, UCL, London, UK), with 1nm resolution. Owing to the relatively low concentration of PPIX, 30 seconds excitation time was used, however, no significant photobleaching was observed under these conditions.
6.3. Results

Figure 16 shows the kinetics of PPIX fluorescence in the normal pancreas and pancreatic tumour following administration of 200mg/kg ALA only (Fig. 16a) or 200mg/kg ALA with 100mg/kg CP94 (Fig. 16b). The PPIX fluorescence in the normal pancreas and the pancreatic tumour remains low in both groups. The peak of PPIX level is observed early, at 2 hours after given ALA alone or with CP94, in both pancreatic tumour and in the normal pancreas with the exception of the normal pancreas in the ALA and CP94 group which shows PPIX maximum at 1 hour. After reaching the maximum, PPIX fluorescence falls rapidly (especially in the tumour tissue) and returns to the virtually background level at 4 hours.

![Graph showing PPIX fluorescence over time for normal pancreas and pancreatic tumour](diagram.png)

**Fig. 16a.** Mean PPIX fluorescence (arbitrary units) (+/- SEM) of the normal pancreas and pancreatic tumour following oral administration of 200mg/kg ALA, as a function of time (hours). Background fluorescence has been subtracted. Value at each time point represents mean from 3-9 animals.
**Fig. 16 b.** Mean PPIX fluorescence (arbitrary units) (+/- SEM) of the normal pancreas and pancreatic tumour following oral administration of 200mg/kg ALA with 100mg/kg CP94, as a function of time (hours). Background fluorescence has been subtracted. Value at each time point represents mean from 3-6 animals.

Co-administration of ALA and 100mg/kg CP94 increases the maximum PPIX fluorescence in the normal pancreas by 30% (ALA only- 8.14 a.u., ALA + 100mg/kg CP94 -11.20 a.u.), (p<0.24), which is not statistically significant and by 100% in the pancreatic tumour which achieves statistically significance (ALA only-6.84 a.u., ALA + 100mg/kg CP94 -14 a.u.), (p<0.007). At 200mg/kg CP94, PPIX fluorescence is further enhanced; by 60% in the normal pancreas (ALA +200mg/kg CP94 -13.43), (p<0.04) and by 200% in the pancreatic tumour (ALA + 200mg/kg CP94 -20.66), (p<0.06).
Simultaneous administration of 200mg/kg ALA and higher doses of CP94 (400 or 800mg/kg) induces the highest PPIX fluorescence in the normal pancreas; 154% and 264% PPIX level increase respectively (ALA only- 8.14 a.u., ALA +400mg/kg CP94-20.91) (p<0.0021) (ALA +800mg/kg CP94 -28.83 a.u.), (p<0.00016).

These doses were poorly tolerated (hypersalivation) and however, were not further investigated in the pancreatic tumour model.

Figure 17 summarises how the administration of the 200mg/kg ALA and increasing doses of CP94 affect the PPIX fluorescence of the normal pancreas (Figure 17a) and pancreatic tumour (Figure 17b) at 2 hours after administration (time of peak fluorescence).

![Graph](image)

**Fig. 17a.** Mean PPIX fluorescence of the pancreatic tumour (+/-SEM) at the fluorescence peak time (2 hours) following administration of 200mg/kg ALA, as a function of CP94 dose (mg/kg). Value at each point represents the mean from 3 animals.
Fig. 17b. Mean PPIX fluorescence of the normal pancreas (+/−SEM) at the fluorescence peak time (2 hours) following administration of 200mg/kg ALA, as a function of CP94 dose (mg/kg). Value at each point represents the mean from 3-7 animals.

100 and 200mg/kg CP94 without ALA produces very low, not significantly different from the background fluorescence of the normal pancreas at the investigated time (2 hours).

No significant PPIX selectivity between tumour and normal pancreatic tissue is seen in either 200mg/kg ALA alone or 200mg/kg ALA with 100 or 200 mg/kg CP94 groups. In fact, in several cases in the ALA only group the PPIX fluorescence was significantly higher in the normal pancreas than in the tumour.

The tumour to pancreas ratio increases slightly by the addition of 200mg/kg CP94 (from 0.84- ALA only to 1.53- ALA + 200mg/CP94), however, this is far too low to be practically useful for achieving selective necrosis.
Fluorescence emission spectra obtained from the representative normal pancreas and tumour specimens from the animals given ALA alone or ALA with CP94 showed the characteristic of PPIX spectra profile with the maxima at 635nm +/- 1nm. There were no differences in the emission spectra of ALA only and ALA with CP94 groups, nor in the normal pancreas and pancreatic tumour (Figure 18). Images obtained by fluorescence microscopy are predominantly due to PPIX and can be directly compared.

**Fig. 18.** Representative fluorescence emission spectra of the normal pancreas following administration of 200mg/kg ALA only (Fig A) and pancreatic tumour following administration of 200mg/kg ALA with 200mg/kg CP94 (Fig B).
Fig. 19. Fluorescence image of the normal pancreas (Photo A) following administration of 200mg/kg ALA. Photo B- matching H&E stained histology section.
Fig. 20. Fluorescence image of the normal pancreas following administration of 200 mg/kg ALA with 200 mg/kg CP94 (Photo A). Photo B- matching H&E stained histology section.
Fig. 21. Fluorescence image of the normal pancreas following administration of 200 mg/kg ALA with 800 mg/kg CP94 (Photo A). Photo B- matching H&E stained histology section.
Fig. 22. Fluorescence image of pancreatic tumour following administration of 200 mg/kg ALA (Photo A) and matching H&E stained histology section (Photo B).
Fig. 23. Fluorescence image of pancreatic tumour following administration of 200 mg/kg ALA with 200 mg/kg CP94 (Photo A), below (Photo B) matching H&E stained histology section.
6.4 Discussion

The results of this study show that coadministration of iron chelating agent CP94 significantly increases fluorescence level produced by ALA both in the normal pancreas and in the pancreatic tumour. Because fluorescent emission spectra were characteristic for PPIX (Ilinuama et al., 1994) and similar in either normal and tumour tissue in all experimental groups, it is possible to conclude that PPIX was the predominant porphyrin generated following administration ALA alone or in combination with CP94. These findings are consistent with the results reported in the previous work, which showed significant increase of PPIX fluorescence following administration of CP94 in several cell lines (Bech et al., 1997), in human skin explants (Casas et al., 1999) and in vivo, in the normal rat urothelium (Chang et al., 1997) and colon (Curnow et al., 1998). However, the data describing the efficacy of any iron chelator for PPIX enhancement in tumour models is very limited.

EDTA applied with ALA topically failed to significantly increase PPIX in the subcutaneously transplanted colon carcinoma in mice, probably due to its poor penetration through the skin (Malik et al., 1995). Curnow found that, CP94 given intravenously doubled PPIX fluorescence in the normal rat colon mucosa and increased it by approximately 50% in the colonic tumour model. However, no full pharmacokinetics studies of iron chelator in the colonic tumour model was performed (Curnow, 1998, thesis).

At a dose of 100mg/kg, CP94 induced higher increase of PPIX in the normal colonic mucosa than in the tumour. That is consistent with the result of the study by Berg et al. (1996) in which, greater accumulation of PPIX following administration of desferoxamine was found in a hamster lung fibroblast than in primary adenocarcinoma cell line. On the contrary, in the study by Ilinuama et al. (1994) desferoxamine enhanced PPIX content in all malignant cell lines tested with respect to normal cells.

In this study, oral administration of ALA and 200mg/kg CP94 produced much higher (60% versus 200%) increase of PPIX fluorescence in the tumour then in the normal pancreas.
It is so possible that PPIX accumulation following iron chelator administration will be different in every tissue and cell type, depending on many factors such as activity of ferrochelatase and other haem biosynthesis enzymes, amount of available iron and ALA absorption and penetration into tissue and of course can be modulated by ALA and CP94 doses used.

Due to greater increase of PPIX in the tumour than in the normal pancreas, the PPIX tumour to normal pancreas selectivity ratio slightly increased following the administration of CP94 from 0.84 (ALA only) to 1.54 (ALA+200mg/kg CP94). Such a degree of selectivity is too low for achieving selective necrosis but does not confirm previous results, which suggest that iron chelators may diminish the selectivity in the PPIX concentration between tumour and normal tissue, as observed in the colonic tumour model (Cumow-thesis, 1999).

The lack of significant selectivity between normal pancreas and tumour found in this study, is in contrast to a study by Régula et al (1994) reporting up to 8:1 selectivity ratio in the same pancreatic tumour model. However, these differences might be caused by the administration of the relatively low (200mg/kg orally) dose of ALA in this study. In a short experiment with double ALA dose-(400mg/kg orally), notable accumulation of PPIX in the tumour was found and similar to the cited study (using the same drug dose and route of administration) 5:1 selectivity ratio has been achieved (data not shown). For study with CP94, much lower ALA dose (200mg/kg) was chosen as iron chelator are only expected to be effective for low ALA doses (Cumow et al, 1998).

The hypothesis that the PPIX selectivity ratio between normal and cancer tissue depends on the administered ALA dose, seems to be confirmed by the results of the study in humans, where 60mg/kg ALA induced significantly greater, selective PPIX built up in gastrointestinal tumours than 30mg/kg of ALA (Regula et al, 1995).

It is suggested that, the increased PPIX accumulation in malignant tissues is caused by the alterations of the haem metabolism in cancer cells, mainly by the reduced activity of the ferrochelatase (Hillgersberg et al, 1992). However, it seems possible, that differences in enzyme capacity between normal and malignant cells become crucial for PPIX accumulation, only if there is significant substrate overload, that is sufficiently high dose of exogenous ALA is administered.
Obviously, the differences in the ferrochelatase activity (if that would be the main reason for preferential PPIX accumulation into the pancreatic tumour) between cancer and normal pancreas were not visible under physiological conditions as the tumour and normal tissue autofluorescence is virtually the same (data not shown). The peak of PPIX fluorescence emerged early in this study at 1-2 hours following administration of ALA with or without CP94. Again different results were achieved in the previous work using the pancreatic cancer model, which showed the maximum PPIX concentration in the normal and tumour pancreas 4 hours after given 200mg/kg ALA iv or 400mg/kg orally (Regula et al, 1994). However, other experiments confirmed that the PPIX fluorescence peaks earlier with lower doses of ALA (Loh et al, 1993b, Loh et al, 1992) and showed that ALA has dose-dependant pharmacokinetics with a linear relationship between the time of the peak PPIX fluorescence and the log of the ALA dose (Kennedy et al, 1996).

It is noteworthy that CP94 did not change significantly the PPIX pharmacokinetics in both the tumour and the normal pancreas, an effect also observed in the previous study in the normal rat colon (Curnow et al, 1998).

Figure 2 shows that there is a positive correlation between CP94 dose and degree of PPIX potentiation. Similar findings were reported in studies on cell lines using desferoxamine for iron therapy (Berg et al, 1996) and on human skin explants where CP94 were applied as an iron chelator (Casas et al, 1999). However, contrary to the aforementioned studies, in this work no CP94 dose was achieved for which PPIX reached a plateau level. The reason for this is that, the maximal CP94 dose (800mg/kg) was close to the LD50 dose for rodents and obviously, should not be exceeded (Porter et al, 1991). Also despite, that no drug related death occurred, doses higher then 200mg/kg were poorly tolerated and as soon as that was noticed, higher doses were not further investigated.

Observed side effects of CP94 included hypersalivation with stained discharge around mouth and hunched posture. Similar adverse effects were seen in rats (Kontoghiorghes et al, 1987), which received another hydroxypyridinone, CP20. The toxicity of hydroxypyridinones may be caused by the rapid inactivation of the iron containing enzymes such as ribonucleotide reductase, which is necessary for DNA synthesis and hence, inhibition cells proliferation (Cooper et al, 1996, Hoyes et al, 1992).
Also, being a bidenate ligands, hydroxypyridinone co-ordinate iron in 3:1 complexes, which may dissociate producing cytotoxic hydroxyl free radicals (Porter, 1997).

In humans, reported complications of prolonged iron chelator therapy with CP20 included nausea, arthritis and most seriously agranulocytosis in 0.6% to 4% of patients (Porter, 1997, Hoffbrand et al, 1998). However, none of the serious adverse effects occurred earlier than 6 weeks following commencing CP20 and single, large drug doses were well tolerated (Hoffbrand et al, 1998, Kontoghiorghes et al, 1990). This suggests that single administration of iron chelator as required for PDT, may be safe even for non iron overloaded individuals.

Little is known about toxicity of CP94 in humans as only CP20 underwent extensive clinical evaluation. However, CP94 may be safer in usage than CP20 as it proved less inhibitory to murine haemopoiesis in vivo and in vitro and seemed to have the best efficacy /toxicity ratio of the whole hydroxypyridinone group tested (Hoyes et al, 1993a, Porter et al, 1991).

Both ALA and CP94 are quickly absorbed from the gastrointestinal tract and rapidly metabolised. The ability of CP94 to access rapidly endosomal (including intramitochondrial) iron pools (Hoyes et al, 1993b) seems to suit it especially for increasing PPIX accumulation. Desferoxamine, which is another clinically effective iron chelating agent, may not be suitable for PPIX elevation in vivo as it is incapable of crossing the cell membrane and enter the cell much more slowly than CP94 by endocytosis, accumulating mainly in lysosomes and cytosol (Hoyes et al, 1993b, Cable et al, 1999).

However, the drawback of using CP94 in humans includes rapid glucorinisation and thus inactivation in the liver (Epemolu et al, 1994), which did not occur in some rodents e.g. rats (Porter et al, 1993). Nothing is known about metabolism of CP94 in hamsters, which were used in this study. However, it is possible that results achieved in humans may be reduced with respect to this study. On the other hand, CP20, which is also quickly metabolised by the same way, was proven to remove iron in humans (Brittenham, 1992), to degree, that may be sufficient for increasing PPIX accumulation following ALA administration.
It has also demonstrated that iron chelating therapy for increasing PPIX accumulation is especially effective for low ALA doses (Cumow-thesis, 1999, Berg et al, 1996), apparently when the haem pathway enzymes are not saturated by the ALA overload. It is possible that iron chelator therapy for enhancing low, clinically tolerable ALA doses could be satisfactory.

In summary, CP94 is effective for elevating ALA induced PPIX fluorescence in the pancreatic tumour model in hamsters. The next step is to investigate if this increased level of PPIX is available for PDT and hence, enhances the PDT effect in the same animal tumour model.
Chapter VII. Iron chelator for enhancing ALA PDT in the experimental pancreatic tumour model

7.1 Introduction

The previous section established that administration of 200mg/kg of the iron chelating agent CP94 enhanced PPIX fluorescence by a factor of three in the hamster pancreatic tumour when compared with ALA alone. The next step is to determine if this increased level of PPIX translates into a greater volume of PDT induced tumour necrosis, as only that will be of clinical value. There are several factors involved as hydroxypyridinones, apart from increasing tissue levels of PPIX, can also have their own effect on tumour tissue, which could be synergistic with PDT action by inhibiting cell growth (Hoyes et al, 1992) or inducing apoptosis (Porter et al, 1994) or, on the contrary, could protect against PDT by an unknown mechanism, as was observed in the blood vessels of the rat bladder (Chang et al, 1997). Other parameters, like light penetration and tissue oxygenation, can also influence the final PDT result and can be more important limiting factors for ALA-PDT than PPIX tissue accumulation.

Previous in vitro works using iron chelating agents for PDT enhancement, had contradictory results. Both Bech and Berg found that there was a strong positive correlation between the increased level of PPIX produced by addition of the iron chelating agent (CP94, desferoxamine or EDTA) and phototoxic cell damage in several cancer and normal cell lines (Bech et al, 1997, Berg et al, 1996). However, work by Linuma and colleagues showed slightly different results. In this experiment greater photoinactivation of the cells treated by desferoxamine was achieved but that seemed to be independent of the cellular PPIX content (Linuma et al, 1994). Another study using a gastric cancer cell line and desferoxamine found that there was a positive relationship between desferoxamine dose and increase of the PPIX level but only low doses of desferoxamine significantly improved PDT induced cell inactivation. Higher concentrations of iron chelator seemed even to protect against PDT photodamage by an unknown mechanism (Tan et al, 1997).
Data from in vivo experiments, in which CP94 was used as the iron chelator, is however, more consistent. In the normal rat colon, the area of PDT necrosis was increased by a factor of three when 100mg/kg CP94 was administered with 50mg/kg ALA iv compared with the same dose of ALA given alone (Curnow et al, 1998). A similar pattern of PPIX enhancement was found in the pharmacokinetics study, although the increase in the PDT effect was greater (Curnow et al, 1998). Even better results were obtained in the colonic tumour model, where simultaneous administration of ALA and CP94 induced seven times the volume of tumour necrosis as that produced by ALA alone using the same light parameters (Curnow-thesis, 1999).

The effect of CP94 could, however, be different in pancreatic cancer due to possible differences in the haem biochemistry, light penetration and tissue oxygenation between experimental pancreatic and colonic cancers. The safety and tolerability of iron chelator enhanced ALA PDT could be also assessed in the pancreatic cancer animal model, which is similar to the human disease. This study on enhancement of PDT induced pancreatic tumour necrosis by administration of CP94 has been conducted under the most promising treatment conditions established in the pharmacokinetics study.

7.2 Material and methods

**Chemicals**

ALA (ALA.HCl, 99% purity powder, DUSA, Pharmaceuticals, Inc., New York, USA) was dissolved in physiological strength phosphate-buffered saline- (PBS) to a concentration of 40mg/ml alone (pH-2.5) or with the iron chelator CP94 (95% purity powder, Department of Pharmacy, Kings College London), which was added at a concentration of 20 or 40mg/ml (pH-2.3 or 2.2). The chemicals were mixed in one solution as the preceding study (Curnow-thesis, 1998) showed no differences in PDT effect when ALA and CP94 were administered either in separate or mixed solutions. Drugs were prepared shortly before administration and given as oral gavages using a long bulb-tip feeding needle (maximum volume < 1ml).
**Tumour model**

The pancreatic tumour model, prepared under the same conditions as for the pharmacokinetics studies, was used for this experiment. However, because the preliminary PDT studies showed variable but mainly very superficial, (probably due to light scattering) PDT necrosis in tumours smaller than 7mm in maximum diameter, only tumours bigger than 7mm were treated further and analysed. Spontaneous necrosis was observed in the centre of the largest tumours and any tumours bearing signs of this in the treated area were excluded from the analysis.

**Photodynamic therapy**

2-3 weeks following an intrapancreatic injection of PC-1 cells at laparotomy, animals received 200mg/kg ALA orally alone or with 100 or 200mg/kg CP94, 2 hours prior to PDT. Laparotomies were performed under general anaesthesia, which was achieved by the intraperitoneal injection of a 1:2:1 mixture of midazolam (Roche), water for injection and Hypnovel (fentanyl and fluanisone, Jansen Pharmaceuticals Ltd) (6-12ml/kg). For longer procedures anaesthesia was maintained by the repeated injection of these drugs or/and inhaled halothane (ICI Pharmaceuticals, Cheshire, UK). The pancreatic tumour was exposed during laparotomy and 635nm+/-2nm (100J, 100mW for 1,000s) light produced by a copper vapour pumped dye laser (Oxford Lasers) was delivered via 200μm bare fibre to the tumour. The fibre was positioned so that it just touched the surface of the tumour. The rest of the abdominal viscera were shielded by opaque paper to prevent any photodynamic effect from scattered light (Fig. 24). The site of treatment was marked with a small stitch after illumination.

Animals were recovered following surgery and buprenorphine (Buprenorphine hydrochloride, Reckitt & Colman Products Ltd, Hull, UK) was given subcutaneously as post laparotomy analgesia. All animals were killed 3 days after PDT at the known time of maximum PDT necrosis. Immediately after killing the animals, the dimensions (depth, width and length) of the tumour necrosis were measured macroscopically using a micrometer. The volume of PDT produced necrosis was calculated using the formula for an ellipsoid, \( \pi abc/6 \) (where \( a, b, c \) are tumour necrosis dimensions) (mm\(^3\)).
The maximum dimension (length, width or depth) of the zone of necrosis was used for further comparisons as in several cases with the combined ALA + CP94 treatments, necrosis extended to the full depth of the tumour. Tumours bearing any signs of spontaneous necrosis in the treated area were excluded from the analysis. Careful examination was made to detect the presence of any other abdominal abnormalities; especially any PDT induced areas of necrosis in adjacent tissues. All findings were recorded and relevant tissues (including tumour specimens) were taken for histological examination. Drug only (ALA alone or with CP94) and light only controls were undertaken.

**Fig. 24.** PDT treatment of the experimental pancreatic tumour. Bare fibres touches the surface of the large tumour. Notice the illumination of the treated area. The rest of the abdominal viscera is shielded.

**Statistical analysis**

Results were analysed using the Student unpaired t-test. Changes were considered to be statistically significant if the p value was less than 0.05. Data are reported as means and the error bars represent the standard error of the mean.
7.3 Results

PDT induced necrosis of the pancreatic tumours was seen macroscopically as creamy, well demarcated areas (Fig. 25). On the contrary, zones of spontaneous necrosis were haemorrhagic, often cystic lesions, which were present in the centre of the largest tumours. Tumours bearing any signs of spontaneous necrosis in the PDT treated region were excluded from the analysis.

Fig. 25. PDT treated and control tumour. Light control tumour (photo A) - no macroscopic signs of necrosis. Photo B. PDT treated tumour. Arrows shows creamy, well demarcated area of the PDT produced necrosis.

The areas of PDT necrosis were always smaller than the tumour dimensions in the ALA only group, although the entire tumour or full depth of tumour was necrosed in several cases in the ALA + CP94 groups. The mean (+/- standard deviation) diameter of the treated tumours (with the exclusion of the tumours smaller than 7mm) in the ALA only, ALA + 100mg/kg CP94 and ALA+ 200mg/kg CP94 groups were 1.2 (+/- 0.5)cm, 1.2 (+/- 0.5)cm and 1.3 (+/- 0.6)cm respectively. Each analysed group consisted of 6-7 separate animals.
The volume of PDT induced tumour necrosis is plotted in Fig 26. 200mg/kg ALA with 100mg/kg CP94 produced three times the volume of PDT necrosis of 200mg/kg ALA alone (109mm$^3$ v. 31 mm$^3$, p=0.004). Addition of 200mg/kg CP94 to 200mg/kg ALA increased the mean volume of PDT induced tumour necrosis by a factor of four (136mm$^3$ v. 31mm$^3$, p=0.01) compared with ALA alone. This was however, statistically not different from that produced by ALA with 100mg/kg CP94 (p=0.44).

**Fig. 26.** Mean volume of PDT induced pancreatic tumour necrosis following administration of 200mg/kg ALA alone and with 100 and 200mg/kg of CP94. Error bars represent standard error of the mean.
The mean maximum diameter of PDT induced tumour necrosis is shown on the fig. 100mg/kg CP94 increased the mean maximum diameter of ALA-PDT induced necrosis by 3mm (4.2 mm in the ALA group versus 7.3 in the ALA + 100mg/kg CP94, p=0.001). Administration of 200mg/kg CP94 with ALA resulted in a further 1.3mm increase in the diameter of tumour necrosis, (4.2 mm ALA only v. 8.6mm ALA + 200mg/kg CP94, p=0.0008).

Control animals (ALA only, ALA with 100 or 200mg/kg CP94 and light only) exhibited no PDT necrosis (Fig. 25). Only a typical area of spontaneous necrosis was visible in the largest tumour.
Histopathological examination confirmed tumour necrosis in the treated areas (Fig. 28) with zones of viable tumour and, sometimes, normal pancreas on the edges of the cancer.

![Histology section of the PDT treated tumour](image)

**Fig. 28.** H&E stained histology section of the PDT treated tumour. TN- PDT induced tumour necrosis. VT- viable tumour at the edges of the treated lesion. NP- normal pancreas.

There was no treatment related mortality in any groups. Complications of PDT are listed in the Table 14. No side effects were observed in the ALA only group other than post inflammatory adhesions in one case.

Areas of PDT necrosis on the colon, small intestine and stomach were seen at the post-mortem examinations of animals receiving ALA + CP94 and the incidence of complications was much greater for the higher (200mg/kg) CP94 dose. PDT necrosis of these organs was full thickness in places in some cases as assessed histologically, although no perforations were detected. A huge, distended colon, suggesting ileus was seen in one animal treated with ALA with 200mg/kg CP94.
Normal pancreas was relatively resistant to PDT. Only a small area of necrosis was observed following unintentional direct illumination of normal pancreatic tissue in the animal with a tumour smaller than 7 mm (so excluded from further analysis) probably due to light scattering. No other abdominal organs seemed to be involved by the PDT.
Table 14. Complications of PDT (Note. One animal, listed here in the ALA +200mg/kg CP94 group, was not included in the previous figures as its tumour disintegrated following treatment and it was not possible to measure the necrosis).

<table>
<thead>
<tr>
<th>PDT treatment group</th>
<th>No. of animal</th>
<th>Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>200mg/kg ALA</td>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Adhesions to the small intestine</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>None</td>
</tr>
<tr>
<td>200mg/kg ALA + 100mg/kg CP94</td>
<td>1</td>
<td>Necrosis of the colon, adhesions to the small intestine</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Necrosis of the small intestine</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Adhesions to the small intestine</td>
</tr>
<tr>
<td>200mg/kg ALA+ 200mg/kg CP94</td>
<td>1</td>
<td>Necrosis of the stomach, colon and small intestine</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Necrosis of the colon</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Distended colon, necrosis of the stomach</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Necrosis of the stomach, adhesions to the colon and small intestine</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>None</td>
</tr>
</tbody>
</table>
7.4 Discussion

This study has demonstrated that oral administration of the iron chelator CP94 significantly enhances the effect of ALA induced PDT in this pancreatic tumour model.

Adding CP94 increased the mean volume of tumour necrosis up to four times and doubled the mean maximum diameter of necrosis produced by ALA PDT compared with ALA alone. The degree of amplification of the PDT result (measured as the tumour necrosis) seems to correlate with the increase of PPIX tumour fluorescence. The same observation was made in the previous studies on cell lines (Berg et al., 1996, Bech et al., 1996). The largest mean volume and diameter of necrosis were achieved with the highest CP94 dose (200mg/kg), which also produced the greatest (up to 3 times) elevation of the tumour PPIX fluorescence. However, PDT-induced tumour damage was not statistically different from that achieved with the lower (100mg/kg) CP94 dose. This lower dose also produced lower (but statistically not significant) levels of PPIX accumulation compared with 200mg/kg CP94 in the pharmacokinetics study. It is likely that co-administration of 400-800mg/kg CP94, which induced substantially higher PPIX fluorescence in the normal pancreas than 100mg/kg CP94, could result in a significantly greater increase of the PDT effect compared with 100mg/kg CP94, but these doses of CP94 were not used in the photodynamic study due to the toxicity found in the pharmacokinetics study.

ALA PDT is a result of the interaction between light and photosensitiser and so is not determined only by the concentration of PPIX in tissues. Other factors like the light dose and light delivery geometry will also influence the volume of PDT induced tumour damage. In this study, very superficial necrosis was obtained in the tumours smaller than 7mm, probably due to light scattering from the convex surface of the small tumours. It is clear therefore, that when the tissue accumulation of PPIX is sufficiently high (e.g. due to administration of the iron chelator) to induce a PDT effect, the volume of tumour necrosis will be limited by the light dose and light tissue penetration (which depends partly on the wavelength of light used).

The incidence of treatment related complications (mainly necrosis of the stomach, colon and small intestine) seemed to be correlated with the dose of CP94.
There were virtually no side effects in the ALA only group but animals receiving ALA with the highest dose of CP94 almost always had necrosis of the organs adjacent to the pancreas. The most likely explanation is that PPIX level was significantly higher in all tissues with the higher doses of CP94, what make them significantly photosensitive. Therefore, even a small dose of light and probably a greatly augmented PPIX accumulation in the organs adjacent to the pancreas exceed the threshold photodynamic dose for damage and produced necrosis in these tissues. As the PPIX increase is positively correlated with the dose of CP94 this effect is also dependent on the CP94 dose. 200mg/kg CP94 did not produce a statistically significant increase of PDT mediated tumour necrosis compared with 100mg/kg, despite the much higher incidence of unwanted damage to surrounding tissues. Thus, for clinical studies, it will be important to establish the CP94 dose which produces the maximum enhancement of PDT necrosis without inducing unacceptable damage to other organs.

Interestingly, in these experiments, the duodenum seemed to be unaffected by PDT, in contrast to the earlier works, where duodenal necrosis with perforations were observed following PDT to the pancreatobiliary region using all studied photosensitising agents including ALA (Ravi et al, 1994, Mlkvy et al, 1996, Mlkvy et al, 1997, Nuutinen et al, 1991). This may have resulted from shielding of the abdominal cavity during illumination and/or from the location of the treated tumours in the gastric lobe of the hamster pancreas, which could be too far away for light to induce any damage in the duodenum. In humans however, the duodenum may be very photosensitive following administration of CP94, and highly prone to photodamage during treatment for pancreatic cancer, as pancreatic tumours are often so close to or involve the duodenum.

As noted in earlier studies (Regula et al, 1994, Mlkvy et al, 1996), normal pancreas is relatively resistant to PDT damage. Only occasionally very small areas of pancreatic necrosis were seen at directly illuminated sites. The technique of shielding the abdominal viscera could effectively limit pancreatic irradiation and thus prevent any damage to the normal pancreas. However, virtually no PDT induced necrosis of the normal pancreas (opposite to the effect observed in the pancreatic tumour) was detected when light was deliberately applied to the normal pancreas (Mang and Wieman, 1987, Chatlani et al, 1992).
In this last study, the calculated threshold photodynamic dose for damage was seven times higher for the normal pancreas than for the pancreatic tumour (Chatlani et al, 1992). Serious damage to the normal pancreas was reported in the only one publication, in which irradiation was performed from a distance of several cm (Schroder et al, 1988). Several protective mechanisms have been suggested for the normal pancreas, including inhibition of PDT photodamage by singlet oxygen scavengers (perhaps glutathione) present in the normal pancreas but not in the cancer (Chatlani et al, 1992).

In the pharmacokinetics study, PPIX fluorescence in the normal pancreas was slightly lower than in the cancer after administration of CP94, but it is doubtful if such low selectivity, which is little more than experimental error, can have any practical meaning. The other factors are more likely to be responsible for the observed therapeutic selectivity between tumour and normal pancreas. It is important however; to emphasise that administration of the iron chelator did not reduce the resistance of the normal pancreas to PDT.

Clinically, the main side effect of the hydroxypyridinones is dose dependent leucopenia (Brittenham, 1992). Nothing is known about whether administration of CP94 with ALA can cause any new complications or augment any adverse effects of ALA or CP94 given alone in humans. It is unclear whether hepatotoxicity, observed following ALA application, is caused by the liver ALA overload or by PPIX itself. If PPIX is a harmful compound for the liver, increased hepatotoxicity could be observed following CP94 administration. Further toxicological studies should be conducted before commencing clinical studies.

The period of skin photosensitivity following ALA and CP94 administration should however, remain short. Both compounds are rapidly metabolised and the pharmacokinetics of the photoactive PPIX (time of the peak PPIX tissue concentration and its tissue elimination) did not change following CP94 administration. Hence, the main benefit of ALA as a photosensitiser will not be lost by adding CP94.

It has been suggested that iron chelators may induce their own antitumour effect by inhibiting DNA synthesis (Hoyes et al, 1993) and inducing cell apoptosis (Porter et al, 1994) and thus further enhance cancer damage (Chang et al, 1997) in addition to increasing tumour photoinactivation.
In this study, the potentiation of ALA-induced PDT effects by CP94 was slightly greater than enhancement of PPIX accumulation in the pancreatic tumour generated by the iron chelator. These findings are in agreement with the results reported in the colonic tumour model by Curnow (Curnow-thesis, 1999) and could be explained by the additive effects of PDT and a direct tumour cytotoxic effect of the iron chelator. In this study, however, no signs of tumour cytotoxicity were observed from CP94 alone. ALA and CP94 drug only control animals exhibited no necrosis with the exception of the largest tumour, which had typical zones of spontaneous necrosis. Also, areas not destroyed by PDT in the treated tumours seemed to grow normally after treatment (as assessed by comparison of the tumour diameter at the time of PDT and 3 days later). Finally, the difference between potentiation of tumour PPIX content and the enhancement of PDT is not significant. Therefore, the hypothesis that CP94 alone or with ALA can induce its own antitumour effect, separate from photoinactivation is not supported on current evidence.

In conclusion, this study has demonstrated that the increased PPIX level observed following administration of CP94 is converted into significantly greater ALA PDT induced necrosis of these experimental pancreatic tumours. Therefore, substantial improvement of the efficacy of ALA-induced PDT is possible without increasing the administered ALA dose, which is considered as a limiting factor for clinical ALA-PDT, due to the hepatotoxicity. Both the iron chelating agent and ALA have been given orally as this is a convenient route of administration of these compounds for patients. More work is, however, needed before commencing clinical studies to assess if any adverse effects could result from the administration of this hydroxypyridinone iron chelating agent and ALA. Also the optimal CP94 dose for humans, which produces the maximum pancreatic tumour necrosis without inducing unacceptable damage to the normal surrounding tissues, should be established.
Chapter VIII. Conclusions and future work

8.1 Summary and conclusions

Pancreatic cancer, with its annual incidence of 100 per million of the population, is one of the common cancers of the gastrointestinal tract and due to its extremely poor prognosis, constitutes the fifth leading cause of cancer deaths in Europe and North America. For this highly lethal disease, five year survivors are exceptional and 80-90% of patients die within one year of diagnosis. The only potentially curative treatment is surgery, although approximately 90% of patients are unresectable at the time of diagnosis. Even in the selected group who are suitable, the results of surgery are unsatisfactory. Therefore for the majority of patients, any palliative treatment with the possible prolongation of survival and improvement of quality of life is of primary importance. Sadly, at the moment, treatment modalities like radiotherapy and chemotherapy do not induce any meaningful impact on survival. Only one agent, gemcitabine, has been shown to produce improvement in the disease-related symptoms and this in only up to 25% of patients.

Photodynamic therapy is an attractive new option. It is a non-thermal laser technique for inducing tissue necrosis, which is mediated by cytotoxic oxygen species resulting from the interaction between a photosensitising agent accumulated in the tissue and light of an appropriate wavelength. Experimental studies have shown that PDT destroys pancreatic cancer with acceptable morbidity and moreover, produces a significant improvement in survival time in these tumour-bearing animals.

The main aim of this thesis was to develop PDT for the management of pancreatic cancer. The aim of the clinical work was to assess the safety and feasibility of interstitial PDT using mTHPC for the treatment of inoperable cancer where the main bulk of the tumour was localised to the immediate vicinity of the pancreas. The aim of the experimental study was to investigate a method of enhancing ALA PDT by the administration of the hydroxypyridinone iron chelating agent CP94, in the pancreatic tumour model in hamsters.
The clinical work in this thesis is the first study applying PDT to pancreatic cancer in humans. In summary, this work has shown that:

1. PDT with mTHPC is technically feasible and effective for producing pancreatic tumour necrosis with light delivered via percutaneously positioned bare optical fibres.
2. There was no treatment related mortality.
3. The most serious PDT related complications were gastrointestinal bleeding, needle track haematoma and duodenal necrosis (which could lead to late duodenal stenosis) but all were manageable.
4. Pancreatic endocrine function was not impaired by the treatment (and was possibly even improved in some cases); pancreatic exocrine function was usually reduced following PDT, necessitating enzyme supplementation.
5. PDT for the treatment of pancreatic cancer is relatively well tolerated, requires a relatively short hospital stay and does not appear to impair the patient’s overall quality of life.
6. Anecdotally, the median survival time of our PDT treated patients was longer than comparable patients treated by best supportive care; nevertheless, randomised studies will be necessary to assess whether PDT can influence the natural history of the disease.

This is the first report of the use of PDT to treat cancers of the pancreas in humans. It has shown efficacy with no mortality and an acceptable level of complications considering the risk of most procedures associated with the treatment of this condition. These promising results open the way for larger, randomised trials, which will determine the role of PDT in the management of pancreatic cancer.
The experimental study investigated the effect of the administration of CP94, an orally active iron chelator, on the PPIX level and ALA PDT outcome on hamsters with a cancer transplanted into their pancreas. This is the first study to assess the complete pharmacokinetics and effect on ALA PDT of this iron chelator in an animal tumour. The results of this work have demonstrated that:

1. Addition of CP94 significantly increases the PPIX fluorescence in the normal pancreas and pancreatic tumour compared with ALA alone.
2. There is a positive correlation between enhancement of the PPIX level and the CP94 dose in the normal pancreas; this hypothesis was not fully investigated in the pancreatic tumour model, due to poor tolerance of the high doses of CP94.
3. Addition of CP94 substantially increases the volume of ALA PDT induced tumour necrosis in the experimental cancer model in hamsters compared with ALA alone.
4. Normal pancreas is highly resistant to ALA PDT regardless of whether CP94 is also administered or not.
5. High doses of CP94 may increase the incidence of unwanted ALA PDT effects on normal tissues surrounding a pancreatic tumour, probably by enhancing tissue photosensitisation.

The results of this experimental study confirmed that administration of CP94, the orally active iron chelating agent, is an effective method for enhancing ALA PDT in malignant tissue without increasing the administered dose of ALA. This could now be tested clinically.
8.2 Future works

The pilot, clinical study has shown the considerable potential of PDT for treating localised cancers in the pancreas of patients who are unsuitable for surgery or in whom the tumour is unresectable. This is, however, only a phase one study, which aimed to assess PDT safety and efficacy. Its major limitations are the small number of treated patients and the lack of a control group. Promising early results justify, however, larger, randomised controlled trials, which would be essential to establish the true role of PDT in the management of inoperable pancreatic carcinoma as a single therapy or in combination with chemotherapy and/or radiotherapy.

There is also a need for optimising treatment conditions to minimise the risk of complications (especially to reduce the incidence of bleeding and duodenal damage) and make the response more predictable. This would involve future studies on the light dosimetry as well as on the drug dose and drug-light interval. Considering the substantial inter patient variations in tissue concentration of photosensitiser and light penetration of tissue, which have been reported previously, there is a need to develop systems for in situ monitoring of drug and light levels or perhaps other measurements of real time changes such as tissue oxygen levels (e.g. by inserting thin fibreoptic sensors into the treatment area), which will ensure adequate adjustment and control of the treatment parameters.

The experimental study has shown enhancement of PPIX levels and ALA-PDT in the pancreatic cancer model by the administration of CP94, the new orally active iron chelating agent. This could now be tested clinically, although before commencing clinical studies it will be necessary to perform toxicological studies to assess whether any serious adverse effect (especially hepatotoxicity) could result from the administration of CP94 and ALA together. The concept of enhancing ALA PDT with CP94 without increasing the administered dose of ALA may be applicable for the treatment of other diseases where a deeper PDT effect is required, but this hypothesis will need confirmation in future experimental studies.
# Appendix 1. Karnofsky Performance Status

**KARNOFSKY PERFORMANCE STATUS**

<table>
<thead>
<tr>
<th>Status Description</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal; no complaints; no evidence of disease</td>
<td>100%</td>
</tr>
<tr>
<td>Able to carry on normal activity; minor signs or symptoms of disease</td>
<td>90%</td>
</tr>
<tr>
<td>Normal activity with effort; some signs or symptoms of disease</td>
<td>80%</td>
</tr>
<tr>
<td>Cares for self; unable to carry on normal activity or to do active work</td>
<td>70%</td>
</tr>
<tr>
<td>Requires occasional assistance but is able to care for most of his needs</td>
<td>60%</td>
</tr>
<tr>
<td>Requires considerable assistance and frequent medical care</td>
<td>50%</td>
</tr>
<tr>
<td>Disabled; requires special care and assistance</td>
<td>40%</td>
</tr>
<tr>
<td>Severely disabled; hospitalization is indicated although death not imminent</td>
<td>30%</td>
</tr>
<tr>
<td>Very sick; hospitalisation necessary</td>
<td>20%</td>
</tr>
<tr>
<td>Moribund; fatal processes progressing rapidly</td>
<td>10%</td>
</tr>
<tr>
<td>Dead</td>
<td>0%</td>
</tr>
</tbody>
</table>
Appendix 2 UICC classification of exocrine pancreatic cancer (1987)

**T: Primary tumour**

TX  Primary tumour cannot be assessed  
T0  No evidence of primary tumour  
T1  Tumour limited to the pancreas  
   T1a  Tumour 2 cm or less in greatest dimension  
   T1b  Tumour more than 2 cm in greatest dimension  
T2  Tumour extends directly to any of the following: duodenum, bile duct, peripancreatic tissues  
T3  Tumour extends directly to any of the following: stomach, spleen, colon, adjacent large vessels

**N: Regional lymph nodes**

NX  Regional lymph nodes cannot be assessed  
N0  No regional lymph nodes metastasis  
N1  Regional lymph node metastasis

**M: Distant metastases**

MX  Presence of distant metastasis cannot be assessed  
M0  No distant metastasis  
M1  Distant metastasis

**Stage grouping**

Stage I  
T1  N0  M0  
   T2  N0  M0

Stage II  
T3  N0  M0

Stage III  
Any T  N1  M0

Stage IV  
Any T  Any N  M1

*Source: Crinnion and Williamson, 1997*
Appendix 3. Publications arising from this work


References


