ENDOVASCULAR PHOTODYNAMIC THERAPY FOR
PREVENTING POST-ANGIOPLASTY AND IN-STENT
RESTENOSIS

A THESIS SUBMITTED TO THE UNIVERSITY OF LONDON IN
FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR
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BY

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Abstract

Cardiovascular disease is the commonest cause of death in the western world and atherosclerosis is the commonest cause of cardiovascular disease. It is characterised by the deposition of intra-luminal plaque leading to arterial stenosis. Balloon angioplasty offers a minimally invasive method of dilating peripheral and coronary arterial stenosis. The results of angioplasty are improved by stenting. However, a significant percentage of these patients have restenosis of their arteries with clinical and resource implications. Restenosis is caused by neointimal hyperplasia (NIH) and negative remodelling the combined effects of which result in vessel re-narrowing. It has previously been shown in animal models that photodynamic therapy (PDT) reduces the restenosis when used as an adjuvant to angioplasty. PDT involves the interaction of light at a particular wavelength with a pre-administered photosensitive agent to produce cell death by apoptosis in the presence of oxygen.

The aims of this thesis are two fold. 1. to optimise the effects of PDT in preventing in-stent restenosis 2. to conduct a randomised controlled trial to show the clinical benefits of PDT in preventing post-angioplasty restenosis.

Experimental project

Balloon injury in the rabbit iliac artery was used as a model of NIH and in-stent restenosis. 5-aminolaevulinic acid (ALA) was used as the photosensitiser with light at 635nm for activation. The rabbit iliac artery was stented and adjuvant PDT was given. Initial studies established that when light is applied before stent deployment there is
almost complete depletion of the vascular smooth muscle cell (VSMC) in the media with loss of endothelium at 3 days. The vessels were harvested at 28 days. Histomorphometric studies showed that in those vessels where light was applied before stent deployment there was significant reduction in NIH and in-stent restenosis with significantly wider lumens. There was repopulation of the media with complete endothelial re-growth. When light was applied after stenting, there was no depletion of VSMC at 3 days and no significant inhibition of NIH.

**Clinical project**

Two clinical projects were run simultaneously. A clinical pilot study had been conducted by my predecessor in the department (Mr. Mike Jenkins) showing safety and efficacy of clinical use of PDT as an adjuvant to angioplasty. Initial 6 months results in 7 patients showed no restenosis. These patients were followed up and at 4 years showed restenosis at a treated site in only one patient with only mild recurrence of symptoms.

A randomised clinical trial was conducted comparing the effect of standard angioplasty with angioplasty with adjuvant PDT in preventing restenosis at 6 months. All patients with symptomatic stenosis/occlusions in the femoro-popliteal artery segment were recruited. While 132 patients were intended to be included in the trial I could recruit only 66 patients in 2 years. The trial is ongoing but currently suspended. Of the 33 patients recruited in each group, 28 patients in the control group and 25 patients in the treatment group completed the 6 month follow-up. At 6 months although the primary patency rate in the treatment group was better than the control
group no significant conclusions can be drawn from my results. There were more complications recorded in the treatment group but these were easily treated.
This work is dedicated to my wife.
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- First generation photosensitisers.
Statement of originality

The majority of the work comprising this thesis was my own. In collaboration with my supervisors Dr Jean McEwan and Professor Stephen Bown I designed the protocols for the experimental animal work. I got home office and ethics committee approval for the study. All experimental work was carried out by me. I acknowledge the valuable advice of Dr Jean McEwan for the angioplasty technique and the assistance of Mr. Waheed Jamal with the procedures. I am indebted to the Central Biological Services at the Royal Free Hospital for their skilful animal care and advice. All the tissue harvesting and sectioning was done by me. I am indebted to the Biomedical Imaging Unit at the University of Southampton Medical School in fixing the stented vessels and for advice with sectioning. All data was collected and analysed by myself.

The initial pilot study using ALA-PDT with angioplasty was done by Mr Mike Jenkins. The long term follow up was done by me and my colleague Dr Richard Mansfield. I am indebted to Nurse Eileen Firman who assisted in the patient follow-up. All the data was analysed by me.

The Randomised Controlled Trial was co-ordinated by me. All patients were recruited and follow-up arranged by me. I am grateful to the departments of vascular surgery at the Middlesex hospital (Mr. Christopher Bishop and Mr. M Adeseshiah consultant surgeons) and the Royal Free Hospital (Mr. George Hamilton and Mr. Daryl Baker) for allowing me to recruit their patients. I am deeply indebted to Nurse Eileen Firman in assisting me with the recruitment and follow-up. The angioplasty was performed by
Drs Maurice Raphael and Jocelyn Brooks and light delivery by Dr Alexander Mosse. Duplex surveillance was performed in conjunction with the vascular laboratories at the Middlesex and Royal Free hospitals. Again, all data collection and analysis was my responsibility. All patients were recruited in the 2 years when I was at UCL. Some patients recruited at the end of my tenure were followed-up by Nurse Eileen Firman for a 6 months period.
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List of Abbreviations

ABPI Ankle Brachial Pressure Index
ACLI Acute Critical Limb Ischaemia
ALA 5-Aminolaevulinic acid
ASOG Anti-sense Oligonucleotides
bFGF basic Fibroblast Growth Factor
CASP Chloraluminium Disulphonated Phthalocyanine
CCF Congestive Cardiac Failure
CCLI Chronic Critical Limb Ischaemia
CFA Common Femoral Artery
CHD Coronary Heart Disease
CIA Common Iliac Artery
CLI Critical Limb Ischaemia
CTA Computerised Tomography Angiogram
DM Diabetes Mellitus
ECM Extra-cellular Matrix
EEL External Elastic Lamina
FDA Food and Drug Administration
H&E Haematoxylin and Eosin
HDL High Density Lipoprotein
HpD Haematoporphyrin Derivative
HPF High Power Field
HRT Hormone Replacement Therapy
HT Hypertension
IEL Internal Elastic Lamina
IGF-1 Insulin-like Growth Factor
IHD Ischaemic Heart Disease
LDL Low Density Lipoprotein
LSV Long Saphenous Vein
MI Myocardial Infarct
MMP Matrix Metallo-Proteinases
MRA Magnetic Resonance Angiography
m-THPC meta Tetrahydroxyphenol
NIDDM Non-Insulin Dependent Diabetes Mellitus
NIH Neointimal Hyperplasia
NPe-6 Mono-L-Aspartyl-Chlorin e6
PDGF Platelet Derived Growth Factor
PDT Photodynamic Therapy
PP9 Protoporphyrin IX
PSVR Peak Systolic Velocity Ratio
PTA Percutaneous Transluminal Angiography
PTFE Poly Tetra Fluoro Ethene
PVD Peripheral Vascular Disease
RCT Randomised Controlled Trial
RIND Reversible Ischaemic Neurological Defect
SFA Superficial Femoral Artery
SSV Short Saphenous Vein
TGFβ Transforming Growth Factor Beta
TIA Transient Ischaemic Attack
<table>
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<tr>
<td>TIMP</td>
<td>Tissue Inhibitors of Metalloproteinases</td>
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<td>TNFα</td>
<td>Tumour Necrosis Factor Alpha</td>
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<td>TUNEL</td>
<td>Terminal deoxynucleotidyl transferase-mediated dUTP biotin Nick End Labelling</td>
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<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
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<td>Very Low Density Lipoprotein</td>
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<td>VSMC</td>
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Mr Christopher Bishop consultant vascular surgeon at the UCL hospital was especially supportive in instigating the project and along with Mr M Adeseshiah and Mr S Barker consultants vascular surgeons provided constant encouragement in recruiting patients. Mr George Hamilton and Mr Daryl Baker consultant vascular surgeons at the Royal Free hospital and Mr Mike Jenkins and Mr David Nott consultant vascular surgeons at the Chelsea and Westminster hospital also helped in the patient recruitment.

I owe much to Dr Alexander Mosse lecturer at the department of Medical Physics who provided constant support and technical assistance in the use and maintenance of the
lasers both in the experimental and clinical projects. Dr David Pickard laser physicist at the NMLC also provided support for the clinical project. I am indebted to the staff of the Central Biological Unit at the Royal Free Hospital and at the Biomedical Imaging Unit at the University Hospital Southampton specifically Dr. Anton Page.

The consultant radiologists at the UCL and Middlesex hospital Dr Jocelyn Brooks and Dr. M Raphael were very accommodating in carrying out the procedures in their vascular suites sometimes at very short notice. I also thank the vascular technologists at the Middlesex Hospital Vascular Laboratory for all their support. I acknowledge the advice provided by Dr. Mark Thomas Consultant statistician at Poole General Hospital.

Finally I would especially like to thank Mr. Waheed Jamal (Research Fellow) who assisted me in all the experimental work and Drs. Dilip Vishwanathan and Andrew Lee with whom I shared my office and along with a lot of trials and tribulations.
Chapter 1. Atherosclerosis

History of arterial disease

Death and taxes, it is said, are the two absolutes of life. As a vascular surgeon however one is almost tempted to add atherosclerosis to these. It is inevitable that anyone who lives into adulthood will have an atherosclerotic plaque somewhere in their vasculature.

Atherosclerosis, derived from the Greek “athero” meaning gruel or paste and “sclerosis” meaning hardening, is responsible, directly or indirectly, for the majority of deaths occurring in the Western Hemisphere. With life styles changing in the Eastern Hemisphere death rates there are also fast approaching western levels.

The ancient Greeks certainly knew of its existence evidenced by the documentation of aneurysms in the writings of Galen. However, it is not clear whether they correlated this with atherosclerosis. Sophisticated histological analysis has shown the presence of atherosclerotic changes in Egyptian mummies. ¹

Although clinicians through the 17 and 18th centuries documented atherosclerosis most of their attention was directed towards aneurysmal disease. It wasn’t until 1829 that the term arteriosclerosis was coined which was later changed to atherosclerosis. Rudolf Virchow was possibly the most significant contributor to the understanding of
the histopathology of atherosclerosis. He published his *Handbuch der Speziellen Pathologie und Therapie* in 1854 in which he has further described the role of embolism and thrombosis.¹

Ignatovski in the early 20th century produced an animal model of atherosclerosis while Adolf Windaus won the Nobel Prize for showing the relationship between cholesterol and atherosclerosis.

In the 20th century 'all hell' it seems has broken loose as our understanding of atherosclerosis and its manifestations has increased manifold. With the increasing life-span of humans characterised by most people, especially in the western hemisphere, living well past their 7th decade it has graduated from being merely a medical curiosity to the one of the biggest concerns of most health care systems.

**Anatomy of an artery**

The arterial wall consists of 3 layers the intima, media and the adventitia. It has been known for several years now that the plaque formation occurs primarily in the media or the interface between the intima and the media. Both of these layers play a significant role in the formation of a plaque. Although the intima is only one cell thick it’s complexity in function can equal any other cell in the body.

Anatomically speaking the endothelial cell forms an interface between the blood
components on the inside and the smooth muscle cells and vascular interstitia on the outside. It provides a surface of several thousand square metres, which can secrete or absorb as required. It is also apparent now that there are differences in the individual cell populations depending on the location.

Functionally it is a major player in most if not all homeostatic mechanisms in the body. It acts as a selectively permeable barrier with the permeability varying both as function of time and location. Given its massive surface it can cause colossal imbalances in circulating volume as for example in shock. It thus plays a vital role in acid-base balance and electrolytic balance. It acts as a balance for the various pro and anti-thrombotic mechanisms by means of regulating the secretion of pro and anti-thrombotic hormones. Similarly it regulates vasomotor tone by maintaining and balancing the secretion of various vasoconstrictor and vasodilator hormones. It also plays an important role in the inflammatory and immune responses and repair mechanisms by regulating the secretion of cytokines and growth factors.

The arterial media consists of a cellular component, which is primarily vascular smooth muscle cell (VSMC), and a complex extra-cellular matrix (ECM). VSMC proliferation is a characteristic feature of plaque formation but very little is known about the mechanisms involved in this. Although the primary function of the VSMC is contraction, it also has a secretory function and is responsible for the secretion of most of the ECM. It is believed that in a normal vessel it remains in a differentiated mature state with plenty of contractile fibres with very little secretory activity.

The ECM consists of various connective tissue components such as collagen fibres
types I-IV, elastic fibres such as elastin, glycoproteins, basement membrane proteins such as laminin and proteoglycans such as hyaluronan. Each of these components plays varying roles in providing structural strength and elasticity to the vessel. Both the composition and the thickness of the ECM varies throughout the vasculature. In areas such as the pulmonary and renal vascular beds they are so thin so as to be almost nonexistent while in some vessels such as the aorta they form a significant proportion of the vessel wall.

**Pathogenesis of atherosclerosis**

Since Virchow described the basic structure of atheroma considerable work has gone into the description of plaque origin and structure. Recent opinions about the pathogenesis of the atherosclerotic plaque have in fact gone back to the original concepts that the plaque was inflammatory in origin. In the 19th century the popularly held view was that it was a degenerative disorder. However in the latter part of the 20th century our understanding of plaque formation has benefited from extremely sophisticated imaging and tissue processing techniques.

In 1994-5 the American heart association committee for vascular lesions proposed a classification for the plaques based on its tissue morphology. Plaques can fall in to 6 categories. Stage I-III included fatty streaks and intermediate lesions while stages IV-VI include plaques with varying degrees of added complications.\(^2\)\(^4\)
**Early lesions**

Fatty streaks are the earliest lesions to be seen in arterial intimas and can be seen even in infants. It is almost universally present in the arteries of young adults. These are said to originate from the deposition of lipid (probably oxidated LDLs) either in the subendothelial plane or between the basement membrane and the endothelium. This induces the migration of monocytes, which phagocytose the lipids to form foam cells. This forms the basic structure of the fatty streak and is the stage I of plaque formation.

In the presence of continuing insults (such as hyperlipidemia and all the risk factors, which will be discussed later) this simple lesion will progress by accumulation of smooth muscle cells from the media and the further deposition of lipids. Thus the presence of smooth muscle cells pushes it up a stage into stage II and extracellular deposition of lipids moves it up to stage III. Not all of these lesions necessarily go on to stage IV or above and some of them are even known to regress.

Progression to an atherogenous plaque is typically seen in specific sites along the vascular tree. These include the:

- Circle of Willis
- Carotid bifurcation
- Coronary arteries
- Abdominal aorta
- Iliac and lower extremity vessels.
Late lesions

Stage III lesions are converted into true atherogenous plaques in stage IV. These are made up of multiple alternating layers of foam cells and SMC surrounded by connective tissue in an uneven complex arrangement. This lesion gets more organised with time so as to be raised from the surface of the intima and project into the arterial lumen. It acquires a fibrous cap of connective tissue. T-lymphocyte appear in the cellular component and the matrix consists of collagen proteoglycans and small elastic fibres. This may be interspersed with necrotic debris and may over time get calcified. This now falls into stage Va. Most uncomplicated plaques would fall into categories IV and Va.

The progression of these plaques into stages Vb, Vc and VI usually leads to some acute manifestation of the disease depending on the affected artery. Stage VI is the development of acute thrombosis in the plaque. This usually leads to a hyper-acute event such as a myocardial infarct or an acutely ischaemic limb. The thrombosis can occur because of 2 reasons. The surface of the plaque being very brittle can easily rupture leading to exposure of an extremely thrombogenic surface to blood and coagulation components. In the presence of an already narrowed lumen with sluggish blood flow this is just the right environment for thrombosis to occur. There is also a theory that the inflammatory response accompanying an atherogenous plaque stimulates the formation of neo-vasogenesis in the outer layers of the vessel. This can cause intra-plaque bleeding leading to thrombosis and occlusion.

Alternatively the plaque may undergo a sub-acute change. This usually involves
some fissuring along the shoulder of the plaque. This leads to bleeding within the plaque and may give rise to symptoms though not enough to cause an acute event. This over time can get organised in 2 ways. If it acquires a proliferation of VSMC and connective tissue then it is classified as a Stage Vb. If however it organises into collagen then it is classified as stage Vc.\textsuperscript{2-4}

**Risk factors**

Risk factor modification has played a key role in reducing the mortality and morbidity rates over the last 25 years.

Atherosclerosis is certainly a disease of ageing with prevalence rate showing a sharp rise from age 45. Male sex has always been viewed as an important cause of atherosclerotic disease. Although this is essentially true in most western countries this is less obvious over the age of 75, as women tend to catch up with men. A similar pattern is seen in cerebro-vascular disease and peripheral vascular disease but is not as marked as in coronary artery disease. Aneurysmal disease however remains a preserve of the male with the ratio being 6:1 in the U.K. (The Office of National Statistics 2000)

Hypercholesterolemia was one of the first risk factors to be directly correlated with atherosclerotic disease. 18\% of all men and 22.4\% of all women in the U.K. had a blood cholesterol level of $>6.5\text{mmol/l}$ in 1998. Treating hypercholesterolemia has
been one of the most effective ways of controlling heart disease in the last 3 decades. It
has been known that LDL-cholesterol is the most important form responsible for
increasing the rate of atherosclerosis. Recent evidence has shown the oxidised form of
LDL-cholesterol is probably the most dangerous. HDL-cholesterol has been shown to
be protective and can sometimes lower the risk of atherosclerosis. Dietary intake of
polyunsaturated fatty acid as opposed to saturated fatty acids is also supposed to lower
LDL-cholesterol.\(^5,6\)

Smoking has probably been directly or indirectly responsible for most of the deaths in
the 20\(^{th}\) century. Smoking affects cardiovascular disease in multiple ways. To begin
with the various components of smoke such as nicotine, nitric oxide, cyanides, carbon
monoxide, polyphenols and multiple other free radicals and antioxidants, each have a
deleterious effect on the vascular system. Further each component seems to affect
different homeostatic mechanisms, which contribute to atherosclerotic disease.
Nicotine and its metabolites have been shown to be cytotoxic to endothelial cells.
Various components in cigarette smoke are directly responsible for increasing serum
LDL-cholesterol, triglyceride and VLDL-cholesterol levels while lowering HDL-
cholesterol levels. These are also responsible for oxidising LDL-cholesterol. This in
turn has been shown to increase local inflammatory response and cause local
vasospasm. Several constituents of smoke have been shown to have chemotactic
properties and also increase leukocyte adherence. It also stimulates the production of a
number of cytokines along with stimulating components of the complement system.
Through its metabolites, it also stimulates a sustained immune reaction with increased
concentrations of all forms of immunoglobulins in the serum. Plasma levels of fibrinogen are elevated and this has been shown to directly relate to increased cardiovascular events. It is also responsible for increased thrombogenicity by inactivating the antithrombotic effects of proteins C and S and tPA, activating factor XII stimulating platelet aggregation and increasing the production of thromboxane A2 and prostacyclin derivatives which preclude the developments of acute events caused by plaque thrombosis.7,8

There is no doubt that hypertension is one of the most important risk factors responsible for atherosclerosis. Both the Framingham study and the North Karelia project showed significant reduction in absolute risks of cardiovascular events after effective control of hypertension.9,10 Moreover blood pressure control is also shown to reduce the incidence of asymptomatic peripheral disease. In spite of these observations it is not clear exactly how hypertension accelerates atherosclerosis. The virtual absence of atherosclerosis in the venous circulation shows that arterial blood pressures are needed to promote atherosclerosis. This is further evidenced by the fact that Long Saphenous Veins (LSV) used for coronary artery bypass promptly develop atherosclerosis. Similarly the pulmonary arteries only develop atherosclerosis in the presence of pulmonary hypertension. Hypertension has been shown to increase the trapping of LDLs in the intima and the media. What is however not clear, is the relationship between hypertension and the predominance of certain sites for atherosclerosis. Atherosclerosis is commonly seen at the arterial wall segments opposite flow-dividers. For example at the carotid bifurcation the plaques are usually
seen in the wall opposite the dividing wall. It has been suggested that it is the slow flow and the turbulence rather than the high shear forces associated with fast flow, that causes the damage required for lipid deposition. Using Bernoulli’s principle, since there are increased flow rates at the dividing wall and slow flow rates at the opposite wall, the pressure differential can be up to 7-8 mm of mercury at normal blood pressure with parallel increases as blood pressure rises. This pressure differential could be one of the causes of accelerated plaque formation. 5,9,10

If atherosclerotic disease is a major cause of death in the general population it is even more so in diabetics. More than 80% of all deaths in diabetics are due to atherosclerotic disease. Diabetes has been shown to accelerate the natural progression of atherosclerosis in all populations. It does so in a number of different ways. It increases circulating levels of VLDLs and LDLs as well as changing the compositions of atherogenous plaque with increased levels of VLDL remnants and small dense LDLs. It also reduces the levels of HDLs. High circulating levels of insulin and insulin resistance in non-insulin dependent diabetics (NIDDM) leads to hypertension and increased plaque formation. Further hyperinsulinemia causes dyslipidemia impaired fibrinolysis, a central pattern of fat distribution and smooth muscle proliferation. Diabetes also leads to a pro-coagulant state with increased levels of clotting factors, increased platelet aggregability, increased levels of fibrinogen and decreased levels of plasminogen activator inhibitor type-I. 11-13

The role of alcohol remains controversial. Small levels of intake could be beneficial as shown in the Edinburgh study, which showed the consumption levels in
claudicants, were lower than that of the general population. However an intake of over 2 units a day is generally considered detrimental. Diet certainly plays an important role in the treatment of hyperlipidemia and diabetes but it is still open to question as to how important a role it plays on its own. Case control studies have shown significantly reduced risk with diets high in crude fibre and polyunsaturated fatty acids. Levels of exercise and personality types are also shown to contribute to atherosclerotic disease. Increased blood viscosity and plasma fibrinogen levels have consistently been shown to be elevated in patients with peripheral arterial disease.

**Manifestations**

Broadly speaking atherosclerotic disease can be categorised into 4 categories.

**Ischaemic heart disease (IHD)**

**Cerebro-vascular disease (CVD)**

**Peripheral vascular disease (PVD)**

**Others**

The manifestations of ischaemic heart disease can range from the relatively benign stable angina to a full-blown myocardial infarct leading to sudden death. Angina can be either precipitated by stress such as exercise or in its more progressive form be
present at rest or in the form of unstable angina. IHD can present itself in the form of congestive cardiac failure, which may be left or right ventricular, acute or chronic. Depending on the vessel(s) involved varying amounts of muscle loss can result from a MI. Significant sequelae of a MI include arrhythmia, acute CCF, ventricular aneurysms and clot formation, peripheral embolisation and sudden death.

Cerebro-vascular events include transient ischaemic attacks (TIAs), amourosis fugax, reversible ischaemic neurological deficits (RINDs) and a full-blown stroke. TIAs can be crescendo in nature while a stroke can be in evolution. A stroke may progress to a hemiplegia with profound sensory loss and depending on the side can produce aphasia.

Although Peripheral vascular disease can affect both upper and lower limb arteries it shows a significant preponderance towards the lower limb vasculature. The symptoms are however similar for both upper and lower limbs and present primarily as claudication or critical limb ischaemia. Critical limb ischaemic includes rest pain or night pain ulceration or gangrene. Leriche syndrome secondary to iliac artery obstruction can present as vascular impotence.

Other manifestations of the disease include renal artery stenosis leading to renal failure and hypertension and mesenteric ischaemia leading to malabsorption syndromes and sometimes infarcted bowel.
Chapter 2. Peripheral Vascular Disease

Presentations

Although the symptomatology of peripheral vascular disease is fairly simple this does not imply that treatment options are equally straightforward. In fact it is not yet clear whether interventional or surgical treatment should be offered at all to the majority of people with peripheral vascular disease.

It is postulated that the vast majority of people with peripheral vascular lesion are asymptomatic. This was first shown in the Basle study which used pulse occilometry to measure occlusive disease and showed that the incidence of asymptomatic disease was 3 times that of patients with claudication.\textsuperscript{14} This has been corroborated in a number of studies since then including the most recent Edinburgh Artery Study which showed 17\% of asymptomatic men had an ABPI of <0.9 or a post-occlusive reactive drop in pressure of >20\%. This was almost 3 times both the prevalence and incidence of claudication in the sample population.\textsuperscript{15}

Claudication

Claudication is considered the early or benign form of the disease. Again it is difficult to estimate the number of claudicants in the population for several reasons. Large sections of claudicants believe it to be part of growing old and do not even approach their doctor for an opinion. Of those who do attend only a small percentage are referred on for expert advice. Further it is also dependent on the degree of disability it cause the patient which in turn is largely dependent on the lifestyle of that patient. For
example, 50 yard claudication could mean loss of a job to a 50 year old forest warden while the same would hardly affect an 85 year old pensioner. The prevalence of claudication has been shown to vary from 0.3% in North American telephone employees to 7.7% in all the male population of Finland. The Edinburgh study reported the prevalence to be 4.6% overall, while the Southampton study showed the prevalence to be 7% in elderly men. The 5-year cumulative incidence in the Edinburgh study was 9% while that in the Basle study was 6%. 14-16

Both of the above 2 categories of patients i.e claudicants and people with asymptomatic PVD are considered benign forms of the disease for good reason. Although there is very little data available on the progression of asymptomatic disease the Basle study showed progression of asymptomatic disease to claudication in only 20% and to CLI in only 8% over a 10-year period. Similarly in the Edinburgh study only 15 % of patients with major asymptomatic disease and only 7% with minor asymptomatic disease progressed to claudication.14,15

Follow up studies in claudicants suggest that at least half are improved at 5 years and less than a third deteriorate. Only 29% of claudicants in the Edinburgh study still had pain at 5 years. Amputation rates, which were low to begin with, have reduced even more in the recent years. Bloor in 1961 reported an amputation rate of 7% for claudicants of over 5 years and 12% for over 10 years. However 2 studies in the 80s have reported a 1-3% amputation rate over 5 years.15,16

This however does not mean that the atherosclerosis does not progress. The Basle study showed a prevalence of 25% of CHD in asymptomatic disease while the
Edinburgh study found IHD in 54% of asymptomatic patients. Similarly, between 25-50% of claudicants had carotid disease on duplex examination. Approximately 30% of claudicants are dead at 5 years, 50% at 10 years and 70% at 15 years the majority of them from cardiac events and stroke.\textsuperscript{14,15}

**Critical Leg Ischaemia (CLI)**

The second category of patients with PVD is those with CLI. The European Working Group in Critical Limb Ischaemia defined CLI as persistently recurring rest pain requiring regular analgesia for more than 2 weeks or ulceration or gangrene of the foot and an ankle pressure of $<50$ mmHg or absence of foot pulses in diabetics. More recently the Audit committee of the Vascular Society of GB & Ireland have redefined it as chronic or acute on chronic ischaemia that endangers the whole or part of the leg.\textsuperscript{17,18} Rest pain is certainly the pre-dominating presenting feature as is seen in 74% of patients with 34% having gangrene and 32% ulceration. Figures published by the Audit Group suggest an annual incidence of 50-100/100 000 in Europe and 40/100000 in the UK.\textsuperscript{17,18} The figures published in Scandinavia were 60-80/100 000.\textsuperscript{16} The annual prevalence is 1 in 2500 with 20 000 admissions per year in GB & Ireland of whom 15000 have major surgery.\textsuperscript{17,18} Wolfe, in the 80s, recorded that 25% of patients presenting with CLI would lose their leg in a year.\textsuperscript{19} The VSS audit however showed a one-year mortality of 13.5% and amputation rate of 21.5%. As is the case with claudicants, concomitant cardiovascular disease usually kills 50% of patients within 5 years. Factors that tend to worsen prognosis include diabetes mellitus and continued smoking. Other factors which predict early mortality are concomitant symptomatic
cardiac or cerebro-vascular disease and initial ABPI <0.5.

Critical leg ischaemia can be further categorised as acute and chronic critical ischaemia (ACLI and CCLI respectively).

By definition acute limb ischaemia progresses from asymptomatic to limb threatening in less than 2 weeks. Pathophysiologically however ACLI can be precipitated by 2 completely separate phenomena with completely different presentation. Although thrombotic occlusion is considered the commonest cause of ACLI embolic arterial obstruction is not far behind. The former usually presents as an acute on chronic event. There is usually some underlying atherosclerotic disease. The acute exacerbation can be precipitated by acute alteration in circulatory dynamics or due to alteration in thrombotic properties. The presence of some pre-existing arterial disease even if asymptomatic usually means that there is some collateral circulation, which tilts the balance in favour of viability of the limb. Embolic events, most commonly from the heart in atrial fibrillation or from an aneurysmal abdominal artery (aorta or iliacs), however are far more precipitous and need immediate attention if there is to be any hope of limb salvage. Although the outcomes both in terms of limb salvage and mortality are poor in both categories mortality is greater in embolic events due to poor cardiac status while limb salvage is worse in thrombotic events.

**Investigations**

Typically a patient who presents to a vascular unit with symptoms suggestive of PVD will have Doppler examination of his pulses and measurement of his ABPI. Although
not as sensitive as more sophisticated tests they have good negative predictive value and are effective in ruling out significant vascular disease. The measurement of ABPI can be made more sensitive by adding an exercise ABPI if the resting ABPI values are normal.

**Vascular Doppler**

Doppler ultrasound in its most basic form relies on the Doppler principle that the frequency of sound reflected by a moving object is changed in proportion to the velocity of the moving object. This frequency shift falls within the audible range if the initial sound frequency is between 5-10MHz. While for a simple outpatient ABPI assessment just the presence or absence of the ‘whoosh’ sound is enough, more sophisticated equipment can convert this into a spectral waveform which can provide more information about the blood flow. For example a normal waveform is tri-phasic in nature coinciding with 1. A strong forward flow in systole, 2. A transient reversed flow and 3. A weak forward flow in diastole. Normal ABPI is >1. Most patients with symptomatic PVD will have an ABPI of <0.9. As mentioned earlier an ankle pressure of <50 mmHg is suggestive of CLI. It is sometimes possible to have normal ABPI in the presence of proximal disease such as aortic or iliac stenosis.

The simple hand held Doppler used in a vascular outpatient clinic has progressed into a far more sophisticated instrument capable of providing both structural and functional information, so that it is questionable now to use angiography in all cases. Contemporary machines are a combination of both Continuous Wave (CW) Doppler and B-mode ultrasound giving both an image of the artery and colour coded
blood flow through it. The Doppler waveform is converted in colour coded waveform and superimposed on the ultrasound image of the artery. The reflected frequency can also be used to calculate the velocity of blood flow within the artery. Thus a complete Doppler ultrasound report would usually include an US image of the artery usually with a colour-coded waveform, spectral waveform, peak systolic and diastolic velocities. Characteristics of the waveform and the degree of stenosis or occlusion are calculated on the basis of the above information. Any stenosis over 50% is considered significant and anything over 75% high grade. More sensitive machines can also provide information on plaque characteristics such as presence of thrombus and an unstable plaque.

The usefulness of a duplex assessment has been proven in a number of studies comparing it with the gold standard of arteriography. Various studies have shown a sensitivity of 71-98% and specificity of 91-100% when comparing the degree of stenosis on US Doppler to arteriography. Newer machines have even been shown to be >90% sensitive and specific for infra-crural vessels. In view of its non-invasive nature, lack of complications, no contraindications and low cost it forms an ideal first investigation in any case of PVD. In selected cases it has been used as the only investigation. In carotid disease it is used as the only imaging in most vascular units. It is especially useful in those patients in whom angioplasty is possible as it can be planned without a preliminary diagnostic angiogram.14,15,20,21

Doppler is also used for Pulse Generated Runoff. This is used to augment a weak or absent Doppler signal in distal vessels which may be poorly filling on
angiograms due to a proximal occlusion. It also helps define continuity with the pedal arches thus helping prognosticate distal bypasses.\textsuperscript{22}

\textbf{Arteriography}

Arteriography remains the gold standard in most vascular units for structural definition of an arterial lesion. The usual approach is a trans-femoral approach using a 4-5F catheter. A standard Seldinger technique is used with guide-wire insertion and guiding the catheter over the guide wire. A pigtail catheter is inserted in to the distal aortic segment and a non-ionic contrast (usually iodine based) is injected. Most procedures are done under image intensification and hard copies are shot at appropriate times. Most units are now computer aided which give digital images. This makes it possible to have multiple views and also to digitally subtract bone and soft tissue artefacts thus providing most if not all the information required in planning treatment. The small calibre of the catheters leaves an extremely small defect in the arterial wall, which usually plugs off with simple pressure. Vaso-seal devices are now available if there is a need for larger catheters. Alternative sites of puncture include the radial and brachial artery in the upper arm or the popliteal or the external iliac artery just above the inguinal ligament. It is also possible to inject dye through a peripheral vein and create a digital subtraction angiogram. An added benefit is the ability to directly measure the pressure gradient across lesions, which is useful in deciding the need for an angioplasty.

While angiograms are a very useful tool in grading PVD the procedure cannot be
carried out in all patients and it has a small incidence of significant complications and even death. Although death rates have gone down considerably a large audit of complication following angiograms in the ‘80s showed a death rate of 0.03% following trans-femoral angiography for non-cardiac pathology. More recent studies such as the one by Egglin et al showed no mortality for non-cardiac and non-cerebral procedures. However major complications occurred in 2.9% cases and minor complications in 9.3% of cases. Specifically for PVD there was a 2% major complication rate and a 7% minor complication rate.\textsuperscript{23} Major complications include arterial dissection, femoral artery thrombosis, pseudo-aneurysm formation and bleeding, distal embolisation and renal failure. There is almost invariably some bruising at the site of puncture and quite often sustained pressure is needed to stop on-site bleeding. Vaso-vagal attacks are common. They however can be especially dangerous given the poor cardio-respiratory reserve of a lot of the arteriopath patients. The contrast used can give rise to hypersensitivity reaction nephrotoxicity and in larger volumes can precipitate CCF. Patients have to lie still and flat for anything ranging from 20 minutes to an hour. Hence orthopnea is a relative contraindication as is renal failure and any bleeding diathesis including patients who are on warfarin. Metformin, an oral hypoglycaemic agent, has to be stopped for 48 before as recommended by the Royal College of Radiologists and restarted only after checking renal function after the procedure as it can precipitate lactic acidosis and renal failure.

**Others**

Because of the problems associated with invasive arteriography a number of non-
invasive imaging techniques have been tried and some of them are proving to be very useful and may in the future replace conventional angiography. Spiral and 3-D reconstructive CT scan has revolutionised the imaging in proximal PVD providing excellent images of the aorto-iliac segment. The obvious disadvantages are the amount of radiation involved and the cost. Magnetic resonance angiography has also recently come into vogue and shows good promise. Gadolinium has been used as a contrast medium and certainly provides very good pictures. Specific arterial segments can be very well visualised and it may be useful in delineating run-off vessels if angiography does not give good images. Again the problems are those of cost and patient compliance with the machine.

Older techniques, no longer commonly used, include plethysmography which measure short-term changes in limb volume. Blood flow measurement using isotope scanning are no longer used routinely and are reserved only for laboratory use. Similarly trans-cutaneous oximetry has been used to assess tissue oxygenation.

Techniques that may have an application in the future include intravascular ultrasound and angioscopy. With fibre-optics getting better and finer there are now angioscopes available for clinical use. They have as yet failed to gain popularity although some units have used them intraoperatively to check anastomotic sites. Intra-vascular ultrasound (IVUS) can prove to be quite exciting, as the quality of images is better than those provided by any other imaging. Cross-sectional images provided by an IVUS can be converted in to a longitudinal image of amazing accuracy and are especially useful in checking lumens after angioplasty or stenting.
The quality of images available today is truly remarkable and certainly there is great promise in the newer techniques available. They can however only supplement a good history and clinical examination. As a very wise physician once said “treat the patient not the investigation”.

**Treatment options**

Broadly speaking treatment of PVD can be divided into medical management and interventional options.

**Medical Management**

As discussed earlier there are a number of risk factors implicated in the causation of PVD. It follows that risk factor modification could play an important role in the treatment of PVD. However most of the evidence that exists in the value of risk factor modification is circumstantial and derived from studies for cardiovascular or cerebrovascular disease.

Stopping smoking is one of the most important recommendations to all patients. Physiologically this will help in multiple ways. Acutely it reduces vasospasm, increases blood and tissue oxygen levels, and lowers blood nicotine and homocystene levels. In the medium and long term it slows and possibly reverses atherosclerosis restores normal thrombogenicity of the blood, improves RBC pliability and opens up capillary bed and collateral circulation thus improving tissue perfusion. By its effects on coronary circulation it also improves cardiac function thus improving output.24

Treatment of hyperlipidemia using statin drugs has been shown to reduce
cardiovascular events in a number of large studies. The risk reduction of cardiovascular events follows an almost parallel course with risk reduction of 30% with a reduction in cholesterol level by 25%. It has been shown to be of most benefit in secondary prevention but also has some benefit in primary prevention. However such strong evidence does not exist for PVD. However since a large proportion of patients of PVD have cardiac disease as well, there is certainly no harm in lowering cholesterol levels using statins.\textsuperscript{25,26}

Control of blood pressure and diabetes mellitus plays as important a role as control of cholesterol levels in the treatment of PVD. Doubts have been expressed as to the adverse effect that lowering blood pressure would have on tissue perfusion. However this is probably of little significance compared to the systemic benefits of good blood pressure control. Control of diabetes is important in more ways than one. Controlling diabetes has been shown to reduce the risk of microangiopathy which contributes significantly to the morbidity of large vessel disease. Similarly because of the added aspect of peripheral neuropathy minor injuries can precipitate the development of CLI early. Good control also reduces infection rates and thus helps in early healing of vascular ulcers.\textsuperscript{27}

Getting out of a sedentary lifestyle is probably as difficult as stopping smoking but a graded exercise programme is one of the few life-style modifications that has been shown to make a significant difference to claudication distance even as compared to angioplasty. In a well-designed study Perkins et al from Oxford compared a graded exercise programme with angioplasty.\textsuperscript{28} While the improvement following
angioplasty was better at 3 months, at assessments at 6, 12 and 15 months those on the walking programme did significantly better. Another study in Edinburgh showed similar results. The early results (6 months) were much better with angioplasty, but at 2 years of follow-up both showed an equal walking distance which was much better than their presenting walking distance.29 A meta-analysis of 21 studies showed that there was mean improvement in walking distance of 105% (SD 56%). Factors which improved results include exercise for at least 30 minutes, 3 sessions or more a week and walking to the limit of pain as the main form of exercise.30 31 A note of caution was produced by the Oxford study which showed that after 70 months of follow up, although only 1/3rd of the patients were available for follow up, their walking distance had deteriorated, although it was still better than their presenting claudication distance.

The data for the use of anti-platelet agents is similar to that for cholesterol lowering drugs and control of hypertension. The Anti-platelets Trialists' Collaboration showed that aspirin lowered the rate of MI and stroke by 25%. Similarly the CAPRIE study has shown that both Clopidogrel and Aspirin significantly lowered the risk of cardiovascular events. Subgroup analysis showed that the relative risk of PVD was reduced by 24%. Ticlopidine is another anti-platelet agent, which has been tried and showed to have beneficial effects.32,33

Prostanoids are probably the most investigated drug in the treatment of CLI. Prostanoids such as Prostacyclin (PCI₂) and Prostaglandin E₁ act at the microcirculatory level due to their anti-platelet, anti-leukocyte, vasodilatory and cytoprotective effects. The stable analogue of PCI₂ Ileoprost is used as an
intravascular infusion in the dose of up to 2mg/kg/minute for upto 2-4 weeks. At present its use is restricted to those patients in whom reconstructive surgery is unsuitable but who nevertheless have rest pain or ulcers. They have also been tried before amputations and have been shown to give better healing rates. A meta-analysis of six controlled studies of iloprost suggested an advantage in terms of reducing mortality and amputation rates.\textsuperscript{34}

Adjunctive therapies that have been tried include HRT in post menopausal women, anti-oxidants, drugs lowering plasma homocysteine levels such as folates.

Gene therapy has been used experimentally to stimulate angiogenesis and certainly shows promise. All the medical therapies are summarised in Table 2.\textsuperscript{10,26,27,32-36}

Surgery and interventional radiology

The advent of angioplasty in the 1980s radically changed the treatment options adding a very powerful weapon in the battle to preserve legs. Certainly a large chunk of a vascular surgeons' PVD workload has been taken over by the interventional radiologist. Given the topic of this thesis, angioplasty deserves a detailed discussion and will be dealt with in greater detail in the next chapter.

Having said that, surgery still plays an important role in the treatment of PVD primarily because it provides the best long-term results. The principles of vascular surgery are fairly simple. For surgery to work there has to be a good inflow, a good outflow and good conduit with sound anastomosis. Surgery can be divided into anatomical and extra-anatomical. It can further be divided into supra and infrainguinal. Supra-inguinal bypasses include aorto-bifemoral, ileo-femoral,
<table>
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<th>Drugs</th>
<th>Clinical benefit</th>
<th>Evidence/Reference</th>
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<td>Anti-diabetic</td>
<td>Insulin</td>
<td>Risk factor modification, improved microcirculation, reduced infection</td>
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<td>Vitamins B₁₂ B₆ and folate</td>
<td>↓ of NO inhibition of VSMC proliferation</td>
<td>Shearman et al</td>
</tr>
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<tr>
<td>Anti-oxidants</td>
<td>Vitamin C &amp; E β carotene</td>
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</tr>
<tr>
<td>Gene-therapy</td>
<td>VEGF</td>
<td>VSMC inhibition</td>
<td>Manninen et al</td>
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Table 2.1 Medical management of peripheral vascular disease
femoro-femoral and axillo-uni or bi-femoral grafts. Results for supra-inguinal grafts
tend on the whole to be very good with long term patency of >90% at 5 years being
quite common. However, if it involves aortic or iliac explorations the morbidity and
mortality is substantially higher.

Infra-inguinal operations include endarterectomies usually at the bifurcation of the
common femoral artery (CFA) and bypasses from the CFA to either the above or
below knee popliteal artery (AK or BK), the calf vessels or the pedal vessels. Bypasses
are also performed from the popliteal artery to the calf or pedal vessels. As is to be
expected, results get worse the lower down the leg the anastomosis is.

The commonly used conduits are autologous vein or synthetic grafts. Veins can be
harvested from ipsilateral or contralateral long or short saphenous veins (LSV or
SSV), deep veins of the leg, or the superficial arm veins. Ipsilateral LSV is the most
commonly used graft and can be used either in-situ by destroying the valves using a
valvulotome or by dissecting out and reversing the vein. The 2 commonly used
synthetic conduits include Polytetraflouroethelene (PTFE) and Dacron. On the whole
for supra-inguinal bypasses there is no proven advantage in using vein over synthetic
graft except in infected fields. In the infra-inguinal bypasses veins are by far better.

The medium term results for femoro-AK popliteal bypasses using vein and synthetic
grafts are the same (70-80% at 1 year). However, long term results are much better
using vein, with synthetic grafts starting to fail at about 3-4 years. In bypasses to below
the knee veins are superior by far and should be used as far as possible. The patency of
synthetic grafts has been shown to be better if a vein cuff such as a Miller’s cuff or a St
Mary’s boot, is used at the lower anastomosis. The reasons for graft failure
include poor anastomosis or preparation of the graft, infection, neo-intimal hyperplasia (NIH) and disease progression.37

Outcomes

As clinicians we see only the tip of the iceberg as far as PVD is concerned. As mentioned earlier a vast majority of patients are asymptomatic. Even among those who are symptomatic only a proportion present to their doctor (Oxford study-50%, London-<10%).16 The estimated annual workload for CLI alone in GB & I estimated by the VSS Audit, is 21450 legs in 20000 patients leading to 12810 revascularisation procedures and 4860 amputations.18 The overall success rate for all revascularisation procedure is only about 75%. However for both claudication and for CLI there is no doubt that the quality of life improvement after successful intervention is remarkable. While surgery does produce a more sustained response there is always an associated mortality and morbidity. It may seem like an excessive risk, especially for claudicants. Angioplasty and its adjuvant treatments hence have the potential to play an important role in filling this need for a low risk procedure. Its biggest drawback is its utility being limited to proximal disease. This is where a lot of effort is being concentrated, as we shall discuss in the next chapter.
Chapter 3. Angioplasty

History

As with so many useful breakthroughs, angioplasty was first performed quite serendipitously by Charles Dotter in the USA in 1963. While performing an aortogram he pushed a catheter through an occluded iliac artery in a retrograde manner thus opening up a channel to the aorta. A year later he carried out the first deliberate procedure in a woman with gangrene who was refusing amputation. He used a coaxial system of catheters. A guide-wire was introduced and an 8F Teflon catheter was guided over it. Further dilatation was achieved by passing a 12F catheter over the indwelling catheter. As can be imagined this left a rather large hole in the artery. There was a lot of enthusiasm for the technique in Europe but not so in the US except for Dotter and his group. Although Dotter is also credited with using a balloon catheter for dilatation his balloon was too thrombogenic and the technique was given up quickly. Porstman first introduced a balloon with a Teflon cage, which gave it added strength. However its uneven outer surface it was still too traumatic to the vessel wall. Gruntzig first developed a double lumen polyvinyl chloride balloon, which could be inflated to up to 5 atmospheres. The forces acting on the plaque were more radial than axial thus leaving a smoother contour and a better result. The balloon system was further improved by the introduction of the polyethylene balloon. Concurrently guide-wire materials have also improved with most peripheral guide-wires 0.035 to 0.038 inch in diameter. They can be soft tipped with high torque, which allows atraumatic passage.
through occluded and tortuous arteries.

Procedure

Percutaneous transluminal angioplasty (PTA) is ideally carried out in a specialised unit specifically equipped for interventional vascular procedures. The concept of an endovascular operating theatre is quite popular in the US but although suggested has not quite caught on here in the UK. This primarily involves a sterile operating room with a ceiling mounted C-arm X-ray unit with a radiolucent operating table made of carbon fibre. The most commonly used imaging system is a high-resolution fluoroscopy unit with road-mapping capability.

The technique used universally is the Seldinger technique. In case the femoral pulse is not felt a smart needle may be introduced under ultrasound guidance. After puncture of the common femoral artery (CFA) a short guide-wire is introduced through the needle antegrade for the SFA and retrograde for the CIA. An introducer sheath is then railroaded over the guide-wire and its position is usually confirmed with a small injection of contrast. After systemic heparinisation a longer guide-wire is introduced through the sheath and the stenosis or occluded segment is passed. Special guide-wires may be needed to traverse difficult occlusions in tortuous vessels especially distally. Once the guide-wire is passed beyond the lesion pressure measurements can be taken to record the gradient across the lesion. The appropriate balloon catheter is then guided over the guide-wire. The radioopaque markers marking the ends of the balloons are used to advance the balloon and position it along the lesion. The balloon is then inflated using dilute contrast. It is usually inflated to a pressure of 6-10 atmospheres
and is usually held inflated for about 60-90 seconds. After deflation of the balloon the catheter is withdrawn and a completion angiogram is done. If the angiographic result is not good enough, the inflation can be repeated one more time. The pressure can be increased in order to fracture the plaque. The result can be measured in two ways. The residual narrowing should be <30%. Alternatively the pressure gradient across the lesion should be abolished or <10 mm Hg. In the presence of vasospasm vasodilators such as papaverine or nitro-glycerine can be flushed down the catheter into the artery. Once satisfied with the result the catheter and guide-wire is gently pulled out. It is important to confirm meticulous haemostasis at the puncture site especially if the patient is anticoagulated. Most oozing stops by simple pressure. A device known as a Vaso-seal can been used if required, to plug the arterial puncture site with good effect.

The mechanism by which angioplasty causes dilatation of the artery, has been well studied. Dotter had initially suggested that it was purely because of compression of the plaque and draining out of all the water and the lipids from the plaque. This has however been shown to be negligible following angioplasty. Animal experiments have shown there to be stretching of the intima media and adventitia. There appears to be straightening of the elastic fibres in the media and a corkscrew deformity of the nuclei of the VSMC. Dilatation of post-mortem atherosclerotic arteries has been shown to cause plaque fracturing and cracking and dehiscence of the intima and media. This may lead to dissection in the media. The disrupted arterial layers heal by formation of neo-intima and scar tissue. There is even a suggestion that the more the calcification, the better the fractures, contributing to a better eventual result.
However a recent study which looked at intravascular US of the lesion before and after angioplasty has shown that the higher the echogenicity of the plaque the worse the outcome because of both acute thrombosis and restenosis. This in physical terms means that the higher the quantity of fibrous tissue and calcification the worse the results. It even suggests that angioplasty should be reserved for plaques with low echogenicity. Work still remains to be done to understand the mechanisms involved in angioplasty, which might have a bearing in preventing restenosis.

**Outcomes**

When Dotter et al first reported their cases in 1965, there was initial apathy to testing this new technique, especially in the US. However since the late 1970s there has been a plethora of publications testing the use of angioplasty. Most papers in the 1980s showed a high rate of long term success. However these rates have shown a downward trend in the 1990s. There are a number of possible reasons. The most likely reason is that angioplasty is attempted for increasingly difficult lesions and more distal lesions. Secondly, the criteria used for assessing patency has shifted from just clinical assessment and measurement of ABPI to duplex scanning of the angioplasty sites. The Standards of Practice Committee of the Society of Cardiovascular and Interventional Radiology with Robert Rutherford laid down guidelines in 1991 for reporting results in PVD. The recommendations were:

1. Patency has to be defined as a.) *Primary patency* as a period of uninterrupted patency without further intervention b.) *Secondary patency* as the outcome following one or more re-interventions to restore blood flow in a
non-functioning PTA or graft and c.) Assisted primary patency whereby patency in a failing graft or PTA was diagnosed and early remedial action taken before the graft or PTA failed completely.

2.Patients in a series should include the whole cohort treated within the time-span of the study in an intention to treat manner, without exclusion of initial technical failures and death from final results.

3. Patients should be graded according to their presenting symptoms:

   Grade I  Claudication mild to severe
   Grade II  Ischaemic rest pain
   Grade III  Tissue loss

4. Outcome measures should confirm to objective assessments of patency including one or more of the following: angiography, duplex and maintenance of improvement in segmental pressure index.

**Iliac angioplasty**

Angioplasty is definitely the treatment of choice for iliac lesions and should be attempted before any surgical procedure is considered. Most recent series have reported an initial success rate of over 90% and 2-year patency of over 80%. Any surgery in this area has a very high morbidity and a peri-operative mortality of at least 5%. Surgery is nowadays considered only when angioplasty fails and some form of intervention is absolutely indicated. Table 3.1 lists the results of iliac angioplasty.\textsuperscript{51,55}
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Table 3.1  Major series reporting outcome of iliac angioplasty.
Femoropopliteal angioplasty

Results for angioplasty to femoro-popliteal segments of the lower limb vasculature have not been quite so successful. Matsi et al who were one of the few investigators to use Duplex to assess the patency of the angioplastied site, reported a 1 year patency of only 47% and a 2 year patency of 42%. Other studies using either clinical assessment or ABPI measurement have reported 1-year patencies from 58-65% and 2-year patencies of 45-53%. There have been only few studies that have followed up patients for longer than 2 years. Johnstone et al reported a 5-year patency of only 38%. The results for femoro-popliteal angioplasty are summarised in table 3.2.56-61

It is immediately clear than results of angioplasty in the fern-pop segment are much worse compared to the iliac. Similarly results in the periphery tend to be worse than coronary vessels. While there is no strong evidence to explain this, it is believed to be due to differences in the lesions and the flow patterns in the 2 circulations. The lesions in the coronary vessels tend to be focal and short lesions while in the periphery they are much longer. Hence longer segments need to be angioplastied which in turn adversely affects the results. The procedure causes more injury and leaves a larger area without endothelial cover. There also tens to be concurrent lesions along other segments of the vessel in the periphery which influence the flow in the vessel. The velocity of flow within the coronary artery is also much higher, again leading to better results and patency.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Indication</th>
<th>No Of Patients/PTA</th>
<th>Assessment</th>
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<th>Patency</th>
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Table 3.2  Major series reporting outcome of femoro-popliteal angioplasty

54
Complications

Lower limb angioplasty is a relatively safe procedure with a very low rate of complications. Moreover the majority of the complications are easily managed. The complications following angioplasty can be divided into puncture site complications, angioplasty site complications and systemic complications.

The commonest puncture site complication is bleeding and haematoma formation. Gardiner et al reported a rate of 4% in 352 patients, of which only one needed surgical intervention. There was only one false aneurysm. These are caused by persistent oozing from the puncture site and formation of an organised haematoma around the vessel. Although these were fairly common in the early series they are rare nowadays. They can be treated non-surgically with thrombin injections into the pseudo-aneurysms or with pressure at the arterial puncture under duplex guidance. Other complications include infection of the puncture site and thrombosis.

The most common angioplasty site complication is thrombosis and acute occlusion. These tend to occur at more distal sites of angioplasty such as the popliteal and the tibial vessels. Thrombolysis is usually sufficient to reverse this. However surgery should be considered if there is no prompt response to the thrombolysis. Similarly one can trigger distal embolisation. However these tend to be minor with no clinical consequences. Matsi et al reported 11 cases in 410 angioplasties most of which did not need any treatment. Dissection and sub-intimal passage of the guide-wire and catheter is another possible complication. However as we will discuss later sub-intimal angioplasty which involves deliberate dissection into the sub-intimal plane is now a
Systemic complications include fluid overload due to the injection of dye leading to CCF especially in patients with pre-existing cardiac problems. Renal failure can be precipitated by the iodine in the contrast media especially in diabetics. It is recommended that Metformin an oral anti-diabetic agent is stopped for 48 hours before angioplasty as it increases the chances of precipitating renal failure.

In a review by Eikelboom et al of 9627 peripheral PTAs the overall complication rate was 11.8% with 4.1% being major complications. Only 2.6% needed surgery. The overall mortality was only 0.28% similar to Gardiner's series with a mortality of 0.3%.

**Adjuvant treatments**

The problems of both early failure and later restenosis following angioplasty have prompted a number of adjuvant and alternative therapies to be tried with varying success.

**Stenting**

When Dotter first described angioplasty he hypothesised that in some cases a frame may be needed to keep the angioplastied site open. He went on to invent a coil stent made of nitinol, which is an alloy of nickel and titanium. This material has a most useful property of expansion on exposure to body temperature and can be compressed by cooling in order to fit around a catheter.

The stents work on the basic principle of providing a frame, which prevents the
recoil and collapse of the angioplastied vessel. This would invariably lead to thrombosis of the vessel and immediate occlusion. There are currently 2 types of stents in use. The Palmaz stent (Cordis Corporation, Warren NJ) and the Strecker stent (Medi-Tech, Watertown MA) are balloon-expanded stents. These either come pre-mounted on an unexpanded balloon or have to be mounted on it and crimped to fit around the balloon. When the balloon expands the stent opens up and fits snugly to the wall of the vessel. The second type of stent used is the self-expanding stent such as the Wall stent (Schneider (USA), Inc., and Minneapolis MN) and the Gianturco Z-stent (Cook, Inc., Bloomington IN). These come mounted on a separate catheter and have a sheath covering it. Once inserted and in place the sheath is withdrawn and the stent opens up and positions itself. These stents are not covered and hence leave the side branches open so that the collateral circulation is not compromised. These stents are available in varying diameters and lengths. Stents are used commonly in CIA disease with good success. A number of studies have shown a 2-year patency of about 80%. In one series by Vorwerk et al the 2-year patency for occlusions was 83%. Although there was a good deal of enthusiasm for the use of stents for iliac disease this is waning because studies by Richter and the Dutch Iliac Stent Trial Study Group showed no benefit in primary stenting. It is now used only in selected cases. Table 3.3 summarises the recent results with iliac stenting.

Stents have been used infra-inguinally as well with varying success rates. In one series by Gray et al the one-year patency was only 22% of 58 patients. However these were long occlusions with poor run-offs in the majority of cases. In general results for infra-
inguinal stenting are poor. Table 3.4 summarises the results from some recent trails. The explanation given for the poorer patency in femoro-popliteal arteries as compared to the iliac is the smaller calibre of the vessel. The coronary vessels are however not much bigger than the femoro-popliteal vessels, but the results in the coronary are significantly better. As discussed earlier this is mainly because of the lesion size and flow characteristics. Short focal lesions with fast flow in the coronary, means that the stents are short and disruption of the intima is reduced. Long lesions in the SFA with presence of disease in other segments of the vessel necessitate the need for longer stents which don’t work. Coupled with slower blood flow and increased intimal injury this leads to platelet aggregation and fibrin deposition and greater restenosis. All of these patients are on aspirin unless otherwise contraindicated. However the results of stents have been further improved with concurrent use of clopidogrel and IIb/IIIa antibody such as Reopro. Similarly with the advent of drug-eluting stents there has been a dramatic improvement in the results of coronary stenting. This has not yet been duplicated in the periphery. In the periphery all patients are on aspirin but there is no evidence that clopidogrel or Reopro improves results.

Sub-intimal angioplasty

This is a recent technique described first by Bolia and associates from Leicester. The technique, done via an ante-grade approach through the ipsilateral CFA, involves creating a deliberate dissection between the intima and the media using a floppy tipped guide-wire. Sometimes a pre-shaped catheter called a Cobra may be needed. Entry into the sub-intimal space is confirmed by injection of contrast.
<table>
<thead>
<tr>
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<th>Patency</th>
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<td>PTA+Selective stenting - 136/59</td>
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Table 3.3  Major series reporting outcome of iliac stenting
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<td>Duplex</td>
<td>91</td>
<td>67 46</td>
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Table 3.4  Major series reporting outcome of femoro-popliteal stenting

60
This usually being the plane of least resistance through the occlusion, the guide-wire usually passes through the occlusion easily. The straight wire is replaced by a J-shaped wire, which is used to re-enter the lumen 2-3 cm. distal to the occlusion. A balloon catheter is passed over the guide-wire and used to perform the angioplasty by inflating it to about 10-12 atmospheres of inflation for about 15 seconds. Bolia et al reported on 200 patients (154 in the femoral and 46 in the popliteal). Primary success was 80%. The 1-year patency was 56%, which was however based only on clinical findings. While results in other centres have not been as good as in Bolia’s group nevertheless they have reported some success. It is especially useful in critically ischaemic limbs where the only alternative is amputations. Bolia’s group have recently published results at 36 months with limb salvage rates of 94% with clinical improvement in 84%. It certainly plays an important part in limb salvage procedures if the only alternative is surgery and has been tried even in infra-popliteal vessels.

Others
Atherectomy is the removal of plaques by cutting, pulverising or shaving them off the arterial wall. A number of devices have been used, which usually consist of two parts, one to actually destroy the plaque and one to collect the debris so that it doesn't embolise distally. The AtheroCath (Devices for Vascular Intervention, Redwood City CA) consisted of a rotary cutting device and a distal collection chamber. The TEC system (Interventional Technologies, San Diego, CA) also used a rotary cutting device but used suction to clear the debris. The Auth Rotabulator (Heart Technologies, Bellevue, WA) consists of a burr rotation at 1,00,000 rpm which causes fine particle
abrasion of the plaque.

However none of these devices have yet proven their efficacy and are not used routinely in clinical practice.

In the early 90's there was a lot of enthusiasm to use lasers to burn a hole through an occlusive plaque. Nd-YAG and argon lasers have been used to create a hot tip at the end of the laser probe. This vaporises the plaque tissue. The passage created was then used for angioplasty of the occlusion. However enthusiasm has since diminished due to an unacceptably high complication rate and poor initial and late results.

Some newer methods which are still in the experimental stage include an intravascular ultrasound device which pulverises the plaque using ultrasonic waves and a modification of the stents to use stent grafts made of PTFE. These stent grafts however have to be put in by an open arteriotomy and can either be left intravascularly or be continued down as a femoropopliteal or distal bypass.

**Summary**

Angioplasty is the first line treatment for iliac arterial obstructive disease. Although not as efficacious in infra-inguinal disease it is still considered first line treatment if interventional treatment is needed. It has reduced the need for surgical intervention in the vast majority of cases and revolutionised the approaches to PVD. But with every new modality arises a new set of problems. Restenosis remains the biggest challenge for vascular biologists and will be investigated in further detail in the next chapter.
Chapter 4. Restenosis

Restenosis is a mechanical obstruction to blood flow that occurs after an initially successful vascular intervention. It can occur after angioplasty, stenting and surgical revascularisation such as a bypass or endarterectomy. Its incidence depends on the method of detection and criteria for assessment. While in coronary disease assessment is based on angiographic findings in peripheral disease it is often clinical. Doppler and Duplex assessment provide an excellent non-interventional alternative to angiography.

The quoted incidence of restenosis is only relevant when the method of assessment and definition of restenosis are stated. Even though it is an ongoing process for clinical comparison the assessments have to take place at specified time points in order to facilitate clinical comparison. The assessment and definition of restenosis will be considered next.

Diagnosis of restenosis

Clinical assessment and ABPI

Traditionally all patients who have had a peripheral vascular intervention are assessed clinically and by Ankle Brachial Pressure Index (ABPI) measurement. Clinical evaluation involves recurrence of symptoms and walking distance. In patients with CLI this is easy. Persistence or recurrence of rest pain, non-healing or recurrence of ischaemic ulcers and new areas of non-viability are easily documented. Early manifestations of return in rest pain includes rest pain on elevation presenting as night pains. Assessment of claudication is more subjective with change in walking distance.
on the flat being a good indicator of outcome. This can however be obscured by co-existing conditions such as arthritis and spinal stenosis. Similarly presence of exercise intolerance and dyspnoea may further influence walking distance. Walking distance can be objectively assessed by a treadmill test. Presence or absence of peripheral pulses is also a subjective assessment as the ability of various clinicians to palpate pulses varies and it only differentiates between patent and occluded vessels.

Measurement of ABPI is a more objective assessment of deterioration in peripheral circulation. This is ideally measured using a hand-held Doppler probe. Any recurrence of symptoms or worsening of ABPI is indicative of a problem and certainly needs investigation. A fall in ABPI of >0.15 is considered significant. The sensitivity can be increased by measuring pre and post exercise values. The biggest drawback is that it is not lesion specific and is unable to differentiate between restenosis at the intervention site and a new lesion.

**Duplex surveillance**

Duplex is an excellent initial investigation in the assessment of restenosis. It has the ability to not only visualise the lesion but also to quantify it. Duplex is the combination of a B-mode ultrasound and a pulsed wave Doppler. The Doppler signal can be converted into a flow image superimposed on the ultrasound image with colour or gray-scale coding to indicate direction and velocity of flow. Thus it demonstrates a restenosis by showing narrowing of the lumen in both the transverse and longitudinal plane and quantifies the narrowing by the alteration in the velocity of flow. This is usually expressed as a ratio of velocity at the area of restenosis and the velocity of
The peak systolic velocity ratio (PSVR) is the most commonly used parameter to objectively measure restenosis. A PSVR of >2 is considered significant restenosis and equates to a narrowing of >50% of the lumen and is the most commonly used criteria to define restenosis.8 8

The biggest problem with Duplex is that it is operator dependent and the greater the skill of the operator the more accurate the results. Assuming that angiography is the gold standard in assessing stenosis, in experienced hands however the sensitivity and specificity of Duplex both in identifying and quantifying arterial stenosis is as good as an angiogram if not better.89-91 Newer machines with increased sensitivity have overcome the old problem of assessment in the face of multiple stenosis and in calcified vessels. It also has the advantage of delineating plaque morphology which may play a role in outcome and prognosis.47

It is however important to differentiate between restenosis and the need for re-intervention. This has to be a clinical decision based on symptoms and the natural history of the problem. Plaque characterisation may help one decide which lesions need re-intervention and which don’t. Duplex surveillance of grafts has been used and is common practice in a number of units. But its usefulness is not yet established. While it picks up treatable lesions earlier it does not affect limb salvage.92-95

**Intravascular ultrasound**

This is a more recent development in the approach to assessing restenosis. While it provides an excellent view of the cross-section of the artery it is not suitable for routine use as it is invasive and expensive. In animal models it has shown increased...
knowledge of changes in the arterial wall after intervention. Clinical studies have shown benefit in predicting restenosis after angioplasty.\textsuperscript{96,97}

**Angiography**

Peripheral arteriography has long been considered the gold standard in evaluating restenosis. It does provide the most accurate operator independent evaluation of the vessel lumen and is easily reproducible to compare with earlier films. However it is an invasive procedure with risk of complications. It provides only uni-planar views a problem which to an extent has been eliminated by using a C-arm to get multiple views. It is also now possible to measure pressure gradients across a lesion, providing good information on flow patterns. Angiography is indicated in patients who are going to have a endovascular re-intervention and will then be done along with the therapeutic procedure. Its use purely as a diagnostic tool in diagnosing and quantifying restenosis is limited. It is however routinely done if surgical intervention is planned.

On angiography restenosis is defined by a >50% loss in the gain in diameter achieved by PTA or an increase in % diameter stenosis by >30% from the post PTA value. These measurement have to be made using standard software in order to avoid inter and intra-observer variation which have been shown to be significant.\textsuperscript{98-100} The volume and rate of contrast injection also has to be standardised.

**MRA and CT angiography.**

These non-invasive techniques have made great strides in the last few years and provide excellent images for both proximal and distal disease. These are especially
useful in assessing distal run-off distal to the occlusion and in proximal vessels which can’t be assessed on Duplex because of body habitus. At present their main disadvantage is cost and availability. The information available on modern Duplex machines is so good that they will remain the first investigation in assessing restenosis. As mentioned in chapter 3 between 35-50% of all femoro-popliteal angioplasties restenose in the first year alone. There are various reasons as to why this can happen. Obviously there is the category where angioplasty fails almost immediately. This is usually due to thrombosis within the vessel. It is important to remember the physical changes occurring in the plaque at the time of angioplasty. It is almost inevitable the plaque fractures during angioplasty. This exposes the highly thrombogenic material within the plaque to blood and it is amazing that thrombosis does not occur in every instance. Obviously the increase in luminal diameter results in an increased velocity of blood flow which contributes to preventing this from happening. Does this mean that it is only those angioplasties that lead to inadequate dilatation in the lumen size that thrombose off? Immediate failure is brought about by such factors as a poor run-off and poor inflow. Ramaswami et al have recently shown that plaque character plays an important role influencing results. They have suggested that the worse the calcification and the more radio-opaque the plaques are the greater the chance of failure. These patients usually need immediate intervention such as thrombolysis or surgery.

Pathogenesis of restenosis

Why do some vessels restenose and others don’t? Is it possible to predict which will need intervention and which won’t? Most work has been done on animal models
with artificially injured artery. The problem with this is that atherosclerotic plaques already have VSMCs in them and are bound to have a different biological response to the injury model. There is however an atherosclerotic model in rabbits which is helping in our understanding of the disease. True restenosis has 2 main causes, intimal hyperplasia and progression of disease. It is commonly believed that recurrence in the first year is primarily due to intimal hyperplasia and recurrence subsequent to that is primarily due to progression of atherosclerosis. Inevitably both processes act in tandem to produce lumen loss. The resulting loss in luminal area is exaggerated by the process of negative remodelling i.e. shrinkage of the vessel in the long term.

**Phases of NIH**

Very simply put, intimal hyperplasia is an inflammatory response to injury to the intima. As in the skin this response can be hyperplastic and can thus do more harm than good. Fuster et al divided this process into 3 phases. Phase I which is basically recoil of the angioplastied vessel occurs in the first 24 hours. Phase II involves the formation of a mural thrombus and reorganisation of the connective tissue in the ensuing 2 weeks. Phase III can be divide into 3 waves and overlaps with Phase II but continues on for 2-3 months.

The first wave seen within 2 days after balloon injury consists of replication of VSMC in the media. This is brought about by the release of basic fibroblast growth factor (bFGF). The bFGF is pre-formed and stored in medial VSMC and released when there is medial injury caused by the angioplasty. It may also be present on matrix
binding sites in the extracellular matrix and released on injury or by stimulation by platelets.

The second wave of response is characterised by the migration of these VSMC from their normal position to the intimal layer. This is brought about by platelet-derived growth factor (PDGF).\textsuperscript{103} Arterial injury stimulates the release of plasminogen activators. These can cause degradation of proteins in the extracellular matrix along with the internal elastic lamina. Injury also releases matrix metalloproteinases (MMP), which not only degrade collagen fragments but also cause lysis of elastic fibres. They also delay the formation of a neo-intima.\textsuperscript{104-106}

Once in the intima, these VSMC replicate to give rise to the third wave of response. It is uncertain as to what exactly causes the mitogenesis. However it is known that large quantities of cytokines and leukotrines such as tumour necrosis factorα (TNFα) are released at the time of arterial injury and these could well lead to the VSMC proliferation.\textsuperscript{45} Simultaneously there is also an increased deposition of extracellular matrix in the intima following injury. In fact it may play quite a major role in NIH given that restenosis usually occurs over months while VSMC proliferation stops within weeks of injury while matrix deposition does not. This theory is further strengthened by the fact that VSMC replication in human models is not as frequent as injury models.\textsuperscript{45}

The various growth factors involved in the above waves are secreted by a variety of different cells such as the endothelial cells, VSMC, platelets and macrophages which are mobilised to the site of injury. Endothelial re-growth also plays a role in
the regulation of NIH by inhibiting both migration and deposition of VSMC.\textsuperscript{103,107,108} It also controls vessel tone and platelet aggregation through the secretion of nitric oxide (NO).\textsuperscript{109,110}

**Remodelling**

To these can be added a 4\textsuperscript{th} stage influenced primarily by arterial remodelling. Glagov et al showed that the human artery responds to the loss in lumen area associated with an atherosclerotic plaque by undergoing adaptive enlargement.\textsuperscript{111} In fact up to a 40% narrowing of the lumen can be compensated by arterial expansion. There is increasing evidence that arterial remodelling plays an important role in the eventual outcome of angioplasty. It is suggested that the eventual lumen area is dependent more on the amount a vessels shrinks after angioplasty than on the extent of NIH. This has been borne out by the fact that stenting an artery does not prevent NIH but maintains luminal size by holding the vessel open. It is likely that matrix remodelling plays a major role in arterial remodelling.

The mechanism causing remodelling has not been clearly demonstrated. It is known that the degree of injury caused by the angioplasty influences the eventual lumen diameter.\textsuperscript{112} The degree of balloon injury has been described by Schwartz et al\textsuperscript{46} as follows:

*Score 0*  Endothelium denuded; IEL intact; Media compressed  
*Score 1*  IEL lacerated; media compressed but not lacerated  
*Score 2*  IEL and Media lacerated; EEL intact  
*Score 3*  Media disrupted; EEL lacerated
The greater the injury score, the more the neo-intima area and larger the whole arterial dimension. When the net result of this remodelling is overall shrinkage of the artery it is called negative remodelling. It is feasible that this is the result of ongoing inflammation and scarring in the adventitia following the injury of angioplasty. Thus if the injury score is higher although the initial result may be a bigger artery the eventual scarring may in fact lead to negative remodelling and restenosis. Remodelling is probably mediated by regulation of elastin and collagen by MMPs and TIMPs. It has been suggested that luminal blood flow post-intervention may be a factor in determining the extent of remodelling. The degree of injury to the vasa vasorum during balloon injury may also affect remodelling.

**Medical and experimental interventions**

**Anti-platelet agents**

Although there are no randomised controlled trials showing an advantage of aspirin over a placebo in preventing restenosis Schwartz et al has shown a small beneficial effect with aspirin. Minar et al randomised 216 patients into 2 groups one getting 1000mg of aspirin, one getting 100mg of aspirin and showed no difference in the 2 groups in terms of restenosis although compliance was better in the low-dose group which also had lesser complications. However, independent of this effect, the proven benefits of preventing cardiovascular morbidity and mortality should prompt every patient to be put on at least 75-150 mg of aspirin daily. Recent additions to this group including ticlopidine and clopidogrel, which again have shown benefit in preventing cardiovascular events both independently and in conjunction with aspirin.
These may also play a role in the prevention of restenosis.32

**Anti-coagulants**

Heparin is widely used both during percutaneous and surgical vascular procedures and its role cannot be overemphasized in preventing thrombosis. However, its long-term role in the prevention of NIH has not been established. There is no evidence to suggest that warfarin, in spite of its anti-thrombin effect, is effective in preventing restenosis.

**ACE inhibitors.**

Although Angiotensin converting enzyme (ACE) inhibitors have been shown to inhibit VSMC proliferation in vitro and in vivo in animals, ACE inhibitors have been shown not to have any effect in preventing restenosis in a clinical setting.

**Antiproliferative therapy and gene therapy.**

As already mentioned, growth factors play an important role in the development of NIH. These growth factors commonly act by stimulating surface receptors, which in turn stimulate the secretion of mitogenic proteins, or by directly modifying oncogenes within the cell. Thus there can theoretically be a 2-pronged attack on this activity. Antiproliferative agents act by blocking the effect of the growth factors either by inhibiting them directly or by blocking the growth factor receptors. The other mode of attack would be to inhibit gene expression. The problems with these methods are two fold. No suitable animal models are available to recreate an atherosclerotic model to test it on. Secondly, presently available delivery systems are very inefficient.118

The systemic therapies used against restenosis are summarised in Table 4.1
<table>
<thead>
<tr>
<th>Generic therapy</th>
<th>Specific examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-platelet drugs</td>
<td>Aspirin, dipyridamole, Ticlopidine</td>
</tr>
<tr>
<td>Anti-coagulants</td>
<td>Heparin, warfarin</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td>Dexamethasone, prednisolone</td>
</tr>
<tr>
<td>Anti-oxidant</td>
<td>Probulol Lazaroids</td>
</tr>
<tr>
<td>Antibodies</td>
<td>Anti-platelet, anti-CD18, Anti-PDGF, Anti-FGF</td>
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<td>ACE inhibitors</td>
<td>Captopril</td>
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<tr>
<td>Calcium-antagonist</td>
<td>Nifedepine, Diltiazem</td>
</tr>
<tr>
<td>Immunosuppressant</td>
<td>Azathioprine, cyclosporine</td>
</tr>
<tr>
<td>Matrix modulators</td>
<td>Collagen synthetase inhibitors</td>
</tr>
<tr>
<td>Endothelial modulators</td>
<td>Prostacyclin E2, L-Arginine</td>
</tr>
<tr>
<td>Peptides</td>
<td>TGF-β, interferon γ, somatostatin</td>
</tr>
<tr>
<td>Receptor antagonists</td>
<td>Prazocin, losartan, ketanserin</td>
</tr>
</tbody>
</table>

Table 4.1  Systemic therapies used to reduce restenosis
Interventional techniques.

We have already talked about the use of stenting in the previous chapter. However it has been shown that stenting does not prevent NIH. The neointima tends to grow over and through the stent and incorporates itself within it. The stent works on the principle of providing a scaffolding to hold the lumen open thus giving credence to the hypothesis of vascular remodelling. Similarly atherectomy devices and laser angioplasty have been attempted to prevent restenosis, none of which have been found to be useful.

**Brachytherapy**

Brachytherapy is the use of intra-luminal radiotherapy as an adjuvant to angioplasty and stenting in order to prevent restenosis. It does so both by inhibition of VSMC proliferation as well as promoting positive remodelling. It prevents late vessel constriction by either reducing collagen and matrix production, or thinning of the residual plaque and media. Intra-luminal radiotherapy is delivered by either catheter-based devises or by radioactive stents. The radiotherapy is provided using radioactive isotopes such as yttrium-90\(^{(90} \text{Y})\) providing \(\beta\)-radiation and iridium-192 \(^{(192}\text{Ir})\) providing \(\gamma\) radiation. While this has been tested extensively in the coronary circulation there have been very few trials in PVD. This is discussed in greater detail in the next chapters.

It is however not without problems. There have been several reports of early late thrombosis at 24-72 hours. Another major problem is that of edge restenosis when there is an increased neointimal response at the edges of the area radiated. The doses
of radiation used are not yet standardised ranging from 5Gy to 25Gy. It is still not clear as to which form of radioactivity is better. Doubts have also been cast as to the long term effects of radiotherapy on the vessel wall, the fear being that there will be aneurysmal change and even vessel blow-out. Further the carcinogenic effect of the radiation is unaccounted for. \(^{119,120}\)

**Coated stents**

In the last five years there has been renewed interest in the use of coated stents as a means of preventing in-stent restenosis. This interest has been stimulated by the use of Rapamycin (sirolimus) and paclitaxel, agents which were previously used as chemotherapy for certain cancers. Sirolimus has weak antibiotic properties but it acts primarily by being an immune modulator and inhibiting the effects of growth factors described in the earlier chapter. It does this by inhibiting kinase pathways responsible for RNA translation thus inhibiting formation of proteins essential for VSMC migration and proliferation.\(^{121}\) Paclitaxel is an anti-proliferative agent which specifically inhibits the G\(_2\)/M phase of cell division.\(^{119,122}\) During the period of writing up of this thesis it has become obvious that drug eluting stents are proving to be extremely effective in inhibiting restenosis. This has been shown in a number of trials and seems to be heading towards first line treatment of coronary artery occlusive disease. They have not yet proven their place in PVD and results for this are forthcoming.

**Photodynamic therapy. (PDT)**

PDT is a novel technique in which application of light at particular frequency to a previously loaded photosensitive drug leads to activation of the drug, release of
highly reactive free radicals and hence cell death. PDT has been used for tumour ablation and palliation in oesophageal, colonic, bronchial and oro-pharyngeal malignancies. In the last 10 years we have pioneered the use of PDT in tackling this problem. PDT has been shown to cause VSMC and endothelial cell death by apoptosis which in turn leads to inhibition of restenosis. The cell loss is temporary and there is endothelial repopulation in 3-7 days and VSMC repopulation with in 14-28 days in animal models. There is no inflammation in the vessel and no apparent long-term effects to the vessel wall. My work in this thesis deals with the application of PDT to restenosis and in-stent restenosis. It is discussed in great detail in the next chapter.

**Stenting and In-stent restenosis**

As already mentioned in the previous chapter stenting is a commonly used adjuvant to PTA. About 85% of coronary angioplasty is accompanied by stenting and over 50% of iliac angioplasty is accompanied by stenting. Stenting is used as a means of preventing restenosis as well as for the treatment of it. However as previously mentioned stenting does not prevent NIH. It acts by maintaining lumen diameter and preventing negative remodelling. It is clear that the processes involved in in-stent restenosis are similar to stenosis following standard angioplasty. It is also apparent that there are clear differences between the two both in pathophysiology and outcome. The main difference in the physiology is the prolonged nature of the injury response in stenting. The greater the reactivity of the stent, the poorer the outcome. All the phases of restenosis last for much longer especially phase 3 i.e. proliferation of the VSMC once they have migrated to the intima. There is also evidence of a more chronic
inflammatory response with the presence of foreign body cell around the stent struts and the presence of more lymphocyte, histiocytes and eosinophils. In addition endothelial regeneration is restricted at the site the stent struts. There is also clear evidence that the expansion and outward pressure created by the stent struts on the vessels wall can create relative hypoxia in the vessels wall which contributes to the inflammatory and repair process. All of these effects are however well countered by its positive effect of wall remodelling. By acting as scaffolding it offsets the lumen narrowing effects of the above processes and hence provides a better clinical result. An ideal stent would be one which can counteract the NIH stimulating properties of ordinary stents.

**Conclusions**

It is clear that restenosis is a significant problem both in the coronary and peripheral circulation accounting for significant morbidity and mortality. The cost of revascularisation in Britain is £8800/patient while that of amputation and the rehabilitation that goes with it, is >£25000/patient. At present there is no completely effective way of treating or preventing restenosis. Stenting reduces restenosis especially in the coronary circulation but does not prevent NIH. Drug eluting stents seem to show very good medium term results, but there have been concerns about long term complications because like brachytherapy, sirolimus causes permanent loss of medial and intimal cells. PDT may provide some of the answers in this battle.
Chapter 5. Photodynamic therapy (PDT)

Photodynamic therapy can be defined as the use of light in order to activate a photosensitive drug in the presence of oxygen in order to treat a disease or disorder.

History of PDT

The therapeutic properties of light have been known to exist for many millennia. For example extract of *Psoralea corylifolia*, now known as furocoumarins, followed by exposure to sunlight was used to treat vitiligo. Hippocrates advocated the use of sunbaths for treating wasted muscles. The solarium for sunbathing was very popular among the ancient Greeks and Romans and there is evidence to suggest that it was used by the ancient Egyptians as well.

The first modern scientist to advocate the use of light for treatment was Niels Finsen who treated lupus vulgaris using light from a carbon arc with a heat filter. Further it was used to treat scarring following small pox. He was awarded the Nobel Prize in 1903 and is regarded as the father of phototherapy.

Oscar Raab a medical student in 1900 was the first to describe the tissue-destroying effects of light in the presence of a photosensitiser. He showed that acridine killed paramecia in the presence of sunlight. Von Tappeiner and Jodlbauer who first showed that oxygen was necessary for this effect coined the term ‘photodynamische Wirkung’ translated into photodynamic action or effect. Through the 20th century there have been a number of contributors to the understanding of PDT, none more than Meyer-Betz, who proved the photosensitivity of porphyrins by injecting
himself with it and exposing himself to light from a Finsen lamp as well as sunlight and showing severe photosensitization due to it.\textsuperscript{125}

It is only since the 1960s that the therapeutic potential of PDT has been exploited. Lipson demonstrated that haematoporphyrin derivative (HpD) produced fluorescence in human tumours and was used for treating a patient with a recurrent ulcerating breast cancer.\textsuperscript{126} Diamond demonstrated tumour necrosis in experimental gliomas in rats using HpD and visible light.\textsuperscript{127} Thomas Dougherty, who is a doyen of PDT treatment, was the first to report a 48% cure rate in rats and mice with sub-cutaneously implanted tumours. He was also the first to report encouraging results in a clinical trial with 25 patients with metastatic, cutaneous and subcutaneous tumours.\textsuperscript{128}

The physics of PDT

PDT requires three essential components to be effective, light, a photosensitiser and oxygen. Application of these three components in an appropriate combination produces tissue destruction. What makes PDT especially useful is that it produces healing without significant scarring.

When a photosensitive drug is subjected to light of a suitable wavelength it becomes activated from a ground state into an unstable excited state. Figure 5.1. This is an electronically excited state called a triplet state. This has an extremely short half life in the range of $10^{-3}$ s. This triplet state can undergo 2 types of reactions: It can react directly with the substrate i.e. the tissues by hydrogen ion or electron transfer to form free radicals, which interact with oxygen to produce oxygenated products. This is
Figure 5.1 Mechanism of PDT
known as a type I reaction. Alternately it can transfer its energy to oxygen directly to form highly reactive singlet oxygen $^1\text{O}_2$, which reacts with the tissue substrates to produce its effects. This is known as a type II reaction.

These 2 reactions may occur simultaneously and to varying extents depending on the photosensitiser substrate and the oxygen concentration. There is however evidence to suggest that most of its effects are caused by singlet oxygen generation. Optimally an oxygen concentration of $>5\%$ is needed. However effects can be seen at concentrations as low as $1\%$.

The first step in PDT is delivery of the photosensitiser to the tissue. The key issues here are the selectivity of the sensitiser for the target tissue and the doses needed to attain optimum concentration. Each photosensitiser has its own pharmacokinetic profile. It has been found that tissues with a high concentration of reticulo-endothelial cells tend to concentrate photosensitisers. Thus the liver has one of the highest concentrations in the body while the muscles have the lowest. Some sensitisers do tend to concentrate in tumour tissue. Selectivity can be achieved by the timing of delivery of light as the drug tends to be concentrated at varying times in varying tissues. Using the pharmacokinetic and pharmacodynamic curves of the drug-light interaction, optimal times for light delivery following drug administration can be selected. One of the ways selectivity can be achieved is by isolation of the tissue to be illuminated. While this is possible in surface PDT such as on skin lesions it is virtually impossible in deep tissues such as gut and blood vessels. Another method that is being tried is tagging the sensitisers with markers such as antibodies to the target tissue. To an
extent however the need for selectivity is superfluous. As mentioned before, one of the key features of PDT is that it heals with minimal scarring, so that even if normal surrounding tissue is affected there are usually no long-term sequelae. Sensitisers can be classified as follows.129

First generation photosensitisers.

**Haematoporphyrin derivative (HpD)**

HpD was one the first agents used for PDT since the 1960s. It is not a pure compound but a mixture of haematoporphyrin (Hp), protoporphyrin and a range of intermediate dehydration products. It is prepared by treating Hp with acetic acid with sulphuric acid as a catalyst. This is then dissolved in a solvent base. Because of the lack of quality control in producing HpD a more standardised and refined derivative is used in practice.

**Photofrin**

Photofrin was the first drug to get FDA approval for use in PDT. It is a refined and standardised form of HpD with the monomers and the unstable component of HpD removed. Light at the wavelength of 630nm in the red end of the visible spectra is used to activate it. It is however not without its drawbacks. Its efficiency in producing cytotoxic effects is low. The wavelength of light it requires has very little tissue penetration. It remains in the skin for 3-4 weeks if not months thus causing the patient to be photosensitive for a long time necessitating the avoidance of direct sunlight or bright lights. However it has the benefit of being time tested. It is commonly used in the treatment of head and neck, oesophageal, bladder and lung cancer. It
has also been successfully used in pre-malignant lesions such as Barrett’s oesophagus and oro-pharyngeal dysplasias. It has also been used in brain tumours such as glioblastomas and astrocytomas.¹³⁰

**Second generation photosensitisers**

These photosensitisers were developed in order to overcome the above mentioned drawbacks of Photofrin. Light sensitisation in the red and infrared region leads to a much higher tissue penetration. This is primarily because of higher absorption of green, blue and ultra-violet light by the haemoglobin in the blood. Most of the second-generation sensitisers are activated most effectively by light in the red and near-infrared wavelength.

*Chlorins and chlorin-type sensitisers*

NPe6, an aspartyl derivative of chlorin-e6, and meta-tetrahydroxyphenol (m-THPC) or Foscan are the commonest 2 used in this group. NPe6 is a highly water-soluble compound with rapid clearance which reduces the incidence of cutaneous photosensitivity. It is activated by light at 664nm. It has been used in the treatment of various cancers such as head and neck, lung cancer, bronchial oesophageal cancer, bone and liver secondaries and in gynaecological conditions. It has more recently been tested for use in vascular PDT with good inhibition of NIH.¹³¹

Foscan is sensitised at multiple wavelengths, but the 652nm is the commonly used one as this provides maximum tissue penetration. It has been used in experimental models of head and neck cancer, skin cancer and oesophageal cancer along with liver secondaries. Photosensitivity of the skin lasts for at least 2-3 weeks. It is now
licensed for use in Europe for advanced head and neck cancers.

**Phthalocyanines**

Aluminium disulphonated phthalocyanines (ASP) and Chloroaluminium disulphonated phthalocyanines (CASP) have both been used as photosensitisers and have shown efficacy in inhibiting VSMC proliferation and NIH. Phthalocyanines are synthetic porphyrins-like compounds which can chelate with a number of heavy ions such as aluminium and zinc which enhance photo-activity while others such as copper and cobalt which reduce it. ASP and CASP have the good absorption at a longer wavelength of 675nm and hence a greater depth of tissue is affected by the PDT effects. Again compared to 1st generation photosensitisers they have better clearance due to higher water solubility and hence less cutaneous photosensitivity.\(^{132,133}\)

**5-aminolaevulinic acid induced protoporphyrin IX**

5-aminolaevulinic acid (ALA) is an endogenous compound formed from glycine and succinyl CoA in the first step of biosynthesis of haem. (Figure 5.2) Protoporphyrin IX (PP9) is a compound further down the process of haem synthesis a step before the formation of haem itself. If ALA is added in excess to this system then the negative feedback loop whereby haem inhibits ALA synthetase is bypassed. The next rate limiting step in the process of haem production is when PP9 is converted to haem by ferrochelatase. Hence when excess ALA is administered there is accumulation of PP9. The amount of PP9 produced in individual cells may vary with accumulation products further up the process accumulating instead. Although PP9 is best activated by light at 410 nm the distinct lack of tissue penetration at this wavelength restricts its
Succinyl CoA + Glycine

\[ \text{ALA synthetase} \]

5-aminolaevulinic acid (5-ALA)

\[ \text{Ferrochelatase} \]

Porphobilinogen

Protoporphyrin IX + Fe

Uroporphobilinogen \[ \rightarrow \] Protoporphyrinogen IX

**Figure 5.2** Pathway of Haem biosynthesis

Haem has a negative feedback affect on the formation of 5-ALA which is bypassed by adding exogenous ALA. Addition of ferrochelatase inhibitors delays the breakdown of PPIX to haem.
use at this wavelength. Optimum tissue effects are produced by using light at 635nm first described by Kennedy et all in 1990 for treatment of 80 basal cell carcinomas. Following these there have been a number of studies showing the efficacy of ALA PDT for cutaneous lesions.

ALA can be administered orally, intra-venously (IV) as well as topically. It has a short drug light interval which can be 1-2 hours if it is administered IV and 4-6 hours orally. It excreted in 24-72 hours with skin photosensitivity lasting only 24 hours. It has favourable tumour: normal tissue fluorescence pattern which together with its photobleaching properties allows limitation of damage to normal tissue. Although it concentrates in normal tissue it does show some selective uptake in mucosa compared to muscle in conditions such as Barrett’s oesophagus and oral cavity lesions. However the depth of its effects is only 2 mm from the surface and hence its use is restricted to mucosal conditions only. This is because of a combination of limited depth of penetration of 635nm light, as well as preferential concentration of PP9 in superficial tissue. This is however an advantage in the blood vessels where its effects are only desired up to 2 mm. In sensitive tissue such as the myocardium this prevents any deeper damage. The limitation of this is that it cannot be used for treatment of invasive cancers.

ALA PDT has been used in number of different applications such as oral lesions, bronchial and oesophageal lesions, gastric lesions, skin lesions, bladder lesions. A number of different agents have been used in order to enhance the effects of ALA. Inhibitors of ferrochelatase and protoporphyrinogen oxidases potentially
increase PP9 concentration along with iron chelation\textsuperscript{135,143,144}

Long chain ALA ester derivatives have recently been used to increase bioavailability.\textsuperscript{145} IV ALA allows higher doses to be used without increasing hepatic toxicity by reducing first pass metabolism.

\textbf{Light delivery and tissue distribution}

Light fluence in tissue is attenuated by optical absorption of both the target tissue (mainly due to haemoglobin), the sensitiser itself and by optical scattering. Hence the haemoglobin content of tissue influences the light penetration in that tissue. Moreover, although in general, absorption of light by the sensitiser itself is much lesser than the tissue itself, for some sensitisers such as phthalocyanines, light absorption can be quite high, leading to a phenomenon known as 'self-shielding'. The wavelength of light used also influences the degree of tissue penetration as lower wavelengths have higher scatter and more absorption and hence lower penetration. Hence tissue penetration is 1-3 mm at 630nm while it is double that at >700nm\textsuperscript{146}. Hence the depth of effects is greater with sensitisers which are activated at longer wavelengths.

Numerous light sources have been used for PDT. Although broad spectrum light sources can be used it is more efficient to deliver light of the specific wavelength. The only absolute is the delivery of the relevant wavelength for the given sensitiser. Lasers and LEDs provide a good source of wavelength specific light while fluorescent tubes and incandescent lamps are broad spectrum. Broad spectrum devices are cheaper and used to be more portable. It is advantageous with ALA as some of the photoproducts produced when PP9 is activated are also photosensitive at 670nm. However their
application is restricted to surface application. Moreover infra-red light is also generated leading to hyperthermia and heat damage.

Lasers are now easily available in a solid-state and portable form and are used very commonly. The biggest advantage of using laser is the application of light at a distant or internal target by use of optical fibres. These have been variously used in gastrointestinal tract and in vascular disease. The biggest drawback is the cost which is high. Old metal vapour dye lasers (copper, gold) are now replaced by diode laser which gives a consistent output and are user-friendly. LEDs are a new development in PDT and could provide a cheaper source of monochromatic light.\(^\text{147}\)

There is now evidence that a break in light transmission allows for greater PDT effects of tissues. This is called fractionation of light and usually involves a window of 1 minute after part of the light delivery. Fractionation allows the re-accumulation of oxygen in the tissue and specifically when using ALA for re-accumulation of PP9. Animal data suggest that the optimum time for fractionation is after 20% of the light has been delivery. It is probably not effective later as micro-vascular damage precludes the mechanism.\(^\text{148-150}\)

**Clinical applications of PDT**

PDT has been used in the treatment of many malignant and pre-malignant conditions and a few benign conditions. The real advantages of PDT are lower morbidity rates, improved functional and cosmetic outcome and relative simplicity of use. There are now Phase IIb studies on large number of patients showing efficacy in both palliative
and curative treatment. However most of the data consists of anecdotal reports or small series. The main difficulty is achieving adequate necrosis of large solid tumours due to limited light penetration. Hence its usefulness as a curative treatment is much better for surface malignant and pre-malignant lesions.

The majority of use is in surface lesion both on the skin and mucosa of hollow organs. Pre-malignant conditions of the oro-pharyngeal cavity, Barrett’s oesophagus with high grade dysplasia, and carcinoma-in-situ of the lung and bladder have been treated effectively with PDT using various sensitisers. Compared to radiotherapy and radical surgery, PDT offers a much better functional result with very little morbidity. It also has the advantage of being used repeatedly with few long term sequelae.

Malignant lesions of the skin such as Basal cell carcinoma are better treated with PDT in areas where wide resection can give a poor functional result. Similarly it has been used successfully in the treatment of Bowen disease. It has been used for curative treatment of early oesophageal and lung cancer as well as superficial bladder cancer. Its role in the palliation of advanced forms of these cancers is also well established. Photofrin PDT has also been shown to extend symptom free survival in cholangiocarinomas. Interstitial PDT with Foscan has also been used with limited success by feeding light fibres into solid tumours through needles placed with image guidance. Using a pull back technique and multiple fibres substantial tumour necrosis can be achieved.

The FDA has approved the use of Visudyne PDT for age-related macular degeneration
of the eye where it has shown excellent results.\textsuperscript{160}

\textbf{PDT in vascular disease}

Both external and endovascular illumination of photosensitised blood vessels causes rapid loss of endothelial and vascular smooth muscle cells\textsuperscript{131,161-163}. As early as 1982 Spears et al demonstrated the concentration of Haematoporphyrin derivative (HpD) in atherosclerotic plaques.\textsuperscript{164} They were the first to demonstrate fluorescence 48 hours following administration of HpD in the aorta of rabbits fed on a high cholesterol diet. The potential implications of this were largely ignored until the early 1990s.

Both Ortu et al, from Boston and Nyamekye et al from our own centre demonstrated significant inhibition of NIH in the rat carotid using different photosensitisers i.e. Chloroaluminium-sulphonated phthalocyanine (CASP), and 5- Aminolaevulenic acid (5-ALA) respectively.\textsuperscript{162,163} Both experiments showed that application of PDT to balloon injured rat carotid arteries showed no NIH at 3 and 7 days. This has been confirmed by a number of articles using different photosensitisers such as Gonschior et al with Photofrin, Woodburn et al with Motexafin Lutetium and Nagae et al with Mono-L-Aspartyl-Chlorin e6 (NPe6).\textsuperscript{131,165,166} Table 5.1 summarises work with different photosensitisers used in vascular PDT.

This was followed by work by Grant et al from our centre looking at the integrity of rabbit carotid arteries after PDT using CASP and 5-ALA.\textsuperscript{167} Rabbits were sacrificed at 3, 7 and 21 days after vascular injury and PDT to the carotid artery. The isolated carotid segment was then subjected to hydrostatic distension till bursting. Compared to controls the PDT treated segment at all 3 time points required higher pressures.
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Photosensitiser</th>
<th>Animal/Clinical</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spears et al</td>
<td>1982</td>
<td>HpD</td>
<td>Rabbits/Monkeys</td>
<td>Concentration of HpD in plaques</td>
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<td>Gonschior et al</td>
<td>1991</td>
<td>Photofrin</td>
<td>Human</td>
<td>Uptake in atherosclerotic plaque</td>
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<td>Ortu et al</td>
<td>1992</td>
<td>CASP</td>
<td>Rat</td>
<td>Inhibition of NIH</td>
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<tr>
<td>Grant et al</td>
<td>1994</td>
<td>ALA</td>
<td>Rat</td>
<td>Loss of endothelium and VSMC</td>
</tr>
<tr>
<td>Nyameke et al</td>
<td>1995</td>
<td>ALSePc</td>
<td>Rat</td>
<td>Inhibition if NIH</td>
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<td>Jenkins et al</td>
<td>1998</td>
<td>ALA</td>
<td>Pig</td>
<td>Inhibition of NIH + positive remodelling</td>
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<tr>
<td>Jenkins et al</td>
<td>1999</td>
<td>ALA</td>
<td>Clinical</td>
<td>Clinical safety and feasibility</td>
</tr>
<tr>
<td>Rockson et al</td>
<td>2000</td>
<td>Antrin</td>
<td>Clinical</td>
<td>Clinical feasibility and safety</td>
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<tr>
<td>Nagae et al</td>
<td>2001</td>
<td>NPe6</td>
<td>Rabbit</td>
<td>Inhibition of NIH Endovascular light application</td>
</tr>
</tbody>
</table>

Table 7.3 Major studies using Vascular PDT for restenosis
before bursting showing that PDT actually increased the structural integrity of the arterial wall. It has subsequently been shown that PDT causes physiological and structural changes in the collagen in the extra-cellular matrix. Various collagenases such as Matrix metalloproteinases and urokinase like collagenases are released at the time of intimal injury occurring during angioplasty. These have been shown to be inhibited by PDT. PDT has also been shown to increase cross-linkages in Type I collagen not only between collagen to collagen but also between collagen and albumin. This is probably the reason the structural strength of the vessel increases after PDT.

PDT has also been shown to be responsible for inhibiting the migration of VSMC from the media after balloon injury. The next step was to show similar effects in higher animals such as pigs where the anatomy and structure is similar to humans. Jenkins et al from our centre showed that these effects could be duplicated in porcine coronary and iliac vessels. In a first set of experiments they demonstrated the concentration of PP9 in the vascular media 4-6 hours after intravenous injection of 5-ALA. They also showed that complete cell ablation could be achieved with a dose of 60mg/kg 5-ALA and a light dose of 50 J/cm² of 635nm red light using a copper vapour pumped dye laser. In a second set of experiments porcine coronary and iliac arteries were balloon injured and exposed to endoluminal PDT. A 0.2mm core diameter optical fibre with a 4cm diffuser tip introduced through the guide-wire channel of a balloon catheter was used for illumination of the angioplasty site under Image-intensifier guidance. Transparent balloons used for brachytherapy were
modified for use in these experiments. Following PDT there was complete cell ablation at 3 days. At 28 days there was complete re-growth of the endothelium with some re-growth of the medial cell. Further at 28 days the area within the External Elastic Lamina and the luminal area in PDT treated arteries was significantly more as compared to controls. The VSMC cell count was also much less in the PDT treated arteries although it just failed to reach statistical significance. This clearly showed that PDT promoted positive arterial remodelling over and above the effects of cell ablation. It is now established that PDT has a number of favourable effects as an adjuvant to angioplasty. As opposed to brachytherapy it does not completely stop regeneration of the cell. In fact the complete regeneration of the endothelium suggests that the risk of late thrombosis is reduced if not absent. Later experiments in our own centre, following pigs to 56 days has shown no late thrombosis. Also compared to brachytherapy where there is a theoretical risk of wall weakening and rupture, PDT actually increases wall strength.
Chapter 6. Aims of Thesis

Models of arterial injury

The advantages, disadvantages and limitation of various animal models of restenosis have been extensively reviewed. While the rat carotid, rabbit carotid and iliac and porcine coronary, carotid and iliac vessels have been used each has its innate advantages and disadvantages.\textsuperscript{173-176}

The rat model has been extensively used as a model of arterial injury and PDT work.\textsuperscript{133,162,163,177} It is cheap, easily supplied and housed and relatively easy to handle. Its small size however precludes the application of and conclusions from this model to humans. The small size creates a vast weight and drug dose differential compared to humans. The small size of the vessels which are in the order of 1-2 mm makes the use of any endovascular devices difficult. Most importantly the response to balloon injury and by corollary stent injury appears to be limited to VSMC proliferation which is overly sensitive to pharmacological and other forms of therapy.

The porcine arteries have provided very good models because of tackling the said disadvantages in the rat model. 3 different arteries can be used the coronary, carotid and iliac allowing the use of more than one vessel in an animal. The size also allows the use of endovascular devices such as angioplasty balloon, stents and light delivery devices with very little modification from the ones used in humans.\textsuperscript{161,165} The response of the vessels is closer to that seen in balloon injury in humans with elements of VSMC proliferation, deposition of extra-cellular matrix and arterial remodelling.
However there are problems with handling of pigs. They are extremely sensitive to any cardiac intervention and very easily develop ventricular fibrillation and asystole leading to death. They are a large animal which makes them expensive and difficult to handle. The incumbent Foot and Mouth disease also made the transfer and handling of pigs more complicated.

The rabbit model is a good compromise. It is a relatively small animal, easy to handle and reasonably cheap. Both the rabbit carotid and iliac have been shown to be useful in creating balloon injury. Recent advances in endovascular devices allow the use of human devices of a smaller size and very little modification to be used in the rabbit. New light delivery devices which are sturdier than ones previously used also make the duplication of these procedures in rabbits easy. The injury response seen in these animals is also similar to the human response. Stenting in the rabbit iliac and carotid is an easy procedure and has been used before to create models of in-stent restenosis. It also possible to predictably create an atherosclerotic plaque in the rabbit model. It is for these reasons we chose the rabbit as an appropriate model for our experiments.

It is important to note that no animal model fully duplicates the complex processes that lead to atherosclerosis and restenosis in humans. The atherosclerotic models alluded to in the previous paragraph do differ from human plaques in issues such a cell make and content, presence of inflammation, deposition of ECM and the progression of the plaque. The oversized stent model that I have used in my work, while providing a good idea of the response of individual cells and matrix in the animal model, does
differ from human models. Not in the least because in humans the presence of a plaques alters the response. Ultimately the proof for all these techniques lies in their effective clinical application.

**Method of injury**

In-stent restenosis has been effectively duplicated in models such as the rabbit iliac and the pig arteries.\(^{173,175}\) Generally over-inflation of the stent by a ratio of 1.2-1.3 with the artery is sufficient to stimulate a consistent response. This includes migration of VSMC in to intima and proliferation leading to a neointimal plaque. The injury response in in-stent restenosis differs from that following balloon injury in being more chronic as that stenting provides a more prolonged stimulus.

**Photosensitisers**

A number of different photosensitisers have been previously used in arterial PDT work including Photofrin, other HpD preparations, phthalocyanines, Antrin, NPe6 and ALA.\(^{131,133,162,166,178,181}\) Both Photofrin and phthalocyanines have the disadvantage of prolonged photosensitivity which would make them less practical for clinical use in the context of restenosis and in-stent restenosis prevention. Both are potent photosensitisers and may cause deeper effects when used endoluminally leading to affects on neighbouring tissue such as cardiac muscle. Local delivery of Photofrin has been investigated in order to eliminate skin photosensitivity but this introduces technical and equipment complexities of local drug delivery prior to light application.\(^{165,182}\)

ALA has a number of advantages which make it ideal for use in arterial PDT. It is
not a highly photosensitive drug. It has a half life of only 10 hours and eliminated in 24 hours. This means direct sunlight should be avoided only for 24 hours. As peak concentrations in the humans are achieved at 5-7 hours in the event of any post-procedure complication, re-intervention such as surgery can be done within 2-3 hours without risk of significant tissue damage from ambient light. The tissue response following endoluminal ALA-PDT is only 2-3 mm deep reducing the risk of any injury to the surrounding structure on light application. ALA has been used both in animal models such as rabbit, rat and pig with success and has also been used safely in human studies as well. The drug can be administered orally or intra-venously simply by dissolving it in distilled water or saline.\textsuperscript{161,162,167}

**Light delivery**

In order to simulate clinical application appropriate sized balloon catheters and optical fibres were essential for use in the rabbit model. While endovascular devices have been used in the porcine vessels in the past there is only one study using endovascular light delivery using optical fibres. These were used in conjunction with angioplasty balloons. As these are deployed flush to the arterial wall they remove any layer of blood between the arterial wall and the optical fibre. Any blood would absorb the light and reduce light delivery to the vessel wall. The key requirements for light delivery in rabbit artery are a balloon catheter of the appropriate size, with a transparent core for diffusion of light and an optical fibre which fits easily in the guide wire channel of the catheter. Occum\textsuperscript{®} made a special catheter with 3 mm X 2cm balloon with a transparent core. The catheter was 3F so as to easily fit within the rabbit femoral artery. It had radio opaque markers at both ends of the balloon to align the catheter.
with an image intensifier. It has guide wire channel of diameter 500 microns capable of accommodating a 200 micron optical fibre. A custom-made 200 micron fibre with a 2 cm diffuser flanked by 2 radio opaque markers was supplied by Pioneer Optics which could be aligned with the markers on the balloon. The optical fibre was preloaded in the catheter and after alignment the balloon was inflated to just over 100mm of mercury to cause occlusion of the artery with out any injury. This also allowed centralisation of the optical fibre within the artery facilitating uniform light delivery to the entire circumference of the artery.

Equipment for light delivery in the clinical project was more easily available. An optical fibre with a core diameter of 400μm was used which fit into the central channel of a conventional angioplasty balloon modified to have a transparent core and balloon.

**Thesis aims**

**Experimental work**

Previous work has established that PDT can inhibit NIH that results from arterial injury. While there is some experimental work in the use of PDT as an adjuvant to stenting it has never been shown to inhibit in-stent restenosis. In my thesis my objective was to apply and optimise the use of PDT as an adjuvant to stenting in order to inhibit in-stent restenosis. My hypothesis is that light for PDT can be delivered percutaneously along with stenting of the artery and that it will inhibit in-stent restenosis when used optimally.

With this in mind ALA was chosen as the photosensitiser as it is converted to PP9 and
can be activated in 3-5 hours and has minimal photosensitivity for 24 hours. ALA PDT has previously been shown to inhibit restenosis and NIH. An experimental small animal – New Zealand White rabbit was proposed as representing an optimum model in terms of size availability and response to stenting. Stenting and light delivery was performed endovascularly under image intensifier guidance. The first series of experiments were aimed at estimating cell loss at 3 days in the intima and media using light delivery before and after stenting. The vessels were subsequently harvested and sectioned and VSMC counts carried out to estimate cell loss. These were followed by experiments to look at neointimal response at 28 days again with light delivery before and after stenting. Stented vessels with no PDT were used as controls. On culling the relevant sections of stented vessels were harvested and appropriately sectioned. Accurate morphometry was performed to estimate arterial dimensions and neointimal area and thickness.

Clinical work

The clinical work included 2 projects.

My predecessors in the department had completed a pilot study looking at the effect of ALA-PDT as an adjuvant to Angioplasty in 7 patients and published the follow-up to 6 months. With this data base available the next step was to establish long-term follow-up. The initial study had shown the absence of any short-term complications and had published excellent results at 6 months. As 4 years had passed since the original study my aim was to follow-up these patients looking at development of any long-term complications and the outcome of the lesions that were treated.
Given the encouraging results of the pilot study and that the long term follow-up also showed good results a randomised controlled trial was conducted. Patients with symptomatic lesions in the superficial femoral artery were divided into 2 groups comparing standard angioplasty with standard angioplasty and adjuvant ALA-PDT. The patients were followed up with regular assessments up to 6 months specifically looking at clinical feasibility, safety, and restenosis rate.
Chapter 7. PDT as an adjuvant to stenting – experimental studies

Photodynamic therapy (PDT) is a technique of achieving cell death and tissue loss by activation of a previously administered photosensitive agent by light of a specific frequency shone upon the target tissue. On application in the artery wall both by endovascular and external sources there is almost complete elimination of medial and intimal cells by apoptosis. This results in significant inhibition of restenosis after angioplasty as well as a significant increase in luminal area. PDT has also been shown to promote positive remodelling. In the USA and Europe almost 70-80% of coronary angioplasties are now stented. In order to make this technique attractive to use along with stents a similar effect needs to be duplicated when PDT is used as an adjuvant for stenting.

The aims of this study were 1. To examine the effects of PDT when used an adjuvant to stenting as a prophylaxis against in-stent restenosis in the rabbit iliac model. 2. To optimise the effects of PDT with stenting in causing intimal and medial cell death and inhibiting NIH.

Materials and methods

Animals and surgical techniques

All animals were treated in accordance with the Animals (Scientific Procedures) Act 1986 under project and personal licences approved by the Home Office (UK Government).
New-Zealand White rabbits (n=32) 2.5-3 kg were used. The arrangement of the operating room is shown in figure 7.1. The rabbits were anaesthetised using Hypnorm (Fentanyl/fluanisone) 0.5ml/kg (Janssen) and Hypnovel (Midazolam) 0.05ml/kg (Roche) for induction and maintained with inhalation anaesthesia using halothane.

Following skin preparation, the superficial femoral artery was exposed and bathed externally in papaverine (Martindale Romford) for 2 minutes. Figure 7.2. An arteriotomy allowed insertion of a 2F cannula and an angiogram delineated the anatomy of the aorta and the iliacs. Angiographic images were obtained using a Siemens S2000 C arm image intensifier with a road mapping facility, which allowed accurate intravascular positioning, and recorded on super VHS video tape. Figure 7.3.

At the end of stenting and light delivery the superficial femoral artery was ligated and the wound repaired. Because of the excellent collateral circulation in the lower limb of rabbits this does not put the leg at any risk of ischaemia. The rabbits were recovered and maintained on standard rabbit chow and daily aspirin (60mgs). We did not feel there was a need to give clopidogrel based on our previous experiments with PDT and angioplasty. Application of PDT with stenting in the coronary circulation may require its use. In the peripheral circulation its use is rare.

Animals were randomly assigned to one of three protocols.

Group 1: (n=8): stent deployed without drug or light application.

Group 2: (n=12): these animals received the ALA three hours before surgery and the iliac artery was illuminated to activate the drug after stent deployment.

Group 3: (n=12) these animals received the ALA three hours before surgery and the iliac artery was illuminated to activate the drug before stent deployment.
Figure 7.1 Arrangement of Animal theatre
Figure 7.2 Exposure of rabbit SFA during stenting and light delivery
Figure 7.3 Rabbit angiograms. (A) normal rabbit angiogram showing aortic bifurcation and lower limb vessels (B) road map of vessels used to position Catheter (C) Deployment of stent mounted on a balloon catheter (D) Deployed stent in the external iliac artery.
Photosensitiser

The delta 5-aminolaevulinic acid (ALA) was dissolved in normal saline neutralised with sodium bicarbonate to pH 7. In accordance with previous pharmacokinetic studies, the ALA was administered intravenously at a dose of 60mg/kg body weight three hours before light delivery.\textsuperscript{167}

Stenting and light delivery.

A unique catheter was used for endovascular illumination. The 3F monorail 20mm X 3mm balloon catheters had a transparent core and transparent balloon (Occam, Netherlands) and a blind ending channel. Light was delivered by an optical fibre with a 200µm core and diameter of 500µm and a distal radial light diffuser, 2 cm in length, marked by radio opaque markers. The entire procedure was done under image intensifier guidance with road mapping facility. This was invaluable in avoiding geometric miss. This was preloaded into the central channel of the balloon catheter. The catheter was introduced into the ipsi-lateral common iliac so that the balloon lay in the target segment just proximal to the iliac bifurcation. The catheter was delivered over a monorail guide-wire, which was pulled back from the balloon when the correct position was obtained, before illumination. During light delivery the balloon was inflated with air sufficiently (100mmHg) to occlude the blood flow but not overstretch the vessel. Figure 7.4 Illumination came from a Diomed 635 laser (wavelength 635nm, 50J/cm\textsuperscript{2} at 80-100mW/cm). Figure 7.5 The total light delivery time in each procedure was 5-600 seconds, excluding a 60 second pause after 1 minute of light delivery. Such a pause during illumination has previously been shown to increase
Figure 7.4  Light delivery in rabbit iliac artery
Figure 7.5  Diode laser box (Diomed®)
efficacy of a drug/light dose. After light delivery the deflated balloon, guide-wire and optical fibre were removed. Either before or after arterial illumination, a 15mm X 3mm stent was loaded on a 3mm balloon catheter and introduced through the femoral arteriotomy and positioned under x-ray guidance, then deployed at the target site at 10 atmosphere pressure.

**Tissue harvesting and processing**

All rabbits were killed by administration of an overdose of 20% phenobarbiturate. The abdomen was opened, the retroperitoneum exposed, and the aorta isolated and tied above its bifurcation. An 18F IV cannula was introduced into the distal aorta and the distal vessels were pressure perfused at 100mm Hg with 4% formalin for 10 minutes. The iliac arteries were then isolated and removed and fixed for a further 16 hours in 4% formalin.

Four rabbits in groups 2&3 were culled at 3 days. In these rabbits the stented vessels were isolated, cut open along the longitudinal axis and peeled from the stent. Vessels from the opposite side i.e. normal un-stented rabbit artery were used for controls. The stent only controls had no count at day 3. The arterial tissues were embedded in paraffin wax, cut into 4µm sections and stained with haematoxylin and eosin (H&E). Microscopy images were transferred onto a personal computer via a colour camera (JVC TK-1281) and morphometric analysis was performed using the Lucia- M (version 3.52a) programme. Three sections from each vessel were examined. Each section was imaged and the number of VSMC nuclei per HPF (20X objective) counted in 8 fields in all quadrants and a mean/HPF was calculated. Table 7.1
<table>
<thead>
<tr>
<th>Vessels with light before stenting</th>
<th>Vessels with light after stenting</th>
</tr>
</thead>
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<tr>
<td>0 0 0 0</td>
<td>91 101 123 121</td>
</tr>
<tr>
<td>0 0 0 0</td>
<td>107 116 128 160</td>
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<td>207 206 191 227</td>
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<td>57 52 57 17</td>
<td>233 228 218 284</td>
</tr>
<tr>
<td>35 35 25 46</td>
<td>293 298 246 293</td>
</tr>
</tbody>
</table>

Table 7.1 Circumferential VSMC/HPF in rabbit iliac at 3 days

Vessel wall was divided into 8 segments.

Measurements were taken from each segment and averaged
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<thead>
<tr>
<th>Rabbit No.</th>
<th>Group</th>
<th>Lumen area mm²</th>
<th>Area IEL mm²</th>
<th>Area EEL mm²</th>
<th>Intimal thickness mm</th>
<th>Intimal area mm²</th>
<th>Medial area mm²</th>
<th>% stenosis</th>
</tr>
</thead>
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<tr>
<td>1</td>
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<td>2.85</td>
<td>4.16</td>
<td>4.62</td>
<td>0.83</td>
<td>1.31</td>
<td>0.46</td>
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<td>4.90</td>
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<td>0.96</td>
<td>0.22</td>
<td>19.70</td>
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<tr>
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<td>0.77</td>
<td>0.79</td>
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<td>4.55</td>
<td>4.82</td>
<td>0.60</td>
<td>0.70</td>
<td>0.27</td>
<td>15.38</td>
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<tr>
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<td>control</td>
<td>4.39</td>
<td>5.14</td>
<td>5.38</td>
<td>0.61</td>
<td>0.75</td>
<td>0.24</td>
<td>14.66</td>
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<td>0.43</td>
<td>0.51</td>
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<td>6.51</td>
<td>6.73</td>
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<td>0.45</td>
<td>0.22</td>
<td>6.90</td>
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<td>5.39</td>
<td>5.77</td>
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<td>1.26</td>
<td>0.38</td>
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<td>0.45</td>
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<td>0.31</td>
<td>0.35</td>
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<td>12</td>
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<td>5.77</td>
<td>6.23</td>
<td>1.12</td>
<td>1.64</td>
<td>0.46</td>
<td>28.38</td>
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<td>0.72</td>
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<tr>
<td>16</td>
<td>Rx</td>
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<td>6.77</td>
<td>7.60</td>
<td>0.45</td>
<td>0.52</td>
<td>0.83</td>
<td>7.68</td>
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<td>control</td>
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<td>2.13</td>
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<td>0.65</td>
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<td>4.59</td>
<td>4.85</td>
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<td>4.35</td>
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<tr>
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<td>3.70</td>
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<td>1.41</td>
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<td>0.52</td>
<td>22.76</td>
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<td>6.09</td>
<td>6.68</td>
<td>0.74</td>
<td>2.10</td>
<td>0.59</td>
<td>34.48</td>
</tr>
</tbody>
</table>

Table 7.2. Morphometric measurements in rabbit iliac at 28 days
The other rabbits (8 in each of the 3 groups) were culled at 28 days after light delivery and stenting. These vessels were embedded in LR White resin using standard techniques with the stent left in situ. Vessels were sectioned into 10μ sections with the stents in situ on a tungsten microtome and stained subsequently with H&E.

Morphometric analysis included the following measurements: luminal area; area within the internal elastic lamina; the area within the external elastic lamina, and the intimal thickness. The intimal area was calculated by subtracting the luminal area from the area within the internal elastic lamina. Similarly the medial area was calculated by subtracting the area with the IEL from the area within the EEL. Intimal thickness was measured at 10 different points around the artery circumference, effectively radii from the vessel centre, approximately 36° apart. The mean of these 10 measurements was used in the subsequent analysis. Table 7.2

**Statistical analysis**

All values were expressed as mean ± SD. Statistical analysis was by the comparison of the means from each group using the unpaired Student t-test. Inter and intra-observer variability was assessed by reanalysis of a representative sample of histological sections. Area and thickness calculations were made by one observer on two separate occasions and by a second independent observer on one occasion. A p value of <0.05 was considered significant.

**Results**

All animals survived to cull and there was no evidence of thrombosis, rupture or
aneurysm formation in either of the PDT treatment groups or in the control group.

At 3 days after stenting and light delivery, Group 3 (PDT light before stenting), VSMC counts/high power field (hpf) were 17±1, while those in Group 2 (PDT light after stenting) were 183±17 (p< 0.006). VSMC counts/hpf in normal rabbit iliacs with no PDT and no stent were 222±41. Thus light treatment before stenting significantly reduced VSMC in the media and intima. (figure 7.6, figure 7.7 a-b) while light after stenting did not.

The results of artery morphometry at 28 days are summarised in table 7.3. These are represented in figure 7.8a-d. At 28 days the stented arteries had all developed a neointimal layer. In group 3 (PDT light before stent deployment) the neo-intimal layer was significantly thinner than that of the other two groups. (figure 7.9a-c) The neointimal area of group 3 was 60% less than that of group 1, but the neointima of group 2 rabbits was no different from that of the Group 1 (stent only) control group. PDT was efficacious in reducing intimal area only when the illumination was before stent. There was no statistical difference in either the area within the EEL or the area within the IEL indicating deployment of the stents to similar diameters in all treatment groups and no expansile remodelling in treated groups. The percent stenosis was calculated as a ratio of the neointimal area to the area within the IEL and was significantly less in Group 3 (figure 7.8e). While no immunohistological confirmation of endothelial integrity was possible with the LR White resin mounted tissue, a single layer of flat endothelium-like cells lined the stented arteries at 28 days (Figure 7.9d). At 28 days there was partial repopulation of the media in group 3 while the other 2 groups retained their cellularity.
Figure 7.6  Histogram of VSMC/HPF in rabbit iliac harvested 3 days after PDT and stenting. (error bars represent standard deviation). There was significant reduction in medial cell count in the arteries where light was given before stenting. p values compared to light before stent group.
Figure 7.7a Rabbit iliac artery peeled from stent which was inserted 3 days previously with PDT illumination after stent deployment (group 2). The tissue was prepared for paraffin embedding and light microscopy and little medial cell depletion is seen.

Figure 7.7b. Rabbit iliac artery peeled from stent which was inserted 3 days previously with PDT illumination before stent deployment (group 3). The tissue was prepared for paraffin embedding and light microscopy and marked medial cell depletion is seen.
<table>
<thead>
<tr>
<th></th>
<th>Stent only controls (Group 1)</th>
<th>Light delivery after Stenting (Group 2)</th>
<th>Light delivery before Stenting (Group 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area within EEL (mm²)</td>
<td>5.43±1.1</td>
<td>5.81±1.3</td>
<td>5.76±1.3</td>
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<td>Area within IEL (mm²)</td>
<td>5.08±0.9</td>
<td>5.28±1.2</td>
<td>5.30±1.3</td>
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<tr>
<td>Luminal Area (mm²)</td>
<td>3.63±0.4</td>
<td>4.04±0.7</td>
<td>4.71±1.1†</td>
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<tr>
<td>Intimal Area (mm²)</td>
<td>1.44±0.5</td>
<td>1.24±0.5</td>
<td>0.60±0.2*</td>
</tr>
<tr>
<td>Intimal thickness (mm)</td>
<td>0.90±0.4</td>
<td>0.88±0.3</td>
<td>0.47±0.1*</td>
</tr>
<tr>
<td>Medial area (mm²)</td>
<td>0.42±0.2</td>
<td>0.56±0.3</td>
<td>0.46±0.2</td>
</tr>
<tr>
<td>Percent stenosis (%)</td>
<td>28.4</td>
<td>24.3</td>
<td>11.9*</td>
</tr>
<tr>
<td>Medial Cell count at 3 days</td>
<td>Not done</td>
<td>184±17</td>
<td>17±1*</td>
</tr>
<tr>
<td>Medial cell count at 28 days</td>
<td>167±13</td>
<td>155±14</td>
<td>56±7*</td>
</tr>
</tbody>
</table>

† Statistically significant compared to Group 1
* Statistically significant compared to Group 1 and 2

Table 7.3 Summary of 3 day cell counts and 28 day morphometry
Fig 7.8(a) intimal area measured in mm$^2$ (error bars represent standard deviation) p values compared to light before stent group

Figure 7.8(b) intimal thickness in mm (error bars represent standard deviation). p values compared to light before stent group
Fig 7.8(c) area within the EEL mm$^2$ (error bars represent standard deviation).

Figure 7.8(d) luminal area in mm$^2$ (error bars represent standard deviation). $p$ value compared to stent only group.
Figure 7.8 (e) % stenosis (Ratio of intimal area to area within IEL expressed as a %) p values compared to light before stent group
Figure 7.9 (a) Section of LR White resin-embedded rabbit iliac with stent in situ, retrieved 28 days after the procedure which involved stent deployment without PDT (group 1).

Figure 7.9(b) Section of LR White resin-embedded rabbit iliac with stent in situ, retrieved 28 days after the procedure which involved stent deployment before PDT illumination (group 2).
Figure 7.9 (c) Section of LR White resin-embedded rabbit iliac with stent in situ, retrieved 28 days after the procedure which involved stent deployment after PDT illumination (group 3).

Figure 7.9 (d) Photomicrograph to a section from a Group 3 animal illustrating a contiguous layer of flattened endothelial-like cells lining the stented vessels, 28 days after the procedure.
Discussion

This study is the first to demonstrate that endovascular PDT can be an effective adjuvant to arterial stenting, significantly reducing intimal hyperplasia without detrimental effects on the vascular integrity or thrombogenicity. However, activation of the photosensitiser must take place before rather than after the stent is deployed.

We used the rabbit iliac artery, a well-established model for in-stent restenosis, although these are disease-free, elastic arteries. There may be a difference in response when PDT is applied to atherosclerotic vessels with the addition of both cellular elements such as macrophages and fibroblasts and a lipid core. Previous studies have shown the in-vivo accumulation of photosensitisers in atherosclerotic plaques both in the cellular and non-cellular elements, although this has not been reported using ALA. Nevertheless, rabbit models of atherosclerosis are quite different from human atherosclerotic lesions and do not mimic complex human coronary disease. We considered that the model we used was appropriate for answering the key question of how PDT before and after stenting influenced cell proliferation. Our studies did only extend to 28 days, but this is a time point frequently used in preclinical studies. Later observations might have revealed a catch up of the lesion, but we think this unlikely as our previous studies following PDT treated arteries in rats for up to 6 months did not show any marked late cell proliferation.

The striking result from this study is the observation that the media remained fully populated with smooth muscle cells with no inhibition of stent-induced intimal
hyperplasia at 28 days if the light was delivered after stent deployment. There are several possible explanations. As the ALA was given at the same time prior to light delivery in both groups 2 and 3, the difference in response is unlikely to be related to the distribution of protoporphyrin IX (the photoactive derivative of ALA). PDT requires oxygen. The simplest explanation is that stent deployment stretches the arterial wall and makes it hypoxic. It has been documented previously in the rabbit that deployment of an oversize stent renders the normal arterial wall hypoxic. This effect is most marked in the media. Oxygenation of the endothelial layer is maintained better by blood flowing through the lumen of the vessel.\textsuperscript{187} This may explain why endothelial loss was seen in arteries illuminated after stent insertion, although this loss may be at least partially attributable to the trauma of the balloon-mounted stent delivery. By 28 days all stented arteries had a layer of endothelial-like cells coating the lumen surface of the artery. Several studies report complete and even accelerated regrowth of normal vascular endothelium after PDT without stenting, with no evidence of thrombosis or any other impairment of blood flow.\textsuperscript{162;188-190} Early re-growth of endothelium after PDT may play a role in the inhibition of VSMC proliferation and intimal hyperplasia and may also play a role in reducing thrombogenic complications after stent placement.

There are other possible explanations for our results. The vascular stent is a metal tube mesh, so optical shadowing from light delivered from within the lumen of the stent must occur to some degree. However, the wire of the stent struts is so thin compared to the penetration depth in tissue of the red light used (typically about 2mm) that
scattering of light within the tissue should make this a minor consideration.
Nevertheless, a recent report describes inhibition of intimal hyperplasia from PDT
given after stenting of the femoral artery, but employing illumination of the external
surface of the artery, thus avoiding strut shadowing. Another possibility is that the
mechanical stretching of the arterial wall by the stent could lead to on-going
stimulation of VSMC proliferation (in contrast to the single insult of a balloon
angioplasty followed by removal of the balloon). However, this is less likely as the
effect would have been expected to be the same in both PDT treated groups.

Several studies report complete and indeed accelerated re-growth of endothelium after
PDT. The complexity of the procedure and the tissue retrieval meant that we
did not define the specific effects on the endothelium of PDT with stenting, since
endothelium-specific staining was not possible in the LR White resin embedded tissue.
Procedure-related physical disruption of the endothelium would be predicted, but since
the stent was stripped from the artery at 3 days prior to processing for histology,
further damage occurs. Nevertheless, we saw an intact endothelium-like layer of cells
when the iliac arteries of all groups were examined 28 days after injury. There were no
thrombotic complications despite only aspirin prophylaxis. Poor endothelial re-growth
with brachytherapy and drug eluting stents has necessitated long term anti-thrombotic
treatment with associated expense and risk of bleeding complications. The
suggestion of rapid re-endothelialisation after PDT is particularly appealing to the
clinical practitioner as it means that adjuvant PDT may have a particular place in the
treatment of diffuse coronary artery disease and long lesions.
In general, the response of tissue to PDT is complex, with inflammation and microvascular damage, but in the arterial wall the development of medial apoptotic bodies, TUNEL staining of residual nuclei and the lack of an early inflammatory infiltrate suggest that the predominant effect is apoptosis.\textsuperscript{194-196} It inhibits VSMC proliferation and migration, increases cross-linking in collagen and extra-cellular matrix and promotes positive remodelling of the artery. The prevention of intimal hyperplasia and restenosis seen in this study is linked closely to ablation of cells in the media. The key role of medial vascular smooth muscle cells in the intimal response to a stent is reflected in the significant reduction in neointimal area and thickness only when the PDT has caused depletion of the cellular elements of the wall i.e. when light delivery was before stent deployment. A correlation between cell depletion/ablation and efficiency is seen also with other techniques now used to prevent or treat in-stent restenosis, such as drug-eluting stents or brachytherapy.\textsuperscript{197,198} The partial medial cell repopulation at 28 days documented here is consistent with repopulation of the media of pig coronaries illuminated endovascularly\textsuperscript{172}, although cell depletion may persist for six months or more in rat carotid arteries\textsuperscript{133}.

How does PDT produce these effects? In a series of experiments Heckenkamp et al looked at the effect of $\gamma$-radiation and PDT on a number of SMC and functions of the extra-cellular matrix (ECM).\textsuperscript{199} When cultured in 100\% calf serum (CS) PDT completely inhibited cellular proliferation compared to controls. In the $\gamma$-radiated group there was significant decrease in proliferation but less than the PDT group. Mitochondrial metabolic activity in the VSMC doubled in control groups when
stimulated with CS compared to no activity being detected in the PDT group. In the 
\( \gamma \)-radiated group there was no difference in activity compared to controls. VSMC, 
grown in media containing mechanically injured VSMC responsible for injury-
associated growth factors, showed a significant increase in mitogenesis. PDT inhibited 
this increase while no effect was seen with \( \gamma \)-radiation. Perhaps most importantly 
apoptotic cell death was found to be the major cytotoxic mechanism causing cell death 
following PDT while no significant apoptosis was seen after \( \gamma \)-radiation. This was 
confirmed by TUNEL Stain, DNA fragmentation ladders, and with characteristic 
changes seen on Electron microscopy. Work from the same centre had also shown that 
migration of VSMC in PDT treated ECM is significantly inhibited. It is 
suggested that PDT causes release of lysosomal enzymes which activate Caspases 
such as Caspase 9. Alternately they could damage mitochondria stimulating the 
release of cytochrome C again activating the caspase cascade. As already explained 
all the effects of PDT are probably mediated through singlet oxygen species. Since the 
half-life of these species is extremely small the effects of PDT are well localised by the 
distribution of drug and illumination of light. What is perhaps most important is that it 
does not stimulate an inflammatory reaction with no evidence of phagocytes seen in 
the areas illuminated. The debris is eliminated by either redistribution in the media or 
by being washed away by the blood.

One of the key attractions of PDT is the nature of the biological effect. Despite its 
increasing use in a range of specialities, there is no evidence of cumulative toxicity and 
it has often been used to treat the same site several times. The photon energy of the
red light used is too low to damage DNA. There is a lack of any effect on the mechanical integrity of arteries or on the blood flow through them and a lack of any chronic changes detectable histologically.\textsuperscript{167,172} This body of evidence makes PDT an attractive option as a possible alternative to drug eluting stents and brachytherapy. However, as PDT only works if the light is given prior to rather than after stent deployment, it may not be of value for the treatment, rather than prophylaxis, of in-stent restenosis. The published data on stent induced hypoxia in the wall of arteries showed that there was some improvement in the hypoxia when the stents had been in situ for several weeks, but further experiments would be required to see if PDT has any potential for treating rather than preventing in-stent restenosis.\textsuperscript{187}

Remodelling with vessel shrinkage is a major contributor to restenosis after balloon angioplasty. Previous studies from our group and others have shown that following angioplasty with adjuvant endovascular PDT but without stenting, there is inhibition of constrictive remodelling.\textsuperscript{172,202} Remodelling is less important in the presence of a stent, but it is reassuring that we saw no evidence of aneurysmal dilatation following PDT, nor signs to suggest stent mal-apposition, which has been a feature of both brachytherapy and drug eluting stents.\textsuperscript{197,198,203} Further, no signs of injury to adjacent tissue have been reported with ALA-PDT, which may reflect the limited tissue penetration (2mm) of the 635nm light used to activate protoporphyrin IX, the active metabolite of ALA. The impaired endothelial re-growth after brachytherapy and drug eluting stents has necessitated long term anti-thrombotic treatment with the associated expense and risk of bleeding complications.\textsuperscript{109,119,204,205} The rapid re-endothelialisation
reported after PDT in animals allows us to speculate that in the clinical setting adjuvant PDT may require less subsequent maintenance drug therapy. The endothelium may not heal so well in atherosclerotic human vessels as it does in non-atherosclerotic animal arteries, but the one clinical study so far reported with extended follow up of 4 years, did not describe any evidence of late thrombotic complications despite only aspirin prophylaxis. A recent report on long term follow up of drug eluting stents described 4 cases in which drug eluting stents in coronary arteries thrombosed more than a year after insertion, when anti-thrombotic therapy was reduced. Two of these patients also had non drug eluting stents, which did not thrombose. There were no thrombotic complications in our experiments. On the evidence currently available from this and other studies, arteries tolerate PDT remarkably well, in the short and long term, although the clinical data available is very limited. No reports of arterial PDT have described any evidence of the chronic changes known to be associated with ionising radiation.

Several other drugs have been used to photosensitise arteries in studies of the effect of PDT on atherosclerosis and restenosis. Chloroaluminum sulfonated phthalocyanines (CASp) PDT is effective in inducing apoptotic cell loss and inhibiting intimal hyperplasia after angioplasty-like injury. However, only external illumination of an exposed artery with optical shielding of adjacent tissue has been described due to the high tissue penetration of the activating light of 675nm wavelength. This would limit its endovascular application. The photosensitiser Motexafin lutetium is activated by far-red light of wavelength 732nm, which is not attenuated by the presence of
blood, so an optical fibre without a blood flow-excluding balloon catheter can be used. Motexafin Lutetium is reported to accumulate preferentially in macrophage rich areas of plaques induced by injury and cholesterol feeding and upon light activation these cells are ablated, raising the potential of plaque specificity\(^{208,209}\). Another sensitiser, NPe6, has the specific advantage of rapid uptake into injured and atherosclerotic arteries, that may allow illumination to follow immediately after local or systemic drug administration\(^{131}\).

ALA was administered systemically and might be expected to cause generalised photosensitivity. However, a pilot study of ALA PDT as an adjunct to femoral angioplasty along with other clinical studies on PDT treatment of cancer and dysplastic lesions have confirmed encouraging results in terms of tolerability\(^{141,161}\). Indeed, as PDT has no long term sequelae in terms of DNA damage and healing, it may be possible, as with other disease applications, to use vascular PDT repeatedly as many times as necessary. Motexafin lutium has also been tested in clinical trials of peripheral and coronary vascular disease\(^{166,209}\).

In practical terms, PDT is more complex than inserting a drug eluting stent, but with the specifically modified balloon catheter described here, it is straightforward to deliver the light endoluminally. The need to administer ALA several hours prior to angioplasty and to take simple precautions against exposure to bright ambient light for 1-2 days are relatively minor inconveniences\(^{183}\). The equipment required for PDT is certainly simpler than the facilities required for safe delivery of the ionising radiation needed for brachytherapy.
On the limited evidence available to date, it would appear that PDT may be able to reduce the incidence of re-stenosis after angioplasty, with or without stenting, with few, if any, long term adverse effects on the arterial wall. This study demonstrates, for the first time, that PDT is an effective adjuvant to arterial stenting, significantly reducing intimal hyperplasia, without detrimental effects on vascular integrity and healing when light is delivered prior to stent deployment.
Chapter 8. Clinical pilot study

Introduction

Percutaneous transluminal angioplasty is well established in the treatment of occlusive and stenotic atheromatous disease of the coronary and peripheral arteries. It is associated with an excellent procedural outcome but is limited by the development of restenosis. In clinical practice, pharmaceutical approaches for preventing this have been disappointing. Stenting has led to a modest reduction in restenosis rates in coronary and iliac angioplasty but has failed to have a major benefit in femoral-popliteal disease. The efficacy of brachytherapy in the treatment and prevention of in-stent and post angioplasty restenosis is recognised. However, there remain concerns about radiation therapy because of reports of late thrombotic occlusion, and the long-term safety of ionising radiation remains unclear. Another promising option is the use of stents, which elute a cytostatic drug such as sirolimus or paclitaxel. The results with drug-coated stents are excellent.

As described in the previous chapters photodynamic therapy involves the activation of a systemically administered photosensitising agent by the application of non-thermal, non-ionising radiation, usually in the form of low power red light from a laser. In both small and large animal models of balloon injury to normal arteries, these histological changes are associated with a decrease in intimal hyperplasia. There is also inhibition of negative remodelling.

In 1999 we reported the short term results of a pilot clinical study of adjuvant
photodynamic therapy, in a total of 8 lesions in 7 patients undergoing repeat superficial femoral angioplasty for restenosis. At six months follow up all patients were asymptomatic, there were no arterial complications and there was no evidence of restenosis. The aim of the present study was to document in detail the long-term outcome in this group of patients.

**Materials and Methods**

All patients gave informed consent to the study, which was approved by the Ethical Committee of The University College London Hospital NHSTrust. In the original study, 7 patients (age range 59-86 years), who had developed symptomatic restenosis of the superficial femoral artery within six months of angioplasty (one with 2 lesions in the same artery) were enrolled. All of these lesions were ≥4cms as this was the size of the balloon. Table 8.1 Three had occlusions of the SFA and the other lesions were severe restenosis (>70%). Risk factors included hypertension (three), diabetes mellitus (two) and continuing cigarette smoking (three). Patients received 60mg/Kg of the sensitising agent 5-aminolaevulinic acid (ALA) by mouth, which is converted *in-vivo* to its photoactive metabolite protoporphyrin IX. Four to six hours later they underwent superficial femoral angioplasty of the re-stenosed or occluded site, using a 4cm length balloon of appropriate diameter, all treatments resulting in a residual stenosis of less than 30%. All patients received 5000U of intravenous heparin before the angioplasty. The angioplasty was immediately followed by the delivery of light (50J/cm² at a wavelength of 635nm from a copper vapour pumped dye laser) to the dilated site via an optical fibre with a 4cm terminal diffuser within the transparent angioplasty
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Risk factors†</th>
<th>Smoker</th>
<th>PTA Balloon Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>M</td>
<td>None</td>
<td>Ex</td>
<td>7</td>
</tr>
<tr>
<td>2a&amp;b*</td>
<td>70</td>
<td>F</td>
<td>H</td>
<td>Ex</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>M</td>
<td>CH</td>
<td>Ex</td>
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<tr>
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<td>66</td>
<td>M</td>
<td>CH</td>
<td>Current</td>
<td>6</td>
</tr>
</tbody>
</table>

† H- Hypertensive, C- Coronary Heart disease, D- Diabetic

* Patient 2 received PDT to two separate lesions in the same leg (2a and 2b)

Table 8.1 Patient demographics in pilot study
balloon. All patients were subsequently treated with long-term aspirin (75-150mg). Follow up was by Duplex scanning of the treatment site and peak systolic velocity ratio (PSVR) at 1, 3 and 6 months and intravenous digital subtraction angiography at six months. As stated above, all treated sites were patent at 6 months.

The long-term safety and efficacy were assessed by clinical review and repeat arterial imaging. The presence or absence of intermittent claudication, ischaemic rest pain and ulceration was established and all patients were offered repeat Duplex imaging of the treatment site by an experienced ultrasonographer. The original lesion site was identified by its distance from the medial femoral condyle, recorded at the time of the original PDT procedure. Doppler velocities were measured immediately proximal to the lesion and within the lesion site to determine the peak systolic velocity ratio. In accordance with the original study protocol, a PSVR greater than 2.0 was defined as indicating restenosis.

**Results**

All seven patients were followed up for a total period of 42-51 months (mean 48 months) after PDT. Table 8.2 No patient had developed critical limb ischaemia or ulceration during the follow up period. There were no arterial complications at the treatment sites in any of the subjects examined at any time during follow up. The results of the PSVR measurement are represented in Figure 8.1 Only one patient (patient 3) had symptoms from recurrent disease at the site treated with adjuvant PDT.
Table 8.2 Results of peak systolic velocity ratio (PSVR) data derived from duplex scanning during the pilot study and at long term follow up

<table>
<thead>
<tr>
<th>Patient</th>
<th>Long term follow up (months)</th>
<th>PSVR</th>
<th>Symptoms at long term follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-PDT</td>
<td>long term</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>51</td>
<td>4.5</td>
<td>2.0</td>
</tr>
<tr>
<td>2a</td>
<td>51</td>
<td>3.7</td>
<td>Declined</td>
</tr>
<tr>
<td>2b</td>
<td>-</td>
<td>5.5</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>Occluded</td>
<td>3.7</td>
</tr>
<tr>
<td>4**</td>
<td>46</td>
<td>Occluded</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
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<td>8.7</td>
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</tr>
<tr>
<td>7</td>
<td>42</td>
<td>Occluded</td>
<td>1.2</td>
</tr>
</tbody>
</table>

* Patient 2 received PDT to two separate lesions in the same leg (2a and 2b) and underwent repeat angioplasty of a new lesion proximal to these after 12 months

** Patient 4 underwent angioplasty and brachytherapy to the contra-lateral femoral artery 2 years after PDT to right femoral artery
Figure 8.1  Individual case data showing PSVR during study period
He had had 2 previous occlusions at this site (at his first presentation and when he was re-treated with adjuvant PDT). He developed mild claudication 18 months after PDT which, unlike his symptoms prior to PDT, was not limiting and which has been stable for the last 2 years. The most recent Duplex and Doppler ultrasound scanning (51 months after PDT) showed evidence of restenosis (PSVR=3.7) with smooth plaque at the treatment site.

The other six patients were asymptomatic at their most recent review. Patient 2 had experienced a recurrence of claudication one year after angioplasty with adjuvant PDT and subsequently underwent repeat angioplasty to two new lesions proximal to one of the two sites in the same artery treated originally. The angiogram at that time showed no evidence of restenosis at either of the PDT-treated sites. (Figure 8.2). This patient remained asymptomatic thereafter as far as her leg was concerned, but declined a further Duplex study as she had had a stroke and was very frail.

Patient 4 had developed symptoms relating to new disease in the contra-lateral leg. This was treated with angioplasty and brachytherapy 26 months after the PDT procedure. He has since developed mild claudication in that limb, but has no symptoms in the PDT treated leg. The bilateral angiogram at the time of brachytherapy showed no evidence of restenosis at the site originally treated with adjuvant PDT and the duplex scan 46 months after PDT showed a PSVR of 0.9. None of the 5 currently asymptomatic patients who agreed to undergo a repeat Duplex examination (including two who had occluded vessels at initial enrolment) demonstrated any evidence of significant restenosis (Table 8.2).
Figure 8.2 Right femoral angiograms from patient 2 illustrating

(a) Two tight irregular stenoses as indicated (arrows) at original angiography.
(b) The arteries after angioplasty with adjuvant PDT of these two lesions in series (arrows).
(c) IV DSA at 6 months showing no restenosis at the treated site (arrows) with suggestion of new lesion proximal to the treated site (arrowhead).
(d) Further angiogram 1 year later showing new lesions (arrowheads) proximal to the previous lesions. There is a moderate degree of concentric narrowing at the site of previous angioplasty with adjuvant PDT (arrows), but the distal vessel is unobstructed (arrow).
Discussion

In this small study, only one of 8 lesions treated by angioplasty with adjuvant PDT developed symptomatic restenosis at the treated site (PSVR 3.7) during the follow up period of nearly 4 years and these symptoms were considerably milder than those prior to PDT. Two others came close to the definition of restenosis (PSVR over 2.0) with PSVR values of 1.9 and 2.0, but both remained asymptomatic. There were no recurrences. Recurrent symptoms in one other patient were due to a new lesion, separate from those treated previously in the same leg. The numbers are small, but these results are most encouraging.

The long-term outlook following angioplasty for femoral and popliteal disease is unsatisfactory with more than most of the failures due to occlusion or restenosis at the treated site. The other symptomatic failures were related to disease progression, as was seen in our patient 2. Our group of patients, with focal disease and good run-off, belong to the group of patients with femoral-popliteal disease who have the most favourable outcome after angioplasty. However, all had restenosis or occlusion after a previous angioplasty and would have been anticipated to have a poor outcome from further intervention.

The most important and reassuring observation from this extended follow up is the finding that there was no evidence of localised arterial complications such as dilatation or aneurysm formation in those scanned or undergoing repeat angiography at a mean
of 4 years after treatment with adjunctive PDT. Further, there was no evidence of sub-
acute thrombosis or occlusion.

The numbers in this study are small and the data is uncontrolled and there is therefore
a need for a randomised controlled trial of femoral balloon angioplasty with and
without adjuvant photodynamic therapy to determine the clinical value of this therapy..
Adjunctive PDT may provide us with a new strategy to prevent restenosis after
angioplasty for occlusive and stenotic vascular disease. In the next chapter we report
the results of our randomised controlled trial.
Chapter 9. Randomised Controlled Trial

Introduction

Evidence from in-vitro work and in small and large animals would suggest that PDT is efficacious in reducing the incidence of restenosis after balloon injury in an artery. (Chapters 5 6 and 7) Pilot studies have also established that with the right combination of drug and light dose and optimum drug light interval the procedure can be applied in a clinical scenario safely and efficiently. They also show lack of significant stenosis at 4 years. (Chapter 8). The work described below is the first randomised controlled trial comparing standard angioplasty with angioplasty with adjuvant PDT.

Justification of methodology

A randomised controlled study using 5-ALA PDT as an adjuvant to angioplasty was deemed to be the best method of proving its efficacy for a number of reasons. Firstly the depth of effects with red light induced PpIX excitation limits extra arterial tissue damage compared to other photosensitisers. Secondly the limited skin photosensitivity period of PpIX makes it ideal for use in a non-malignant condition with short hospital stay. This would allow photosensitisation on the day of the procedure with angioplasty a few hours later, a overnight stay in the hospital and discharge the next day much in line with normal practice for patients undergoing PTA alone.

Thirdly the safety of 5-ALA has been shown in both animal and pilot clinical studies. An oral dose of 60mg/kg was selected as this had been safely used in the pilot study. As in the pilot study, it was given in 3 divided doses to give a longer plateau and
avoid a post bolus peak. In human studies in patients undergoing operation for gastrointestinal and colorectal malignancies there is a peak PP9 fluorescence activity at 4-6 hours after oral administration. Six hours was used in the pilot study with good results and was hence used in my controlled study as well.

Fourthly the clinical pilot study had shown no stenosis at the treated site in 8 lesions at 4 years. The established way of proving the efficacy of a particular form of treatment is in a randomised controlled double blinded study. Hence our study was designed in this manner.

Methods

Patient selection

Full approval from the Ethical Committee of the Royal Free and University College London Hospitals NHS Trust was obtained for the study. Our hypothesis was that we could reduce the rate of restenosis following PTA of the SFA by a significant level by using adjuvant ALA-PDT with PTA. All patients with symptomatic SFA lesions i.e. stenosis or occlusions were offered entry in to the trial and were randomised into 2 arms, one receiving standard PTA and the other group receiving PTA with adjuvant ALA-PDT. We anticipated a restenotic rate of ≈ 50% in patients undergoing routine PTA and a reduction of restenotic rate to 25% in patients under going PTA with adjuvant ALA-PDT. In consultation with a statistician in order to achieve a power of 80% to show a statistically significant difference between the 2 groups (p≤0.05) we would need to recruit 66 patients in each arm, i.e. a total of 132 patients.
All patients were consented following a full explanation of the trial backed up by a
written information sheet. Angioplasty limited to the SFA segment was elected due to
the relatively poor current outcome for PTA in this segment and the ease with which
any salvage bypass surgery could be carried out in the event of an unexpected complication.

Although in the pilot study only patients who had experienced restenosis following
previous angioplasty were selected in the RCT we used patients presenting for both
primary and redo angioplasty. This was because we had established the safety of the
procedure in the pilot study. Including patients for primary angioplasty allowed us to
recruit larger numbers of patients.

All patients were recruited prospectively. They were excluded if they had pre-existing
liver disease (as ALA can cause elevations of liver enzymes). All patients presenting
with technically suitable symptomatic lesions of the SFA were recruited into the trial.
The presenting symptoms included claudication, rest pain, non-healing ischaemic ulcers or ischaemic gangrene of the foot. Following a complete history and examination all patients had a duplex scan of the arteries of the leg. If the duplex scan confirmed a lesion in the SFA suitable for standard PTA the patient was included in the trial. These included stenosis and short occlusions (< 4 cm) of the SFA. Long occlusions, which were not suitable for standard angioplasty, were not included. The degree of stenosis was derived using peak systolic velocity ratio. This is the ratio of velocity of blood flow in the narrowed segment in the velocity of blood flow to the closest normal part of the vessel. A ratio of greater than 2 implies a significant
stenosis of > 50% All patients were started on aspirin and were put on lipid lowering drugs if required. Smoking cessation was advised but was not always complied with. Any pre-existing diabetes mellitus (DM), hypertension (HT) or ischeamic heart disease (IHD) was recorded and its management optimised.

Drug delivery

Patients were then randomised in the 2 arms. The randomisation was done by the pharmacist dispensing the ALA by selection of a pre-labelled envelope. The patients in the treatment arm were given 5-ALA (DUSA Pharmaceuticals. Inc., Tarrytown NY) in the dose of 60mg/kg. This was given in 3 divided doses of 20mg/kg. The first dose was given 5 hours before the procedure and at hourly intervals thereafter. The ALA was dissolved in orange juice and drunk by the patients. The control patients were given sugar dissolved in orange juice. All patients both in the treatment and control arm were housed in subdued light from the time of ingestion of the drug for 24 hours. The patient was blinded as to which arm he or she was in. The primary investigator was also blinded. The drug was delivered by the pharmacist, who along with the laser physicist and the radiologist were the only people who were un-blinded.

Procedure and Light delivery

All patients were given prophylactic analgesia (diclofenac PR or IM) before the procedure. Patients were starved for 6 hours before the procedure apart from the orange juice used to dissolve the ALA or placebo. An angiogram was done before the angioplasty to confirm the suitability of the lesion. If the lesion was found to be unsuitable no light was delivered. If angioplasty was done 5000 IU of heparin
was given intra-arterially. The procedure was done through a femoral approach under local anaesthesia and sedation if required. A standard Seldinger technique was used. After needle puncture of the common femoral artery a guide-wire was introduced and a sheath was introduced over the guide wire. After doing the angiogram the balloon was introduced over the guide-wire and aligned with the lesion to be treated. A special balloon, the Powerflex 3 (Cordis Ltd), with a transparent core allowing light diffusion was used. Figure 9.1 All balloons were 4 cm in length with the diameter ranging from 4-7 mm. The balloon was inflated, maintained for 15-30 seconds and deflated again. In patients in the treatment group the guide wire was replaced by an optical fibre with a 400-μm core (Pioneer Optics) with a 4-cm diffuser. Figure 9.2

The tip of the fibre was marked with a radio-opaque marker visible on an image intensifier. The diffuser was aligned with the balloon and the balloon re-inflated at low pressure to occlude blood flow. Figure 9.3 Light was provided by a 635-nm diode laser (Diomed). Light was given at 50 J/cm² with the fluance kept below 120 mW/cm to avoid any thermal injury. There was a one-minute dark interval after 20% of the light delivery in order to allow re-oxygenation of the arterial wall. Light delivery took 8 minutes including the window. A check angiogram was done at the end of the procedure. Figure 9.4 is an example of a successful treatment in the treatment group.

The patients were kept on a flat bed for 4-6 hours after the procedure and in subdued lighting overnight. All patients who underwent all of these procedures were deemed to have completed the protocol. Even though some patients did not have the full dose of light they were included in the analysis and were deemed to have completed the protocol.
Figure 9.1 Custom made PTA balloon for RCT. It has a transparent central channel to allow light transmission.

Figure 9.2 Custom made 400 μm core Optical light delivery fibre for RCT.
Figure 9.3 Light delivery fibre in angioplasty balloon. The balloon is inflated to occlude blood flow and light is delivered at 635nm.
Figure 9.4 Right femoral angiograms illustrating (a) occlusion of the femoral artery before angioplasty and photodynamic therapy (b) guide wire successfully across the occlusion (c) angiographic appearance after angioplasty and adjunctive PDT and (d) repeat angiogram of the right femoral artery 6 months later demonstrating patency and no evidence of restenosis
Monitoring

All patients had their pulse and blood pressure monitored along with temperature and respiration on an hourly basis on the day of the procedure. All patients had a full blood count with renal and liver function tests done prior to the angioplasty. The liver function tests were repeated on the day following the angioplasty. The patients had a duplex scan on the day before for confirmation of the lesion, measurement of the ankle brachial pressure index (ABPI) and measurement of the degree of stenosis. The efficacy of the angioplasty was recorded by another duplex scan on the day following the angioplasty for re-measurement of the treated site and measurement of the ABPI. In the absence of any complications the patients were discharged following the second scan. The patients were recalled at 1, 3 and 6 months after the procedure for follow-up scans. Patients who showed evidence of restenosis on duplex were re-assessed clinically and if necessary had an angiogram with radiological or surgical intervention. The trial was not run on an intention-to-treat basis. This was so that we could analyse the restenosis rate directly attributable to the ALA-PDT. However I have presented my results taking into consideration all patients in the trial as well as looking at those patients who have completed the protocol.

Results

In the 2 years I co-ordinated the trial, 66 patients were recruited. Only half the anticipated number of patients was recruited. Their demographics are summarised in table 9.1. Of the 33 patients in each arm 26 in the treatment arm and 28 in the control arm completed the protocol. Of the 8 patients in the treatment arm that did not complete the protocol, 5 were found to be unsuitable at angiogram, 1 was
<table>
<thead>
<tr>
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<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total patients</strong></td>
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<td>33</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>68</td>
<td>72</td>
</tr>
<tr>
<td><strong>Male: female ratio</strong></td>
<td>1.2:1</td>
<td>1:1.1</td>
</tr>
<tr>
<td><strong>Risk factors:</strong> (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>12 (36)</td>
<td>8 (24)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7 (20)</td>
<td>10 (32)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>24 (72)</td>
<td>20 (61)</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>8 (24)</td>
<td>5 (14)</td>
</tr>
<tr>
<td>Renal disease</td>
<td>4 (12)</td>
<td>5 (14)</td>
</tr>
<tr>
<td><strong>Previous arterial surgery</strong></td>
<td>9 (28)</td>
<td>6 (18)</td>
</tr>
<tr>
<td><strong>Previous PTA</strong></td>
<td>14 (40)</td>
<td>9 (28)</td>
</tr>
<tr>
<td><strong>Smoker:</strong> (%)</td>
<td></td>
<td></td>
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<tr>
<td>Present</td>
<td>10 (32)</td>
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</tr>
<tr>
<td>Ex</td>
<td>18 (55)</td>
<td>18 (55)</td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
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<td></td>
</tr>
<tr>
<td>Claudication alone</td>
<td>17 (52)</td>
<td>18 (55)</td>
</tr>
<tr>
<td>Rest pain</td>
<td>8 (24)</td>
<td>8 (24)</td>
</tr>
<tr>
<td>Tissue loss</td>
<td>8 (24)</td>
<td>7 (21)</td>
</tr>
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</table>

Table 9.1 Patient demographics in RCT
withdrawn because of cardiac instability just before the procedure and in 1 patient the lesion could not be crossed by a guide-wire for angioplasty. 1 was lost to follow-up. In the placebo group, of the 5 patients who did not complete the protocol 4 lesions were found to be unsuitable at the angiogram and 1 could not be negotiated with a guide-wire. Light delivery was cut short in 3 patients in the treatment arm because of pain during light delivery. One of them received 90% of the light dose one received 60% and one 40%. As mentioned in the methods these patients were deemed to have completed the protocol and analysed as part of the treatment arm. This has been summarised in the CONSORT flow diagram. Table 9.2 and 9.3 summarise the findings at follow-up at 3 and 6 months. Briefly of the 25 patients in the treatment group who completed the protocol 3 occluded immediate post-procedure and 5 more developed restenosis at 6 months. In the control group of the 28 patients who completed the protocol 1 occluded immediate post-procedure and 12 more developed restenosis at 6 months. There is no statistical difference between these outcomes. The PSVR was lower and the ABPI was higher in the treatment group at 6 months but again there was no statistical difference.

The complications recorded are summarised in the table 9.4. A fall of greater than 20mm Hg in the mean pressure or a systolic blood pressure of >100mm Hg was considered a significant drop in blood pressure. 6 patients in the treatment group recorded a significant drop in blood pressure while only 2 in the control showed this. (p<0.05 Fisher exact test) On the recommendation of the risk assessment reviewer a safety review was carried out after 30 patients during which the hypotension
Assessed for eligibility based on Duplex (n=66)

Randomised (n=66)

Allocated to PTA with PDT (n=33)
- Received allocated intervention (n=26)
- Did not receive allocated intervention:
  - Unsuitable at angiogram: 5
  - Unsuccessful angioplasty: 1
  - Pre-op cardiac instability: 1

Allocated to standard PTA (n=33)
- Received allocated intervention (n=28)
- Did not receive allocated intervention:
  - Unsuitable at angiogram: 4
  - Unsuccessful angioplasty: 1

Follow-up
- Lost to follow-up; Non-compliance: 1
  - Analysed;
    - Intention to treat: 33
    - Treatment received: 26
    - Per protocol: 25
- Lost to follow-up; 0
  - Analysed;
    - Intention to treat: 33
    - Treatment received: 28
    - Per protocol: 28

Figure 9.5 CONSORT statement for RAAPT trial
<table>
<thead>
<tr>
<th></th>
<th>Treatment group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number randomised</td>
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<td>33</td>
</tr>
<tr>
<td>Successful PTA ± PDT</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>Lost to follow up</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Acute Post procedure thrombosis</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Patent vessels at day 1</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>Patent vessels at 3 months</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Patent vessels at 6 months</td>
<td>18</td>
<td>15</td>
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Table 9.2 Summary of Follow-up -1
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<tr>
<td><strong>PSVR: median (range)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Pre procedure</td>
<td>2.2 (0-4.6)</td>
<td>2.9 (0-7.8)</td>
</tr>
<tr>
<td>Post-procedure</td>
<td>1.32 (0-2.7)</td>
<td>1.58 (0-9.5)</td>
</tr>
<tr>
<td>3 months</td>
<td>1.35 (0-3.3)</td>
<td>1.51 (0-3.8)</td>
</tr>
<tr>
<td>6 months</td>
<td>1.54 (0-3.4)</td>
<td>1.7 (0-3.6)</td>
</tr>
<tr>
<td><strong>ABPI: median (range)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre procedure</td>
<td>0.7 (0.2-1)</td>
<td>0.7 (0.4-1)</td>
</tr>
<tr>
<td>Post-procedure</td>
<td>0.87 (0.4-1)</td>
<td>0.9 (0.4-1)</td>
</tr>
<tr>
<td>3 months</td>
<td>0.9 (0.5-1)</td>
<td>0.89 (0.5-1)</td>
</tr>
<tr>
<td>6 months</td>
<td>0.91 (0.6-1)</td>
<td>0.88 (0.5-1)</td>
</tr>
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Table 9.3 PSVR and ABPI in patients who had successful PTA ± PDT. N=26 in the treatment group and 28 in the control group. One patient was lost to follow-up in the treatment group at 3 and 6 months.
<table>
<thead>
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<tbody>
<tr>
<td>Pain during procedure</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Significant hypotension</td>
<td>6*</td>
<td>2</td>
</tr>
<tr>
<td>Haematomas</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Post procedure thrombosis</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 9.2 Recorded complications in RCT**

* $p = 0.03$ (Fisher exact test) compared to control arm
caused by ALA was investigated further and found to be potentiated by Anti-hypertensives and diuretics. The protocol was changed so that the last dose of these drugs was omitted before the procedure and the patients were pre-filled with a litre of crystalloid. No hypotension was recorded after these changes. There were no instances of fluid overload.

Of the 2 patients in the control group who developed haematomas at the puncture site one had to be explored for a repair of pseudo-aneurysm.

The raw data of PSVR and ABPI in all patients completing the protocol is documented in tables 9.5 and 9.6 at the end of the chapter.

**Discussion**

This is the first clinical trial comparing Standard PTA with PTA with adjuvant PDT. An initial pilot study of PTA with adjuvant PDT conducted in our department had shown only one site of partial restenosis at the treated site at 4 years in 8 lesions. It had also shown the treatment to be safe and clinically feasible. A randomised controlled double blinded trial was essential to prove the efficacy of ALA PDT in preventing restenosis.

The trial highlights a number of problems which are encountered while running a randomised trial of this nature. The first striking problem is the number of patients recruited into the trial. While the initial intention was to recruit 132 patients into the trial I could recruit only 66 in the 2 years I co-ordinated it. During the planning of the trial it was indeed anticipated that we would be able to recruit the required
number (132) into the trial within this 2 year period. The funding for the trial which was provided for by industry was for 2 years. There a number of reasons I could not recruit. To begin with although all the personnel and ethics approval was in place there was a delay in the procurement of the necessary equipment by 2-3 months. Every patient which was thought to be suitable on the duplex was offered entry in to the trial and every patient who was offered the trial agreed to participate in the trial. Hence there just were not enough patients presenting to us who were suitable for the trial. Part of the reason there was a discrepancy between the anticipated number was that only a percentage of SFA lesions were suitable for entry into the trial i.e. only those with lesions smaller than 4 cms. SFA angioplasty is certainly extended to lesions far longer than these. Moreover of the patients who were initially thought to be suitable for the trial, 20% did not complete the protocol. This was because of a variety of reasons but the most common was because the lesions were found unsuitable on angiogram. About 6-8 months into the trial we realised that we were not going to get enough patients from a single centre i.e. UCL and Middlesex hospital. Hence we got ethics approval and stared recruiting from a second centre i.e. Royal Free Hospital and after a few more months from the Chelsea and Westminster hospital. In spite of this we were not close to getting the required numbers. Expansion to any more centres would deem the trial a multi-centre trial which come its own added set of problems. Multi-centre trials need special Ethics Committee permission requiring a central ethics committee with resubmission of the proposal as opposed just needing Chairman’s actions at the Royal Free and Chelsea and Westminster Ethics Committees. Specifically for this trial it would have been a logistical problem in terms of equipment and personnel. Since
we had only 2 diode laser machines and only one laser physicist a multi-centre trial
would have been impossible with the current arrangement. The special catheters we
were using for the trial would have either to be ordered by the procurement department
of the respective hospitals or provide for by us, raising the issue of funding for these
catheters as well approval of use of this non-standard equipment at these hospitals. The
use of lasers would also require approval of the health and safety departments with the
satisfaction of their rules. The smooth conduct of this procedure with ALA
administration and light delivery at the appropriate time would involve the cooperation
of a number of different departments and skilled personnel and equipment at the same
time and place. Finally at the end of 2 years we had no more funding for the trial and
hence we suspended the trial.

The second problem is one of co-ordination of all the personnel involved in the
administration of the treatment. As already mentioned the smooth running of the trial
needed a radiologist, vascular surgeons, specialised nurses and wards (given the
patients needed to avoid sunlight in peri-procedural period), laser physicist,
pharmacist, vascular technologist, the nurse co-ordinator and myself. We feel lucky in
obtaining co-operation from all these people sometimes at short notice in running the
trial. Anyone working in NHS hospital will realise the difficulty in getting beds, but
we seem to have managed to not only get a bed when we wanted but one where it
would be possible to shield the patient from sunlight.

The third problem is one of non-suitablity of potential patients at the time of their
angiogram. As we have shown we lost about 20% of our patients mainly because
the lesion was found to be unsuitable on angiogram. This is due to both over and under estimation of the lesions on Duplex. This is bound to happen in any trial involving angioplasty and hence should be taken into account when estimating numbers. This was however accounted for when the number need to treat were calculated. In fact if all patients recruited were suitable we would need only 45 in each arm to achieve statistical significance at 0.05 level with a power of 80% with at least a 25% difference in stenosis rates in the 2 groups.

Finally one has to take into account the statistics involved in drawing any conclusions from this trial. The patients were unblinded twice, once at the time of the safety analysis, and then at the end of my tenure as we had run out funds and time. The first time it was done at the request of the risk-reviewer. It was important to do so as hypotension in this group of patients with significant cardiovascular disease could have significant consequences. It could be suggested that somebody other than the main trial co-ordinator should do this, but this would involve extra personnel who were not available. In my defence however it is important to note that that the Duplex sonographer always remained blinded and hence the PSVR and ABPI measurements were done in an unblinded manner.

At the end my appointment as the coordinator we had run out of funds to continue the trial and recruit more patients. Hence it was decided to break the code and analyse the data we already had. It is impossible to get more funding without the interim results. The implications of this on the statistics is that in order to achieve the same power i.e. 80% we would now have to get more numbers that originally estimated. After
consulting with a statistician the best way to counteract the greater chance involved in
a significant result, would be to increase the level of significance needed to \( p < 0.025 \)
which necessitates an increase in the numbers needed in each group by 10%.

Having said all of the above there are I feel a few lesions we can learn from the trial
data. The trial confirmed that the treatment is indeed feasible and practical in a clinical
setting. Although there would still be a lot of different people involved in providing
the treatment experience from application of PDT in other fields such as esophageal
and oral cancers suggests this. The absence of any blinding and randomisation process
and application of the PDT as standard treatment makes this easier.

I have shown that ALA can cause hypotension in patients with significant
cardiovascular morbidity. Although the mechanism of this is not completely clear it
appears to respond consistently to simple remedies. The hypotension is seen in patients
with pre-existing cardiovascular disease. This has been reported before in elderly
patients who received ALA-PDT for bladder cancer.\(^{214}\) In our study vasodilators and
diuretics appeared to potentiate the hypotensive effects of ALA-PDT. This has not
been previously reported. Although all patients in the study whether receiving
treatment or placebo showed some drop in BP, the drop was significantly more in the
ALA-PDT group. While the exact cause of this is not known, a previous studies using
Swan-Ganz monitoring suggested that the hypotension is caused by peripheral
vasodilatation and possibly a volume shift out of circulation.\(^{215}\) That study showed that
both systolic and diastolic pressures fell along with a fall in peripheral vascular
resistance. However the pulmonary capillary wedge pressure remained
normal in the same study. It is not clear whether it is ALA, PpIX or one of its metabolites which causes these effects. The timing of the hypotension suggests that it would be either the PpIX or another metabolite. Herman et al reported hypotension 2-4 hours after oral administration of ALA. However hypotension in our series was slightly later at a mean of 5 hours after 1st fraction of ALA. (Figure 9.6) This may be partly because in their study ALA was administered in a single bolus dose of 60 mg/kg while in our study we administered it in three divided doses. It has previously been shown that blood levels of PPIX peak between 8-10 hours after administration. However, high levels of PPIX are seen from about 4 hours. This is consistent with the timing of the hypotension. No previous study has addressed the issue of how to prevent or treat this hypotension. Among the first 30 patients, those who developed hypotension responded immediately to the fluid infusion. All of them recovered their blood pressure within 30-60 minutes. Only one patient was symptomatic with the hypotension. This was a patient of known significant ischaemic heart disease who developed angina. This responded to GTN and the fluid infusion. All the other patients were asymptomatic. No patients suffered any lasting consequences from the hypotension. By 9 hours after oral intake the blood pressure in all the patients had returned to normal. Protocol changes were made as a result of this finding on the safety review. All patients were preloaded with a litre of crystalloid (normal saline) and their anti-hypertensives were omitted on the day of the treatment. Once the protocol changes were put into place none of the patients developed any significant fall in blood pressure. i.e. no systolic pressure < 100 mm Hg. was recorded. None of the patients have had any cardiovascular symptoms. We recommend that all
Figure 9.6  Bar Histogram showing time taken in hours for the blood pressure to fall to its lowest recording after ingestion of ALA. Most patients recorded maximum drop at 5-6 hours which corresponded with time for maximum concentration in the vessel wall. This drop was prevented by preloading patients with a litre of crystalloid.
patients with significant cardiovascular risk factors should have these measures put into place during ALA PDT.

Besides these effects no other significant complications were seen. Any pain, which was encountered with light delivery, can be prevented by prophylactic analgesics and reducing the light delivery rate to 80 mW/cm. The mild photosensitivity seen with some patients was self-limiting. In spite of taking all precautions to prevent exposure to direct sunlight some exposure could not be avoided in these patients as they did not necessarily stay in their bed during the 24 hours after ALA intake.

ALA had no effect on renal or liver function tests.

The laser source used in the pilot study was a copper vapour dye at 635 nm. For this study we used a diode laser at 635 nm. This is solid state and very consistent in its output. Being compact it is easy and safe to transport. The balloon used in the study was custom-made with a transparent core in order to allow the laser light to shine through. It was however very similar in all other respects to a standard balloon catheter. Since red light is absorbed by blood the lumen had to be occluded during light delivery. The balloon pressures were kept low during light delivery for 2 reasons. Firstly distension of the arterial wall might cause some hypoxia in the arterial wall compromising the PDT effect. Secondly keeping the balloon and the artery distended for 8 minutes might compromise the mechanical integrity of the vessel. It is important that an appropriate dose of Heparin is administered intra-arterially during the procedure in order to prevent thrombosis post procedure. Stasis of blood in the artery distal to the balloon will inevitably lead to thrombosis unless adequately
heparinised. The 3 patients who did have post procedure thrombosis in the treatment group were not given adequate doses of the heparin which probably lead to the poor result.

When the follow-up results at 6 months are analysed it is difficult to draw any strong conclusions from it. The bottom line is that 18 patients in the treatment arm had a patent angioplasty site at 6 months compared to 15 in the control arm. If one analyses this in an intension to treat manner the primary patency is 55% in the treatment are and 45% in the control arm. However I do not feel this is a true reflection of the result and these results are better looked at as group who received full treatment or completed protocol. In the control arm this number is the same i.e. 28 but in the treatment arm 26 received treatment, but 1 was lost to follow-up and hence only 25 completed protocol. Hence the primary patency in the treatment arm in all patients who received treatment is 69% and 53% in the control arm. Further in the treatment arm the primary patency in the patients who completed the protocol is 72%. Just to complicate these results further acute post-procedure thrombosis is a different problem from restenosis and hence should not be analysed as the same outcome. This is one of the main reasons this data cannot be presented and analysed as an actuarial life table or Kaplan Meier analysis. (This is the view of the consultant statistician). Another reason this data cannot be analysed as a life-table analysis is that this assumes that once the event has taken place it cannot reverse back. However in my data there are patients who have restenosis demonstrated on PSVR at 3 months or immediate post-procedure who have patent vessels at 6 months. The only conclusion one can draw from this data is that the
application of PDT as an adjuvant to PTA is possible in the clinical setting and there is a trend for reduced restenosis in the treatment group. This is not statistically significant and should be interpreted accordingly.

One has to look at these results compared to the other approaches in vogue for treatment and prevention of restenosis. Brachytherapy and drug eluting stents are 2 methods currently in use.

There have been a number of trials showing the efficacy of brachytherapy in the coronary circulation.\textsuperscript{216-224} Brachytherapy is licensed for use in treatment of in-stent stenosis. Radiation can be sourced either from $\beta$ emitting sources such as yttrium or phosphorous beads or $\gamma$ irradiation from iridium 192 wires. It however has not completely eliminated restenosis and rates between 15 and 58\% have been recorded at 6 months. This compares very well to our study. Its usefulness in the femoro-popliteal artery has recently been shown in a randomised controlled trial comparing PTA with brachytherapy with PTA alone. Patency at 6 months and one year were significantly better in the former.\textsuperscript{218} Since the work on my trial was completed, Brachytherapy has been withdrawn as licenced treatment for restenosis because of the efficacy of DES.

The most common complications seen with brachytherapy are edge stenosis, late thrombosis and aneurysm formation. This is due to lack of re-endothelialisation and a prolonged inflammatory response. This has necessitated the use of long term anticoagulation. All of these problems are potentially avoidable with adjuvant PDT. Animal studies have confirmed re-endothelialisation within a few days of treatment.
and no late problems were recorded either in the pilot study or in our study.

Since this trial was suspended Drug eluting stents (DES) have all but revolutionised the prevention and treatment of restenosis. In 2002 when I finished my study the DES was just being trialled. Published results now suggest a restenosis rate of between 5-9% at 1-2 years, effectively reducing restenosis compared to stent only patients by 60-90%.225-228 The stents are coated with agents such as sirolimus and paclitaxel both of which are anti-proliferative agents which prevent restenosis by inhibiting VSMC proliferation. A recent study has also documented its use in the treatment of in-stent restenosis. DES are now licensed for use for de-novo angioplasty and it is anticipated that they will be used for restenosis as well. There are still a few words of caution with drug-eluting stents. High concentration gradients of the drug at the stented site could lead to lack of vessel healing especially re-endothelialisation and thinning of the vessel wall. As with brachytherapy this could lead to late thrombosis, stent displacement and aneurysm formation. It is currently recommended that patients stay on anticoagulation (fractionated heparin or warfarin) for up to 6 months after brachytherapy which obviously adds to both the morbidity and cost of treatment. It is as yet unclear as to how much anti-platelet treatment is needed for patients with drug eluting stents. Current recommendation are 6-12 months. As far as PDT is concerned, the lack of any thrombotic complications and good evidence to show endothelial re-growth fairly quickly after the procedure indicates no need for anticoagulation. Finally there is the question of cost. With out doubt all of these treatments are expensive. The cost of every treatment of brachytherapy has been put at over $3000 and that of Sirolimis
stents is approximately $2500. While at present the PDT treatment was subsidised it is estimated that each dose of ALA would be about £500 while the extra equipment needed would also add to the cost. These have to be balanced out against the savings made by the reduced need of re-intervention and the reduced need of surgery.

This study shows that PTA with adjuvant PDT is clinically feasible and safe with simple precautions. The study also highlights the problems associated with running a randomised controlled trial. The smooth running of a RCT requires a concerted and meticulous approach to sticking to the protocol of the trial and considerable cooperation from all the personnel involved. Although the study has not been completed my results show a trend toward benefit in reducing restenosis when ALA-PDT is used in conjunction with PTA. We eagerly await the final results of the study.
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Table 9.5   Follow-up PSVR and ABPI data in treatment group in RCT
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Table 9.6 Follow-up PSVR and ABPI data in control group in RCT
Chapter 10. Conclusions and the future

Restenosis and NIH are brought about by a combination of complex biological mechanisms which interact in a manner which is not fully understood. Review of current literature has led me to conclude that restenosis is a diverse process brought about by a combination of different pathophysiology. The extent and mechanism of these changes are not fully understood and because of this a number of different biological and mechanical approaches have been used to combat this with varying success. At the time of writing drug-eluting stents seem to be the flavour of the month with very good medium term results.

Conclusions:

The main aims of this thesis were to optimise the use of PDT with stents and to conduct a randomised controlled study to establish its efficacy as an adjuvant to PTA.

In summary I can draw the following conclusions from my thesis.

Animal studies:

1. Systemic ALA PDT is feasible as an adjuvant to stenting in the rabbit iliac models using appropriate sized versions of standard clinical equipment. This is the first study to demonstrate this.

2. The optimum effects are achieved by intravenous delivery of ALA 3 hours before the procedure, and light application before stent delivery.

3. When PDT is given before stent delivery there is almost complete loss of medial
VSMC at 3 days. There is no significant loss when PDT is given after light delivery.

4. When PDT is given before stent delivery there is significant inhibition of in-stent restenosis at 28 days. This leads to a wider lumen in these vessels. As these vessels are stented there is no effect on remodelling. Changing the dosimetry may improve the results further.

Clinical study:

1. Long term follow-up has shown restenosis in only one patient at 4 years with only minimal symptoms and no limb loss. It is important to note that these were a group of patients who were at high risk of restenosis.

2. Our randomised study using PTA with adjuvant PDT suggests that the incidence of restenosis could be reduced by our technique. While this failed to reach statistical significance with the number of patients recruited there is a trend toward reducing restenosis and we are optimistic that on completion of the trial we will achieve results that will show statistical significance.

Future work:

However while answering a lot of questions these studies also raises new ones. While the rabbit iliac model is a suitable model for studies on restenosis the pig model mimics human anatomy and pathophysiology more closely. Both the iliac and coronary arteries in the pig are easily accessible and would provide an ideal model to test clinical techniques with almost the same equipment as for clinical use. It also
provides a good model to test the applicability of the technique in the coronary circulation and consequently its effect on myocardium both intra and post procedure. This is especially important given light delivery times of 8-10 minutes. Once safety and efficacy is established in the pig model the next step will be to test it in a clinical pilot study followed if successful in a randomised controlled study. The other issue to be considered is the use of alternative photosensitisers which have faster concentration time, appropriate level of effects and less photosensitivity. This last factor is especially important in vascular surgery in case patients need emergency surgery because of complications of angioplasty and stenting.

Although the results of the randomised controlled trial are encouraging they are not conclusive. The trial is still ongoing although currently suspended due to lack of funds. It is hoped that on conclusion it will prove the efficacy of PDT as an adjuvant to PTA. However one has to consider the other techniques being used for preventing restenosis especially drug-eluting stents which have shown excellent results in the short to medium term. The results with DES are so good that the licence for use brachytherapy for in-stent restenosis has very recently been withdrawn. While it is highly likely the DES with the standard first line treatment for coronary occlusive disease its role in PVD has not yet been defined. PDT may have a particular role to play in the peripheral vessels where stenting has not produced good results. It may also play a role in failures of DES. It has the particular advantage of being safe to use repeatedly with no apparent adverse effects. While the use of PDT, drug-eluting stents and brachytherapy concurrently has not yet been tested, it may provide another direction of attack.
As briefly mentioned in the previous chapter cost still remains an issue. None of the treatments currently under investigation or in use are cheap. But they are expensive precisely because they are in the developmental stage. Common clinical use will certainly bring the costs down because of mass production. This is paramount in widespread application of these techniques, although companies will have to recoup their research costs to make a profit. Certainly cheaper sources of light such as LEDs are currently being investigated and may prove useful.

In conclusion PDT is effective in preventing in-stent and angioplasty restenosis, but a lot more work needs to be done to establish its best role in everyday clinical use.
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Protocols

RAAPT TRIAL
RANDOMISED ANGIOPLASTY AND ADJUVANT PHOTODYNAMIC THERAPY TO REDUCE RESTENOSIS

Patient Number: ______  name: _____

Telephone no:  Trial number

GP name and telephone no:

SCREENING VISIT – MEDICAL HISTORY

Has the patient any significant medical history?

☐ Yes (Specify)  ☐ No

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<th>Description</th>
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<td>Diabetes mellitus</td>
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<td>Renal impairment</td>
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Has the patient had previous PTA?

☐ Yes (Specify)  ☐ No

Patient Name  Date of Visit:  _____ / ___ / ____
RAAPT TRIAL
RANDOMISED ANGIOPLASTY AND ADJUVANT PHOTODYNAMIC THERAPY TO REDUCE RESTENOSIS

Number: ______ Initials: _____ Trial number: __________

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LFTs (including AST, ALT): ________________________________

SCREENING VISIT – PHYSICAL EXAMINATION

Sex: ☐ Male ☐ Female

Date of Birth: ____ / ____ / ____

Month Day Year

Weight: ______ kg

Blood Pressure: _____ / _____ mm Hg

Heart Rate: _____ bpm

Smoking? ☐ Yes ☐ No ☐ Ex

Intermittent claudication? ☐ Yes ☐ No

(If “Yes” distance before claudication = _____ meters)

Ischaemic rest pain? ☐ Yes ☐ No

Tissue loss ☐ Yes ☐ No

Pulses: Right:

Fem ______ Pop ______ DP ______ PT ______

Left:

Fem ______ Pop ______ DP ______ PT ______

☐ PE NORMAL
RAAPT TRIAL
RANDOMISED ANGIOPLASTY AND ADJUVANT PHOTODYNAMIC THERAPY TO REDUCE RESTENOSIS

Pre-angioplasty assessment

Patient Initials: Patient Number: Trial Number:

Date of Visit:

Stenotic Segment:

Side: R/L Length:

Site:

Distance from Medial Femoral Condyle (cms):

Other lesions:

Same side: Other side:

Run Off:

Luminal Diameter (mm): Angle 1 Angle 2

(Transverse)

(Longitudinal)

Lesion 1

Lesion 2

Adjacent artery- Proximal Distal

Velocities (m/s)- Proximal Lesion Distal

Ankle Brachial Pressure Index (ABPI) at rest:
## RAAPT TRIAL
### RANDOMISED ANGIOPLASTY AND ADJUVANT PHOTODYNAMIC THERAPY TO REDUCE RESTENOSIS

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<td>Angiographic result:</td>
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<td>Complications at time of PTA?</td>
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Nausea/vomiting after procedure?  Yes (Specify)  No  □
RAAPT TRIAL
RANDOMISED ANGIOPLASTY AND ADJUVANT PHOTODYNAMIC THERAPY TO REDUCE RESTENOSIS

Patient Number: ______  Initials: _____
Date of Visit: ____ / ____ / ____
Trial number: _____

DAY 1 FOLLOW-UP

Arterial complication? □ Yes (Specify) □ No

Surgery (emergency/elective)? □ Yes (Specify) □ No

Repeat PTA? □ Yes (Specify) □ No

Pulses: Right: Fem____ Pop____ DP____ PT____
        Left: Fem____ Pop____ DP____ PT____

Access site:

Stenotic Segment:
Side: R/L  Length:

Site:
Distance from Medial Femoral Condyle (cms):

Other lesions:
Same side:  Other side:

Run Off:

Luminal Diameter (mm):

Angle 1
(Transverse)  Angle 2
(Longitudinal)

Lesion 1

Lesion 2

Adjacent artery-
Proximal
Distal

Velocities (m/s)-
Proximal
Lesion
Distal

Ankle Brachial Pressure Index (ABPI) at rest:

197
RAAPT TRIAL
RANDOMISED ANGIOPLASTY AND ADJUVANT PHOTODYNAMIC THERAPY TO REDUCE RESTENOSIS

Patient Number: _____ Patient Initials: _____ Date of Visit: _____ / _____ / ____

MONTH 1 FOLLOW-UP

Arterial complication? □ Yes (Specify) □ No

Surgery (emergency/elective)? □ Yes (Specify) □ No

Repeat PTA? □ Yes (Specify) □ No

Pulses: Right: Fem _____ Pop _____ DP _____ PT _____
Left: Fem _____ Pop _____ DP _____ PT _____

Access site: ____________________________

Stenotic Segment:
Side: R/L Length:
Site:
Distance from Medial Femoral Condyle (cms):
Other lesions:
Same side: Other side:
Run Off:
Luminal Diameter (mm): Angle 1 Angle 2
Lesion 1 (Transverse) (Longitudinal)
Lesion 2
Adjacent artery-
Proximal Distal
Velocities (m/s)-
Proximal Lesion Distal

Ankle Brachial Pressure Index (ABPI) at rest:

198
## MONTH 3 FOLLOW-UP

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### Stenotic Segment:
- Side: R/L
- Length: 

### Other lesions:
- Same side: 
- Other side: 

### Run Off:
- Luminal Diameter (mm):
  - Angle 1 (Transverse) 
  - Angle 2 (Longitudinal)
- Lesion 1
- Lesion 2

### Adjacent artery:
- Proximal
- Distal

### Velocities (m/s):
- Proximal
- Lesion
- Distal

### Ankle Brachial Pressure Index (ABPI) at rest:
- Patient
- Date of Visit: ___ / ___ / ___
**RAAPT TRIAL**
**RANDOMISED ANGIOPLASTY AND ADJUVANT PHOTODYNAMIC THERAPY TO REDUCE RESTENOSIS**

Number: ____  Initials: ____  Trial number: ____

---

### MONTH 6 FOLLOW-UP

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Pulses:
- **Right:** Fem____ Pop____ DP____ PT____
- **Left:** Fem____ Pop____ DP____ PT____

Access site:

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**Stenotic Segment:**

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Site:

Distance from Medial Femoral Condyle (cms):

Other lesions:

Same side:  
Other side:

Run Off:

Luminal Diameter (mm):
- **Lesion 1**
- **Lesion 2**

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Ankle Brachial Pressure Index (ABPI) at rest:

200
RAAPT TRIAL
RANDOMISED ANGIOPLASTY AND ADJUVANT PHOTODYNAMIC THERAPY TO REDUCE RESTENOSIS

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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

None
# Adverse Experiences

Did the Patient report any new problem that was not present at the initial visit, or that has worsened since initial visit?

- [ ] Yes (Specify)
- [ ] No

<table>
<thead>
<tr>
<th>Adverse experience</th>
<th>Date of onset</th>
<th>Duration</th>
<th>Severity</th>
<th>Study Drug relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

Cutaneous photosensitivity?  
- [ ] Yes (Specify)  
- [ ] No
RAAPT TRIAL
RANDOMISED ANGIOPLASTY AND ADJUVANT PHOTODYNAMIC THERAPY TO REDUCE RESTENOSIS

Patient Number: _______  Patient Initials: _______

<table>
<thead>
<tr>
<th>UNBLINDED LASER PHYSICIST WORKSHEET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date: ___________  Physicist: ___________</td>
</tr>
<tr>
<td>Laser used: ___________  Wavelength: _______ nm</td>
</tr>
<tr>
<td>Fibre used: ___________  Previous uses: ___________</td>
</tr>
<tr>
<td>Calibration factor: _______ %</td>
</tr>
<tr>
<td>Dose of ALA: _______ mg  Time of first dose: _______</td>
</tr>
<tr>
<td>Time of light delivery for PDT: _______</td>
</tr>
<tr>
<td>Power used: _______ mW/ cm²  Duration: _______ sec</td>
</tr>
<tr>
<td>Laser energy: _______ J/cm²  Number of applications: _______</td>
</tr>
<tr>
<td>Diffuser diameter: _______ mm</td>
</tr>
<tr>
<td>Diffuser area: _______ cm²</td>
</tr>
</tbody>
</table>

Note: 4cm long, 4 mm balloon, 50 J/cm², area = 5.02 cm² → 251 J, 0.4 W
4cm long, 5 mm balloon, 50 J/cm², area = 6.28 cm² → 314 J, 0.5 W
4cm long, 6 mm balloon, 50 J/cm², area = 7.54 cm² → 377 J, 0.6 W
4cm long, 7 mm balloon, 50 J/cm², area = 8.80 cm² → 440 J, 0.7 W

50 J at 80 mW implies 625 s (about 10 1/2 minutes)

DO NOT FORGET FRACTIONATION

1 minute when time counted down to 520 s.
CONFIDENTIAL
PATIENT CONSENT

RANDOMISED CONTROLLED TRIAL OF ADJUVANT PHOTODYNAMIC THERAPY TO REDUCE RESTENOSIS AFTER PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY TO THE SUPERFICIAL FEMORAL ARTERY


Have you read the information sheet about this study? YES/NO

Have you had the opportunity to ask questions and discuss the study? YES/NO

Have you received satisfactory answers to your questions? YES/NO

Have you received enough information about the study? YES/NO

My questions have been answered by.................................................................
I agree to take part in this study and I understand that I may withdraw at any time without giving a reason and without it affecting my subsequent care.

Signed.............................................. Print Name..............................................

Witness.............................................. Print Name..............................................

Investigator................................. Date..................................................
SYMPTOMS ARISING FROM A BLOCKAGE IN THE LEG ARTERIES MAY REQUIRE AN OPERATION BUT CAN OFTEN BE TREATED BY A SIMPLER PROCEDURE TO STRETCH THE ARTERY OPEN (ANGIOPLASTY). ALTHOUGH ANGIOPLASTY CAN BE VERY EFFECTIVE IN RE-OPENING THE BLOCKAGE THE PROBLEM CAN RECUR IN UP TO ONE THIRD OF PATIENTS.

PHOTODYNAMIC THERAPY (PDT) IS A RELATIVELY NEW TREATMENT THAT IS ALREADY USED IN OTHER BRANCHES OF MEDICINE AND IS ASSOCIATED WITH FEW SIDE EFFECTS. OUR PRELIMINARY STUDIES SHOW THAT PDT IS ALSO OF VALUE IN PREVENTING THE LEG ARTERIES RE-BLOCKING AFTER ANGIOPLASTY.

IN ORDER TO ASSESS THE TECHNIQUE THOROUGHLY WE NEED TO COMPARE STANDARD ANGIOPLASTY (CONTROL GROUP) WITH STANDARD ANGIOPLASTY COMBINED WITH PDT (TREATMENT GROUP). ON ENROLMENT INTO THE STUDY YOU WOULD BE RANDOMLY Assigned TO EITHER THE CONTROL OR TREATMENT GROUP. EITHER WAY YOU WOULD STILL RECEIVE ANGIOPLASTY TREATMENT.

THE STUDY WOULD INVOLVE YOU TAKING A DRINK THAT MAY CONTAIN ALA (5-AMINOLAEVULINIC ACID). ALA OCCURS NATURALLY IN THE BODY, BUT BY GIVING THE BODY MORE WE CAN MAKE YOUR ARTERIES SENSITIVE TO LASER LIGHT. DURING YOUR ANGIOPLASTY WE MAY (IF YOU WERE ASSIGNED TO THE TREATMENT GROUP) EXPOSE YOUR ARTERY TO LASER LIGHT TO ACTIVATE THE ALA. EXPOSURE TO LASER LIGHT SHOULD BE PAINLESS.

THE MAIN SIDE EFFECT FROM ALA IS SKIN SENSITIVITY AND SO YOU WOULD BE KEPT IN SUBDUED LIGHT FOR 24 HRS TO PREVENT THE POSSIBILITY OF SUNBURN. SOMETIMES THE BLOOD PRESSURE FALLS DURING THE HOURS AFTER DRINKING THE ALA. WE KNOW THAT INCREASING THE FLUID INTAKE CAN AVOID THIS. YOU WILL BE GIVEN IV FLUIDS IN THE PERIOD AFTER DRINKING THE ALA AND WILL BE ENCOURAGED TO DRINK PRETTY CLEAR FLUIDS. MOREOVER IF YOU ARE ON ANY TABLETS FOR HYPERTENSION OR IF YOU ARE ON WATER TABLETS WE WILL OMIT THE LAST DOSE BEFORE YOU GO DOWN FOR YOUR TREATMENT.

AFTER YOUR ANGIOPLASTY TREATMENT YOU WILL BE FOLLOWED UP BY THE RESEARCH TEAM ONE MONTH, THREE MONTHS AND SIX MONTHS LATER. THIS WILL INVOLVE AN ULTRASOUND SCAN (WHICH IS PAINLESS) OF THE ARTERY THAT WAS TREATED.

YOU HAVE ABSOLUTELY NO OBLIGATION TO TAKE PART IN THIS STUDY AND IF YOU DO TAKE PART THEN, OF COURSE, YOU CAN WITHDRAW AT ANY TIME WITHOUT GIVING A REASON AND WITHOUT IT AFFECTING YOUR SUBSEQUENT TREATMENT.

IF YOU DO AGREE TO TAKE PART THEN YOU WILL BE ASKED TO SIGN A CONSENT FORM. THE WHOLE DESIGN OF THE STUDY AND THE PROCEDURE WILL BE EXPLAINED TO YOU.
All studies such as this are reviewed by an ethics committee at the hospital before they can proceed. This study was reviewed by the joint UCL/UCLH Committees on the Ethics of Human Research.
Publications arising from this dissertation

Abstracts and presentations


Femoral artery angioplasty with adjuvant photodynamic therapy (PDT) using 5-aminolaevulinic acid (ALA): early findings. Circulation supplement 2001; 104 (17): II-369 (Abstract)


Prospective randomised trial of superficial femoral artery (SFA) angioplasty with adjuvant photodynamic therapy (PDT) using 5-aminolaevulinic acid (ALA): safety and tolerability. British Journal of Surgery 2002; 89(supp 1): 29


26 month review of the pilot study of adjuvant Photodynamic Therapy (PDT) with angioplasty for restenosis of the Superficial Femoral Artery (SFA) European Radiology 2002; 12 (Supp 1): 223

**Pai ML, Jamal W, Moss A, Bishop C, Bown S, McEwan J**

Photodynamic therapy for vascular disease: inhibition of in-stent restenosis in a rabbit model BJS 2003 ; 90 suppl 1:117

**Pai ML, Jamal W, Moss A, Bishop C, Bown S, McEwan J**

Photodynamic therapy for vascular disease: inhibition of in-stent restenosis in a rabbit model World Congress of Photodynamic Therapy Miyazaki Japan May 2003

**Pai ML, Mansfield R, Bishop C, Bown S, McEwan J**

ALACL ALA causes significant
hypotension in patients with cardiovascular risk factors. World Congress of Photodynamic Therapy Miyazaki Japan May 2003

Prospective randomised trial of superficial femoral artery (SFA) angioplasty with adjuvant photodynamic therapy (PDT) using 5-aminolaevulinic acid (ALA): safety and tolerability. World Congress of Photodynamic Therapy Miyazaki Japan May 2003


Prospective randomised trial of superficial femoral artery (SFA) angioplasty with adjuvant photodynamic therapy (PDT) using 5-aminolaevulinic acid (ALA): safety and tolerability. ASGBI Dublin May 2002

26 month review of the pilot study of adjuvant Photodynamic Therapy (PDT) with
angioplasty for restenosis of the Superficial Femoral Artery (SFA) European congress of Radiology Vienna March 2002

Femoral artery angioplasty with adjuvant photodynamic therapy (PDT) using 5-aminolaevulinic acid (ALA): early findings Scientific Sessions of the American Heart Association Anahein California Nov 2001


**Publications**

**Book Chapter** D3.2.6 'Applications: Case Studies: Medical: Therapeutic Applications: Lasers in Vascular Surgery' M Pai. Book Chapter in Handbook of Laser Medicine
