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MECHANISMS OF THROMBOSIS AND RESTENOSIS AFTER VASCULAR INJURY

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To my parents

Cogito Ergo Seco

CONTENTS

ABSTRACT

Atherosclerosis is the underlying cause of about 50% of all deaths in the western world. Peripheral vascular disease commonly affects the arteries supplying the leg and is mostly caused by atherosclerosis. When medical treatment of lower extremity ischemia has failed, percutaneous transluminal angioplasty (PTA) and bypass surgery are two major therapeutic options. The advances in vascular surgery and endovascular techniques over the past half-century have greatly expanded the number of arterial lesions that can be treated. The major limitations of a successful revascularisation are thrombosis and the later development of restenosis. This thesis has explored the mechanisms of thrombosis and restenosis after vascular injury, focusing on the interaction between coagulation, inflammation, and oxidative stress.

The long-term outcome of infrainguinal PTA was evaluated in 77 patients. Cumulative primary and secondary patency rates, respectively, were 81% and 86% at 1 year, 65% and 73% at 5 years, and 12% and 17% at 10 years. Patency rates were better for patients with claudication than critical ischemia. Stenoses had better primary patency than occlusions. Generalised femoral artery disease and diabetes mellitus predicted poor survival. Although the overall long-term patency of infrainguinal PTA is poor, the technique has a low morbidity and can be performed in selected patients with a reasonable long-term result. If conservative treatment has failed infrainguinal PTA should be considered, when lesions and patients are suitable, because of its minimal invasive nature. It is also important when treating patients with peripheral arterial disease to give attention to their general cardiovascular condition.

In an experimental study a specific direct thrombin inhibitor, inogatran, reduced neointimal hyperplasia after arterial injury in rats. A more prolonged administration of the thrombin inhibitor gave a further reduction of the neointimal hyperplasia. It seems that inhibition of thrombin activity is not only important early after injury, but also later. This could have clinical implications in the treatment of restenosis.

Inflammation and oxidative stress in the vessel wall may play important roles in the development of restenosis after angioplasty. In patients with peripheral arterial disease, a much more prolonged inflammatory response than previously noted was observed after angioplasty, but only minor changes in coagulation activity. C-reactive protein was elevated the day after angioplasty and peaked after one week. Coagulation and inflammatory markers were not significantly related to restenosis. The redox-active protein, thioredoxin, was significantly elevated 4 hours after angioplasty and returned to baseline within 24 hours. Circulating thioredoxin could theoretically impair the chemotactic response at local sites of inflammation. An association in patients with elevated levels of thioredoxin after angioplasty and reduced restenosis needs to be further evaluated.

This thesis has discussed the intimate relation between thrombosis, inflammation, oxidative stress, and restenosis. Further studies are needed to delineate the molecular mechanisms behind these observations and their involvement in thrombosis and restenosis. It is not only important to be able to understand the individual pathways of these processes, but also the ways they intersect and interact. If these pathways are further defined, improved treatment strategies, including antithrombotic treatments, statins, and thioredoxin, to modulate postprocedure inflammation could be tailored.

Key words: *Restenosis, neointimal hyperplasia, thrombin inhibition, angioplasty, PTA, outcome analysis, coagulation, inflammation, CRP, thioredoxin, oxidative stress*

LIST OF ORIGINAL PAPERS

The thesis is based on the following studies, which will be referred to by their Roman numerals.

I. Wahlgren CM, Kalin B, Lund K, Swedenborg J, Takolander R. **Infrainguinal percutaneous transluminal angioplasty: Long-term outcome.** *Journal of Endovascular Therapy* 2004; 11: 287-293.

II. Wahlgren CM, Frebelius S, Swedenborg J. **Inhibition of neointimal hyperplasia by a specific thrombin inhibitor.** *Scandinavian Cardiovascular Journal* 2004; 38: 16-21**.**

III. Wahlgren CM, Sten-Linder M, Egberg N, Kalin B, Blohmé L, and Swedenborg J. **The role of coagulation and inflammation after angioplasty in patients with peripheral arterial disease.** *Submitted.*

IV. Wahlgren CM, Pekkari K. **Elevated thioredoxin after angioplasty in peripheral arterial disease.** *European Journal of Vascular and Endovascular Surgery* 2005; 29:281-6.

List of Abbreviations

ABI	Ankle-brachial index
ADP	Adenosin diphosphate
AP-1	Activator protein-1
APC	Activated protein C
aPTT	Activated partial thromboplastin time
AT	Antithrombin
bFGF	Basic fibroblast growth factor
CLI	Critical limb ischemia
CRP	C-reactive protein
EPCR	Endothelial protein C receptor
ERK	Extracellular signal regulated kinase
GP	Glycoprotein
Grx	Glutaredoxin
GSH	Glutathione
hsCRP	High-sensitivity C-reactive protein
IC	Intermittent claudication
ICAM-1	Intracellular adhesion molecule-1
IFN-γ	Interferon-γ
IGF	Insulin-like growth factor
IL	Interleukin
JNK	Jun N-terminal kinase
LDL	Low-density lipoprotein
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein-1
M-CSF	Macrophage colony stimulating factor
MMPs	Matrix metalloproteases
NADPH	Nicotine adenine dinucleotide phosphate
NF-κβ	Nucleotid factor-κβ
NO	Nitric oxide
PAD	Peripheral arterial disease
PAI	Plasminogen activator inhibitor
PAR	Protease activated receptor
PDGF	Platelet derived growth factor
PSGL-1	P-selectin glycoprotein-1

PT	Prothrombin time
PTA	Percutaneous transluminal angioplasty
PTCA	Percutaneous transluminal coronary angioplasty
ROS	Reactive oxygen species
SMCs	Smooth muscle cells
TAFI	Thrombin activated fibrinolysis inhibitor
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor
Trx	Thioredoxin
t-PA	Tissue plasminogen activator
u-PA	Urokinase plasminogen activator
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VSMCs	Vascular smooth muscle cells
vWF	von Willebrand factor
WIQ	Walking impairment questionnaire

BACKGROUND

Introduction

Peripheral arterial disease (PAD) is a manifestation of systemic atherosclerosis. It is defined by progressive stenosis or occlusion within the arteries of the lower extremities. The earliest and the most frequent presenting symptom in patients with PAD is intermittent claudication (IC), defined as pain in the muscles of the leg when walking. Critical limb ischemia (CLI) occurs when PAD progresses to critical impairment of blood flow to the leg, and may be considered the end stage of the disease. The natural history of IC is considered relatively benign. Symptoms remain stable or improve in 50 % to 75 % of patients, 15 % to 20 % of patients progress to CLI over the course of their disease, and only approximately 1 % require amputation annually [Ouriel 2001, Dormandy 1991, Weitz 1996]. Ultimately, 25 % to 30 % of patients with IC will need intervention for worsening symptoms. Despite the benign nature of IC, patient mortality is three to four times higher in claudicants than in non-claudicants of a similar age because of an increased prevalence of cardiovascular disease at other sites [Leng 1993].

The prevalence of PAD in populations depends on the criteria used for diagnosis. The prevalence of IC at around the age of 60 years in men is 2 % to 6 % [Schroll 1981, Reunanen 1982, Fowkes 1991]. The prevalence of men is greater than that for women at all ages. There is little direct information on the incidence and prevalence of CLI. A national survey in United Kingdom concluded that there were 20 000 patients with CLI in the population, which gives an annual incidence of 400 per million per year [The Vascular Surgical Society of Great Britain and Ireland 1995].

When medical treatment of lower extremity ischemia has failed, percutaneous transluminal angioplasty (PTA) and bypass surgery are two major therapeutic options. The advances in vascular surgery and endovascular techniques over the past half-century have greatly expanded the number of arterial lesions that can be treated. These reconstructions do not last indefinitely, because continuing atherosclerosis, stenosis, and ultimately spontaneous thrombosis frequently occur. This represents a significant clinical and economic burden upon the health care system. Exactly why arterial reconstructions are unsuccessful is not known. Acute closure of the artery after angioplasty is usually caused by dissection, spasm, or embolism and frequently complicated by thrombosis [Pentecost 2003]. Also technical factors and poor in- or outflow have an effect on the outcome. Thrombotic occlusion of venous grafts soon after surgery arises from endothelial and medial injury during surgical preparation and implantation [Mehta 1997]. It has been known for a long time that injured arteries respond with a pathologic healing response that can lead to luminal narrowing. Restenosis is the narrowing or occlusion of a vessel that was previously stenotic and has undergone a therapeutic procedure to open it [Larson 2004]. It most frequently occurs from 1-2 months to 1 year after intervention, and is followed after 1-2 years by the development of superimposed atherosclerotic changes [Bryan 1994]. There has been extensive efforts to control the restenotic process, but none have proved successful in preventing it from occurring. Drug-eluting stents seem to show promising initial results. The multifactorial nature and the complexity of the events leading to restenosis and thrombosis after vascular injury implicates why it is still an unsolved problem.

This thesis will further explore the mechanisms of thrombosis and restenosis after vascular injury, focusing on the interaction between coagulation, inflammation, and oxidative stress.

The artery wall

Arteries are traditionally divided into three types: large or elastic arteries, medium or muscular arteries, and small arteries and arterioles [Ross M 1989]. Aorta and the larger branches of aorta are elastic arteries. There is no sharp dividing line between elastic and muscular arteries. Arteries are often intermediate between the two types and difficult to classify. The artery wall consists of three distinct layers: the intima, the media, and the adventitia (Fig. 1). The intima, the innermost layer, is composed of a monolayer of endothelial cells, extracellular connective tissue matrix (primarily collagen and proteoglycans), and the internal elastic lamina. The media, the middle layer, is made up of smooth muscle cells (SMCs) and extracellular connective tissue matrix. The exact composition of the media depends on the size and location of the artery. One of the features that distinguishes muscular arteries from elastic arteries is the presence of a prominent internal elastic lamina and an external elastic lamina in muscular arteries [Ross M 1989]. The external elastic lamina separates media from adventitia, the outer layer. The adventitia consists of connective tissues with interspersed fibroblasts and SMCs. This outer layer contains nerves and small blood vessels, *vasa vasorum,* which supply the adventitia and outer part of the media, while the inner part of the artery wall depends on diffusion from the lumen for nourishment [MacSween 1992].

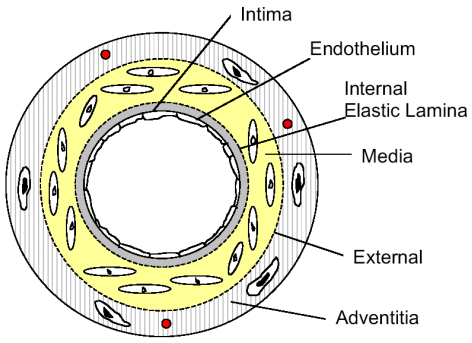

Fig. 1. *Anatomy of the muscular artery wall.*

Atherosclerosis

Atherosclerosis is the underlying cause of about 50% of all deaths in the western world. It has been identified, sometimes in advanced degree, in ancient Egyptian mummies [Lyons 1987]. Atherosclerosis is a complex and diffuse disease that starts early in childhood and progresses through adult life. Fatty streak, the earliest type of lesion, has been found to be present already in the intima of infants [Stary 1994]. The lesions of atherosclerosis occur principally in large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, brain, or extremities [Ross 1999]. The lesions tend to develop at branch points and at areas of major arterial curvature [Gimbrone 1999]. These regions show increased permeability to macromolecules such as low-density lipoprotein (LDL). There are numerous well-known risk factors for atherosclerosis, including smoking, hypertension, hyperlipidemia, diabetes, obesity, and lack of exercise [Assmann 1999].

The pathogenesis of atherosclerosis involves a complex series of events with the formation of the atherosclerotic plaque as the end result (Fig. 2). It is now clear that atherosclerosis is not simply an inevitably degenerative consequence of ageing, but rather a chronic inflammatory condition that can be converted into an acute clinical event by plaque rupture and thrombosis [Lusis 2000, Libby 2002]. The response to injury

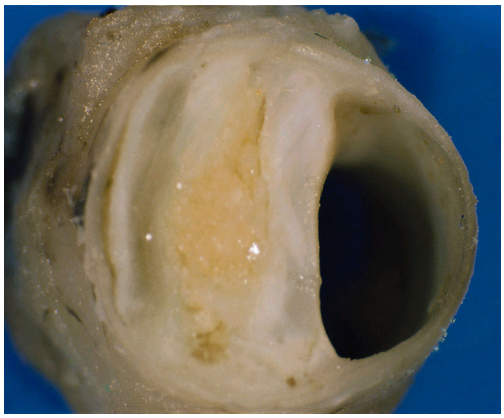

Fig. 2. *An atherosclerotic coronary artery. (with permisssion from Remedica Medical Education and Publishing Ltd)*

hypothesis of atherosclerosis, described by Ross, has become the cornerstone of our current understanding of the pathogenesis of atherosclerosis [Ross 1973]. The revised hypothesis emphasises endothelial dysfunction rather than denudation as the crucial first step in atherosclerosis [Ross 1999]. Injury to the endothelium, from atherosclerotic risk factors, leads to endothelial cell dysfunction with increased permeability to lipoproteins and other plasma constituents. An important initiating event is the retention of LDL and other apolipoprotein B-containing lipoproteins (lipoprotein a and remnants) in the subendothelial matrix [Lusis 2000]. The LDL undergoes oxidative modification as a result of interaction with reactive oxygen species (ROS). Oxidized LDL stimulates endothelial cells to produce adhesion molecules, chemotactic proteins such as monocyte chemotactic protein-1 (MCP-1), and growth factors such as macrophage colony stimulating factor (M-CSF) [Libby 2002]. This leads to migration of monocytes into the subendothelium, where they begin to accumulate lipid and become foam cells. Foam cells are formed when macrophages via scavenger receptors take up oxidized LDL [Berliner 1995]. Foam cells along with T-lymphocytes and SMCs form the fatty streak, the early lesion of atherosclerosis. Fatty streaks progress to intermediate lesions after further accumulation of lipids, macrophages and T-lymphocytes, and proliferation of SMCs. A fibrous plaque is subsequently formed. It is characterised by a growing mass of extracellular lipid, and by the accumulation of SMCs and SMC-derived extracellular matrix [Lusis 2000]. The interaction of CD40 with its ligand CD40L, a specific proinflammatory cytokine, has drawn attention lately [Libby 2003]. The ligation of CD40 seems to have several vascular functions e.g. stimulation of T-lymphocytes and macrophages to express cytokines such as IFN-γ that can influence inflammation, SMC growth, and matrix accumulation [Schonbeck 2000]. Also induction of tissue factor in macrophages and in VSMCs has been shown [Mach 1997, Schonbeck 2000].

Intimal SMCs secrete extracellular matrix and give rise to a fibrous cap that walls off the lesion from the lumen. A complex plaque is typically characterised by a fibrous cap that overlies a necrotic core. The necrotic core consists of cell debris, cholesterol, and a high concentration of tissue factor. Plaque rupture with superimposed thrombus formation is determined by the plaque composition, rather than luminal stenosis [Corti 2001]. A vulnerable plaque (rupture-prone) consists, histologically, of a large core of extracellular lipids, a dense accumulation of macrophages, reduced numbers of SMCs, and a thin fibrous cap [Viles-Gonzales 2004]. Activated macrophages can secrete several mediators, such as various proteinases, making the fibrous cap weak [Libby 2003].

Plaque rupture is a major cause of acute coronary syndromes. That plaque rupture contributes to acute exacerbations of peripheral arterial disease, or to its gradual progression, is very likely but has not yet been definitively demonstrated [Hiatt 2000]. Rupture usually occurs at the lesion edges, ”shoulders”, where the cap is often thinnest, most heavily infiltrated with inflammatory cells, and subjected to greatest hemodynamic stress [Falk 1992]. When the plaque rupture, tissue factor in the necrotic core is exposed to the blood and initiates the formation of a thrombus that could cause ischemic symptoms distal to it (Fig. 3).

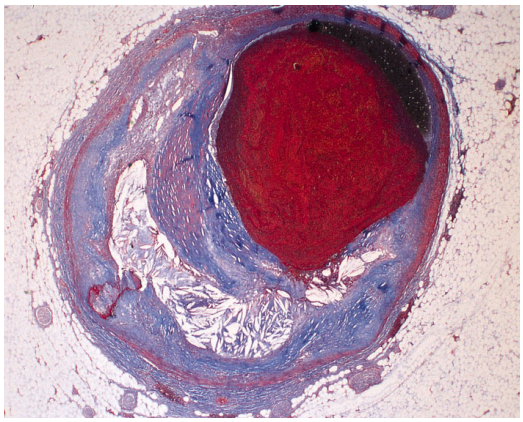

Fig. 3. *An acute thrombosed coronary artery. (with permisssion from Remedica Medical Education and Publishing Ltd)*

Haemostasis

Haemostasis is the arrest of blood loss from injured blood vessels and is essential to life [Rang 1991]. Perfect haemostasis means no bleeding and no thrombosis. Haemostasis involves three main processes: primary haemostasis, the coagulation system, and the fibrinolytic system. Arterial thrombosis, usually associated with atherosclerosis, is the unwanted formation of a haemostatic plug or thrombus within the artery. A thrombus forms *in vivo* and should be distinguished from a blood clot, which can form in static blood *in vitro*. Rudolf Virchow (1821-1902) described that the occurrence of arterial thrombosis depends on what is known as the "Virchow triad": Changes in composition of the arterial vessel wall and in the blood, and alteration in rheology [Virchow 1856, Rauch 2001].

Primary haemostasis

Primary haemostasis includes vasoconstriction and formation of a platelet plug after vessel injury. Vasoconstriction is mediated by a complex interaction of the autonomous nerve system, humoral responses, and VSMCs [Calaitges 2000, Kolde 2001]. Local production of thrombin and release of adenosin diphosphate (ADP), serotonin, and thromboxane A_2 from adhered platelets, stimulate SMC contraction in arteries. The loss of endothelial cells from the damaged artery also promotes vasoconstriction, because the production of vasodilators, such as prostacyclin and nitric oxide (NO), are reduced.

Platelets serve a primary role in haemostasis by forming a plug after vessel injury. Endothelial denudation exposes subendothelial components such as collagen and von Willebrand factor (vWF). Collagen interacts with glycoprotein Ia/IIa (GPIa/IIa) and vWF interacts with GPIb/IX complex on the platelets resulting in adhesion of the platelets to the subendothelial matrix [Cassar 2003]. Platelet aggregation involves links formed by fibrinogen between complexes involving GPIIb/IIIa on adjacent platelets [Kolde 2001]. Platelet activation also results in degranulation of alpha and dense granules content, releasing factors such as different growth factors, ADP, adrenaline, serotonin, β-thromboglobulin, and platelet factor 4 [Cassar 2003]. These substances have multiple roles in the vasculature including platelet function, local vasoconstriction, progression and development of atherosclerosis, and potent chemoattractants, causing cellular migration of inflammatory cells. The aggregated platelets form a plug which, together with vasoconstriction, maintains haemostasis in the vessel until the platelet plug is reinforced by fibrin [Rang 1991].

Blood coagulation

The coagulation cascade is a series of reactions involving activation of serine proteases that eventually leads to the production of fibrin by thrombin. The activation process of circulating inactive coagulation factors is primarily a sequence of proteolytic cleavages at specific sites [Kolde 2001]. The cascade scheme or waterfall model of the coagulation system was proposed nearly simultaneously by two groups [Davie 1964, MacFarlane 1964]. The classic coagulation system with the external and internal pathway was established 1975 [Austen 1975]. These two pathways interact on several steps. The physiological activation of blood coagulation is initiated almost exclusively via tissue factor and the extrinsic pathway [Conde 2003]. The physiologic relevance of the initial complex of the intrinsic or contact activation system in hemorrhage control is unclear. Contact activation plays of course an important role when blood is exposed to nonbiological surfaces, such as during cardiopulmonary bypass surgery.

The coagulation cascade model describes very well the screening coagulation laboratory tests, the prothrombin time (PT) and activated partial thromboplastin time (aPTT), but is clearly inadequate to explain the pathways leading to haemostasis *in vivo.* This had led to a revision of the coagulation model. A cell-based model of coagulation focusing on the role of different cell surfaces in mediating coagulation has been proposed [Hoffman 2001] (Fig. 4). According to this model coagulation occurs in three overlapping phases:

1. Initiation, which occurs on a TF-bearing cell.
2. Amplification, which occurs on the platelet surface. Platelets and cofactors are activated to set the stage for large scale thrombin generation.
3. Propagation, in which large amounts of thrombin are generated on the activated platelet surface and subsequent fibrin polymerisation.

Tissue factor (TF) is a transmembrane glycoprotein, normally located on the surface of a variety of extravascular cells, that initiates the clotting cascade [Nemerson 1987]. TF activity can also be induced in various cells in blood and plasma. The TF:FVIIa complex activates protease activated receptor-2 (PAR-2) [Camerer 2000], suggesting that TF, in addition to its role in coagulation, may contribute to other biological processes. TF exhibits a nonuniform tissue distribution with high levels in the brain, lung, and placenta, intermediate levels in the heart, kidney, intestine, uterus, and testes, and low levels in the spleen, thymus, skeletal muscle, and liver [Mackman 2004]. The higher levels of TF in the brain, lung, placenta, heart, and uterus would provide additional hemostatic protection to these vital organs [Drake 1989]. Thus, tissues that express low levels of TF rely more on the FVIIIa:IXa complex of the intrinsic pathway to prevent bleeding. An additional source of TF, known as blood-borne TF or plasma TF, may also contribute to thrombosis. Circulating TF on microparticles has been shown to incorporate into arterial thrombi *ex vivo* [Giesen 1999, Rauch 2000]. Leukocytes could be the main source of circulating blood TF in the form of cell-derived microparticles. Platelets are also a possible source of TF [Muller 2003].

Vascular injury results in exposure of TF to the blood, whereupon it binds factor VII/VIIa with very high affinity and specificity. About 99% of factor VII is circulating in plasma as a zymogen, inactive precursor, and about 1% as active factor VIIa [Morrissey 2001]. There are two ways the

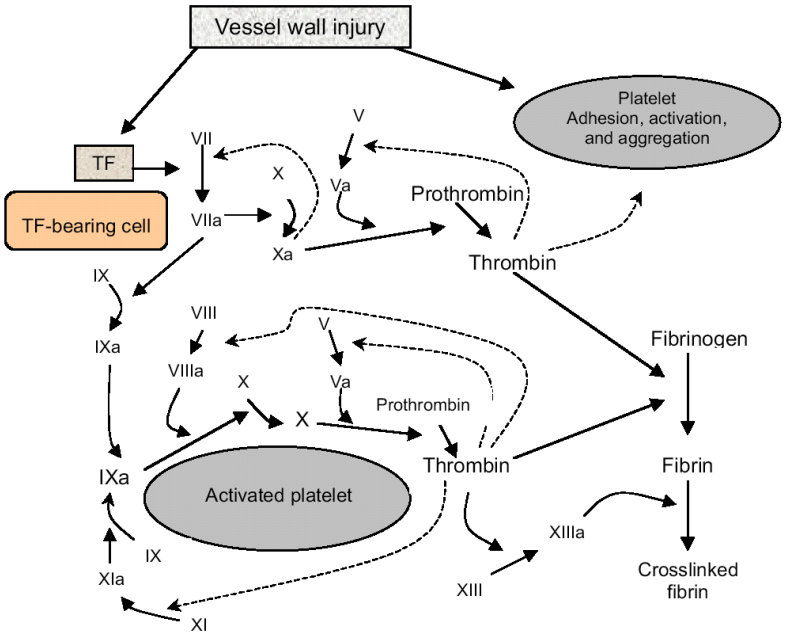

Fig. 4. *A cell-based model of coagulation. The dotted arrows = positive feedback reactions.*

TF:VIIa complex can form. Either through direct capture of circulating factor VIIa by TF or through capture of factor VII by TF followed by conversion of bound factor VII to VIIa [Morrissey 2001]. During this initiation phase, the complex then activates factor IX and factor X, although factor X activation is more efficient. Factor Xa converts small amounts of prothrombin to thrombin. This initial thrombin production is essential for propagation of coagulation by serving as the activator for platelets, factor V, and factor VIII [Mann 2003]. This amplification of the thrombotic response occurs as the action is moved from the surface of TF-bearing cells to the surface of activated platelets [Hoffman 2001]. There are three procoagulant complexes: prothrombinase (F Xa, F Va, phospholipids, calcium), intrinsic tenase (F IXa, F VIIIa, phospholipids, calcium), and extrinsic tenase (F VIIa, TF, phospholipids, calcium) [Jenny 2003]. Massive amounts of thrombin are generated on the platelet surface during the propagation phase. F IXa binds to F VIIIa on the activated platelet and forms the intrinsic tenase, that becomes the major activator of F X. The intrinsic tenase is much more efficient than the TF:VIIa complex in catalysing F X activation [Mann 2003]. F Xa binds to F Va on the activated platelet surface to form the prothrombinase complex that converts prothrombin to thrombin.

In the final phase of the coagulation cascade, thrombin converts fibrinogen to fibrin. This leads to the formation of an insoluble polymer or fibrin clot [Kolde 2001, Jenny 2003]. Thrombin activates F XIII which stabilises the fibrin clot by cross-linking the fibrin network. Thrombin remains bound in the clot and is still active.

Thrombin

Thrombin is a multifunctional serine protease which plays a central role in haemostasis and also has effects on virtually every aspect of vascular wall biology (Fig. 5). Thrombin is generated in large amounts at the site of injury and is amplified by formation of the prothrombinase complex both in humans and animals [Marmur 1994, Barry 1996]. The resultant thrombus and exposed extracellular matrix can serve as a reservoir of active thrombin. Thrombin also promotes numerous cellular and physiological effects including regulation of vessel tone, chemotaxis, smooth muscle cell proliferation, extracellular matrix turnover, release of cytokines, atherogenesis, and angiogenesis [Baykal 1995, Fager 1995, Goldsack 1998, Patterson 2001].

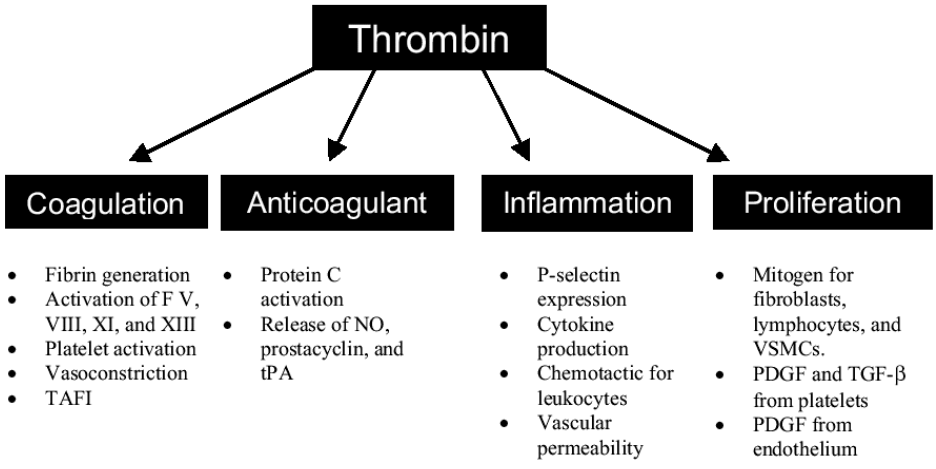

Fig. 5. *Thrombin is a multifunctional serine protease generated at sites of vascular injury. Thrombin generates procoagulant, anticoagulant, inflammatory, and proliferative responses on blood cells and blood vessels.*

Thrombin is formed from its precursor prothrombin, at sites of vascular injury, by cleavage at two sites by factor Xa [Goldsack 1998]. The resultant 39 kDa thrombin converts fibrinogen to fibrin in the final step of the clotting cascade. The activity half-life of thrombin in serum is 14 s as it is rapidly bound by inhibitors (see *endogenous inhibitors*). The thrombin-antithrombin complex is transported to the liver and undergoes degradation in Kupfer cells. Thrombin signalling is mediated at least in part by a family of protease activated receptors (PARs) [Coughlin 1999]. PARs are G-protein-coupled receptors which use diverse downstream intracellular signalling events [Coughlin 2000, Patterson 2001]. There are four identified PARs. PAR1, PAR3, and PAR4 can be activated by thrombin [Vu 1991, Ishihara 1997, Kahn 1998]. PAR 2 is activated by trypsin as well as factor VIIa and Xa, but not by thrombin [Nystedt 1994, Camerer 2000]. PAR1-3 has been found in human vascular cells and PAR4 in rat aorta.

The multiple actions of thrombin are mediated by unique structural features of the thrombin molecule [Eisenberg 1996] (Fig. 6). The molecule has several distinct receptors (recognition) sites, including the catalytic binding site, an anion-binding exosite (exosite-1), an apolar binding site, and separate sites where binding of heparin (exosite-2) and fibrin occur [Stubbs 1994, Eisenberg 1996]. The catalytic binding site is the active center, located in a deep narrow slot of the molecule, and involved in enzymatic activity [Stubbs 1993]. Fibrinogen, PAR1, thrombomodulin, heparin cofactor II, and the inhibitor hirudin bind at exosite-1 [Fenton 1991, Mathews 1994]. Heparin binds to exosite-2 [Sheehan 1994]. Heparin coupled with antithrombin (AT) cannot inactivate clot-bound thrombin, likely because of a conformational change in thrombin's structure once bound to fibrin. This change makes the exosite-2 binding site on clot-bound thrombin inaccessible for heparin [Weitz 1990]. Several direct thrombin inhibitors bind to the apolar binding site adjacent to the catalytic site. These inhibitors are smaller than heparin, need no cofactors, and can reach their site on thrombin within the thrombus. The apolar binding site is involved in substrate recognition as well as the interaction of thrombin with platelets, leukocytes, endothelial cells and SMCs [Moliterno 2003]. Fibrin binds to another part of the thrombin molecule, separated from the other binding sites mentioned.

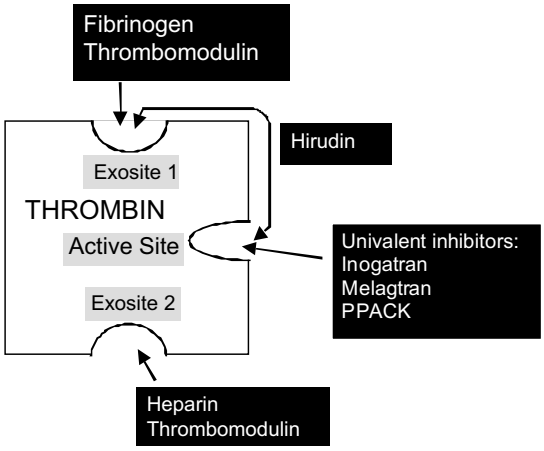

Fig. 6. *Different binding sites on the thrombin molecule. Exosite 1 and 2 are involved in binding substrates, fibrin, heparin, thrombomodulin, and bivalent inhibitors such as hirudin. The active or catalytic site is the binding site for univalent inhibitors and is also involved in enzymatic activity.*

Fibrinolytic system

The fibrinolytic system is essential for the lysis of fibrin clots and important in regulating thrombus formation in the vessel. The system is as complex as the coagulation system. A fibrinolytic cascade is initiated concomitantly with the coagulation cascade, resulting in formation of the enzyme plasmin. Plasmin is generated from the zymogen plasminogen through the action of tissue plasminogen activator (t-PA) or urokinase plasminogen activator (u-PA) [Jenny 2003]. Plasminogen, tightly bound to fibrin, is activated by a protelytic cleavage mediated by t-PA. The u-PA dependent pathway is not fully understood [Kolde 2001]. Plasmin is a very powerful enzyme which cleaves the fibrin network and releases fibrin degradation products. Inhibitors of fibrinolysis includes α2-antiplasmin, plasminogen activator inhibitor type 1 and 2 (PAI-1 and 2), α2-macroglobulin, and thrombin activated fibrinolysis inhibitor (TAFI).

Endogenous anticoagulants

The coagulation cascade has to be controlled and balanced. Endogenous anticoagulants maintain haemostatic balance by shutting down further thrombin formation and platelet recruitment. Endogenous anticoagulants include antithrombin (AT), heparin cofactor II, tissue factor pathway inhibitor (TFPI), and activated protein C (APC). AT is a member of the serpin family of protease inhibitors and is the most important inhibitor for the activated coagulation factors [Rosenberg 1984]. The binding of AT to thrombin inhibits coagulation not only by limiting fibrin formation, but also by inhibiting positive feedback pathways that normally amplify coagulation, including platelet activation [Boules 2004]. AT also directly inhibits factors VIIa, IXa, Xa, XIa, and XIIa [Vinazzer 1999]. The inhibition of thrombin by AT is significantly enhanced in the presence of heparin.

Heparin cofactor II is also a serine protease inhibitor. Its inhibitory activity is specific for thrombin [He 2002] and augmented by both heparin and dermatan sulfate [Yamanaga 2000]. There is data supporting that the inhibitory activity of heparin cofactor II may be located extravascularly. The physiological importance of its anticoagulant role is still unknown.

TFPI is synthesised by endothelial cells and is the most important inhibitor of the activation of coagulation via the extrinsic pathway. TFPI binds to the TF:FVIIa complex through one of its domains and to F Xa through another [Esmon 2001]. It can only inactivate TF:FVIIa after a previous reaction with F Xa. Heparin can competitively inhibit the TFPI binding to the surface of endothelial cells and increase the concentration of plasma TFPI [Hansen 1997, Sandset 1988].

The generation of APC is directly proportional to that of thrombin [Conde 2003]. It is produced on the surface of the endothelium when thrombin binds to thrombomodulin. The thrombin-thrombomodulin complex not only inhibits the actions of thrombin, but also activates protein C to APC [Esmon 2001]. Protein C activation is augmented by the endothelial protein C receptor (EPCR) [Stearns-Kurosawa 1996]. APC bound to EPCR does not appear to be able to function as an anticoagulant [Regan 1996]. When APC dissociates from EPCR, it binds to its cofactor protein S and the complex inactivates factors Va or VIIIa [Esmon 2001]. This results in inhibition of the intrinsic tenase and prothrombinase complex on the surface of activated platelets.

Atherosclerosis, inflammation, and thrombosis

Atherosclerosis, inflammation, and thrombosis are intimately intertwined. There is a complex interplay among these three processes. Atherosclerosis involves, as previously mentioned, inflammation at every stage of the disease, from initiation to progression and eventually plaque rupture [Libby 2002]. Thrombosis is also involved in all stages of atherosclerosis, except maybe the earliest lesion of atherosclerosis. At last, there is growing evidence that thrombosis and inflammation are tightly interrelated.

1) Atherosclerosis and inflammation

Atherosclerosis is an inflammatory disease [Ross 1999]. The term inflammation used here does not mean the classical signs with

rubor, calor, dolor, and *tumor*, but rather implies a chronic "micro-inflammation". Several risk-factors, e.g. oxidised lipoproteins, hypertension, diabetes, obesity, and intravascular infection (*Chlamydia pneumoniae* and cytomegalovirus), trigger inflammation in atherogenesis [Libby 2002]. These riskfactors facilitate attachment and migration of leukocytes into the subintimal space. Virtually, every step in atherogenesis is believed to involve cytokines and cells that are characteristic of inflammation [Pearson 2003].
Several acute phase proteins are associated with atherosclerosis and cardiovascular disease [Tracy 2003]. They are usually produced in the liver in response to IL-6. The most promising inflammatory biomarker for clinical purpose appears to be C-reactive protein (CRP) [Pearson 2003]. Numerous studies have now confirmed that high-sensitivity CRP (hsCRP) levels in normal volunteers predict cardiovascular events [Ridker 2001]. CRP belongs to the pentraxin protein family and consists of five identical nonglycosylated 23-kd subunits that are synthesised mainly by hepatocytes under the control of IL-6 [Pepys 2003]. However, IL-1 and TNF-α may also contribute to hepatic synthesis and secretion of CRP. CRP was discovered and named because of its binding to the C-polysaccharide of *Streptococcus pneumoniae* [Tillett 1930]. Serum concentrations of CRP peak within 24-48 h in response to tissue injury, infarction, and inflammation [Volanakis 2001]. The plasma half-life is about 19 h and this appears to be constant in both health and disease.

The main biological function of CRP appears to be host defense against bacterial pathogens and clearance of apoptotic and necrotic cells by recruiting the complement system and phagocytic cells [Volanakis 2001]. However, there is recent data supporting an active role for CRP in atherogenesis [Jialal 2004, Yeh 2004, Labarrere 2004]. CRP is not exclusively produced in the liver, but also in the atherosclerotic lesion, especially by SMCs and macrophages [Calabro 2003, Kobayashi 2003]. Several proinflammatory and proatherogenic effects of CRP, largely derived from *in vitro* studies, have been documented in endothelial cells, monocytes/macrophages, and SMCs [Jialal 2004].

2) Atherosclerosis and thrombosis

The relation between atherosclerosis and thrombosis has been recognised for a long time. However, the mechanisms by which these two pathological processes are associated have only recently emerged. Atherothrombosis, characterised by atherosclerotic plaque disruption with superimposed thrombus formation, is a major cause of cardiovascular death [Viles-Gonzalez 2004]. There are several thrombotic factors, like platelets, TF, thrombin, fibrinogen, plasminogen, and tPA, that are associated with atherosclerosis [Loscalzo 1992, Wilcox 1994]. Thrombin and fibrinogen could contribute to atherogenesis by their chemotactic and mitogenic properties [Falk 1995]. Increased expression of PAR1 has been observed in atherosclerotic plaques from human arteries [Nelken 1992]. Thrombin may here play a role in the progression of atherosclerosis by mediating inflammatory and proliferative processes. Fibrinogen has effects on the permeability properties of the endothelium and may itself contribute to the process of intimal thickening associated with atherogenesis [Loscalzo 1992]. Plasmin cleaves components of the extracellular matrix and basement membrane, and activates matrix metallo-proteases (MMPs) [Liotta 1981, Gross 1982].
During progression of atherosclerosis with accumulation of macrophages in the intima, small areas of endothelial injury occur. Microthrombosis occurs at these sites with adhesion of platelets and release of growth factors that promotes SMC migration (PDGF and thrombin), collagen synthesis (TGF-β), and inhibit fibrinolysis (PAI-1) [Falk 1995, Libby 2001]. As one final example highlighting the link between thrombosis and atherosclerosis, the plaque rupture is mentioned. Foamy macrophages in the lipid core express TF in human atheroma [Wilcox 1989]. When the plaque ruptures, coagulation factors in blood gain access to TF which triggers thrombus formation.

3) Thrombosis and inflammation

Studies during the last years have demonstrated an increasingly tight interplay between inflammation and the coagulation system. Several coagulation factors have structural similarities to components involved in inflammation. Tissue factor, for instance, has structural homology to the cytokine receptors [Morrissey 1987]. Systemic inflammation is a potent prothrombotic stimulus. Septic shock is a dramatic example of systemic inflammatory activation, where bacterial endotoxin potently stimulates the expression of TF and initiation of the clotting cascade. Inflammatory mechanisms upregulate procoagulant factors, downregulate natural anticoagulants, increase platelet reactivity, and inhibit fibrinolytic activity [Esmon 2003]. Thrombin generates several inflammatory responses via augmentation of leukocyte adhesion and activation, stimulation of platelet-activating factor formation (neutrophil agonist) [Bar-Shavit 1986], and release of CD40 ligands from platelets (induction of TF and cytokines) [Miller 1998, Henn 1998]. In addition, thrombin stimulates production of the proinflammatory cytokines IL-6 and IL-8 from monocytes and endothelial cells [Johnson 1998] and upregulation on endothelial cells of adhesion molecules such as P-selectin, intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) [Sugama 1992, Kaplanski 1998].

Thrombomodulin and the EPCR are both downregulated by inflammatory cytokines like TNF-α [Fukudome 1994, Conway 1988]. TNF-α can also inhibit fibrinolysis by suppressing the release of tPA and inducing expression of tPA inhibitors such as PAI-1 [Lansink 1988]. The drop in levels of protein C and S in sepsis is associated with increase risk of death [Fisher 2000]. Clinical studies have shown improvement in septic patients following protein C infusion [White 2000]. The three major endogenous anticoagulants: antithrombin, TFPI, and APC; seem to have unique anti-inflammatory qualities like reduction of cytokine formation and leukocyte activation [Esmon 2001 and 2003].

Treatment of peripheral arterial disease

Vascular surgery has undergone a technical explosion since the 1950s. The first reversed saphenous vein by pass graft for a superficial femoral artery occlusion was described 1949 [Kunlin 1949]. Synthetic materials, as alternative bypass conduits to autologous vein, have been developed since 1955 [Edwards 1955]. In 1964 Dotter and Judkins described percutaneous radiological techniques for treating vascular disease [Dotter 1964]. However, it was not until Grüntzig and Hopff 1974 introduced a coaxial balloon catheter, which inflated to a fixed diameter, that angioplasty came to be used with any frequency [Grüntzig 1974].

Patients with IC and CLI clearly show a different natural history for their disease, as previously described. Risk factor modification, antiplatelet therapy, and regular exercise training are all the treatment that most patients with IC will need and is all that most should be offered in the current state of our knowledge [TASC 2000]. Preventative measures directed at decreasing cardiovascular complications, which result from widespread atherosclerosis, should be implemented. This includes antihypertensive, antiplatelet, and antihyperlipidaemic therapy, blood sugar control, exercise, weight loss, and smoking cessation [Hiatt 2002]. A minority of claudicants will deteriorate, with symptoms interfering with work or lifestyle, and their handicap will become sufficiently severe to justify some local intervention for their PAD. In patients with CLI, the primary aim is limb salvage with revascularisation to provide sufficient blood flow to relieve rest pain, nonhealing ulcers, and/or infection or gangrene [Weitz 1996]. The medical treatment for patients with IC, also applies to patients with CLI, although the urgency for the latter group will alter the emphasis. The optimal form of re-vascularisation, normally surgery or endo-vascular techniques, has to be decided. That decision can be complex with a number of factors requiring consideration. The second decision is whether to apply this form of revascularisation or proceed to a primary amputation [TASC 2000].

In the most general terms, bypass surgery is best undertaken in patients with advanced arterial disease who are reasonable surgical risk. PTA is an attractive alternative to open surgical procedures because of low morbidity and short hospital stay. Thus, percutaneous techniques have their greatest application in less advanced disease and in those patients who are poor operative risk. The success of angioplasty is influenced by lesion and patient characteristics, such as the lesion severity/location, arterial runoff, and clinical manifestation [TASC 2000].

Iliac artery disease

In patients with IC, surgery is today less common. The patency rates, in a meta-analysis, for aortobifemoral bypass are at 5 years 91% and at 10 years 87% [De Vries 1997]. The use of PTA in the aortoiliac segment produces better results than when applied in the femoropopliteal region. The initial technical and clinical success of PTA of iliac stenoses exceeds 90% and long-term results have improved after stent placement [Bosch 1997]. A literature review showed that average primary patency rates after angioplasty in patients with IC were 61% for iliac stenoses (5 years), 72% for stented iliac stenoses (5 years), 60% for iliac occlusions (3 years), and 64% for stented iliac occlusions (3 years) [Hunink 1995]. A 10-year follow-up after iliac artery stent placement (93% IC and 48% occlusions) showed 46% primary stent patency rate and 55% secondary patency rate [Schürmann 2002]. In patients with CLI, the meta-analysis from de Vries *et al* showed that the limb-based primary patency results for aortobifeomral bypass were 88% and 82% at 5 and 10 years, respectively [De Vries 1997]. Endovascular procedures in the aortoiliac segment have predominantly been performed for claudication. The adjusted 4-year primary patency rates after PTA for CLI were 53% for stenoses and 44% for occlusions [Bosch 1997]. After stent placement the primary patency rates were 67% for stenoses versus 53% for occlusions.

Suprainguinal PTA and stent placement seem to be a relevant treatment option for suitable lesions in the aortoiliac region. Bypass surgery with greater durability and symptom relief may be a reasonable trade-off for immediate morbidity in patients with reasonable surgical risk whose aortoiliac lesions are not suitable for PTA and stent [TASC 2000].

Femoropopliteal disease

Lesions are often multiple, long, and ulcerated in the femoropopliteal region (Fig. 7). Therefore, the long-term patency rates of femoropopliteal endovascular interventions are somewhat disappointing when compared to bypass surgery. Available data suggest that long-term patency is greater with surgical revascularisation compared with endovascular procedures [Ouriel 2001]. Long-term patency rates of infrainguinal by pass surgery for claudication are about 62% to 90% at 4 to 5 years [Belkin 1996, Byrne 1999]. A meta-analysis showed that the 5-year primary patency results after bypass surgery in patients with IC and CLI were 80% and 66% for vein bypass, 75% and 47% for above-knee PTFE, and 65% and 33% for below-knee PTFE, respectively [Hunink 1994].

The TASC document indicates endovascular therapy as the treatment of choice for short single stenoses (< 3cm) [TASC 2000]. Technical progress with new endovascular material, such as self-expanding stents, has made it possible to treat more complex lesions.

However, the efficacy of infrainguinal PTA and stenting remains controversial. There are few studies with a long follow-up time after infrainguinal PTA [Martin 1999, Jämsén 2002]. A meta-analysis showed that the combined 3-year patency rates after infrainguinal PTA were 61 % for stenoses and claudication, 48 % for occlusions and claudication, 43 % for stenoses and critical ischemia, and 30 % for occlusions and critical ischemia [Muradin 2001]. The 3-year patency rates after stent implantation were 63-66 % and were independent of clinical indication and lesion type. Self-expandable nitinol stents appear to perform well for the treatment of short femoral lesions with a primary patency rate of 84% after 1 year [Vogel 2003] and a 3-year primary patency rate of 67% in stenotic lesions > 8 cm of length [Henry 2003]. However, there are no long-term results, beyond a period of 3 years, with nitinol stents. Techniques like drug-

eluting stents [Duda 2002] and subintimal angioplasty [Bolia 1990 and 1995, Flørenes 2004] are therapeutic methods that are not yet established.

Vascular response to injury

An arterial injury, e.g. dilatation with a balloon catheter or placement of a suture, responds with a pathological healing process that could lead to luminal narrowing. The occurrence of restenosis, the renarrowing of a vessel, is one of the most important factors preventing long-term patency after vascular interventions. Restenosis is a complex and multifactorial vascular wound healing process that involves several different and overlapping mechanisms (Fig. 8). The primary function of the vessel injury response during evolution was to limit tissue damage from trauma, especially to assist the coagulation pathways to limit blood loss [Berk 2003]. It is therefore logical with an overlap between mediators involved in blood coagulation and vessel repair.

Restenosis is not a case of accelerated atherosclerosis but rather a characteristic and distinct pathophysiological process [Orford 2000]. Studies of the process in humans are limited by the fact that direct tissue examination is only rarely possible. Thus, most of our knowledge of the vascular response to injury comes from studies in animal models. The bulk of literature is also directed at the prevention of restenosis after PTCA, conclusions regarding applicability of these data to the peripheral

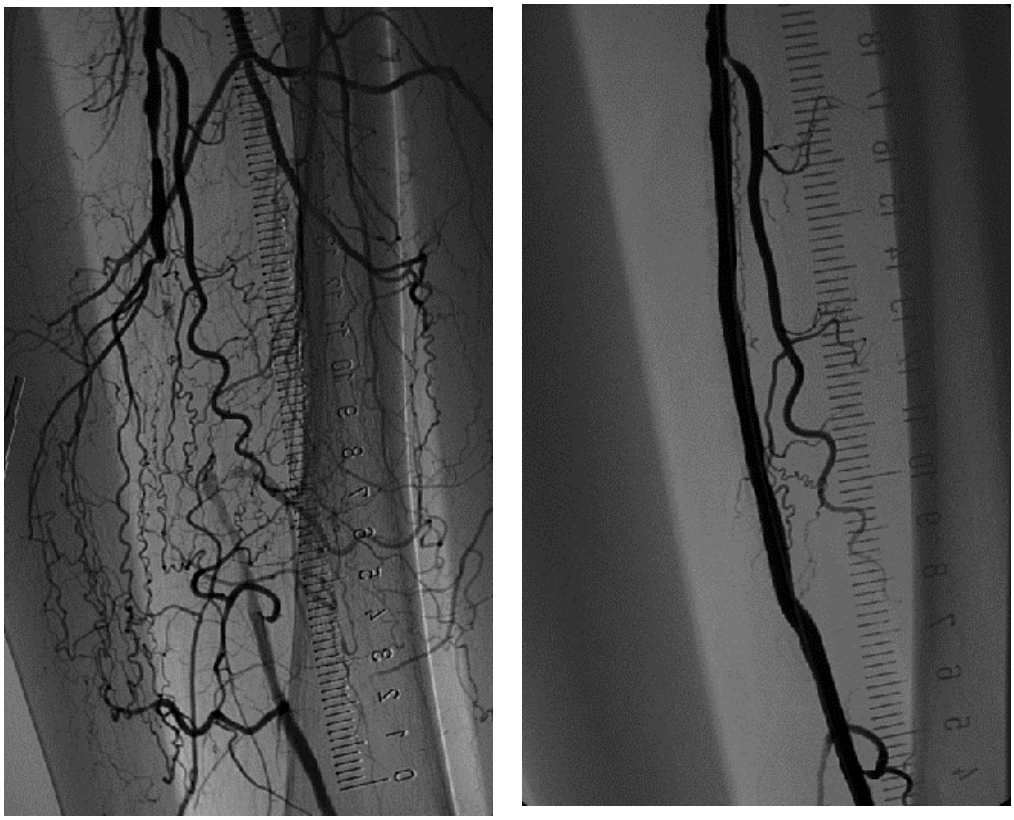

Fig. 7. *Patient with arterial occlusive disease in the superficial femoral artery. Percutaneous transluminal angioplasty with placement of a covered stent (Viabahn) was performed.*

vascular surgery patient are therefore limited. The lack of efficacy of prior drugs in human trials of restenosis could implicate either an incomplete understanding of the pathophysiology behind the vascular response to injury, use of the wrong drug or drugs, or an incomplete understanding of pharmacokinetics and pharmacodynamics.

Restenosis can schematically be divided into four interrelated processes [based on Fuster 1995, Schwartz S 1995, Schwartz R 1997]: elastic recoil, inflammation and thrombus formation, neointimal hyperplasia, and negative vessel remodelling. These processes will be further discussed.

Clinical significance

Negative remodelling is the principal restenosis mechanism following balloon angioplasty [Andersen 1996]. Stenting reduces elastic recoil and negative remodelling but causes instead in-stent restenosis [Mach 2000]. Neointimal hyperplasia is considered to be the primary mechanism of restenosis after stenting [Mach 2000]. Approximately 30-50% of the patients undergoing PTCA will develop restenosis within the first year [Serruys 1988, Bauters 1999]. Stent restenosis rates are reported to be 15-20% in ideal coronary lesions, but may occur in over 30-60% of patients with complex lesions [Fattori 2003].

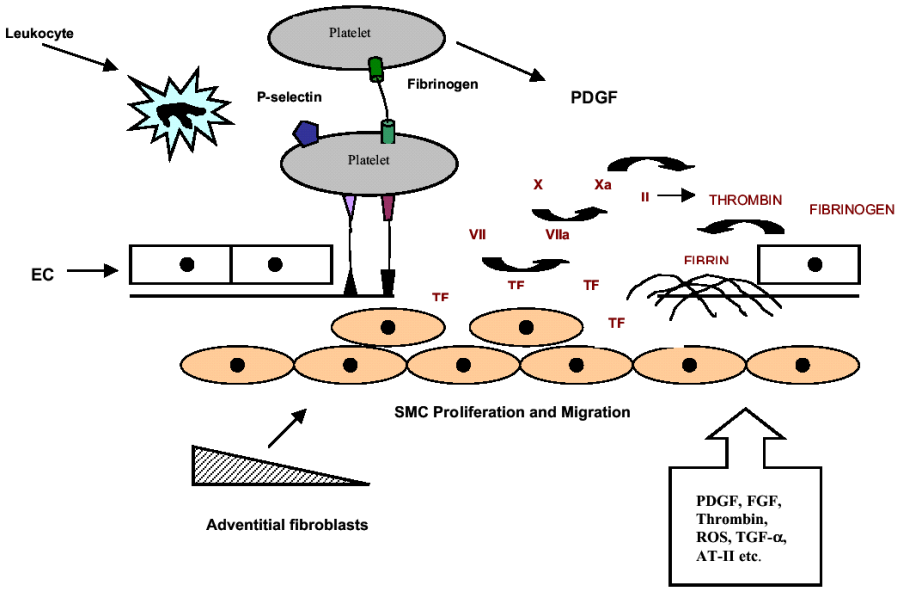

Fig. 8. *Multiple, complex cellular and hormonal reactions occur after vascular injury. The coagulation system, inflammatory reactions, and oxidative stress are all involved in the vascular response to injury. Subendothelial matrix and smooth muscle cells are exposed after injury to the endothelial cells. Platelets are activated, degranulate and release vasoactive mediators and growth factors. Coagulation is initiated with deposition of fibrin. Leukocytes infiltrate and release inflammatory mediators. Vascular smooth muscle cells proliferate and migrate to the neointima where extracellular matrix is produced. Also, adventitial fibroblasts are activated, dedifferentiate, and migrate into the neointima*

The restenosis rate at 6-12 months after angioplasty in the iliac arteries is around 20 % [Schillinger 2002] and in the femeropopliteal arteries 30-50 % [Tschopl 1997, Kovacevic 2004]. Vein graft failure after lower extremity bypass averages 20% to 30% in long-term follow-up [Taylor 1990], and the problem of neointimal hyperplasia is even worse with prosthetic grafts. After carotid endarterectomy , about 20% of patients will develop a hemodynamically significant lesion [Healy 1989]. However, few of these patients become symptomatic.

Inflammation

Inflammation plays, as previously described, an essential role in the development of atheroclerosis. There is also now support for a central role of inflammation in the biological repair response to vascular injury. Mechanical injury denudes the endothelial lining leading to platelet deposition that is rapidly followed by leukocyte recruitment and infiltration at the site of injury (Fig. 9). Leukocytes attach loosely and roll on platelets in an interaction mediated by P-selectin glycoprotein-1 (PSGL-1) and platelet P-selectin [Diacovo 1996, McEver 1997]. The activated leukocyte promotes adhesion, transplatelet migration, and vessel wall invasion (diapedesis). This process depends on leukocyte integrin Mac-1 [Diacovo 1996], and platelet receptors, including GP Ibα [Simon 2000] and ICAM-2 [Diacovo 1994]. The recruitment of leukocytes across the platelet-fibrin layer and into the tissue is also driven by chemoattractant cytokines, chemokines, released from SMCs, leukocytes and endothelial cells. Monocyte chemoattractant protein (MCP-1) participates in the recruitment of monocytes [Rollins 1996]. Another example of a chemokine recruiting neutrophils to the site of injury is IL-8 [Webb 1993]. Leukocytes release a wide range of potent vasoactive substances, such as reactive oxygen species (ROS), proteolytic enzymes, growth factors, cytokines (IL-1, IL-6, and TNF-α), and chemokines, all of which could further perpetuate injury [Wainwright 2001]. Monocytes specifically produce a number of cytokines, including PDGF, IL-1, IL-6, bFGF, TNF-α, and TGF-β [Epstein 1994]. In stented arteries, the inflammatory response is prolonged and rich in monocytes/macrophages compared to balloon injury alone [Horvath 2002]. Also levels of neutrophils are higher in patients undergoing stent implantation compared with patients undergoing only balloon injury [Inoue 2000]. Neutrophils are not known to produce growth factors, however they can secrete cytokines such as IL-1, IL-6, and TNF-α, which can induce growth factor production [Lloyd 1992]. This increased inflammatory response may help to explain the larger neointimal growth in stented arteries [Welt 2002].

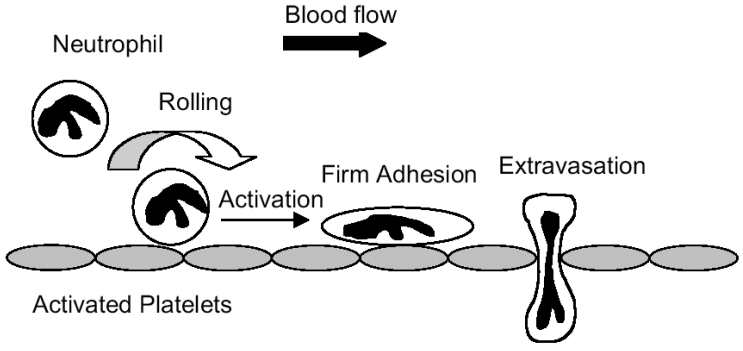

Fig. 9. *Leukocyte-platelet interactions at the site of vessel wall injury. Leukocytes roll on the activated platelets through an interaction between P-selectin glycoprotein-1 (PSGL-1) and platelet P-selectin. Firm adhesion and leukocyte extravasation are mediated by integrins and adhesion molecules.*

Arterial injury results in upregulation of cell adhesion molecules. There are three major classes of cell adhesion molecules: selectins (P-selectin, E-selectin, and L-selectin), integrins, and the immunoglobulin superfamily (e.g. ICAM-1 and VCAM-1) [Menger 1996]. These cell adhesion molecules have all distinct roles in inflammatory cell recruitment to the damaged vessel wall [Davis 2003]. P-selectin is expressed in the α-granules of platelets [Stenberg 1985] and the Weibel-Palade bodies of endothelial cells [Bonfanti 1989], with a soluble form in plasma. Upon platelet stimulation, P-selectin is expressed on the platelet surface where it is rapidly shed. This shedding from platelets could be the main source of the soluble form found in plasma following thrombotic events [Michelson 1996] and may have its own physiological activity. PSGL-1 is the primary ligand for P-selectin and is expressed on most leukocytes. P-selectin plays critical roles in the interaction between platelets, endothelial cells, and leukocytes, resulting in leukocyte recruitment to the injured site [André 2004]. Inhibition of P-selectin or PSGL-1 with a monoclonal antibody, at the time of arterial injury in a mouse model, limited neointima formation [Philips 2003].

Adventitial inflammation could play an important role in the development of neointimal hyperplasia and negative remodelling. A recent study showed that E-selectin controls adventitial inflammation through leukocyte adhesion and contributes to the process of intimal hyperplasia in the late stage after balloon injury [Gotoh 2004]. A monoclonal antibody against E-selectin attenuated intimal hyperplasia after balloon injury, with significantly reduced infiltration of leukocytes in the adventitia.

Thrombus formation

Platelets adhere to the injured vessel wall via platelet glycoprotein Ib binding with von Willebrand factor in the subendothelial matrix. The deposition of platelets results in the release of PDGF, TGF-β, and thromboxane A_2 [Chandrasekar 2000]. PDGF is the most important growth factor released by activated platelets. The primary effect of PDGF on VSMCs could be the induction of migration, as PDGF is a strong chemoattractant for VSMCs [Bornfeldt 1994]. Platelets, when activated, can enhance thrombin generation 5-6 times. Thrombin stimulates proliferation of SMCs both directly and indirectly by inducing platelet release of PDGF [McNamara 1996].

Vascular injury results in the exposure of tissue factor to the blood and initiation of the coagulation cascade resulting in thrombin production with formation of a fibrin rich thrombus. There is increasing support of a major role for TF in thrombosis and development of neointimal hyperplasia after arterial injury [Taubman 1999]. Inhibition of TF activity has been shown to reduce thrombosis and neointimal hyperplasia in several animal models [Hasenstab 2000, Huynh 2001]. Circulating TF, in the form of cell derived microparticles, has been shown to incorporate into arterial thrombi [Giesen 1999]. This incorporation appears to be mediated by the binding of PSGL-1 on the microparticle to P-selectin on the surface of the activated platelet [Mackman 2004].

Thrombin is generated in large amounts at the site of injury and is amplified by formation of the prothrombinase complex both in humans and animals [Marmur 1994, Barry 1996]. It is known that arterial wall associated thrombin activity remains elevated for at least 48 hours after injury and returns to baseline after one week. Also PAR1 is upregulated in the proliferating neointima [Major 2003]. The effects of thrombin on vascular lesion formation seem to be mediated primarily via direct effects of thrombin on vascular cells. It is a SMC mitogen and several animal studies have examined the effect of thrombin inhibition on neointimal formation after vascular mechanical injury [Sarembock 1991, Gerdes 1996, Gallo 1998]. Thrombin promotes leukocyte transmigration by upregulating endothelial cell adhesion molecules through activation of NF-κβ [Strukova 2001]. Thrombin also potently stimulates IL-6 and MCP-1 production from SMCs [Kranzhofer 1996].

Neointimal hyperplasia

Intimal hyperplasia strictly means an increase in the number of cells in the intima. A more correct term would be intimal thickening, since the intimal cells are accompanied by an increase in the amount of extracellular matrix (ECM) [Newby 2000]. The term neointima is used to describe the intima that forms in response to vessel wall injury [Schwartz 1995]. The term is also used for intimal hyperplasia in general. The SMC response after balloon injury in the rat carotid artery can be divided into three phases [Schwartz 1995]. The first phase is the burst of medial SMC proliferation (0-3 days). The second phase is the migration of SMCs from the media into the intima (3-14 days). The third phase is the proliferation of SMCs within the neointima and deposition of large amounts of extracellular matrix, which contributes to the rapid growth of the lesion (7 days-1 month).

The first consequence of SMC activation is proliferation, which means hyperplasia with an increase in cell number. The SMCs undergo a phenotypic modulation from a contractile to a synthetic phenotype (dedifferentiation) [Berk 2001]. The percentage of dividing SMCs increases in the rat from a basal level of 0.06% per day to 10-30% per day [Allaire 1997]. The cellular mechanisms that regulate SMC proliferation are not fully understood but involve complex regulation of entry into the cell cycle at multiple levels [Newby 2000, Davis 2003]. At the time of SMC injury, bFGF is released and stimulates the initial phase of SMC proliferation. Antibodies against bFGF, given at the time of vascular injury, inhibit SMC proliferation by 80% [Lindner 1991]. Growth factors, including PDGF, IGF, thrombin, FGF, VEGF, and TGF-β, and cytokines, such as IL-1 and IL-6, are released from platelets, leukocytes, and SMCs, which influence the proliferation and migration of SMCs from the media into the neointima [Berk 2001]. Mechanical forces, such as stretch and wall tension, and chemicals, such as ROS, may also have important roles in regulating SMC growth. PDGF appears to be the critical factor responsible for SMC migration [Bendeck 1994]. Besides expression of chemotactic factors, activation of the plasminogen activator system and activation of matrix metalloproteinases (MMPs) seem to be essential for migration. MMPs are thought to be important for resorption of ECM to facilitate SMC migration. VSMC proliferation ceases once the site of injury is re-endothelialised and the normal antiproliferative actions of NO and heparin exert their influence on these activated SMCs [Clowes 1983]. An autopsy study of the tissue response to coronary stent implantation suggested that re-endothelialisation may take at least 3 months in humans [Grewe 2000].

ECM, composed of collagen, elastin, and proteoglycans, makes up a majority of the neointimal volume. ECM is mainly produced by SMC and fibroblasts, and primarily regulated by TGF-α and PDGF [Madri 1991]. Adventitial fibroblasts may have an important role after vascular injury [Gutterman 1999]. Adventitial proliferation occurred within 3 days in a pig balloon injury model with continued migration and proliferation in the neointima [Wilcox 1997]. Adventitial fibroblasts may be an important source of autocrine and paracrine factors, like TGF-β [Shi 1996] and ROS [Shi 2001]. The fibroblasts produce NADPH oxidase-derived ROS that appear to be involved in fibroblast proliferation, connective tissue deposition, and perhaps vascular tone [Rey 2002].

Remodelling

Vascular remodelling is a physiologic response to alterations in flow, pressure, injury, and atherosclerosis [Ward 2000]. Glagov *et al* described that human coronary arteries often enlarge in response to plaque formation as a compensatory response that limits narrowing of the vessel lumen [Glagov 1987]. Positive remodelling (outward remodelling) denotes an increase in vessel size and negative remodelling (inward remodelling) denotes a reduction in vessel size. Restenosis, with the exception of in-stent restenosis, after angioplasty is determined primarily by negative remodelling rather than by intimal hyperplasia [Ward 2000]. The precise mechanisms responsible for remodelling after arterial injury are unknown. Endothelial dys-

function due to inactivation of NO by oxidative stress after vascular injury [Janiszewski 1998] and low blood flow [Krams 1998] seem to be important. Negative remodelling occurs predominantly between 1 and 6 months after angioplasty, thus distinguishing it from elastic recoil [Kimura 1997]. Elastic recoil usually occurs within the first 24 to 48 hours [Casterella 1999]. Stenting reduces both elastic recoil and negative remodelling.

Oxidative stress

Aerobic metabolism generates ROS, required for normal cell function in physiological concentration, against which protective antioxidants have evolved. Oxidative stress is defined as an imbalance between production of ROS and the antioxidant defenses leading to tissue injury [Halliwell 1994]. Oxidative stress is associated with cardiovascular disease [Griendling 2003]. The two terms ROS and free radicals, which mean free low molecular weight molecules with an unpaired electron, are commonly used as equivalents. There are many ROS that play central roles in vascular physiology and pathophysiology. Several cytokines, growth factors, and hormones use ROS as secondary messengers in the intracellular signal transduction [Thannickal 2000]. Many functions of the endothelium and the VSMCs are affected by ROS [Taniyama 2003]. Higher amounts of ROS can cause damage to various biomolecules, including DNA, lipids, and proteins, significant toxicity, or even apoptosis [Marnett 2000, Stadtman 2000]. The major ROS are superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), nitric oxide (NO), and peroxynitrite ($ONOO^-$) [Nordberg 2001, Griendling 2003]. A stepwise 1-electron reduction of oxygen produces the ROS molecules (Fig. 10). Several enzyme systems seem to be important in this process, including NADPH oxidase, xanthine oxidase, NO synthase, and cytochrome P450 monooxygenase [Harrison 2003]. ROS are generated intracellularly, extracellularly, or in specific intracellular compartments. Virtually all types of vascular cells produce $O_2^{\bullet -}$ and H_2O_2 [Griendling 2003]. Macrophages are perhaps the major vascular source of $O_2^{\bullet -}$ in disease states. NO is normally produced by endothelial NO synthase (eNOS) in arterial, venous or capillary endothelial cells, but in inflammatory states, inducible NOS (iNOS) can be expressed in macrophages, monocytes, and SMCs [Vural 2001]. $ONOO^-$ is an important mediator of lipid peroxidation and protein nitration, including oxidation of LDL.

There are accumulating evidence indicating that oxidative stress in the vessel wall is involved in atherosclerosis [Harrison 2003, Leite 2004]. The common risk factors for atherosclerosis, including hypercholesterolemia, hypertension, and smoking, increase production of ROS by endothelial cells, SMCs, and adventitial cells [Cai 2000]. ROS have been shown to initiate several processes involved in atherogenesis, such as expression of adhesion molecules, SMC proliferation and migration, apoptosis in the endothelium, oxidation of lipids, activation of MMPs, and altered vasomotor activity [Harrison 2003].

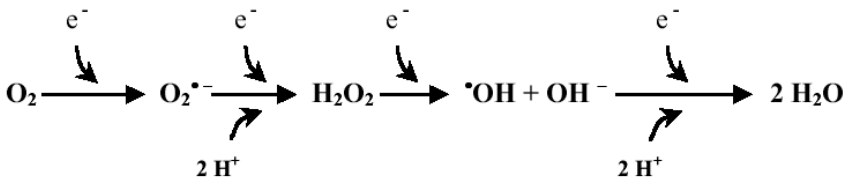

Fig. 10. ***n****ROS production. A step-wise reduction of molecular oxygen via 1-electron transfers.*

Angiotensin II, TNF-α, thrombin, and PDGF all increase oxidase activity and raise intracellular levels of O_2^- and H_2O_2 in VSMCs [Griendling 2003]. There are several intracellular signalling targets of ROS, including mitogen-activated protein kinase (MAPK), p38 MAPK, and NF-κβ [Irani 2000]. MAPK is activated by exogenous H_2O_2 and by endogenously generated ROS in SMCs stimulated with growth factors [Sundaresan 1995]. The signalling proteins may not be direct targets of ROS. It is very likely that one or more intermediary proteins are involved, e.g. tyrosine phosphatases [Irani 2000].

Oxidative stress and restenosis

There is increasing evidence suggesting that oxidative stress and inflammation in the vessel wall plays an important role in the development of restenosis after angioplasty [Azevedo 2000, Leite 2004]. Vascular injury after balloon dilatation rapidly increases the local concentration of ROS [Nunes 1997]. $O_2^{\cdot -}$ production is especially increased in medial and neointimal SMCs and adventitial fibroblasts after balloon injury [Azevedo 2000]. After PTA in patients with PAD, there is an increase of oxidative stress, as measured by peroxide levels, within 48 hours [Roller 2001]. Increased oxidative stress within the vessel wall may result from direct damage of the vessel, leading to activation of NF-κβ, as well as genes controlling cellular growth [Edelman 1998, Braun-Dullaeus 1998]. NF-κβ is a ROS-sensitive transcription factor and has a central role, as previously mentioned, in the expression of proinflammatory genes, including MCP-1 and IL-6 [Li 2002]. ROS can induce endothelial dysfunction and macrophage activation, resulting in the release of cytokines and growth factors that stimulate matrix remodelling and SMC proliferation [Taniyama 2003].

In animal models treatment with a variety of antioxidants have reduced neointimal proliferation and promoted vessel remodelling [Ferns 1992, Freyschuss 1993]. The oxidases responsible for ROS production after balloon injury have not been fully characterised. A specific peptide inhibitor for NADPH oxidases has been reported to inhibit restenosis, suggesting a mechanistic role for these enzymes in restenosis [Jacobson 2003]. In clinical trials the antioxidant and lipid-lowering drug probucol has shown promising results in preventing restenosis after coronary artery balloon angioplasty [Tardif 1997].

Thioredoxin

There are several cellular antioxidant enzyme systems, including superoxide dismutases, catalases, glutathione peroxidases and thioredoxin (Trx), serving to protect cells and organisms from the lethal effects of excessive ROS formation. Human Trx is a 12 kDa protein catalysing redox (reduction/oxidation) reactions. Trx contains a conserved active site (-Cys-Gly-Pro-Cys-), essential for the function as a general and potent protein disulfide oxidoreductase. A protein disulfide reduction is catalysed by Trx in combination with Trx reductase and NADPH [Holmgren 1985] (Fig. 11). Trx exerts its effects by this mechanism in numerous different cellular

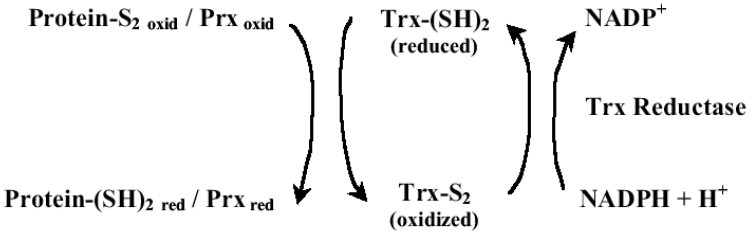

Fig. 11. *The Trx system (Trx, Trx reductase, and NADPH). TRX reductase reduces the active site disulfide in Trx directly under consumption of NADPH. Reduced Trx is efficient in reducing disulfides in proteins, including peroxiredoxins (Prx). Prx catalyzes the reduction of hydrogen peroxide (H_2O_2).*

systems. Trx can limit oxidative stress directly via antioxidant effects, mainly through Trx peroxidase, and indirectly by protein-protein interaction with key signalling molecules [Yamawaki 2003] (Fig. 12). Intracellular Trx is a general antioxidant and activates several transcription factors like NF-κβ and AP-1 [Arnér 2000]. In the extracellular milieu Trx is a co-cytokine and chemokine for monocytes, lymphocytes, and neutrophils [Bertini 1999]. The half-life of Trx in blood is approximately 1 hour [Nakamura 2001]. Human Trx is released by or secreted by lymphocytes and other kinds of cells, e.g. hepatocytes, fibroblasts and endothelial cells, through a leaderless pathway [Rubartelli 1992, Nakamura 1997]. Red blood cells, leukocytes, and platelets contain Trx which they may release in response to oxidative stress. Plasma levels of Trx are changed in both neoplastic diseases as well as in inflammatory diseases [Nakamura 1997]. Increased plasma levels of Trx are a sensitive indicator of oxidative stress [Sumida 2000 and 2001]. A protective effect of Trx against ROS-induced cellular damage has been demonstrated in vivo [Fukuse 1995]. Also circulating Trx can block neutrophil extravasation into inflammatory sites [Nakamura 2001].

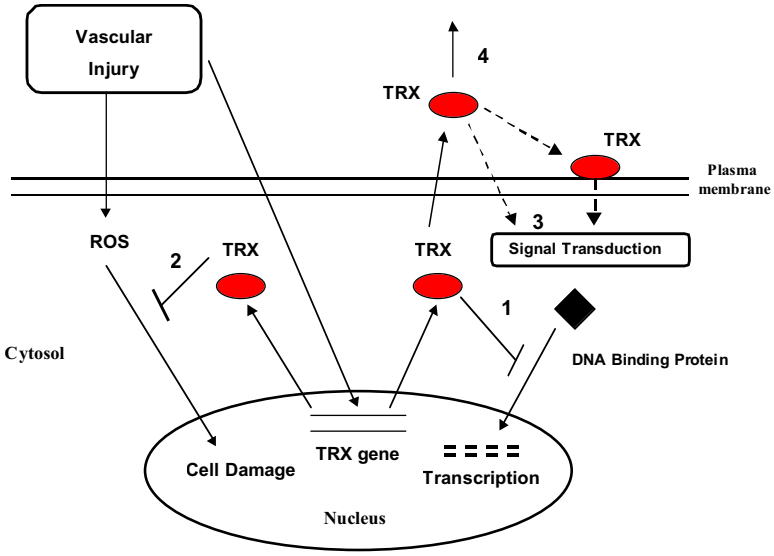

Fig. 12. *Vascular injury induces immediate oxidative stress in the arterial wall with oxidative damage to DNA. Thioredoxin (Trx) has both intracellular and extracellular activities:*

1. *Regulation of protein-nucleotide interactions. Trx promotes DNA binding of transcription factors such as activator protein-1 and nuclear factor-*κβ
2. *Antioxidant. Cytoprotection against reactive oxygen species (ROS).*
3. *Signal transduction. Trx negatively regulates activation of p38 mitogen-activated protein (MAP) kinase and apoptosis signal-regulating kinase-1 (ASK-1).*
4. *Chemoattractant and cytokine-like effects. Trx can augment the expression of several cytokines and is considered to be extracellularly secreted.*

(Modified from Nakamura H et al. Redox regulation of cellular activation. Annu. Rev. Immunol. 1997; 15:351-69.)

In vitro data from the same study show that Trx inhibits activation of p38 MAPK in neutrophils but not in lymphocytes.

Glutaredoxin (Grx) was originally discovered as the second hydrogen donor for ribonucleotide reductase in *E. coli* lacking Trx [Holmgren 1976]. Grx has, as Trx, a molecular weight around 12 kDa, but catalyses protein disulfide reductions coupled with glutathione, glutathione reductase, and NADPH. Grx is distinguished from Trx by its ability to be reduced by glutathione (GSH), association with apoptosis, and to control DNA binding of transcription factors [Holmgren 1985, Hirota 2000, Chrestensen 2000].

Several studies support the idea that Trx could play a major role in the development of atherosclerosis and in the pathology of reperfusion injury. In human aortic SMC, adenoviral gene transfer of Trx enhanced Trx enzyme activity and significantly increased DNA synthesis, suggesting a role for Trx in SMC proliferation [Schulze 2002]. In addition, Trx is upregulated in atherosclerotic plaques [Takagi 1998]. Secretion of Trx is stimulated by a wide variety of stimuli, all having in common that they induce an oxidative burst in the cell. Oxygen and H_2O_2 induce secretion of Trx from cells, including endothelial cells [Nakamura 1994, Higashikubo 1999]. ROS play a major role in the pathology of ischemia-reperfusion injury and studies have found that Trx could have a protective role in this scenario. Trx has been shown to lower levels of ROS. Intravenous administration of human recombinant Trx has been shown to attenuate ischemia-reperfusion injury in animal models [Yagi 1994, Wada 1995, Okubo 1997].

The role of Trx in restenosis is not fully established. In balloon injured rat carotid arteries Trx immunoreactivity begins to increase from two weeks after injury and is upregulated until six weeks in the neointimal endothelium [Takagi 1998]. The sources of Trx and Grx after vascular injury are not clearly defined. Human Trx was first detected in platelets which is a rich source of Trx [Blombäck 1974]. Trx is enhanced in endothelial cells and macrophages in atherosclerotic plaques from human carotid arteries [Takagi 1998]. In human coronary artery specimens, Grx and Trx are expressed in endothelial cells, fibroblasts in the adventitia, and most intensely in medial VSMCs [Okuda 2001].

AIMS OF THE STUDY

The principal objective of this thesis was to investigate mechanisms of thrombosis and restenosis after vascular injury.

The specific aims were:

1. To evaluate the long-term outcome after infrainguinal PTA.

2. To determine the effects of a specific thrombin inhibitor on neointimal hyperplasia after balloon injury in a rat carotid artery injury model and to investigate if a prolonged inhibition of thrombin prevents neointimal hyperplasia.

3. To investigate the response of inflammation and blood coagulation, and its significance for thrombosis and restenosis, in patients with peripheral arterial disease undergoing PTA.

4. To evaluate the response of thioredoxin and glutaredoxin in patients with peripheral arterial disease undergoing PTA and to correlate the plasma levels of these redox-active proteins with the occurrence of restenosis.

MATERIAL AND METHODS

Study design

Study I

The records of 77 patients (45 men, mean age 70 years, range 41-85) undergoing infrainguinal PTA between 1991 and 1994 at the department of surgery, Karolinska University Hospital, were evaluated retrospectively. All patients had been referred to the vascular clinic and examined by a vascular surgeon at their initial presentation. Low molecular weight heparin (Enoxaprin 20 mg subcutaneously) was given 6-8 hours and 18-20 hours after the procedure. In general, a daily dose of 75 mg acetylsalicylic acid was started the day after the procedure. Patients stayed overnight in the ward. After discharge, they were followed with periodic evaluation by physical examination and measurement of the ankle-brachial index (ABI). Patients were routinely scheduled for a control to a vascular surgeon 1 month after PTA; subsequent controls were scheduled according to the clinical situation. Patients were followed until a major amputation or their death.

The records were reviewed to retrieve patient data such as demographics and risk factors, clinical manifestation, lesion characteristics, and the long-term result. Reports regarding cause of death were obtained through the National Board of Health and Welfare. Patients undergoing PTA of grafts were excluded from the analysis.

In 2003, surviving patients (34 patients) were called for a follow-up examination (mean follow-up 9.3 years, range 8.2-12.0). Data collection at this visit included history, completion of a walking impairment questionnaire (WIQ), physical examination of a vascular surgeon, ABI measurement, and a duplex ultrasound scan performed by a clinical physiologist.

Study II

Forty-seven male Sprague-Dawley rats were divided in five groups (Fig. 1). Four groups received different doses of the thrombin inhibitor inogatran (Astra Hässle, Mölndal, Sweden), starting at the time of injury. An additional group was injured without treatment and served as a control group (n=8). Inogatran has a molecular weight of 439 Dalton and in vitro studies have shown that inogatran is a classical competitive inhibitor at the active site of thrombin[Teger-Nilsson 1997]. Animal studies have shown a short half-life of inogatran after intravenous administration, due to a small volume of distribution and a relatively high clearance. Doses of inogatran were based on previous in vitro studies and antithrombotic animal studies [Eriksson 1998, Uriuda 1996].

Table 1. Overview of study I-IV.

Study	Recruitment period	Study design	Numbers, *n*	Men/women
I	1991-1994	Retrospective study	77	45/32
II	1998-2000	Experimental study	47	47/0
III	Nov 2002-Jan 2004	Prospective clinical study	44	26/18
IV	Nov 2002-May 2003	Prospective observational study	29	16/13

A low dose injection (1 mg/kg) (n=6) and a high dose injection (5 mg/kg) (n=6) were given at 0, 1 and 2 hours after the injury. A short-term continuos intravenous infusion (5mg/kg/h) (n=7) was given for three hours following injury. Finally, the last group (n=20) was given an intravenous bolus (0.5mg/kg) after injury followed by a continuos intravenous infusion (2 mg/kg/h) maintained for one week through an implantable osmotic infusion pump (Alzet 2ML1, Alza Corporation, Palo Alto, CA, USA). The animals were sacrificed after two weeks. After tissue preparation, the extent of carotid neointima formation was calculated.

Study III

Thirty-four patients (20 men, mean age 69 years, range: 54-81) admitted to the department of vascular surgery at Karolinska University Hospital, between November 2002 and January 2004, for elective PTA were enrolled in the study. Ten patients (6 men, mean age 72 years, range 55-84) undergoing a diagnostic angiography were included in the study as controls. Patients undergoing angioplasty of bypass grafts, redo procedures, or primary local thrombolysis were excluded from the study. Patients with wounds were also excluded. Venous blood was drawn from an antecubital vein before, 1, 4, and 24 hours, 1 week, and 1 month after PTA into tubes containing 0.5 ml of 0.129 M trisodium citrate , EDTA or nothing for serum samples (Becton Dickinson Vacutainer Systems, Myealn Cedex, France). In the control group, blood samples were drawn before and 1 and 4 hours after PTA. Plasma levels of prothrombin fragment 1+2 (F 1+2), D-dimer, soluble P-selectin, and fibrinogen were measured at the different time points, as well as serum levels of CRP, measured with a highly sensitive assay. Soluble tissue factor (TF) was analysed in a pilot study, including ten patients. Antithrombin, Factor V Leiden, homocysteine, and anticardiolipin antibodies were measured before PTA. Clinical examination and ABI were performed before and after the procedure as well as after one month. Patients were followed up with angiography after 6 months to evaluate restenosis.

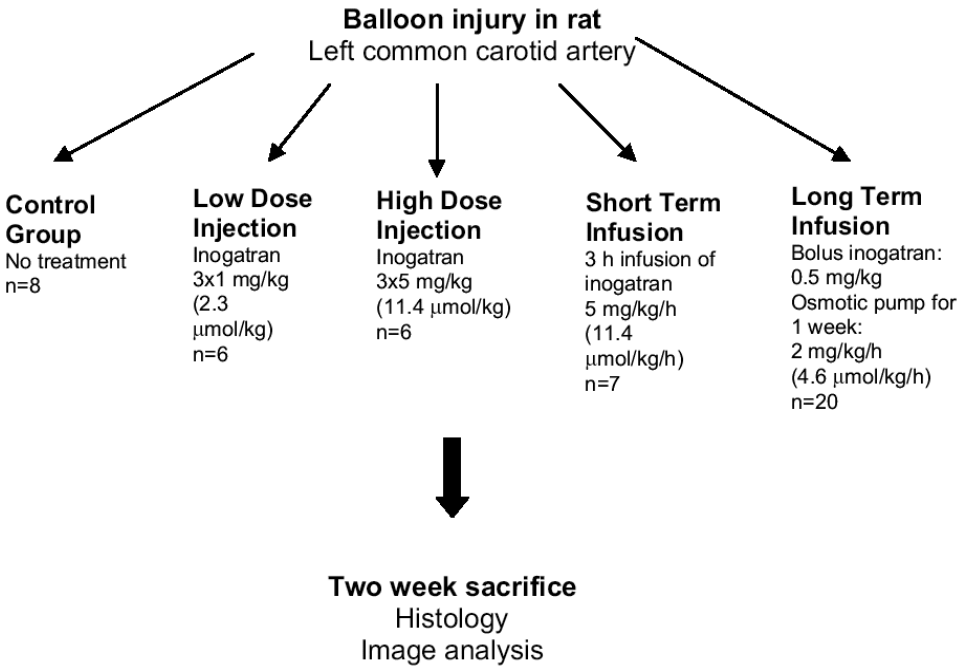

Fig. 1. *Study design in* ***study II.***

Study IV

Nineteen patients (10 men, mean age 68 years, range: 54-81) admitted to the department of vascular surgery at Karolinska University Hospital, between November 2002 and May 2003, for elective PTA were enrolled in the study. Ten patients (6 men, mean age 72 years, range 55-84) undergoing diagnostic angiography were included in the study as controls. Patients undergoing angioplasty of bypass grafts, redo procedures, or primary local thrombolysis were excluded from the study. Venous blood was drawn from an antecubital vein before and 1, 4, and 24 hours, and 1 week after PTA into tubes containing 0.5 ml of 0.129 M citrate (Becton Dickinson Vacutainer Systems, Myealn Cedex, France). In the control group, blood samples were drawn before and 1 and 4 hours after PTA. Plasma levels of thioredoxin (Trx) and glutaredoxin (Grx) were measured at the different time points. Clinical examination and ABI were performed before and after angioplasty. Patients were followed up with angiography after 6 months to evaluate restenosis.

Percutaneous transluminal angioplasty

(Study I, III, and IV)

PTA was performed in an angiography suite by an interventional radiologist or a vascular surgeon. An ipsilateral antegrade or a contralateral retrograde percutaneous puncture of the femoral artery was performed. Suitable lesions were treated with balloon angioplasty. Only stenoses above 50 % and/or a hemodynamically significant pressure gradient were accepted for angioplasty. The pressure gradient across the stenosis was measured in the iliac arteries. A peak systolic resting pressure gradient > 10 mmHg was considered hemodynamically significant. The diameter of the artery was measured digitally after calibration. The balloon diameter was selected to be equal or 1 mm more than the non-stenosed artery. Patients received 5000 U of heparin intraarterially before angioplasty. A balloon diameter ranging from 5 to 9 mm was used. Inflation pressure was 6-14 atmosphere during approximately 30 seconds. Technical success was defined as PTA resulting in less than 30 % residual stenosis after dilatation. Hemodynamic improvement was defined as an increase in ankle/brachial index > 0.10 [Pentecost 2003]. Automated vessel analysis was performed on Philips Integris V software to assess arterial lumen diameter reduction.

Stent placement was done for residual stenosis due to elastic recoil or residual gradients exceeding 10 mm Hg after PTA. A highly eccentric stenosis or dissection as a result of PTA was also an indication for stent placement. In *study I,* stents were not in general used during the period in which patients were treated.

Low molecular weight heparin (Enoxaprin 20 mg subcutaneously) was given 6-8 hours and 18-20 hours after PTA in *study I*. In *study III* and *IV,* no low molecular weight heparin was given after the procedure.

Table 2. Baseline patient characteristics in study I, III, and IV.

Study	No. of patients	Mean age	Indication: Intermittent claudication	Critical ischemia	Location of PTA, %: Iliac arteries	SFA	Popliteal	Stents
I	77	70 (range: 41-85)	51 (66%)	26 (34%)	0	70 (70%)	20 (20%)	1
III	34	69 (range: 54-81)	31 (91%)	3 (9%)	29 (85%)	5 (5%)	0	28
IV	19	68 (range: 54-81)	18 (95%)	1 (5%)	17 (89%)	2 (11%)	0	17

SFA= Superficial femoral artery

Rat carotid balloon injury model

(Study II)

The rats were anaesthetized with an intramuscular injection of fentanyl/fluanisone (Hypnorm®, Janssen Pharmaceutica, Beerse, Belgium) before surgery. The dose was 0.1ml/100g body weight. Balloon injury of the left common carotid artery in the rats was performed as previously described [Clowes 1983]. Briefly, the left common carotid artery and the left external carotid artery were exposed. A 2F Fogarty balloon embolectomy catheter (Baxter Healthcare, Santa Ana, CA, USA) was introduced into the external carotid artery and advanced to the aortic arch. The balloon was inflated and then pulled back to the carotid bifurcation. This procedure was repeated three times. The catheter was then removed and the external carotid was ligated. An osmotic pump, with an attached polyethylene catheter, was implanted subcutaneously between the scapulae in the rats receiving continuos intravenous infusion of inogatran for one week. The catheter was then subcutaneously brought to the front of the neck and inserted into the left jugular vein.

Definitions

Intermittent claudication and critical ischemia were defined according to the criteria proposed by the Society for Vascular Surgery and International Society for Cardiovascular Surgery (SVS/ISCVS) [Rutherford 1997]. Diabetes mellitus and hypertension were defined according to the criteria from the World Health Organization (WHO) and the International Society of Hypertension (ISH) [Alberti 1998, Whitworth 2003]. Cardiac disease was defined as active class II to IV angina pectoris according to the Canadian Cardiovascular Society (CCS) classification, history of myocardial infarction, or congestive heart failure class II to IV according to the New York Heart Association (NYHA) classification. Renal insufficiency was defined as a serum creatinine level of 120 μmol/L or higher.

Primary patency was considered if the treated vessel had uninterrupted patency with no procedure performed on it. Secondary patency was considered if patency was restored after occlusion by PTA, surgery or thrombolysis. The extent of atherosclerotic disease in the superficial femoral artery (SFA) was divided into a localised disease with only one occlusion/stenosis localised to the arterial segment or a generalised disease with two or more occlusions/stenoses per arterial segment. Restenosis was defined as ≥ 50 % lumen diameter reduction at the site of PTA (*Study III)* or as more than 20 % further lumen diameter reduction at the site of PTA (lumen diameter reduction at 6 months minus lumen diameter reduction post-PTA) (*Study IV).*

Quality of life assessment

(study I)

The walking impairment questionnaire (WIQ), briefly, is a validated questionnaire used to measure the walking ability of patients with peripheral arterial disease [Regensteiner 1990, Hiatt 1995]. The questionnaire quantifies the patient's walking capability in terms of defined distance, speed, and stair climbing. It also rates the severity of claudication pain during usual walking activities. The questions are expressed on a scale of 0% (unable to perform because of severe claudication) to 100% (no impairment). A question was added regarding the effect of walking impairment on the patient's quality of life, where 0 % represented great effect on quality of life and 100 % was no effect

Laboratory methods

Blood samples

The blood samples were centrifuged within 30 minutes at 2000 rpm for 20 minutes. The plasma fraction was immediately frozen in aliquots and stored at -70 °C until analysis. The coagulation tests were all performed on citrate plasma, unless otherwise stated.

Laboratory Analyses

Study II

The plasma levels of inogatran were measured by Astra Hässle, Mölndal, Sweden.

Study III

CRP in serum and fibrinogen in plasma were determined immunochemically by a nephelometric assay, using N High Sensitivity CRP kits

(Dade Behring, Marburg, Germany)(detection limit 0.02 mg/L) and N antiserum to Human Fibrinogen in combination with N supplementary Reagent/Precipitation (Dade Behring). Plasma concentrations of F 1+2 were determined using the Enzygnost F1+2 micro enzyme immunoassay (Dade Behring). Human Soluble P-Selectin ELISA Kit (R&D Systems, Abingdon, UK) was used for the quantitation of soluble P-Selectin in plasma. The amount of soluble tissue factor in plasma was determined with Imubind Tissue Factor ELISA Kit (American Diagnostica Inc., Stanford, CT, USA). D-dimer, Activated Partial Thromboplastin Time (APTT), International Normalized Ratio (INR) and antithrombin were analysed by the Sysmex CA 1500 instrument. D-dimer was analysed with the quantitative assay Tinaquant D-dimer (Roche Diagnostics GmbH, Mannheim, Germany). APTT was measured using the PTT Automate test (Diagnostica Stago, Asnieres, France). The prothrombin time was determined using the STA – SPA 50 kit (Diagnostica Stago) and expressed as INR. The antithrombin concentration in plasma was determined using the Berichrom Antithrombin III kit (Dade Behring, Marburg, Germany). Genomic DNA preparations were obtained from 0.2 mL of EDTA whole blood samples, using QIAamp DNA Blood Mini Kit columns (Qiagen Inc., Valencia, CA, USA). The Factor V Leiden genotype was determined using the Factor V Leiden Kit on the LightCycler Instrument (Roche Diagnostics GmbH, Mannheim, Germany), according to the manufactorer's instructions. The concentration of total L-homocysteine in EDTA plasma was determined using the IMx Homocysteine Fluorescence Polarization Immunoassay (Abbott, Wiesbaden, Germany). Serum anticardiolipin antibodies were studied with an ELISA procedure in accordance with the recommendations of the Standardisation Workshop [Harris 1994].

Study IV

Trx was measured in duplicated plasma samples as previously described [Pekkari 2000]. Hemolysis in plasma samples was negligible. Briefly a sandwich ELISA was conducted using a monoclonal anti-Trx antibody as the primary antibody, ensuring no unspecific binding to truncated Trx (Trx80) [Sahaf 1997]. Subsequently, Trx standards and plasma samples were added in duplicates and a secondary biotinylated polyclonal goat anti-Trx antibody was added. Finally, alkaline phosphatase-conjugated streptavidin was added and the reaction was developed by p-nitrophenyl phosphate. Absorbance was recorded at 405 nm by a microplate reader (Thermomax, Molecular Devices). Data were analysed using the accompanying SOFTmax version 2.31 software. Detection limit for the Trx sandwich ELISA was 0.5 ng/ml. Intra-assay and inter-assay coefficients of variation were 4.5% and 4.7%, respectively.

Plasma levels of Grx were measured in a sandwich ELISA with a primary affinity purified goat anti-Grx1 antibody [Lundberg 2004]. Hemolysis in plasma samples was negligible. The secondary antibody was the same as the primary but biotinylated. Finally alkaline phosphatase-conjugated streptavidin was added and the reaction was developed by p-nitrophenyl phosphate. Absorbance was recorded, as for Trx, at 405 nm by a microplate reader (Thermomax, Molecular Devices). Data were analysed using the accompanying SOFTmax version 2.31 software. Detection limit for the Grx sandwich ELISA was 0.2 ng/ml. Intra-assay and inter-assay coefficients of variation were 7.3%, respectively.

***Tissue Analysis** (Study II)*

The rats were euthanatized by an overdose of pentobarbital two weeks after balloon injury. At a perfusion pressure of 100 mmHg, the carotid arteries were flushed with a physiological saline solution followed by perfusion fixation with 4 % paraformaldehyde. The left and right common carotid arteries were excised and stored in paraformaldehyde. Three different sections of the vessels were selected and embedded in paraffin for sectioning. Cross sections were stained with hematoxylin and Masson's trichrome.

Image Analysis
(Study II)
The histological sections were analysed under a Nikon Eclipse E800 microscope, where each cross section was captured as a digital image with a camera. A single section in each slide, from three different levels of the vessel, showing the maximal degree of neointimal formation was chosen for analysis. Cross-sectional areas of lumen, neointima and media were measured by use of NIH Image software (National Institute of Health, Bethesda, MD, USA). Intimal and medial areas were defined by internal and external elastic laminae respectively. The extent of neointima formation was expressed as the neointima/media ratio. The percentage of luminal narrowing was also calculated (100 x neointimal area / [luminal area + neointimal area]).

Statistical methods

In *study I* survival analyses were constructed using the life table method. The statistical difference between patency and survival curves was determined with the Wilcoxon signed rank test. Fisher's pairwise least-significant-difference (PLSD) method or the Wilcoxon rank sum test was used as appropriate to compare differences between groups. In *study II* the statistical difference between treatment groups was determined by using analysis of variance (ANOVA). In *study III* and *IV* the statistical significance of differences between plasma levels at different time points was determined by using repeated measures ANOVA. Paired t-test with Bonferoni adjustment (*study III)* or Fisher's PLSD method (*Study II* and *IV*) was used for *post hoc* tests. Mann-Whitney U or Wilcoxon signed rank test was used as appropriate to compare differences between groups. Statistical significance was accepted if $p<0.05$. Values were expressed as mean ± standard deviation (*study I* and *II*) or as mean and ranges (*study III* and *IV*). StatView 5.0 (SAS institute Inc., Cary, NC, USA) was used for all statistical calculations.

Ethical considerations

The local ethics committee approved all the studies. All patients gave written informed consent to participate. Reports regarding cause of death were approved and obtained from the National Board of Health and Welfare.

RESULTS

Study I

Long term outcome after infrainguinal PTA

During the 3-year treatment period, 77 patients underwent infrainguinal PTA of 100 lesions in 81 limbs. Fifty-one (66%) patients presented with intermittent claudication and 26 (34%) had critical ischemia. PTA procedures were located mainly in arterial segments above the knee. Thirty-one limbs had localised SFA disease, and 32 limbs had generalised disease of the SFA. There were 27 occlusions with a mean length of 4.9 cm (range 0.5-20) and 73 stenoses with an average of 77% lumen diameter reduction (mean 2.8 cm length, range 0.5-15). Distal runoff was good (≥2 vessels) in 46 (57%) limbs and poor (0 or 1 patent vessel) in 35 (43%) limbs.

The overall immediate technical success was 93% in the 100 procedures; for stenoses, the technical success was 95% (69/73) and 89% (24/27) for occlusions. Local thrombolysis was performed in 6 patients. The mean postintervention runoff was 1.8. There were 18 (18%) dissections (not treated with stents at the time). However, 1 stent was placed at the end of the study period for suboptimal angioplasty. The ABI, which was 0.58±0.25 before intervention, rose to 0.79 ± 0.21 after intervention (p=0.0001). The 30-day mortality rate was 0 %. There were 3 occlusions and 2 reinterventions within 30 days. In total during the follow-up period, nine patients underwent a secondary endovascular procedure, nine underwent open surgery, and seven were amputated.

Cumulative primary and secondary patency rates, respectively, were 81% and 86% at 1 year, 65% and 73% at 5 years, and 12% and 17% at 10 years (Table 1). There were significant differences in both primary and secondary patency rates between patients with claudication and critical ischemia (p=0.02) (Table 2). There was also a significant difference in primary patency between patients with stenoses and occlusions (p=0.001), but no difference in terms of secondary patency (p=0.09). In the subgroup analysis of stenoses <2 cm versus ≥2 cm, differences in primary patency rates did not achieve statistical difference (p=0.50). Generalised atherosclerotic SFA disease did not significantly affect patency (p=0.09).

Altogether, 43 (56%) patients died during follow-up (Table 3). Cardiovascular disease was the cause of 19 (44%) deaths and cancer the cause of 5 (12%) patients. Cumulative survival rates after primary PTA were 90 % at 1 year, 67 % at 5 years, and 9 % at 10 years. Patients with local SFA disease had a better survival than those with generalised SFA disease (p=0.03). Diabetes mellitus was a negative predictor of survival (p=0.03). The cumulative survival rates for patients with claudication were 89 % at 1 year, 73 % at 5 years, and 8 % at 10 years compared to patients with critical ischemia: 86 % at 1 year, 54 % at 5 years, and 11 % at 10 years (p=0.18).

At long-term follow-up (mean 9.3 years, range 8.2-12.0), 34 (44%) patients were alive. Twenty-eight patients (82%) returned their questionnaire and 22 (79 %) patients accepted to be examined at the vascular clinic. After duplex examination total patency was 50 % (11/22). According to the WIQ, patients with patent vessel segments had significantly less pain, aching, or cramps in their calves compared to patients with non-patent vessels (p=0.04). There was no significant difference between these 2 groups regarding walking distance, walking speed, stair climbing, or the effect of walking impairment on quality of life.

Table 1. Life-table analysis of patency after infrainguinal angioplasty

Interval, y	Limbs at Risk	Failed	Withdrawn	Interval Failure	Cumulative Patency, %	Standard Error, %
Primary Patency						
0–0.5	81	10	1	0.123	87.7	3.42
0.5–1	70	5	1	0.071	81.4	4.20
1–2	64	5	2	0.078	75.0	4.69
2–3	57	8	5	0.140	64.5	5.09
3–4	44	0	5	0.000	64.5	5.79
4–5	39	0	4	0.000	64.5	6.15
5–6	35	1	0	0.029	62.7	6.47
6–7	34	0	5	0.000	62.7	6.57
7–8	29	2	2	0.071	58.2	6.99
8–9	25	7	6	0.318	39.7	6.16
9–10	12	8	1	0.696	12.1	3.27
10–11	3	0	3	0.000	12.1	6.54
Secondary Patency						
0–0.5	81	8	1	0.099	90.1	3.15
0.5–1	72	3	0	0.042	86.4	3.76
1–2	69	4	3	0.058	81.4	4.23
2–3	62	6	6	0.097	73.5	4.81
3–4	50	0	5	0.000	73.5	5.35
4–5	46	0	4	0.000	73.5	5.58
5–6	41	1	0	0.024	71.7	5.96
6–7	40	0	5	0.000	71.7	6.03
7–8	35	2	3	0.060	67.4	6.50
8–9	30	10	4	0.357	43.3	5.96
9–10	16	9	2	0.600	17.3	3.94
10–11	5	0	5	0.000	17.3	7.05

Table 2. Primary patency rates by variable at different time intervals.

	1 Year	5 Years	10 Years	p
Clinical category				0.02
Claudication	87	73	9	
Critical ischemia	70	47	0	
Diabetes mellitus				0.68
Yes	75	67	0	
No	83	63	13	
Gender				0.84
Male	89	65	9	
Female	71	65	15	
Postoperative runoff				0.64
≥2 patent vessels	86	68	11	
0 or 1 patent vessel	77	64	14	
Type of lesion				0.001
Occlusion	65	42	0	
Stenosis	88	74	17	

Table 3. Causes of death in infrainguinal angioplasty patients.

Cause	**No. of patients**
Cardiovascular	19 (44 %)
Cerebrovascular	7 (16 %)
Pneumonia / septicemia	5 (12 %)
Cancer	5 (12 %)
Respiratory	2 (5 %)
Miscellaneous	2 (5 %)
Unknown	3 (7 %)

Study II

Inhibition of neointimal hyperplasia by a specific thrombin inhibitor

Plasma concentrations of inogatran were measured to ensure presence of the drug in the circulation. After the treatment had started no bleeding complications were observed in any of the animals. Injured rats with no treatment (controls) showed a significant neointimal thicke ning after injury. They had an intima/media ratio of 1.18 which was significantly reduced by all treatment forms (Fig. 1). Inogatran given with a continuos osmotic pump for one week had the lowest neointima/media ratio (0.23), which was significantly lower than the control group and also significantly lower than the other treatments (Fig. 2). The analysis of the percentage of lumen narrowing indicated a statistically significant larger lumen in all the treatment groups compared with the control group (Fig. 3). There was no significant difference between the treatment groups regarding lumen narrowing.

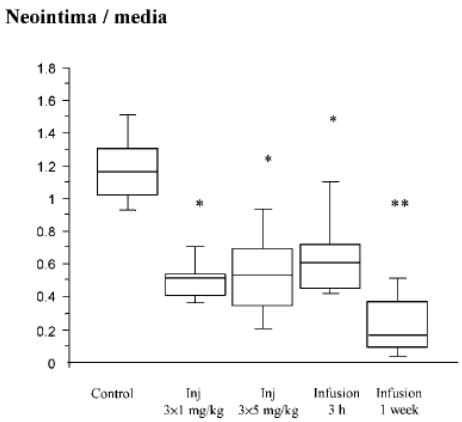

Fig. 1. *Inhibition of neointimal formation by inogatran after balloon injury of the rat carotid artery. Neointima/media ratios were measured in the control rats and in the four treatment forms two weeks after injury. Values are expressed in mean ± SEM.*
** P=0.001 vs control group, ** P=0.001 vs control group and inf 3h 5mg/kg/h, P=0.002 vs inj 3x1mg/kg, and P=0.005 vs inj 3x5mg/kg.*

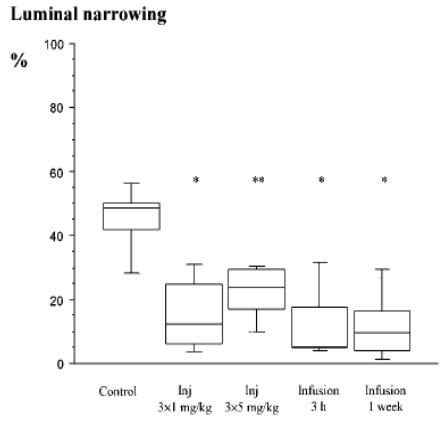

Fig. 3. *Percent of luminal narrowing at two weeks after balloon injury of the rat carotid artery for the control and the four inogatran-treated groups. Values are expressed in mean ± SEM.*
** P=0.001 vs control group,*
*** P=0.003 vs control group.*

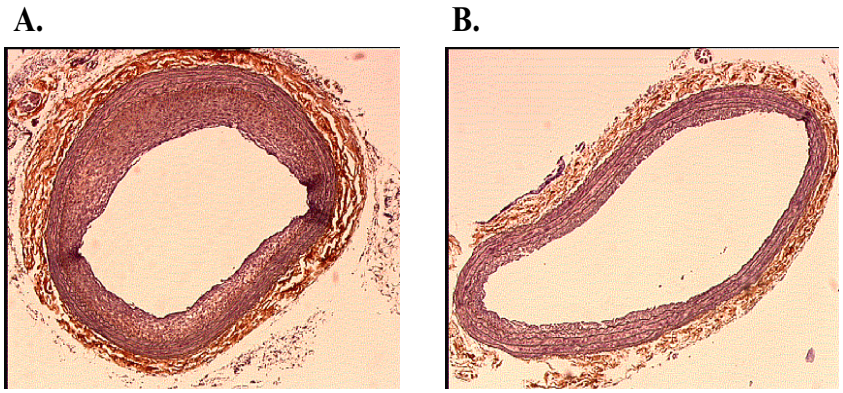

Figure 2. Photomicrographs of representative carotid artery cross sections obtained from Sprague-Dawley rats two weeks after balloon injury. Cross sections were stained with Masson's trichrome stain for collagen. **A** No treatment (control). **B** Intravenous infusion of a thrombin inhibitor, inogatran, maintained for one week through an implantable osmotic infusion pump.

Study III

The role of coagulation and inflammation after angioplasty in patients with peripheral arterial disease

The indication for PTA was intermittent claudication in 91 % of the patients and critical ischemia in 9 %. The location of the procedure was the iliac arteries in 85 %, SFA in 15 %. The overall immediate technical success rate was 88 %. The mean degree of stenosis was 71 % (50-100 %) before and 9 % (0-50 %) after PTA (p=0.0001). The ABI, which was 0.54 (0-1.0) before intervention, rose to 0.73 (0.3-1.1) after intervention (p=0.0001), and 0.74 (0-1.2) (p=0.0002) after 1 month. Neither minor nor major complications related to the procedure occurred. There was no mortality during the 6-month follow-up period. Two stents (6 %) thrombosed acutely but underwent successful local thrombolysis.

Repeated measures of ANOVA showed that CRP, D-dimer, fibrinogen, and P-selectin varied significantly over time after PTA (p<0.001, df=5) (Table 4). There were no significant changes of plasma levels of F 1+2 or TF after PTA (p=0.43 and p=0.78, respectively). *Post hoc* tests showed that CRP was elevated the day after PTA (before vs. 24 h, p=0.001), peaked after one week (before vs. 1 week, p=0.02), and decreased to baseline after one month (1 week vs. 1 month, p=0.02). There was a significant increase of D-dimer and P-selectin one to four hours after PTA in comparison to baseline (p=0.0005 and p=0.05, respectively). D-dimer was elevated for at least a week after PTA and decreased to baseline after one month. Fibrinogen peaked one week after PTA. In the control group, there were no statistical significant differences during the first four hours for P-selectin, and F 1+2. However, D-dimer was significantly elevated four hours after angiography (p=0.005). A subgroup analysis showed that elevated levels of CRP before intervention were predictive of acute stent thrombosis (3 vs. 9 mg/L, p=0.04). Patients on statin therapy seemed to have lower levels of CRP before intervention (2 vs. 5 mg/L, p=0.05) and one week after intervention (6 vs.15 mg/L, p=0.06).

Twenty-nine patients (85 %) were followed-up with angiography after 6 months. Only three patients with restenosis (10 %) and nine patients with a pressure gradient above 10 mmHg (31 %) were diagnosed. The mean reduction of the lumen diameter at the site of PTA was 32 % (0-100) after 6 months. The mean reduction of the lumen diameter after intervention (lumen diameter reduction at 6 months minus lumen diameter reduction post-PTA) was 24 % (0-100) at 6 months. None of the biochemical markers were statistically significant associated with the development of restenosis. Lesion length above 10 cm and occlusion were associated with restenosis (p=0.03 and p=0.04, respectively).

Table 4. Plasma levels of biochemical markers in venous blood after angioplasty. Values are expressed as mean and ranges. # before vs. 1h, 4h, 24h, and 1 week, p<0.001; ## before vs. 1 week, p=0.16; 1h vs. 1 week, p=0.01; 4h vs. 1 week, p=0.001; ¶ before vs. 1h, p=0.05; * before vs. 24h, p=0.001; ** before vs. 1 week, p=0.02.

	Before PTA	1 hour	4 hour	24 hour	1 week	1 month
D-Dimer, mg/L	0.3 (0.1-1.3)	0.4 (0.2-1.1) #	0.4 (0.2-2.2)#	0.4 (0.1-1.4) #	0.4 (0.2-1.4) #	0.3 (0.1-1.4)
F 1+2, nmol/L	1.0 (0.4-1.7)	1.0 (0.4-2.6)	1.2 (0.5-3.3)	1.2 (0.5-6.2)	1.1 (0.4-2.3)	1.0 (0.5-2.0)
TF, pg/ml	332 (100-1296)	303 (95-1068)	314 (90-1000)	272 (122-846)	266 (75-661)	287 (102-811)
P-Selectin, ng/ml	61 (29-332)	68 (35-307)¶	68 (25-317)	62 (26-324)	60 (23-246)	55 (17-238)
CRP, mg/L	3.7 (0-16)	3.6 (0.1-20)	4.0 (0.1-21)	6.6 (0.9-24)*	11 (0-53)**	3.7 (0.21-10)
Fibrinogen, g/L	4.2 (2.4-6.8)	3.8 (1.2-6.2)	3.7 (1.1-7.1)	4.2 (1.4-7.9)	4.8 (0.2-8.0)##	4.3 (1.5-6.4)

Study IV

Elevated thioredoxin after angioplasty in peripheral arterial disease

The technical success after PTA was 100 %. There were four occlusions (21%) and fifteen stenoses (79%). Seventeen stents were placed (89%). The mean peak systolic pressure gradient over the stenoses before intervention was 36 (12-83) mmHg and after 12 (1-40) mmHg (p=0.008). ABI before intervention was 0.60 (0-1.0) and rose to 0.75 (0.4-1.1) (p=0.01). After one month ABI was 0.75 (0-1.2) which was still increased compared to before intervention (p=0.05). There were no minor or major complications and there was no mortality at follow up after 6 months. No low-molecular-weight heparin was given after PTA. Nine patients were on acetyl-salicylic acid (ASA) before PTA. Five further patients received 75 mg of ASA post-PTA and in four patients 75 mg of Clopidogrel was added.

After PTA plasma levels of Trx were elevated at four hours compared to baseline (p=0.02) (Table 5). The levels then decreased to baseline after 24 hours (p=0.02) and stayed at that level after 1 week (p=0.006*).* In contrast, plasma levels of Grx remained unchanged when Grx levels before and after PTA were compared (p=0.60). There were also no changes in plasma levels of Trx or Grx after angiography in the control group (p=0.39 and p=0.17, respectively). There was also no correlation between the balloon pressure at dilatation and the release of Trx or Grx.

Angiography six months after PTA showed a mean lumen reduction at the site of PTA of 23 % (0-60 %). The mean peak systolic pressure gradient over the stenoses was 10 (1-25 %) mmHg. There were 6 patients (32 %) with restenosis (defined as more than 20 % further diameter reduction at the site of PTA). The mean lumen reduction at the site of PTA was 38 % (26-60) in the restenosis group compared to 14 % (0-20 %) in the group without restenosis. We observed elevated levels of Trx at four hours after PTA in the group of patients without restenosis. The statistical significance of this observation was rather weak (p=0.12). There was no such observation in the Grx group. There was no obvious difference between the restenosis and the non-restenosis group regarding risk factors and medication.

Table 5. Plasma levels of Thioredoxin (Trx) and Glutaredoxin (Grx) before and after angioplasty (PTA) and angiography (controls) in patients with peripheral arterial disease. Values are mean and ranges.

* p=0.02 vs. before, p=0.02 vs. 24 h, and p=0.006 vs. 1 week.

	Before	1 hour	4 hours	24 hours	1 week
Trx, ng/ml (PTA)	1.1 (0.5-3.6)	1.6 (0.5-5.6)	2.3 (0.5-14) *	1.1 (0.5-3.1)	0.9 (0.5-2.2)
Trx, ng/ml (control)	5.6 (0.5-21)	2.2 (0.5-7.3)	3.9 (0.5-15)	-	-
Grx, ng/ml (PTA)	4.1 (0.3-12)	2.5 (0.3-9.4)	3.8 (0.3-19)	3.0 (0.3-7.4)	3.8 (0.3-14)
Grx, ng/ml (control)	16 (3.1-72)	8.3 (1.8-23)	6.1 (1.5-11)	-	-

GENERAL DISCUSSION

The mechanisms underlying the response of the arterial wall to injury still remain an unsolved problem. Interrelated components, such as coagulation factors, growth factors, inflammatory mediators, and chemokines, all contribute to the development of neointimal hyperplasia. If this process could be either prevented or controlled, the long-term results of endovascular therapy or bypass surgery would be greatly improved. This thesis has explored the mechanisms of thrombosis and restenosis after vascular injury, focusing on the interaction between coagulation, inflammation, and oxidative stress.

Long-term outcome of infrainguinal PTA

Today, restenosis is the most important limitation related to femoropopliteal interventions [Schainfeld 2002]. In *study I*, the long-term outcome of infrainguinal PTA was evaluated. The primary patency rates correlated well with other published data, although there are few studies with a longer follow-up than 5 years (Table 1). Lesion and patient characteristics, such as the lesion severity/location, arterial runoff, and clinical manifestation, seem to influence the treatment and prognosis of PAD [TASC 2000, Pentecost 2003]. *Study I* showed better primary and secondary patency rates for patients with intermittent claudication than critical ischemia. Stenoses had significantly better patency rates than occlusions, but there was no difference between short (<2 cm) and lengthy lesions.

Only one stent was placed at end of the study period for suboptimal angioplasty. Previously, it has been shown that the general use of stents for infrainguinal lesions provides unacceptably poor results and is therefore not advocated [Vroegindeweij 1997]. More recently, the TASC working group recommended a limited use of stents in the treatment of patients with acute PTA failures or complications. New devices have developed for percutaneous treatment of infrainguinal arterial disease. These devices hold promise for improvement in the technical success and patency rates. However, there are no long-term data yet available supporting a more routine use. Drug-eluting stents in the coronary arteries have been proven to be effective [Morice 2002, Fajadet 2005], but there exists only a limited amount of data on their application in peripheral arteries. The randomised prospective SIROCCO I trial, with implantation of a sirolimus coated nitinol stent into the superficial femoral arteries, showed only 6% restenosis after 6 months [Duda 2002]. However, after 18 months, the restenosis

Table 1. Litterature reports of long-term patency after femoropopliteal angioplasty.

Study	Year	No. of patients	Patients with IC (%)	Patency: 1 year	5 years	10 years
Stanley et al	1996	176	74	58	26	NA
Martin et al	1999	88	74	78	37	NA
Clark et al	2001	205	58	87	55	NA
Jämsén et al	2002	173	100	46	25	14
Wahlgren et al	2003	77	63	81	65	12

IC: Intermittent Claudication, NA: Not available.

rate was similar to the uncoated stents. A slow release form of sirolimus appeared to show better results in the subgroup evaluation. Therefore, the ongoing SIROCCO II trial was started. Preliminary results from that trial indicate a lack of benefit from sirolimus stents after 9 months (unpublished data).

If endovascular intervention and open surgery give equivalent short-term and long-term benefit, the technique with the least morbidity and mortality must be used first [TASC 2000]. The cost should also be considered. Angioplasty and surgery seem to be complementary rather than competitive therapeutic options. In clinical practice, endovascular and surgical treatments are often combined over time to achieve the best outcome. It has been shown that a combination of open and endovascular surgery, when necessary, improved the overall outcome of infrainguinal treatment for claudication [Jämsén 2003]. In patients with chronic critical ischemia, bypass surgery is more effective than PTA because the occlusive disease is characteristically much more extensive and the degree of hemodynamic improvement required is greater [Cooper 1991]. Nevertheless, PTA has become increasingly used as first line treatment for critical lower limb ischemia despite no evidence of durable patency. A randomised controlled trial is underway [Bradbury 2002], and subintimal techniques [Bolia 1995] need further evaluation.

In conclusion, although the overall long-term patency of infrainguinal PTA in *study I* is poor, the technique has low morbidity and a short hospital stay. It can be performed in selected patients with a reasonable long-term clinical result. When conservative treatment has failed, infrainguinal PTA should be considered for suitable lesions because of its minimally invasive nature. Open surgery should be considered for more advanced disease in patients who are candidates for surgery.

Peripheral arterial disease and cardiovascular disease

PAD is a known marker for systemic atherosclerosis and is associated with a markedly increased risk of cardiovascular events. In *study I* a high cardiovascular mortality was observed. Patients with claudication had a better tendency to survival than those with critical ischemia. It seems as if the spread of atherosclerotic disease in the SFA is a predictor of survival after infrainguinal PTA. Diabetes mellitus was also found to predict survival negatively. Previously, the extent of atherosclerotic lesions in the trifurcation of the popliteal artery has been shown to be associated with a higher mortality rate after arterial reconstructive surgery [Källerö 1985].

CRP is clearly a risk marker for cardiovascular disease and a decrease in CRP levels has been associated with a decrease in major coronary events [Ridker 2001]. The heart protection study (HPS) was the first large, randomised trial of statin therapy to demonstrate that lipid modification, simvastatin 40 mg, was associated with a marked reduction in cardiovascular events (myocardial infarction, stroke, and vascular death) [HPS group 2002]. A limitation of the HPS was that the evidence in PAD was derived from a subgroup analysis, and we still do not have a trial exclusively evaluating patients with PAD. There are studies showing both short- and long term effects of statin therapy on CRP levels [Jialal 2003]. A subgroup analysis in *study III* indicates anti-inflammatory effects of statins after angioplasty. Patients on statins had lower levels of CRP before intervention and it seemed that statins could inhibit the CRP peak 1 week after intervention. However, no multivariate analysis was performed because of a relatively small study population. Other studies have documented that statins decrease the secretion of pro-inflammatory cytokines, like IL-6 and IL-8, and inhibit the expression of adhesion molecules on leukocytes and endothelial cells [Laws 2004]. Statins could also have effects on thrombosis by attenuating platelet activation and reducing macrophage expression of tissue factor [Colli 1997, Laufs 2000]. There were two

patients with acute stent thrombosis in *study III*. Both had significantly higher levels of CRP before intervention. This has, of course, to be interpreted cautiously because of the low number of patients. Theoretically, CRP induced procoagulant activity and monocyte tissue factor secretion could be an explanation of the increased risk for thrombosis [Cermak 1993]. The current recommendation regarding management of lipid disorder in patients with established cardiovascular disease or diabetes according to the European Society of Cardiology (ESC) is a total cholesterol <4.5 mmol/l and an LDL cholesterol level <2.5 mmol/l [de Backer 2003].

Animal models of vascular injury

The healing response after arterial injury has been well characterised in various animal models of balloon injury. This includes replication in the media and migration of SMCs into the intimal layer, and later intimal proliferation of the SMCs and extracellular matrix synthesis. The main animal models used to study vascular balloon injury are the rat carotid, the rabbit iliac, and the pig coronary and carotid models. Each model has a particular suitability to investigate different aspects of the balloon injury process. In the rat carotid model previously normal vessels are injured rather than atherosclerotic vessels in the human situation. The rat artery has a single layer of endothelium lining the internal elastic lamina, no vasa vasorum, and the thrombus formation and the leukocyte infiltration after injury are minimal. However, the rat carotid artery injury model is perhaps the most commonly used and has the advantages of availability, low cost, and the ability to develop a rapid reproducible response to balloon injury. The model is a good start to prove the effects of potential therapies in vivo before moving on to more complex models. The drug used in *study II*, inogatran, may not have the same effect in another animal model considering the differences between the species. Rabbits reliably develop hypercholesterolemia when fed a diet high in cholesterol. This animal model is more complex than the rat and more closely approximating human disease. The pigs develop arterial wall calcifications and respond to arterial injury with a thrombotic process similar to that seen in humans. There is a risk of overestimating the ability of study drugs to reduce neointimal formation in animal models in general, which may be partially due to the usage of higher doses. It is also uncertain whether the mechanisms responsible for restenosis in anatomically distinct vascular beds are similar. Major arteries are preferable used for vascular injury in small animal models. It is likely that vessels of different developmental origins have different responses to injury, since VSMCs derived from different developmental origins vary in regard to their growth and transcriptional responses to growth factors [Majesky 1996, Johnson 1999].

Thrombin and the vascular response to injury

Study II showed that a direct thrombin inhibitor, inogatran, reduces neointimal hyperplasia in the rat carotid balloon injury model. Early administration of the thrombin inhibitor resulted in a significant reduction of neointimal hyperplasia. The low dose injection did not differ from the high dose injection or the 3-hour infusion. Apparently the low dose of inogatran was enough to significant reduce the neointima and shows the importance to inhibit thrombin early after injury. The continuos infusion for one week caused a further reduction of the neointima. This emphasises the importance of both an early and a prolonged inhibition of thrombin. There is today a lot of evidence that different thrombin inhibitors reduce neointimal hyperplasia in different animal models (Table 2). It has been suggested that thrombosis may play a key role in the early events leading to restenosis [Libby 1997]. Thrombin has a central role in coagulation and haemostasis. It is a smooth muscle cell mitogen and it is generated in large amounts at sites of arterial injury [Marmur 1994, Barry 1996, Goldsack 1998]. Thrombin seems to participate in the vascular response to injury [Harker 1995]. These responses seem to be primarily mediated via direct effects of thrombin on vascular arterial cells rather than indirectly through its haemostatic effects. It is known that arterial wall associated thrombin activity remains elevated for at least 48 hours after injury and

Table 2. The effects of direct thrombin inhibition on neointimal formation after vascular injury in animal and human studies.

Study	Study drug	Administration	Model	Results
Sarembock 1991	Hirudin	IV bolus + 2-h inf	Rabbit	↓ cross-sectional narrowing
Serruys 1995	Hirudin	IV bolus + 24-h inf ± 2 additional days sc	Human PTCA	No difference in event-free survival
Bittl 1995	Bivalirudin	IV bolus	Human PTCA	No difference in event-free survival
Gerdes 1996	Hirudin	IV bolus + 2-h inf + 3 or 14 days in rats	Minipig, rabbit, and rat	↓ neointimal area in rabbits after short-term inf and in rats after 3-day and 14-day inf
Thome 1998	Hirudin	IV bolus + 2-h inf ± IV bolus after 24 h	Rabbit	Combination of early and late treatment ↓ neointimal formation
Gallo 1998	Hirudin	IV bolus + 2-week inf	Pig	↓ neointimal formation
Wahlgren 2004	Inogatran	IV bolus + 1-week inf	Rat	↓ neointimal formation

returns to baseline after one week [Barry 1996]. The kinetics of thrombin-induced SMC proliferation *in vitro* are delayed relative to other known SMC growth factors [McNamara 1996]. Several mechanisms such as thrombin-induced upregulation of its own receptor, secondary growth factor production, and gradual recruitment into the cell cycle seem to be involved in this delay. Thrombin can also remain in the clot and bound to extracellular matrix for prolonged periods of time, and thus serve as a continuous stimulus. This, altogether, could explain why a more prolonged thrombin inhibition reduces neointima even further than only a short term administration. It could also explain, if extrapolated to humans, the lack of effect on restenosis observed in clinical trials with short term administration of antithrombin agents [Bittl 1995, Serruys 1995]. Maybe a locally delivered thrombin inhibitor, for example via impregnated stents, could be a possible approach to inhibit thrombin effectively, locally, and for prolonged periods.

The mechanisms by which a thrombin inhibitor reduces the development of restenosis remain unclear. Early inhibition of thrombin induced thrombus formation at the site of vascular injury seems to be important. Further explanations may include effects on limiting matrix production, inhibition of cellular migration rather than proliferation, and modulation of growth factors and adhesion molecule expression and production. Thrombin inhibition could have effects on the inflammatory response and the oxidative stress generated after vascular injury. Thrombin stimulates secretion of IL-6 and MCP-1 from human VSMC [Kranzhofer 1996] and IL-8 and 6 from blood monocytes and vascular endothelial cells [Johnson 1998]. It also activates and upregulates heat shock proteins in VSMC [Madamanchi 2001]. Thrombin stimulated VSMC migration is associated with increased generation of ROS and activation of mitogen-activated protein kinases [Wang 2004]. Thrombin stimulates H_2O_2 and superoxide production in VSMC. Suppression of these ROS by treatment with catalase or superoxide dismutase inhibits thrombin-induced mitogenesis [Patterson 1999].

The role that thrombin receptor blockade may have on restenosis may also be an important clue to find out more about underlying mechanisms. An antibody to the thrombin receptor inhibited neointimal hyperplasia after balloon injury in the rat [Takada 1998]. This could indicate that the cellular response to thrombin may be determined at least in part by the presence of different combinations of thrombin receptors on different cell types [Patterson 2001]. There is data suggesting that the thrombin receptor may mediate SMC growth independent of activation by thrombin, raising the possibility that thrombin receptor blockade may have effects more profound than thrombin inhibition [Chaikof 1995].

In conclusion, *study II* has shown that a direct thrombin inhibitor reduces neointimal hyperplasia after arterial injury. Early administration of the thrombin inhibitor gave a significant reduction of neointimal hyperplasia and continuos infusion for one week gave even a further reduction. This indicates that inhibition of thrombin activity is not only important early after injury, but also during the first week in this model. Expanding knowledge of vascular thrombin receptors and thrombin-mediated signalling has thrown a new light on thrombin. More studies are needed to further evaluate the underlying mechanisms by which thrombin inhibitors reduce the development of restenosis.

The inflammatory response after vascular injury

Inflammatory processes play a critical role in the vascular response to injury [Davis 2003]. Several inflammatory mediators (e.g. cytokines and cell adhesion molecules) have multiple roles in this process. Leukocytes adhere to the injured arterial wall and finally transmigrate through the endothelial cell layer. Platelets are not only involved in thrombus formation but also produce a number of important inflammatory mediators. Inflammation can cause local thrombosis, which can amplify inflammation [Libby 2001]. The vascular smooth muscle cells not only produce procoagulants but can also undergo inflammatory activation. Thrombin stimulation causes VSMC production of IL-6, which in turn, induces CRP [Kranzhofer 1996].

Study III showed significantly elevated levels of CRP one week after angioplasty. This finding confirms the inflammatory response after vascular injury [Schillinger 2002]. The inflammatory peak, however, seems to occur much later than previously noted. Elevated plasma levels of CRP after angioplasty reflect a prolonged inflammatory reaction that might causally be involved in patophysiological mechanisms leading to thrombosis and restenosis. Previously baseline and up to 48-hour CRP levels have shown to be associated with an increased risk of restenosis after peripheral PTA [Tschopl 1997, Schillinger 2002]. A high pre-procedure fibrinogen level has also been reported to be a risk factor for restenosis after endovascular treatment of the iliac arteries [Schillinger 2002]. Fibrinogen, well known as an acute phase protein and as a component of the coagulation cascade, confirms that vascular inflammation and disturbed coagulation are intimately intertwined in the patophysiology of restenosis. There was no association in *study III* between CRP or fibrinogen and restenosis. This could be a reflection of a small study population. However, a clinically useful predictor of restenosis should be able to find patients at risk even in this population. Long lesions (above 10 cm) and occlusions had elevated levels of CRP after one week and were associated with restenosis after 6 months.

What could cause the prolonged inflammatory response after vascular injury ? Stented arteries have a more prominent inflammatory reaction compared to balloon angioplasty alone [Inoue 2000]. Their early neutrophil recruitment is followed by a prolonged macrophage accumulation [Welt 2002]. In *Study III,* the amount of stents in an arterial segment did not show any difference in the inflammatory response. The neutrophil accumulation has been observed as early as 6-24 h [Welt 2000] after vascular injury, while T-lymphocytes [Tanaka 1993] and monocytes [Miller 1996] appear after 2-14 days and 3-8 days, respectively. A sustained elevation of MCP-1 has been observed as late as 14 days [Welt 2003]. Adventitial and perivascular inflammation [Okamoto 2001] could also contribute to the prolonged inflammation.

Atherothrombotic markers after vascular injury

Epidemiological studies have identified several potential hemostatic risk markers for atherothrombotic events, e.g. D-dimer and fibrinogen, but their causal role in atherothrombosis remains incompletely established [Folsom 2001]. Fibrinogen and D-dimer have been positive correlated to the severity of atherosclerosis in the lower limbs

[Lassila 1993]. Increased levels of thrombin-antithrombin III complexes, prothrombin fragment 1+2 (F1+2) and D-dimer were found up to 48 hours after PTA [Tschopl 1997]. Several factors, e.g. TF and thrombin, involved in thrombosis are also of importance in the development of restenosis [Hasenstab 2000, Patterson 2001]. In the coronary circulation, expression of TF seems to be a prognostic factor for restenosis after percutaneous transluminal coronary angioplasty (PTCA) [Mizuno 2001].
Study III found elevated levels of D-dimer and P-selectin after PTA, but they seemed to have no impact on the clinical outcome. Expression of TF had not been analysed previously after peripheral angioplasty. The pilot study did however not find any changes in TF response after PTA, and TF was therefore not further evaluated.

Oxidative stress after vascular injury

Oxidative stress is associated with cardiovascular disease [Griendling 2003]. In addition, there is increasing evidence suggesting that oxidative stress and inflammation in the vessel wall also plays an important role in the development of restenosis after angioplasty [Azevedo 2000, Leite 2004]. Inflammation and oxidative stress are closely interrelated. ROS induce inflammatory reactions with a secondary amplification of ROS production [Hakim 1993]. They activate DNA binding factors, i.e. AP-1 and NFκβ, that in turn lead to transcription of several proinflammatory genes, including VCAM-1, MCP-1, ICAM, and E-selectin [Kunsch 1999]. Human umbilical cord vein endothelial cells treated with ROS showed increased binding to neutrophils, which seemed to be due to ICAM-1 [Sellak 1994]. Neutrophils produce hydrogen peroxide (H_2O_2), which diffuse into the cytosol of endothelial cells and may cause cytotoxicity [Lentsch 2000]. Studies strongly suggest that ROS mediate the proliferative phenotype in VSMC [Irani 2000].

The role of Trx and Grx after angioplasty in patients with peripheral artery disease has never been assessed before. However, several studies support the idea that Trx could play a major role in the development of atherosclerosis and in the pathology of reperfusion injury. There is also support for Trx as a sensitive indicator of oxidative stress [Sumida 2000, Sumida 2001].
Study IV showed an increase in plasma levels of Trx four hours after angioplasty in patients with PAD, with levels returning to baseline after 24 hours. Although Grx has some overlapping functions with Trx, a similar pattern of Grx levels in plasma after angioplasty was not found. This supports the notion that the increase of Trx after angioplasty and reperfusion is specific for Trx, and not a general increase of proteins involved in the pathology in oxidative stress. The plasma levels of Trx in healthy individuals have been reported to 30 ng/ml [Pekkari 2000]. Trx levels in *study IV* were about 10-fold lower. However, plasma Trx levels in patients with PAD have never been measured before. We have previously seen that patients with neurological disease can have low Trx levels (3-6 ng/ml) (unpublished data). The values of Trx and Grx in the control group had a tendency to be higher compared to the PTA group. It could be an expression of a more severe arterial disease in the PTA group.

A correlation between plasma levels of Trx and development of restenosis may exist. Patients with elevated plasma levels of Trx after angioplasty could develop restenosis to a lesser extent after 6 months compared to patients with unchanged plasma Trx level. This observation must be interpreted very cautiously due to low patient numbers and no multi-variant analysis. A protective role of Trx in development of restenosis could be explained by the antioxidative effect of Trx but also by the effect of Trx in modulating the inflammatory response after vascular injury. Trx is a chemoattractant for neutrophils, monocytes and T cells [Bertini 1999] and elevated Trx in atheriosclerotic lesions enhance inflammation [Takagi 1998]. In contrast, increased plasma Trx impairs the chemotactic responses to local sites of inflammation [Nakamura 2001]. The peak of plasma Trx after angioplasty could inhibit the migration of leukocytes to the lesion. This would lead to decreased inflammation which could results in decreased restenosis.

The observations in *study IV* give support to the idea of Trx playing a role after vascular and reperfusion injury. The role in the pathology of restenosis needs to be further evaluated. A study of the effect of infused recombinant Trx, lowering inflammation at the site of injury, on restenosis is underway. There is also a need in future studies of new markers to assess the degree of oxidative stress and to monitor the effect of antioxidant therapy. In conclusion, Trx has promising qualities both as a marker of oxidative stress and as a therapeutic agent.

Future directions

The vascular response to injury includes multiple, complex cellular and hormonal reactions. Thrombosis, inflammation, and oxidative stress are involved in the development of restenosis (Fig. 1). Based on this thesis the following pathway, induced by thrombin after vascular injury, is proposed (Fig. 2). Thrombin activates platelets, leukocytes, endothelium, and VSMCs by cleaving G protein-coupled protease-activated receptors. Arterial injury induces an immediate profound vascular oxidative stress [Souza 2000]. There is also a significant oxidative damage to DNA after vascular injury [Zhang 2004]. Mitogen activated protein (MAP) kinases (including extracellular signal regulated kinase [ERK], Jun N-terminal kinase [JNK], and p38) transmit signals from the cell surface to transcription factors and other intracellular target proteins. Thrombin stimulates ROS production in endothelial cells via NADPH oxidase [Holland 1998]. In VSMCs, thrombin by activating NADPH oxidase elicits ROS generation and activation of p38 MAP kinase as well as the expression of MCP-1 [Patterson 1999, Brandes 2001]. It has been showed that thrombin stimulates VSMC migration and VEGF expression through this ROS-sensitive p38 MAPK pathway [Wang 2004]. Thus, ROS are used as messengers for thrombin activation of p38 MAPK and p38 MAPK is an important pathway mediating the pro-migratory action of thrombin.

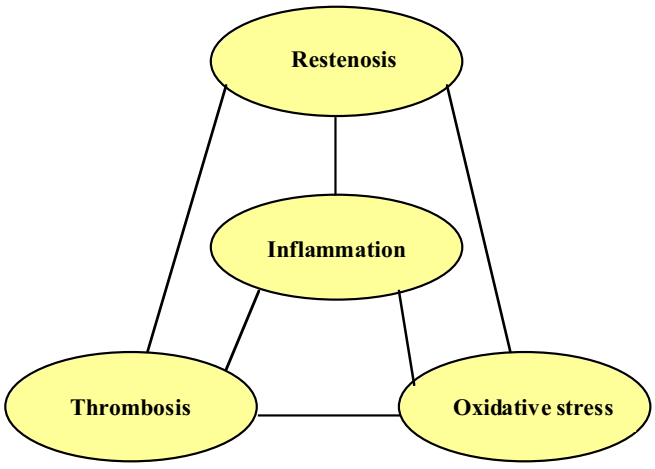

Fig. 1. *Thrombosis, inflammation, and oxidative stress are interrelated and involved in the development of restenosis.*

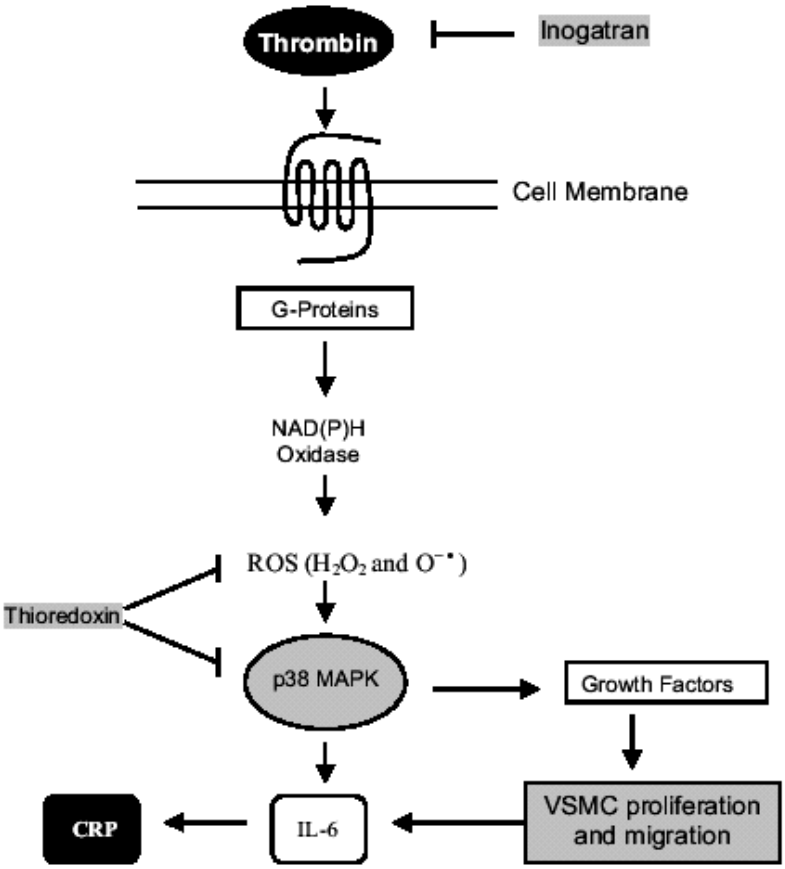

Fig. 2. *A simplified signalling cascade induced by thrombin after vascular injury.*

Thrombin induces IL-6 expression in VSMCs, where both the cAMP response element and p38 MAPK seem to play an important role [Tokunou 2001]. IL-6 has been shown to be a potent stimulator of platelet production [Carrington 1991], and inhibition of IL-6 function by a neutralizing antibody was reported to attenuate endotoxin-induced blood coagulation [van der Poll 1994]. IL-6 can also induce the acute phase response with increased plasma concentration of fibrinogen, PAI-1, and CRP. Thus, a local thrombotic stimulation of VSMCs in the artery wall can amplify the inflammatory response and promote a systemic procoagulant effect due to increased fibrinogen and PAI-1 levels in the circulation [Libby 2001]. Trx and the redox system modulated by Trx have an important role in cellular defense against oxidative stress in cardiovascular disease [Shioji 2003]. Trx seems to inhibit the p38 MAP kinase signalling cascade and suppresses TNF-α induced IL-6 production [Hashimoto 1999].

Further research of the downstream thrombin-mediated signals after vascular injury may lead to further knowledge of the role of thrombin in restenosis and new therapeutic approaches, for example via drug-eluting stents. Further research in thrombin receptor biology to understand the complex mechanisms of PAR activation is needed. Considering the multiple functions of thrombin, PARs may well have additional roles in vascular injury including events in the inflammatory reaction, such as leukocyte rolling, adhesion, and extravasation.

Atherosclerosis is an inflammatory disease [Ross 1999]. Patients with a high-sensitive CRP >3.0 mg/L have a high risk of cardiovascular disease [Pearson 2003, Labarrere 2004]. The predictive value of CRP for occlusive PAD requires additional study. Thus, CRP is clearly a risk marker for cardiovascular disease, but there are now data also supporting a role for CRP in atherogenesis [Jialal 2004]. In vitro studies have shown effects of CRP on monocytes-macrophages, endothelial cells, and SMCs [Jialal 2004]. CRP influences the production of ROS by macrophages and VSMC [Tebo 1991, Wang 2003], induces production of TF on monocytes/macrophages and endothelial cells [Cermak 1993], and also facilitates the release of cytokines, such as IL-6 and TNF-α [Ballou 1992, Verma 2002]. The role of CRP as a predictor of an increased risk of restenosis or whether it could causally contribute to its occurrence is not clear.

It seems that the degree of atherosclerosis and vascular damage cause changes in coagulation status, not *vice versa* [Tracy 2003]. Thus, it does not appear that a preexisting hypercoagulable state is the causal pathway of atherosclerotic disease and cardiovascular events [Lowe 2001]. Fibrinogen, FVIII, and several other proteins, proven to be risk factors for cardiovascular disease, instead reflect a chronic inflammation [Tracy 2003]. These proteins, acute phase reactants, respond to inflammatory stimulation via the effects of proinflammatory cytokines, such as IL-6. In *study III* a prolonged inflammatory response but only minor changes in coagulation activity after PTA was observed. CRP could causally be involved in atherothrombosis after angioplasty. Further studies are needed to delineate the molecular mechanisms behind these observations and their involvement in thrombosis and restenosis. If these pathways are further defined, improved treatment strategies, including antithrombotic treatments, ACE inhibitors, statins, and antioxidants, to modulate postprocedure inflammation could be tailored

Aspirin has clear benefits in patients with cardiovascular disease [Antithrombotic Trialists' Collaboration 2002]. The direct effects of aspirin on CRP levels are still controversial, where some studies report a decrease in levels with aspirin [Ikonomidis 1999], while others report no effect [Feldman 2001]. Patients who have low CRP levels after statin therapy seem to have better cardiovascular outcomes than those with higher CRP levels, regardless of the resultant level of LDL cholesterol [Ridker 2005]. However, there are concerns regarding the effects of statins in the process of angiographic restenosis, despite the beneficial clinical outcome after PCI [Serruys 1999]. Statins seem to have antioxidant effects such as inhibition of ROS through interference with NADPH oxidase [Stoll 2004]. Risk factor modifications, such as weight reduction and smoking cessation, may also reduce CRP.

Several systemically administered drugs, including antiplatelet agents, antioxidants, antithrombotics, antihypertensive drugs, glucocorticoids, and statins, have successfully inhibit arterial narrowing after vascular injury in animal models, but have been almost universally unsuccessful in inhibiting restenosis in human studies. Restenosis is a multifactorial process and drugs that target only one pathway for a restricted period may have limited value. The lack of efficacy in human studies may be in part due to insufficient concentration of drug at the injury site or lack of chronic dosing [Fattori 2003]. It is possible that pharmacodynamic and pharmacokinetic issues may lie behind the failure of thrombin inhibitors in human trials. The concentrations of thrombin inhibitors needed to prevent restenosis may not be achievable by

systemic infusions without producing unacceptable hemostatic complications. Local drug delivery strategies, providing prolonged, potent, and locally active therapies, will probably have a major impact in peripheral vascular interventions in the future. Caution should be taken with the early data from the sirolimus stents. The initial failure of sirolimus stents in peripheral vascular interventions might be explained by the fact that peripheral arteries are larger than the coronaries and the lesions are likely to be more complex [Ruef 2004]. The pathophysiology of restenosis might also be different in the peripheral arteries and the timing and duration of the given drug might be different. Potential systemic or local toxic effect of the drug or a delayed inflammatory response induced by the polymer itself may cause restenosis or damage to the distal vascular bed [Fattori 2003]. Cost-effectiveness and long-term reliability remain also to be defined. Improving the biocompatibility of polymer coatings with improved loading capacity and sustained-release capabilities are important future considerations for drug-eluting stents [Huang 2002]

This thesis has discussed the intimate relation between thrombosis, inflammation, oxidative stress, and restenosis. Several mediators are in common of these entities and involved both in physiological and pathological activities. An understanding how to interfere with these pathological activities without disturbing physiological activities will be a future challenge. It is a need for an integral approach for the study of restenosis. It is not only important to be able to understand the individual pathways of coagulation, inflammation, and oxidative stress, but also the ways they intersect and interact.

CONCLUSIONS

1. Although the overall long-term patency of infrainguinal PTA in patients with peripheral arterial disease is poor, the technique has low morbidity and a short hospital stay. It can be performed in selected patients with a reasonable long-term clinical result. When conservative treatment has failed, infrainguinal PTA should be considered for suitable lesions because of its minimally invasive nature. It is also important when treating patients with peripheral arterial disease to give attention to their general cardiovascular condition.

2. A specific direct thrombin inhibitor, inogatran, reduces neointimal hyperplasia after arterial injury in rats. A more prolonged administration of the thrombin inhibitor gives a further reduction of the neointimal hyperplasia. It seems that inhibition of thrombin activity is not only important early after injury, but also later.

3. There is a prolonged inflammatory response but only minor changes in coagulation activity after PTA in patients with peripheral arterial disease. CRP could causally be involved in atherothrombosis after angioplasty. The inflammatory response was not associated with later restenosis.

4. There is an increase in plasma levels of thioredoxin four hours after PTA in patients with peripheral arterial disease with levels returning to baseline after 24 hours. This observation gives support to the idea of thioredoxin and oxidative stress playing a role after vascular injury. The role of thioredoxin in the pathology of restenosis needs to be further evaluated.

POPULAR SCIENCE SUMMARY IN SWEDISH

Åderförkalkning (ateroskleros) i kroppens pulsådror (artärer) är den dominerande orsaken till hjärtkärlsjukdom och svarar för mer än hälften av dödsfallen i västvärlden. Åderförkalkning som drabbar benens pulsådror leder ofta till förträngningar i blodkärlen med ett minskat blodflöde till muskler och andra vävnader som följd. Vid gång kan detta leda till värk i benen och detta kallas då för fönstertittarsjuka eller claudicatio intermittens. Det årliga antalet nyinsjuknande i fönstertittarsjuka i Sverige kan grovt uppskattas till 10 000. Det är viktigt att hejda den fortsatta utvecklingen av åderförkalkningssjukdomen genom bland annat rökstopp, motion och kontroll av blodfetter, blodtryck samt blodsocker. Hos ca 15% av patienterna med fönstertittarsjuka kommer dock symptomen att förvärras till vilovärk och/eller sår på benet. Detta tillstånd med kritisk blodbrist till benet är allvarligt och bör handläggas skyndsamt.

De viktigaste behandlingsmetoderna för att förbättra cirkulationen i benet är ballongvidgning (PTA: Perkutan Transluminal Angioplastik) av kärlet eller bypass kirurgi. De största hindret för en framgångsrik kärlrekonstruktion utgörs av att blodet kan levra sig i kärlet (trombos) och senare utveckling av en ny förträngning i pulsådern (restenos). Denna avhandling har studerat effekter av den kärlskada som uppkommer vid ballongvidgning både vad avser trombos och senare utveckling av restenos. Även det retningstillstånd som uppkommer efter vävnadsskada, inflammation, och skyddet mot skadliga instabila små ämnen (fria radikaler) som frisätts har studerats.

Patienter som genomgått ballongvidgning av pulsådern på låret har studerats i 8-12 år efter sin behandling. Långtidsresultatet är relativt dåligt då 65% av patienterna har ett primärt öppetstående kärl efter 5 år och endast 12% efter 10 år. Riskerna med behandlingen är dock låga och resultatet är tillfredsställande hos selekterade patienter. Patienter med fönstertittarsjuka har en generaliserad åderförkalkningssjukdom i kroppens pulsådror. Dödsorsaken hos 60% av patienterna i denna behandlingsgrupp är åderförkalkningsrelaterad sjukdom i hjärta och hjärna.

Efter skada på kärlväggen sker en kaskad av olika processer. Den normala skyddsfunktionen mot att blodet levrar sig i kärlet förloras. Trombin är en mycket potent och viktig molekyl för blodets levring (koagulation). Denna molekyl har även andra viktiga funktioner och är bland annat inblandad i den inflammatoriska processen samt vid tillväxtstimulering av muskelceller i kärlväggen. Experimentellt har vi kunnat visa att genom att tillsätta en specifik hämmare av trombin efter kärlskada erhålls en minskad utveckling av restenos. Det verkar som att inte bara tidig trombinhämning efter kärlskada är viktig utan även senare, vilket kan vara viktig information vid en eventuell framtida medicinsk behandling. Koagulationssystemet är aktiverat de första timmarna efter ballongvidgning. Det sker också en signifikant inflammatorisk reaktion redan efter 1 dygn och som kvarstår åtminstone efter 1 vecka. Dessa initiala reaktioner tycks inte vara associerade med senare utveckling av restenos. En ökning av proteinet thioredoxin i blodet sker under första dygnet efter ballongvidgning hos patienter med kärlsjukdom i benen. Detta protein kan ha betydelse för att minska den inflammation som uppstår i kärlväggen.

Vid alla former av kärlrekonstruktion, såväl ballongvidgning som bypass kirurgi, sker någon form av kärlskada som startar en läkningsprocess. Detta leder till risk för blodpropp och eventuell senare utveckling av restenos som orsakar nya symptom. En förutsättning för att kunna behandla dessa två kliniskt viktiga komplikationer är att deras bakomliggande orsak klarläggs. Föreliggande avhandling har klarlagt samband mellan koagulation och inflammation efter kärlskada. Detta kan i en förlängning erbjuda patienter möjlighet till medicinsk behandling för att minska risken för blodpropp och nya förträngningar i blodkärlen.

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"Science is the servant and not the master in the treatment of patients"

Inscription at the research laboratory of Radiumhemmet,
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