PERTTU ROSSI

Eicosanoids and Lower Limb Ischemia

ACADEMIC DISSERTATION
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LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, referred to in the text by their Roman numerals I - V.


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ABSTRACT

BACKGROUND: This study sought new theoretical support for the pharmacotherapy of lower limb ischemia. Urinary metabolites of eicosanoids: prostacyclin (PGI2), thromboxane (TXA2), leukotriene E4 (LTE4) and isoprostanes (8-iso-PGF2α), were measured in lower limb ischemia, after balloon angioplasty (PTA) and after surgical revascularisation.

PATIENTS AND METHODS: Each group comprised patients suffering from acute or chronic lower limb ischemia. Urine samples were overnight morning urine (12 h) samples. Urinary 2,3-dinor-6-keto-PGF1α, 11-dehydro-TXB2, LTE4 and 8-iso-PGF2α were measured by radioimmunoassay (RIA) and their excretions were correlated to that of urinary creatinine.

RESULTS: PGI2 metabolite and TXA2 metabolite excretions were increased 2-fold in acute compared to chronic lower limb ischemia. After PTA PGI2 metabolite excretion was not affected, but TXA2 metabolite excretion increased 2-fold. LTE4 excretion was increased 7-fold in both acute and chronic lower limb ischemia, and surgical revascularisation did not affect it. 8-Iso-PGF2α excretion was raised in chronic lower limb ischemia and decreased to normal level after revascularisation.

CONCLUSIONS: PGI2-analogues and TXA2-inhibitors would appear to be useful in both acute and chronic lower limb ischemia. The same applies after PTA. Leukotriene antagonists may also be useful in both acute and chronic lower limb ischemia. Chronic lower limb ischemia is also associated with oxidative stress and surgical revascularisation normalises this stress.
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>ASA</td>
<td>Acetylsalicylic acid</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>IMA</td>
<td>Internal mammary artery</td>
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<td>8-iso-PGF2α</td>
<td>8-Isoprostaglandin F2α</td>
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<tr>
<td>LOX</td>
<td>Lipoxygenase</td>
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<td>LT</td>
<td>Leukotriene</td>
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<td>PGI2</td>
<td>Prostacyclin</td>
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<tr>
<td>PTA</td>
<td>Percutaneous transluminal angioplasty</td>
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<tr>
<td>PTCA</td>
<td>Percutaneous transluminal coronary angioplasty</td>
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<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>SRS-A</td>
<td>Slow reacting substance-A</td>
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<tr>
<td>TXA2</td>
<td>Thromboxane A2</td>
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<td>TXB2</td>
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INTRODUCTION

Peripheral arterial occlusive disease is a common medical problem associated with considerable morbidity, amputation frequency and mortality. It is a manifestation of systemic atherosclerosis and its incidence is increasing with the aging of the population. Acute lower limb ischemia is still accompanied by a 25 % mortality and a 20-40 % major amputation rate, and chronic lower limb ischemia is associated with a 30-42 % mortality rate at five years. The prevention and treatment of lower limb ischemia is thus a growing problem in a society with an aging population.

The pathophysiology of lower limb ischemia is a complex process influenced by many factors, among them the eicosanoids. These are compounds formed from arachidonic acid, and include prostaglandins, thromboxanes and leukotrienes. There are two enzymes which act on arachidonic acid: cyclooxygenase and lipoxygenase. The first forms the series of prostaglandins and thromboxane and the second forms leukotrienes.

Eicosanoids exert many actions on the vascular system. Prostacyclin (PGI2) is a potent vasodilator and inhibitor of platelet aggregation. It is released from the vascular endothelium by interactions with platelets or leukocytes or by mechanical injury. Thromboxane (TXA2) is formed from platelets and it has the opposite effects: vasoconstriction and thrombosis. The balance between these two compounds (PGI2/TXA2 ratio) has thus been shown to be disturbed in various cardiovascular diseases.
Leukotrienes are formed by activated leukocytes. They constrict smooth muscle cells - as best known in asthma - and promote vasospasm and edema formation. The role of eicosanoids in the pathophysiology of cardiovascular diseases has been investigated (FitzGerald et al. 1981, 1984). Most series deal with cardiac events, and lower limb ischemia has awakened only minor interest. However, the prostanoids form the basis for pharmacotherapy of many ischemic conditions. Acetylsalicylic acid (ASA), which is a TXA2 synthesis inhibitor, has long been the medication of choice for lower limb ischemia. PGI2-analogues (iloprost, taprostene) have more recently gained a role in the treatment of lower limb ischemia, but leukotriene antagonists or synthetase inhibitors have not yet been used in these conditions.
Eicosanoids

Eicosanoids are biologically active compounds derived from 20 carbon unsaturated fatty acids (eicos: Greek twenty), among which arachidonic acid is the most important substrate (Baker 1990). Arachidonic acid (AA), the precursor of proinflammatory eicosanoids, is released from membrane phospholipids by the action of phospholipase A2 and C (Homaidon et al. 2002). It is metabolised to prostaglandins and leukotrienes by the action of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, respectively.

Knowledge of eicosanoids dates back to the time when new biologically active compounds were being found in human seminal plasma - hence “prostaglandins” (von Euler 1935). More were found in the 1970s, including thromboxane A2, a potent platelet aggregating agent, and prostacyclin, which is an antagonist to thromboxane A2 (Bunting et al. 1983). At this time the inhibitory actions of acetylsalisylic acid (ASA) on eicosanoid synthesis were also discovered.

A new group of eicosanoids, leukotrienes, was found by Samuelsson in 1979 (see Figure) and these were subsequently shown to evince many biological activities, one of which is bronchoconstriction in patients with asthma (Patrono et al. 1992). Eicosanoid biosynthesis has been widely investigated in human vascular diseases (FitzGerald et al. 1987). Especially the importance of the balance between prostacyclin and thromboxane A2 has been widely acknowledged since 1980 (Moncada and Vane 1980). The role of leukotrienes in vascular diseases has been also known since 1986 (Ford-Hutchinson and Letts 1986) but less research has been devoted to this aspect.
To the author’s knowledge no previous studies are available which evaluate the leukotrienes in association with acute or chronic lower limb ischemia or in conjunction with surgical revascularisation of an ischemic lower limb.

5-Lipoxygenase pathway and leukotriene formation in human leukocytes
Prostacyclin

Prostacyclin is prostaglandin I2 (PGI2). It is a product of arachidonic acid generated in the vessel wall and released from arterial endothelium by interactions with platelets or leukocytes or by mechanical injury. Prostacyclin is also regarded as a circulating hormone generated by the lungs (Gryglewski 1980) but the pulmonary formation of prostacyclin is low (Edlund et al. 1981) and plasma levels are negligible. Prostacyclin is a potent vasodilator and inhibitor of platelet aggregation (FitzGerald et al. 1987). This latter function proceeds by stimulation of adenylate cyclase, which leads to an increase in cyclic AMP in the platelets. This prevents the formation of platelet aggregates and the growth of thrombi in both arteries and the microcirculation.

Prostacyclin is also an inhibitor of leukocyte activation and adhesion. It inhibits the human leukocyte aggregation induced by activated platelets, thus preventing plugging of the capillaries (Belch et al. 1987). It also reduces the release of harmful substances such as leukotrienes, free oxygen radicals and proteolytic enzymes.

Prostacyclin also exerts protective effects at cellular level in hypoxic conditions by limiting tissue edema and maintaining capillary flow (Vischer 1982). Plasma levels of prostacyclin in healthy volunteers are equal to or less than 5 pg/ml (FitzGerald et al. 1987). Prostacyclin production is commonly measured in urine as the hydrolysis product 2,3-dinor-6-keto-prostaglandin F1α (Riutta et al. 1994).

Thromboxane A2

The term “thromboxanes” was introduced because these compounds were first isolated from thrombocytes (platelets). The biologically active compound is thromboxane A2 (TXA2),
which is hydrolysed to the inactive metabolite thromboxane B2 (TXB2). The formation of TXA1 is low and it thus constitutes the most important of the thromboxanes.

TXA2 is formed in human platelets from arachidonic acid. Minor amounts are also formed in the lungs, in monocytes and in blood vessels. Major actions of TXA2 comprise stimulation of platelet function and contraction of vascular and nonvascular smooth muscles. Thus, it contracts arteries, veins and lymphatic vessels (Chan et al. 1986). Enhanced TXA2 generation occurs during platelet stimulation, e.g. in atherosclerosis, hypercholesterolemia, diabetes mellitus, unstable angina pectoris and acute myocardial infarction (Henriksson et al. 1986). Inhibition of exaggerated thromboxane formation constitutes the rationale for the clinical use of acetylsalicylic acid.

The balance between PGI2 and TXA2 is of clinical importance. TXA2 generation at the site of vessel injury promotes closure of the vessel and thrombus formation, thereby preventing uncontrolled blood loss. PGI2 formation prevents thrombus growth beyond the immediate area of damage and limits thrombus formation to the site of vascular lesion.

Thromboxane formation can be estimated from urinary measurement of 11-dehydro-TXB2 (Riutta et al. 1992). Determination of TXB2 in blood or plasma samples might result in artificially high levels by reason of additional thromboxane generation during sampling and assay procedures.

Leukotrienes

The term “leukotrienes” was introduced to describe a group of arachidonic acid metabolites originally detected in leukocytes (“leuko”) and carrying three (“tri”) conjugated double bonds (“enes”). First to be found were “the slow reacting substances of anaphylaxis” (SRS-A), which were later shown to be cysteiny1 leukotrienes (Samuelsson 1983).

Leukotrienes are formed from arachidonic acid via the lipoxygenase pathway, mostly in
neutrophils (see Figure). They (LTB4) are important mediators of inflammatory and immune reactions as well as potent spasmogens (cysteinyl leukotrienes) of vascular and nonvascular smooth muscle. The nomenclature is based on their general chemical structure and the number of double bonds (LTA4, LTh4, LTC4, LTD4, LTE4 and LTF4). LTC4, LTD4 and LTh4 are also called cysteinyl leukotrienes. LTE4 formation is regarded as an index metabolite for cysteinyl leukotrienes (Barnes et al. 1999).

8-Isoprostaglandin F2α

8-Isoprostaglandin F2α (8-iso-PGF2α) is an isoprostane produced from arachidonic acid in membrane phospholipids. It is present in normal human urine at concentrations of 200-300 pg/ml and its excretion is increased in conditions of oxidative injury (Pratico et al. 2001). 8-iso-PGF2α is considered an ideal index marker of the isoprostane family. It is a potent vasoconstrictor and a platelet activator and also a mediator of free radical induced oxidative injury (Roberts et al. 2000).

Pathophysiological aspects of eicosanoids

Eicosanoids are important modulators for tissue and organ function. Decreased secretion of prostacyclin or increased secretions of thromboxane A2, leukotrienes and 8-iso-PGF2α have been detected in various vascular diseases. These deleterious sequelae in inflammatory and allergic reactions, in ischemia and in thrombosis have stimulated an intensive search for selective antagonists of these compounds. Best known among these are non-steroidal anti-inflammatory drugs (NSAIDs) such as ASA, which inhibits nonselectively but irreversibly the cyclooxygenase enzyme (Chan et al 1986). Also inhibitors of lipoxygenase enzyme are available, many of them nonselective antioxidants (Barnes et al. 1999). Prostacyclin analogues (iloprost and taprostene) have been used in the treatment of lower limb ischemia (Dormandy 1996). While beneficial effects have been reported, the clinical situation appears to be more...
complex, and more information is called for to consolidate the basis of pharmacotherapy.

Prostacyclin and thromboxane A2 in vascular diseases

PGI2 is an unstable compound with a plasma half-life of only about 3-5 minutes. This has prompted the development of stable prostacyclin analogues, of which iloprost is the best known. Intravenous administration of PGI2 results in dilatation of arterioles seen e.g. in an elevated skin temperature (Pessi et al. 1986, Quilmot et al. 1991). These responses are followed by a reduction of diastolic blood pressure, headache and a reflective increase in heart rate. Higher doses lead to restlessness, abdominal spasms, nausea and vomiting, which disappear within one hour after cessation of infusion (Kaukinen et al. 1984). However, it also has therapeutic effects such as the disappearance of ischemic pain in peripheral arterial occlusive disease (Pessi et al. 1986), and the effect may persist over several days (Gaetano et al. 1990). Watson and associates (2000) showed that intragraft iloprost used in femorodistal bypass surgery reduces the distal vascular resistance for a clearly longer period than the half-life of iloprost. Dormandy (1996) analysed the European experience of prostanoid therapy for peripheral arterial occlusive disease in 2000 patients, who were treated with intravenous iloprost for 2-3 weeks. He found some of the trials have to yield benefits compared to placebo in terms of major amputation or death during the 6 months following the treatment. However, oral iloprost administered for a year showed no clear benefit in patients with advanced severe leg ischemia (Eur J Vasc Endovasc Surg 2000). In contrast in vasospastic diseases such as Raynaud’s syndrome and Buerger’s disease, intravenous iloprost has proved beneficial (Belch et al. 1981).

FitzGerald and colleagues (1984) have noted that PGI2 metabolite excretion is increased in lower limb ischemia. However, it is seen to be decreased locally in atheromatous plaques (D’Angelo et al. 1978). Thus, healthy endothelial cells seem to compensate the local deficit of PGI2 by increasing total PGI2 production. This may be one explanation for the controversial results of the clinical trials.
Apart from total PGI2 production, even more important seems to be the balance between PGI2 and thromboxane A2 (Bunting et al. 1983). The PGI2 / TXA2 ratio is disturbed in many cardiovascular diseases, for example in unstable angina (FitzGerald et al. 1986), deep vein thrombosis (Vesterqvist et al. 1987) and peripheral arterial disease (Chan et al. 1986). In coronary artery disease the most substantial rise in TXA2 synthesis is seen during unstable angina, whereas the greatest rise in PGI2 synthesis occurs during myocardial infarction, which reflects the fact that PGI2 biosynthesis is probably a compensatory response to limit the deleterious effects of TXA2. In healthy persons the excretion of PGI2 metabolite is thus low, since there is almost no stimulus for its production. However, atherosclerosis, thrombosis or endothelial injury leads to platelet activation and TXA2 excretion, which is accordingly a stimulus for PGI2 synthesis (FitzGerald et al. 1981). In essential hypertension the PGI2 / TXA2 ratio has been shown to correlate to blood pressure (Minuz et al. 1990) and recently a correlation between the level of blood glucose in diabetic patients and the PGI2 / TXA2 ratio has also been recognised (Hishinuma et al. 1999). The authors showed that the PGI2 / TXA2 ratio is lower in diabetic than in healthy persons and they suggest that this ratio could be a useful marker of the severity of diabetes.

In conclusion, in future the PGI2 / TXA2 ratio may provide a marker of various vascular diseases.

Prostacyclin and thromboxane A2 in percutaneous transluminal angioplasty (PTA)

Since PGI2 is released mainly from the vascular endothelial cells, its production is disturbed by any vascular trauma. PGI2 production is locally diminished in an atheromatous plaque (D’Angelo et al 1978). If a plaque is dilated with a balloon catheter, the procedure leads to the activation of both endothelial cells (PGI2 synthesis) and platelets (TXA2 synthesis)(Mehta et al. 1982). The balance between these two agents (PGI2 / TXA2 ratio) has not previously been measured. However, Onohara and associates (2000) studied platelet activation after angioplasty of the iliac artery in seven patients. They collected blood samples locally from the iliac artery before and after PTA and observed that platelet activation was decreased after the
angioplasty. This was probably attributable to the normalised blood flow in the previously stenosed artery, but the total excretion (urinary metabolites) of TXA2 was not measured. In animals the phenomenon has been studied in one experiment. Mattson and colleagues (1990) measured the local TXA2 excretion in ten rabbits after dilatation of the aorta and found no increase in TXA2 production.

Other studies have focused on coronary artery angioplasties (PTCA). A group under Peterson (1986) collected blood samples from 10 patients during PTCA. They noted no change in PGI2 and TXA2 excretions in uncomplicated PTCAs, but in two patients who required an emergency bypass operation for occlusion, a marked increase of TXA2 excretion was seen. Uncomplicated PTCAs were also analysed by groups under Korovesis (2000) and Kesmarky (2001), who collected blood samples from the coronary sinus and observed platelet activation to be reduced after the procedure.

The effect of pure catheterisation (without any procedure) on PGI2 excretion has been analysed in one study. Roy and associates (1985) measured PGI2 production during cardiac catheterisation and saw an increase in PGI2 excretion, which remained elevated for 2-4 hours. Furthermore, they administered radiocontrast medium intravenously to healthy volunteers and this also temporarily increased PGI2 excretion.

In conclusion, PGI2 / TXA2 ratio in man has not hitherto been measured.

Prostacyclin and thromboxane A2 in bypass operations

PGI2 and TXA2 productions in conjunction with surgical operations have been little investigated. However, as early as 1987 a group under Vesterqvist noticed that a synthetic arterial graft increased the excretions of the metabolites of both PGI2 and TXA2 in humans. They analysed patients who had a synthetic graft replacement of the abdominal aorta and a control group with patients undergoing cholecystectomy. They found a forty-fold increase in PGI2 excretion and a tenfold increase in TXA2 excretion on the first postoperative day after
the aorta operation. This elevated excretion persisted for 6-10 days. The authors concluded that a synthetic graft is a foreign surface for platelets, causing platelet activation and TXA2 synthesis, which is followed, as a compensatory mechanism, by PGI2 formation.

No studies focused on lower limb revascularisation and PGI2 / TXA2 excretions are available. However, these compounds have been analysed in conjunction with cardiac bypass operations. Teoh and colleagues (1987) measured both systemic and cardiac (from the coronary sinus) secretions of PGI2 and TXA2 during a coronary bypass operation. Both systemic formations were increased after cardiac cannulation and remained elevated during cardiopulmonary bypass. The cardiac synthesis was also elevated, but only TXA2 excretion remained elevated over the postoperative period. Thus, the PGI2 / TXA2 ratio in the myocardium was altered in the direction of TXA2 formation, which could contribute to the reperfusion syndrome. The authors proposed that PGI2 could be useful in the treatment of this syndrome. This was tested by a group under Chelly (1982), who infused PGI2 intravenously in 12 patients after coronary revascularisation. PGI2 reduced vascular resistance and improved cardiac function and the authors suggested that PGI2 could be useful in preventing the reperfusion syndrome.

In conclusion, bypass operations probably alter the PGI2 / TXA2 ratio in the direction of TXA2 formation, which may have a therapeutic implication.

Leukocytes and leukotrienes in vascular diseases

White blood cells (leukocytes) contribute to arterial disease by atherogenesis, thrombosis and ischemia (Lowe 1987). Studies have shown that in an ischemic organ the impactation of leukocytes on the microcirculation causes plugging of the nutritive capillaries (Ernst et al. 1987). Leukocytes have more rigid nuclei than erythrocytes and are thus less deformable, which leads to their adhesion to the capillary endothelium. As a consequence, leukocytes migrate to tissues, causing swelling and an increase in microvascular flow resistance.

Patients with critical lower limb ischemia have been shown to evince leukocyte activation
Nash and colleagues (1988) collected blood samples from both ischemic lower limb veins and nonischemic arm veins. They observed marked leukocyte activation in ischemic lower limbs. These activated leukocytes can produce leukotrienes, which contract smooth muscle cells and increase edema formation (Goldstein et al. 1990). Activated leukocytes can also activate platelets, and conversely, activated platelets can activate leukocytes (Dinerman et al. 1988). Klausner and associates (1988) investigated the increased permeability and edema following lower limb ischemia and noted that it is mediated by leukocytes and leukotrienes. They also recognized that treatment with the leukotriene antagonist diethylcarbamazine prevented the increase in permeability and also reduced the white blood cell count.

Most studies of leukotrienes have focused on their effects on the heart. Coronary artery constriction caused by leukotrienes is seen both in animals (Ezra et al. 1983 and Michelassi et al. 1982) and in humans (Allen et al. 1993 and Goldstein 1990). However, when Goldstein and his group (1990) administered leukotrienes intracoronarily, it led first to a profound vasoconstriction, but this was followed by the complete disappearance of these effects, even though leukotrienes continued to be infused at a constant rate. The authors propose that the effects of leukotrienes are possibly transient, but the nature of the mechanism remains unclear. Recently again it has been suggested that myocardial ischemia is possibly mediated by leukotrienes (Szczeklik et al. 2002). Allen and colleagues (1998) compared the contractile responses of leukotrienes in atherosclerotic and nonatherosclerotic human coronary arteries. They observed that nonatherosclerotic coronary arteries did not respond to leukotrienes, whereas atherosclerotic arteries evinced concentration-dependent contractions. Also earlier (Ezra et al. 1983, Michelassi et al. 1982 and Roth et al. 1983) it has been noted, that leukotrienes reduce myocardial contractility. Mugge and colleagues (1991) saw the same phenomenon in monkeys: the response to leukotrienes was greater in atherosclerotic arteries
than in healthy vessels. These investigations have led to the conclusion that in atherosclerosis there exist specific leukotriene receptors. This has stimulated wide research on leukotriene receptors (Haeggstrom and Westerholm 2002) and specific antagonists (Funk 2001). Apart from vascular diseases, currently leukotrienes have also been connected to many other pathophysiological states, for example juvenile rheumatoid arthritis (Fauler et al. 1994), atopic eczema (Adamek-Guzik et al. 2002), dysmenorrhea (Harel et al. 2000), interstitial cystitis (Bouchelouche et al. 2001) and, in view of bronchoconstriction, naturally to asthma (Drazen 1999) and chronic obstructive pulmonary disease (Shindo et al. 1997). It is proposed that in these pulmonary diseases the arterial blood level of leukotrienes may reflect the severity of the disease. It is also suggested that some of the adverse effects of smoking are related to increased leukotriene synthesis (Fauler et al. 1997).

In conclusion, leukotrienes appear to cause vasoconstriction. Thus their antagonists may also have a role in the therapy of vascular diseases.

Leukotrienes and bypass operations

Patients with critical limb ischemia have been shown to have leucocyte activation (Nash et al. 1988). The same authors also found a bypass operation to normalise the leucocyte count. Furthermore, Dormandy and colleagues (1989) observed a positive correlation between white cell count and reocclusion of a distal bypass reconstruction. However, studies evaluating leukotrienes and lower limb revascularisation have not as far as we know previously been available.

Allen and associates (1993) measured leukotrienes before and after coronary artery bypass surgery. They noted that before the operation urinary leukotriene E4 excretion was elevated in patients with stable coronary artery disease and further increased after the operation. The peak of excretion occurred on the second postoperative day and the levels decreased on the third. They suggested that this finding may have some therapeutic implications and offer possibilities for new treatment strategies. Later the same authors (Allen et al. 1994) compared
the effects of leukotrienes between the human internal mammary artery (IMA) and the saphenous vein to find possible reasons for better graft patency. They observed that leukotrienes had no effect on IMA, but they significantly contracted the saphenous vein. They thus suggested that this difference in smooth muscle reactivity may be an important factor in graft function and that this may explain the better patency of IMAs compared to saphenous veins in cardiac surgery.

Likewise Jeremy and colleagues (1996) studied leukotrienes and the failure of saphenous vein bypass grafts. They compared the effects of leukotrienes on grafted saphenous veins and saphenous veins left intact. There was significantly increased leukotriene formation in saphenous veins prepared for use in a bypass operation. The same was seen by Allen and group (1992) and the authors speculate that the intra-operative preparation of the saphenous vein may cause intimal damage, which leads to leukocyte activation and possibly leukotriene excretion. This may contribute to subsequent intimal proliferation and, as a consequence, graft failure. The difference between artery and vein was also seen in studies by a group under Schellenberg et al. (1984). They compared the responses of leukotrienes between human pulmonary artery and vein. Leukotrienes were potent contractants of the pulmonary vein while minimal contractions were seen in the pulmonary artery. They concluded that pulmonary veins have specific leukotriene receptors not found in pulmonary arteries. These studies have led to an intensive search for leukotriene receptors and antagonists (Johnsson 1998, Devillier et al. 1999, Gompertz et al. 2002 and Haeggstrom and Wetterholm 2002).

Isoprostanes and vascular diseases

Isoprostanes comprise a recently discovered family of compounds formed from arachidonic acid during oxidative injury (Oguogho et al. 2000). They are stable compounds, and they are present in detectable quantities in all biological fluids and tissues. Their formation increases dramatically under oxidant injury and is modulated by antioxidant status (Roberts et al. 2000). Thus they provide a reliable index for oxidative injury in vivo and serve as a basis for studies of antioxidants (Pratico 2001). 8-Isoprostaglandin F2α (8-iso-PGF2α) is a major isoprostane in
plasma and urine (Helmersson et al. 2001). Thus urinary measurement of this compound has been shown to reflect the oxidative stress of the body (Helmersson and Basu 1999).

Ischemia is a common clinical event. The major part of the tissue damage involved is shown to occur upon reperfusion (Zimmerman 1994, Ward et al. 1994). Reperfusion injury is manifested by an increased microvascular permeability and edema. Ward and colleagues (1995) studied isolated rat hearts in reperfusion and observed endothelial swelling of capillaries under electron microscopy. This syndrome is mediated by activated leukocytes (Bengisun et al. 1997) and the degree of leukocyte activation is related to the extent of skeletal muscle infarction sustained during reperfusion (Cambria et al. 1991). Thus interventions, which inhibit leukocyte activation, have been shown to reduce muscle infarct size (Williams 1996).

8-Iso-PGF2α excretion is increased, in addition to reperfusion, also in association with vascular risk factors, including diabetes, hypercholesterolemia and cigarette smoking (Patrano et al. 1997). Other factors such as sex, age, hypertension and obesity are of minor importance (Oguogho et al. 2000). Cigarette smoking increases 8-iso-PGF2α excretion significantly (Chehne et al. 2001), but after cessation of smoking this excretion shows a rapid decrease within a few days (Oguogho et al. 2000). Thereafter, a further continuous decrease is seen, reaching a steady state after about 4 weeks (Pilz et al. 2000).

Isoprostanes have also been found in human atherosclerotic lesions. Pratico and associates (1997) observed them in plaques but not in vascular tissue devoid of atherosclerosis. Oguogho and colleagues (1999) report the same finding. They suggest that oxidation injury, which is detected by an increased 8-iso-PGF2α excretion, may play a relevant role in atherogenesis.

8-Isoprostaglandin F2α and bypass operations

No studies evaluating 8-iso-PGF2α excretion in association with bypass operations are available. However, in animal studies the restoration of blood flow after lower limb ischemia
is seen to be followed by the reperfusion syndrome. Goldman and group (1992) analysed the limbs of rabbits after 3 hours of ischemia and found high plasma levels of leukotriene B4 during reperfusion. They and other authors (Kirschner et al. 1997, Dempsey et al. 1986) have proposed that leukotriene B4 is one of the main mediators of the reperfusion syndrome. Also decreased nitric oxide production has been proposed to contribute to impaired blood flow during reperfusion (Bleba et al. 1996), but 8-iso-PGF2α excretion is considered to be the most reliable index for oxidative damage (Pratico 2001).

The available studies are focused on treatments designed to avoid the revascularisation syndrome. These include the administration of diuretics, bicarbonate, free radical scavengers or dialysis. Also so-called “controlled limb reperfusion” has been used (Defraigne et al. 1997). This means that after embolectomy the blood is mixed with a crystalloid solution to obtain hyperosmolar and alkalotic perfusate. The solution is injected with a pump for 30 minutes into the superficial and deep femoral arteries to avoid the reperfusion syndrome. Dialysis was tested by Magnoni and associates (1996). They had 5 patients with severe lower limb ischemia due to aortic occlusion. After embolectomy the patients received local hemodialysis, which reduced mortality, the amputation rate and the incidence of the reperfusion syndrome.

As a whole, 8-isoprostaglandin F2α excretion in association with lower limb ischemia constitutes a practically unstudied area.
AIMS OF THE PRESENT STUDY

The main purpose of this study was to evaluate the role of eicosanoids in the pathophysiology of lower limb ischemia. The specific aims were to investigate:

1) Whether prostacyclin and thromboxane A2 productions and their ratio are altered in acute and chronic lower limb ischemia (Study I).

2) How percutaneous transluminal angioplasty affects prostacyclin and thromboxane A2 productions in lower limb ischemia (Study II).

3) Whether leukotriene E4 production is altered in acute and chronic lower limb ischemia (Study III).

4) How surgical revascularisation of an ischemic lower limb affects leukotriene E4 production (Study IV).

5) Whether 8-isoprostaglandin F2α excretion, as a marker of oxidative stress, is altered in chronic lower limb ischemia and how surgical revascularisation affects its production (Study V).
PATIENTS AND METHODS

Patients

The characteristics of patients are seen in the table below:

<table>
<thead>
<tr>
<th>Study</th>
<th>n (acute ischemia)</th>
<th>males</th>
<th>females</th>
<th>age</th>
<th>ABI</th>
<th>measurements</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>58-66</td>
<td>-</td>
<td>PGI2 and TXA2</td>
</tr>
<tr>
<td></td>
<td>10 (chronic ischemia)</td>
<td>10</td>
<td>-</td>
<td>63-82</td>
<td>0.40-0.71</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>10 (chronic ischemia)</td>
<td>9</td>
<td>1</td>
<td>58-84</td>
<td>0.33-0.60</td>
<td>PGI2 and TXA2 after PTA</td>
</tr>
<tr>
<td>III</td>
<td>11 (acute ischemia)</td>
<td>5</td>
<td>6</td>
<td>61-93</td>
<td>-</td>
<td>LTE4</td>
</tr>
<tr>
<td></td>
<td>8 (chronic ischemia)</td>
<td>8</td>
<td>-</td>
<td>60-84</td>
<td>0.40-0.71</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>10 (chronic ischemia)</td>
<td>4</td>
<td>6</td>
<td>61-78</td>
<td>0-0.64</td>
<td>LTE4 after revascularisation</td>
</tr>
<tr>
<td>V</td>
<td>10 (chronic ischemia)</td>
<td>4</td>
<td>6</td>
<td>61-78</td>
<td>0-0.64</td>
<td>8-iso-PGF2α after revascularisation</td>
</tr>
</tbody>
</table>
Study I

Patients with acute (n = 10) and chronic (n = 10) lower limb ischemia were evaluated. The acute group consisted of 4 males and 6 females (age 58-86, mean 72). They had sustained a sudden onset of symptoms (duration 3 -48 h) and urine samples were collected in the emergency room before any medical procedures. The etiology was evaluated as an embolus in four patients and thrombosis (acute on chronic ischemia) in six. All patients underwent either an endovascular (thrombolysis) or a surgical (embolectomy) intervention.

The chronic group comprised ten males (age 63 - 82, mean 68), who were electively hospitalised in the department of vascular surgery for a diagnostic angiography because of claudication. Walking distance was 10 to 500 meters and ankle-brachial indices varied between 0.40 - 0.71.

In both groups patients with a positive history of anti-inflammatory analgetics (also low-dose ASA) within 2 weeks were excluded.

Study II

The material here comprised 10 patients who suffered claudication (9 males, one female, aged between 58 -86 years, mean 74). Walking distance was 10 to 500 meters and ankle-brachial indices varied between 0.33 - 0.60. All patients attended the unit of vascular surgery for diagnostic angiography. Four patients had a pure diagnostic angiography and six had angiography followed by PTA. The first urine sample was taken before the angiography and the second the next morning.
Study III

The material in this study consisted of patients both with acute (n = 11) and chronic (n = 8) lower limb ischemia, and healthy controls (n = 17). In the acute group there were 5 males and 6 females (age 61 - 93, mean 78) who suffered a sudden onset of symptoms with a duration of 3 to 48 hours. Urine samples were collected in the emergency room before any medical measures. The chronic group comprised 8 male patients (age 60-84, mean 77) who suffered claudication. Ankle-brachial indices varied between 0.40 - 0.71 and walking distance was from 10 m to 500 m. Overnight urine (12 h) samples were taken before angiography. Healthy controls were males aged 22-31 years and their samples were likewise overnight urine samples.

Study IV

This material consisted of patients who had chronic lower limb ischemia (n = 10, four males, six females, aged 61 - 78, mean 72). They were hospitalised in the department of vascular surgery to undergo surgical revascularisation. Five patients had a painful toe ulcer, two had toe gangrene and three had claudication. All had a stenosis or an occlusion either in the aortoiliac or the femoropopliteal region. Ankle-brachial indices ranged between 0- 0.64. Six patients were operated by femoropopliteal reconstruction, two by femorofemoral reconstruction, one by aortobifemoral reconstruction and one femoral endarterectomy.

Preoperative urinary samples were overnight urine (12 h) taken a day before the operation. Postoperative samples were taken on the second postoperative day (35 -48 hours after the end of surgery).
Study V

The material in this case comprised the same 10 patients with lower limb ischemia as in Study IV and there was also a control group (n = 10, healthy volunteers, 6 males, 4 females, age 60 - 82, mean 69).
Methods

Urinary 2,3-dinor-6-keto-PGF1α and 11-dehydro-TXB2 were measured after selective solid-phase extraction by RIA (Riutta et al. 1994, Riutta et al. 1992). The values were correlated to urinary creatinine excretion, measured by the picric acid method based on the Jaffe reaction using a commercial assay kit (Orion Diagnostic, Espoo, Finland).

Urinary leukotriene E4 and 8-iso-PGF2α were measured after solid-phase and HPLC purifications by radioimmunoassay (RIA) and their excretions were correlated to urinary creatinine excretion.

Statistical analysis was by the Mann-Whitney U-test and Pearson’s correlation coefficient as appropriate. The values are expressed as mean (SEM). Probabilities less than 0.05 were accepted as significant. Analyses were made using SPSS software package (SPSS incorporation, USA). The studies were approved by the Ethical Committee of Tampere University Hospital.
### RESULTS

The main findings are presented in the following tables:

#### Study I: PGI2 and TXA2 in acute compared to chronic lower limb ischemia

<table>
<thead>
<tr>
<th></th>
<th>PGI2</th>
<th>TXA2</th>
<th>PGI2 / TXA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>acute ischemia</td>
<td>increased 2-fold</td>
<td>increased 2-fold</td>
<td>not changed</td>
</tr>
<tr>
<td>chronic ischemia</td>
<td>comparison group</td>
<td>comparison group</td>
<td>not changed</td>
</tr>
</tbody>
</table>

#### Study II: PGI2 and TXA2 in association with PTA

<table>
<thead>
<tr>
<th></th>
<th>PGI2</th>
<th>TXA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-PTA</td>
<td>comparison group</td>
<td>comparison group</td>
</tr>
<tr>
<td>post-PTA</td>
<td>not affected</td>
<td>increased 2-fold</td>
</tr>
</tbody>
</table>

#### Study III and IV: Leukotrienes in association with lower limb revascularisation

<table>
<thead>
<tr>
<th></th>
<th>LTE4</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-operative</td>
<td>increased</td>
</tr>
<tr>
<td>post-operative</td>
<td>not affected</td>
</tr>
</tbody>
</table>

#### Study V: 8-iso-PGF2α in association with lower limb revascularisation

<table>
<thead>
<tr>
<th></th>
<th>8-iso-PGF2α</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-operative</td>
<td>increased</td>
</tr>
<tr>
<td>post-operative</td>
<td>decreased to normal level</td>
</tr>
</tbody>
</table>
Prostacyclin and thromboxane A2 in acute and chronic lower limb ischemia (Study I)

Both 2,3-dinor-6-keto-PGF1α and 11-dehydro-TXB2 syntheses were increased about twofold in patients with acute lower limb ischemia compared to chronic lower limb ischemia, 2,3-dinor-6-keto-PGF1α excretion being 669 ± 124 ng/g creatinine in patients with acute lower limb ischemia and 327 ± 97 ng/g creatinine in those with chronic lower limb ischemia (p = 0.028). Total 11-dehydro-TXB2 excretion was 2510 ± 907 ng/g creatinine in acute ischemia and 1201 ± 454 ng/g creatinine in chronic ischemia (p = 0.082); PGI2 / TXA2 -ratio did not differ between the acute and chronic lower limb ischemia (0.57 versus 0.42, p = 0.82).

Prostacyclin (PGI2) and thromboxane A2 (TXA2) in association with percutaneous transluminal angiopisty (PTA) (Study II)

The excretion of 2,3-dinor-6-PGF1α was not affected by PTA (pre 263 ± 94, post 352 ± 87 ng/g creatinine, p = 0.46), but 11-dehydro-TXB2 excretion showed a twofold increase after PTA (pre 1770 ± 1285, post 3569 ± 1535 ng/g creatinine, p= 0.03). Thus the PGI2 / TXA2 ratio was altered in the direction of TXA2 formation after PTA (pre 0.77, post 0.41, p= 0.05). Pure angiography did not affect either 2,3-dinor-6-PGF1α (pre 204, post 221 ng/g creatinine, p = 0.47) or 11-dehydro-TXB2 excretion (pre 802, post 1285 ng/g creatinine, p = 0.14).

Leukotriene E4 excretion in acute and chronic lower limb ischemia (Study III)

Leukotriene E4 excretion was increased in both acute (152 ± 38 pg/mg/creatinine, p = 0.001) and chronic (140 ± 45 pg/mg/creatinine, p = 0.001) lower limb ischemia about 7-fold compared with healthy volunteers (22 ± 3 pg/mg/creatinine).

No correlation was found between leukotriene E4 excretion and walking distance and ankle pressures.
Leukotriene E4 excretion in association with revascularisation of an ischemic lower limb (Study IV)

Leukotriene E4 excretion was not significantly altered (preoperative 34.9 ± 7.1 pg/mg/creatinine, postoperative 24.5 ± 4.7 pg/mg/creatinine, p = 0.238) by surgical revascularisation. Revascularisation raised ankle-brachial indices but there was no correlation between leukotriene E4 excretions and these figures.

8-Iso-prostaglandin F2α excretion in association with revascularisation of an ischemic lower limb (Study V)

All revascularisations were successful, and ankle-brachial indices increased from 0-0.6 to 0.4-0.8. The excretion of 8-iso-PGF2α decreased 2.5-fold in the urine samples collected on the second postoperative day, i.e. to the level measured in ten healthy volunteers.
DISCUSSION

In this study we examined eicosanoids in association with lower limb ischemia. Our aim was to establish a theoretical basis for possible new strategies of pharmacotherapy in this condition. Our main findings are summarised in the table below:

<table>
<thead>
<tr>
<th></th>
<th>acute ischemia</th>
<th>chronic ischemia</th>
<th>after PTA</th>
<th>after revascularisation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PGI2</strong></td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>NA</td>
</tr>
<tr>
<td><strong>TXA2</strong></td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>NA</td>
</tr>
<tr>
<td><strong>LTE4</strong></td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>±</td>
</tr>
<tr>
<td>8-iso-PGF2α</td>
<td>NA</td>
<td>+</td>
<td>NA</td>
<td>-</td>
</tr>
</tbody>
</table>

Lower, limb ischemia is the most common problem in a vascular surgeon’s practice (Salenius et al. 1992). Acute lower limb ischemia, which is caused by embolism or local thrombosis in atherosclerotic vessels, continues to carry high morbidity and mortality rates. Mortality rates vary between 15 and 40 % (Cambria et al. 1984, Jivegård et al. 1986) and after operative treatment amputation rates of 30-40 % are reported (Dryiski et al. 1984). Chronic lower limb
ischemia is a common disease and its prevalence in the whole population is estimated to be about 5% in men over 50 years. These patients have a more than doubled mortality rate within five years compared to the general population (Dormandy et al. 1989).

Endovascular and surgical treatments of lower limb ischemia have developed markedly during the last decade. Chronic lower limb ischemia can be managed in many cases by non-invasive treatment, whereas acute lower limb ischemia requires active, invasive intervention. It has been estimated that 35% of patients with acute lower limb ischemia need an emergency operation for limb salvage (Cambria et al. 1984). Thrombolysis is regarded as the initial treatment in many patients (Barr et al. 1991) and it has been shown to reduce the need for surgery (DeMaioRibus et al. 1993), but it alone suffices in fewer than 20% of patients, additional endovascular or surgical interventions being called for (Giddings et al. 1993). In contrast to the development of invasive methods, pharmacotherapy of lower limb ischemia has served only few new strategies. Low-dose acetylsalicylic acid has long been the recommended medication for this condition. However, antiplatelet drugs have no proven effect on limb ischemia itself and no pharmacological agent has proved superior to others (Eur J Vasc Endovasc Surg 2000). Oral anticoagulants are recommended only in hypercoagulable states and the prostacyclin analogue iloprost can be used if revascularisation is not possible and early amputation is still avoidable (Eur J Vasc Endovasc Surg 2000). There is a need to determine whether pharmacotherapy is useful in lower limb ischemia and possibly to find a theoretical basis for new treatments.

Eicosanoids and lower limb ischemia

Eicosanoid biosynthesis (prostacyclin, thromboxane and leukotrienes) has been shown to be altered in many vascular diseases. However, the role of these agents in lower limb ischemia has been little investigated; most studies concern their cardiac functions. In our material (Study I) both prostacyclin (PGI2) and thromboxane (TXA2) excretions were increased in acute lower limb ischemia compared to chronic lower limb ischemia. Other groups have studied chronic lower limb ischemia (FitzGerald et al. 1984, Reilly et al. 1986, Knapp et al. 1988), the excretion of PGI2 has been the same as here and increased compared to healthy volunteers. Thus, PGI2 and TXA2 excretions seem to be enhanced in chronic lower limb
ischemia and this increase is markedly more conspicuous in the acute phase of lower limb ischemia.

This notwithstanding, the balance between these two agents remains unchanged in both types of lower limb ischemia, which would imply the existence of a strong relationship between platelets (TXA2 production) and vessel wall (PGI2 production) even in atheromatous vessels. This may partly explain the controversial results of pharmacotherapy with prostacyclin analogues and thromboxane inhibitors in this condition. On the other hand, since TXA2 excretion is markedly more increased in acute than chronic lower limb ischemia, pharmacotherapy could be more useful in acute cases. To determine the usefulness of PGI2 analogues and TXA2 inhibitors, a study measuring the concentrations of these agents also during and after medication, would be helpful.

The main problem in this study was to find patients with chronic lower limb ischemia, who had not used acetylsalicylic acid within two weeks prior to the study. Near all claudicants in Finland receive antiplatelet medication and when asked about it, an old person cannot be totally sure of his or her medication. Thus, the exclusion criteria are the restricting point in this study.

Leukotrienes (Study III) have not previously been analysed in association with lower limb ischemia. In coronary artery disease their production is seen to be increased (Allen et al. 1998). This parallels to our findings its production being increased in both acute and chronic lower limb ischemia. The situation may be the same in all atherosclerotic diseases. What is reason and what is consequence, remains unclear. However, leukotriene antagonists are suggested at least during reperfusion (Homer-Vanniasinkam et al. 1994, Aktan et al. 1991). More studies evaluating leukotriene antagonists or synthetase inhibitors and possibly measuring the concentrations of leukotrienes during and after medication would be useful. Exclusion criteria in this leukotriene study were easy, because antiplatelet drugs do not affect the lipoxygenase enzyme.

Isoprostanes are a newly found family of compounds, which have not previously been analysed in the context of lower limb ischemia. They are found in atheromatous plaques
(Pratico et al. 1997) but are not seen in vessel wall devoid of atherosclerosis. Thus they are
supposed to have some role in atherogenesis and at least, they are an index of oxidative stress
in the body (Pratico 2001). In our material (Study V) isoprostane excretion was increased in
patients with chronic lower limb ischemia. This may reflect the fact that chronic lower limb
ischemia is associated with some degree of oxidative stress, which would mean that this stress
could be modulated by antioxidant therapy. A study measuring isoprostane excretion during
and after antioxidant therapy would be clarifying.

The weakness of this study was that many cardiovascular risk factors such as diabetes,
hypercholesterolemia and smoking, have been shown to be associated with increased
isoprostane excretion. Our material included both smokers and non-smokers, diabetics and
non-diabetics and patients with high lipid values. Nonetheless the fact that isoprostane
excretion was decreased after revascularisation means that the high isoprostane excretion was
due to lower limb ischemia itself, not due to risk factors.

Eicosanoids and percutaneous transluminal angioplasty (PTA)

Percutaneous transluminal angioplasty (PTA) always causes local endothelial damage, which
has been shown to lead to the activation of platelets (TXA2 excretion) and the endothelium
(PGI2 excretion) (Bride et al. 1988). Restenosis at the site of a dilated plaque is an important
limitation to the long-term benefits of this procedure (Holmes et al. 1984). Platelet activation
is regarded as an important stimulus for subsequent restenosis (Packham et al. 1986). In spite
of this, studies focusing on PTA of peripheral arteries and PGI2 / TXA2 excretions have not
hitherto been available. However, studies concerning coronary artery angioplasties (PTCA)
have been made. Korovesis and associates (2000) and a group under Kesmarky (2001)
collected blood samples from the coronary sinus after PTCA and observed that platelet
activation was decreased after the procedure. Also earlier, Peterson and colleagues (1986)
collected blood samples from patients during PTCA. They observed no change in PGI2 and
TXA2 excretions in uncomplicated patients, but in two patients who required an emergency
coronary bypass operation, a marked increase in TXA2 excretion was seen. This parallels our
own finding (Study I), where the highest increase in TXA2 formation was seen in patients
with acute occlusion of the aorta and the common iliac artery. Mehta and associates (1982)
dilated the umbilical vein and measured an increase in both PGI2 and TXA2 excretions. Recently a group under Onohara studied PTA in two experiments. In 1999 they measured PGI2, but not TXA2, excretion before and after PTA of the common iliac artery in eight patients and saw an increase in PGI2 production. Later, in 2000, they also measured TXA2 excretion and detected decreased platelet activation.

In our material there was an approximately twofold increase in TXA2 excretion, but no significant increase in PGI2 excretion after PTA. Thus, compared to the above-mentioned groups, the results are controversial. The explanation may be that Onohara’s group collected blood samples locally from the common iliac artery, in contrast to our approach where we measured the total urine output of PGI2 and TXA2.

Other confusing factors may also obtain in these studies. In our patients pure angiography (without PTA) did not affect either PGI2 or TXA2 excretions, but Roy and colleagues (1985) measured temporarily increased PGI2 excretion when they administered X-ray contrast media intravenously to healthy volunteers. They also observed that cardiac catheterisation without other interventions increased PGI2 excretion for 2-4 hours. Our urinary samples were collected the next morning (about 18 hours after the procedure) and thus we cannot estimate the immediate effects of PTA on PGI2 and TXA2 excretions. Accordingly, a study with more frequent measurements would clarify the situation.

Eicosanoids and bypass operations

Eicosanoids in conjunction with bypass operations have been evaluated in only a few studies, most of which have dealt with cardiac operations. Only one study (Vesterqvist et al. 1987) has been concerned with non-cardiac operations. The group analysed patients who had a graft replacement of the abdominal aorta and a control group of patients undergoing cholecystectomy. They found a forty-fold increase in PGI2 and a tenfold increase in TXA2 excretions on the first postoperative day in the aorta group. The excretion remained elevated for 6-10 days and the authors concluded that a synthetic graft is a foreign surface for platelets, which are activated to form TXA2 and, as a consequence, also PGI2. The study does not reveal which type of graft (PTFE / Dacron) was used and further studies evaluating different
types of synthetic grafts would clearly be useful.

In cardiac operations both PGI2 and TXA2 excretions are seen to be increased (Teoh et al. 1987). The investigators in question observed that cardiac cannulation caused the increased output, which continued throughout the cardiopulmonary bypass procedure. The cardiac excretion (measured from the coronary sinus) of TXA2 was increased over the postoperative period but PGI2 excretion ceased postoperatively. This led the PGI2 / TXA2 ratio in the direction of TXA2 production, which may have therapeutic implications. We did not measure PGI2 or TXA2 excretions after surgical bypass operations and such studies would be useful.

Leukotrienes in conjunction with a bypass operation have been previously evaluated in one study. Allen and associates (1993) found increased leukotriene excretion before coronary artery bypass surgery and this excretion increased further after the operation. The highest excretion was measured on the second postoperative day and the levels of leukotrienes were decreased on the third postoperative day. Also in our material preoperative values were increased in patients with lower limb ischemia. The mechanism underlying this is unclear, but apparently increased leukotriene excretion is associated with leukocyte activation, which is accordingly a consequence of platelet activation, as Dinerman and colleagues (1988) have shown. Platelets have probably been activated due to atherosclerosis and hence leukotriene excretion may be one marker of atherosclerosis. However, its clinical significance remains obscure and a further study with leukotriene antagonists would be needed.

Isoprostanes and surgical revascularisation

Isoprostanes (8-isoprostaglandin F2α) have not previously been measured in the context of bypass operations. In our material surgical revascularisation significantly reduced their excretion. Our measurements were made on the second postoperative day, which means about 35-48 hours after the end of surgery. As previously shown (Goldman et al. 1992, Kirschner et al. 1997), the reperfusion syndrome is manifested in the first hours of reperfusion. Thus we can draw no conclusions as to the reperfusion syndrome and from the prevention of it. This syndrome is highly complex and its prevention is one of the main objects of current medical studies.
The conclusion from our isoprostane study is that apparently lower limb ischemia itself causes oxidative stress to the body and this stress is reduced by revascularisation. Theoretically this would mean that interventions, which diminish oxidative stress, could be helpful in chronic lower limb ischemia. Antioxidants could thus have some role in the therapy of chronic lower limb ischemia. It has recently been suggested that measurement of isoprostanes can provide a basis for dose selection in studies of antioxidants (Pratico et al. 2001). Further measurements of isoprostanes before and after antioxidant therapy in chronic lower limb ischemia would thus be clarifying.
SUMMARY AND CONCLUSIONS

The purpose of this study was to evaluate the role of eicosanoids in the pathophysiology of lower limb ischemia and possibly find theoretical support for the treatment of this condition. Eicosanoids were evaluated in acute and chronic lower limb ischemia, in conjunction with percutaneous transluminal angioplasty (PTA) and with surgical revascularisation.

The main findings and conclusions were as follows:

1) Prostacyclin and thromboxane A2 synthesis are increased in acute lower limb ischemia compared to chronic lower limb ischemia. The balance between prostacyclin and thromboxane A2 remains unchanged in both acute and chronic lower limb ischemia. Prostacyclin analogues and thromboxane A2 synthase inhibitors may be more useful in acute than in chronic lower limb ischemia.

2) PTA increases thromboxane A2, but not prostacyclin production. This tends theoretical support to the use of prostacyclin analogues and thromboxane A2 synthase inhibitors in conjunction with this procedure.

3) Leukotriene E4 production is increased in both acute and chronic lower limb ischemia, but leukotriene E4 production is not affected by surgical revascularisation in the chronic form. Leukotriene antagonists or synthesis inhibitors could be useful in these conditions.

4) Chronic lower limb ischemia is associated with oxidative stress, which is reduced by surgical revascularisation. Means of preventing oxidative stress may be helpful in chronic lower limb ischemia.
ACKNOWLEDGEMENTS

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I am grateful to former Professor of Surgery Sam T. Lindholm, M.D. for his encouragement to undertake this study and to Professor Markku Järvinen, who made it possible to complete it.

My warmest thanks go to my supervisor Docent Juha-Pekka Salenius, M.D., who introduced me to scientific work and, during all these years, also reminded me to finish this study. Without his support the work would probably never have been completed. Especially I wish to thank Docent Juha Alanko, M.D. who ten years ago took me into his study group and introduced me to leukotrienes, of which I had previously scarcely heard about.

Docent Asko Riutta is one of the few investigators in Finland who has made analyses of eicosanoids, and without his support this study would have been impossible. I also wish to thank Docent Pekka Kuukasjarvi, M.D., who taught me how to write a scientific article and who came up with new ideas on how to investigate eicosanoids.

I am also grateful to Docent Matti Tarkka, M.D., Head of the Department of Thoracic and Cardiovascular Surgery, who provided the facilities for the undertaking.

Finally I owe my gratitude to my wife and colleague Anna-Maija for her encouragement throughout this work.

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