Early outcome and restenosis after coronary stenting:

- Intervention with stent-coatings, antioxidants and radiation

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Contents

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2. List of papers

1. Kuiper KKJ, Robinson KA, Chronos NA, Cui J, Palmer SJ, Nordrehaug JE. Phosphorylcholine-coated metallic stents in rabbit iliac and porcine coronary arteries. Scand Cardiovasc J 1998;32:261-8.

2. Kuiper KKJ, Nordrehaug JE. Early mobilisation after protamine reversal of heparin following implantation of phosphorylcholine-coated stents in totally occluded coronary arteries. Am J Cardiol 2000;85:698-702.

3. Kuiper KKJ, Muna ZA, Erga KS, Dyrøy E, Svendsen E, Berge RK, Nordrehaug JE. Tetradecylthioacetic acid reduces stenosis development after balloon angioplasty injury of rabbit iliac arteries. Atherosclerosis 2001;158:269-275.

4. Kuiper KKJ, Salem M, Gudbrandsen OA, Muna ZA, Berge RK and Nordrehaug JE. Dose-dependent increase of coronary artery intimal thickening after local delivery of the anti-oxidant tetradecylthioacetic acid from stents. Submitted.

5. Kuiper KKJ, Salem M, Rotevatn S, Mills J, Nordrehaug JE. Implementing a best treatment strategy with intracoronary brachytherapy for in-stent restenosis in patients at high risk for recurrence. Cardiovasc Revasc Med In press.

3. List of abbreviations

ACT: activated clotting time

AIFAI: anti-inflammatory fatty acid index

CABG: coronary artery bypass surgery

ICBT: intracoronary brachytherapy

ISR: in-stent restenosis

LAD: left anterior descending artery

LCx: left circumflex artery

MACE: major adverse cardiac events

QCA: quantitative coronary angiography

PC: phosphorylcholine

PCI: percutaneous coronary intervention

RCA: right coronary artery

SMC: smooth muscle cell

TTA: tetradecylthioacetic acid

4. Introduction

Since its introduction in 1977 (1;2), percutaneous coronary intervention (PCI) has become the most frequent used treatment of symptomatic coronary artery disease with high procedural success (3) and advantages of short hospital stay and quick return to normal daily life activities. In selected cases PCI is performed as an outpatient procedure.

Initial treatment with balloon angioplasty was limited by restenosis (35-45%) due to residual stenosis, early elastic recoil, negative remodeling and to some degree neointima formation. The high number of acute closure (5-8%) was another limitation, but this was dramatically reduced by the introduction of coronary stents, nearly abandoning the need for emergency coronary artery bypass surgery (CABG).

Two important clinical problems still remain with the use of stents: subacute stent thrombosis and in-stent restenosis (ISR). Subacute stent thrombosis is a life threatening condition, but has been reduced to about 0.5% of patients using modern antithrombotic medication (4). Stents also reduce restenosis compared to balloon angioplasty (5-11), but restenosis rates in patients receiving stents may still be 20-40%. In-stent restenosis is caused by proliferation of intima inside the stent and effective treatment requires prevention or suppression of this intima response.

4.1 Pathophysiology of restenosis

The vascular response to balloon angioplasty injury consists of four phases: thrombus formation, inflammation, proliferation and arterial remodeling. The luminal enlargement is caused by stretch of the entire arterial wall, while there is little compression of the atherosclerotic plaque (12;13). As a consequence of this stretch, variable degrees of dissection and dehiscence of the media as well as fracture and separation of the media from the adventitia evolve. Subsequent to the angioplasty injury there is an exposure of thrombogenic surface. Circulating inflammatory cells

(neutrophils, lymphocytes and monocytes) adhere to the injury site and migrate into the thrombus (14;15). These cells release growth factors like platelet derived growth factor, promoting smooth muscle cell (SMC) migration and proliferation and modulating the transition of SMC from a quiescent into mitotic phase. Macrophages produce matrix-degrading metalloproteinases. Inhibition of matrix-degrading metalloproteinases reduce the number of SMCs migrating into the intima (16;17). Several studies have demonstrated that proliferating adventitial cells also migrate into the intima (18;19). The role of the adventitia and collagen in the remodeling of the arterial wall has been increasingly recognized and geometric remodeling is accepted as a more important determinant of late loss than neointimal formation after balloon angioplasty (20).

4.2 Treatment of restenosis: pharmacological approach

Several animal models have been used to study a variety of pharmaceutical agents to reduce restenosis after angioplasty. Most often used are the rabbit iliac and porcine coronary models. An overstretch injury is performed by balloon angioplasty or stent implantation to either the iliac arteries in rabbit or coronary arteries in swine. A number of agents have been reported to have a beneficial effect on restenosis in experimental studies (21;22). In human trials most of these agents failed to influence on restenosis (23-27). This lack of effect may be explained by dissimilarity between the experimental models and the clinical situation and by differences in concentrations of the agents used in animal studies (28).

4.3 Treatment of restenosis: mechanical devices

A larger final minimal lumen diameter after angioplasty is related to a lower restenosis rate. Mechanical removal of the plaque (atherectomy) can be achieved by directional coronary atherectomy (DCA), by rotational atherectomy (RA)

(Rotablator) or by laser-assisted angioplasty. Implantation of stents provides a larger final lumen diameter by compressing the atherosclerotic obstruction.

DCA removes tissue by directional cutting of the plaque, followed by balloon angioplasty. DCA is superior to balloon angioplasty in selected patients with discrete lesions when a final stenosis less than 10 % is obtained (29). A study comparing DCA prior to stent implantation with stent implantation alone showed advantages of the DCA treatment only in bifurcational stenosis (30). Overall restenosis rate was not reduced by DCA, while complications were reported to be more frequent (29). Therefore, routine use of DCA before stent implantation is not recommended.

The rotational atherectomy device removes tissue by pulverizing inelastic tissue like calcified plaques by using a high speed rotating burr. Adjunctive angioplasty is used to optimize final lumen diameter. Several studies comparing RA and angioplasty have been carried out, but restenosis rate after 6 month was not significantly different (31). Rotational atherectomy is nowadays restricted to cases of heavily calcified lesions, ostial lesions or selected bifurcational lesions (32).

Laser atherectomy devices ablate tissue by delivering laser energy. Two types of laser systems exist: the ELCA system and the Ho:YAG system, using laser light with a different wavelength. Reduction in restenosis rates have not been demonstrated using laser atherectomy combined with angioplasty compared to balloon angioplasty alone (31;33;34).

Coronary stents are endoluminal devices, initially introduced to treat threatening acute closure of the vessel by scaffolding large dissections. Stents have also shown to reduce restenosis not only in non-complex lesions (5;6), but their superiority over balloon angioplasty is also evident in saphenous vein graft lesions (7), chronic total occlusions (8;9), and ST-segment elevation myocardial infarction (10;11).

The vascular response after stent implantation is different from balloon angioplasty. Stenting results in larger acute gain in luminal diameter and prevents

elastic recoil and negative remodeling of the vessel (35). On the other hand, stents provoke a greater absolute late luminal loss than angioplasty and carry the additional risk of thrombosis. Stents lead to chronic and prolonged damage of the vessel wall, resulting in pronounced neointimal hyperplasia (36). The degree of intimal hyperplasia is dependent on the extent of injury and of the type of stent (36).

4.4 Treatment of in-stent restenosis: intracoronary brachytherapy

In-stent restenosis is caused by neointimal proliferation and effective treatment requires prevention or suppression of this intima response. Current treatments like balloon angioplasty, atheroablative techniques and repeated stenting have not been effective in treating ISR. Intracoronary brachytherapy (ICBT) in patients with prior restenosis undergoing repeat coronary angioplasty has been shown to successfully reduce restenosis (37-41). ICBT given immediately after stent implantation does not prevent the restenosis rate (42;43). Serious concerns of this therapy like late thrombosis, an increased rate of myocardial infarction and restenosis at the edges of the irradiated zones have prevented its widespread use. However, there is clearly a beneficial short-term effect of brachytherapy after in-stent restenosis. Follow-up after the early experience studies suggested a reduced long-term effect (44), but these studies did not include the possible benefits of a broader clinical experience with brachytherapy.

4.5 Prevention of in-stent restenosis: local drug-delivery, stent-coatings

An alternative way of counteracting in-stent restenosis is by reducing the development of neointima. Systemic pharmacological treatment demands high concentrations necessary to obtain sufficient effect locally at the dilated area and this may cause serious side-effects. Local drug delivery has the advantage of achieving high local concentration without systemic side-effects. It offers substantial benefit since the treatment can be delivered over time. However, initial clinical studies with

local drug delivery catheters (InfusaSleeve, Dispatch, Microporous Infusion Catheter) did not show benefit and initial enthusiasm for local drug therapy declined.

Since stents are the only devices to reduce restenosis rates, it seemed logical to deliver agents by stents. Drugs can be attached to bare stents but this concept has not yielded promising results (45). A lot of efforts have been made to develop stents which were coated either by a biocompatible coating reducing neointima by itself or by a coating of synthetic polymers which could serve as a drug carrier. The early development of coated stents was hampered by the exaggerated inflammatory response and neointimal hyperplasia in animal models of synthetic polymers (46-48). Phosphorylcholine (PC) is a naturally occurring phospholipid in plasma membranes of blood cells, rendering them non-thrombogenic. PC derivatives have been reported to be non-thrombogenic coating medical devices (49-51). Phosphorylcholine is a zwitter ion and binds water, which makes the coated surface non-thrombogenic (fig. 1). The biocompatibility of PC makes it interesting as a stent-coating to reduce intimal hyperplasia itself or serve as a drug carrier. The coating binds strongly to metal.

Coatings like carbon, gold and silicon count also as biocompatible, of which gold (52) and carbon have been tested in animal models (53).

4.6 Antioxidants

The angioplasty injury causes expression of reactive oxygen species. They may act as vascular smooth muscle cell mitogens. Animal studies have shown that antioxidants inhibit both cell proliferation and negative arterial remodeling after angioplasty (54-57). Recently it has been shown that the antioxidant probucol reduces restenosis after percutaneous balloon angioplasty in patients (58;59). Probucol also has been reported to reduce restenosis after stent implantation when given as combined treatment with cilostazol (60), while probucol alone does not affect restenosis rate after stenting (61).

Tetradecylthioacetic acid (TTA){CH_3-$(CH_2)_{13}$-S-CH_2-COOH} is a 3-thia fatty acid analogue in which a sulfur atom is located in the 3rd position in the carbon chain from the carboxyl end of a normal saturated fatty acid. The chemical structure of TTA is shown in fig. 2. TTA has been shown to possess antioxidant and antiinflammatory effects, inhibiting the oxidative modification of low-density lipoprotein (LDL) particles in vitro (62). A potential anti-proliferative effect of TTA has been reported (63). This fatty acid analogue may therefore have important actions on the arterial wall and modify the pathogenesis of restenosis.

4.7 Early ambulation

Coronary stents have reduced the risk for acute occlusion after PCI to <1%. Stent thrombosis occurs most often during the first week after implantation but has been reported later during the first weeks and year (64-66). The risk of subacute stent thrombosis is higher in patients with acute coronary syndromes than in elective patients (67). Prolonged hospital stay after the procedure is therefore not indicated in elective patients, provided low risk of bleeding at the puncture site. Bleeding complications are associated with choice of entry site, the diameter of the arterial sheath used, systolic hypertension, the dose of acetylsalicylic acid and the dose of heparin administered during the procedure (68-70). Because of early mobilisation and low bleeding risk, the radial approach has been proposed as the routine entry site. However, loss of pulse after radial cannulation has been described in 3% of the patients, compared to 0% using the femoral artery (71). Radial spasm is another, more frequent complication, causing discomfort and reducing procedural success rate. Heparin reduces intracoronary thrombosis and complications during invasive procedures (72;73). However, prolonged treatment does not improve outcome after angioplasty (74;75) and increases puncture site complications even at low doses (69;70). A number of vascular hemostasis devices (Vasoseal) have been developed to shorten immobilisation after femoral procedures.

Heparin effect can be monitored by activated clotting time (ACT). ACT <250 seconds is associated with an increased risk for complications, which are rare in patients who have an ACT >300 seconds (72;76). After the procedure, femoral sheath removal is not recommended before ACT <175 seconds. Protamine sulphate antagonises heparin and has been used to treat bleeding complications after coronary interventions (77;78). The routine use of protamine after PCI at the end of the procedure could be a non-expensive and easy way to rapid sheath removal, provided it does not increase the number of coronary events such as subacute stent thrombosis. The development of a non-thrombogenic stent coating would in theory reduce the risk of thrombosis in conjunction with the use of protamine at the end of the procedure.

5. Aims of the studies

The two major limitations of bare metal coronary stents have been thrombosis and restenosis. Acute and subacute stent thrombosis are life threatening conditions and its prevention often causes delayed hospital discharge. In-stent restenosis has been difficult to treat in the past. Consequently the aims of these studies were:

5.1 To examine the arterial wall reaction and stenosis development after implantation of phosphorylcholine-coated stents in rabbit iliac and porcine coronary arteries.

5.2 To assess the feasibility of reversing the heparin effect with protamine sulphate after implantation of a phosphorylcholine-coated coronary stent in order to avoid stent thrombosis and to permit future outpatient procedure.

5.3 To assess the vessel wall reaction after balloon angioplasty injury in rabbits receiving oral supplements of the antioxidant TTA and to evaluate the myocardial contents and ratio of omega-3 and omega-6 fatty acids as an inflammatory index.

5.4 To investigate the phosphorylcholine-coating as a carrier for TTA, its uptake and retention of TTA into the vessel wall and the vessel wall reaction to TTA in a porcine coronary model.

5.5 To examine the long-term efficacy of a best-treatment strategy for intracoronary brachytherapy in patients with in-stent restenosis at high-risk for recurrence, in view of the shortcomings of phosphorylcholine, TTA or both to reduce restenosis.

6. Methods

Paper 1, 3 and 4 describe experimental studies, paper 2 and 5 describe clinical studies.

6.1 Experimental studies

To study different treatment strategies interfering with the vessel wall healing response after the angioplasty injury, we used both the rabbit iliac and the porcine coronary model. Injury was performed either by balloon angioplasty or by implantation of a stent.

Procedures were performed at the Vivariet, the animal laboratory of the Haukeland University Hospital. Animals were fed on standard chow diet without cholesterol supplementation and kept under standard environmental conditions.

All animal study protocols were approved by the local ethical committee for animal care and use.

6.1.1 The rabbit iliac model

Chinchilla rabbits Chbb:CH were used in paper 1and 3. Anesthesia was induced by administering diazepam 4 mg/kg intraperitoneally after premedication of Hypnorm® 0.5 mL intramuscularly. Repeat doses of 0.3-0.4 mL of a 1:1 mixture of Hypnorm® and diazepam were administered intraperitoneally if necessary. Heparin (100 IU/kg) was administered intra-arterially to achieve anticoagulation. Balloon angioplasty catheters were advanced to the iliac arteries over a 0.035 mm angioplasty guide wire via an introducer in the carotid artery. Overstretch injury was performed with a handcrimped stent on a balloon catheter (diameter 2.5 mm) or by an oversized angioplasty balloon (diameter 2.5 mm) to both iliac arteries using a pressure manometer to assure accurate and comparable pressures. Angiography was performed

before and after angioplasty using Hexabrix to confirm artery patency. The animals were allowed to recover and follow-up angiography was performed after 4 weeks (paper 1) or 10 weeks (paper 3). Analgetics (Buprenorfin (Temgesic®)) were given for the first 2 to 3 days, penicillin for 3 to 4 days to prevent post-procedure infections. The animals were killed by giving overdose pentobarbital intraarterially and the iliac arteries perfusion-fixed with 2% glutaraldehyde.

6.1.2 The porcine coronary model

In paper 1, both full-grown Göttingen minipigs and juvenile domestic crossbred swine were used, in paper 4 only domestic crossbred pigs. A peroral dose of 330 mg of acetylsalicylic acid was given the night before the procedure Anesthesia was induced by giving ketamine 10 mg/kg (Ketalar®), medetomadine 0.1 mg/kg (Dormitor®) and atropine 0.03 mg/kg intramuscularly. Isoflurane was given in a mixture of O_2/NO_2 (1:1) by nose cone until the animal was sufficiently relaxed to allow endotracheal intubation. Anesthesia was maintained by 1-1.5% isoflurane in O_2/air. Blood pressure, heart rate, ECG and temperature were monitored continuously. During all procedures heparin (100 IU/kg) was administered intra-arterially to achieve anticoagulation. Stents were delivered to the proximal parts of the coronary arteries on balloon catheters of diameter 3.0 mm (paper 1) or 3.5 mm (paper 4) at identical pressures using a pressure manometer. Analgetics (Buprenorfin (Temgesic®)) were given for the first 2 to 3 days, antibiotics (Penicillin/streptomycin combination) for 3 to 4 days to prevent post-procedure infections. Follow-up angiography was performed after 4 weeks. Animals were killed by an overdose of potassium chloride, the heart excised after a midline thoracotomy. The coronary arteries were perfusion-fixed at 100 mmHg using 2% glutaraldehyde or 4% formaldehyde.

6.1.3 Phosphorylcholine-coating

The biocompatibility of the PC-coating was studied in paper 1. The PC-coating is composed of methacrylate polymers which serve as a backbone to which phosphorylcholine is attached. This coating was attached to disarticulated slotted tube stainless steel Palmaz-Schatz stents®, PS 153 (Johnson & Johnson) by BCP Dilatec Inc., UK. A PC-coated and a non-coated stent were randomized to implantation in either iliac artery of 8 rabbits and to the proximal part of the left anterior descending artery (LAD) or the left circumflex artery (LCx) of 10 pigs. Stents were deployed in a standard manner.

6.1.4 The antioxidant Tetradecylthioacetic acid (TTA)

Fourteen rabbits received either peroral supplementation of 800 mg TTA daily or standard diet (paper 3). The animals were pretreated with TTA for three weeks to ensure accumulation in the tissues, according to earlier studies with the antioxidant probucol (58;59). A dose of 800 mg daily was chosen based on earlier experiences with TTA in rats. In these rats a daily dose of 150 mg/kg changed the fatty acid composition of plasma significantly. TTA was dissolved in aceton and sprayed on food pallets. Blood samples were collected at baseline, at the time of the procedure and the time of euthanasia. Balloon angioplasty injury was performed to both iliac arteries in a standard manner.

6.1.5 Local delivery of TTA using phosphorylcholine-coated stent as carrier

The uptake, retention and vessel wall response to TTA-loaded PC-coated stainless steel stents was studied in a pocine coronary model. The drug loading and elution of these stents was also determined in vitro (paper 4). Stainless steel Bio*divYsio*® stents were coated with PC-coating of increased thickness of ca. 100nm (BCP Ltd Inc) (79), necessary to provide a reservoir to hold therapeutical loadings of drugs. The TTA content per stent was determined by gas chromatography after immersion in solutions

of different concentrations of TTA. TTA-loaded stents were then perfused in a dissolution bath to determine the release of TTA.

Bare Bio*divYsio*® stents and Bio*divYsio*® stents loaded with TTA (25, 50 and 100 mg/mL) were randomized to implantation in the right coronary and left circumflex artery in the same pig in 3 series of 6 pigs. Uptake of TTA into and the fatty acid composition of the coronary wall was determined for upto 28 days using the same model and compared to a non-dilated control vessel in the same animal.

6.2 Clinical studies

Two clinical studies were performed on different patient populations (paper 2 and 5). Studies were performed at the Coronary Intervention Center of the Haukeland University Hospital. Patients signed the informed consent and the study protocols were approved by the local ethics committee.

6.2.1 Patient population

In paper 2, all patients undergoing angioplasty to a single totally occluded coronary artery were evaluated for implantation of PC-coated Divysio stents and reversal of heparin by protamine. After succesful opening of the occlusion, fifty patients were included in the study meeting the following inclusion criteria: stable or medically stabilised unstable angina pectoris, reference artery diameter >2.5 mm and the length of the occlusion <15 mm. Patients with a left ventricular ejection fraction below <30%, occlusion of a vein graft or known adverse effects to protamine sulphate were excluded.

In paper 5, patients with symptomatic in-stent restenosis in native coronary arteries or in aortocoronary venous bypass grafts were screened for treatment with intracoronary brachytherapy (ICBT). Patients were selected if they at least had one increased risk criterium for in-stent restenosis: vein graft lesion, ostial lesion, at least one previous treatment for in-stent restenosis in the same location, lesion length >20

mm, reference vessel diameter <3.0 mm or diabetes mellitus. Angiographic entry criteria included diameter stenosis ≥50% within the stent treatment site in vessels that were ≥2.5 to 5.0 mm in diameter. Exlusion criteria were unprotected left main lesion, unsuccessful PCI, acute myocardial infarction within 5 days, severe heart failure (EF <20%), refractory ventricular arrhythmias, and cardiogenic shock. Based on these criteria 48 patients were included in the study. Patients were referred for ICBT also from other interventional laboratories in Norway.

6.2.2 Angioplasty procedure

All angioplasty procedures were performed using 8F catheters via the femoral approach. Patients were pretreated with acetylsalicylic acid for at least 24 hours. After insertion of the arterial sheath, a bolus dose of 7.500 IE of heparin was administered to assure ACT > 300 sec. Clopidogrel treatment was started after conclusion of the procedure in study 2 (heparin reversal) to mimick a fast-track angioplasty as close as possible. Patients referred to brachytherapy started with clopidogrel at least 24 hours before the procedure and continued for 6 months or more to decrease the risk of late stent thrombosis. Standard angioplasty technique was used in both studies. Follow-up angiographies were performed using 6F catheters due to calibration for quantitative coronary angiography (QCA) (80).

6.2.3 Divysio stent

In study 2, a 15 mm PC-coated Divysio stent was implanted at a pressure of >12 atmospheres after predilatation of the occluded artery. The Divysio® stent is a slotted tube laser cut stent composed of stainless steel comprising interlocking arrowhead design. The expanded stent covers 15% of the wall surface and is coated with phosphorylcholine, which adheres strongly to stainless steel. The PC-coating is described under 6.1.3. All the stents were handcrimped on commercially available angioplasty balloon catheters. Additional stents of the same type were implanted to the same lesion if clinically indicated at the operator's discretion.

6.2.4 Protamine sulphate

To study the antithrombotic property of the Divysio® stent, protamine sulphate 25 mg was administered intravenously after successful implantation of the stent. Special care was taken to inject protamine slowly to prevent side-effects.

6.2.5 Intracoronary brachytherapy

Brachytherapy was given as β-radiation, using The Galileo™ Intravascular Radiotherapy System (Guidant Corp). The stenotic segment was dilated carefully by using non-compliant balloon catheters at high pressure. Before delivering radioactive treatment, the angioplastic result was evaluated by angiography and by intravascular ultrasound to eliminate edge dissections, significant residual stenosis and incomplete stent apposition. Radiation therapy was given with a fixed dose of 20 Gy, radiation time was dependent on reference lumen diameter as measured by QCA and intravascular ultrasound (IVUS).

6.3 Imaging methods

6.3.1 Quantitative coronary angiography and intravascular ultrasound

In the rabbit model (paper 1 and 3), angiography was performed in the anterior-posterior view. Frames with optimal opification were selected for quantification. A digital electronic caliper (Sandhill, model EC-1) was used to measure lumen diameter of the iliac artery before dilatation, while maximal lumen diameter, reference diameter and balloon-to-artery ratio were measured after dilatation (81). A balloon angioplasty catheter dilated to rated pressure served for calibration. The contrast-filled balloon was placed on the lower part of the abdomen in the same level of the dilated arteries. This method was validated by repeated measurements of the same balloon catheter expanded to rated pressures (table 1). At follow-up, minimal luminal diameter, reference diameter and stenosis were determined using the same method.

In the pig model (paper 1), single matched 'best views' from the angiography images were selected for digital quantitative analysis. Stenosis diameter (minimal luminal diameter in the stent) and reference vessel diameter were measured, and percent stenosis was calculated. The guiding catheter served for calibration (82).

In both clinical studies QCA was performed using the CAAS II analysis system (Pie Medical BV, Maastricht, Netherlands). Calibration was achieved by measuring the guiding catheter.

IVUS was performed with the Endosonics system, and quantitation performed by a technician blinded to the clinical data (paper 5).

6.3.2 Preparing of the injured vessel segments in the animal studies

To maintain the vessel structure post-mortem, the injured arteries were perfusion-fixed at 100 mmHg with 2% glutaraldehyde (paper 1 and 3) or 4% formaldehyde (paper 4) after flushing with prewarmed (37 ^{0}C) and heparinized saline to clear the blood. Non-stented vessels were embedded in paraffin after dehydration in graded ethanol series, cut into sections and stained with hematoxylin-eosin or Verhoef-van Giesen elastin. Stented vessels were processed in methyl-methacrylate to solid blocks. A diamond-tipped rotary saw (Isomet 4000, Buehler) was used to cut the blocks into 100 μm cross-sections throughout the whole length of the stent. The sections were glued to slides and ground to 30 μm with the Metaserv 2000 grinder and grounded further to 10 μm and polished with the Biothin grinder (Buehler) (83).

In paper 4, stented vessels were excised to determine uptake of TTA into and fatty acid composition of the coronary wall. The stents were then cut longitudinally and the tissue on both sides of the stent was removed and put in separate tubes for further analysis. Non-dilated vessels served as control. The samples were immediately frozen in liquid nitrogen and stored at –70 ^{0}C.

Myocardium and liver tissue (paper 3) were excised to study the effects of peroral substitution of TTA on fatty acid composition in rabbits. All samples were immediately frozen in liquid nitrogen and stored at –70 ^{0}C.

6.3.3 Histomorphometry

Histomorphometry was performed blinded to the randomisation after digital transition using computer-assisted planimetry (Leica Q500MC, software Qwin 01.02 in paper 3 and analySIS vs 3.2, Soft Imaging System in paper 4). In balloon injured vessels (paper 3), areas surrounded by endothelium (lumen area), the internal elastic lamina and by the external elastic lamina were traced. Neointima was defined as the area between the lumen and the internal elastic lamina. Vessel area was defined as the area within the external elastic area. In sections of the stented segments (paper 1 and 4), neointima was defined as the area between the lumen and the stent struts. The extent of arterial injury at each stent strut was determined according to the score proposed by Schwartz et al (84). Morphologic area stenosis was calculated as 100 x (1-Stenotic lumen area/Original lumen area). Stenotic lumen area was defined as the lumen vessel area, original lumen area as the area within the internal elastic lamina (paper 4).

6.4 Statistics

All data were presented as mean ± S.D for continuous variables and as n (%) for categorical data.

Student's t-test for unpaired data was used to compare groups with normally distributed data. This test was used in paper 1 to compare angiographic measurements between the PC-coated and non-coated stents. In paper 3 and 4 these observations were tested for significant differences between the TTA-group and the control group.

Non-normally distributed data were analyzed by Mann-Whitney's test, f.i. to compare histological data in paper 1.

Student's t-test for paired variables was used to compare changes within the groups. Examples are changes in luminal diameter for a stent after dilatation and at follow-up (paper 1, paper 3) and changes in left ventricular ejection fraction and exercise tests before and after PCI (paper 2).

A causal connection between variables was tested by Pearson's correlation test or linear regression analysis. An example is the relation between angiographic minimal lumen diameter and histological lumen area (paper 3) in rabbits.

Logistic regression analysis was performed to assess independent risk factors for restenosis and major adverse coronary events (paper 5) during follow-up after intracoronary brachytherapy.

In the same study, survival analysis was conducted using Kaplan-Meier method.

All data analysis was performed using the SPSS program for Windows (versions 8.0, 11.0 and 13.0).

7. Summary of the results

Paper 1

In this paper the vessel wall response to PC-coated stents was studied in the rabbit iliac model (n=8) and porcine coronary model (n=10). A PC-coated or a non-coated stent was deployed in either iliac artery of rabbits and in the left anterior descending artery and left circumflex artery of pigs. During follow-up no thrombotic stent occlusion occurred in the rabbits, but occlusion of a non-coated stent caused sudden death in one pig. After 4 weeks angiography in rabbits showed stent minimal luminal diameter 2.25±0.20 mm for the PC-coated stents versus 2.20±0.18 mm for the non-coated stetns (p=NS), while histomorphometry showed no significant differences in intimal area, maximal intimal thickness nor luminal area between the two groups. Follow-up angiography in swine showed stent minimal luminal diameter was 2.74±0.65 mm for the PC-coated group versus 2.55±0.73 mm for the non-coated stent group (p=NS) at 4 weeks. Intimal area was 1.51±0.97 mm^2 versus 1.34±0.55 mm^2, maximal intimal thickness 0.44±0.27 mm versus 0.47±0.38 mm, luminal area 2.67±1.03 versus 2.42±1.27 mm^2 (all p=NS) for coated and non-coated stent groups. In these two animal models, the PC-coating does not provoke arterial neo-intima formation nor decrease luminal diameter compared to stainless steel, while the coating does not seem to reduce restenosis. Its potential as a drug carrier should be further explored.

Paper 2

Immediate removal of the femoral artery sheath after coronary angioplasty may allow rapid mobilisation and reduces the number of in-hospital days. We studied the early and one month clinical and angiographic follow-up of patients having heparin antagonised with protamine after implantation of PC-coated metal (Divysio®) stents, followed by removal of the femoral artery sheath. Fifty patients (37 male, mean age

59±10 years) with stable angina pectoris and a single totally occluded artery (1 unprotected left main stem, 15 LAD, 11 LCx, 23 RCA) underwent coronary angioplasty. Angiography was performed after 30 minutes and 1 month. One stent thrombus was detected after 30 minutes and was treated by balloon dilatation. One patient underwent emergency bypass surgery for non-stent related problems. Fourty-six patients were mobilised after 5 hours, 2 patients after >5 hours. At one month there had been no major coronary endpoints, rehospitalisations, groin bleeding, or more thrombi. One episode of transient pulmonary oedema occurred after protamine injection. In conclusion, heparin may be reversed by protamine sulphate after the implantation of PC-coated stents without increased risk of stent thrombosis. It is not known if this also applies for uncoated metal stents.

Paper 3

Tetradecylthioacetic acid (TTA) is a synthetic long-chain fatty acid analogue that inhibits the oxidative modification of low-density lipoprotein particles *in vitro*. We examined the influence of TTA on the arterial wall response after balloon angioplasty injury in a rabbit iliac model. Fourteen rabbits were randomised to receiving either TTA fatty acids 800 mg daily perorally or to normal diet. Angioplasty was performed via right carotidotomy on both iliac arteries using an oversized balloon catheter, the TTA group being pretreated for 3 weeks. At 10 weeks follow-up angiography, minimal luminal diameter was 1.64±0.27 versus 1.13±0.52 mm for the TTA and control groups respectively ($p<0.05$). Histomorphometry did not show significant differences in intimal hyperplasia between the two groups (maximal intimal thickness 0.19±0.10 versus 0.22±0.04 mm, p=NS and intimal area 0.32±0.12 versus 0.36±0.23 mm^2, p=NS for the TTA and the control groups respectively). Remodeling was 0.52±0.32 versus 1.03±0.47 mm respectively ($p<0.05$). In the heart, the sum of the n-3 fatty acids was 8.9±2.7 in the TTA group versus 4.3±0.2 mol% in the control group ($p<0.05$). The anti-inflammatory fatty acid index, calculated as (22:5 n-3 + 22:6 n-3 + 20:3 n-6)/ 20:4 n-6, was 0.76±0.10 versus 0.25±0.03 for the TTA and control groups

respectively ($p<0.05$). *In vitro* TTA (100 μM) reduced the proliferation of human smooth muscle cell by more than 50%.

Paper 4

In this report the coronary wall response of a phosphorylcholine-coated stent loaded with the antioxidant Tetradecylthioacetic acid (TTA){CH_3-$(CH_2)_{13}$-S-CH_2-COOH} and the uptake of TTA in a porcine coronary artery injury model were studied. Eighteen-millimeter long premounted phosphorylcoline-coated stents were loaded with 3 different concentrations of TTA (25, 50 and 100 mg/mL) in series of 6 pigs each (*Sus scrofa*, weight 38.5±7.2 kg). A PC-coated and a TTA-loaded PC-coated stent were randomised to implantation in the right coronary and left circumflex artery in the same pig. The stents were removed after 4 weeks and prepared for histomorphometry. Uptake of TTA into the coronary wall was measured after 24 hours, 7 days, 14 days or 28 days (2 pigs at each time point, *Sus scrofa*, weight 44.3±7.3 kg) using the same model. TTA content per stent ranged from 32±20 μg/stent using a solution of 25 mg/mL TTA concentration to 173±17 μg/stent using a 100 mg/mL TTA concentration. The total amount left on the stent decreased from 100 μg/stent to 40 μg/stent after 1 hour, to 6 μg/stent after 8 hours, whereas nearly all of the loaded TTA was eluted after 48 hours. During follow-up no death or subacute stent thrombosis occurred. Percent area stenosis was significantly higher in the TTA-eluting stent group, 35.2±20.9 vs 27.5±17.0 %, p=0.03). Dose-related comparison showed a significantly increased intimal thickness (0.66±0.53 vs 0.29±0.26 mm, p=0.008) and intimal area (2.83±1.61 versus 1.58±0.91 mm2, p=0.004) for the 100 mg/mL TTA stent group compared to the non-eluting stent group. TTA could be demonstrated in the vessel wall for up to 4 weeks. The anti-inflammatory fatty acid index (AIFAI), calculated as (docosapentaenoic acid + docosahexaenoic acid + dihomo-linolenic acid)/arachidonic acid, was suppressed to 0.65±0.27 compared to 1.13±0.23 in control vessels ($p<0.001$). This was mainly due to a significant increase of arachidonic acid. TTA caused a dose-dependent intimal thickening and reduced

anti-inflammatory index contrary to expectations. Antioxidants may change property to pro-oxidants when used as a stent coatings.

Paper 5

In this study the efficacy of best treatment with intracoronary brachytherapy for in-stent restenosis was evaluated in patients at high-risk for recurrence. Forty-seven patients with symptomatic in-stent restenosis with at least one or more increased risk criterion for recurrence were treated with beta-radiation. The patients received best treatment based on avoidance of previously reported procedural risk factors for recurrence (incomplete stent apposition, dissection, geographical miss, damage to the non-injured vessel segment), deferring brachytherapy when provisional stenting was performed, the use of beta-radiation dose of 20 Gy and clopidogrel for at least 6 months**.** Treatment was successful for all patients without in-hospital complications. Brachytherapy increased the total intervention procedure-time by 15±10 minutes. The in-stent restenosis length was 25.4±11.5 mm. The angiographic minimal luminal diameter after the intracoronary brachytherapy was 2.24±0.43 mm versus 0.75±0.58 mm at baseline ($p<0.05$). At 9-month follow-up minimal luminal diameter was 1.93±0.48 mm ($p<0.05$ versus minimal luminal diameter at baseline). Binary restenosis was detected in six patients (13%). Target lesion revascularisation or target vessel (non-lesion) revascularisation was performed in seven patients (15%). No death, new myocardial infarctions or subacute stent thrombosis were observed. During 29.7±9.3 months follow-up, target lesion revascularisation or target vessel (non-lesion) revascularisation was performed in 17 patients (36%). Only one patient (2%) suffered from new myocardial infarction and no deaths were observed. Adoption of a best-practice protocol for the use of ICBT to treat in-stent restenosis can result in a safe and effectice clinical and angiographic outcome. However, long-term results may be a limitation to a widespread use of this method.

8. General discussion

The implantation of a stent is widely accepted as the most important way of reducing restenosis after percutaneous catheterbased treatment of stable or unstable coronary artery syndromes. In this thesis experimental and clinical studies assessed various interventions to further improve the results after metal stent implantation. The first step was to evaluate phosphorylcholine as a stent coating because of its two potential advantages, biocompatibility and reduced thrombogenicity. However, we found that it had no potential to reduce restenosis, but may be used with intravenous protamin sulphate injection to facilitate early arterial sheath removal and patient ambulation, without increased risk for stent thrombosis.

Phosphorylcholine also has the potential to carry other agents that may be effective to prevent restenosis. With this in mind, an antioxidant with antinflammatory action (TTA) was assessed for potential action on both main mechanisms of restenosis, remodeling and wall thickening. TTA was effective to prevent restenosis as demonstrated by its effect on remodeling, but had no efficacy as a stent coating.

Finally, brachyterapy does not prevent restenosis, but reduces restenosis during short-term follow-up, however the effect seems to diminish long-term. Studies are often performed when many investigators are in a learning phase, and the technique improves with time. Our best treatment strategy using brachytherapy did however not reduce cardiac events during follow-up.

8.1 Methodological aspects

8.1.1 Animal models

In study 1 and 4 we used the porcine coronary injury model, the animal model of angioplasty and stenting most often applied to study restenosis (84;85) and to predict

response of new treatments in humans. The porcine coronary artery vasculature resembles that of humans in distribution and diameter, although pigs have fewer collaterals and the right coronary artery is less often dominant. The healing process after vascular injury in swine is comparable to humans, but the time course is much shorter in pigs. Neointima formation is formed typically during the first 28 days, whereas the same process takes 6-12 months in humans (86;87). The atherosclerotic process, causing coronary obstructions might explain the delayed healing process in humans. Stents are placed overlying plaques, which contain necrotic material with few smooth muscle cells and therefore might prolong recruitment of smooth muscle cells (88). Inflammation may play an additional role. In contrast, stents are implanted in normal arteries of non-adult pigs. The difference in life span might also play a role: human beings have an expected life age/span of more than 70 years, pigs approximately 16 years and rabbits 5-6 years.

The rabbit iliac injury model was the other model we applied (paper 1 and 3) to study vessel wall healing. The rabbit model is easier to handle, has lower cost and has also been studied extensively. As in the porcine model the healing process after injury is usually completed after 4 weeks, but less intima and more matrix is formed (paper 1). Breaks in the internal elastic lamina after angioplasty are uncommon since iliac arteries are elastic arteries while coronary arteries in human and pigs are muscular arteries.

A comparison of the neointimal area, as found in the papers 1, 3, 4 and another study published by our group (89), elucidates this clearly (fig. 3). The intimal response after balloon angioplasty in rabbits (paper 3) is less than after balloon angioplasty in pigs (89). Stenting provokes a stronger intimal reaction, but is most pronounced in pig coronaries (paper 1 and 4).

Injury with an oversized balloon angioplasty catheter causes disruption of the internal elastic lamina with dissection into the media. After implantation of a stent, penetrating struts into the media cause a chronic inflammatory reaction (90). The increase of intimal thickness depends on the depth of the injury (36).

Animal models have been shown to be valuable in predicting clinical outcome of new treatment modalities. Negative responses in pigs correspond well to negative clinical trials, suggesting the model has good specificity. On the other hand, promising experimental studies do not always predict positive clinical trial results, indicating less high sensitivity of the model. Favourable results of inhibition of neointima in pigs at 28 days after brachytherapy (91), were followed by positive clinical findings after 6 months (40;41). Sirolimus and paclitaxel reduced formation of neointima at 28 days in pigs, this correlated well with promising results in humans (92-97). Many clinical trials of systemic pharmacological treatment have failed after positive experimental results. The large PRESTO-study is a recent example, tranilast not being effective in preventing restenosis, in contradiction to good preclinical and initial clinical results (24;98).

8.1.2 Imaging methods

Angiography has been the gold standard for visualizing vessels but wall pathology can be pronounced before lumen is decreased (99). Intravascular ultrasound depicts all the layers of the vessel wall, using high frequency piezoelectric transducers mounted on a 2.9 Fr catheter. Spasm is the most frequent complication related to IVUS in both animal models, although intravasal nitroglycerine reduces spasm. Potential damage to the stented segment is another concern in performing IVUS in all objects in experimental projects (100). Angiography is therefore a useful supplement to histological measurements, evaluating the degree of dilatation after balloon and stent injury and measuring late loss at follow-up. Negative remodeling is the most important factor in reducing vessel lumen after balloon angioplasty (20), while intimal hyperplasia causes lumen reduction after stent implantation (101). Combining angiographic late loss and histological measured intimal thickness gives information about lumen reduction of the vessel. Remodeling can be defined as angiographic late loss minus intimal thickness measured in the histology sections. This method was used in paper 3, validated by the significant correlation between the angiographic and histologic lumen (paper 3, fig. 1).

The concept of remodeling is well demonstrated in our studies. Angiographic late loss is largest in the balloon-injured segments, while it is minimal in the stented segments. Intimal thickness cannot alone explain lumen loss in the angioplasty studies, the difference is explained by negative remodeling of the vessel. In the stented rabbit and porcine arteries, angiographic late loss is comparable with intimal thickness, confirming that stents prevent either positive or negative remodeling (fig. 4).

The injured arteries were perfusion-fixed at 100 mm Hg with the purpose of maintaining the vascular structure as optimal as possible. Two types of fixatives were used. Glutaraldehyde, used in paper 1 and 3, penetrates slowly into the arterial wall but reacts rapidly with proteins and produces a stable fixation. Formaldehyde penetrates quicker into the tissue but reacts slower and more reversible with proteins. By perfusing the vessel with fixatives, both alternatives resulted in good tissue preservation due to the short distances for the fixatives to penetrate from the vessel lumen. Generally, fixation with glutaraldehyde impairs subsequent immunohistochemical staining due to altered properties of the antigens. Thus, formaldehyde fixation was preferred in paper 4 to enable subsequent immunohistochemical analysis, since most proteins preserve their immunoreactivity after fixation with formaldehyde.

Non-stented vessels were embedded in paraffin, which gives a rather weak support to the tissue. This introduces possible artifacts due to compression of the tissue during cutting and to subsequent stretching of the sections, which may result in altered shape and cross-sectional volumes of the studied structures. However, by handling all material in the same manner, the risk for observing false differences between the groups should be minimized.

Stented vessel specimens were embedded in methyl-methacrylate to achieve solid blocks, to enable cutting the stents with a diamond saw.

When assessing histomorphometry it is important to notice that balloon angioplasty injury provokes asymmetrical injury response, especially in pigs. Intimal area is therefore a more correct measure than intimal thickness. Both measurements were used in our studies.

8.2 In-stent restenosis

8.2.1 Shortcomings of coronary stents in clinical use

Coronary stents are effective scaffolding devices to treat large dissections and thereby preventing acute closure in conjunction to angioplasty (102). Stents reduce restenosis in selected cases (5;6), but implantation is complicated with recurrent stenosis after 6 months. To evaluate the long-term effects of coronary stents, we performed a prospective complete follow-up of all patients who had implanted a Palmaz-Schatz bare metal stent at Haukeland University Hospital (103). Patients treated by plain balloon angioplasty with a stent-like result during the same time period served as a control. A single 15 mm length Palmaz-Schatz stent was implanted in 167 patients (group 1), 336 patients underwent balloon angioplasty only (group 2), and 331 patients had deployed a single disarticulated (7 mm length) ("half") Palmaz-Schatz stent (group 3). The indication for stenting was the absence of a stent-like result (dissection or residual stenosis >20%). There were no baseline differences between the groups with respect to age, gender, target vessel, target vessel reference diameter or % stenosis. Lesion length was shorter in group 3. Major adverse cardiac events (MACE) during follow-up were defined as death, acute myocardial infarction (MI), or revascularisaton with PCI or CABG. Total MACE at a mean of 6.6±1.0 years was 34.1%, 31.3% and 33.8% in group 1, 2 and 3 respectively (p=NS) (fig. 5). The percentage of target lesion revascularisation with PCI was 11.4%, 11.9% and 12.1%; CABG 6.0%, 4.8% and 6.6%; acute MI 7.2%, 9.2%, and 7.6%; and death 9.0%, 5.7%, and 7.6%, respectively (p=NS). Thus, long-term prognosis is not different for patients with stents versus a stent-like result after balloon angioplasty. Short

(disarticulated) stents neither improved nor worsened the long-term MACE. These data indicate that stents need to be improved to perform better than balloon angioplasty during long-term follow-up. Effective stent-coatings could be the answer to this.

8.2.2 Stent-coatings: PC-coating (paper 1)

In the first study of this thesis, we evaluated the ability of phosphorylcholine to reduce the formation of neointima when used as stent coating in two animal models. Phosphorylcholine was applied as coating because of its, at that time, theoretical biocompatibility and antithrombotic properties. Many previous stent coatings provoked an intense inflammatory reaction (46). Our findings showed that phosphorylcholine did not have a pro-inflammatory effect, on the other hand it did not reduce neointima formation. An important conclusion therefore was that PC-coating could serve as a carrier of an anti-restenotic drug. Other preclinical (104;105) and clinical studies (106) later confirmed our findings that PC-coated stents did not reduce restenosis rate compared to bare metal stents. Subsequently, PC-coating has only been promoted as a basic stent coating and as a drug carrier.

8.2.3 PC-coated stents and early mobilization (paper 2)

In theory, PC-coating should have favourable non-thrombogenic properties. Study 2 was a prospective, study of consecutive patients having a total coronary occlusion. This was the first study performed in patients in whom heparin was antagonized with protamin after PCI, therefore only patients with a total coronary occlusion were included. Already existing collaterals would reduce the risk of myocardial damage in case of acute stent thrombosis. Patients with an acute coronary syndrome were excluded, since documentation of the favourable effects of continued antithrombotics in these patients is overwhelming (107).

Only one stent thrombosis occurred during 1-month follow-up. No bleedings or major hematomas were observed, while 48 of 50 patients were mobilized the same

day. In a subsequent comparable study of 429 patients, the authors also used protamine to enable immediate femoral sheath removal. Only 6 patients had minor hematomas and 1 patient required repair of pseudoaneurysm (108). Stents (bare-metal) were used in 85% of the patients in this non-randomized study. Eight patients (1.9%) required emergency PCI or CABG prior to 48 hours, but details (e.g. stent thrombosis) were not given. Randomized studies with protamine have not been performed, thus it is not possible to conclude if the use of PC-coated stents would have reduced the number of revascularizations. While treatment effects always have to be confirmed by randomized clinical trials, complications to treatment may be assessed in observational studies. Our observational feasibility study shows that the rate of complications (stent thrombosis) was low using PC-coated stents with protamine. We cannot be sure that PC-coated stents perform better than bare metal stents, a randomized study would have required a very large number of patients. On the other hand, there was no indication of an increased rate of stent thrombosis with this regimen.

Alternative methods of early femoral sheath removal include arteriotomy closure devices like Angio-seal, VasoSeal, Duett and Perclose. The safety of these closure devices versus mechanical compression was newly assessed in 2 meta-analysis both including 30 studies published between 1991 and 2003 (109;110). In none of these meta-analyses the closure devices were more effective than mechanical devices. Groin complications after Vasoseal® vary from 10-34% (111), while only 50% could be mobilised within a few hours after Vasoseal® (112). An important point of concern is the higher cost related to these devices.

8.2.4 Inflammation and restenosis (paper 3 and 4)

Balloon angioplasty and stent implantation both confer injury to the vessel. The endothelial surface is disrupted and denuded, promoting platelet activation, adhesion and deposition causing a fibrin-rich thrombus (113). It is quite clear that inflammation plays an important role in the subsequent wall thickening and stenosis

formation. Platelet activation expresses P-selectin, which mediates the adhesion and infiltration of monocytes into the vessel wall. β2-integrin Mac-1 plays a critical role in the adhesion of leukocytes and platelets. In a rabbit iliac stent model, inflammatory cells were seen immediately after stenting. The concentration of monocytes in the media was found to be proportional with the degree of neointimal thickening (114). Inhibition of β2-integrin Mac-1 reduced intimal thickening after both angioplasty and stenting in rabbits (115). In P-selectin-deficient mice inflammatory cells were not present in the carotid artery wall 4 weeks after ligation (116). Oxidative stress occurs early after angioplasty. Reactive oxygen species like free radicals and hydrogen peroxide are generated by the injured endothelium, activated platelets and neutrophils, leading to release of cytokines and growth factors which stimulate matrix remodeling and smooth muscle cell proliferation. Reactive oxygen species regulate the activity of matrix metalloproteinase, which are involved in matrix remodeling. Oxidative stress is also involved in cross-linking of collagen fibers, which is an important factor in negative remodeling. We demonstrated a reduction in late lumen loss in balloon injured iliac arteries of rabbits that got supplements with the antioxidant TTA.

The anti-inflammatory fatty acid index (AIFAI), calculated as (docosapentaenoic acid + docosahexaenoic acid + dihomo-linolenic acid)/arachidonic acid, was significantly higher in the myocardium of the control group compared to the TTA group. This index reflects the relation between the omega-3 fatty acids possessing anti-inflammatory and anti-thrombotic properties and the omega-6 fatty acids being proinflammatory and prothrombotic (117). A higher index is indicative for an increased anti-inflammatory status. We propose that the AIFAI seems to be a reliable marker of inflammation after PCI as this index was increased in relation to positive remodeling in paper 3 and decreased with increased vessel wall thickening in paper 4.

As demonstrated in paper 1, PC-coating could be used as a drug carrier. Local drug delivery has the advantage of administering high concentration of drugs at the site of injury over time without systemic side-effects. In vitro, TTA had anti-

proliferative properties while TTA reduced the negative remodeling in vivo (paper 3). In the same model, TTA was shown to have anti-inflammatory activity. In vitro testing showed that TTA could easily be bound to and released from PC-coating (paper 4). TTA was demonstrated in the arterial wall for up to 4 weeks, but local delivery of TTA did not reduce the formation of neointima after stent implantation. At the highest concentration, TTA increased intima proliferation compared to PC-coating only. The AIFAI was suppressed in the coronary arterial wall after implantation of TTA-eluting stents compared to controls (paper 4). Thus, when used as a stent coating on a phosphorylcholine-coating, TTA seems to act proinflammatory.

8.2.5 Intracoronary brachytherapy (paper 5)

Intracoronary brachytherapy has been shown to reduce neointimal proliferation in patients with in-stent restenosis, either administered as β-radiation or as γ-radiation. The adverse effect of late stent thrombosis is prevented by the long-term use of clopidogrel as we showed in our study (paper 5). We postponed brachytherapy for a month when a new stent was implanted to permit endothelialization and avoid acute stent thrombosis. By applying careful dilatation within the stent, using IVUS and appropriate positioning of the radiation source, we reduced restenosis to 13%, despite the patients were at high risk of recurrence. The favourable effects of intracoronary brachytherapy have been confirmed after 3 years (38), but an increasing number of target vessel revascularization has been described at 5 years, although the total number of MACE remained lower than in the placebo group (118). This late increase of target vessel revascularization (“late catch-up”) is a point of concern, also because it is known that vessel injury can occur many years after therapeutic radiation exposure (119;120). In animal models, brachytherapy inhibits neointimal formation for up to three months, with evidence of incomplete healing. At six month healing is completed and brachytherapy failed to show benefit, due to neointimal growth (121). Another point of concern is the logistics linked to the intracoronary brachytherapy, requiring the presence of nurses, cardiologists, oncologist and radiophysicist.

Study 5 was designed to test the efficacy of intracoronary brachytherapy in patients with in-stent restenosis at high risk of recurrence eliminating all the factors which can contribute to late failure. An appropriate control arm would have been patients with in-stent restenosis treated with a drug-eluting stent. However, drug-eluting stents were not available in daily clinical practice at the start of the study. On the other hand, a great advantage of this study was that we implemented all the previous published experience with pitfalls of brachytherapy in our protocol (best treatment strategy).

Very recently published studies have shown a favourable effect of both sirolimus and paclitaxel eluting stents compared to intracoronary brachytherapy on clinical as well as angiographic end-points in patients treated for in-stent restenosis (122;123). The rate of MACE after brachytherapy was higher in both studies (21.6% and 20.1%) than in our study (14.9%). Rates of MACE after sirolimus and paclitaxel eluting stents were 12.4% and 11.5%. Thus, the future place for ICBT may still be undecided.

Intima inhibiton of sirolimus-eluting stent is not sustained in the pig model (124). Bearing in mind the diminishing effects of brachytherapy on the long-term, caution is necessitated using drug-eluting stents in human-beings unlimited. Delayed endothelialization might cause late stent thrombosis (125;126), while there is a tendency to use longer stents. Stents initiate a chronic inflammation, but the anti-proliferative effect of drugs could be time-limited. Recent analyses also from registries have in fact suggested a significantly increased rate of late stent thrombosis compared to metal stents (127).

9. Conclusions

The conclusions of the studies in this thesis were:

9.1 PC-coating of stainless steel stents does not provoke arterial neo-intima formation in rabbit iliac and porcine coronary arteries. The coating does not reduce stenosis compared to stainless steel.

9.2 After the implantation of a PC-coated stent, reversal of heparin with protamine sulphate does not cause stent thrombosis in patients with single vessel total occlusions and permits the angioplasty to be performed as an outpatient procedure.

9.3 Peroral supplements with TTA is associated with less neointima formation due to positive arterial remodeling after balloon angioplasty injury in rabbit iliac arteries. Increase of the anti-inflammatory index in the myocardium suggests an anti-inflammatory action of TTA.

9.4 TTA was easily loaded onto a PC-coated stent. TTA loaded Bio*divYsio*® stents did not reduce the vessel wall reaction after implantation in the coronary arteries. There was a significant association between the TTA dose and vessel intimal area. TTA seems to act proinflammatory when delivered to the vessel wall via a metal stent.

9.5 Intracoronary brachytherapy performed according to best treatment criteria confers short-term benefits for the treatment of in-stent restenosis in patients at high risk for recurrence, but an increased long-term recurrence rate may be a limitation.

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Table 1 Repetitive measurements of angioplasty balloon catheter with diameter of 3.0 mm, inflated at 8 atm.

	Day 1	**Day 2**
1st inflation	3.04	3.03
	2.99	2.97
	2.92	2.94
	2.96	3.00
	3.00	2.97
Mean	2.98±0.05	2.98±0.03
Coefficient of variation	1.5%	1.1%
2nd inflation	2.96	2.95
	3.02	2.98
	3.02	2.97
	2.97	2.98
	3.01	3.00
Mean	3.00±0.03	2.98±0.02
Coefficient of variation	0.6%	0.6%
Mean of all measurements	2.98±0.03	
Coefficient of variation	1%	

Figure 1

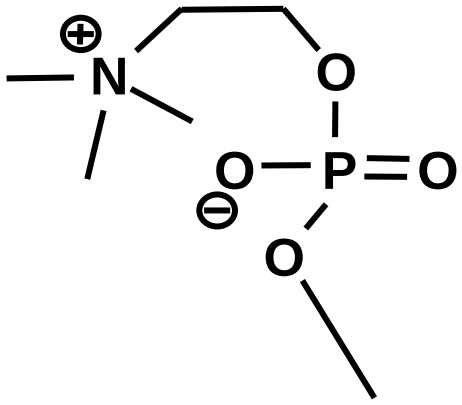


Figure 2

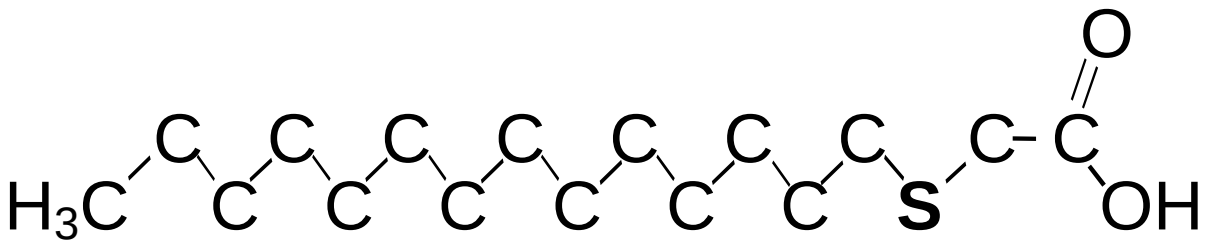
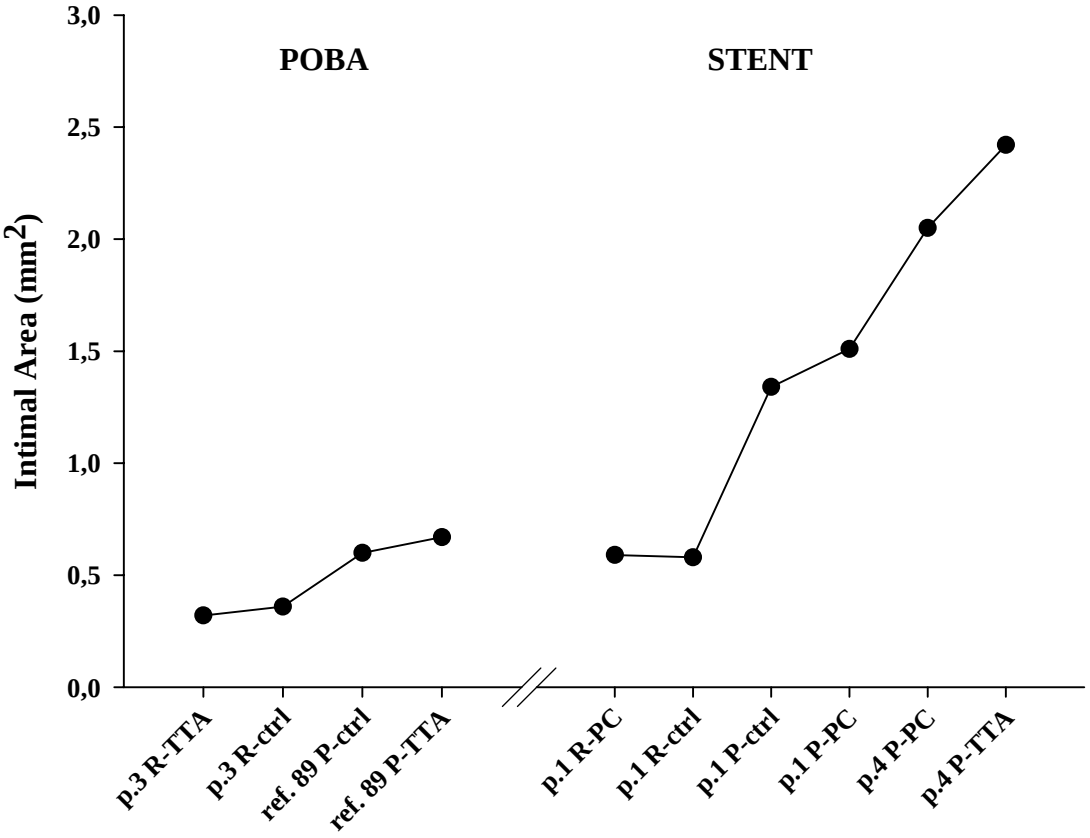


Figure 3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0,0
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p.3 R-ctrl
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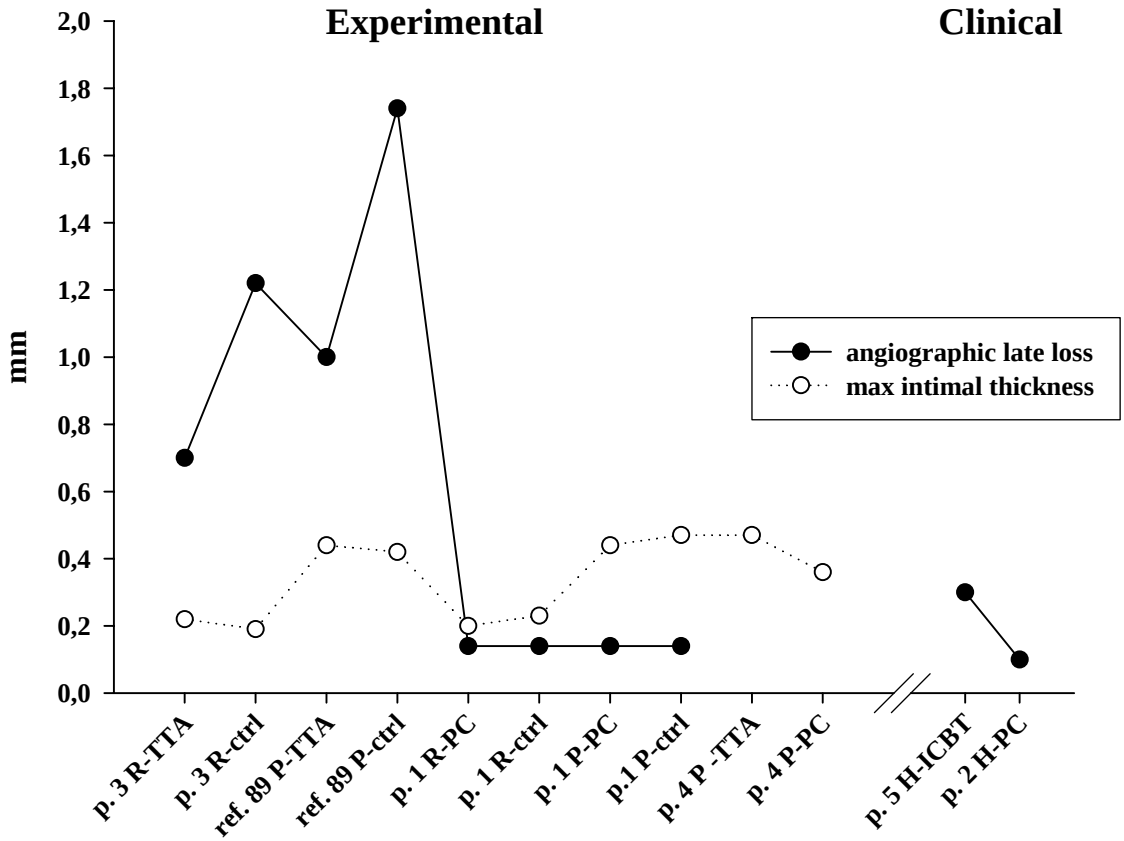
ref. 89 P-TTA
p.1 R-PC
p.1 R-ctrl
p.1 P-ctrl
p.1 P-PC
p.4 P-PC
p.4 P-TTA

Figure 4


Experimental
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p. 3 R-TTA
p. 3 R-ctrl
ref. 89 P-TTA
ref. 89 P-ctrl
p. 1 R-PC
p. 1 R-ctrl
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p.1 P-ctrl
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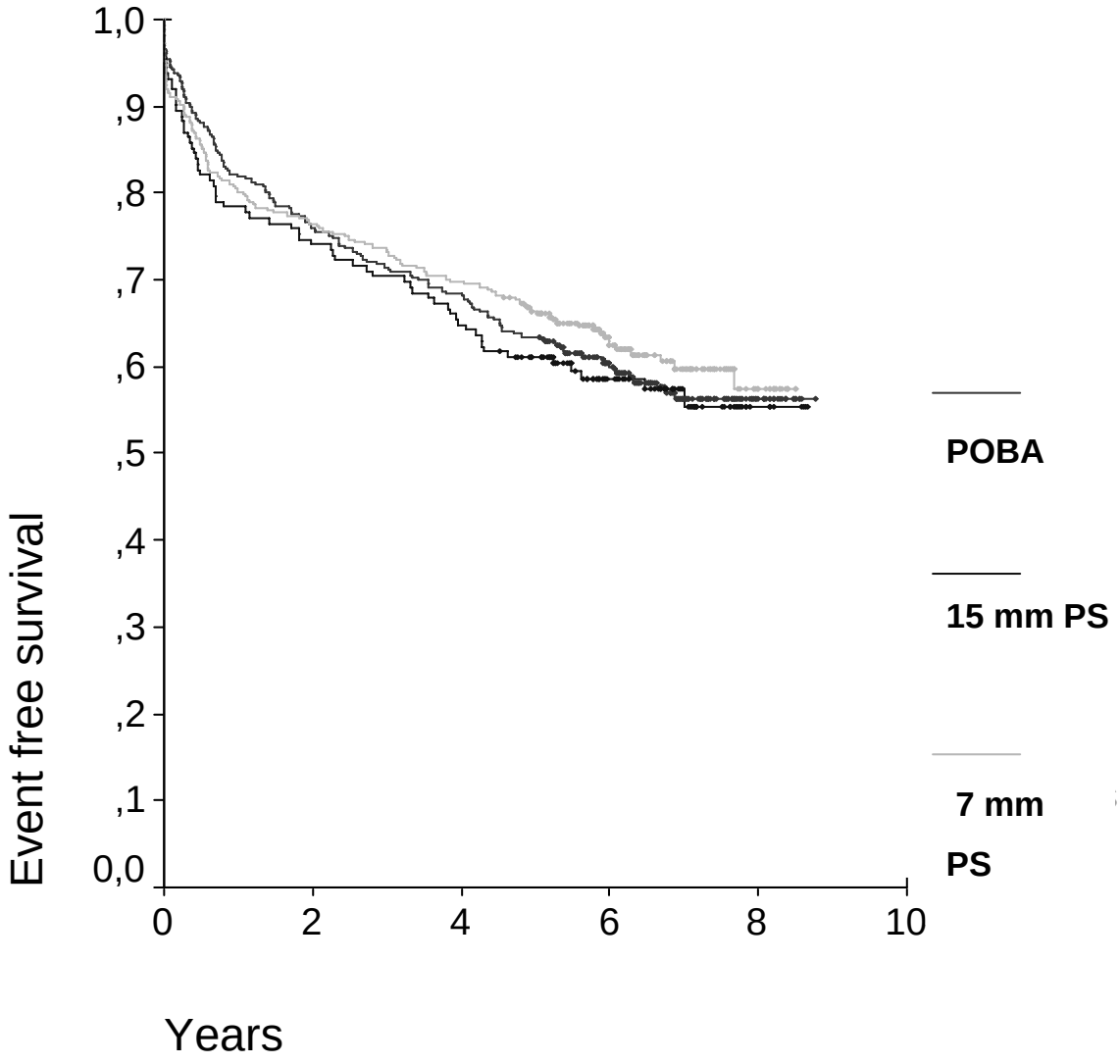
p. 4 P-PC
p. 5 H-ICBT
p. 2 H-PC

Figure 5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Years
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7 mm PS

Figure Legend

Figure 1

The chemical structure of the phosphorylcholine head group. The molecule is a zwitterion with a positive loaded charged quaternary nitrogen-group and a negative charged phosphate-group within the same molecule. Overall the molecule is neutral.

Figure 2

The chemical structure of *Tetradecylthioacetic Acid.*

Figure 3

Graphical summary of data from the 3 experimental studies in the thesis (study 1, 3 and 4) and another study published by our group (89). Data markers represent averages of intimal area from these studies. On the left side of the break balloon angioplasty studies, on the right side stent studies. The intimal response after balloon angioplasty in rabbit iliac arteries (paper 3) is less than after balloon angioplasty in pig coronary arteries. Stenting provokes a stronger intimal reaction, but is most pronounced in pig coronary arteries (paper 1 and 4).

R= rabbit, P= pig, TTA= Tetradecylthioacetic acid, PC= phosphorylcholine.

Figure 4

Graphical summary of data from the 5 studies in this thesis and another study published by our group (89). Data markers represent averages of angiographic late loss and maximal intimal thickness as measured by histomorphometry. Angiographic loss is largest in the balloon-injured segments, while it is minimal in the stented segments. Intimal thickness alone cannot explain late lumen loss in the balloon angioplasty studies (paper 3 and (89)), the difference is explained by negative remodeling of the vessel. In the stented rabbit and porcine arteries (paper 1 and 4),

angiographic late loss is comparable with intimal thickness, confirming that stents prevent remodeling. On the right side of the break angiographic late lumen loss 9 month after balloon angioplasty and brachytherapy of in-stent restenosis (paper 5) and 1 month after implantation of a Divysio stent after opening of a totally occluded coronary artery (paper 2).

R= rabbit, P= pig, H=human, TTA= Tetradecylthioacetic acid, PC= phosphorylcholine, ICBT= intracoronary brachytherapy.

Figure 5

Kaplan-Meier survival curves for major cardiac events (death, myocardial infarction or revascularisaton with coronary artery bypass surgery or percutaneous coronary intervention) for patients treated with balloon angioplasty (POBA), implantation of a disarticulated Palmaz-Schatz stent (7 mm PS) or a 15 mm Palmaz-Schatz stent (15 mm PS).