Therapeutic and mechanistic explorations of in-stent restenosis in the rat aortic stenting model
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CHAPTER 8
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In recent years therapeutic advances of PCI such as antiplatelet drugs given before and after the procedure, the stent and later the drug eluting stent have improved mortality and morbidity for patients with CHD and this has led to a marked rise in the number of PCI procedures. Nowadays in the Netherlands every year around thirty thousand PCI procedures are performed. However PCI is still troubled by two major limitations: in-stent restenosis and stent thrombosis. In-stent restenosis is the narrowing of the vessel wall after stenting. In-stent restenosis usually does not lead to acute occlusion of the vessel with myocardial infarction and death, however recurrence of angina pectoris is often seen which in practice requires revascularization of the stented vessel. Stent thrombosis is the occurrence of thrombus formation in or near the stent resulting in an acute occlusion of the vessel. Although its incidence is much lower than in-stent restenosis nevertheless it is a serious complication because in contrast with in-stent restenosis it leads more often to acute myocardial infarction and has a much higher mortality rate. In this thesis the rat aortic stenting model was used to study several new treatments to reduce in-stent restenosis and some aspects of the pathophysiology of in-stent restenosis. We also adapted our rat aortic model for studying in-stent restenosis in diabetics by developing two different (type 1 and type 2) diabetic models for in-stent restenosis. Furthermore we tried to identify a possible mechanism of late stent thrombosis by comparing the neointima of bare metal and drug eluting stents.

Chapter 2 describes a novel type 1 diabetic model for in-stent restenosis after rat abdominal aortic stenting. Diabetic animal models are useful for studying the mechanisms of increased in-stent restenosis in diabetic populations. We demonstrated increased neointimal formation in diabetic BBDP (Bio-Breeding Diabetes-Prone) and significant proteinuria, polyuria and glycemia compared to non-diabetic thymectomized BB-DP rats. These results validate this novel type 1 diabetic rat abdominal aortic stenting model for studying the mechanism of increased in-stent restenosis in diabetic populations and more specific in the type 1 diabetes population. Furthermore because of the genetic similarity of the diabetic and normoglycemic rats in this model the mechanisms responsible for increased in-stent restenosis in diabetic populations can more easily be identified in future studies.

In Chapter 3 we describe the contribution of circulating bone marrow cells to neointimal formation after in-stent restenosis and transplant arteriosclerosis. In the specimens of both stented and transplanted vessels no bone marrow-derived neointimal smooth muscle and endothelial cells were detected. A few bone marrow-derived cells were found in the neointima but they were infiltrating leukocytes. In conclusion, non-bone marrow-derived cells are the predominant source of neointimal cells in stented and transplanted vessels. Vascular wall-derived progenitor cells may rather be the source of smooth muscle cells that contribute to in-stent restenosis and transplant arteriosclerosis which may have implications for our quest for new therapeutic targets to treat these vasculopathies.

In Chapter 4 we discuss the effect of the AT1-receptor candesartan on neointimal formation after stenting. Systemic candesartan cilexetil treatment did not result in reduction of neointimal formation however angiotensin II did result in an increase in neointimal formation. It is likely that AT1-receptor stimulation by Ang II is not a major contributor to in-stent restenosis and only high doses lead to neointimal formation explaining the inability of the AT1-receptor blocker candesartan to reduce neointimal formation after systemic treatment. The current study examined systemic AT1-receptor blockade, limited by its systemic side-effects like hypotension. However, local delivery could allow higher dosing, and could be more successful than systemic AT1-receptor blockade.
The effect of treatment with rosuvastin on in-stent restenosis was analyzed in Chapter 5. Rosuvastatin treatment reduced neointimal formation both after Ang II infusion stimulated neointimal formation and in the absence of Ang II infusion stimulated neointimal formation. Furthermore we found rosuvastatin improved systemic endothelial function in the presence and absence of high levels of Ang II and reduced inflammation. Retrospective analysis in patients suggest statins also slightly reduced in-stent restenosis in humans and that the pleiotropic effects (involve improving endothelial function, decreasing oxidative stress and inflammation, and inhibiting the thrombogenic response of statins) are responsible for the reduction of neointima formation rather than the lipid-lowering effects. The improvement of endothelial function by rosuvastatin as described in chapter 5 also suggest involvement of the pleiotropic effects. Although the high dose of rosuvastatin greatly reduced neointimal formation in our study, systemic treatment in patients is not feasible in these doses due to hepatotoxicity. A rosuvastatin-eluting stent could circumvent this problem. In a recent study in the porcine coronary stenting model a cerivastatin statin-eluting stent proved successful in reducing in-stent restenosis. Lipophilic statins like cerivastatin penetrate muscle cells at a higher degree than hydrophilic statins like rosuvastatin. Therefore in theory cerivastatin will inhibit smooth muscle proliferation and in-stent restenosis more strongly than rosuvastatin. However the potential for local tissue toxicity is also greater in lipophilic statins. Indeed cerivastatin was withdrawn from the market for systemic treatment in patients because of myopathy and rhabdomyolysis. Therefore both lipophilic and hydrophilic statin-eluting stents should be tested in animal models for both efficacy and safety.

In Chapter 6 and 7 we describe quantitative and qualitative differences in neointimal formation between bare metal stents and drug eluting stents in normoglycemic (chapter 6) and diabetic setting (chapter 7). The drug eluting stents reduced neointimal formation in the normoglycemic setting compared with the bare metals stent. However in the diabetic setting only the sirolimus eluting stent successfully reduced in-stent restenosis in comparison with the bare metal stent. The qualitative differences we found were low cell neointimal density, low neointimal collagen content and increased low-grade inflammation. These differences developed in time: after 1 week cell density and inflammation did not differ between bare metals stent and drug eluting stents but after 4 weeks they differed.

We conclude that drug eluting stents were superior in reducing neointimal formation, although the paclitaxel-eluting stent did not reduce neointimal formation as well as the sirolimus-eluting stent in diabetic setting. In clinical trials the sirolimus-eluting stent seems also to decrease restenosis better than the paclitaxel stent. Our results suggests that the decreased efficiency of the paclitaxel-eluting stent in reducing stent thrombosis may be related to the stent design of the paclitaxel-eluting stent rather than the drug itself. Second-generation paclitaxel-eluting stents with different stent design may thus be better in reducing in-stent restenosis.

We found notable incomplete healing in both non-diabetic rats and diabetic rats. This suggests that incomplete healing is not specific for diabetic animals but rather a generic effect possibly due to the inhibiting effect of both sirolimus and paclitaxel on proliferating cells which may induce low neointimal cell density, impaired extracellular matrix formation and prolonged chronic inflammation. A link between late stent thrombosis and incomplete endothelial healing was suggested by Joner et al: incomplete reendothelization and high fibrin content in drug-eluting stents could be a potent thrombogenic stimulus. Our results which demonstrate low collagen content in neointimal areas with incomplete healing suggest a second hypothesis: the subendothelial neointimal tissue of drug-eluting stents is weaker and could rupture more easily thereby exposing tissue factor and inducing thrombus. The presence of hemorrhages found within the neointima of drug-eluting stents seems to support this hypothesis. The low neointimal cell density could be correlated to tissue strength: there are not enough cells to produce extracellular matrix and ensure a strong neointima. Both sirolimus and

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paclitaxel have a direct effect on the extracellular matrix production in cells. Sirolimus inhibits collagen synthesis in rat vascular smooth muscle cells and paclitaxel also reduces tenascin (an extracellular matrix glycoprotein) in human arterial smooth muscle cells\textsuperscript{14}.

Future perspectives: Further reduction of in-stent restenosis will remain the most important goal in stent development. However this reduction of in-stent restenosis should be accomplished while minimizing the risk of stent trombosis. Several concepts need to be developed to reach these goals.

The prevailing concept to achieve those goals at the moment is that of the drug eluting stent. The concept of the drug eluting stent is to use high local drug doses to inhibit the arterial healing response after stenting and thus reduce in-stent restenosis. Indeed current first and second generation drug eluting stents have been successful in reducing in-stent restenosis, however at the moment it is not clear if they present a long-term risk of stent trombosis and this is currently being addressed in clinical trials. Furthermore pre-clinical research will be needed to find the pathological substrate of stent trombosis. It is possible that the aggressive inhibition of the arterial healing response predisposes to stent trombosis. If the pathological substrate of stent trombosis is related to the strong anti-proliferative drugs used on current drug eluting stents, a critical reevaluation of the advantages and disadvantages of the use of these drugs on stents will be needed. In addition the search for new more ‘physiological’ acting drugs will start. Statins as discussed in chapter 5 may be a candidate for such drug eluting stents. However it remains to be seen if these more ‘physiological’ acting drug eluting stents are as efficient in reducing in-stent restenosis as the older ones.

Another concept is to reduce the vascular injury induced by the stent, resulting in a smaller arterial healing response and less in-stent restenosis. An optimal stent design should combine maximum final stent diameter with little arterial trauma\textsuperscript{15} ref. Significant improvements in stent design have already been made by abandoning long, self-expandable, thick strutted, coiled stents for shorter, balloon expandable, thin strutted stents of tubular design\textsuperscript{15}. Especially the thickness of the struts seems important for reducing vascular injury and in-stent restenosis\textsuperscript{16}. At the moment high grade hard steel and kobalt alloys are the preferred material for stents enabling thin struts which minimize arterial trauma. However improvements in material sciences and nanotechnology may lead to stronger and more elastic metal alloys. Stents made of these new alloys would enabling even thinner struts while also being more flexible. Dense and radio-opaque elements such as hafnium and tantalum might also be used in these alloys to maintain radiopacity of the thin stent struts.

Biocompatibility may also be important for stent design because increased thromboresistance may reduce in-stent restenosis and are likely to reduce stent thrombosis. The thromboresistant substances pyrolytic carbon and phosphorylcholine have been tested as coatings for hard steel and kobalt alloys stents. These studies did not show a reduction in restenosis\textsuperscript{17,18}. The effect on stent thrombosis was not evaluated and should be evaluated in new studies. A controlled registry of the biocompatible iridium oxide-coated stent showed in-stent restenosis rates somewhat in between current drug-eluting stents and bare metal stents. However if this difference is due to the better biocompatibility of the iridium oxide coating or due to the thinner struts of the iridium oxide coated stents in comparison with first-generation bare metal stents is not clear. More promising is the polymer polybistrifluoroethoxyphosphazene (Polyzene-F). The haemocompatibility of a Polyzene-F-coated stainless steel was much better than that of uncoated bare metal stents\textsuperscript{19}. More interestingly the Polyzene-F nanocoated cobalt-chromium stent demonstrated similar rates of in-stent restenosis as the paclitaxel and sirolimus-eluting stents in a porcine coronary model\textsuperscript{20}. 
Still randomized controlled trials comparing biocompatible coated stents with drug-eluting and bare metal stents are needed to demonstrate the relevance of biocompatible stents for reducing stent thrombosis and in-stent restenosis in humans.

A relatively new concept is that of the drug eluting balloon\textsuperscript{21}. In this concept drug-eluting balloon dilatation is followed by bare metal stent implantation. It is similar to the drug-eluting concept in that it aims to inhibit the arterial healing response after stenting. However this concept seems more attractive: the drug itself is not present for long periods because no physical connection between drug and stent exists. Another theoretical advantage of the drug eluting balloon over the drug eluting stent is in the treatment of in-stent restenosis. Treatment of in-stent restenosis with a drug eluting stent creates an undesirable amount of four metal layers whereas the drug eluting balloon does not.

Another promising concept is that of the resorbable stent. Directly after PCI a stent acts as a stabilizing scaffolding device: it prevents collapsing of intimal flaps and plaque dissections leading to a smoother surface and a larger lumen with more blood flow\textsuperscript{22}. Furthermore a stent prevents abrupt vessel closure and reduces restenosis rates by eliminating elastic vessel recoil and negative vessel remodeling\textsuperscript{22}. However on the long term the permanent nature of a stent has also negative effects: it prevents positive remodeling and the stent may continue to evoke inflammatory responses and thus stimulate neointimal formation. The resorbable stent acts in the early phase successfully as a scaffolding device like any other stent. However after several months the stent dissolves into the body theoretically allowing positive remodeling to occur and preventing neointimal formation due to the continued presence of the stent. Preclinical and human studies with a magnesium resorbable stent have shown the safety of the resorbable stent, although they had high restenosis rates due to both negative remodeling and chronic wall recoil\textsuperscript{23,24}. Still the magnesium stent was almost completely resorbed and it successfully functioned as a scaffolding device in the early phase after stenting. The rate of resorption of a resorbable stent may be critical in preventing increased in-stent restenosis due to negative remodeling and premature recoil. Recent preclinical research suggests that biocorrodible iron stents may be better in preventing premature recoil than magnesium resorbable stents\textsuperscript{25}. Future research on resorbable stents will learn us more about the ideal resorption interval and material.

It is likely that in the near future less in-stent restenosis and stent thrombosis will be achieved by developing new drug eluting stents with superior (less injury) stent design and possibly also with new less aggressive drugs (e.g. statins) or with a polymer biocoating (e.g. Polyzene-F). The drug eluting balloon concept although interesting has yet to prove itself in large clinical trials. The resorbable stent is very promising but still in an experimental phase. In the long run combinations of these concepts (e.g. a resorbable drug eluting or biocoated stent) may reduce in-stent restenosis and stent thrombosis even better.
SUMMARY

References


