Inhibition of Neointima Formation After Experimental Coronary Artery Stenting
A New Biodegradable Stent Coating Releasing Hirudin and the Prostacyclin Analogue Iloprost

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Background—To minimize acute stent thrombosis and development of restenosis, stents coated with biodegradable and nonbiodegradable polymers have been proposed to serve as sustained-release drug carriers.

Methods and Results—In both a sheep and a pig model, we examined the vascular response to standard and high-pressure implantation of coronary Palmaz-Schatz stents coated with a 10-μm layer of polylactic acid (MW 30 kDa) releasing recombinant polyethylene glycol (r-PEG)—hirudin and the prostacyclin analogue iloprost, both drugs with antithrombotic and potentially antiproliferative effects. Study observation time was 28 days. Between the corresponding stent groups, no differences were observed with regard to preplacement and postplacement implantation parameters. The morphometric analysis demonstrated that the coating was associated with a greater lumen diameter through a reduction in the mean restenosis area by 22.9% (P<0.02) in the standard-pressure model (sheep) and by 24.8% (P<0.02) in the overstretch pig model compared with uncoated control stents without inducing a local inflammatory response.

Conclusions—The results from this study demonstrate beneficial effects of a polymeric stent coating with polylactic acid releasing r-PEG—hirudin and iloprost on the development of restenosis after coronary stent placement at 4 weeks, independent of the extent of vascular injury. Future studies are proposed to investigate the integration of other substances to further enhance the potential of the stent coating on reducing neointimal formation. (Circulation. 2000;101:1453-1458.)

Key Words: angioplasty ▪ stents ▪ restenosis

The introduction of stent implantation in coronary lesions had a substantial impact on improving early and late outcome compared with coronary angioplasty alone, providing mechanical scaffolding that reduces the impact of early elastic recoil or dissection and eliminates late lumen loss by circumferential remodeling.1,2 Still, implantation of coronary stents is not free of complications. In addition to wall injury at the site of stent deployment, which provides a powerful stimulus to platelet activation and thrombus formation, the surface of the stent itself constitutes a thrombogenic foreign body. Thus, without treatment, a high rate of early stent thrombosis may be expected. Furthermore, together with the impact of the arterial wall injury, a multifactorial process is initiated, leading to neointimal hyperplasia and restenosis.

A number of studies have addressed these problems with aggressive anticoagulant and antiplatelet therapies and application of systemic antiproliferative strategies to inhibit neointimal growth. Although acute thrombosis has been reduced with new antithrombotic therapeutic approaches in humans, inhibition of neointimal hyperplasia is still of concern. Studies that demonstrated a significant reduction of restenosis were performed only in animals, applying much higher systemic doses than used in clinical practice.3,4 Application of comparable doses in humans is associated with toxicity and unwanted side effects, thus leading to the concept of local treatment strategies to achieve considerably higher drug concentrations at the site of the vessel wall injury than attainable by systemic administration.5

Aside from different devices to introduce drugs, stent coating with synthetic polymers has been proposed to reduce the thrombogenicity of the metal backbone and to serve as a sustained-release drug reservoir, allowing a pharmacological interaction with the vascular wall at the site of intervention. Several potential materials have been investigated so far, although without consistent results. Recently, a multicenter investigation of 8 different polymers coated on Wiktor stents and implanted into porcine coronary arteries found a marked...
inflammatory reaction with subsequent exaggerated neointimal thickening.6

The aim of this investigation was to establish the applicability and biocompatibility of a homogeneous yet thin layered stent coating with a polylactic acid (PLA) polymer, releasing 2 antithrombotic analogues with potentially antiproliferative effects, hirudin and the prostacyclin (PGI2) analogue iloprost. The vascular responses to implantation of the coated stents in 2 different animal models and whether the effect of the coating would be achieved independently of the pressure of deployment and the species were studied.

**Results**

**Stent Placement Parameters**

Angiographic vessel diameters before (not shown) and after stent deployment did not show any differences between uncoated and coated stents in both animal models, nor did stent deployment characteristics (Table).

**Arterial Injury Score**

As shown in Figure 1, the extent of damage inflicted on the vessel wall by the stent placement, represented by the numerical injury score, was similar for the coated and uncoated groups in both animal models. However, because of the greater stent-to-vessel ratio with higher inflation pressures in the pig overstretch model, higher injury score values were reached, which were associated with a greater range.

**Histological Examination**

Histological examination demonstrated that all struts were completely covered with endothelium/neointimal cells (Figure 2A). The stented vessel segments showed a neointimal thickening by smooth muscle cell proliferation in all examined cross sections, with an abundance of extracellular matrix with collagen and elastic fibers and some fibroblasts. Most importantly, histological examination corresponding to biocompatibility parameters revealed no apparent acute inflammatory reaction with infiltrates of lymphocytes, histiocytes, or eosinophils in coated-stent samples compared with uncoated stents and nonstented control segments (Figure 2B). Adjacent to stent struts cutting deeply into the arterial wall, there was frequently hemosiderin pigment deposition and sometimes signs of hemorrhage, thrombus formation, and invasion of macrophages and multinucleated giant cells, suggesting a foreign-body type of reaction after severe arterial injury (Figure 2C), but with no apparent difference between coated and uncoated stents (not shown).

**Histomorphometry**

Morphometric analysis of stent diameters, equivalent to the postprocedural lumen diameter, revealed no apparent differences between uncoated and coated stents in the standard-pressure group (2.8±0.13 versus 2.90±0.18 mm, P=NS) or in the overstretch group (2.71±0.17 versus 2.73±0.15 mm, P=NS). Representative photomicrographs of sections with coated and uncoated stents in both animal models are shown in Figure 3. These figures demonstrate that in both animal models, the PLA stent coating eluting r-PEG–hirudin and iloprost resulted in a significant reduction of the neointimal formation compared with the control groups, equaling −22.9% in the standard-pressure sheep model and −24.8% in the overstretch pig model in neointimal area (from 2.50 to 1.92 mm² in sheep and from 4.13 to 3.11 mm² in pigs, both P<0.02; Figure 4A). This newly formed tissue was composed primarily of cells of smooth muscle cell origin, shown by a positive staining for smooth muscle cell α-actin. The quantitative comparison of coated and uncoated stents corresponding to the restenosis rate is depicted in Figure 4B. This beneficial effect was independent of the applied balloon pressure, as shown by a comparable reduction of the restenosis in the standard-pressure model as well as the high-pressure overstretch model.

**Discussion**

In this study, we demonstrated that a very thin (10-μm) biodegradable stent coating with a high-molecular-weight PLA (30 kDa), serving as a sustained-release reservoir for r-PEG–hirudin and the PGI2 analogue iloprost, significantly reduced the neointimal formation after stent placement after 4 weeks. In contrast to most other polymers investigated so far, no local inflammatory reaction was induced by the PLA polymer used in this study, in agreement with its well-described biocompatibility.14 Also, van der Giessen and colleagues implanted stents with the same coating for a
follow-up period of 3 months and found good biocompatibility (W.J. van der Giessen et al, unpublished data, 1999).

Local inflammation caused by foreign-body implants or degradation of biomaterials can cause a remarkable degree of intimal hyperplasia and therefore increase the extent of the evolving restenosis, even causing complete arterial occlusion after polymer-coated-stent implantation. This has been attributed to the initial polymer tissue load as well as the speed of its degradation, which correlates inversely with the molecular weight. After implantation, the specific degradation pattern is connected to the polymer thickness diameter: thin coatings degrade continuously, whereas thicker coating layers tend to bulk-degrade because of water penetration into the matrix. This not only increases the total surface area of coating material to be processed by the surrounding tissue but also causes uncontrolled drug release during breakup of the matrix. In a recently published study, PLA (80 and 321 kDa) was used as a drug carrier for dexamethasone at a total concentration of 800 μg per 400-μg carrier. This allowed a fast release of the drug within several days. However, at least with 80-kDa PLA, an intense local inflammatory neointimal response was induced as a result of a local tissue "overload" with the polymer degradation products by dramatically increasing the carrier surface available for erosion. We have shown in extensive in vitro studies that the 30-kDa PLA can carry an additional "drug load" of at most 20% (wt/wt) without compromising its degradation pattern. In this study, the thickness of the PLA coating was only 10 μm, resulting in an overall weight per stent of ~200 μg, much less than that used in other studies with less favorable results from implanting coated stents because of marked inflammatory infiltration.

The rationale for selecting the drugs to be released from the coating was based on a combination of strong antiplatelet action and a potential effect on proliferation as well. Hirudin is a specific, highly potent, direct inhibitor of thrombin.
Thrombin plays an important role in thrombus formation after vessel injury and acts as a potent mitogen and chemotactic agent for monocytes. In particular after a prolonged application of hirudin, neointimal thickening was significantly reduced after balloon angioplasty in 2 animal studies. One of these studies provided evidence that thrombin formation at the site of vascular injury is maintained for 2 weeks, supporting the approach in our study for the sustained release of hirudin.

Iloprost is a PGI₂ analogue with a prolonged action profile. PGI₂ has been shown to inhibit platelet aggregation markedly and to limit both platelet and leukocyte activation after interaction with artificial surfaces. Prostaglandins also possess a certain antiproliferative effect. Even though in vivo application of prostaglandins to control smooth muscle cell proliferation in response to arterial injury has produced conflicting results, clinical studies with short-term administration demonstrated a beneficial effect on neointimal proliferation.

The measurements of the PLA carrier degradation clearly demonstrated a slow and continuous erosion pattern, which accounts for the favorable biocompatibility features of the stent coating described in this 4-week study. In contrast to the release of iloprost, which was similar to the degradation of the PLA carrier, r-PEG–hirudin was eluted up to 59% of the total amount loaded during the first 24 hours. This was a result of the incorporation of crystals into the carrier, which allowed a fast antithrombotic effect during the most critical phase after stent implantation. Both drugs were still released as pharmacologically active compounds for 3 months (Figure 5).

Strong evidence has been presented, at least in studies in animal models, that postinjury neointimal hyperplasia and therefore the development of restenosis are linked to platelet adhesion, aggregation, and thrombus formation. Sirois et al demonstrated a suppression of restenosis after balloon angioplasty of the rat carotid artery by platelet depletion. The neointimal hyperplastic potential was fully restored by infusion of fresh platelets even 14 days after the initial injury. This is even more important after stent implantation, which provides a continuous trigger of extensive arterial wall stress.

Figure 3. Photomicrographs showing representative samples of neointimal hyperplasia 28 days after implantation of uncoated (A) and coated (B) stents in standard-pressure sheep model and uncoated (C) and coated (D) in overstretch pig model. Bar=500 μm.
GP IIb/IIIa inhibitors on neointimal hyperplasia, however, ticlopidine. The conflicting data on the long-term benefit of logical onset of orally administered antiplatelet drugs, such as may therefore serve as a bridge covering delayed pharmaco-
both drugs is maintained for delayed onset of the pharmacological effects. We previously
ticlopidine or clopidogrel, although it is encumbered by a stent implantation includes the combination of aspirin and
implantation, a study in patients randomized to either a combined antiplatelet therapy with aspirin and ticlopidine or
contrary to the general application in humans to treat atherosclerotic lesions, which may respond differently to the stent
coating with drug release. And finally, even though many drugs have proved to be effective in animal models with
regard to prevention or reduction of restenosis, they later failed in human studies.

Limitations of the Study
The sheep animal model has not been widely used to evaluate restenosis after experimental coronary interventions, although the activity of the ovine coagulation and fibrinolytic system has more similarities to humans than other species. This is considered particularly important for a representative animal model of the development of restenosis, because the dog animal model with a particularly high fibrinolytic activity showed a diminished response to vascular injury. The implantation of coronary stents in pigs has been used more extensively because of the similar histological appearance of the proliferative neointimal tissue to human restenosis. However, to generate comparable amounts of restenosis, an oversizing of 30% to 50% is required. Although the similarities between animal models and humans in this respect might be adequate, it is not clear whether the beneficial results from this study might translate acceptably to humans. In this study, normal coronary arteries were used for stent implantation, in contrast to the general application in humans to treat atherosclerotic lesions, which may respond differently to the stent coating with drug release. And finally, even though many drugs have proved to be effective in animal models with regard to prevention or reduction of restenosis, they later failed in human studies.

Conclusions
The results presented in this study showed a significant reduction of neointimal hyperplasia and hence restenosis after experimental implantation of Palmaz-Schatz stents coated with a PLA polymer, releasing r-PEG–hirudin and iloprost. The histopathological examination revealed no apparent inflammatory reaction after 28 days. Considering the failure of oral pharmacological treatments to reduce restenosis after interventional stent implantation in humans, the method of local drug delivery deserves further attention.

References
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*Circulation*. 2000;101:1453-1458
doi: 10.1161/01.CIR.101.12.1453

*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/101/12/1453

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