Paclitaxel Balloon Coating, a Novel Method for Prevention and Therapy of Restenosis

Bruno Scheller, MD; Ulrich Speck, PhD; Claudia Abramjuk, DVM; Ulrich Bernhardt, PhD; Michael Böhm, MD; Georg Nickenig MD

Background—Drug-eluting stents have shown promising antirestenotic effects in clinical trials. Non–stent-based local delivery of antiproliferative drugs may offer additional flexibility and also reach vessel areas beyond the immediate stent coverage. The aim of the present study was to evaluate a novel method of local drug delivery based on angioplasty balloons.

Methods and Results—Stainless steel stents (n=40; diameter, 3.0 to 3.5 mm; length, 18 mm) were implanted in the left anterior descending and circumflex coronary arteries of domestic pigs. Both conventional uncoated and 3 different types of paclitaxel-coated, percutaneous transluminal coronary angioplasty balloons (contact with vessel wall for 1 minute) were used. No difference in short-term tolerance between coated and uncoated balloons and no signs of thrombotic events were observed. Quantitative angiography and histomorphometry of the stented arteries asserted the statistical equality of the baseline parameters between the control and the 3 treatment groups. Paclitaxel balloon coating led to a marked, dose-dependent reduction of parameters characterizing in-stent restenosis (reduction of neointimal area up to 63%). Despite the marked reduction in neointimal proliferation, endothelialization of stent struts was present in all samples. There was no evidence of a significant inflammatory response in the neighborhood of the stent struts.

Conclusions—Paclitaxel balloon coating is safe, and it effectively inhibits restenosis after coronary angioplasty with stent implantation in the porcine model. The degree of reduction in neointimal formation was comparable to that achieved with drug-eluting stents. (Circulation. 2004;110:810-814.)

Key Words: restenosis ■ angioplasty ■ paclitaxel

Coronary stent implantation has been proven to be an effective technique for the prevention of restenosis in native coronary vessels compared with angioplasty alone. However, the restenosis rates after bare-metal stent implantation are still as high as 20% to 40% at 6 months. Numerous initially promising approaches that included systemic antiproliferative agents have failed to prevent restenosis.1 Coronary brachytherapy has been considered a breakthrough treatment against in-stent restenosis but depends on the availability of the radiotherapeutic armamentarium.2

Drug-eluting stents (DES) were shown to be safe and feasible in reducing restenosis.3-7 However, their efficacy and safety have not been confirmed in all clinical settings, especially with regard to treating in-stent restenosis. Concerns have been raised that the polymeric matrix on the stent in which the antiproliferative drug is embedded might induce inflammation and thrombosis.8 Another important limitation of DES is the fact that the drug concentration is highest at the stent struts, where healing is most important. On the other hand, incomplete suppression of neointimal hyperplasia at the stent margins or between the struts may limit the efficacy of DES.4

Non–stent-based local delivery of antiproliferative drugs may offer additional flexibility and efficacy in the entire range of applications. It may also deliver drugs to vessel areas not directly covered by the stent, which could be of special interest for small and tortuous vessels. Furthermore, healing and reendothelialization of stent struts that do not carry antiproliferative agents may be facilitated.

Paclitaxel has already been investigated in previous studies that included a variety of catheter-based, local drug-delivery approaches. The “double-balloon” catheter,9 the “porous balloon,”10 and even intrapericardial administration11 have been used. Although all of these approaches showed efficacy in preclinical trials, they require special and sometimes cumbersome devices, involve blockage of coronary blood flow, or induce additional vascular injury.

The aim of the present study was to test a novel method of intracoronary local drug delivery in the porcine coronary overstretch model: paclitaxel coating of conventional percutaneous transluminal coronary angioplasty (PTCA) balloon catheters with a new coating technique that allows for immediate drug release on inflation.
Methods

Balloon Coating

Conventional PTCA angioplasty balloon catheters (length, 20 mm; diameters, 3.0 and 3.5 mm; Bavaria Medizin Technologie) were coated with paclitaxel by 2 different procedures: procedure EEE used ethyl acetate as the solvent and resulted in ~2 µg paclitaxel per square millimeter of balloon surface, and procedure Ac used acetone as the solvent and was completed with 2 concentrations of paclitaxel: a low dose (AcL), resulting in 1.3 µg/mm², and a regular dose (AcR), resulting in 2.5 µg/mm². The paclitaxel dose in EEE and AcR catheters was the maximum achievable at the time of the experiments. After coating, uncoated bare stents (MeoFlex, MeoMedical) were crimped onto all balloons. No decomposition products of paclitaxel were detected by high-performance liquid chromatography (HPLC) after ethylene oxide sterilization. Pressure resistance of the balloons and stent delivery were not affected by the coating. Adhesion of paclitaxel to the surface of standard balloons was fully satisfactory, with no loss due to normal handling, significantly <1% of the dose adhering to the close-fitting, balloon-protecting tube, and very little loss in blood unless the balloon was expanded (see Results). The coating resulted in a very slight increase in profile but no recognizable change in flexibility.

All experiments were conducted in accordance with the guidelines for animal experiments set forth by the local animal protection committee. Twenty-six domestic pigs were premedicated by intramuscular injection of 6 mL ketamine and 4 mL xylazine. A venous access was provided, and anesthesia was induced by intravenous injection of propofol, followed by orotracheal intubation, which was maintained with 1.0% to 2.0% isoflurane, 70% N₂O, and 30% oxygen (all vol/vol). All animals received 5000 IU heparin, 250 mg aspirin, and 200 µg intracoronary nitroglycerin. The coronary arteries were imaged by a standard angiographic technique via the left carotid artery. Target segments were selected in the left anterior descending (LAD) and circumflex (CX) arteries, and vessel diameters were estimated by comparison with the diameter of the angiographic catheter.

Paclitaxel Loss in Blood and Coronary Tissue Concentration After Inflation

To determine the uptake of paclitaxel into the vessel wall and various tissues, 4 domestic pigs (25 to 30 kg body weight) received paclitaxel-coated and uncoated balloon catheters without or with premounted stents. Coated balloon catheters without or with stents were advanced into the coronary arteries, where they were left uninfated in the bloodstream for ~5 minutes and then withdrawn. The paclitaxel content of the balloons was determined by HPLC after extraction and compared with the paclitaxel content of unused catheters of the same batch. In the same animals, 3 different treatments were performed in randomized order: (1) implantation of a bare stent premounted on a paclitaxel-coated balloon catheter (AcR); (2) angioplasty with a paclitaxel-coated balloon catheter (AcR) without stent implantation (to "label" the site of dilatation, a short stent was implanted distal to it before PTCA was performed); and (3) bare-stent implantation with an uncoated balloon and then postdilatation of the stented segment with a paclitaxel-coated balloon catheter (AcR).

After 40 to 60 minutes, treated coronary artery segments from the LAD, CX, and right coronary arteries were dissected. Paclitaxel was extracted from homogenized tissue samples with dimethyl sulfoxide and from plasma samples with ethyl acetate, with docetaxel as an internal standard. Samples were analyzed by HPLC on a Waters Symmetry C-18 column (5 µm, 25 cm × 4.6 mm) and an acetonitrile/0.005 mol/L potassium phosphate buffer, pH 3.3. With 40:60 (vol/vol) acetonitrile/potassium phosphate, paclitaxel retention time was 35 to 45 minutes; with 55:45, paclitaxel retention time was ~7.5 minutes. Detection was performed by ultraviolet photometry at 230 nm.

Restenosis Study

Stents were implanted with an oversize ratio of ~1.2 (implantation pressure, 12 atm). According to the randomization list, the 20 pigs (body weight, 30.9±2.3 kg) received a total of 40 identical stents into LAD and CX arteries. The stents were premounted on identical balloon catheters, which were either uncoated or coated in 3 different ways: (1) control, consisting of bare stents crimped onto conventional, uncoated PTCA balloon catheters (n=13); (2) consisting of bare stents crimped onto paclitaxel-coated PTCA balloon catheters with use of the EEE coating technique and a regular paclitaxel dose (n=10); (3) AcL, consisting of bare stents crimped onto paclitaxel-coated, PTCA balloon catheters with use of the Ac coating technique and low-dose paclitaxel (n=10); and (4) AcR, consisting of bare stents crimped onto paclitaxel-coated, PTCA balloon catheters with use of the Ac coating technique and regular paclitaxel dose (n=11).

After stent implantation, prophylactic intramuscular streptomycin and penicillin were given; pigs were allowed to recover and were monitored for 5 weeks. Oral aspirin 100 mg and ticlopidine 250 mg per day were administered starting 3 days before the procedure and continuing until sacrifice. After 5 weeks, the animals were euthanized with 20 mL pentobarbital while under deep anesthesia. Hearts were rapidly excised, the coronary system was flushed with 0.9% saline, and the arteries were fixed by perfusion with 4% buffered formalin under physiological pressure and overnight immersion. The target segments were then dissected and samples for histology obtained.

Quantitative Coronary Analysis

Coronary artery imaging was performed with a Philips PolyArc fluoroscope connected to a digitizer and an Apple Macintosh Power PC. The CAAS II System (Pie Medical) was used for quantitative coronary analysis by 2 experienced cardiologists blinded to the treatment groups. Discrepancies were resolved by mutual consensus.

Histology

Stented coronary arteries were dissected from the formalin-fixed hearts and immersed in methylmethacrylate (Merek). Three representative cross sections per stent were cut from the blocks with a coping saw, polished, and glued onto acrylic plastic slides. Final specimens were stained with hematoxylin eosin. After digitizing, histomorphometric measurements were taken with the NIH Image program (PC version Scion Image, Scion Corp). The evaluated parameters were luminal diameter, external elastic lamina (EEL) diameter, maximal neointimal thickness, EEL area, luminal area, and neointimal area. Injury scores were assigned as previously described by Schwartz et al. and the inflammation score for each individual strut was graded as described by Kornowski et al. Statistical Analysis

Histomorphometric variables of the 3 cross-sectional planes were averaged to obtain a mean value per stent. Continuous variables of quantitative coronary angiography were compared by 1-way, repeated-measures ANOVA (3 or 4 factors) and Student’s t test after a normalized distribution was assured by a statistical program (SPSS 11.5 for Windows, SPSS Inc). Data are presented as mean±SD.

Results

Paclitaxel Loss in Blood and Coronary Tissue Concentration After Inflation

Introduction of coated balloon catheters without stents through the delivery sheath to the coronary arteries and retraction without inflation resulted in 6% loss of the active ingredient (paclitaxel content, 94±10% of initial dose; n=4). Folded balloons with mounted stents showed no loss of paclitaxel during 5 minutes of floating (paclitaxel content, 102±6% of initial dose; n=3). After coronary artery dilatation, most of the drug was released during balloon inflation, resulting in the transfer of effective doses to the vessel wall (Table 1).

Restenosis Study

There were no acute or subacute thrombotic complications and no significant adverse events in terms of ECG parame-
angiography revealed no edge effects. Histomorphometry showed a statistically significant increase in lumen diameter and lumen area and a corresponding decrease in maximal neointimal thickness and neointimal area in the vessels treated with AcL- and AcR-coated balloons. The AcL paclitaxel coating led to a reduction of the neointimal area by 42% and the AcR coating, by 63%. Despite the marked reduction in neointimal proliferation, endothelialization of stent struts was present in all samples (Figure 2). There was no evidence of a significant inflammatory response in the neighborhood of the stent struts (Table 2).

### Discussion

In the present study, we tested a new concept of paclitaxel-coated balloon catheters as a lesion-specific alternative for transferring the drug to the vessel wall. Drug-coated balloons do not require the use of stents to inhibit restenosis. This new coating technique of conventional angioplasty balloon catheters does not involve any chemical bonding to the balloon surface or a sustained-release mechanism but allows for immediate drug delivery on inflation, administration of a

<table>
<thead>
<tr>
<th>Balloon Catheter Use</th>
<th>Percentage of Dose Recovered on Balloon After PTCA or Stent Implantation</th>
<th>Percentage of Dose in Vessel Wall 40 to 60 min After PTCA or Stent Implantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel-coated 3.0–20 or 3.5–20 mm PTCA with coated balloon</td>
<td>7.9±2.6 (n=4)</td>
<td>8.7±4.9 (n=4)</td>
</tr>
<tr>
<td>Paclitaxel-coated 3.0–20 or 3.5–20 mm Stent + postdilatation with coated balloon</td>
<td>11.0±4.3 (n=4)</td>
<td>15.6±13.1 (n=4)</td>
</tr>
<tr>
<td>Paclitaxel-coated 3.0–20 or 3.5–20 mm with stent Premounted stent on coated balloon</td>
<td>6.1±1.8 (n=4)</td>
<td>17.3±8.5 (n=4)</td>
</tr>
</tbody>
</table>

Values are mean±SD.

**TABLE 2.** Results of Quantitative Coronary Angiography and Histomorphometry of Stented Porcine Coronary Arteries After 35 Days

<table>
<thead>
<tr>
<th>Method</th>
<th>Control (n=12)</th>
<th>EEER (n=9)</th>
<th>AcL (n=10)</th>
<th>AcR (n=9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative coronary angiography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference diameter, mm</td>
<td>2.81±0.23</td>
<td>2.82±0.10</td>
<td>2.82±0.29</td>
<td>2.75±0.22</td>
<td>0.905</td>
</tr>
<tr>
<td>Stent diameter, mm</td>
<td>3.33±0.20</td>
<td>3.38±0.12</td>
<td>3.35±0.23</td>
<td>3.30±0.22</td>
<td>0.895</td>
</tr>
<tr>
<td>Overstretch ratio</td>
<td>1.19±0.08</td>
<td>1.20±0.06</td>
<td>1.19±0.08</td>
<td>1.21±0.08</td>
<td>0.978</td>
</tr>
<tr>
<td>Minimal lumen diameter at 5 wk, mm</td>
<td>1.43±0.79</td>
<td>1.98±0.70</td>
<td>2.48±0.89</td>
<td>2.81±0.78</td>
<td>0.005</td>
</tr>
<tr>
<td>Late lumen loss, †mm</td>
<td>2.02±0.77</td>
<td>1.84±1.06</td>
<td>0.87±0.92</td>
<td>0.49±0.83</td>
<td>0.002</td>
</tr>
<tr>
<td>Histomorphometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injury score</td>
<td>0.68±0.20</td>
<td>0.77±0.16</td>
<td>0.63±0.15</td>
<td>0.58±0.10</td>
<td>0.127</td>
</tr>
<tr>
<td>Vessel diameter, mm</td>
<td>2.88±0.20</td>
<td>3.01±0.24</td>
<td>2.82±0.22</td>
<td>3.02±0.23</td>
<td>0.213</td>
</tr>
<tr>
<td>Lumen diameter, mm</td>
<td>1.91±0.32</td>
<td>1.88±0.36</td>
<td>2.31±0.22</td>
<td>2.66±0.22</td>
<td>0.001</td>
</tr>
<tr>
<td>Maximal neointimal thickness, mm</td>
<td>0.89±0.29</td>
<td>0.98±0.46</td>
<td>0.50±0.27</td>
<td>0.33±0.15</td>
<td>0.001</td>
</tr>
<tr>
<td>Vessel area, mm²</td>
<td>6.91±0.74</td>
<td>7.13±0.77</td>
<td>6.32±1.06</td>
<td>7.11±0.85</td>
<td>0.189</td>
</tr>
<tr>
<td>Luminal area, mm²</td>
<td>2.85±0.82</td>
<td>2.72±1.11</td>
<td>3.96±0.93</td>
<td>5.62±1.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Neointimal area, mm²</td>
<td>4.05±1.07</td>
<td>4.40±1.32</td>
<td>2.35±0.87</td>
<td>1.49±0.41</td>
<td>0.001</td>
</tr>
<tr>
<td>Inflammation score</td>
<td>1.51±0.31</td>
<td>1.54±0.35</td>
<td>1.25±0.35</td>
<td>1.20±0.64</td>
<td>0.219</td>
</tr>
</tbody>
</table>

Implantation of bare metal stents using conventional PTCA catheters (control), paclitaxel-coated PTCA catheters with EEE coating or Ac coating. Values are mean±SD. n=40.

*Overstretch ratio = stent diameter/reference diameter.

†Late lumen loss = lumen after stent implantation minus minimal lumen diameter 35 days) at 35 days’ follow-up.
controlled dose, and homogeneity of vessel wall exposure. Paclitaxel-coated balloons lose only $\approx 6\%$ of the dose on the way to the coronary arteries and back. About 80% of the drug is released during inflation. We tested 2 different types of coating. According to the histomorphometry results, the EEE procedure was ineffective in the coronary arteries of our animal model. The use of paclitaxel-coated balloons prepared with the Ac procedure was associated with a marked reduction of neointimal proliferation. Endothelialization of stent struts was present in all samples. The difference in efficacy of the 2 coating procedures may be explained by the presence of a hydrophilic x-ray contrast-medium substance in the coating preparation in the case of the Ac version.

The results of this study confirm that short-term exposure of injured arteries is sufficient to inhibit restenosis during a critical period of time after angioplasty in a recognized animal model. This model used a normal coronary artery with a very thin intima and moderately sized media, which is different from an atherosclerotic human coronary artery. Despite the differences in clinical application, this new approach may offer several advantages: There is no need for radiation, polymers, or other sustained-release techniques. This new technique allows for drug delivery to the total injured vessel area. Potential clinical indications are the prevention of restenosis after angioplasty, with or without stent implantation, and the treatment of in-stent restenosis (as an alternative to brachytherapy or stent-in-stent).

According to current concepts and the results of clinical trials, effective inhibition of restenosis requires local drug administration by implantation of DES. The use of stents, however, is not always possible or desirable. Restenosis inhibition is required in vessel areas beyond the immediate stent coverage, between stents, in small and tortuous vessels, and in restenotic, stented vessels. Furthermore, healing and reendothelialization of stent struts not carrying antiproliferative agents may be facilitated if the drug supply does not have its origin near the stent struts.
On the other hand, drug-releasing stents are unique in allowing sustained release for days and weeks after a single, short intervention. Restenosis due to neointimal hyperplasia is a slow process, suggesting the need for prolonged or repeated drug administration. Sustained drug release is considered to be essential for preventing restenosis by local drug delivery. However, in the TAXUS II trial, both slow- and moderate-release paclitaxel-eluting stents prevented neointimal formation to the same degree.16

Our concept of non–stent-based, local paclitaxel delivery was stimulated by the surprising observation that the short period of exposure during the passage of contrast media through the coronary arteries allows for taxane uptake sufficient to inhibit restenosis. A short incubation time (3 minutes) with paclitaxel added to the contrast agent iomodi (Ultravist) almost completely inhibited vascular smooth muscle cell proliferation for up to 12 days. Creel et al reported that uptake of the lipophilic paclitaxel into the arterial wall was increased 20-fold over heparin after 15 minutes of exposure. Although a process that requires 15 minutes is far too long to be applicable during PTCA, the experiment indicates that lipophilic drugs such as paclitaxel or sirolimus are rapidly taken up by the tissue. In 2 animal studies, a dose-dependent reduction of neointimal area and of restenosis by intracoronary iomodi taxane administration was demonstrated.17,18

If sustained release of antiproliferative drugs should indeed prove to be unnecessary for inhibiting biological processes that result in restenosis, alternative new methods of drug supply during the interventional procedure are conceivable. Application of drugs on the surface of balloon catheters is one possible option. The antiproliferative drug stays on the balloon until it is inflated. During inflation it is pressed against the stenotic vessel wall, and a sufficiently large proportion of the drug is dissolved and penetrates the vessel wall.

Sustained drug release from stent coatings results in long-lasting, low tissue concentrations over a longer period until the healing process is well advanced. In the case of coated balloons, higher tissue concentrations may initially be expected. In the present study, paclitaxel tissue concentration in the treated coronary arteries was measured 40 to 60 minutes after balloon insufflation. In combination with stents, about 16% of the total paclitaxel dose was transferred to the injured vessel area. In a prior animal trial that used contrast media–paclitaxel formulations for restenosis prevention, we found that the tissue concentration half-life of paclitaxel in the coronary artery wall ranged from 45 minutes (LAD) to 85 minutes (CX). Twenty-four hours later, paclitaxel was still detected in the LAD of 2 of 3 pigs. Paclitaxel concentrations were almost identical in the vessel wall proximal and distal to the stent and in the stented segment.17 Because of the half-life of about 1 hour, prolonged drug action due to persisting high tissue concentrations seems unlikely.

Therefore, short-term exposure to paclitaxel obviously blocks early growth-initiating events that are crucial for subsequent neointimal formation. Importantly, exclusive abrogation of this premature proliferation is sufficient to prevent restenosis. Our data indicate that the paclitaxel balloon coating is safe in the porcine overstretch stent model. The degree of reduction in neointimal formation is at least comparable to that achieved with sustained drug release from stents.

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