Local paclitaxel delivery after coronary stenting in an experimental animal model

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Summary

The goal of this study was to test the safety and efficacy of local paclitaxel delivery via a newly designed application catheter in an experimental animal study. Drug-eluting stents reduce restenosis in comparison to bare-metal stents. The drug-eluting polymer, however, may exert potential thrombogenic and inflammatory effects. A catheter-based local paclitaxel delivery offers further advantages, particularly a homogenous drug transfer into the vessel wall and a pharmacotherapy of the stent edges. In 30 pigs, both bare-metal stent (3.0 x 13mm) implantation and balloon angioplasty were performed. Ten pigs received subsequent local delivery of paclitaxel-solution via a newly designed catheter (Genie™, ACROSTAK corp., Switzerland), 10 animals served as a sham group and received vehicle (0.9% NaCl solution) and 10 animals were used as a control group. All animals were treated with aspirin and clopidogrel to prevent stent thrombosis. After final angiography the vessels were excised 42 days after intervention and prepared for histological and histomorphometric analysis. All coronary arteries showed complete endothelialization 42 days following treatment. Paclitaxel treatment led to a marked reduction of neointimal proliferation either post stent implantation (neointimal area: 1.04 ± 0.10 mm² vs. 2.37 ± 0.23 mm², p<0.001) or post balloon dilatation (neointimal area: 0.35 ± 0.14 mm², vs. 0.68 ± 0.24 mm², p<0.01). There were no significant angiographic or histomorphometric differences between the control and the sham group. In both paclitaxel groups neither angiographic edge phenomena nor a significant histomorphometric inflammatory response were found in the treated vessel segments. In conclusion, the local application of paclitaxel via the Genie™ catheter is safe and effective to significantly reduce the proliferative response post-stent implantation or balloon dilatation in an experimental animal model.

Keywords
Restenosis, angioplasty, paclitaxel, local drug delivery, Genie™

Introduction

The principal cause of restenosis after coronary stenting is neointimal hyperplasia resulting from the proliferation and migration of smooth-muscle cells and extracellular matrix production (1). Recently, the site-specific delivery of agents capable of interrupting cellular replication has shown promising results in inhibiting neointimal hyperplasia (2, 3). In particular, sirolimus and paclitaxel eluting stents have proven to be effective in reducing the risk of restenosis in large randomized studies (4, 5). Stent-based local drug delivery provides sustained drug release since the drug is embedded in a polymer that serves as a drug depot. However, cell-culture experiments indicate that even brief contact between vascular smooth muscle cells and lipophilic taxane compounds can inhibit the proliferation of such cells for a long period, which questions the concept of a polymer-mediated taxane release. Moreover, concerns have been raised that the polymeric matrix on the stent, in which the drug is embedded, might induce sustained inflammation (6), subsequently leading to an increased rate of late stent thrombosis, particularly in those patients with aspirin or clopidogrel resistance (7, 8).

The aim of the present study was to evaluate the safety and efficacy of a novel catheter-based method of passive intracoronary local paclitaxel drug delivery in porcine coronary arteries following direct bare metal stent implantation for the reduction of neointima formation.
Methods

Study protocol
In 30 crossbred domestic pigs of either gender (24.7 ± 3.6 kg) two individual coronary arteries were treated with either bare-metal stent implantation or balloon angioplasty. Bare-metal stents (Multi-Link Penta 3.0x13 mm; Guidant Inc., USA) were implanted using an overstretch ratio of 1.2 (mean implantation pressure 12.2 ± 1.3 atm) to induce neointimal hyperplasia. Immediately post-stent implantation, 10 pigs were treated in the stented vessel area with a continuous local application of paclitaxel solution for 120 seconds (sec) using the new local delivery device (Genie™, Acrostak, Switzerland), 10 animals served as a sham group and received vehicle (0.9% NaCl solution) and 10 animals were used as a control group without local delivery. The same protocol was performed following percutaneous transluminal coronary angioplasty (PTCA) without the implantation of a stent. All animals received aspirin (Aspirin, Bayer, Leverkusen, Germany) and clopidogrel (Iscover, Bristol-Meyer Squibb GmbH, Munich, Germany) treatment to prevent stent thrombosis. On the day before stent implantation the pigs were loaded with 600 mg aspirin and 300 mg clopidogrel, which was replaced by 100 mg aspirin and 75 mg clopidogrel once daily thereafter. To reduce the absolute number of animals needed for these experiments two different coronary arteries were treated in each animal.

Final angiography was performed after six weeks, the animals were sacrificed using an overdose of pentobarbital (60 mg/kg Nembutal, CEVA, Hannover, Germany), and the treated coronary arteries were excised, pressure-perfusion fixed and prepared for histopathological evaluation.

Instrumentation
Pigs were sedated with ketamine 10–20 mg/kg (Ketanest, Sanofi-CEVA, Germany) using an intramuscular injection. Afterwards, general anaesthesia was induced using an intravenous application of ketamine and midazolam (2 mg/kg Dormicum, Roche, Germany). Fluid balance was maintained using a constant intravenous infusion of sodium chloride (0.9%) at a rate of 2–3 ml per minute (min). All animals were treated in accordance with the National Institute of Health guidelines for the care and use of laboratory animals.

After arteriotomy of the right carotid artery a 7F sheath was introduced and 5,000 IU of heparin and a bolus of 250 mg acetylsalicylic acid were given intravenously. A 7F-guiding catheter was then advanced under fluoroscopic guidance. Selective coronary angiography was performed using at least two orthogonal projections for each coronary artery. Balloon dilatation or direct stent implantation were performed using mean pressures of 12 ± 1.3 atm. The interventional procedures were undertaken in the proximal segments of the left anterior descending artery (n: 9/9), the circumflex artery (n: 12/11) or the right coronary artery (n: 9/10) in the stented group or the balloon dilatation only group, respectively. Stent placement was avoided in angulated vessel segments and at the origin of side branches. After the intracoronary injection of 200 µg nitro-glycerine (Perlinganit, Schwarz Pharma D. GmbH, Germany) a final angiogram was obtained, the arteriotomy was closed, and the animals recovered from an-
aesthesia. Immediately following surgery the animals were administered a single dose of enrofloxacin 2.5 mg/kg (Baytril, Bayer Healthcare, Germany). The pigs were fed on a standard natural-grain diet without lipid or cholesterol supplementation throughout the study.

**Local drug delivery device**

A 3.0 mm application catheter (Genie™, Acrostak Corp., Switzerland) was used for local paclitaxel delivery. In comparison to former local drug delivery devices this balloon offers the advantage of drug transfer by jets that are directed coaxially to the vessel wall. Basically this system consists of a balloon having a distal and proximal segment with occlusive function and a central segment (length 18 mm) that allows for homogenous transfer of paclitaxel to the vessel wall. Holes in the distal ramp allow for filling of the drug depot without hydrojets due to an almost parallel flooding of the central segment (Fig. 1A and B). The balloon was continuously inflated at a low pressure of 2 atm that allows for distal and proximal occlusion of the vessel while simultaneously forming a central drug depot. A continuous pressure of 2 atm was maintained throughout application of the drug.

After inflation, stent deposition, and withdrawal of the PTCA balloon through the guiding catheter, the double balloon catheter was advanced and either paclitaxel or 0.9% saline solution were applied. To maintain an average pressure of 2 atm, which was necessary for complete balloon blockage, an average of 2.9 ± 1.6ml paclitaxel or normal saline solution were administered.

**Preparation of the paclitaxel solution**

Since paclitaxel (Sigma/Fluka Cat.No.86346, Germany) has a poor solubility in water, it was dissolved in a mixture of 50% Cremophor EL (Sigma/Fluka Cat.No.27963, Germany) and 50% anhydrous ethanol. This solution was then further diluted with physiological saline yielding a final concentration of 10 µM paclitaxel. We applied an average 2.9 ± 1.6 ml of the 10 µM paclitaxel solution containing 24.8 ± 13.7 µg of paclitaxel (9).

**Histological examination**

After sacrificing the animals, their hearts were excised, and a tube, which was connected to a fluid reservoir, was fixed within the ascending aorta. The coronary arteries were perfused with PBS (phosphate buffered saline) for 0.5 min and thereafter with 4% paraformaldehyde for 10 min using a pressure of 1.2 m water column. The stented and dilated coronary artery segments were carefully dissected from the epicardial surface and stored in a 4% paraformaldehyde solution over night. The segments were embedded in 2-hydroxyethylmetacrylate (Technovit 7100; Heraeus Kulzer, Germany). All specimens were sectioned into 200 µm slices with a rotating diamond-coated saw (Leica, Germany) with the struts remaining in situ. Sont slices, from which the struts had been removed, were re-embedded in 2-hydroxyethylmetacrylate and were cut into 6-µm sections. All sections were either stained with hematoxilin-eosin solution or with toluidine blue solution. At least three slices from each treated vessel were analysed. With minor modifications the measurements of the following histomorphometric parameters were performed according to Schwartz et al. (10) using a light microscope (Olympus, Germany) connected to a Sony DXC- 750P video camera (Sony, Germany) and a computer-based digitizing image analyser (VFG-1, Pavlov Engineering, Bulgaria): luminal area (mm²), neointimal area (mm²), internal elastic lamina area (IEL area; mm²), external elastic lamina area (EEL area; mm²) and maximal neointimal thickness. The % area stenosis was defined as neointimal area / IEL area expressed as a percentage. Inflammation and endothelialization were evaluated using slices of 200 µm and 6 µm thickness. The inflammation score for each individual strut was graduated as described by Kornowski et al. (11).

**Angiographic analysis**

Coronary angiograms were obtained pre-intervention, immediately following intervention, and after 42 days. Quantitative coronary analysis was performed using comparable coronary pro-

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Figure 2: Angiography six weeks after stent implantation with and without paclitaxel application. Control group (A), paclitaxel group (B), arrows indicate the area of treatment
Reference diameter and minimal lumen diameter (MLD) were immediately measured after stenting and at day 42. Late lumen loss was calculated as the difference between the MLD immediately after stenting and the MLD at 42 days follow-up using a standard software program (12).

Statistical methods
Data are presented as mean ± SD. Multiple comparisons between different groups were performed by use of ANOVA. Continuous variables of quantitative coronary angiography were compared by one-way, repeated measures by ANOVA (3 or 4 factors) and Student’s t-test after a normalized distribution was assured by a statistical program (SigmaStat for Windows Version 3.0, SPSS Inc). Statistical significance performed with ANOVA was inferred for a value of P<0.05.

Results
There were no acute or subacute thrombotic complications and no significant adverse events in terms of electrocardiographic (ECG) parameters or blood pressure during or after the coronary interventions.

Table 1: Results of quantitative coronary angiography of stented porcine coronary arteries immediately after stenting and 42 days following treatment.

<table>
<thead>
<tr>
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<th>S-P</th>
<th>S-NaCl</th>
<th>S-control</th>
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<tbody>
<tr>
<td>Reference diameter (mm)</td>
<td>2.81 ± 0.14</td>
<td>2.85 ± 0.23</td>
<td>2.88 ± 0.26</td>
</tr>
<tr>
<td>Stent diameter (mm)</td>
<td>3.29 ± 0.22</td>
<td>3.33 ± 0.12</td>
<td>3.32 ± 0.16</td>
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<tr>
<td>Overstretch ratio (mm)</td>
<td>1.17 ± 0.25</td>
<td>1.16 ± 0.37</td>
<td>1.15 ± 0.14</td>
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<tr>
<td>Minimal lumen diameter (mm)</td>
<td>2.37 ± 0.07</td>
<td>1.26 ± 0.05</td>
<td>1.15 ± 0.13</td>
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<tr>
<td>Late lumen loss (mm)</td>
<td>0.92 ± 0.08</td>
<td>2.07 ± 0.15</td>
<td>2.17 ± 0.19</td>
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</table>

S-P: stent and paclitaxel; S-NaCl: stent and 0.9% saline solution; S-control: stent only. Overstretch ratio= stent diameter/reference diameter. Values are given as mean ± SD. P-value between treated group (S-P) and control group.

Figure 3: Histological specimens of stented arterial segments (A and B) and dilated arterial segments (C and D) with (right) and without (left) treatment. Hematoxilin-eosin staining.
Angiographic findings
There were no significant angiographic differences between the control and the sham group, and no edge effects, such as proliferation, persistent dissection or aneurysm formation were observed in all three groups. Immediately post-stent deployment and after 42 days, angiographic analysis showed that all stents were fully expanded. In the treatment group paclitaxel resulted in a marked reduction of late lumen loss (Fig. 2), which was more than halved as compared to the control and the sham group (Table 1).

Histomorphometric parameters
The histomorphometric evaluation showed a statistically significant increase in lumen area and a corresponding decrease in maximal neointimal thickness and neointimal area in the stented vessels treated with paclitaxel (Fig. 3). Paclitaxel treatment led to a reduction of the neointimal area by 55% in the stented group (1.04 ± 0.10 mm² vs. 2.37 ± 0.23 mm²; P<0.001) (Table 2a). The neointima of all stented coronary segments was covered by endothelial cells (Fig. 4). There was no evidence of an increased inflammatory response around the stent struts in the paclitaxel group. The inflammation score was 1.43 ± 0.43 in the paclitaxel group versus 1.88 ± 0.21 and 1.83 ± 0.36 in the control and in the sham group, respectively. In particular no thrombus deposition nor necrosis nor hemorrhagia of the vessel wall were seen.

In the balloon dilatation only group the control group not having received treatment and the group treated with 0.9% saline solution showed no significant differences, whereas the group treated with paclitaxel presented with a reduction of neointima formation post dilatation (Table 2b). Following PTA neoimal formation was reduced by 59% by subsequent paclitaxel delivery, which translated into a significant increase in lumen area and a reduction in neointimal area of 0.35 ± 0.14 mm² versus 0.68 ± 0.24 mm²; P<0.01.

Discussion
Local antiproliferative therapy with drug-eluting stents (DES) has proved effective in a large number of clinical trials (4, 5, 13). However, concerns exist, in particular with respect to the stent coating material, since it has potential thrombogenic and inflammatory effects (6, 14–17). Furthermore, the fate of the coating itself, after the drug has been released, remains unclear. The problem of late thrombosis, which is related to incomplete endothelialization (18) and sustained inflammation is still pending, and like in brachytherapy before (19), the question about long-term safety cannot be answered within a few years of experience.

Paclitaxel has shown to exert long-lasting effects even after short single-dose application because of its irreversible effects on the cytoskeleton of smooth muscle cells (9). This makes paclitaxel a promising candidate for local intracoronary delivery independent of the stent itself by using catheter techniques. Besides the fact that no coating as a drug carrier has to be used, this technique offers further advantages, such as the possibility to treat the whole circumference of the vessel area and not only the area around the stent struts. Even more importantly, the stent

<table>
<thead>
<tr>
<th>Luminal area mm²</th>
<th>S-P</th>
<th>S-NaCl</th>
<th>S-control</th>
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<tr>
<td>5.24 ± 1.02</td>
<td>3.15 ± 0.32</td>
<td>3.0 ± 0.25</td>
<td>p&lt;0.001</td>
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<td>Internal elastic lamina (IEL) mm²</td>
<td>6.28 ± 1.1</td>
<td>5.48 ± 0.11</td>
<td>5.38 ± 0.24</td>
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<td>External elastic lamina (EEL) mm²</td>
<td>7.72 ± 1.09</td>
<td>6.89 ± 0.19</td>
<td>6.65 ± 0.57</td>
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<tr>
<td>Media area mm²</td>
<td>1.44 ± 0.17</td>
<td>1.41 ± 0.12</td>
<td>1.27 ± 0.36</td>
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<tr>
<td>Neointimal area mm²</td>
<td>1.04 ± 0.10</td>
<td>2.33 ± 0.25</td>
<td>2.37 ± 0.23</td>
</tr>
<tr>
<td>Neointima thickness mm</td>
<td>0.12 ± 0.008</td>
<td>0.32 ± 0.04</td>
<td>0.33 ± 0.04</td>
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<tr>
<td>% area stenosis</td>
<td>17 ± 2</td>
<td>43 ± 5</td>
<td>44 ± 4</td>
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Table 2a: Histomorphometric results of stented porcine coronary arteries 42 days following treatment.

<table>
<thead>
<tr>
<th>Luminal area mm²</th>
<th>B-P</th>
<th>B-NaCl</th>
<th>B-control</th>
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<tr>
<td>3.23 ± 0.45</td>
<td>1.7 ± 0.65</td>
<td>1.72 ± 0.57</td>
<td>P&lt;0.001</td>
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<tr>
<td>Internal elastic lamina (IEL) mm²</td>
<td>3.58 ± 0.45</td>
<td>2.41 ± 1.03</td>
<td>2.40 ± 0.78</td>
</tr>
<tr>
<td>External elastic lamina (EEL) mm²</td>
<td>4.43 ± 0.53</td>
<td>3.35 ± 1.15</td>
<td>3.33 ± 0.99</td>
</tr>
<tr>
<td>Media area mm²</td>
<td>0.85 ± 0.14</td>
<td>0.94 ± 0.29</td>
<td>0.93 ± 0.22</td>
</tr>
<tr>
<td>Neointima area mm²</td>
<td>0.35 ± 0.14</td>
<td>0.71 ± 0.26</td>
<td>0.68 ± 0.24</td>
</tr>
<tr>
<td>Neointima thickness mm</td>
<td>0.054 ± 0.022</td>
<td>0.14 ± 0.028</td>
<td>0.13 ± 0.028</td>
</tr>
<tr>
<td>% area stenosis</td>
<td>10 ± 4</td>
<td>30 ± 4</td>
<td>28 ± 4</td>
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Table 2b: Histomorphometric results of dilated porcine coronary arteries 42 days following treatment.
edges and adjacent vessel segments can be covered by pharmacotherapy to avoid a possible “edge-effect” as seen in brachytherapy and also in DES (20).

The results of this study indicate that a catheter-based local paclitaxel delivery is safe and effective in reducing restenosis in an experimental animal model. Although the delivery efficiency of a catheter-based therapy is generally low, the pharmacological properties of the applied drug play a crucial role. It has been shown that the delivery efficiency of the lipophilic compound paclitaxel is 10–20 times higher than with hydrophilic drugs (21, 22). Studies in cell culture indicate that an increased concentration of paclitaxel in the culture medium compensates for a shorter incubation time and that the duration of inhibition of cell proliferation exceeds the time during which the cells are exposed to the drug (9, 21). The dose of 2.9 ± 1.6 ml 10 µM paclitaxel in our study equals 24.8 ± 13.7 µg paclitaxel. This was found to be the optimal compromise between efficacy and toxicity in cell culture experiments (9) and earlier proved effective in the rabbit model (21). A local delivery of 10 ml 10 µM paclitaxel will likely not result in measurable systemic plasma levels. Although no side effects were detected in our animal studies, these cannot be generally excluded. Yet again they are unlikely, since even a local intracoronary infusion of 80 ml 200 µM paclitaxel added to contrast media did not cause side effects in an experimental animal model (23).

According to a consensus paper recently published in Circulation (24) the efficacy of drug eluting stents should be evaluated at different time points in preclinical studies. It is suggested that at day 28 neointimal hyperplasia should be determined and that 2–5 months later long-term effects should be examined when “healing” is assumed to be complete. To reduce the absolute number of animals needed we used a time point of six weeks following paclitaxel drug transfer to measure both neointimal hyperplasia and long-term effects, which were subsequently compared to a sufficiently sized sham and control group for the detection of differences. While possible edge effects were excluded by angiography, only the reduction of neointimal hyperplasia was documented both angiographically and histologically. Focal edge effects, however, such as inflammation or localized lacerations, cannot be excluded, which constitutes a limitation of the study.

The results presented in this study are in accordance with the recently published work of Scheller et al. who used a concept of balloon coating with paclitaxel showing that this technique was highly effective in the pig model (25). Comparable to our study this group also chose a time point of five weeks for the detection of neointimal growth. Even more importantly they conducted a randomised clinical study in patients with in-stent stenosis in which a paclitaxel-coated balloon showed excellent results (26).

The conceptual advantages of local paclitaxel delivery independent of a polymer-carrying stent are quite comparable in the study of Scheller et al. and the work presented here. Paclitaxel coated onto a balloon as well as a catheter-based delivery of paclitaxel solution distribute the drug more evenly on the vessel surface than a drug-eluting stent, where the drug is bound to the stent struts. Moreover, a short application time led to long lasting effects using both application techniques, which again questions the need for a drug depot that elutes paclitaxel over a longer period of time. Furthermore, the drug that is delivered via the Genie™ balloon can be applied several times to the target area using low pressures if this is clinically deemed to be necessary. These preclinical data have now led to a prospective, randomised, blinded clinical study in 204 patients, which will be completed within the year 2007.

**References**