Do Pharmacokinetics Explain Persistent Restenosis Inhibition by a Single Dose of Paclitaxel?

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Background—The purpose of this study was to investigate the elimination of paclitaxel from the arterial wall after a single short administration with a coated balloon.

Methods and Results—Slightly oversized paclitaxel-coated balloons (dose 3 or 9 μg/mm²) without or with premounted stents were inflated in nonatherosclerotic coronary arteries of either young domestic pigs or adult Goettingen minipigs. The paclitaxel content of plasma, arterial segments, and residual hearts (without treated arteries) was measured for up to 180 days using high-performance liquid chromatography/ultraviolet detection or mass spectrometry. Angiograms were evaluated for lumen narrowing. The paclitaxel concentration in plasma remained <10 ng/mL. In arteries of domestic pigs and minipigs treated with paclitaxel-coated balloons with premounted stents, 10%±5% or 21%±8% of dose, respectively, was initially detected and decreased to 3.5%±3.1% of dose (domestic pig) by Day 7. Within 6 months it fell with a half-life of 1.9 months to 0.40%±0.35%. After 3 months the concentration in the arterial wall was 17±11 μmol/L. Without a stent, drug transfer to the vessel wall was somewhat reduced and elimination faster. Immediately after treatment up to 26%±4% of dose was detected in the residual whole hearts. It dropped with a half-life of 45 days to 1.5%±0.6% of dose (0.3 μmol/L) within 6 months.

Conclusions—After a single local administration with coated balloons, paclitaxel stays in the vessel wall of pigs long enough to explain persistent inhibition of neointimal proliferation. The pharmacokinetics of paclitaxel does, however, not exclude other reasons for sustained efficacy such as early blocking of processes initiating excessive and prolonged neointimal proliferation. (Circ Cardiovasc Interv. 2012;5:00-00.)

Key Words: drug-eluting balloon ■ long-term efficacy ■ pharmacokinetics ■ porcine coronary overstretch model ■ restenosis

Approximately 10 years ago drug-eluting stents were introduced for efficacious prophylaxis of restenosis due to neointimal proliferation. As permanent implants, stents provide an attractive platform for sustained-release formulations aimed at selectively treating the stented vessel segment with high drug concentrations without risking systemic side effects. Animal experiments1-2 and clinical trials3 indicated that sustained release and the choice of a suitable drug were mandatory for effective inhibition of restenosis during the months and years after angioplasty.4-5 It is surprising that a single immediately released dose of a drug requiring sustained release when coated on a stent inhibits neointimal proliferation in animals for weeks and restenosis in patients for years.6 Paclitaxel concentrations in the vessel wall shortly after treatment with the dissolved drug or drug-coated balloons have been reported7-13 but little is known about the residence time of the drug, which may be relevant for the persistent effect if administered only during the short time of balloon inflation.

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WHAT IS KNOWN?

- Sustained release is mandatory for drug-eluting stents.
- Drug-coated balloons have a limited period of drug release, yet, surprisingly, paclitaxel released during the short inflation time inhibits restenosis without late catch-up.
- Studies of the pharmacokinetics of paclitaxel indicate a half-life in the bloodstream of approximately 1 day.
- Limited data exist pertaining to the pharmacokinetics and paclitaxel content of the vessel wall after treatment with paclitaxel-coated balloons not extending beyond 1 to 4 weeks.

WHAT THIS ARTICLE ADDS

- A single inflation of a paclitaxel-coated balloon catheter in the coronary artery of a healthy swine results in measurable drug concentration in the vessel wall that is detectable up to 6 months after the balloon inflation.
- The long residence time of paclitaxel in the vessel wall may explain persistent efficacy of the drug-coated balloon but does not exclude early inhibition of processes initiating excessive and prolonged neointimal proliferation.

Methods

Study Design

In a first study with a follow-up of up to 4 weeks, 21 pigs (provided by the Institute of Medical Technology & Research, Rottmersleben, Germany) were randomly assigned to the following treatment groups (Figure 1): drug-coated balloons, regular dose of 3 μg paclitaxel/mm² balloon surface with premounted stent, n=13; drug-coated balloons, regular dose, no stent, n=5; and high-dose balloons coated with 3 times the regular dose of 3 μg/mm² with premounted stent, n=3. The high-dose group was added because an identical high-dose balloon was tested in a previous efficacy and tolerance study to explore the margin of safety related to the regular dose. Each pig received 3 coated balloon catheters with or without premounted bare metal stents, 1 each into the right coronary artery, left ramus circumflex, and left anterior descending artery. Shortly thereafter (10–30 minutes), 2 animals per group were euthanized for measurement of remaining paclitaxel in the treated artery segment: domestic pigs, regular dose with premounted stent—2 animals at 1, 6, 24, and 48 hours and 7 days and 1 animal 4 weeks posttreatment; domestic pigs, regular dose without stent—2 animals at 24 hours and 1 animal 4 weeks posttreatment and domestic pigs, triple dose with premounted stent 1 animal 4 weeks posttreatment.

Because results indicated persistence of paclitaxel in the vessel wall 4 weeks after treatment, a second study was started in additional 9 pigs with drug-coated balloons, regular dose with a premounted stent. In 3 pigs each, the paclitaxel content of coronary arteries was investigated 10 to 30 minutes after treatment and after 3 and 6 months to obtain a valid starting point and longer-term follow-up through 6 months.

Balloon Catheters

Falcon Bravo 3.5×20-mm percutaneous transluminal coronary angioplasty balloon catheters, 15-mm stainless steel Coro large stents suitable for expansion between 3.0 and 4.0 mm were provided by Invatec Technology Center, Frauenfeld, Switzerland. Balloons were coated with the regular dose of 3 μg paclitaxel/mm² balloon surface or with the 3-fold dose applying the composition used in Invatec’s FreePac coating. The total dose administered to an animal with 3 balloon catheters amounted to approximately 2.5 mg or 7.5 mg, respectively. On part of the coated balloon catheters, stents were mounted. All catheters were EO-sterilized. Small Invastent Volo stents (no paclitaxel) were implanted distal to the treated segment as “marker stents” immediately before introducing the paclitaxel-coated balloons without premounted stents.

Animals and Treatment

All animal experiments were conducted in accordance with the guidelines for animal experiments set forth by the animal protection committee of the Sachsen-Anhalt government, Germany. The studies with follow-up periods of up to 4 weeks were performed in castrated male domestic pigs, approximately 3 months old and weighting 28.5±2.1 kg.

Because usual domestic pigs would grow too large within 6 months, female or male Ellegaard Goettingen minipigs (provided by the Institute of Medical Technology & Research, Rottmersleben, Germany), approximately 2 years old and weighting 44.6±3.8 kg, were used for the additional study with follow-up periods of 3 and 6 months.

Pigs were anesthetized as previously described. All animals received 5000 IU heparin, 250 mg aspirin, and 200 μg intracoronary nitroglycerin. The coronary arteries were imaged, and target segments for treatment were selected in left ramus circumflex, left anterior descending artery, and right coronary artery. In each of the 2 studies, animals were randomized to the treatments, and treatments were performed in a randomized order. Balloons were inflated to an oversize ratio of approximately 1.2 (implantation pressure 8–16 atm, domestic pigs 1.2±1.4 atm, minipigs 1.2±1.3 atm) and left expanded exactly for 1 minute. Electrocardiography and oxygen saturation were monitored during the procedure and blood pressure before and after the intervention. Balloons of used (inflated) balloon catheters were cut off and stored at −20°C for drug analysis (Table 1). For follow-up angiography ≥28 days after the intervention, the animals were anesthetized as described previously and angiography was performed in the same way as during treatment. At the time intervals indicated in Table 2, the animals were euthanized. Hearts were rapidly excised, the treated segments of the coronary arteries plus the portions 5 mm distal and proximal to the stent edges were dissected, and placed in preweighed vials.

Aspirin (100 mg/day; Bayer) and ticlopidine hydrochloride (250 mg/day; Tiklyd; Sanofi Winthrop) were orally administered starting 2 days before the procedure and continuing until euthanasia. During the whole in-life phase of the studies, the animals were observed for clinical abnormalities.

Angiographic Data

Coronary imaging was done after intracoronary administration of 200 μg nitroglycerin using a Siemens AXIOM Artis zee fluoroscope. A 6-Fr Judkins L3.5 catheter served as a reference. Quantitative analysis of coronary arteries was performed with the CAAS II System (Pie Medical).

Paclitaxel Analysis

Paclitaxel on used balloons was measured after extraction with ethanol. After addition of 1 μg of docetaxel as an internal standard, plasma samples were extracted with tert-butyl methyl ether. The organic solvent was evaporated and the residue dissolved in the mobile phase used for high-performance liquid chromatography analysis.

For extraction of arteries, a defined volume of ethanol was added. The samples were treated with ultrasound for 30 minutes at room temperature and then centrifuged. After dissection of the treated arteries, the residual hearts were homogenized in methanol. Homogenates were treated as described for the arteries.
Paclitaxel was determined by high-performance liquid chromatography with ultraviolet or mass spectrometry detection. Paclitaxel extracted from balloons was analyzed on a Waters Symmetry column, C18, 5-μm, 25 cm × 4.6 mm using: 45% phosphate buffer 0.005 M (pH 3.5), and 55% acetonitrile as mobile phase, for biological samples the same column was used but the mobile phase was 60% phosphate buffer and 40% acetonitrile; flow rate was always 1 mL/min, and the detection at 230 nm.

Plasma and tissue extracts with low paclitaxel content were analyzed by high-performance liquid chromatography with mass spectrometry detection (API 2000, Applied Biosystems, Darmstadt, Germany) in the multiple reaction monitoring mode. Plasma extracts were directly injected, 150 μL of the tissue extract was mixed with 150 μL of 0.1% formic acid and injected into the high-performance liquid chromatography system. The column was Waters Symmetry, C18, 3.5-μm, 2.1×100 mm. Mobile phase was 50% of 0.1% formic acid and 50% acetonitrile, the flow rate 0.2 ml/min resulting in a retention time of 8 min. Paclitaxel (and internal standard docetaxel) was ionized by electrospray ionization (TurboIonSpray) and detected at 854.3 (808.3 for docetaxel) average mass units with a transition in the collision cell to 105.1 (226.0 for docetaxel) average mass units.

**Statistical Analysis**

Data are presented as mean values±SD. Statistical analysis is exploratory. Continuous variables were compared by 2-sided Student’s t test; no multiple comparison adjustments to the significance level were made across the multiple variables tested. The analyses refer to balloons or treated arterial segments. Different treatments and animal strains were compared at identical time points. An additional analysis referring to pigs was not performed due to the small sample size; within-pig correlation was not taken into account. Probability values <0.05 were considered to indicate statistical significance. Half-lives of drug were estimated assuming first-order kinetics, Pearson correlation coefficients, and probability values were calculated using SPSS 10 (SPSS Inc).

**Results**

In the first study, 3 coronary arteries were treated in each of 21 domestic pigs with paclitaxel-coated balloon catheters with or without premounted stents at the clinically tested dose of 3 μg/mm² balloon surface or with balloons coated with 3 times this dose. The interventional procedure was well tolerated by all animals. During the observation period, including balloon inflation and stent implantation, short electrocardiographic disturbances were observed during balloon inflation in 2 animals. In the majority of the animals, vessel spasms proximal and/or distal to the implanted stents or within the treated segment occurred, which may be explained by overstretch during stent implantation/balloon dilatation. All animals survived without clinical signs of...
disease until euthanasia. The same applied to the minipigs used in the second study except that 1 of them did not recover from anesthesia. A blood clot was found in the stented segment of the left anterior descending artery. The left anterior descending artery of this animal was very narrow. It had been overdilated by 40%. The animal was assigned to the group for immediate analysis. The paclitaxel content of the affected artery was not different from that of the other arteries of this and the other pigs of the same group. No device failures occurred, and treatment and stent implantation were successful in all arteries.

The paclitaxel content of the balloons before use (1) represents the dose. We measured (2) the paclitaxel content of balloons after inflation in a coronary artery and retraction (= proportion of dose not released during the procedure); (3) the amount of paclitaxel in the vessel wall (assumed to be identical with the transfer to the vessel wall if measured 10–30 minutes after balloon inflation); (4) paclitaxel in the vessel wall later points in time up to 6 months postadministration indicates the residence time; (5) concentration in plasma which depends on the systemic exposure; and (6) the amount of paclitaxel in the myocardium after dissection of the treated vessel segments.

Approximately 20% of the regular dose of the 3-μg/mm² balloon surface was retrieved on the used balloons (Table 1), which means that 80% was released during treatment. However, when the balloons were coated with 3 times this dose, paclitaxel was almost completely released, which may be explained by firm adherence of only a fixed amount of the drug, which is a smaller proportion of a large dose than of a smaller dose.

However, when catheters coated with a dose of 3 μg/mm² were used with premounted stents in young domestic pigs 10 to 30 minutes posttreatment, only approximately 9.9±5.1% of the dose was found in the coronary arteries resulting in a mean concentration of approximately 200 μmol/L in the dissected segment (Table 2). The amount of drug initially found in the vessel tended to be reduced when no stent was applied (5.5±4.7% of drug) or when the dose on the balloon was increased (6.5±4.1%). After 24 hours and 28 days, statistically significantly less drug (percent of dose) was found in the vessel segments without stent compared with those with stent. In the adult minipigs of the second study, the proportion of dose found in the coronary arteries shortly after treatment using the drug-coated balloons with premounted stents was approximately twice as high as the proportion of dose transferred from the same balloons with stent to the arteries of young domestic pigs (last column of Table 2; P<0.05). From the beginning, plasma levels in all treatment groups including the high-dose group remained distinctly below the limit of quantification of our method, that is, 10 nmol/L. Paclitaxel was slowly eliminated from the treated arteries of the domestic pigs (Figure 2A). Four weeks after treatment with the dose of 3 μg/mm², a mean concentration...
of 34.1±8.4 µmol/L was found. Therefore, the second study was started to investigate the drug content up to 6 months after treatment. The results indicate a further slow decrease in paclitaxel concentration to approximately 5.4±4.8 µmol/L.

The paclitaxel content of whole hearts after the excision of the treated segments of the coronary arteries was also determined. Because of the small number of hearts, individual values are given in Table 3. Initially, approximately 20% to 25% of the total dose on the 3 catheters used per pig was detected in the hearts. The drug concentrations were far lower in the hearts than in the treated arteries but they decreased at a similar rate (Figure 2B–C). Table 4 shows the overall balance of retrieved paclitaxel. Depending on dose, premounted stents and strain of swine, 5.5% to 21.1% of dose on the balloons were retrieved in the treated vessel segments, 17.7% to 25.9% in the myocardium, and 5.0% to 20.9% remained on the balloons. Overall, 33.2% to 56.4% of dose was not retrieved. They may be lost in the hemostatic valve and guiding catheter or in the general circulation. Table 5 summarizes the quantitative angiographic data. The latter confirms the overstretch during treatment, inhibition of neointimal proliferation in the few arteries, which were investigated 4 weeks after treatment with the lowest late lumen of 0.34±0.03 mm in domestic pigs treated with the regular dose of 3 µg/mm² and premounted stents, and the lack of persistent inhibition of neointimal proliferation thereafter, that is, late lumen loss of 1.31±0.82 mm after 3 months and 1.24±0.60 mm after 6 months (Figure 3).

**Discussion**

**Animal Model**

The studies were performed in healthy young domestic pigs or adult minipigs. In this animal model, pronounced neointimal proliferation of coronary arteries is induced by injury caused by overstretch of the vessel wall and stent implantation, which stabilizes the overstretch and adds continuous irritation. It has been developed for preclinical testing of drug-eluting stents. The coronary injury model in healthy pigs used in our studies is a disease model recommended by experts despite the obvious difference to human atherosclerosis. It is a standardized model with which extensive experience has been gained. Large animal models closer to human atherosclerosis have been explored. Although desirable, these models do not reflect the spectrum of human atherosclerotic lesions, and the incidence and quality of lesions in these models are variable or the reproducibility has
not yet been investigated. Vessel wall retention, and possibly the amount of drug retrieved in the myocardium, will certainly be altered if atherosclerotic arteries are treated. The increased transfer of the drug into the arterial wall of adult minipigs compared with young domestic pigs may be due to age-related increasing strength of the arteries, which results in higher pressure during drug release. This finding emphasizes the importance of the choice of the animal model.

After administration by a coated balloon, pharmacokinetics of paclitaxel in the arterial wall are remarkably different from those of paclitaxel in plasma. After intravenous infusion, the terminal half-life usually reflecting metabolic inactivation and/or excretion was 21 ± 14 hours (range, 4–65 hours).17,18 This is within the range of many drugs and justifies the assumption that the drug leaves the tissue at approximately the same rate. Accordingly, our first study was designed to extend up to 7 days after treatment with a final control in just 3 vessels per treatment group at 28 days. Treatments were performed using paclitaxel-coated percutaneous transluminal coronary angioplasty catheters either with premounted stents because stents were shown in animal efficacy studies to enhance neointimal proliferation14,19–22 or with coated balloons with no stent in the treated segment, which is closer to the preferred clinical application.23–27 A high-dose group was added to explore if uptake and elimination are dose-dependent. Because considerable concentrations of paclitaxel were still detected in all treated arterial segments after 4 weeks, a second study using minipigs was performed with follow-up for up to 6 months after local drug delivery. In this study, only the catheters 3 μg/mm² with premounted stents were investigated.

As far as comparable, the results of the current study are in agreement with previously published data. The release of the drug from the balloons used in the current study with urea as an additive was slightly less complete than for a coating composition with a contrast medium,19 similar to that recently reported by Posa et al12 but more complete than from a paclitaxel-coated balloon tested by Cremers et al,22 which was found to be less effective. The uptake in the vessel wall and its concentration in the tissue was similar to published data12,19 and significantly higher than concentrations achieved by exposing arteries to paclitaxel solutions10 or paclitaxel-coated balloon catheters, which did not inhibit restenosis to a satisfactory degree.13 Surprisingly, after an initial drop in concentration, paclitaxel stayed much longer in the arterial wall than expected. After 3 months, approximately 3% of the initial concentration was detected, and after 6 months, it was still 1%.

### Paclitaxel Concentration, Efficacy, and Toxicity
The water solubility of paclitaxel is extremely low. Depending on the physical state of solid paclitaxel (ie, crystalline or

### Study Design and Comparison of Results to the Literature
According to the literature, the half-life of paclitaxel in plasma is in the order of hours. After intravenous infusion, the terminal half-life usually reflecting metabolic inactivation and/or excretion was 21 ± 14 hours (range, 4–65 hours).17,18 This is within the range of many drugs and justifies the assumption that the drug leaves the tissue at approximately the same rate. Accordingly, our first study was designed to extend up to 7 days after treatment with a final control in just 3 vessels per treatment group at 28 days. Treatments were performed using paclitaxel-coated percutaneous transluminal coronary angioplasty catheters either with premounted stents because stents were shown in animal efficacy studies to enhance neointimal proliferation14,19–22 or with coated balloons with no stent in the treated segment, which is closer to the preferred clinical application.23–27 A high-dose group was added to explore if uptake and elimination are dose-dependent. Because considerable concentrations of paclitaxel were still detected in all treated arterial segments after 4 weeks, a second study using minipigs was performed with follow-up for up to 6 months after local drug delivery. In this study, only the catheters 3 μg/mm² with premounted stents were investigated.

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### Paclitaxel Balance Shortly After Balloon Angioplasty, Percent of Dose (Mean or Mean±SD*)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Percent of Dose Not Released/Retrieved on the Used Balloons</th>
<th>Percent of Dose in the Treated Segment of the Coronary Artery</th>
<th>Percent of Dose in the Myocardium (Excluding Treated Arterial Segments)</th>
<th>Percent of Dose Not Retrieved (Lost on the Way to the Treatment Site or Released Into the General Circulation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic pig</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard dose, premounted stent</td>
<td>17.5±6.0</td>
<td>9.9±5.1</td>
<td>24.8</td>
<td>47.8</td>
</tr>
<tr>
<td>Standard dose, without stent</td>
<td>20.9±4.0</td>
<td>5.5±4.7</td>
<td>17.2</td>
<td>56.4</td>
</tr>
<tr>
<td>Triple dose, premounted stent</td>
<td>5.0±1.6</td>
<td>6.5±4.05</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>Minipig</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard dose, premounted stent</td>
<td>19.8±5.3</td>
<td>21.1±7.6</td>
<td>25.9±3.7</td>
<td>33.2</td>
</tr>
</tbody>
</table>

*For *P* values, see Tables 1 and 2.
amorphous), it is in the range of <1 μg/mL to a few micrograms per milliliter. The high concentrations detected in the vessel wall in the current and previous investigations indicate that the drug is not fully dissolved but is either still in a particulate state or bound to tissue constituents. Low solubility is also a problem in determining the effective concentration in cell culture. To achieve higher than 1 to 10 μmol concentrations in the culture medium, organic solvents such as dimethyl sulfoxide have been added, which display their own toxicity. Furthermore, dimethyl sulfoxide stabilizes the microtubules like paclitaxel. In cell culture, inhibition of cell proliferation has been observed at an extremely wide range of concentrations (i.e., <1 nmol/L, >10 μmol/L). The efficacy of low concentrations may be

Table 5. Summary of Quantitative Coronary Angiography: Domestic Pig 28 D and Goettingen Minipig 90 and 180 D

<table>
<thead>
<tr>
<th></th>
<th>Standard Dose Premounted Stent</th>
<th>Standard Dose Without Stent</th>
<th>Triple Dose Premounted Stent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic pigs, at treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (analyzed vessels)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>RFD, mm</td>
<td>2.79±0.03</td>
<td>2.32±0.56</td>
<td>2.95±0.31</td>
</tr>
<tr>
<td>Diameter post-PTCA, mm</td>
<td>3.13±0.22* (stent diameter)</td>
<td>2.52±0.20 (mean lumen diameter)</td>
<td>3.19±0.12* (stent diameter)</td>
</tr>
<tr>
<td>Domestic pigs, at 4-wk control angiography</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RFD 4 wk, mm</td>
<td>2.66±0.08</td>
<td>2.22±0.36</td>
<td>2.71±0.30</td>
</tr>
<tr>
<td>MLD 4 wk, mm</td>
<td>2.79±0.19*</td>
<td>1.98±0.45</td>
<td>2.64±0.22</td>
</tr>
<tr>
<td>LLL, mm</td>
<td>0.34±0.03</td>
<td>0.54±0.37</td>
<td>0.55±0.33</td>
</tr>
<tr>
<td>Minipig, at treatment, no follow-up</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No. (analyzed vessels)</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RFD, mm</td>
<td>2.30±0.28</td>
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<td></td>
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<tr>
<td>Lumen diameter post-PTCA, mm</td>
<td>2.81±0.17</td>
<td></td>
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<tr>
<td>Minipig, 3 mo, at treatment</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RFD, mm</td>
<td>2.51±0.33</td>
<td></td>
<td></td>
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<tr>
<td>Lumen diameter post-PTCA, mm</td>
<td>2.74±0.25</td>
<td></td>
<td></td>
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<tr>
<td>Minipig, 3 mo, at follow-up</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RFD, mm</td>
<td>2.58±0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLD, mm</td>
<td>1.49±0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLL, mm</td>
<td>1.31±0.82</td>
<td></td>
<td></td>
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<tr>
<td>Minipig, 6 mo, at treatment</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RFD, mm</td>
<td>2.42±0.23</td>
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<td></td>
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<tr>
<td>Lumen diameter post-PTCA, mm</td>
<td>2.81±0.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minipig, 6 mo, at follow-up</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>RFD, mm</td>
<td>2.41±0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLD, mm</td>
<td>1.57±0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LLL, mm</td>
<td>1.24±0.60</td>
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</table>

Mean±SD; exploratory P values indicate the following statistically significant differences (P<0.05): *post-PTCA greater in the nonstented vessels. RFD indicates reference diameter; PTCA, percutaneous transluminal coronary angioplasty; MLD, minimal lumen diameter; LLL, late lumen loss.

Figure 3. Angiographic presentation of right coronary arteries of a domestic pig (4 weeks) and minipigs (3 months and 6 months) after overdilatation with paclitaxel-coated balloon catheters (3-μg/mm² balloon surface) with premounted stents. Arrows indicate stented segments.
explained by the uptake of the lipophilic paclitaxel from the culture medium, usually present at large excess, into the cells over time, resulting in much higher intracellular concentrations, that is, 20- to 50-fold as reported by Kuh et al.\textsuperscript{31} Survival at permanent postmitotic arrest may explain the tolerability of high concentrations of paclitaxel.\textsuperscript{32}

The distribution of paclitaxel in the arterial wall\textsuperscript{7–9,33,34} has been the subject of experiments and theoretical considerations. It depends on a large number of parameters, some of which are changed by the effects of the drug, which makes predictions even more difficult. Irreversible binding to microtubules\textsuperscript{17} and the detection of paclitaxel in solid tumors\textsuperscript{1} have been the subject of experiments and theoretical considerations, that is, 20- to 50-fold as reported by Kuh et al.\textsuperscript{31}

The current study was not intended to investigate inhibition of neointimal proliferation. Late lumen loss after 4 weeks was low. Contrary to human arteries, restenosis inhibition in pigs does not persist significantly beyond 4 weeks after treatment\textsuperscript{15} even if slow-release drug-eluting stents are implanted. Accordingly, after 3 and 6 months, late lumen loss was comparatively high.

Do Pharmacokinetics Explain Persistent Restenosis Inhibition by a Single Dose of Paclitaxel?

Taking the high potency of paclitaxel into account, the concentration in the vessel wall of healthy pigs remains at a level that might be sufficient to inhibit vascular smooth muscle cell proliferation for >3 months. The long-lasting persistence of paclitaxel in the tissue is a very unusual property for a drug. It may explain the persistent inhibition of neointimal proliferation caused by paclitaxel-coated balloon catheters. Although at first glance tempting, this explanation may be misleading. High concentrations shortly after balloon inflation suggest that a significant portion of the drug is not dissolved. After paclitaxel is dissolved, the protection from distribution (from the treated tissue to distant organs), excretion, and decomposition is most likely attributable to the binding to cell constituents. It is questionable if paclitaxel when bound in a way which prevents its excretion and metabolic decomposition can be pharmacologically active and inhibit cell proliferation. Therefore, an alternative or additional explanation for restenosis inhibition by drug-coated balloon catheters or short infusion of paclitaxel solutions\textsuperscript{37} cannot be excluded, namely the inhibition of a process that initiates vascular smooth muscle cell proliferation just after the vessel wall injury caused by angioplasty has occurred. It has been reported that intimal smooth muscle cell proliferation increased from Day 2 to Day 7 after injury and declined thereafter.\textsuperscript{38} If this is correct, it is further assumed that cell proliferation will not begin later when healing has reached an advanced stage. In this case, persistent drug supply or retention is not required.

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Disclosures

Dr Speck and Dr Scheller are coinventors of a patent application for various methods of inhibiting restenosis including the formulation used in this trial; Silvio Schaffner was an employee of Medtronic Invatec Technology Center GmbH.

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