The long-term clinical efficacy of intracoronary stenting is limited by restenosis, which occurs in 15–30% of patients and is caused solely by neointimal hyperplasia. Stent-induced mechanical arterial injury and a foreign body response to the prosthesis incite acute inflammation in the vessel wall, with elaboration of cytokines and growth factors that induce multiple signaling pathways for activation of smooth muscle cell proliferation and migration, whereas probucol is a vascular protectant and reduces stent restenosis by improving the lumen dimension at the stent placement site.

**Background** The long-term clinical efficacy of intracoronary stenting is limited by restenosis, which occurs in 15–30% of patients and is caused solely by neointimal hyperplasia. Stent-induced mechanical arterial injury and a foreign body response to the prosthesis incite acute and chronic inflammation in the vessel wall, with elaboration of cytokines and growth factors that induce multiple signaling pathways for activation of smooth muscle cell proliferation and migration. Therefore, the delivery of agents inhibiting cell cycle progression within the stent platform should produce an effective inhibition of in-stent neointimal hyperplasia. Carvedilol is an antioxidant that inhibits smooth muscle cell proliferation and migration, whereas probucol is a vascular protectant and reduces stent restenosis by improving the lumen dimension at the stent placement site.

**Methods and Results** Biodivysio phospholipid-coated stents were dip-coated with carvedilol (5 mg/ml) or probucol (50 mg/ml) by immersion in respective methanol solutions. Twenty-four stents (carvedilol=8, probucol=8, control=8) were placed in 12 pigs and histopathologic analysis was done 4 weeks later. Histomorphometry of the carvedilol-coated stent group compared with the control groups showed that the neointimal area decreased by 42% (1.12±0.55 mm² in the carvedilol group vs 1.92±0.52 mm² in the control, p=0.004) and the lumen area increased by 20% (5.15±0.90 mm² vs 4.17±0.87 mm², p=0.008), resulting in a 43% reduction of the percent area stenosis (18.22±9.6% vs 31.9±9.2%, p=0.002). In the probucol-coated stent group, the lumen area, neointimal area, and %area stenosis did not differ significantly from the control group. There were 7.7±2.97% proliferating nuclear cell antigen-positive cells in the carvedilol-coated stent group compared with 17.8±1.45% in the control group (p=0.0001) and 15.9±1.91% in the probucol group (vs control, p=NS).

**Conclusions** The carvedilol-coated stent, but not the probucol-coated one, inhibited neointimal hyperplasia in a porcine stent restenosis model.

**Key Words:** Carvedilol; Probucol; Restenosis; Stents

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(Received June 18, 2004; revised manuscript received September 14, 2004; accepted October 7, 2004)

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**Effect of Anti-Oxidant (Carvedilol and Probucol) Loaded Stents in a Porcine Coronary Restenosis Model**

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BiodivYsio® drug delivery stents (Biocompatibles Ltd, Farnham, UK) in concentrations of 5 mg/ml and 50 mg/ml, respectively, which were prepared by dissolving carvedilol or probucol powder (Roche Diagnostics GmbH) in methanol. Five stents were immersed in each drug solution for 5 min and then removed and air dried. The amount of drug loaded onto the stents was assessed by high-performance liquid chromatography (HPLC). The stents were placed into individual vials containing HPLC mobile phase solvent (acetonitrile [60%] and 0.25 mol/L ammonium acetate [40%]), which were then placed in an ultrasound bath for extraction for 30 min.

To assess the release kinetics of drug from the stents, the stents were placed in glass vials and immersed in 100 ml of phosphate-buffered saline. At different time intervals up to 60 min, the drug concentration in the buffer solution was measured by HPLC and converted to a cumulative release curve. The loading and release kinetic studies were performed in an in vitro system at a commercial reference laboratory (Biocompatibles Ltd).

Stent Preparation and Implantation

The animal study was carried out with approval of the institutional animal care and use committee and conformed to the guidelines of the American Heart Association on animal research. As previously described, juvenile farm pigs (25–30 kg body weight) were given oral acetylsalicylic acid (100 mg/day) and ticlopidine (250 mg/day) one day preoperatively and then daily thereafter until death. The animals were anesthetized with ketamine (12 mg/kg im) and xylazine (8 mg/kg im) and additional midazolam was injected intravenously during the procedure. Using sterile surgical technique, the left carotid artery was cannulated with an 8Fr hemostatic sheath through a midline cervical incision. Heparin (250 IU/kg) was administered through the arterial sheath and baseline coronary angiography was performed using guiding catheters.

Immediately before stenting, 3.0×15 mm BiodivYsio® drug delivery stents were coated by dipping the stents into the carvedilol, probucol or control solutions for 5 min and evaporating the solvent at room temperature for another 5 min. The segment of coronary artery to be stented was selected to allow more than 1.2-fold oversizing by visual estimation. The stents were placed using an inflation pressure of 8–12 atmosphere for 20–30 s. After intracoronary administration of 200 μg nitroglycerin, angiography was repeated to confirm adequate stent expansion and vessel patency. Twelve pigs underwent successful placement of 24 stents (control, n=8; carvedilol, n=8; probucol, n=8). The carotid arteriotomy site was ligated and the neck wound closed. Follow-up angiography was performed 28 days after stent implantation and then the animals were killed.

Histomorphometry

After euthanasia, the stented coronary arterial segments were carefully dissected with 1 cm of the vessel both proximal and distal to the stent and then fixed in a 10% formalin solution. Specimens were paraffin-embedded, sectioned, and stained with hematoxylin-eosin. All sections were examined with a light microscope by an experienced pathologist (J.T.P.) who had no knowledge of the treatment assignment of the segments. Morphometric analysis was performed using a computerized morphometry program (Visus 2000 Visual Image Analysis System). A minimum of 3 sections for each segment were analyzed and the results were averaged. The lumen, neointima, and total vessel cross-sectional areas were measured and recorded. The areas of external elastic lamina (EEL), internal elastic lamina (IEL), and lumen were measured by digital planimetry to obtain the neointimal area (IEL area–lumen area). Morphometric area of stenosis was calculated as 100 (1–lumen area/IEL area). The extent of arterial injury was...
Drug-loaded stents were implanted into the coronary arteries of rabbits, and the in vivo release kinetics of carvedilol and probucol were studied. The amount of loaded drug was determined by HPLC, and the release was monitored over time. The amount of drug released was expressed as a percentage of the total amount loaded.

**Table 1** Quantitative Analysis of Coronary Angiography Before and After Placement of Carvedilol- and Probucol-Loaded Stents and at 28-Day Follow-up

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Carvedilol Stent</th>
<th>Probucol Stent</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline RD (mm)</td>
<td>2.91±0.17</td>
<td>2.92±0.24</td>
<td>2.94±0.12</td>
<td>0.72</td>
</tr>
<tr>
<td>Post-stenting MLD (mm)</td>
<td>3.43±0.10</td>
<td>3.44±0.25</td>
<td>3.37±0.12</td>
<td>0.94</td>
</tr>
<tr>
<td>Follow-up RD (mm)</td>
<td>2.92±0.25</td>
<td>2.93±0.69</td>
<td>2.93±0.42</td>
<td>0.82</td>
</tr>
<tr>
<td>Follow-up MLD (mm)</td>
<td>2.60±0.13</td>
<td>2.79±0.14</td>
<td>2.68±0.44</td>
<td>0.09</td>
</tr>
<tr>
<td>Follow-up DS (%)</td>
<td>11.07±3.10</td>
<td>4.77±3.51</td>
<td>8.74±2.68</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Table 2** Histomorphometry and Immunocytochemistry at 28 Days After Implantation of Carvedilol- and Probucol-Loaded Stents

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Probucol Stent</th>
<th>Carvedilol Stent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injury score</td>
<td>2.12±0.50</td>
<td>1.95±0.49</td>
<td>2.05±0.42</td>
</tr>
<tr>
<td>EEL area, mm²</td>
<td>7.69±0.60</td>
<td>7.68±0.75</td>
<td>7.58±0.48</td>
</tr>
<tr>
<td>IEL area, mm²</td>
<td>6.09±0.76</td>
<td>6.27±0.83</td>
<td>6.28±0.59</td>
</tr>
<tr>
<td>Neointimal area, mm²</td>
<td>1.92±0.52</td>
<td>1.79±0.41</td>
<td>1.12±0.55*</td>
</tr>
<tr>
<td>Lumen area, mm²</td>
<td>4.17±0.87</td>
<td>4.15±0.61</td>
<td>5.15±0.90*</td>
</tr>
<tr>
<td>Area stenosis, %</td>
<td>31.9±9.2</td>
<td>32.2±10.4</td>
<td>18.2±9.6*</td>
</tr>
<tr>
<td>Inflammation score</td>
<td>1.13±0.30</td>
<td>1.09±0.31</td>
<td>1.19±0.21</td>
</tr>
<tr>
<td>Re-endothelialization score</td>
<td>3.0±0.0</td>
<td>3.0±0.0</td>
<td>3.0±0.0</td>
</tr>
<tr>
<td>PCNA index, %</td>
<td>17.8±1.45</td>
<td>15.9±1.91</td>
<td>7.78±2.97</td>
</tr>
</tbody>
</table>

**Results**

**Carvedilol and Probucol Loading and In Vitro Release Pharmacokinetics**

The amount of carvedilol loaded onto the stents from the 5 mg/ml solution was 7±1 mg/stent, and from the 50 mg/ml probucol solution was 52±16 mg/stent. In vitro release study showed that approximately 50% of the loaded carvedilol was released by 5 min, 77% by 30 min, and 85% by 60 min (Fig 1A). Approximately 15% of the carvedilol remained on the stents after 60 min. Therefore, carvedilol seemed a suitable candidate for delivery using the PC-coated stents in terms of its in vitro release kinetics. In contrast, the probucol loaded polymer-coated stent showed no evidence of release by 72 h after loading (Fig 1B).

**In Vivo Effect of Carvedilol and Probucol Loaded Onto the PC-Coated Stents**

All stent implantations were successful and all animals survived until euthanasia. Quantitative coronary angiography before and after the implantation, and at the 28-day follow-up demonstrated similar baseline lumen diameter, stent-to-artery ratio, and post-procedural and follow-up minimal lumen diameters among the 3 groups (Table 1). Pathologic examination at 28 days showed no evidence of myocardial infarction on gross inspection and all stented vessels were patent. On morphometric analysis, the injury score and the EEL and IEL areas were similar among the 3 groups. As compared with the 1.92±0.52 mm² of the control group, the neointimal area was reduced to 1.12±0.55 mm² in the carvedilol group (p=0.004), and 1.79±0.41 mm² in the probucol group (Table 2, Fig 2). There was a 42% reduction in the neointimal area in the carvedilol group compared with the control group, contrasting with a modest and nonsignificant decrease in the probucol group. Area stenosis was, as compared with the 31.9±9.2% of the control group, reduced to 18.2±9.6% in the carvedilol group (p=0.002), and 32.2±10.4% in the probucol group, resulting in an overall 43% reduction in the carvedilol group.

There was no evidence of an increased cellular inflammatory response in the stented vessels (Table 2). The neointimal tissue overlying the stents was identical in appearance in the 3 groups, consisting of SMCs and extracellular matrix without inflammatory cells (Fig 2). No cases of aneurysm or thrombosis were observed. Complete and equivalent healing and re-endothelialization were seen in the 3 groups.
The percentage of PCNA-positive SMCs in the neointima was, as compared with the 17.8±1.4% of the control group, reduced to 7.7±2.9% in the carvedilol group (p=0.0001), and 15.9±1.9% in the probucol group (Table 2, Fig. 2). The extracellular matrix of the neointima was proteoglycan-rich and less collagenous in the carvedilol group (Fig. 2).

**Discussion**

Carvedilol or probucol can be loaded onto PC-coated stents, but the in vitro release kinetics of the stent-based drug delivery were more suitable with carvedilol. The carvedilol-coated stent produced a 42% inhibition of neointimal hyperplasia in this porcine model of restenosis. The inhibition of neointimal hyperplasia by carvedilol is associated with inhibition of mitogen-activated protein kinase activity and regulation of cell cycle progression,17,18 and our result is comparable to other experimental stent-delivery studies using rapamycin (50%),32 paclitaxel (39.5%),33 and estradiol (40%).34 Our finding supports the feasibility of carvedilol stent coating using the PC polymer and demonstrated that stent-based delivery of carvedilol has a potential therapeutic benefit for the prevention of stent restenosis. In contrast, although probucol could be loaded onto the polymer-coated stent, it was not released for nearly 72 h after loading in the in vivo experiment. Approximately 52μg of probucol, loaded from a 50 mg/ml probucol solution onto a randomly selected stent, did not reduce restenosis in the stented porcine coronary arteries.

**Carvedilol or Probufol Stent Coating**

A drug-eluting stent is a device releasing single or multiple bioactive agents that will deposit in or affect tissues adjacent to the stent. Phosphorylcholine occurs naturally on the external surface of the cell membrane lipid bilayers, so PC-coated metal stents are biocompatible and well tolerated in porcine coronary arteries35 and humans36 and can act as a drug reservoir capable of controlled release of an agent.34,37 The principle of drug loading on the PC coating has been investigated using a variety of pharmaceutical compounds including a growth factor inhibitor (angiopep-2),37 an anti-coagulant (dipyridamole), an anti-inflammatory agent (dexamethasone), and an enhancer for re-endothelialization (estradiol).34 Loading of drugs occurred via absorption of the drug solution into the PC coating by swelling of the polymer matrix. Once loaded, the release of the therapeutic agents took place in a controlled and sustained manner (diffusion) from the PC coating.

In our preliminary experiment, the amount of carvedilol taken up onto the PC-coated stents was dependent on the concentration of the carvedilol solution. The in vitro release kinetics showed a steady but slower release curve over 60 min compared with the other drugs described.
earlier, all of which had release curves that terminated before 40 min. Carvedilol appears to be a suitable candidate for delivery using the PC-coated stents in terms of its release kinetics. The amount of probucol released from the BiodivYsio™ DD stent after 72 h was not as much as the other drugs, which does not seem plausible because the drugs are released from the polymer by diffusion, so we presumed the lipopholic property of probucol must have been involved in its release from the polymer-coated stent.

**Inhibitory Effect of Carvedilol on SMC Proliferation and Migration**

Carvedilol inhibits vascular SMC proliferation and migration. It has a greater inhibitory effect at concentrations as low as 1 μmol/l and, at 10 μmol/l, inhibited basal mitogenesis by approximately 65% and endothelin-1-stimulated mitogenesis by approximately 95%. Those in vitro and in vivo studies collectively demonstrate that carvedilol has a unique capacity to preserve vascular integrity even under conditions of profound vascular injury.

Nevertheless, no data are available to document whether carvedilol inhibits neointimal hyperplasia after stent implantation, although oral administration failed to reduce restenosis after successful atherectomy in the EUROCAR trial. A significant antiproliferative effect in ex vivo studies was observed with a high concentration of carvedilol (>1 μmol/l, and profound inhibition of neointimal growth has been achieved in rat at a concentration of approximately 5 μmol/kg per day by intraperitoneal delivery of 1 mg/kg twice daily for 17 days; however, the maximum recommended oral dose (50 mg/day) of carvedilol in humans provides a maximum plasma concentration of 60 μg/ml (0.157 μmol/l).

In our preliminary experiment, carvedilol loading onto the PC-coated stents was assessed with 3 concentrations of carvedilol (5, 25 and 40 mg/ml) before deciding the optimal dose. However, the 25 and 40 mg/ml solutions precipitated and had to be warmed to melt completely. Thus, a higher concentration of carvedilol does not correlate with a greater inhibitory effect on the neointima. In fact, a high concentration of carvedilol may precipitate the drug onto the surface layer of the PC polymer with less loading into the deep layer of the polymer. Furthermore, the carvedilol that has crystallized on the surface of the polymer may be easily washed off in the bloodstream before it reaches the tissue, similar to the dip-coating technique of a bare stent. Another concern is the local toxicity at higher concentrations.

There was a significant reduction of neointimal hyperplasia in the carvedilol stented artery, although no significant difference was observed between the carvedilol and control stents on quantitative coronary angiography. These pathologic and angiographic findings should be considered in future clinical trials.

**Effect of Probucol on SMC Proliferation and Migration**

Probucol can reduce restenosis after PTCA by its major mechanism of an antioxidant effect on vessel remodeling with an additional vasodilatory effect. Its antioxidant effect also reduces atherosclerosis and vascular SMC proliferation.

There are few studies of probucol’s effect on stent restenosis. Most recently, Kim et al could not prove the effect of probucol, but a multicenter study (CART-1) showed that probucol lowered stent restenosis to 32% (probucol group: 25.5%, control group: 37.5%) after administration from 2 weeks before stenting to 4 weeks after stenting. That preliminary study triggered our presumption that if probucol could be delivered to the vessel wall by a stent after being loaded onto a polymer and then released slowly because of its lipopholic property, it could replace the necessity for administration at least 2 weeks before stenting. However, as the present study has shown in a porcine coronary artery, even if probucol is loaded onto a polymer-coated stent and released slowly, there is no reduction of in-stent restenosis. We consider that there are several reasons why the probucol-loaded stent did not prevent in-stent restenosis. First, there is its action. The general improvement of the luminal dimensions of the stented segment as shown in the CART-1 study is not the same effect as that produced by probucol loaded onto a stent. Second, there is the duration of exposure to probucol. The probucol loaded onto the stent was released slowly over a long time, but not for 4 weeks after stenting. Third, there is the possible impediment to probucol being released from the polymer and finally there is the issue of the appropriate dosage. The present study assessed only one randomly chosen stent loaded with 52 μg probucol from a 50 mg/ml solution, so the effect of probucol loaded in either larger or smaller amounts was not assessed.

**Study Limitations**

This study was an observation of an experimental model of restenosis the relevance of which to human clinical circumstances is uncertain. Long-term studies may be necessary to elucidate whether the drug is simply delaying neointimal formation. The release profile of the drugs from the PC-coated BiodivYsio stent is not always suitable for humans (usually too short) and so this stent may be appropriate for the porcine model because the restenotic process of the pig is much faster than that of humans. Because probucol was not released from the stent, this model may not be the best for animal experiments. Neither the carvedilol nor the probucol concentration in tissue was estimated. We could not use more stents to extend the drug-loading test.

**Conclusion**

This is the first experimental study to demonstrate that carvedilol and probucol can be loaded onto PC-coated stents and have in vitro release kinetics suitable for stent-based delivery. The carvedilol-coated stents profoundly reduced neointimal hyperplasia in porcine coronary arteries, but probucol did not reduce in-stent stenosis.

**Acknowledgments**

This work was supported by grants from the Dong-A University Hospital (No. 111–2001) and the Chonnam National University Hospital (No. CUHRI-Y-200302), Korea. The authors express their thanks to Michael Juran and his laboratory personnel at Biocompatibles Ltd for performing the in vitro experiments.

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