Circ J 2005; **69:** 101 – 106

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

Weon Kim, MD; Myung Ho Jeong, MD; Kwang Soo Cha, MD*; Dae Woo Hyun, MD**; Seung Ho Hur, MD**; Kwon Bae Kim, MD**; Young Joon Hong, MD; Hyung Wook Park, MD; Ju Han Kim, MD; Young Keun Ahn, MD; Moo Hyun Kim, MD*; Jeong Gwan Cho, MD; Jong Tae Park, MD†; Jong Chun Park, MD; Jung Chaee Kang, MD

Background The long-term clinical efficacy of intracoronary stenting is limited by restenosis and delivery by the stent of agents inhibiting cell cycle progression should prevent 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® phosphorylcholine-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 mm^2 in the carvedilol group vs 1.92±0.52 mm^2 in the control, p=0.004) and the lumen area increased by 20% (5.15±0.90 mm^2 vs 4.17±0.87 mm^2, 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 different 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. (*Circ J* 2005; **69:** 101 – 106)

Key Words: Carvedilol; Probucol; Restenosis; Stents

The long-term clinical efficacy of intracoronary stenting is limited by restenosis, which occurs in 15–30% of patients[1,2] and is caused solely by neointimal hyperplasia[3–5] 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 (SMC) migration and proliferation[3–5] Therefore, the delivery of agents inhibiting cell cycle progression within the stent platform should produce an effective inhibition of in-stent neointimal hyperplasia[6–11]

Carvedilol is a neurohumoral antagonist with multiple actions[12,13] It was originally discovered to be a β-adrenoreceptor antagonist,[14] but subsequent research revealed that it has potent antioxidant and free radical scavenger properties[15] In addition, carvedilol inhibits vascular SMC proliferation induced by a broad group of mitogens, such as platelet-derived growth factor, fibroblast growth factor, endothelin-1, serum and thrombin,[15,16] and it has produced 84% suppression of neointimal hyperplasia in rat carotid injury model[17] Recently, the mechanism of the antimitogenic action of carvedilol on vascular smooth muscle was shown to be inhibition of mitogen-activated protein kinase activity and regulation of cell cycle progression[18,19] Carvedilol is highly lipophilic, which promotes a rapid cellular uptake,[20] and a stent containing carvedilol should result in a high local concentration in the vessel walls with which it is in contact.

Since probucol was discovered, it has been developed and sold as a lipid-lowering agent[21] It is also an antioxidant[22] and reduces restenosis after percutaneous transluminal coronary angioplasty (PTCA),[23–26] and has recently been identified as a vascular protectant[27] A multicenter study recently reported that post-stenting restenosis was reduced by probucol via a mechanism improving the dimension of the vessel's lumen[28] The combination of probucol and candersatan has the potential effects of both anti-oxidant and vascular protectant[29]

In the present study, we tested whether loading of carvedilol or probucol onto phosphorylcholine (PC)-coated stents was feasible and which one most effectively inhibits neointimal hyperplasia in a porcine model of stent restenosis.

Methods

Carvedilol and Probucol Loading and in Vitro Release Kinetics

Carvedilol or probucol was loaded onto 3.0×15 mm

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

*Heart Center of Chonnam National University Hospital, Gwangju, *Department of Cardiology, Dong-A University Hospital, Busan, **Department of Internal Medicine, Keimyung University Dongsan Medical Center, Daegu and †Department of Pathology, Chonnam National University Medical School, Gwangju, Korea*

Mailing address: Myung Ho Jeong, MD, PhD, FACC, FESC, FSCAI, Professor of Medicine, Chief of Cardiovascular Medicine, Director of Cardiac Catheterization Laboratory, Heart Center of Chonnam National University Hospital, 8 Hak-dong, Dong-gu, Gwangju 501-757, Korea. E-mail: myungho@chollian.net

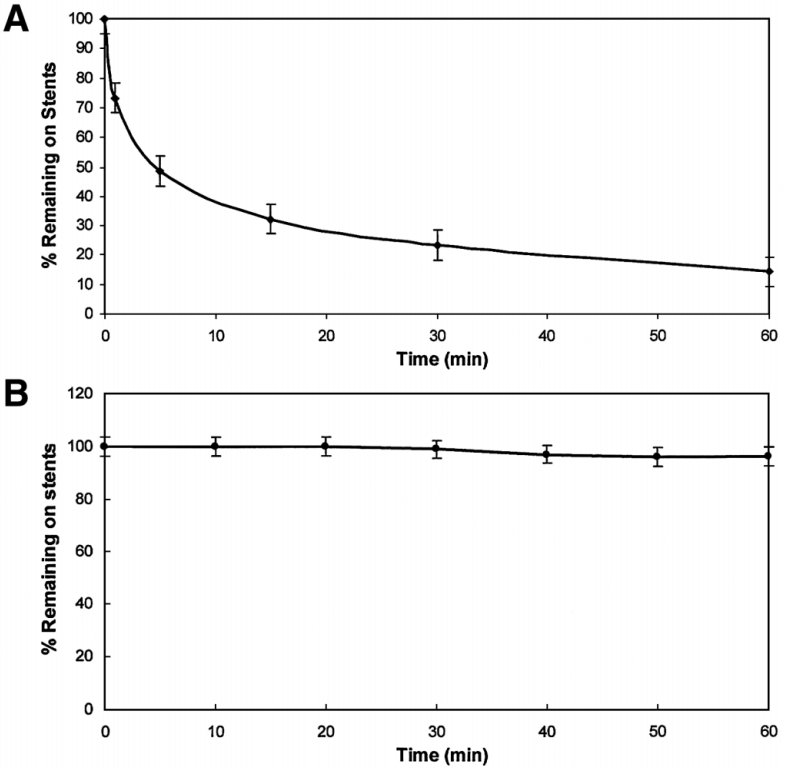

Fig 1. Cumulative curve of in vitro carvedilol release from the stents. (A) Approximately 50% of the loaded carvedilol was released by 5 min, 77% by 30 min, and 86% by 60 min. Approximately 15% of the carvedilol remained on the stent from 60 min onward. (B) Probucol-loaded polymer-coated stent showed no evidence of release for 72 h after the probucol was loaded.

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,[30] 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).[30] The extent of arterial injury was

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

	Control	*Carvedilol stent*	*Probucol stent*	*p value*
Baseline RD (mm)	2.91±0.17	2.92±0.24	2.94±0.12	0.72
Post-stenting MLD (mm)	3.43±0.10	3.44±0.25	3.37±0.12	0.94
Follow-up RD (mm)	2.92±0.25	2.93±0.69	2.93±0.42	0.82
Follow-up MLD (mm)	2.60±0.13	2.79±0.14	2.68±0.44	0.09
Follow-up DS (%)	11.07±3.10	4.77±3.51	8.74±3.68	0.06

RD, reference diameter; MLD, minimal lumen diameter; DS, diameter stenosis.

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

	Control	*Probucol stent*	*Carvedilol stent*
Injury score	2.12±0.50	1.95±0.49	2.05±0.42
EEL area, mm²	7.69±0.60	7.68±0.75	7.58±0.48
IEL area, mm²	6.09±0.76	6.27±0.83	6.28±0.59
Neointimal area, mm²	1.92±0.52	1.79±0.41	1.12±0.55*
Lumen area, mm²	4.17±0.87	4.15±0.61	5.15±0.90†
Area stenosis, %	31.9±9.2	32.2±10.4	18.2±9.6‡
Inflammation score	1.13±0.30	1.09±0.51	1.19±0.21
Re-endothelialization score	3.0±0.0	3.0±0.0	3.0±0.0
PCNA index, %	17.82±1.45	15.9±1.91	7.78±2.97§

EEL, external elastic lamina; IEL, internal elastic lamina; PCNA, proliferating cell nuclear antigen.
**p=0.004 vs control stent, †p=0.008 vs control stent, ‡p=0.002 vs control stent, §p=0.0001 vs control stent.*

assessed according to the numeric score described by Schwartz et al.[31] The degree of both inflammation and re-endothelialization was assessed according to the scoring systems used by previous studies.[5, 31]

Immunocytochemistry

After the stent filaments were removed without distorting or damaging the artery, the sections were treated with 1:100 diluted mouse anti-proliferating cell nuclear antigen (PCNA) antibody (clone PC 10, DAKO) to study SMC proliferation in the neointima. The samples were incubated, developed, and counterstained with hematoxylin. The SMC density of 5 randomly chosen areas (0.1 mm^2) of the neointima was measured with computer assistance at 400 magnification. The percentage of proliferating SMCs was obtained by dividing the number of PCNA-positive SMCs by the total number of SMCs in each field and the results were averaged. In addition, a modification of Movat's pentachrome stain using a saffron/Alcian blue combination was applied to evaluate the extracellular matrix of the neointima.

Statistical Analysis

The data are expressed as mean ± SD. The angiographic, morphometric, and cytochemistry data for each group were compared by ANOVA with posthoc Tukey analysis for multiple comparisons. Significance was established by a value of $p<0.05$.

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 μg/stent, and from the 50 mg/ml probucol solution was 52±16 μg/stent. The 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 mm^2 of the control group, the neointimal area was reduced to 1.12±0.55 mm^2 in the carvedilol group ($p=0.004$), and 1.79±0.41 mm^2 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

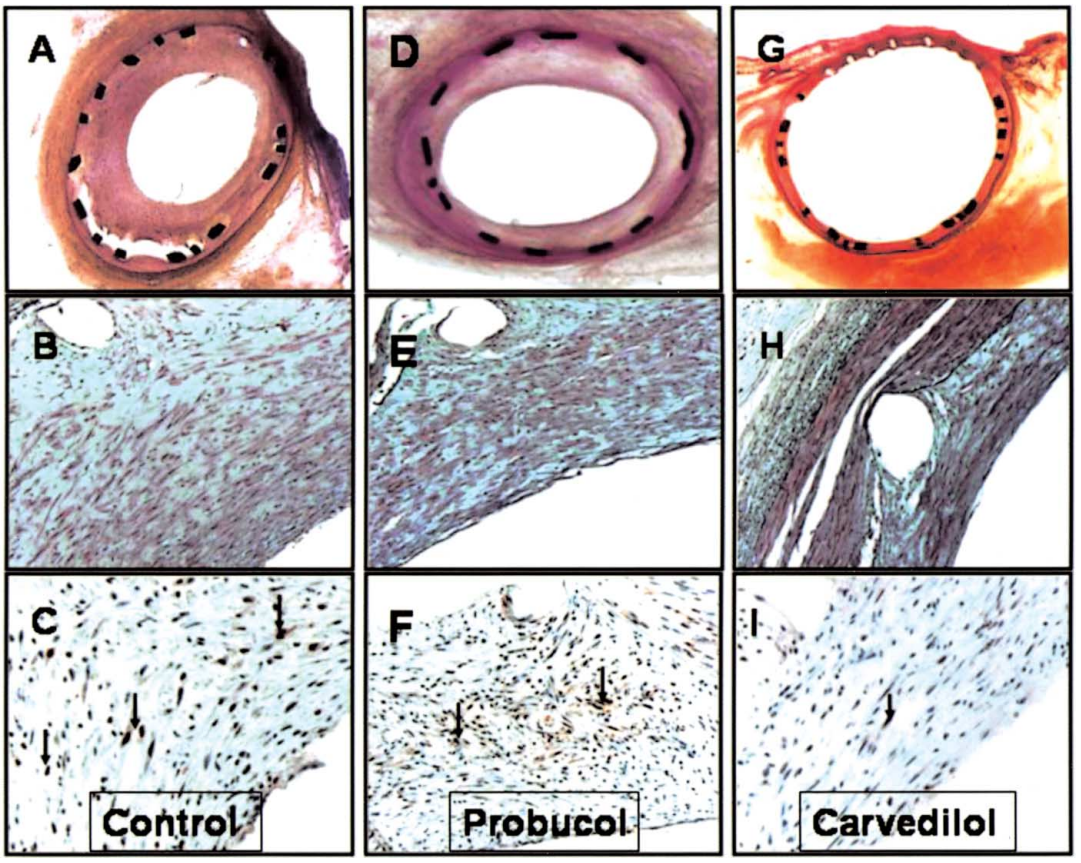

Fig 2. Photomicrographs of histological sections 28 days after implantation of carvedilol and probucol-coated stents. As compared with the control (A), neointimal hyperplasia is profoundly reduced in the carvedilol group (G), but modestly in the probucol group(D). There are less proliferating cell nuclear antigen (PCNA) -positive cells (arrows) in the carvedilol (I), control (C), and probucol (F) groups. There is less collagen (dark yellow) in the proteoglycan-rich neointima (blue) in the carvedilol group (H), compared with the control (B). (A, D, G: H&E, ×40), (C, F, I: PCNA stain, ×400), (B, E, H: Movat stain, ×200).

(Table 2). 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 can be loaded onto the polymer-coated stent, it was not released for nearly 72 h after loading in the in vivo experiment. Approximately 52 μg 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 Probucol 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 arteries[35] and humans,[36] 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 (angiopeptin),[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 same as the other drugs;[35,37] 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.[14–16] It has a greater inhibitory effect at a concentration as low as 1 μmol and, at 10 μmol, inhibited basal mitogenesis by approximately 65% and endothelin-1-stimulated mitogenesis by approximately 95%.[17] 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 EUROCARE trial.[38] A significant antiproliferative effect in ex vivo studies[16,39] was observed with a high concentration of carvedilol (>1 μmol), 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;[15] however, the maximum recommended oral dose (50 mg/day) of carvedilol in humans provides a maximum plasma concentration of 66 μg/ml (0.157 μmol).[40]

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[23,24,41] by its major mechanism of an antioxidant effect on vessel remodeling with an additional vasodilatory effect. Its antioxidant effect also reduces atherosclerosis[42] and vascular SMC proliferation.[25,43]

There are few studies of probucol's effect on stent restenosis. Most recently, Kim et al[44] could not prove the effect of probucol, but a multicenter study (CART-1)[28] 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 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.

References

1. Williams DO, Holubkov R, Yeh W, Bourassa MG, Al-Bassam M, Block PC, et al. Percutaneous coronary interventions in the current era compared with 1985–1986: The National Heart, Lung, and Blood Institute Registries. *Circulation* 2000; **102:** 2945–2951.
2. Mehran R, Dangas G, Abizaid AS, Mintz GS, Lansky AJ, Salter LF, et al. Patterns of in-stent restenosis: Angiographic classification and implications for long-term clinical outcome. *Circulation* 1999; **100:** 1872–1878.
3. Farb A, Sangiorgi G, Carter AJ, Walley VM, Edwards WD, Schwartz

RS, et al. Pathology of acute and chronic coronary stenting in humans. *Circulation* 1999; **99:** 44–52.
4. Grewe P, Peter H, Deneke T, Machraoui A, Barmeyer J, Muller KM. Acute and chronic tissue response to coronary stent implantation: Pathologic findings in human specimen. *J Am Coll Cardiol* 2000; **35:** 157–163.
5. Kornowski R, Hong MK, Tio FO, Bramwell O, Wu HS, Leon MB. In-stent restenosis: Contributions of inflammatory responses and arterial injury to neointimal hyperplasia. *J Am Coll Cardiol* 1998; **31:** 224–230.
6. Braum-Dullaeus RC, Mann MJ, Dzau VJ. Cell cycle progression: New therapeutic target for vascular proliferative disease. *Circulation* 1998; **98:** 82–89.
7. Sousa JE, Costa MA, Sousa AG, Abizaid AC, Seixas AC, Abizaid AS, et al. Two-year angiographic and intravascular ultrasound follow-up after implantation of sirolimus-eluting stents in human coronary arteries. *Circulation* 2003; **107:** 381–383.
8. Hong MK, Mintz GS, Lee CW, Song JM, Han KH, Kang DH, et al. Paclitaxel coating reduces in-stent intimal hyperplasia in human coronary arteries: A serial volumetric intravascular ultrasound analysis from the ASian Paclitaxel-Eluting Stent Clinical Trial (ASPECT). *Circulation* 2003; **107:** 517–520.
9. Kim KI, Bae J, Kang HJ, Koo BK, Youn TJ, Kim SH, et al. Three-year clinical follow-up results of intracoronary radiation therapy using a rhenium-188-diethylene-triamine-penta-acetic-acid-filled balloon system. *Circ J* 2004; **68:** 532–537.
10. Kim W, Jeong MH, Park OY, Rhew JY, Bom HS, Choi SJ, et al. Effects of beta-radiation using a Holmium-166 coated balloon on neointimal hyperplasia in a porcine coronary stent restenosis model. *Circ J* 2003; **67:** 519–524.
11. Ahn YK, Jeong MH, Kim JW, Kim SH, Cho JH, Cho JG, et al. Preventive effects of the heparin-coated stent on restenosis in the porcine model. *Catheter Cardiovasc Interv* 1999; **48:** 324–330.
12. Feuerstein GZ, Ruffolo RR. Carvedilol, a novel multiple action antihypertensive agent with antioxidant activity and the potential for myocardial and vascular protection. *Eur Heart J* 1995; **16:** 38–42.
13. Bril A, Slivjak M, DiMartino MJ, Feuerstein GZ, Linee P, Poyser RH, et al. Cardioprotective effects of carvedilol, a novel beta adrenoceptor antagonist with vasodilating properties, in anaesthetised minipigs: Comparison with propranolol. *Cardiovasc Res* 1992; **26:** 518–525.
14. Yue TL, Cheng HY, Lysko PG, McKenna PJ, Feuerstein R, Gu JL, et al. Carvedilol, a new vasodilator and beta adrenoceptor antagonist, is an antioxidant and free radical scavenger. *J Pharmacol Exp Ther* 1992; **263:** 92–98.
15. Ohlstein EH, Douglas SA, Sung CP, Yue TL, Louden C, Arleth A, et al. Carvedilol, a cardiovascular drug, prevents vascular smooth muscle cell proliferation, migration and neointimal formation following vascular injury. *Proc Natl Acad Sci USA* 1993; **90:** 6189–6193.
16. Sung CP, Arleth AJ, Ohlstein EH. Carvedilol inhibits vascular smooth muscle cell proliferation. *J Cardiovasc Pharmacol* 1993; **21:** 221–227.
17. Feuerstein GZ, Ruffolo RR. Carvedilol, a novel vasodilating beta-blocker with the potential for cardiovascular organ protection. *Eur Heart J* 1996; **17:** 24–29.
18. Sung CP, Arleth AJ, Eichman C, Truneh A, Ohlstein EH. Carvedilol, a multiple-action neurohumoral antagonist, inhibits mitogen-activated protein kinase and cell cycle progression in vascular smooth muscle cells. *J Pharmacol Exp Ther* 1997; **283:** 910–917.
19. Fattori R, Piva T. Drug-eluting stents in vascular intervention. *Lancet* 2003; **361:** 247–249.
20. Ruffolo RR, Feuerstein GZ. Pharmacology of carvedilol: Rationale for use in hypertension, coronary artery disease and congestive heart failure. *Cardiovasc Drugs Ther* 1997; **11:** 247–256.
21. Pfuetze KD, Dujovne CA. Probucol. *Curr Atheroscler Rep* 2000; **2:** 47–57.
22. Parthasathy S, Young SG, Wiztum JL, Pittman RC, Steinberg D. Probucol inhibits oxidative modification of low density lipoprotein. *J Clin Invest* 1986; **77:** 641–644.
23. Tardif JC, Cote G, Lesperance J, Bourassa M, Lambert J, Doucet S, et al. Probucol and multivitamins in the prevention of restenosis after coronary angioplasty. Multivitamins and Probucol Study Group. *N Engl J Med* 1997; **337:** 365–372.
24. Watanabe K, Sekiya M, Ikeda S, Miyagawa M, Hashida K. Preventive effects of probucol on restenosis after percutaneous transluminal coronary angioplasty. *Am Heart J* 1996; **132:** 23–29.
25. Cote G, Tardif JC, Lesperance J, Lambert J, Bourassa M, Bonan R, et al. Effects of probucol on vascular remodeling after coronary angioplasty. Multivitamins and Probucol Study Group. *Circulation* 1999; **99:** 30–35.
26. Schneider JE, Berk BC, Gravanis MB, Santoian EC, Cipolla GD, Tarazona N, et al. Probucol decreases neointimal formation in a swine model of coronary artery balloon injury: A possible role for anti-oxidants in restenosis. *Circulation* 1993; **88:** 628–637.
27. Wasserman MA, Sundell CL, Kunsch C, Edwards D, Meng CQ, Medford RM. Chemistry and pharmacology of vascular protectants: A novel approach to the treatment of atherosclerosis and coronary artery disease. *Am J Cardiol* 2003; **91:** 34–40.
28. Tardif JC, Gregoire J, Schwartz L, Title L, Laramee L, Reeves F. Effects of AGI-1067 and probucol after percutaneous coronary interventions. *Circulation* 2003; **107:** 552–558.
29. Wakeyama T, Ogawa H, Iida H, Takaki A, Iwami T, Mochizuki M, et al. Effects of candersatan and probucol on restenosis after coronary stenting: Results of insight of stent intimal hyperplasia inhibition by new angiotensin II receptor antagonist (ISHIN) trial. *Circ J* 2003; **67:** 519–524.
30. Varenne O, Pislaru S, Gillijns H, Van Pelt N, Gerard RD, Zoldhelyi P, et al. Local adenovirus-mediated transfer of human endothelial nitric oxide synthase reduces luminal narrowing after coronary angioplasty in pigs. *Circulation* 1998; **98:** 916–926.
31. Schwartz RS, Huber KC, Murphy JG, Edwards WD, Camrud AR, Vlietstra RE, et al. Restenosis and the proportional neointimal response to coronary artery injury: Results in a porcine model. *J Am Coll Cardiol* 1992; **19:** 267–274.
32. Suzuki T, Kopia G, Hayashi S, Bailey LR, Llanos G, Wilensky R, et al. Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. *Circulation* 2001; **104:** 1188–1193.
33. Heldman AW, Cheng L, Jenkins M, Heller PF, Kim DW, Ware M Jr, et al. Paclitaxel stent coating inhibits neointimal hyperplasia at 4 weeks in a porcine model of coronary restenosis. *Circulation* 2001; **103:** 2289–2295.
34. New G, Moses JW, Roubin GS, Leon MB, Colombo A, Iyer SS, et al. Estrogen-eluting, phosphorylcholine-coated stent implantation is associated with reduced neointimal formation but no delay in vascular repair in a porcine coronary model. *Catheter Cardiovasc Interv* 2002; **57:** 266–271.
35. Lewis AL, Tolhurst LA, Stratford PW. Analysis of a phosphorylcholine-based polymer coating on a coronary stent pre- and post-implantation. *Biomaterials* 2002; **23:** 1697–1706.
36. Galli M, Bartorelli A, Bedogni F, DeCesare N, Klugmann S, Maiello L, et al. Italian BiodivYsio open registry (BiodivYsio PC-coated stent): Study of clinical outcomes of the implant of a PC-coated coronary stent. *J Invasive Cardiol* 2000; **12:** 452–458.
37. Armstrong J, Gunn J, Arnold N, Malik N, Chan KH, Vick T, et al. Angiopeptin-eluting stents: Observations in human vessels and pig coronary arteries. *J Invasive Cardiol* 2002; **14:** 230–238.
38. Serruys PW, Foley DP, Höfling B, Puel J, Glogar HD, Seabra-Gomes R, et al. Carvedilol for prevention of restenosis after directional coronary atherectomy: Final results of the European Carvedilol Atherectomy Restenosis (EUROCARE) trial. *Circulation* 2000; **101:** 1512–1518.
39. Patel MK, Chan P, Betteridge LJ, Schachter M, Sever PS. Inhibition of human vascular smooth muscle cell proliferation by the novel multiple-action antihypertensive agent carvedilol. *J Cardiovasc Pharmacol* 1995; **25:** 652–657.
40. Neugebauer G, Akpan W, Mollendorf EV, Neubert P, Reiff K. Pharmacokinetics and disposition of carvedilol in humans. *J Cardiovasc Pharmacol* 1987; **10**(Suppl 11)**:** S85–S88.
41. Yokoi H, Daida H, Kuwabara Y, Nishikawa H, Takatsu F, Tomihara H, et al. Effectiveness of an antioxidant in preventing restenosis after percutaneous transluminal coronary angioplasty: The Probucol Angioplasty Restenosis Trial. *J Am Coll Cardiol* 1997; **30:** 855–862.
42. Kita T, Nagano Y, Yokode M, Ishii K, Kume N, Ooshima A, et al. Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. *Proc Natl Acad Sci USA* 1987; **84:** 5928–5931.
43. Freyschuss A, Stiko-Rahm A, Swedenborg J, Henriksson P, Bjorkhem I, Berglund L, et al. Antioxidant treatment inhibits the development of intimal thickening after balloon injury of the aorta in hypercholesterolemic rabbits. *J Clin Invest* 1993; **91:** 1282–1288.
44. Kim MH, Cha KS, Han JY, Kim HJ, Kim JS. Effect of antioxidant probucol for preventing stent restenosis. *Catheter Cardiovasc Interv* 2002; **57:** 424–428.