

Local Delivery of Nitric Oxide from an Eluting Stent to Inhibit Neointimal Thickening in a Porcine Coronary Injury Model

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To assess the effect of a NO-eluting stent on reducing neointimal thickening in a porcine coronary artery stent injury model, sodium nitroprusside (SNP), a NO donor, was incorporated into polyurethane (PU) polymer and coated onto metallic coil stents, and two types of stents with thin and thick barrier coatings were characterized. *In vivo* biological activity of the NO-eluting stents was assessed by measurement of coronary arterial cGMP levels in 32 pigs/64 arteries at days 1, 2, 7 and 14. Morphometric analyses were performed in 16 pigs to determine the effect of NO-eluting stents on neointimal hyperplasia 28 days following arterial injury. The SNP-coated stents released NO in a controlled manner for up to 4 weeks in the *in vitro* experiments and an increase in local tissue cGMP levels was demonstrated for up to 14 days. The neointimal area at 28 days was not diminished, however, by NO eluted from either stents of thin or thick barriers (control bare stent - 0.66 mm², control PU stent - 0.68 mm², SNP-PU thin coating stent - 0.78 mm², SNP-PU thick coating stent - 0.85 mm², all *p*=NS). In conclusion, the SNP-coated polymer stent exerted a local biological effect on the arterial wall, with sustained elevation of cGMP level. Although local delivery of NO from this device did not reduce neointimal hyperplasia in this porcine model, this polymer-coated stent might be a promising tool for administration of other agents that may modify the reparative tissue responses leading to restenosis.

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INTRODUCTION

Local drug delivery is an attractive therapeutic option for the prevention of restenosis, given that restenosis is a localized pathologic process. This technique may allow high local tissue concentrations of drug without side effects of systemic drug administration.^{1,2} The tissue reparative processes that lead to restenosis after angioplasty may be sustained for a time frame of days to weeks, and a method of releasing drug for a prolonged period after arterial injury may be needed. A drug-eluting polymer-coating stent is one potential technique to achieve high local tissue concentrations of an effective drug for a prolonged period after arterial injury, in addition to the beneficial effects of the metallic stent on arterial remodeling.

Besides a primary role in regulating the vascular tone,³ nitric oxide, one of the important products of normal endothelium, has been postulated to reduce vascular lesion formation by inhibiting platelet adhesion,⁴ leukocyte adhesion,^{5,6} vascular smooth muscle cell migration and proliferation,^{7,8} and protein and collagen synthesis of the vascular smooth muscle cells.⁹ In several animal models, dietary supplementation of L-arginine, local delivery of a nitric oxide synthetase gene, and local delivery of nitric oxide donors restored endothelial function and reduced neointimal thickening.¹⁰⁻¹⁴

Endothelial dysfunction has been demonstrated

to last up to 4 to 8 weeks after injury, even after the endothelium is regenerated completely,^{15,16} with local deficiency of nitric oxide during that time. A means of prolonged delivery of nitric oxide would likely be required if local administration were to inhibit the neointimal hyperplastic response to arterial injury. In the current study, we used sodium nitroprusside (SNP) as a nitric oxide donor, and impregnated it into a polyurethane (PU) polymer and coated onto a metallic stent. We evaluated the efficacy of this local delivery system on neointimal thickening in the porcine coronary artery stent overexpansion injury model.

MATERIALS AND METHODS

Nitric oxide eluting stents were used for arterial injury and local drug delivery. Sodium nitroprusside (SNP), a nitric oxide donor, was incorporated into a polyurethane (PU) polymer and coated onto tantalum coil wire stents. Characterization of the SNP-PU stent device was performed by an *in vitro* nitric oxide-elution kinetics study. The *in vivo* biological activity of nitric oxide eluted from this stent was assessed by measurement of tissue cyclic GMP levels. Morphometric studies were performed to determine the efficiency of nitric oxide stent on neointimal hyperplasia following arterial injury.

Nitric oxide-eluting stent

SNP releases nitric oxide either spontaneously or in the presence of sulphhydryl groups.¹⁷⁻¹⁹ The nitric oxide eluting stent consisted of a 125- μ m diameter tantalum wire configured into a 16-mm long balloon-expandable coil stent (Wiktor, Medtronic, Inc., Minneapolis, MN, U.S.A.) and coated with a monolithic matrix of PU (Medtronic proprietary formulation) and SNP covered by PU barrier layer (Fig. 1). A 2% (w/w) solution of PU in tetrahydrofuran was prepared, in which a 3% suspension of SNP was formed. This mixture was sprayed onto the stent wires to form the SNP eluting polymer coating. The ratio of SNP to PU in the base coating was 3:2, with 1.9 - 2.0 mg SNP per stent. This SNP coating was covered with a barrier layer of either -0.7 mg (thin coating stent)

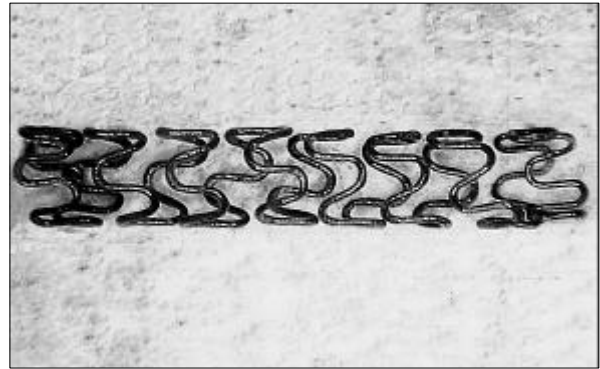


Fig. 1. Photograph of SNP-PU thick coating stent (7 \times magnification).

or -2.2 mg (thick coating stent) of PU. A PU-coated control stent (2.9 mg of PU) was prepared using same method. The thickness of the coating for these stents ranged from -20 μ m for the controls to -80 μ m for the thick barrier coating stents. The polymer coating was demonstrated by microscopy to be sufficiently flexible to allow balloon expansion of the coil wire stent without cracking or peeling from the wire. Stents were sterilized using a conventional ethylene oxide gas technique and hand-mounted on the commercial angioplasty balloons before stent implantation. We tested 4 different stent designs: control-bare stents, control PU polymer stents without impregnation of SNP, SNP-PU thin coating stents, and SNP-PU thick coating stents.

In vitro elution kinetics

The elution kinetics of SNP from sterilized polymer-coated stents were characterized in an *in vitro* system using a colorimetric assay based on the nitrosation of famotidine. Stents were placed in glass vials and immersed in 4.0 ml of phosphate-buffer saline. At time points ranging from two hours to 39 days, aliquots of the elution buffer were taken and SNP concentration measured using a famotidine assay.²⁰ Famotidine reacts with SNP to give absorbance peaks at 394 and 498 nm. Absorbance was measured at 394 nm and 498 nm by ultraviolet spectrophotometry (model 8452, Hewlett-Packard, Palo Alto, California, U.S.A.) and converted to cumulative elution curves. The absorbance at 394 nm is indicative of "total" SNP. The peak at 498 nm, although smaller, represents

the nitrosated famotidine and is indicative of "active" SNP. SNP concentration was determined by comparison to standard curves generated on the same day.

Animal procedures and study groups

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Juvenile domestic farm pigs, weighing 20-25 kg, were treated with oral aspirin 325 mg preoperatively and daily thereafter until sacrifice. General anesthesia was induced with intramuscular injection of ketamine (22 mg/kg; Fort Dodge Laboratories Inc., Fort Dodge, Iowa) and maintained with inhalation of isoflurane (Abbott Laboratories, Chicago, IL, U.S.A.) during the procedure. The right carotid artery was exposed through a midline neck incision, and a 9 Fr hemostatic arterial sheath was introduced over a guidewire through a carotid arteriotomy. Heparin (300U/kg; Elkins-Sinn Inc., Cherry Hill, NJ, U.S.A.) was administered as a single intravenous bolus. Left and right coronary angiography was performed using an 8 Fr guiding catheter (Hockeystick, Cordis, Miami, FLA, U.S.A.). Based upon the baseline angiography, coronary arterial size was estimated and suitable epicardial sites were chosen to allow deployment of a stent, over expanded by 15-20%, using a 3.0-4.0 mm angioplasty balloon catheter. Stents were hand-crimped onto the selected balloon catheter and deployment was performed under fluoroscopic guidance over a standard 0.014-inch angioplasty guidewire. Animals were randomly assigned to receive control bare stents, control polymer stents, SNP-PU thin coating stents, or SNP-PU thick coating stents. Two or three coronary arteries per animal underwent stent over-expansion injury using the same stent type in each artery. The balloon was inflated once for 30 seconds with inflation pressure of 8 atm to deploy the stent. Follow-up angiography was performed following stent implantation to confirm adequate stent expansion and vessel patency. The arteriotomy site was ligated and the neck wound closed with continuous interrupted sutures. Animals were kept alive on a standard laboratory chow

diet throughout the study period.

For determination of tissue cGMP levels, animals were sacrificed at day 1, 2, 7, or 14 following the stent implantation. Under general anesthesia with intramuscular injection of ketamine (50 mg), 10,000 U of heparin was given intravenously as a bolus injection, and euthanasia was induced with intracoronary injection of potassium chloride (40mEq). The heart was immediately explanted, and peri-arterial fat and connective tissue were carefully removed. Stented and normal coronary arterial segments (about 1 cm in length) proximal and distal to the stented segment of the coronary artery were excised. Harvested specimens were immediately frozen in liquid nitrogen and kept at -70°C until analysis of tissue cGMP levels.

For the morphometric analysis, follow-up coronary angiography was performed under general anesthesia 28 days after stent implantation. Pigs were euthanized by over-dose of intracoronary injection of potassium chloride and the heart was removed and perfusion-fixed at 70 mmHg for 24 hours with 10% neutral buffered formalin. Stent-containing coronary arterial segments were removed and sectioned at 2-mm intervals perpendicular to the vessel axis. Coronary arterial segments were embedded in paraffin blocks, sectioned and stained with conventional hematoxylin-eosin and Lawson's elastic van Giesson stains.

Determination of coronary arterial cGMP levels

Determination of local arterial cGMP levels was performed to evaluate the biological activity of the stent-based nitric oxide delivery. Stented arteries were divided into proximal, stented, and distal segments. Each segment was minced using a scalpel and homogenized in 750 μ L of ice cold 6% trichloroacetic acid using a motorized tissue homogenizer (VirTis Handishear with 6-mm shaft; VirTis Company, Gardiner, N.Y., U.S.A.). After centrifugation at 10,000 g for 15 minutes at 4°C, pellets were kept for the protein analysis in the refrigerator and supernatants were extracted three times with 1 vol. water-saturated diethylether (Sigma, St. Louis, MO, U.S.A.). The ether portion was totally removed and the water-extracted

portion was lyophilized at -52°C in a vacuum state using freeze dry system (Lyph 4.5, LABCONCO Corporation, Kansas City, MO, U.S.A.).

The concentration of cGMP in each supernatant sample was determined using a commercially available cGMP enzyme-immunoassay (EIA) kit (Amersham, Life Science, Chicago, IL, U.S.A.) with the addition of acetylation step to increase the sensitivity. Pellets from the initial homogenization step were digested in 1 ml 0.1 N NaOH overnight at 60°C to extract protein, which was assayed by using a Pierce BCA kit (Pierce Chemical Co., Rockford, IL, U.S.A.).

Histomorphometric analysis

Morphometric analyses were performed using light microscopy and a computerized digital microscopy algorithm (Image-1/MetaMorph; Universal Imaging Corporation, West Chester, PA, U.S.A.). All coronary segments were qualitatively inspected by observers (J.Y. and W.C.) blinded to study group who assessed for the presence of thrombus and inflammatory cell infiltration and evaluated the depth of arterial injury by each stent struts. In every stented arterial segment, the single histologic section showing the most severe luminal narrowing was used for measurements of lumen area, internal elastic lamina (IEL) area, and external elastic lamina (EEL) area. Medial area was calculated as EEL area minus IEL area. Intimal area was calculated as IEL area minus luminal area. Maximal intimal thickness was measured at each stent wire site, and an injury score at each stent wire site was assigned by the depth of the stent wire disruption of the vessel wall structure: 0 = intact IEL; 1 = IEL lacerated; 2 = IEL and media lacerated; and 3 = EEL lacerated.²¹

Statistical analysis

The Kruskal-Wallis test was used to determine the statistical significance of differences in coronary arterial cGMP levels among 4 different stent groups, and the Man-Whitney test was applied to the 6 sets of 2 group combinations to assess statistical significance.

Continuous variables of morphometric measurements are expressed a mean +/- standard

deviation. The ANOVA test was used for comparison of luminal areas, intimal areas and intimal thicknesses among 4 different groups. A p value < 0.05 was considered to be statistically significant. To determine the influence of SNP-PU coated stents on the extent of intimal hyperplasia 28 days after stent implantation over control bare and control PU stents, linear regression curves relating intimal thickness to injury scores were plotted for each treatment group.^{21,22} A reduction in the neointimal thickness to arterial injury due to therapy would result in a decrease in the slope or the intercept of this regression relation, or both.² Thus, these slope and intercept values serve as end points for comparing study groups. Linear regression analysis for mean neointimal thickness versus mean injury score was performed using arterial segments obtained from four study groups (control bare stents, control PU stents, SNP-PU thin coating stents, and SNP-PU thick coating stents). Three binary variables, S_P , S_{S1} , and S_{S2} (value 0 or 1) representing the groups of PU coated stents, SNP-PU thin coating stents or SNP-PU thick coating stents, respectively, and their interaction terms, injury score* S_P , injury score* S_{S1} , and injury score* S_{S2} , were added to the regression equation to evaluate whether any stent coating produced a statistically significant change in slope or intercept. The following multiple regression model was generated:

$$\text{Mean neointimal thickness} = [\text{Slope} + (\alpha_P \times S_P) + (\alpha_{S1} \times S_{S1}) + (\alpha_{S2} \times S_{S2})] \times \text{mean injury score} + \text{Intercept} + (\beta_P \times S_P) + (\beta_{S1} \times S_{S1}) + (\beta_{S2} \times S_{S2}),$$

where α_P , α_{S1} , and α_{S2} are the coefficients of S_P , S_{S1} , and S_{S2} and β_P , β_{S1} and β_{S2} the coefficients of their interactions, injury score* S_P , injury score* S_{S1} , and injury score* S_{S2} estimated by multiple regression. A statistically significant effect of stent coating on the slope of the linear relation between neointimal thickening and injury score was considered if the p value for α_P , α_{S1} , or α_{S2} was < 0.05 . A significant effect of stent coating on the intercept of the linear regression curve was considered if the p value for β_P , β_{S1} or β_{S2} was < 0.05 .

RESULTS

***In vitro* SNP-elution kinetics**

Elution of SNP started immediately and lasted for at least 2 weeks in the buffered bath with both types of stents (Fig. 2). The SNP-eluting stent with thin barrier coating eluted drug during the first 2 weeks, and while the thick barrier stent eluted drug over approximately 4 weeks. Approximately 85 percent of the total SNP mass in the SNP-PU stents was eluted by 30 days.

Coronary arterial cGMP levels

Stent were implanted in 2 epicardial arteries of each of 33 pigs. A total of 69 stents were used; three stents were embolized to the descending thoracic aorta, and the remaining 66 stents were successfully deployed. One animal died suddenly 2 hours after stent implantation. A total 64 stented coronary arteries were available for tissue cGMP determination.

At 1 day after stent injury (Fig. 3A), cGMP levels in stented segments for all stents were lower than in proximal or distal normal segments in general. cGMP levels in SNP stented segments were significantly higher than those in control stented segments ($p < 0.05$).

At 2 days after stent injury (Fig. 3B), cGMP levels in stented segments remained lower than those in proximal and distal normal segments except for SNP-PU thin coating stents. cGMP levels in stented segments with SNP-PU thick coating stents were significantly lower than those in control stents and SNP-PU thin coating stents. cGMP levels in stented segments with SNP-PU thin coating stent were significantly higher than those in other stents.

At 7 days after stent injury (Fig. 3C), cGMP levels in stented segments of control-bare and control-PU stents remained lower than those in

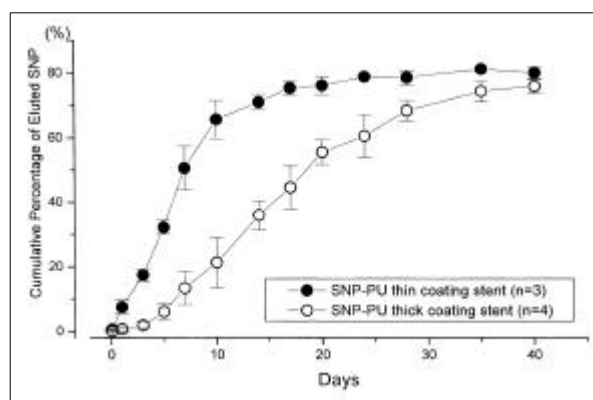


Fig. 2. *In vitro* sodium nitroprusside (SNP) elution kinetic studies using three SNP-PU stents of thin barrier coating and four SNP-PU stents of thick barrier coating. Values are mean \pm SE.

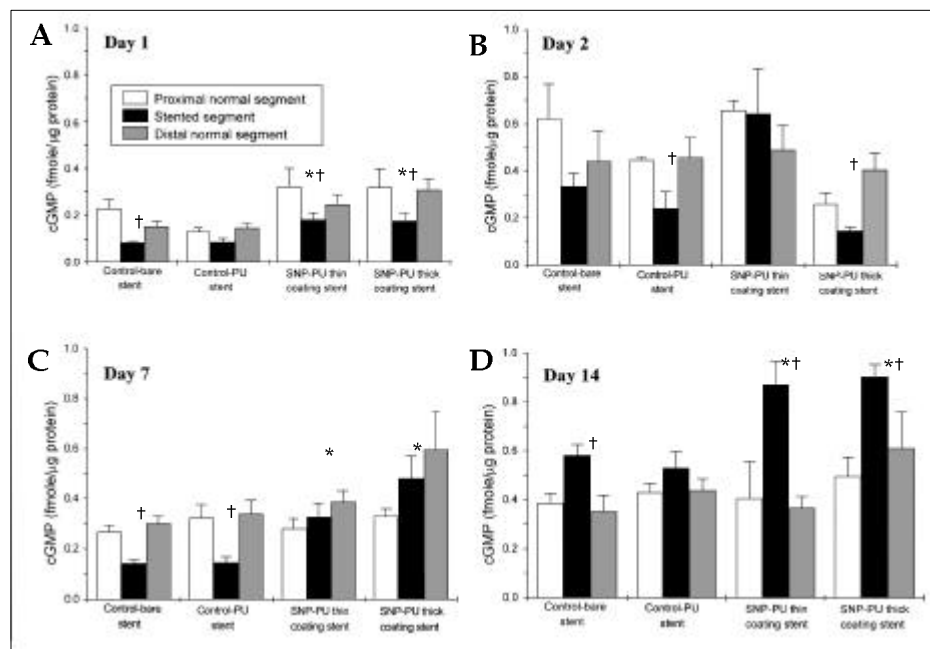


Fig. 3. cGMP levels in stented, proximal, and distal normal segments among 4 treatment groups at (A) day 1, (B) day 2, (C) day 7, and (D) day 14 after stent implantation. The error bar represents 1 standard error. The "*" denotes statistical significance of cGMP levels in stented segments comparing SNP-PU stents with control stents and "+" denotes statistical significance of cGMP levels between stented segments and adjacent non-stented segments. Values are mean \pm SE. "*" and "+" $p < 0.05$.

proximal and distal normals ($p < 0.05$). However, cGMP levels in stented segments of both SNP-PU stents were normalized relative to those in proximal and distal normal segments and significantly higher than those in control stented segments ($p < 0.05$).

At 14 days after stent injury (Fig. 3D), all stented segments trended to have higher cGMP levels than proximal and distal normal segments ($p < 0.05$). cGMP levels in stented segments of both SNP-PU stents were significantly higher than those of controls ($p < 0.05$).

Chronic study - neointimal hyperplasia

Stent implantation was attempted in 21 pigs, 3 vessels per pig. A total of 63 stents were used, with 2 stents embolized to the descending thoracic aorta and 1 stent to the left common carotid artery. Thus, 60 stents were successfully im-

planted in 60 arteries of 21 pigs. Five pigs died 2 to 5 hours after stent implantation, likely due to acute stent occlusion and ischemia (3 in control bare stent, 1 in control PU stent, and 1 in SNP-PU thin coating stent). The remaining pigs survived the 28-day follow-up period. A total of 46 coronary arterial segments in 16 pigs were available for chronic morphometric analysis: 12 in control-bare stent group, 11 in control-PU stent group, 11 in SNP-PU thick coating stent group, and 12 in SNP-PU thin coating stent group.

Histopathologic examination of porcine coronary artery cross-sections 28 days following implantation of the stents showed no inflammatory responses in specimens in which stent struts did not penetrate the external elastic lamina (Fig. 4, left panel). In contrast, there was intense inflammatory cell infiltration whenever the external elastic lamina was penetrated with stent struts regardless of the stent type (Fig. 4, right panel).

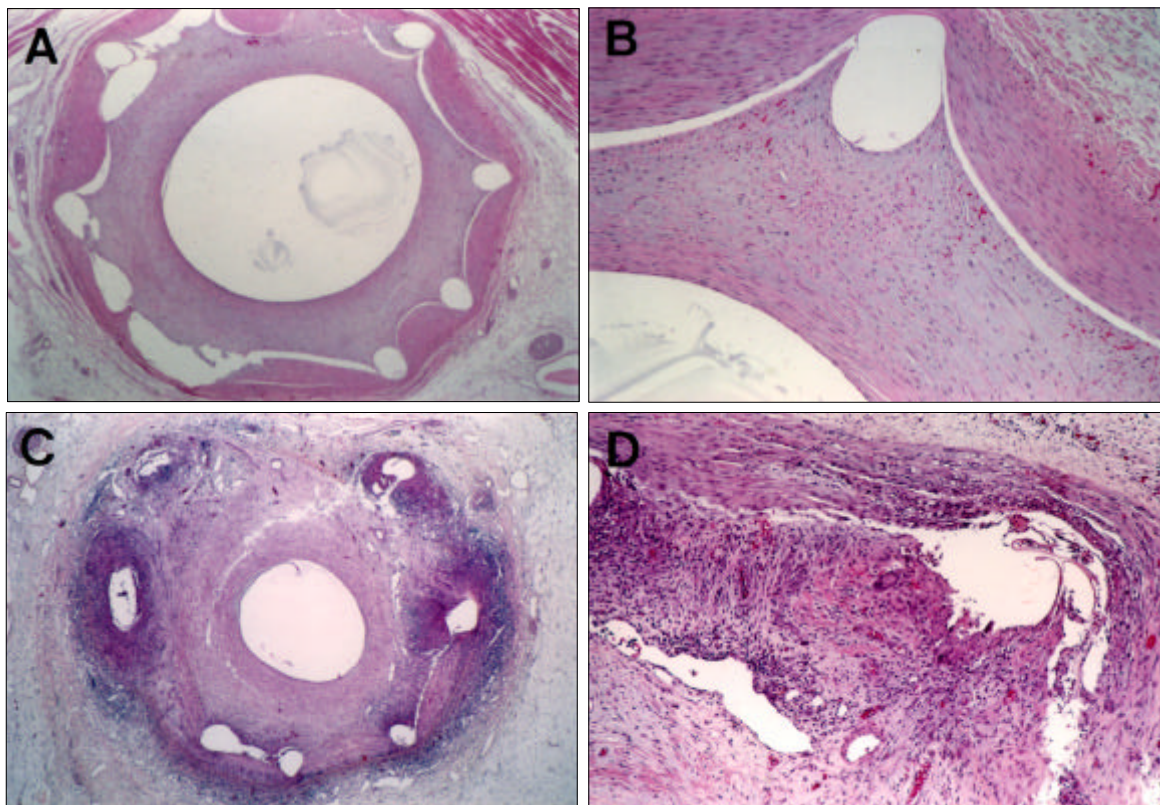


Fig. 4. Histopathologic findings of porcine coronary artery cross-section 28 days following control-bare stent or control-PU coating stent. 4-A (2.5X magnification) and 4-B (24X magnification) show no inflammatory responses in specimens in which stents were implanted without penetrating external elastic lamina. In contrast, 4-C (2.5X magnification) and 4-D (24X magnification) show intense inflammatory cell infiltration in specimens in which external elastic lamina was penetrated with stent struts. Hematoxylin and eosin stains.

There were no qualitative or quantitative differences in the inflammatory response to the 4 different stent types.

There were no significant differences in injury scores, luminal areas, intimal areas, medial areas, or intimal thicknesses among the 4 study groups (Table 1). Linear regression analysis of the relationship between neointimal thickness and injury score revealed no differences in the slope or intercepts of the regression lines for the 4 treatment groups (Table 2).

DISCUSSION

In the current study, we used sodium nitroprusside (SNP) as a nitric oxide donor, and impregnated it into a polyurethane (PU) polymer coated onto a metallic stent to evaluate the efficacy of sustained local delivery on reducing neointimal thickening in the porcine coronary artery stent overexpansion injury model. We demonstrated sustained biological effect of the

eluted agent for up to 2 weeks in the porcine coronary artery, although neointimal hyperplasia was not reduced.

Besides the beneficial effects of metallic stents on recoil and adverse arterial remodeling, these devices could also serve as a drug-eluting reservoir. Several drugs such as heparin,²³⁻²⁵ forskolin,²⁶ dexamethasone,² and taxol²⁷ have been assessed with stent coatings. A heparin-coated stent, in which heparin is covalently bonded to the stent and does not elute, may have reduced thrombogenicity, but did not reduce neointimal hyperplasia in a porcine coronary model.^{24,25,28} Another polymer-coated stent, which delivered forskolin, was tested in the rabbit carotid injury model.^{1,26} This device used a polyurethane polymer as a reservoir on a removable metallic stent and was able to deliver forskolin to the arterial wall in high local concentrations relative to the blood or other tissues. Although the delivered forskolin demonstrated vasodilating and antiplatelet biological activities, the tissue half-life was only 5.8 hours after removal of stent. In another

Table 1. Injury Scores and Morphometric Results in 4 Study Groups

Group	Control-bare (n=12)	Control-PU (n=11)	SNP-PU thin coating (n=12)	SNP-PU thick coating (n=11)
Injury score	2.14 ± 0.40	2.07 ± 0.32	2.36 ± 0.37	2.34 ± 0.36
Luminal area (mm ²)	0.48 ± 0.31	0.71 ± 0.38	0.44 ± 0.31	0.49 ± 0.34
Medial area (mm ²)	0.46 ± 0.20	0.46 ± 0.18	0.50 ± 0.23	0.57 ± 0.35
Intimal area (mm ²)	0.66 ± 0.20	0.68 ± 0.29	0.78 ± 0.23	0.85 ± 0.23
Intimal thickness (mm)	0.36 ± 0.14	0.35 ± 0.14	0.43 ± 0.12	0.46 ± 0.12

n, number of vessels used for morphometric analysis; all measurements are expressed as mean ± standard deviation. SNP, sodium nitroprusside; PU, polyurethane; There were no significant differences among 4 study groups in injury scores, luminal areas, intimal areas, or intimal thicknesses.

Table 2. Linear Regression Analysis of Chronic Morphometric Measurements in Control-Bare Stents, Control-PU stents, and Nitric Oxide Eluting PU Stents on the Chronic Neointimal Thickening

	PU Control Stents		SNP-PU Thin Coating Stents		SNP-PU Thick Coating Stents	
	Coefficient	p value	Coefficient	p value	Coefficient	p value
Alpha (slope)	0.143	0.371	0.392	0.596	0.338	0.840
Beta (intercept)	-0.206	0.315	-0.326	0.629	-0.288	0.981

PU, polyurethane; SNP, sodium nitroprusside; Slope = 0.302; intercept = -0.290; R² = 0.791.

study, a high molecular weight PLLA polymer was demonstrated to be well tolerated in porcine coronary arteries and was an effective means of providing sustained drug delivery for up to 4 weeks, although dexamethasone eluted from this stent did not reduce the neointimal hyperplasia in the porcine coronary injury model.² Several *in vitro* and *in vivo* studies of local paclitaxel delivery using polymer coating stent-based techniques to inhibit proliferation and lumen renarrowing have been performed with encouraging results.²⁷

Although several synthetic polymers have been proposed to serve as vehicles for local drug delivery combined with metallic stents, a major issue raised by previous work using currently available biodegradable or non-biodegradable polymers is that of tissue-blood incompatibility.²⁹⁻³¹ Marked inflammatory cell responses were observed with several polymers implanted in porcine coronary arteries.³⁰ To be applicable to humans, long-term biocompatibility of a polymer would need to be assured. In the current study, no inflammatory response to the PU coated stents was observed relative to the bare stents. Deep penetration of stent struts to the adventitial layer, however, elicited marked inflammatory responses around the penetrating stent struts regardless of stent types, bare or polymer coated, while there was minimal or no inflammatory cell infiltration with lesser degrees of arterial injury.

Various agents that increase local nitric oxide production have been studied to restore normal endothelial function after arterial injury and reduce neointima formation.¹⁰⁻¹⁴ Long-term dietary supplementation of L-arginine, the nitric oxide precursor, improved endothelium-dependent vaso-relaxation and reduced atherosclerotic lesion formation in rat¹¹ and hypercholesterolemic rabbit models.^{10,12,13} Transfection of the endothelial nitric oxide synthetase gene in rats not only restored local nitric oxide production and vascular reactivity of the injured vessel, but also attenuated neointima formation.¹⁴

In this current study using a non-biodegradable PU coating, drug was uniformly dispersed within polymer forming a "monolithic matrix". Elution of nitroprusside by diffusion through pores of polymer was too rapid, occurring over only a few

days.^{26,32} Barrier coatings of two different thicknesses were therefore added to serve as a diffusion barrier, creating a polymeric "reservoir" to obtain controlled, sustained elution kinetics. By *in vitro* studies, these SNP-PU coating stents showed nearly zero order kinetics without an initial lag time; increased barrier thickness decreased the rate and prolonged the duration of elution. One design, the thick barrier coating, was used to prolong release of nitroprusside for the entire experimental period (4 weeks), while the thin barrier coating was used to maximize delivery to the first 2 weeks after injury, during which time the stimulus for proliferation would presumably be most intense.

The biological effect of the nitric oxide-eluting PU stents was demonstrated by measuring coronary arterial tissue cGMP levels. At time points of 1 day, 2 days, and 7 days after stent implantation, control (bare or PU) stented segments had lower cGMP levels than proximal or normal distal vessels, likely due to endothelial denudation, medial necrosis and cell loss at the sites of stent injury. SNP stented segments generally had higher levels than control stents, but cGMP levels were not normalized relative to proximal and distal normal segments at day 1 and 2. The failure of these stents to normalize cGMP levels at these time points may be related to a reduced target cell mass due to endothelial and medial injury, loss of some drug from polymer surface facing arterial lumen and thus not apposed to vessel wall, or a uniform elution rate with no early "burst" of nitric oxide release. By day 7, however, cGMP levels were normalized relative to proximal and distal normal artery in SNP stented segments, but remained low in control stented segments. This finding suggests regrowth of medial and intimal tissue by this time point, providing target cells for nitric oxide and encapsulating the stent wires, "capturing" most of the nitric oxide released from the polymer matrix. Despite probable reconstitution of medial and intimal tissue layers, these layers did not appear to be functionally normal by 7 days, as evidenced by continued suppression of cGMP levels in control stented segments compared with proximal and distal normals. By day 14, control stented segments showed restoration of cGMP levels relative to proximal or distal

normals. The greater cGMP levels in control stented segments may be due to tissue healing response and neointimal hyperplasia. SNP stented segments showed supranormal levels of cGMP, which were significantly higher than control stented segments, suggesting continued release of nitric oxide from the stents and its biological action on hyperplastic tissue. At all time points, proximal and distal normal segments did not show increased cGMP levels in arteries treated with SNP stents compared with controls, confirming the local nature of delivery of nitric oxide to the stented arterial wall by the SNP stents.

Neither SNP stent design reduced neointimal hyperplasia, despite prolonged biological activity demonstrated through elevation in tissue cGMP levels. Potential explanations include an inadequate dose of nitric oxide delivered to the tissue, particularly during the first few days, when cGMP levels were increased but not normalized. Modification of the stent design, with SNP dispersed within the barrier coating to provide an early "burst", might improve efficacy in this regard. The degree of arterial injury may have been too great in some vessel segments with inflammatory response due to penetration of the EEL in many segments which may have "overwhelmed" the effect of nitric oxide.³³ Finally, the hypothesis that nitric oxide is an important modulator of arterial response to injury, at least in this species and model, may be incorrect.

In conclusion, stent-based local delivery of sodium nitroprusside from an eluting stent was effective in delivering nitric oxide in sustained fashion for up to 4 weeks *in vitro* experiments and demonstrated biologic effectiveness through increased local tissue cGMP levels for up to 14 days after stent implantation. However, this sodium nitroprusside-eluting stent failed to reduce chronic neointima thickening in the porcine coronary stent injury model. This technology may prove more efficacious, however, with improved drug elution characteristics or another choice of eluted drug.

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