Stent-Based Delivery of Sirolimus Reduces Neointimal Formation in a Porcine Coronary Model

Takeshi Suzuki, MD; Greg Kopia, PhD; Shin-ichiro Hayashi, MD, PhD; Lynn R. Bailey, AA; Gerard Llanos, PhD; Robert Wilensky, MD; Bruce D. Klugherz, MD; George Papandreou, PhD; Pallassana Narayan, PhD; Martin B. Leon, MD; Alan C. Yeung, MD; Fermin Tio, MD; Philip S. Tsao, PhD; Robert Falotico, PhD; Andrew J. Carter, DO

Background—The purpose of this study was to determine the efficacy of stent-based delivery of sirolimus (SRL) alone or in combination with dexamethasone (DEX) to reduce in-stent neointimal hyperplasia. SRL is a potent immunosuppressive agent that inhibits SMC proliferation by blocking cell cycle progression.

Methods and Results—Stents were coated with a nonerodable polymer containing 185 μg SRL, 350 μg DEX, or 185 μg SRL and 350 μg DEX. Polymer biocompatibility studies in the porcine and canine models showed acceptable tissue response at 60 days. Forty-seven stents (metal, n=13; SRL, n=13; DEX, n=13; SRL and DEX, n=8) were implanted in the coronary arteries of 16 pigs. The tissue level of SRL was 97±13 ng/artery, with a stent content of 71±10 μg at 3 days. At 7 days, proliferating cell nuclear antigen and retinoblastoma protein expression were reduced 60% and 50%, respectively, by the SRL at 3 days. After 28 days, the mean neointimal area was 2.47±1.04 mm² for the SRL alone and 2.42±1.04 mm² for the combination of SRL and DEX compared with the metal (5.06±1.88 mm², P<0.0001) or DEX-coated stents (4.31±3.21 mm², P<0.001), resulting in a 50% reduction of percent in-stent stenosis.

Conclusions—Stent-based delivery of SRL via a nonerodable polymer matrix is feasible and effectively reduces in-stent neointimal hyperplasia by inhibiting cellular proliferation. (Circulation. 2001;104:1188-1193.)

Key Words: sirolimus ■ stents ■ restenosis

The long-term clinical efficacy of intracoronary stenting is limited by restenosis, which occurs in 15% to 30% of patients. In-stent restenosis is due solely to 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 to activate smooth muscle cell (SMC) migration and proliferation. Experimental data suggest that inhibition of cell cycle progression with sirolimus (SRL) may be an effective strategy to prevent restenosis.

Gregory et al demonstrated that intraperitoneal administration of SRL, a potent immunosuppressive agent, resulted in a dose-dependent inhibition of arterial intimal thickening caused by either chronic alloimmune or mechanical injury in a rat model. Subsequent studies by Poon et al and Marx et al reported that SRL inhibited both human and rat vascular SMC proliferation in vitro by blocking the G1/S transition. The inhibition of proliferation was mediated by reduced cdk2 activity and retinoblastoma protein (pRb) phosphorylation. Gallo et al recently demonstrated that systemic SRL therapy significantly reduces the proliferative response after coronary angioplasty in the porcine model. The antiproliferative effects of SRL after PTCA were attributed to an inhibition of the pRb phosphorylation and prevention of the downregulation of p27kip1. Thus, the antiproliferative activity of SRL after balloon arterial injury in conjunction with its immunosuppressive properties suggests that this drug could also be useful for the prevention of in-stent restenosis.

The potential for untoward side effects such as infection, leukopenia, thrombocytopenia, and hyperlipidemia, however, limits the use of systemic administration of this agent for the prevention of in-stent restenosis. Local delivery using a stent platform, however, might allow for deposition of a therapeutic SRL concentration in the arterial wall, with a substantially reduced risk of systemic toxicity. The purpose of the present study was to determine the efficacy of stent-based delivery of SRL and to explore the synergistic effects of SRL in combination with dexamethasone (DEX) to reduce neointimal formation. In addition, we also characterized polymer biocompatibility, the in vivo pharmacokinetics, and the mechanism by which an SRL-coated stent inhibits neointimal hyperplasia.
Methods

Polymer- andDrug-Coated Stents
Stainless steel balloon-expandable tubular stents (Cordis Co), 18 mm long, were coated with a thin layer of a poly-n-butyl methacrylate and polyethylene–vinyl acetate copolymer containing ~185 µg SRL (Wyeth-Ayerst). In addition, the effects of DEX (350 to 700 µg/stent) alone and in combination with SRL (~185 µg) were evaluated to identify potential synergism with combined drugs. The total drug and polymer weight was ~500 µg for the SRL, 1000 µg for the DEX, and 1500 µg for the SRL+DEX stents (ratio of drug to polymer ~30%). For the biocompatibility studies, stents were coated with 600 to 750 µg or 1300 to 1850 µg of the copolymer. All stents were individually packaged, coded with a serial number on the packaging label, and sterilized with ethylene oxide. The identity of each serial number was known only by the sponsor so as to permit deployment and analysis of each stent in a blinded fashion.

Polymer Biocompatibility Studies
Animal research was completed after approval by the institutional animal care and use committees. The protocols conformed to the guidelines of the American Heart Association on animal research.

Porcine Studies
Nine juvenile swine (25 to 35 kg) underwent placement of 25 stents (bare metal, n=8; 750 µg polymer–coated, n=8; 1300 µg polymer–coated, n=9) in the left anterior descending, circumflex, or right coronary artery. The methods of stent implantation have been published previously. A grading scheme was developed to assess arterial wall and cellular parameters that determine the maturity of vascular repair.

Canine Studies
Fourteen purpose-bred mongrel dogs (20 to 30 kg) underwent placement of 42 stents (bare metal, n=14; 600 µg polymer, n=14; 1850 µg polymer, n=14) in the left anterior descending, proximal, or distal left circumflex coronary artery. The guiding catheter was used as a reference to obtain a 1:1 ratio between the stent-to-artery diameter compared with the baseline vessel diameter. Animals were allowed to recover and received postoperative care as previously described. At 60 days, the animals (n=9) were euthanized after completion of coronary angiography.

In Vivo Pharmacokinetics of Drug-Coated Stents
Four stents were coated with SRL as previously described and mounted on 3.5-mm-diameter angioplasty balloons. The stents were deployed in the coronary arteries of 4 pigs (1 stent per pig) by the same techniques as described above. Blood samples were obtained at 10 minutes; 1, 6, 24, and 48 hours; and 3 days to determine systemic SRL levels. The animals were euthanized and vessels harvested at 3 days after stent implantation. Stents were removed from freshly isolated arterial segments, and all tissue was frozen in liquid nitrogen. SRL levels in whole blood, arterial wall, and the stent were determined by high-performance liquid chromatography.

Efficacy Studies
Sixteen juvenile swine (25 to 35 kg) underwent placement of 47 stents (bare metal, n=13; SRL, n=13; DEX, n=13; SRL and DEX, n=8) in the left anterior descending, circumflex, or right coronary artery. The guiding catheter was used as a reference to obtain a 1:2:1 to 1:4:1 stent-to-artery ratio compared with the baseline vessel diameter. Animals were allowed to recover and were returned to care facilities, where they received a normal diet, aspirin 325 mg/d, and ticlopidine 250 mg/d. At 7 (n=4) or 28 (n=10) days, the animals were euthanized after completion of coronary angiography for quantitative analysis.

Pathological Evaluation
Immediately after euthanasia, the hearts were harvested, and the coronary arteries were perfusion-fixed with 10% buffered formalin at 60 to 80 mm Hg for 30 minutes via the aortic stump. In the efficacy studies, the vessels from the 7-day group placement (n=4; bare metal; n=4; SRL; n=4; DEX) were dissected from the heart after perfusion with lactated Ringer’s solution, cleaned of excess perivascular tissue, and frozen in liquid nitrogen. Vessel wall expression of proliferating cell nuclear antigen (PCNA) (Santa Cruz Biotechnology), pRb (Pharmingen), monocyte chemotactic protein (MCP)-1 (R&D Systems), or interleukin (IL)-6 (R&D Systems) was evaluated by Western blot analysis. In the 28-day studies, the stented coronary artery segments were processed for plastic embedding, staining, and histomorphometric analysis of 6 sections from the proximal aspect through the distal margin of the stent by use of published methods. A grading scheme was developed to assess arterial wall and cellular parameters that determine the maturity of vascular repair.

The stent endothelialization score was defined as the extent of the circumference of the arterial lumen covered by endothelial cells and was scored from 1 to 3 (1 = 25%; 2 = 25% to 75%; 3 = 75%). The minimal SMC content was graded as 1, focal residual fibrin involving any portion of the artery and for moderate fibrin deposition adjacent to the strut involving <25% of the circumference of the artery; 2, moderate fibrin deposition involving >25% of the circumference of the artery or heavy deposition of fibrin adjacent to and between stent struts involving <25% of the circumference of the artery; or 3, heavy deposition of fibrin involving >25% of the circumference of the artery. The intimal SMC content was scored as 1, sparse SMC density involving any portion of the artery and for moderate SMC infiltration less than the full thickness of the neointima involving <25% of the circumference of the artery; 2, moderate SMC infiltration less than the full thickness of the neointima involving >25% of the circumference of the artery or dense SMC content the full thickness of the neointima involving >25% of the circumference of the artery; or 3, dense SMC content the full thickness of the neointima involving >25% of the circumference of the artery.

Statistical Analysis
The mean angiographic, histological, morphological, and densitometric data for each stent were compared by ANOVA with post hoc analysis for multiple comparisons. Significance was established by a value of P<0.05. The data are expressed as mean±SD except as noted. All statistics were calculated with Statview 4.5 software.

Results

Polymer Biocompatibility Studies
In the porcine model, the bare metal and the 750 µg polymer–coated stents had a similar histological appearance. The mean vessel area, neointimal area, and percent area stenosis were similar for the bare metal and the 750 µg polymer–coated stents. The 1300 µg polymer–coated stents, however, had greater neointimal area compared with the 750 µg polymer–coated stents. The 1300 µg polymer–coated stents, despite identical deployment techniques.

In the canine model, the bare metal, the 600 µg polymer–coated, and the 1850 µg polymer–coated stents each had a similar appearance on histological sections. The mean vessel.
area, neointimal area, and percent stenosis were similar for the bare metal, the 600 µg polymer–coated, and the 1850 µg polymer–coated stents. Furthermore, the strut-associated inflammation scores were similar for the bare metal (28 days, 0.33±0.12; 56 days, 0.25±0.13), the 750 µg polymer–coated (28 days, 0.63±0.11; 56 days, 0.08±0.06), and the 1300 µg polymer–coated (28 days, 0.33±0.12; 56 days, 0.25±0.13) stents. The strut-associated inflammation scores declined at 56 days for each group compared with 28 days (P=0.0021). The mean vessel injury score were similar for the bare metal, the 600 µg polymer–coated, and the 1850 µg polymer–coated stents at 28 days. The mean injury score at 56 days was greater for the bare metal (1.84±0.25) than the 600 µg polymer–coated (1.28±0.15, P=0.047) and the 1850 µg polymer–coated stents (1.09±0.07, P=0.10).

**Pharmacokinetic Studies**

Whole-blood concentration of SRL peaked at 1 hour (2.63±0.74 ng/mL) after stent deployment and declined below the lower limit of detection (0.4 ng/mL) by 3 days. The total arterial tissue level of SRL was 97±13 ng/artery, and the residual stent content was 71±10 µg at 3 days. The amount of residual SRL on the stent at 3 days was 43% of the initial quantity loaded on the stent.

**In-Stent Neointimal Formation and SRL Therapy**

Quantitative analysis of the coronary angiograms at implantation from the 28-day efficacy studies demonstrated similar baseline lumen diameter, stent-to-artery ratio, and postprocedure minimal lumen diameter for each of the stent groups (data not shown). The histomorphometry data for each of the stent groups are summarized in the Table and Figure 1. Strut-associated inflammation was significantly reduced for the SRL (0.13±0.34) compared with metal stents (0.97±1.10, P<0.0001). SRL alone or combined with DEX resulted in a 50% reduction in neointimal area compared with bare metal stents, whereas DEX alone had a modest and nonsignificant effect on neointimal formation. The mean neointimal area was 2.47±1.04 mm² for the SRL alone and 2.42±1.04 mm² for the SRL+DEX compared with the metal (5.06±1.88 mm², P<0.0001) or DEX-coated (4.31±3.21 mm², P<0.001) stents. Thus, the percent area stenosis was significantly less for the SRL (24±10%) and the SRL+DEX (24±13%) than for the bare metal (47±19%, P<0.0001) or DEX-coated (45±31%, P<0.001) stents.

**Arterial Wall Morphology With SRL-Eluting Stents**

The arterial wall morphologies at 28 days for the SRL-coated and the bare metal stents are demonstrated in Figure 2. The morphologies of nonstented reference arterial wall sections, including the vessel area, neointimal area, and % area stenosis, were similar for the metal and each of the drug-coated stents. The appearance of the neointima was more variable for each of the drug-coated stent groups than the bare metal stents. In general, the neointima of the SRL-coated stents consisted of SMCs, matrix proteoglycans, and focal regions of residual fibrin adjacent to the strut struts. Focal medial necrosis or intimal hemorrhage was not observed within any of the bare metal or drug-coated stents.

A semiquantitative histological grading system demonstrated less SMC colonization and more residual fibrin deposition for the SRL-eluting stents than the bare metal stents (Figure 3). The SMC content score was less for the drug-coated stents than the bare metal stents (metal, 2.09±0.30 versus SRL, 2.50±0.51, P=0.002). Intimal fibrin scores were higher for the SRL (1.09±0.73) versus metal stents (0.44±0.56, P<0.0001), whereas the DEX stents exhibited a similar degree of residual fibrin deposition.
(0.50±0.69). Endothelialization scores were identical for the metal (2.9±0.4) and the SRL stents (2.9±0.4, \(P=0.66\)).

Cellular Proliferation and Inflammation

Figures 4 and 5 show the differences in protein expression observed between the bare metal and drug-coated stents. At 7 days, stainless steel stent placement was associated with increased expression of PCNA and pRb. These markers of neointimal formation were dramatically reduced by the SRL-eluting stents (38% and 48% of bare metal control, respectively) but not with the stents coated only with DEX. The SRL-eluting stents also inhibited the phosphorylation of the pRb protein. Stent-induced arterial injury was associated with enhanced production of MCP-1 and IL-6. Interestingly, exposure of vessels to stents coated with either SRL or DEX resulted in lower expression of both MCP-1 and IL-6.

Discussion

This study demonstrates that SRL-coated stents inhibit strut-associated inflammation and neointimal formation in the porcine coronary model. Our results show a 50% reduction in neointimal hyperplasia with a stent coated with a nonerodable copolymer matrix containing ≈185 \(\mu\)g SRL compared with a bare metal stent. The profound reduction in neointimal formation with an SRL-eluting stent is associated with an inhibition of pRb phosphorylation and suppression of inflammatory cytokines. Stent-based delivery of DEX alone, however, was insufficient to inhibit neointimal formation and also failed to produce any synergism in combination with SRL. Our findings document the feasibility of an SRL-eluting stent, and the efficacy data support the notion that stent-based SRL delivery via a nonerodable copolymer matrix is a promising approach for the prevention of restenosis.

Drug-Eluting Stents

Drug-eluting stents have been proposed as a means of preventing stent thrombosis and restenosis.\(^7,14\) Immobilized heparin surface coating of stents appears to favorably reduce stent thrombogenicity.\(^15\) The efficacy of drug-eluting stents for the prevention of restenosis has been limited by polymer biocompatibility, suitability of pharmacological agents, suboptimal in vivo pharmacokinetic properties, and local drug toxicity.\(^7,14\)

Biodegradable and nonbiodegradable polymers have been used as passive surface coatings or as a matrix for drug loading of stents.\(^14\) In the present study, a nonbiodegradable methacrylate and ethylene-based copolymer was applied to the surface of a stent to serve as a matrix for drug loading.
Figure 5. Representative Western blots demonstrating effect of SRL-coated stent at 7 days, with reduction in PCNA, increase in hypophosphorylated pRb vs hyperphosphorylated (ppRb) form of pRb, and inhibition of MCP-1 expression.

Methacrylate polymers have proven biocompatibility when used as a passive surface coating on stents. The histological data in the porcine and canine models suggest that this nonerodible polymer surface coating is biocompatible at 60 days. The porcine data, however, indicate a possible bulk effect, with more severe strut-associated inflammation and neointimal formation for the 1300 µg polymer-coated than the 750 µg polymer-coated stents. This “bulk response” to the polymer coating was not observed in the canine model. Others have observed similar differential responses to endovascular prosthesis in the porcine and canine models. Our data indicate a more severe and persistent inflammatory response to bare metal and polymer-coated stents in the porcine than the canine model. A 750-µg polymer coating, which exceeds the total polymer mass for the SRL-coated stents, was well tolerated in both the porcine and canine models at 2 months after implantation. These data indicate that the polymer appears to be a suitable candidate to serve as a matrix for drug delivery.

SRL-Eluting Stent

SRL is a potent immunosuppressive agent with anti-inflammatory and antiproliferative effects. SRL is a hydrophobic drug that has low solubility in aqueous solutions. Because of its lipophilicity, the drug passes easily through cell membranes, enabling intramural distribution and prolonged arterial tissue retention. Cellular uptake is enhanced by binding to the cytosolic receptor FKBP 12, which also may enhance chronic tissue retention of SRL. Thus, the known biological effects and pharmacokinetic properties of SRL suggest that the agent is an ideal candidate for a stent-based delivery to prevent restenosis.

In vivo pharmacokinetic studies demonstrated an arterial wall drug level of 97 ng/artery after 72 hours, with <0.4 ng/mL in the systemic circulation. Furthermore, modification of the coating has provided similar arterial tissue levels at 28 days and 3 days for the present drug coating. These data document the ability to deliver a potentially therapeutic arterial tissue concentration of SRL and insignificant levels in the systemic circulation with the nonerodible copolymer matrix.

The efficacy studies demonstrated a profound reduction in strut-associated inflammation, with a 50% reduction in in-stent neointimal hyperplasia for each of the SRL coating formulations. Histological assessment revealed the presence of typical cellular components of the neointima and a similar degree of endothelialization for the SRL compared with the bare metal stents. Therefore, critical reparative events, such as endothelialization and SMC colonization of the neointima, with SRL-eluting stents occur in a temporal sequence similar to that observed with bare metal stents. The focal remnants of residual fibrin deposition observed in the vessels with SRL-coated stents may reflect a delay in arterial repair or impaired fibrin degradation secondary to the local effects of the drug. Long-term studies are necessary to elucidate whether the drug is simply delaying the formation of neointima or subtly impairing fibrin degradation without late neointimal formation.

The analysis of arterial wall protein expression at 7 days suggests that the mechanism of action by which stent-based delivery of SRL reduces in-stent neointimal formation is similar to systemic treatment with the agent. A Western blot demonstrated a profound reduction in PCNA expression in the vessel wall for the SRL-eluting compared with bare metal stents. We also documented reduced phosphorylation of pRb by an SRL-eluting stent, which is consistent with the proven effects of the agent on cell cycle signaling and proliferation. Furthermore, a significant reduction in strut-associated inflammation was observed at 28 days for the SRL compared with bare metal stents, suggesting the potential for additional mechanisms of action to inhibit neointimal formation. Analysis of the vessel wall protein expression documented a 70% reduction in the inflammatory cytokine MCP-1 for the SRL-eluting compared with a bare metal stent. Unlike cyclosporine and tacrolimus, SRL is a weak inhibitor of cytokine production. The potent immunosuppressive effect of SRL is directed toward inhibiting the proliferation of T cells by blocking IL-2 activation of p70 kinase. The observed reduction of MCP-1 in the present study may be secondary to the effects of SRL on cellular proliferation and the production of cytokines by SMCs.
Limitations
This study is limited to observations in experimental models of restenosis whose relevance to human clinical circumstances is uncertain. The long-term effects of the polymer and drug-polymer formulation are unknown. The observed efficacy at 28 days may not be sustained after the drug concentration wanes to a subtherapeutic level. The dose-response effects for this SRL-eluting stent are incompletely characterized, although we have demonstrated a dose-dependent reduction in intimal hyperplasia with 60 μg to 200 μg SRL–coated stents in the rabbit model. Finally, stent-based SRL delivery may delay maturation and normal endothelial function, thus increasing the potential for a late thrombotic event. Recent clinical data, however, demonstrate inhibition of neointimal hyperplasia without any thrombotic events at 4 months after SRL-coated stent placement in patients with focal native coronary arterial lesions.17

Despite the limitations, our data provide sufficient evidence to conclude that stent-based delivery of SRL via a nonerodable polymer matrix is feasible and effectively reduces in-stent neointimal formation. An SRL-coated stent, unlike other potent antiproliferative restenosis therapies, does not induce stimulatory “edge” phenomena.18 Local stent-based delivery of SRL profoundly suppresses neointimal hyperplasia by inhibiting cell cycle progression and expression of inflammatory cytokines.

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