Rapamycin-Eluting Stents in the Arterial Duct: Experimental Observations in the Pig Model

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Background—Maintaining arterial duct patency by stent implantation may be advantageous in congenital heart disease management algorithms. Rapamycin, an immunosuppressant drug that demonstrates antiproliferative properties and inhibits smooth muscle cell migration, may deter the intimal hyperplasia that occurs during spontaneous closure and after-stent implantation of the arterial duct.

Methods and Results—Twenty-eight Yorkshire piglets (7 to 11 days old; weight, 2.2 to 4.9 kg) underwent stent implantation of the arterial duct (rapamycin-eluting (n=14) or bare metal (n=14) stents, 3.5-mm diameter) and were euthanized at 2, 4, and 6 weeks. Dissected arterial ducts were analyzed for lumen diameter, smooth muscle cell, and extracellular matrix components. Isolated arterial duct–derived smooth muscle cells were cultured in the presence or absence of rapamycin. Cellular proliferation rates were assessed by Ki-67 detection and [3H]-thymidine incorporation. No significant neointimal proliferation was present in either stent type at 2 weeks. At 4 weeks, the median luminal diameters of the bare metal stents were 87% (P=0.009), 54% (P=0.004), and 77% (P=0.004) that of the drug-eluting stents at the middle and aortic and pulmonary artery ends, respectively. At 6 weeks, the median luminal diameters of the bare metal stents were 0% (P=0.18), 5% (P=0.25), and 61% (P=0.13) that of the drug-eluting stents at the same respective levels. Complete histological occlusion was found in at least 1 level of the lumen in 9 pigs: 1 (17%) in the BMS group at 4 weeks, 5 (83%) in the BMS group at 6 weeks, and 3 (50%) in the DES group at 6 weeks. In vitro studies demonstrated 50%-lower proliferation rates in rapamycin-treated cultures of duct-derived smooth muscle cell cultures (P<0.001).

Conclusions—Rapamycin has antiproliferative actions on the arterial duct. Drug-eluting stents may be a more efficient tool than current palliative options for maintaining patency in critically duct-dependent states, but there may be a finite time-related benefit. (Circulation. 2009;119:2078-2085.)

Key Words: ductus arteriosus, patent ▪ rapamycin ▪ stents

Patency of the arterial duct is critical for maintaining pulmonary blood flow in many neonatal congenital heart disease states; however, it is programmed for spontaneous anatomic closure. This complex process involves an interplay of modulations within the extracellular matrix and smooth muscle cells (SMCs), resulting in contraction and migration of the SMCs of the tunica media into the subendothelial space in a process described as neointimal proliferation.1–4 Clinical Perspective p 2085

Continued ductal patency relies on active intervention, and in its absence, alternative sources of pulmonary blood flow are necessary. Important shortcomings exist with each of the current palliative options.5–12 Continuous prostaglandin E1 infusion involves prolonged intravenous access and hospitalization, high drug cost, and medical complications. Balloon dilation of the arterial duct has a high rate of reocclusion.5,9 A surgical shunt may be complicated by luminal compromise, pulmonary artery distortion, phrenic nerve and thoracic duct injuries, and seroma formation.8,10,11 In a multicenter report of 1004 infants with shunt-dependent pulmonary blood flow, the incidence of shunt compromise was 12%, and mortality at 1 year was 26%.11 In another series, shunt narrowing >50% was noted in 23% examined at the time of elective takedown, with the predominant mechanism being neointimal proliferation.8

More effective than balloon dilatation, ductal stenting with a bare metal stent (BMS) has been reported in a variety of conditions with duct-dependent systemic and pulmonary blood flows.6,9,12 The initial technical challenges of ductal stenting have been overcome by the use of coronary stents with a low profile and easy deliverability.6 Although acutely...

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taxel.17,18 Rapamycin is an immunosuppressive agent with cell-cycle arrest, ultimately resulting in inhibition of SMC proliferation.16,19,20

In this study, we examined whether the rapamycin drug-eluting coronary stent (DES) implanted in the neonatal porcine arterial duct demonstrated a reduction in neointimal proliferation translating to increased luminal diameter compared with the conventional BMS. To further assess the mechanism by which rapamycin may affect this unique vessel, we tested the influence of the drug in cultures of arterial duct—derived SMCs.

Methods

Stent Properties
The DES implanted was the CYPHER Select; the BMS was the BX Sonic (Cordis Inc, Miami, Fla). Both stents, 23 mm in length, were mounted on 3.5-mm-diameter balloon platforms. The DES was loaded with 140 μg rapamycin per 1 cm² or a total of 245 μg. A drug-free polymer layer was applied on top of the drug-polymer matrix as a diffusion barrier to prolong the release of the drug, with 50% of the rapamycin eluted over the first week, 80% over 30 days, and 100% over 90 days.18

Procedure/Stenting Protocol
Twenty-eight Yorkshire piglets (7 to 11 days old; median weight, 3.6 kg; range, 2.2 to 4.9 kg) were anesthetized with isoflurane and nitrous gases, intubated, and mechanically ventilated. A 4F sheath (Cordis Corp) was placed across the arterial duct into the pulmonary artery. Premounted stents (14 DES, 14 BMS) were implanted in the arterial duct over the wire with balloon inflation to 18 atm to achieve a stent diameter of 3.7 mm. The stents were placed so that the implant protruded into the main pulmonary artery but was flush with the aorta, ensuring complete ductal coverage (Figure 1). Penicillin 17 000 IU/kg IM was administered. For both stent groups, 2 pigs were euthanized at 2 weeks and 6 pigs at 4 and 6 weeks after the implantation. Weight ranges at the time of death were 5.1 to 5.8 kg; range, 2.2 to 4.9 kg; median weight, 3.6 kg. Heparin sulfate 1000 U IM was administered to limit acute thrombus formation during harvesting. The arterial duct was excised and fixed in 10% buffered 10% formalin.

Cellular proliferation rates of control and rapamycin-treated SMCs, derived from 3 intact (not stented) arterial ducts by collagenase digestion as previously described22 and cultured for 7 days in α-minimum essential medium supplemented with 10% FBS in the presence or absence of 100 nmol/L rapamycin. The drug was added twice, at time 0 and at day 4, together with fresh medium.

Assessment of Cell Proliferation
Cellular proliferation rates of control and rapamycin-treated SMCs, derived from 3 separate arterial ducts, were compared by [$^3$H]-

![Figure 1. Two-dimensional (A) and color (B) echocardiographic images demonstrating flow through stented arterial duct. Gross specimens of the arterial duct showing lack of thrombosis on the stent portion protruding into the main pulmonary artery (C), widely patent aortic end of the stented arterial duct (D), nonocclusive neointimal growth of the stented arterial duct at the aortic end (E), and complete occlusion at the aortic end (F). PA indicates pulmonary artery; Ao, aorta.](http://circ.ahajournals.org/69251)
thymidine incorporation and immunohistochemical detection of the proliferative antigen Ki-67 in quadruplicate cultures within each experimental group. Briefly, 1 μCi [3H]-thymidine per 1 mL media was added to the 4-day-old cultures at the time of the second rapamycin treatment, and the cultures were incubated for an additional 72 hours. The cultures were then washed twice with cold 5% trichloroacetic acid at 4°C and incubated with 0.5 mL of 0.3N NaOH for 30 minutes. Then, 200-μL aliquots from each well were added to 5 mL liquid scintillation cocktail and counted with a Win Spectral 1414 liquid scintillation counter (Wallac, Turku, Finland) as previously described.21 The parallel 7-day-old cultures were fixed in cold 100% methanol and exposed to antibody detecting the Ki-67 antigen in proliferating cells and then to peroxidase-labeled secondary antibody. The cultures were then counterstained with hematoxylin. For each of the quadruplicate cultures in each experimental group, the numbers of positively and negatively stained cells were counted under ×200 magnification in 30 separate fields. The percent of positively staining cells was determined within each field and averaged over the 30 fields examined.

Assessment of Elastin Production
The parallel 7-day-old cultures were probed with specific antibodies to elastin, collagen type I, and chondroitin sulfate–containing glycosaminoglycans, and the results were evaluated quantitatively by morphometry. The presence of elastic fibers was detected with polyclonal antibody to tropoelastin. The immunoreactions were visualized with fluorescein isothiocyanate–conjugated goat anti-rabbit secondary antibody. Nuclei were counterstained red with propidium iodide.21,22 The production of a new (metabolically labeled) insoluble elastin also was assessed in parallel 7-day-old cultures that were incubated for 72 hours with [3H]-valine.21 Deposition of insoluble elastin was reflected by levels of radioactive valine present in residues remaining after the cell layers of the same cultures were boiled in 0.1N NaOH for 45 minutes. This procedure removes all cellular and extracellular components except the cross-linked elastin. Quadruplicate cultures within each experimental group were analyzed. For each culture, 30 fields (magnification ×200) were randomly selected, and the area occupied by the particular immunodetectable component was quantified. The presence of each component was expressed as a percentage area of the entire analyzed field.

Statistical Analysis
Descriptive statistics are reported as medians and median absolute deviation and first and third quartiles. Comparison of BMS and DES parameters was performed with the Wilcoxon rank-sum test. Data from the cultures of arterial duct–derived SMCs were analyzed by Student t test and are reported as means and SDs. Statistical significance was defined as P<0.05. All data analysis was performed with R 2.8.1.23

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Echocardiographic assessment of ductal patency corresponded with gross and histological examination findings in all piglets except 1 BMS piglet at 4 weeks, in which the lumen was very small on histological examination but appeared occluded on gross inspection and had no demonstrable flow by echocardiography. Effective luminal diameter throughout the length of the stent could not be measured accurately with echocardiography. For example, even in the widely patent stented arterial duct with minimal neointimal proliferation, the diameter of the color flow was not uniform throughout the stent length.

Insertion of both the DES and BMS prevented the rapid natural contraction and consequent closure of piglet arterial duct (Figure 2). At 2 weeks, no significant neointimal hyperplasia was found in either stent type resulting in a lumen diameter similar to that of the stented arterial duct harvested immediately after implantation (Figures 2 and 3). At 4 weeks, the median luminal diameters of the BMS were 87% (P=0.009), 54% (P=0.004), and 77% (P=0.004) that of the DES at the same respective levels (Figure 3). At 6 weeks, the median luminal diameters of the BMS were 0% (P=0.18), 5% (P=0.25), and 61% (P=0.13) that of the DES at the same respective levels (Figure 4). Despite the striking differences in median luminal diameter at 6 weeks, no statistical difference resulting from the scatter of the values was found. Complete histological occlusion occurred in at least 1 level of the lumen in 9 pigs: 1 (17%) in the BMS group at 4 weeks, 5 (83%) in the BMS group at 6 weeks, and 3 (50%) in the DES group at 6 weeks. Occlusion involved at least the middle level of the stent in all cases. Nine (50%) of the 18 segments at 6 weeks in the BMS...
Histological analysis revealed that neither stent type induced significant inflammatory infiltration of the arterial wall. Neither implant induced any progressive remodeling of tunica adventitia, and luminal compromise was a result of neointimal formation. Immunostaining with α-actin antibody showed relative abundance of activated SMCs in the neointima, particularly in the BMS group (Figure 5). Histochemical evaluation of transverse sections indicated that the milder and mostly focal intraluminal outgrowth found in the DES-treated arterial ducts, particularly in close proximity to the stent struts, contained more elastic fibers and less collagen than observed in BMS-treated ducts (Figure 6). At 2 weeks, evidence of endothelialization was found in the tissue between stent struts; however, the struts themselves were not covered because there neointimal proliferation was minimal. By 4 to 6 weeks, intimal coverage of stent struts had begun but was not complete in all sections (Figure 6).

Results from the in vitro studies demonstrated that rapamycin-treated cultures of arterial duct–derived SMCs demonstrated 50%-lower proliferation rates \( (P < 0.001) \) than untreated counterparts as demonstrated by \([^3H]\)-thymidine incorporation and immunohistochemical detection of the proliferative antigen Ki-67 (Figure 7). The rapamycin-treated arterial duct SMCs also tripled their production of elastin (Figure 8).

Figure 3. Top, Luminal diameter of BMS (-) and DES (+) at 2, 4, and 6 weeks at the aortic end and middle and pulmonary artery ends. Time 0 weeks reflects measurements of arterial duct with BMS with tissue harvested immediately after implantation (-). Medians are represented as solid lines for BMS and dashed lines for DES. Number of animals: time 0, n = 1; time 2 weeks, n = 4 (BMS, n = 2; DES, n = 2); time 4 weeks, n = 12 (BMS, n = 6, DES, n = 6); time 6 weeks, n = 12 (BMS, n = 6, DES, n = 6). Bottom, Median diameters at 4 and 6 weeks. Ao indicates aorta; PA, pulmonary artery; Q1, first quartile; and Q3, third quartile.

Figure 4. Movat pentachrome staining of stented arterial duct in the BMS and DES groups at the center and pulmonary artery and aortic ends at 6 weeks after stent implantation showing the most widely patent and complete occlusion within both groups.
Discussion
The newborn arterial duct is a unique blood vessel programmed for spontaneous closure within the first few hours to days of postnatal life. Stent implantation of blood vessels also incites a reactive cellular response. Thus, the stented arterial duct provided a good model of a highly proliferative milieu of neointimal hyperplasia to compare the effects of the DES and BMS. This may have important clinical implications for the management of the neonate with duct-dependent blood flow.

Rapamycin: Clinical Applications
Clinical experience with rapamycin has been predominantly as an immunosuppressive agent in posttransplant recipients and as the rapamycin-eluting stent implanted into adult coronary arteries. Only sporadic reports exist of successful rapamycin treatment in other settings of intimal hyperplasia. Pulmonary vein stenosis acquired from radiofrequency ablation to cure atrial fibrillation has been treated successfully with endovascular stenting and adjunctive oral rapamycin. Recently, a rapamycin DES was implanted in the arterial ductal component of an isolated left pulmonary artery in a 7-week-old infant with follow-up stent patency confirmed at 7 months. Clinical studies of coronary artery stents demonstrated substantially reduced in-stent intimal hyperplasia with the DES compared with the BMS, translating to a reduction in the need for repeat revascularization procedures. This initial enthusiasm and change in practice toward the DES have been tempered by safety concerns around emerging evidence of late thrombosis (1.3%) associated with the DES compared with BMS. The strongest independent predictors of stent thrombosis were premature discontinuation of and inadequate responsiveness to antiplatelet therapy. Delayed endothelialization after DES implantation is hypothesized as a causative factor. The issue of late thrombosis of the DES in an arterial duct is of limited concern because patency rates beyond 3 to 6 months are usually not necessary.

Local delivery via drug-impregnated stents allows localized therapeutic concentrations within the vessel walls with...
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generally larger, particularly with prostaglandin therapy. It is possible that the mechanical injury of balloon dilation was magnified in the smaller animal vessels, inciting a more hyperplastic response. However, such effects would be similar in both the DES and BMS groups and would reinforce the antiproliferative benefits of rapamycin.

The number of animals in our study was small but larger than in published animal studies of coronary stenting.20,34 Further studies in animals are needed before this technology can be applied to infants.

No antiplatelet or anticoagulation treatment was administered during this study other than at time of euthanasia. This study does not draw any conclusions regarding the presence or role of thrombosis.

The contribution of thrombus to subsequent neointima formation is not thought to be significant, an observation supported by the lack of reduction in in-stent restenosis by antithrombotic agents.35 Luminal thrombosis was a rare finding on gross and histological assessments; however, its potential importance is underscored by our observation of incomplete endothelialization of stent struts at all time periods. In the clinical setting, antithrombotic therapy is strongly recommended after ductal stenting.

Conclusions

BMS implantation counteracts the immediate contraction-driven closure of the newborn arterial duct. Its effectiveness appears to be enhanced when impregnated with rapamycin, which inhibits the neointimal proliferation that is intrinsic to ductal closure and BMS implantation, proposing it as a potentially more efficient tool for maintaining patency in critically duct-dependent states. However, there may be a finite time-related benefit.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Maintaining arterial duct patency by stent implantation may be advantageous in congenital heart disease management algorithms. Rapamycin has antiproliferative properties and inhibits smooth muscle cell migration. In this study, bare metal and rapamycin-eluting stents were implanted into the arterial duct of 28 newborn pigs. At 2 weeks after stent implantation, minimal neointimal proliferation was observed in both the bare metal and rapamycin-eluting groups. At 4 weeks, luminal diameters were significantly increased in the rapamycin-eluting stent group. The difference at 6 weeks was not statistically significant. In vitro studies examining the cultures of duct-derived smooth muscle cells in the absence or presence of rapamycin demonstrated 50%-lower proliferation rates in the rapamycin group. This study demonstrates that rapamycin has antiproliferative actions on the arterial duct. Drug-eluting stents may be a more efficient tool than current palliative options for maintaining patency in critically duct-dependent states, but there may be a finite time-related benefit.
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