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Basic Science Review

Pimecrolimus and Dual Pimecrolimus-Paclitaxel Eluting Stents Decrease Neointimal Proliferation in a Porcine Model

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Objectives and Background: The purpose of this study was to determine the effectiveness and vascular response of a pimecrolimus drug eluting stent and a combination (pimecrolimus + paclitaxel) stent as compared with bare metal controls in the porcine coronary model. **Methods and Results:** In the first phase of the study, cobalt chromium stents were loaded with an erodible polymer and either a slow release or a fast release formulation of pimecrolimus. Thirty stents (metal, $n = 10$; pimecrolimus slow, $n = 10$; pimecrolimus fast, $n = 10$) were implanted in the coronary arteries of 10 pigs. At 30 days, neointimal proliferation and inflammation were both significantly less in the pimecrolimus fast release group as compared with the bare metal controls. Endothelialization was complete and equal in all three groups of stents. In the second phase of the study, stents were loaded with an erodible polymer with alternating reservoirs of paclitaxel and pimecrolimus. Twenty stents (8 control stents and 12 dual stents) were implanted in the coronary arteries of seven pigs. At 30 days, neointimal proliferation was significantly less in the dual drug group as compared with the bare metal controls. Endothelialization was complete in both groups of stents, suggesting complete healing of the arteries. **Conclusions:** In a 30-day porcine stent model, pimecrolimus inhibits neointimal proliferation as compared with bare metal stents. Also, the proof of concept of a dual drug eluting stent was established showing both safety and efficacy.

Key words: restenosis; animal models of human disease; vascular biology

INTRODUCTION

Drug eluting stents (DES) have significantly decreased restenosis rates after percutaneous coronary intervention [1,2]. However, in patients with diabetes and other high risk attributes, clinical and angiographic restenosis rates above 10% may be seen [3,4]. A further potential limitation of current DES designs is the phenomenon of delayed stent thrombosis, the exact frequency of which is unknown. Therefore, the opportunity exists for the development of DES devices with improved safety and efficacy.

To date, human efficacy has been demonstrated with only two classes of pharmaceutical agents. Rapamycin (sirolimus) and its derivatives have both anti-inflammatory and direct anti-proliferative properties [5]. The anti-inflammatory effects are mediated via inhibition of iNos and cox2 gene expression [6] and the anti-proliferative effects derive from inhibition of the mTOR oncogene. The relative importance of these two actions is incompletely understood. Paclitaxel inhibits mitosis

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by stabilizing microtubules and preventing mitosis. Paclitaxel has a narrow toxic-therapeutic window, and can induce vascular inflammation or necrosis if this threshold is approached or exceeded [7]. Both paclitaxel and rapamycin can interfere with complete vascular healing. Neither of these agents have specificity for vascular smooth muscle cells, and consequently both may inhibit endothelial cell proliferation, an important modulator of vascular healing [8,9].

Despite its name, pimecrolimus is not functionally a rapamycin analogue. It is best classified as a macrolactam ascomycin derivative (tacrolimus analogue) that exerts multiple anti-inflammatory effects including inhibition of IL-2 synthesis via calcinuerin inhibition, inhibition of IL-4, interferon γ, and release of inflammatory cytokines from mast cells. It does not bind to the mammalian target of rapamycin (mTOR) and therefore does not work in directly effecting cell cycle regulation but may do so indirectly via IL-2 inhibition [10–14].

The Conor DES is differentiated by virtue of its reservoirs [15]. A drug that is dissolved in a bioerodible polymer is placed within these reservoirs and released with both directional and kinetic control. There is no surface coating with either drug or polymer. Both preclinical and clinical results using a paclitaxel-based Conor stent have been promising. Efficacy at both 30 and 10 μg has been demonstrated in the longer release (30 day in vitro) formulations but faster release formulations were less effective [7,16]. The reservoir stent design also permits the simultaneous delivery of more than one agent simply by placing different polymer-drug combinations in alternate, adjacent reservoirs. This provides the opportunity to test drug eluting stents with combination therapy. It is possible that by combining two agents with differing modes of action, improved safety and/or efficacy may be achieved as is the case with dual agent cancer chemotherapy. We hypothesize that there is a synergistic decrease of neointimal formation by directly blocking the process of neointimal formation at two critical pathway points, inflammation and mitosis. We further hypothesize that this can be achieved without the expense of vascular toxicity or impaired vessel healing.

The goals of our study were three-fold; First, to determine if pimecrolimus, a predominant anti-inflammatory agent, prevents neointimal hyperplasia in the porcine model; Second, to determine if a combination of pimecrolimus plus a direct anti-mitotic, paclitaxel, act synergistically to inhibit intimal proliferation in this model; Third, to study the effect of these agents on vascular healing, endothelial cell regeneration, and inflammation.

MATERIALS AND METHODS

Stent Preparation

3.0 mm × 16 mm cobalt–chromium coronary stents were used for all parts of the study. These were provided by Conor Medsystems. Each stent has 590 individual reservoirs (each 100 μm in diameter) embedded in the stent. The first group of stents was loaded with ~325 μg of pimecrolimus dissolved in a polylactide-*co*-glycolide (PLGA) erodible polymer. A 325 μg dose was chosen as this was the maximum dose that could be loaded onto the stent due to stent reservoir volume constraints. Different comonomer ratios of PLGA (50:50 and 85:15) were used to vary the release kinetics of pimecrolimus as has been previously described for paclitaxel [7,15,16]. The PLGA 50:50 dissolves 5–6× as fast as the PLGA 85:15. The slow release stent uses only the PLGA 85:15 polymer whereas the fast release stent uses only the PLGA 50:50 polymer. Both stents lack a polymer cap, so that both formulations provide an initial burst release. The faster release kinetic stent was designed for ~50% release in the first 48 hr, with the majority of the drug released over ~10 weeks. The 'slow release' kinetic stent was designed to have a lower initial burst with ~25% of the drug released in the first 48 hr and the remainder to be released over approximately a 6-month period. Release kinetics were initially tested with in vitro studies. Following this in vivo studies were done with explanted stents removed and analyzed to ascertain remaining drug left on the stent. Release kinetics were comparable between the in vitro and in vivo groups, which matched the designed kinesis. A second group of stents were loaded with a combination of pimecrolimus and paclitaxel (Fig. 1). Because of manufacturing constraints only previously programmed formulations could be applied to the stent. Since every other well alternated the drugs, the stents were made such that half of the 325 μg pimecrolimus dose was used per stent, and half of the 30 μg paclitaxel dose (previously shown to be efficacious in the PISCES study) was used per stent. Therefore, the total stent dose of pimecrolimus was 162 μg, and the total dose of paclitaxel used was 15 μg. In terms of kinetics, for pimecrolimus the fast release formulation was used (as this had shown better efficacy than the slow release group in our initial analysis). For paclitaxel, the same polymer formulation was used as in the 30-day release group in the PISCES study, as the 30-day release groups showed the best efficacy in follow-up analyses of the PISCES study [16].

Porcine Studies

In the first phase of the study, 10 juvenile farm swine, 25–35 kg in weight, 3–6 months in age were

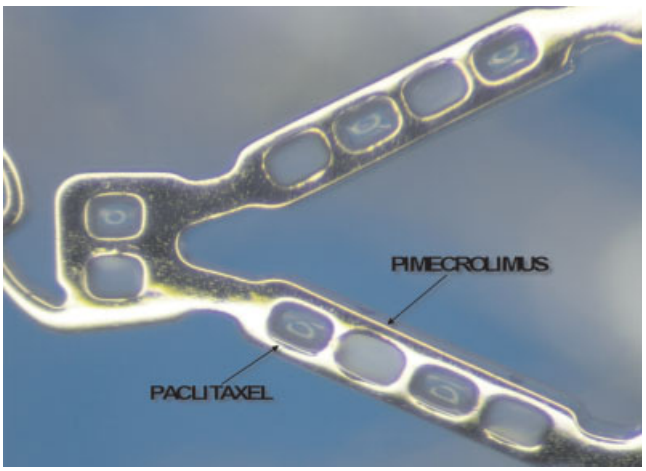

Fig. 1. Close up view of reservoir stents with alternating wells filled with paclitaxel and pimecrolimus. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

enrolled. The two pimecrolimus formulations and a control group consisting of bare metal cobalt stents were evaluated at a follow-up interval of 1 month. In the second phase of the study, seven juvenile farm swine, 25–35 kg in weight, 3–6 months in age were enrolled. One bare metal group and one combination stent group were evaluated at a follow up interval of 1 month. The stents were evenly and randomly distributed throughout each of the three major branches of the coronary arteries (RCA, LAD, and LCX). The artery segment was selected based on vessel diameter and ability to accommodate the length of the stent without excessive curvature. Vessels with baseline diameter ranging from 2.4 to 3.1 mm along the length of the implant (based on on-line QCA analysis) were selected, and the implantation pressure was varied according to the balloon compliance curve to achieve a stent/vessel ratio of 1.1:1 to 1.2:1. Repeat angiograms were conducted just prior to euthanasia and vessel harvest. QCA analysis was performed. All animals were pretreated with 325 mg aspirin, 75 mg clopidogrel, and amiodarone 75 mg daily for 3 days prior to the day of the procedure. All animals were post-treated with aspirin 325 mg and clopidogrel 75 mg p.o. daily until termination.

Angiographic Analysis

Angiograms were performed at the time of the initial catheterization and at 30 days of follow up. Quantitative Coronary angiography was performed (GE medical systems). Typical measures of angiographic neointimal formation were measured including reference vessel diameter, balloon to artery ratio, minimal luminal diameter (MLD), late loss (LL), and percent diameter of stenosis. LL is defined as MLD follow-up − MLD baseline. Percent Diameter stenosis is defined as the follow-up average reference vessel diameter (mean 5-mm diameter proximal to stent + mean 5-mm distal to stent/2) − MLD follow up, all divided by follow up average reference vessel diameter.

Histomorphometric Analysis

After euthanasia of the pig, coronary arteries were perfusion fixed at 100–120 mm Hg with ~500 ml of formalin. After overnight immersion–fixation, the hearts were sent to the CV Path, International Registry of Pathology. The stented vessel segments were dehydrated in a graded series of ethanol and embedded in methylmethacylate plastic. After polymerization, 2–3 mm sections were sawed from the proximal, mid, and distal portions of each single stent. Sections from the stents were cut on a rotary microtome at 4–5 μm, mounted and stained with hematoxylin and eosin and elastic Van Gieson stains. Segments of the native coronary arteries proximal and distal to the stents were taken for paraffin histology. Sections of the vessels were cut on a rotary microtome at 4–5 μm, mounted and stained with hematoxylin and eosin and Movat pentachrome stains. All sections were examined by light microcopy for the presence of inflammation, thrombus, neointimal formation, and vessel wall injury. Myocardial sections were taken from the anterior, lateral, posterior, and septal walls of the left ventricle distal to the sent and from the apical region of the left ventricle and examined for the presence of infarct, thromboembolus, and inflammation. To determine localized affects of the drug, the myocardium was also sampled beneath the area of stent placement. Histomorphometric analysis was performed including vessel injury score (Schwartz method), cross sectional areas (external elastic lamina [EEL], internal elastic lamina [IEL] and lumen), neointimal thickness, percent stenosis, neointimal inflammation, adventitial inflammation, fibrin coverage, and percent endothelialization. Neointimal thickness was measured as the distance from the inner surface of each stent strut to the luminal border. Area measurements were used to calculate vessel layer areas with the following formulas: Media = EEL − IEL, Neointima = IEL − Lumen, and % Stenosis = 100 × (Neointimal Area/IEL). Ordinal data were collected on each stent section and included strut apposition to the vessel wall, fibrin deposition, granuloma reactions, and hemorrhage around the stent struts and were expressed as a percentage of the total number of struts in each section. An overall neointimal inflammation, adventitial inflammation, and fibrin score were measured for each section. For neointimal inflammation, a score of 0 is less than 25% of struts with fewer than 10 inflammatory cells, a score of 1 is up to 25% of struts with greater than 10 inflammatory cells, a score of 2 is 25–50% of struts with greater

TABLE I. Summary of Vessel Injury

Stent type	Balloon/artery ratio	Injury score
Bare metal	1.19 ± 0.04	0.77 ± 0.46
Pimecrolimus slow release	1.18 ± 0.07	0.62 ± 0.26
Pimecrolimus fast release	1.21 ± 0.08	0.47 ± 0.38

$P = 0.22$ when comparing injury scores between groups (ANOVA).
$P = 0.60$ when comparing B/A ratio between all groups (ANOVA).

than 10 inflammatory cells, a score of 3 is greater than 50% of struts with greater than 10 inflammatory cells, and a score of 4 is two or more struts with granulomatous inflammatory reactions. A fibrin score of 0 is absent to focal residual fibrin involving any portion of the artery, a score of 1 is mild fibrin deposition involving less than 10% of the circumference of the artery or around less than 25% of the stent struts, a score of 2 is moderate fibrin deposition involving 10–25% of the circumference of the artery or around 25–50% of the stent struts, and a fibrin score of 3 is heavy deposition of fibrin involving greater than 25% of the circumference of the artery or surrounding greater than 50% of the stent struts. Adventitial inflammation was scored a 0 for no inflammation to minimal interspersed inflammatory cells anywhere in the adventitia, a 1 for mild peripheral inflammatory infiltration or focally marked in less then 25% of adventitial area, a 2 for moderate peripheral inflammatory infiltration or focally marked in 25–50% of adventitial area, and a 3 for heavy peripheral inflammatory infiltration or focally marked in greater than 50% of adventitial area. Endothelial coverage was semi quantified and was assessed microscopically on 6-μm sections stained with hematoxylin and eosin. The artery was divided into quadrants as a guide, and percent coverage is reported based on total luminal circumference.

Statistical Analysis

Angiographic and histological data were analyzed by comparing control and pimecrolimus eluting stents and control and combination drug stents by use of a one way ANOVA and Student's *t*-test. When ANOVA was used for multiple groups, pair-wise comparisons involving the control and different treatment groups were performed according to the post hoc Dunnett test. The level of significance was taken as $P < 0.05$. Results are reported as mean ± SD. All statistics were calculated with SPSS software.

RESULTS

Phase One: Pimecrolimus Study

There were ten pigs that each received three single stents deployed in the LAD, LCX, and RCA. The animals received 30 stents in a total of 30 coronary arteries (10 stents in each group [bare metal control, fast-release pimecrolimus, and slow-release pimecrolimus]). All pigs survived to completion of the 30-day study without evidence of myocardial infarction on gross inspection. No myocardial changes of ischemia or healed infarction were observed in any of the animals. The myocardium appeared healthy without changes of myocardial necrosis or fibrosis. Twenty-nine stents were analyzed. One of the slow-release pimecrolimus stents was not included in the QCA or histomorphometric analysis. Histologic analysis showed that the lumen was totally occluded by acute thrombus. There was no significant difference in injury between the three groups as seen by the similarity in the balloon to artery ratio and injury scores (Table I).

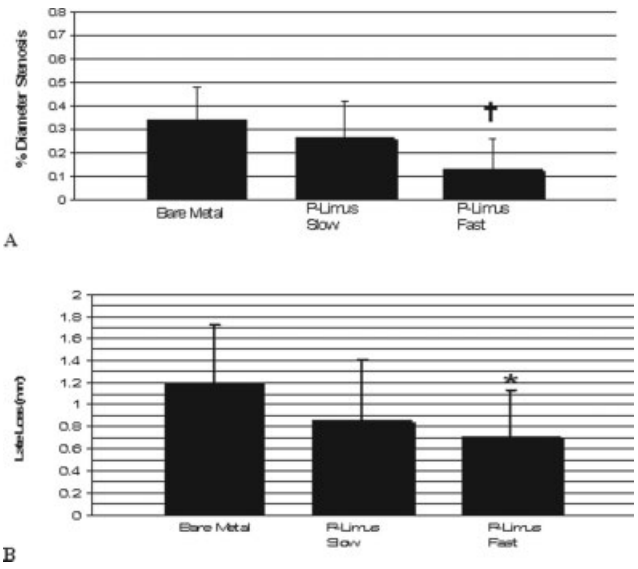

Fig. 2. A: Percent diameter stenosis versus stent type. B: Late loss versus stent type. †*P* = 0.004 versus control (Dunnettt's Test). **P* = 0.07 versus control (Dunnettt's test).

Angiographic Analysis

The angiograms of the pimecrolimus treated pigs showed a reduction in neointimal formation when compared with the bare metal controls. Angiograms were evaluated for LL and percent diameter stenosis at 30-day follow up (Fig. 2). There was a near-significant trend toward decreased LL in the fast release pimecrolimus treated group, with the bare metal stent having a LL of 1.20 ± 0.52, and the fast release pimecrolimus group having a LL of 0.71 ± 0.43 ($P = 0.07$ by Dunnett's test). There was a similar trend seen in percent diameter stenosis, with statistical significance seen between the bare metal group (34% ± 14%) and the fast release pimecrolimus group (13% ± 13%, $P = 0.004$ by Dunnett's test). Although there was a trend

towards decreased LL (0.86 ± 0.55) and percent diameter stenosis (26% ± 15%) in the slow release pimecrolimus group, there was no statistically significant difference as compared with the bare metal control.

Histomorphometric Analysis

Arterial cross sections of the different stent groups are shown in Fig. 3. On gross inspection, a decrease in neointimal formation in the slow and fast release pimecrolimus groups is seen when compared with control. The histomorphometry data for each of the stent groups are summarized in Table II and Fig. 4. In terms of efficacy, there was significantly less neointimal thickness in the fast release pimecrolimus group as compared with bare metal stent ($P = 0.02$ by Dunnett's), while statistical significance was not seen between the slow release pimecrolimus and controls ($P = 0.10$ by Dunnett's). A regression analysis of strut injury versus neointimal thickness was also performed. This demonstrated a significant reduction of neointimal thickness for both pimecrolimus groups as compared with control and no significant difference between the two treatment groups (Fig. 5). There was significantly less neointimal inflammation ($P = 0.004$ by ANOVA) and adventitial inflammation ($P = 0.005$ by ANOVA) in both pimecrolimus groups as compared bare metal controls. To evaluate stent healing, intimal fibrin scores and percent endothelialization of the lumen were measured. Fibrins scores and percent endothelialization showed no statistically significant difference across all groups (see Table II).

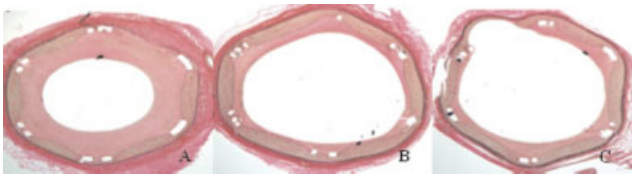

Fig. 3. Coronary artery morphology 30 days after stent placement. All arteries are from the same animal. Section A is from the RCA with a bare metal stent. Section B is from the LCX with a fast release pimecrolimus stent. Section C is from the LAD with a slow release pimecrolimus stent. Low power views (×2) show increased neointimal formation in the bare metal cross section (A) when compared with the pimecrolimus cross sections (B and C). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Phase 2: Dual Drug Study

Seven pigs each received three single stents deployed in the LAD, LCX, and RCA, except for the RCA of animal p5-47, which was too small for implantation. The animals received twenty stents in a total of twenty coronary arteries (12 stents in dual drug group, 8 stents in the bare metal control group). All pigs, except for animal p5-43, survived to completion of the 30-day study. Animal p5-43 died after 2 weeks after stent implantation. Gross inspection revealed aneurismal dilation around the LAD stent, which was confirmed by light microscopy to be a healing medial dissection, which occurred at the time of initial implantation as evidenced on angiography. The rest of the animals were without evidence of myocardial infarction on gross inspection. No myocardial changes of ischemia or healed infarction were observed in any of the animals. The myocardium appeared healthy without changes of myocardial necrosis or fibrosis. A total of 17 stents were analyzed by quantitative angiography and histomorphometry (10 dual drug stents and 7 bare controls). There was no significant difference in injury between the three groups as seen by the similarity in the balloon to artery ratio and injury scores (Table III).

Angiographic Analysis

The angiograms of the dual-drug treated pigs showed a reduction in neointimal formation when compared with the bare metal controls. Angiograms were evaluated for LL and percent diameter stenosis at 30-day follow up (Fig. 6). There was a statistically significant decrease in LL in the dual drug treated group as compared with the bare metal group (0.26 ± 0.15 vs. 0.94 ± 0.40, $P = 0.003$ by t-test). There was a similar significant decrease in percent diameter stenosis, with statistical significance seen between the bare metal group (28% ± 15%) and the dual drug group (8% ± 5%, $P = 0.01$ by t-test).

TABLE II. Summary of Histologic Markers of Vessel Healing at 30 Days

Stent type	% Endothelialization	Neointimal inflammation score	Adventitial inflammation score	Fibrin score
Bare metal	98.67 ± 2.69	1.40 ± 1.09	0.87 ± 1.00	0.63 ± 0.71
Pimecrolimus slow release	97.80 ± 2.99	0.33 ± 0.58[a]	0.07 ± 0.15[b]	0.70 ± 0.51
Pimecrolimus fast release	98.67 ± 2.09	0.27 ± 0.41[c]	0.00 ± 0.00[d]	0.70 ± 0.94

$P = 0.66$ for % endothelialization between all groups (ANOVA).
$P = 0.97$ for fibrin score between all groups (ANOVA).
[a]$P = 0.009$ vs. control (Dunnett's).
[b]$P = 0.004$ vs. control (Dunnett's).
[c]$P = 0.01$ vs. control (Dunnett's)
[d]$P = 0.006$ vs. control (Dunnett's).

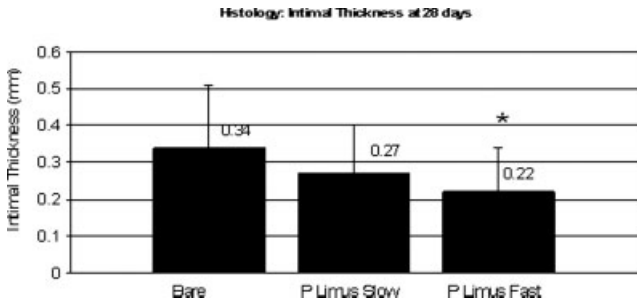

Fig. 4. Intimal thickness at 28 days. *P = 0.02 versus control (Dunnett's Test).

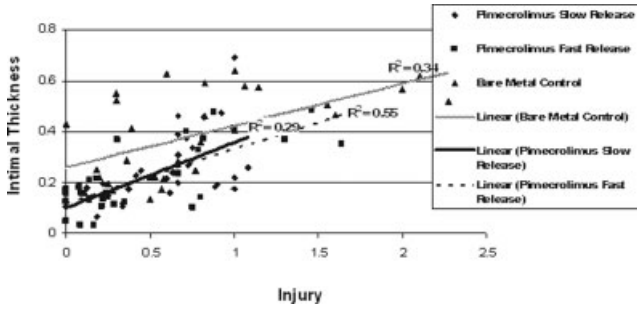

Fig. 5. Regression analysis of injury versus intimal thickness. For each given amount of injury, both slow release and fast release pimecrolimus show lower neointimal thickness as compared with bare control.

Histomorphometric Analysis

Arterial cross sections of the different stent groups are show in Fig. 7. On gross inspection, a decrease in neointimal formation in the dual drug group is seen when compared with the bare metal control group. The histomorphometry data for each of the stent groups are summarized in Table IV and Fig. 8. In terms of efficacy, there was significantly less neointimal thickness in the dual drug group as compared with bare metal stent (0.16 ± 0.07 vs. 0.33 ± 0.18, $P = 0.01$). Neointimal inflammation was similar between the dual drug and control stents (0.57 ± 0.57 vs. 0.48 ± 0.47, $P = 0.73$) and no significant adventitial inflammation was seen in either group. To evaluate stent healing, intimal fibrin scores and percent endothelialization of the lumen were measured. Percent endothelialization showed no statistically significant difference between the dual drug and control stents ($93\% \pm 10\%$ vs. $98\% \pm 2\%$, $P = 0.22$). There was greater neointimal fibrin seen in the dual drug stents as compared with the bare metal stents (1.23 ± 0.32 vs. 0.36 ± 0.34, $P = 0.0001$).

DISCUSSION

This proof of concept study is significant in several respects. First, in this porcine model, we have demonstrated the efficacy of a pimecrolimus drug eluting stent. This establishes pimecrolimus as a potential new therapeutic agent for drug eluting stents. Second, since pimecrolimus has no direct anti-proliferative properties, these data may have important implications with respect to mechanisms of poststent intimal hyperplasia. If borne out in human studies, the efficacy of pimecrolimus would tend to support the role of inflammation as central to the biologic response to stent injury as proposed by some investigators [17–23]. Second, to the best of our knowledge, this is the first study to describe simultaneous sustained release of dual agents from a stent with independent programmable release kinetics. This development establishes the framework for a wide variety of potential new therapeutic combinations. Finally, we did show both safety and efficacy with this dual drug eluting stent in this porcine model, but we failed to show superiority as compared with the pimecrolimus only stent configuration, despite our hypothesis that we expected a synergistic superiority of the dual drug eluting stent.

In the first phase of the study we examined the safety and efficacy of a pimecrolimus drug eluting

TABLE III. Summary of Vessel Injury

Stent type	Balloon/artery ratio	Injury score
Bare metal	1.18 ± 0.06	0.47 ± 0.29
Pimecrolimus/paciltaxel combination stent	1.21 ± 0.10	0.37 ± 0.17

$P = 0.41$ when comparing injury scores between groups (ANOVA).
$P = 0.37$ when comparing B/A ratio between all groups (t-test).

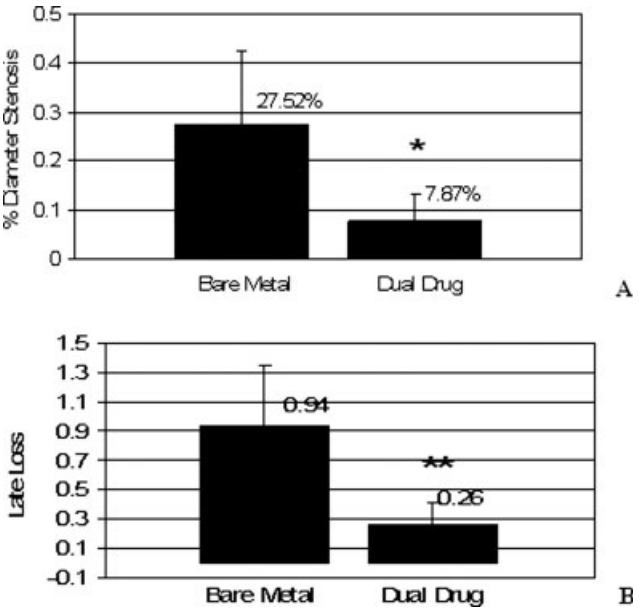

Fig. 6. A: Percent diameter stenosis versus stent type. B: Late loss versus stent type. *P = 0.01 versus control (t-test). **P = 0.003 versus control (t-test).

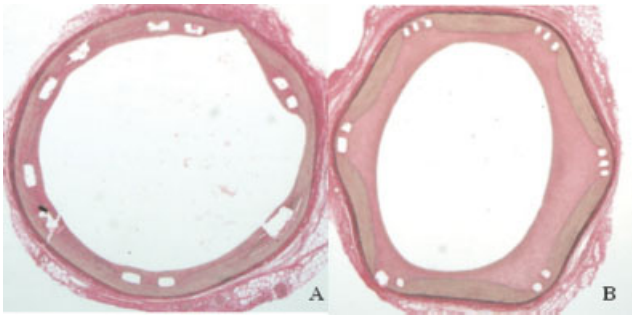

Fig. 7. Coronary artery morphology 30 days after stent placement. Section A is from the LAD with a paclitaxel/pimecrolimus combination stent. Section B is from the LAD with a bare metal stent. Low power views (×2) show increased neointimal formation in the bare metal cross section (B) when compared with the dual drug eluting stent (A). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE IV. Summary of Histologic Markers of Vessel Healing at 30 Days

Stent type	% Endothelialization	Neointimal inflammation score	Fibrin score
Bare metal	98.67 ± 2.69	0.48 ± 0.47	0.36 ± 0.34
Dual drug stent	97.80 ± 2.99	0.57 ± 0.57	1.23 ± 0.32[a]

$P = 0.22$ for % endothelialization between groups (ANOVA).
$P = 0.73$ for neointimal inflammation score between groups.
[a]$P < 0.0001$ (ANOVA) dual vs. bare for fibrin score between groups.
There was no significant adventitial inflammation in either group.

stent. On overall quantitative analysis of histologic sections the fast release pimecrolimus formulation appeared to be more effective than the slower one. This difference appeared to disappear with regression analysis comparing intimal thickness corrected for variation in strut injury. The porcine model is known to be injury dependent [24]. Whether a larger study would bring out clearer differences between the two formulation groups is unknown. Both groups demonstrated reduction in vascular inflammation as compared with bare metal stents. Both pimecrolimus formulations resulted in excellent vascular healing as demonstrated by a lack of excessive intimal fibrin and completed endothelialization by 30 days. Since pimecrolimus does not directly effect cell proliferation, the finding that pimecrolimus did not inhibit endothelial regeneration is not unexpected.

Other anti-inflammatory agents such as dexamethasone have been tried without success as coatings of drug eluting stents [25–27]. One might question why corticosteroids were ineffective while pimecrolimus was effective in the present study. These drugs work by markedly different mechanisms. Steroids act by much broader immunosuppressive effects at the level of the nucleus. They inhibit all known cytokines, inhibit cell migration of neutrophils, eosinophils, and macrophages, inhibit production of cox-2, and cause a rapid depletion of circulating T-cells [28–34]. As noted previously, pimecrolimus acts primarily as a calcineurin inhibitor and therefore mainly interferes with the synthesis of IL-2. This difference of a broad versus narrow spectrum of immunosuppressive response may be important in the modulation of the pathways responsible for stent induced intimal proliferation.

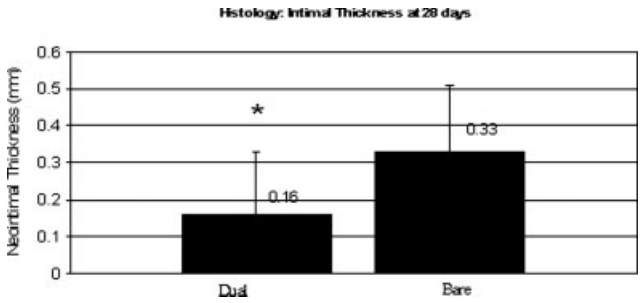

Fig. 8. Neointimal thickness at 28 days between dual drug eluting stents and bare metal controls. *$P = 0.014$ (ANOVA).

In the second phase of the study we examined the safety and efficacy of a combination drug eluting stent. This dual stent showed less neointimal formation by quantitative angiography and by histomorphometry as compared with the bare metal stents. Anecdotally, the neointimal thickness of the dual drug stent appeared to be less than previously shown with even 30 μg of paclitaxel in our laboratory, although no conclusions with respect to enhanced inhibitory effects versus paclitaxel alone may be drawn since we did not perform direct comparison in the present study [7]. The dual drug stents showed no significant difference in inflammation as compared with bare metal stents. This may be the result of the halved dose of pimecrolimus used as compared with the pimecrolimus alone study or to the presence of paclitaxel, which even at low doses may produce subtle inflammatory findings [7,22,35].

With respect to arterial healing, endothelialization was near complete in both the dual drug group and the bare metal group. There was no statistically significant difference between the groups. Unlike the pimecrolimus only stents, the dual drug stents did show a statistically significant increase in neointimal fibrin formation as compared with the bare metal controls. This increased intimal fibrin is likely due to the addition of paclitaxel to the stent. Intimal fibrin is a marker for overall stent healing, and one does expect delayed stent healing with paclitaxel [7,35].

Besides showing safety and efficacy in the dual drug eluting stent analysis, the second goal of that study was to see a synergistic decrease in neointimal formation with the dual drug eluting stent compared with the pimecrolimus stent. While there was a trend

towards decreased neointimal thickness (0.16 ± 0.07 in the dual drug group compared with 0.22 ± 0.13 in the fast release pimecrolimus group), this was not statistically significant. Therefore, we were unable to prove this hypothesis. It is possible with a larger sample size, we might have seen a statistically significant difference. We were also hampered by manufacturing constraints at the time to only use half the dose of pimecrolimus (162 μg) on the dual drug eluting stent as compared with that used in the pimecrolimus only study. This decreased dose of pimecrolimus was likely not as effective at decreasing inflammation as the full 325 μg dose was.

The possibilities for dual drug stents are intriguing. In the present case the agents were directed at inhibition of neointimal formation. One can easily imagine combinations with other therapeutic goals. For example, combining an anti-proliferative or anti-inflammatory with a PPAR agonist in diabetics, combining an anti-proliferative with an anti-thrombotic, or combining an anti-proliferative with an agent to promote myocardial salvage and/or regeneration following myocardial infarction may one day be possible.

The present study is subject to limitations. It was designed as a proof of concept for a novel agent with specific anti-inflammatory effects and for dual agents. It is not intended as a definitive preclinical evaluation. Any consideration for clinical trial would necessarily include more animals with control groups of polymer only stents as well as those with each of the two drugs alone. Follow-up at both 30 and 90 days would be required so as to assure no deleterious long-term findings. This not withstanding, the present polymers have been demonstrated as safe in both long-term animal and human studies [7,16,36]. As such, the probability of significant and unpredictable adverse effects at 90 days seems diminished. The porcine model is best suited for determination of safety. Relative human efficacy is less predictable in this model and can only be definitively ascertained in clinical trials [37].

CONCLUSION

In conclusion, this study demonstrated that in a 30-day porcine stent model, pimecrolimus inhibits neointimal proliferation as compared with bare metal stents. Pimecrolimus, currently approved for the treatment of atopic skin disorders, is worthy of further study as a potential agent for use in drug eluting stents. The concept of sustained dual drug delivery from stents is established. The possibility of combination therapy for improving the safety and efficacy of drug eluting stents as well as expanding their indications is worthy of further research and development.

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