EDITORIAL COMMENT

Do We Really Understand Pimecrolimus?*

Marc Vorpahl, MD,† Aloke V. Finn, MD,‡
Masataka Nakano, MD,† Renu Virmani, MD†

Gaithersburg, Maryland; and Atlanta, Georgia

Pimecrolimus was specifically developed for the topical treatment of inflammatory skin diseases. Like tacrolimus, it binds to the cytosolic receptor FK506 binding protein (Macrophilin-12). This complex inhibits the calcium-dependent phosphatase calcineurin and the translocation of the nuclear factor of activated T-cells transcription factor (NF-AT), which leads to an inhibition of inflammatory cytokines in T cells and mast cells. Pimecrolimus does not directly affect cell cycle regulation and therefore does have distinct differences from sirolimus or its analogue everolimus, zotarolimus, or biolimus A9, the most commonly used agents used in drug-eluting stents. The antirestenotic efficacy of mammalian target of rapamycin (mTOR) inhibition by sirolimus is based in part on its ability to prevent the degradation of $p27^{Kip1}$, a cyclin-dependent kinase inhibitor that plays an important role in smooth muscle cell (SMC) proliferation and migration. The use of pimecrolimus as an antirestenotic agent then relies on targeting inflammation rather than cell proliferation.

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In this issue of *JACC: Cardiovascular Interventions*, Ormiston et al. (1) present results from clinical and preclinical studies of pimecrolimus-eluting stents. Pimecrolimus was sprayed in a solvent at a dose of 400 mg/stent, and a top-coat of paralene C was applied to the stent. The results of the phase I clinical trial performed in noncomplex lesions showed an unexpectedly high in-stent binary restenosis (54%), with greater in-stent late loss (1.44 mm) in the pimecrolimus-eluting stent in relation to bare-metal stent (BMS) historical controls (0.91 mm) p = 0.03. Preclinical studies carried out in a porcine model failed to predict this excessive neointimal growth.

On first glance, the reasons for the failure of the preclinical studies to foreshadow these results are puzzling and raise questions about the reliability of preclinical models for predicting outcomes in humans. However, a closer examination of these results reveals some important insights. It is clear the results actually might have been misinterpreted by the authors. In fact, these studies showed safety but not efficacy of the pimecrolimus stent system. The artery size was significantly smaller (p = 0.04) in the group with 400-μg pimecrolimus and polymer than in all other groups. This flaw in the study design would tend to favor less neointimal area in the experimental arms compared with control. Therefore, the borderline significant reduction in neointimal area measurement (p = 0.06) might be a result of size differences rather than of drug efficacy. This is reinforced by the fact that the percent stenosis, a measurement that is independent of vessel size, was not significantly less in the pimecrolimus arm, despite the fact that there was a definite trend toward less stenosis (25 ± 6% vs. 34 ± 0% for BMS, and 30 ± 12% for polymer only), with injury and inflammatory scores being uniformly low in all groups. In addition, the 90-day study failed to raise any concern that no excessive neointimal formation or inflammation was observed. Further supporting our contention about the lack of efficacy is the finding that the neointima in the treated arm relative to control did not show delayed healing or even a decrease in inflammation, which would have suggested a biological effect of the drug.

The preclinical study also failed to raise any concern that there might be an increase in the neointimal formation that would lead to greater restenosis. However, we are not given the basic information regarding the release kinetics of the drug, the tissue concentration in the arterial wall, or the solvent used to dissolve the drug, all essential pieces of information for estimating its potential efficacy.

Given the lack of efficacy seen in both the preclinical model and humans, the question arises why calcineurin inhibitors might not be the optimal drugs for local drug delivery on a stent in human coronary arteries? In light of these data, the concept of modulating the inflammatory response to reduce restenosis needs to be re-examined. In human and animal models it has been suggested that inflammation plays an important role in in-stent neointimal growth and restenosis (2–4). In stented rabbit arteries early neutrophil infiltration is followed by prolonged macrophage infiltration and interleukin (IL)-8 and monocyte chemotactic protein 1 levels remain high beyond the 14-day period. Blockage of early monocyte recruitment with anti-inflammatory agents has been shown in animal models to result in reduction in neointimal formation. This study also demonstrated a linear relationship between tissue macrophage content and neointimal area (3). Until recently the role of T-cell in the proliferation of intimal cells was unknown. Evidence has emerged suggesting that recruit-

ment and activation of T cells directly activates mTOR complex, which is also essential for intimal proliferation of SMCs (5). Although pimecrolimus has been shown to inhibit T-cell activation in vitro and is highly effective when applied locally or given systemically for the treatment of psoriasis and other exfoliating skin conditions (6), there is no doubt in the pig model that the pimecrolimus-eluting stent had at best a weak effect on retarding intimal growth. Therefore, in man we cannot assume that T-cell activation—Th1 (IL-2), and Th2 (IL-4, IL-10) and proliferation, which is suppressed by pimecrolimus—necessarily plays an important role in restenosis.

It is well-known that sirolimus and everolimus have a profound effect on SMC suppression at very low inhibitory concentrations (IC)—IC_{50} (7). Both drugs have been successfully applied to stent surfaces and have shown minimal late loss in clinical trials (8,9). The IC_{50} of pimecrolimus as well as tacrolimus, also a calcineurin inhibitor for smooth muscle and endothelial cells suppression, are several thousand times less effective as compared with sirolimus (6).

Pimecrolimus, when applied on stents as reported by Ormiston et al. (1), is not effective in preventing the in-stent neointimal growth but in fact seemed to have stimulated SMC proliferation, with greater late loss as compared with historic controls. This result seems counterintuitive at first glance. Tacrolimus is structurally related to sirolimus. The FKBP12 is the intracellular ligand of both FK506 and sirolimus. The interaction between FKBP12 and FK506 results in a complex that inhibits calcineurin phosphatase, which controls lymphocytic activation, whereas the binding of FKBP12 to sirolimus produces a complex that inhibits autophosphorylation of the mTOR kinase and its downstream effectors that control SMC proliferation (10,11). A potential answer for the pro-proliferative characteristics of pimecrolimus can be found in the work of Giordano et al. (10) who recently demonstrated that the immunosuppressant FK506 or "tacrolimus" acts as a growth factor for vascular SMCs by activating transforming growth factor-β signaling in SMCs. This finding along with the clinical data reported by Ormiston et al. (1) emphasizes the importance of understanding thoroughly the complexity of biologic effects of newer pharmacologic agents before their use in drug delivery devices.

Although the porcine coronary model failed to predict the phenomenon of excessive neointimal formation after pimecrolimus-eluting stents, we believe animal models still hold predictive value in most cases, because the sequence of biological events associated with arterial repair are remarkably comparable in animals and man (12). For instance, pre-clinical data of the Actinomycin D-eluting stent showed media necrosis and varying degree of acute inflammation at 28 days and a thick neointima with adventitial fibrosis at 90 days (13). These data also predicted the results seen in the clinical ACTION (Actinomycin-Eluting Stent Improves Outcomes by Reducing Neointimal Hyperplasia) trial, which was interrupted prematurely because of a high incidence of repeat target vessel revascularization. In fact, we also learned the dose-dependent effects of paclitaxel that result in tissue necrosis and inflammation in the porcine animal model (14). Long-term data in the porcine model with a sirolimus-eluting stent (Cypher) demonstrated lack of efficacy at 90 and 180 days but showed a greater degree of inflammation with significant eosinophilic infiltration (15), which suggested that cases of polymer-induced hypersensitivity might also be seen in man.

Finally, the results with pimecrolimus are consistent with the findings of multiple clinical studies using stents that elute pimecrolimus. The GENESIS (Randomized, Multicenter Study of the Pimecrolimus-Eluting and Pimecrolimus/Paclitaxel-Eluting Coronary Stent System in Patients with De Novo Lesions of the Native Coronary Arteries) trial was the first large clinical trial of pimecrolimus-eluting stents in a bioerodable polymer, which also tested dual releasing pimecrolimus- and paclitaxel-eluting stents as well as only a paclitaxel-eluting stent in man. The enrollment of the GENESIS trial was suspended early, due to an increase in target vessel revascularization in the pimecrolimus-eluting-stent group (35.5%) (16). The efficacy of the drug-eluting stent was only observed in patients receiving paclitaxel stents (2%), and those receiving pimecrolimus- and paclitaxel-eluting stents was in between (14.4%). Both the GENESIS and First-In-Man phase I clinical trials with the pimecrolimus-eluting stent lacked BMS control groups; the usage of a different polymer in the 2 trials makes it impossible to implicate pimecrolimus as the culprit for the induction of increased late loss as compared with historical control BMS.

In conclusion, although the results of this work involving pimecrolimus to prevent restenosis were unsuccessful in man, they help us to understand the limitations of our models as well as the processes that are important in neointimal proliferation. It seems clear that strategies aimed at reducing inflammation alone need to be re-evaluated, while antiproliferative drugs targeting inhibition of smooth muscle cell proliferation pathways (i.e., mTOR) warrant further consideration as newer agents are developed that will impact the future of stent design.

Author Disclosures

Inc.; Atritech, Inc.; Atrium Medical Corp.; Avantec Vascular; Bard Peripheral Vascular, Inc.; B-Balloon Ltd; Biotronik AG; Biogen IDEC; Biotegra, Inc.; Biomerix; BioPAL, Inc.; Biosensors International; Biomer Technology LTD; Boston Scientific Corp.; ByPass Medical Technologies, Ltd; CardioDex, LTD; Cardica, Inc.; CardioKinetix, Inc.; CardioFocus, Inc.; Cardiovascular Research Foundation-Korea; CardioMind, Inc.; Cardiovascular Research Foundation; Cierra, Inc.; CoAptus Medical Corp.; Coherex Medical, Inc.; Concentric Medical; Conor Med Systems; CorAssist Cardiovascular LTD; Cordis Corporation; CoRepair, Inc.; Correx, Inc.; Corindus, Inc.; CorNova Inc.; CVRx, Inc.; CyberHeart Inc.; Devax, Inc.; Edwards Lifesciences, LLC; Elixir Medical Corp.; Elutex, Inc.; ev3, Inc.; Evalve, Inc.; Gardia Medical Ltd; Gem Biosystems, GlaxoSmithKline; HemCon; InfraReDx, Inc.; Invatec Technology Center GmbH; Jerini AG; Kaneka Corp.; Laax, Inc.; Lumen Biomedical, Inc.; Lutonix, Inc.; Maquet Cardiovascular; Medtronic AVE; Medtronic Heart Valves; Meril Life Sciences Pvt Ltd; Microvention, Inc.; Minnow Medical, LLC; Miravant Medical, LLC; Neovasc Medical Ltd; Novartis Pharmaceuticals Corporation; NovoStent Corp.; OrbusNeich Medical, Inc.; Oregon Medical Laser Center; Paragon Intellectual Properties, LLC; Prescient Medical, Inc.; Probiodrug AG; ReLeaf Medical; Relisys Medical Devices Ltd; ReValve Vascular LTD; Revascular Therapeutics; Sahajanand Medical Technologies Pvt. LTD; Sorin Biomedica Cardio S.r.l; Surmodics, Inc.; Takeda Pharmaceuticals North America; Terumo Corp.; Theregen, Inc.; TissueGen, Inc.; Top Spin; Toray Industries, Inc.; Transluminal Technologies; Vascular Therapies, LLC; VIA Pharmaceuticals, Inc.; Volcano Therapeutics, Inc.; X-Cell Medical, Inc.; and Xtent, Inc. He is also a consultant for Medtronic AVE; Abbott Vascular; W.L. Gore; Volcano Therapeutics, Inc.; Prescient Medical; CardioMind, Inc.; Direct Flow; and Atrium Medical Corporation.

Reprint requests and correspondence: Dr. Renu Virmani, Medical Director, CVPath Institute, Inc., 19 Firstfield Road, Gaithersburg, Maryland 20878. E-mail: rvirmani@cvpath.org.

REFERENCES

1. Ormiston JA, Webster MWI, Schwartz RS, et al. Feasibility, safety, and efficacy of a novel polymeric pimecrolimus-eluting stent: traditional pre-clinical safety end points failed to predict 6-month clinical angiographic results. J Am Coll Cardiol Intv 2009;2:1017–24.
2. Farb A, Weber DK, Kolodgie FD, Burke AP, Virmani R. Morphological predictors of restenosis after coronary stenting in humans. Circulation 2002;105:2974–80.
3. Welt FG, Rogers C. Inflammation and restenosis in the stent era. Arterioscler Thromb Vasc Biol 2002;22:1769–76.
4. Kornowski R, Hong MK, Virmani R, Jones R, Vodovotz Y, Leon MB. Granulomatous 'foreign body reactions' contribute to exaggerated in-stent restenosis. Coron Artery Dis 1999;10:9–14.
5. Schwarz JB, Langwieser N, Langwieser NN, et al. Novel role of the CXC chemokine receptor 3 in inflammatory response to arterial injury: involvement of mTORC1. Circ Res 2009;104:189–200, 8p following 200.
6. Grassberger M, Baumruker T, Enz A, et al. A novel anti-inflammatory drug, SDZ ASM 981, for the treatment of skin diseases: in vitro pharmacology. Br J Dermatol 1999;141:264–73.
7. Schuler W, Sedrani R, Cottens S, et al. SDZ RAD, a new rapamycin derivative: pharmacological properties in vitro and in vivo. Transplantation 1997;64:36–42.
8. Morice MC, Wijns W, DiMario C, et al. Direct stenting of de novo coronary stenoses with tacrolimus-eluting versus carbon-coated carbostents: the randomized JUPITER II trial. Eurointervention 2006;2:45–52.
9. Stone GW, Midei M, Newman W, et al. Comparison of an everolimus-eluting stent and a paclitaxel-eluting stent in patients with coronary artery disease: a randomized trial. JAMA 2008;299:1903–13.
10. Giordano A, Romano S, Mallardo M, et al. FK506 can activate transforming growth factor-beta signalling in vascular smooth muscle cells and promote proliferation. Cardiovasc Res 2008;79:519–26.
11. Donners MM, Daemen MJ, Cleutjens KB, Heeneman S. Inflammation and restenosis: implications for therapy. Ann Med 2003;35:523–31.
12. Virmani R, Kolodgie FD, Farb A, Lafont A. Drug eluting stents: are human and animal studies comparable? Heart 2003;89:133–8.
13. Schwartz RS, Chronos NA, Virmani R. Preclinical restenosis models and drug-eluting stents: still important, still much to learn. J Am Coll Cardiol 2004;44:1373–85.
14. Heldman AW, Cheng L, Jenkins GM, et al. Paclitaxel stent coating inhibits neointimal hyperplasia at 4 weeks in a porcine model of coronary restenosis. Circulation 2001;103:2289–95.
15. Carter AJ, Aggarwal M, Kopia GA, et al. Long-term effects of polymer-based, slow-release, sirolimus-eluting stents in a porcine coronary model. Cardiovasc Res 2004;63:617–24.
16. Verheye S, Agostoni P, Dawkins KD, et al. The GENESIS (Randomized, Multicenter Study of the Pimecrolimus-Eluting and Pimecrolimus/Paclitaxel-Eluting Coronary Stent System in Patients with De Novo Lesions of the Native Coronary Arteries) trial. J Am Coll Cardiol Intv 2009;2:205–14.

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