

Intramural Delivery of Recombinant Apolipoprotein A-I_{Milano}/Phospholipid Complex (ETC-216) Inhibits In-Stent Stenosis in Porcine Coronary Arteries

Sanjay Kaul, MD; Vladimir Rukshin, MD; Raul Santos, MD; Babak Azarbal, MD; Charles L. Bisgaier, PhD; Jan Johansson, MD; Vivian T. Tsang, BS; Kuang-Yuh Chyu, MD; Bojan Cercek, MD, PhD; James Mirocha, PhD; Prediman K. Shah MD

Background—We have previously demonstrated vasculoprotective effects after repeated intravenous administration of recombinant apolipoprotein A-I_{Milano} (apoA-I_m)/phospholipid complex. In this study, we sought to determine the effects of local recombinant apoA-I_m/1-palmitoyl,2-oleoyl phosphatidylcholine complex (ETC-216) delivered intramurally via the Infiltrator catheter on luminal narrowing in a porcine coronary artery stent overstretch injury model.

Methods and Results—In twelve domestic swine (≈ 25 kg), two arteries each were infiltrated with 0.4 mL ETC-216 (14 mg/mL) or vehicle control immediately before deployment of GFX stents (stent:artery ratio=1.3:1). Animals were euthanized at day 28, and evaluation by QCA revealed a significant improvement in mean lumen loss index with ETC-216 treatment ($21 \pm 22\%$ versus $43 \pm 13\%$ lumen loss; $P=0.01$). Histomorphometric analysis showed that ETC-216 treatment significantly reduced the intimal area (6.7 ± 1.5 versus 5.2 ± 1.4 mm², -22% ; $P=0.02$) and the stenosis index (0.76 ± 0.15 versus 0.59 ± 0.15 ; $P=0.01$), and increased the lumen area (2.1 ± 1.4 versus 3.7 ± 1.8 mm², $+76\%$; $P=0.02$). Regression analysis showed significant differences in lumen area ($P=0.004$), neointimal area ($P=0.003$), stenosis index ($P=0.001$), and neointimal thickness ($P=0.003$) adjusted for injury score in favor of ETC-216.

Conclusions—A single intramural administration of ETC-216 significantly inhibited injury-induced luminal narrowing in the porcine stent overstretch model through reduction of intimal hyperplasia. These data show that local intracoronary delivery of ETC-216 may be useful to prevent restenosis after coronary stenting. (*Circulation*. 2003;107:2551-2554.)

Key Words: lipoproteins ■ restenosis ■ stents

Apolipoprotein A-I_{Milano} (apoA-I_m) is a naturally occurring variant of apoA-I, with arginine to cysteine substitution at position 173, that is associated with low rates of vascular disease in its carriers, despite markedly reduced HDL and elevated triglyceride levels.¹ We and others have previously demonstrated vasculoprotective effects of recombinant apoA-I_m/phospholipid complex in rabbits^{2,3} and mice.^{4,5} However, this protective effect was evident only after repeated intravenous administration of high-dose apoA-I_m. Because apoA-I and HDL are known to have antiinflammatory effects²⁻⁶ and inflammation is implicated in restenosis,⁷ we examined the effects of recombinant apoA-I_m/1-palmitoyl,2-oleoyl phosphatidylcholine complex (ETC-216) on luminal narrowing in a porcine coronary artery stent overstretch injury model utilizing a local intramural drug delivery approach via the Infiltrator catheter. We hypothesized that intramural delivery into the coronary vessel wall provides for enhanced delivery efficiency, minimizing agent loss into the systemic circula-

tion, thereby resulting in lower doses, longer duration of activity, and improved overall effectiveness in modulating coronary arterial response to injury.

Methods

Test Substances

Test substance (ETC-216) is a synthetic HDL complex composed of 14 mg/mL recombinant apoA-I_m and 13 mg/mL 1-palmitoyl-2-oleoyl phosphatidyl choline (POPC) in sucrose-mannitol-phosphate buffer solution (Esperion Therapeutics, Inc). Control animals received sucrose-mannitol vehicle (sterile 6.4% sucrose, 0.8% mannitol in 6 mmol/L phosphate buffer, pH 7.4; Esperion Therapeutics, Inc). Treatments were randomized, and the investigators were blinded with regard to treatment group until data analysis was completed.

Animal Preparation

All animal care and handling were performed in accordance with the guidelines specified by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the

Received January 29, 2003; revision received March 20, 2003; accepted April 11, 2003.

From the Vascular Physiology and Thrombosis Research Laboratory of the Atherosclerosis Research Center (S.K., V.R., R.S., B.A., V.T.T., K.-Y.C., B.C., J.M., P.K.S.), the Division of Cardiology, Cedars-Sinai Medical Center, Los Angeles, Calif; and Esperion Therapeutics, Inc (C.L.B., J.J.), Ann Arbor, Mich.

Correspondence to Sanjay Kaul, MD, Division of Cardiology, Rm 5314, Cedars-Sinai Medical Center, 8700 Beverly Blvd, Los Angeles, CA 90048. E-mail kaul@cshs.org

© 2003 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

DOI: 10.1161/01.CIR.0000074042.19447.B1

Institutional Animal Care and Use Committee. Adult farm swine weighing approximately 25 kg were treated with aspirin 325 mg, ticlopidine 250 mg, and diltiazem 120 mg PO beginning 3 days before surgery. After overnight fasting, swine were immobilized by an intramuscular injection of acepromazine (0.5 mg/kg), ketamine (20 mg/kg), and atropine (0.05 mg/kg); anesthesia was induced with intravenous thiopental (5 to 8 mg/kg), and maintained by 1% to 2% isoflurane after endotracheal intubation. Heparin was given as a bolus of 8000 IU intravenously.

After baseline coronary angiography, the ETC-216 or sucrose-mannitol vehicle was injected into two out of three coronary arteries of each animal with an Infiltrator catheter (InterVentional Technology Inc). The Infiltrator is a triple-lumen balloon catheter allowing intramural drug delivery via 3 longitudinal strips of 7 low-profile 0.254-mm injector ports capable of penetrating the internal elastic lamina (IEL).⁸ The catheter allows local drug delivery without perforation, dissection, or hemorrhage of the arterial segment.⁸ It has been successfully used for local drug⁹ and gene delivery¹⁰ to reduce luminal narrowing after coronary angioplasty in swine.

After intramural drug delivery, a clinical grade GFX stent (3.0, 3.5, or 4.0 mm×18 mm) was deployed at each infiltrated segment with a final stent: artery ratio of ≈1.3:1. Follow-up coronary angiogram was obtained at 28 days, after which the animals were euthanized by intravenous injection of 10% potassium chloride, and the injured coronary arteries were harvested and prepared for analysis.

Angiographic Measurements

Using the computerized quantitative coronary angiography analysis (QCA) system (Advantx DX, GE Medical Systems), the following measurements were obtained: preinjury vessel lumen diameter, postinjury in-stent lumen diameter, in-stent mean and minimum lumen diameter at 28 day follow-up, and reference diameter proximal to stent.

Tissue Preparation and Histomorphometric Analysis

Special histological processing was performed to maintain the vascular architecture of the stented segments. Tissue blocks were cut with a diamond-wafering blade and embedded in methyl methacrylate (CLB Enterprises). Three radial cross sections containing 12 struts were cut; one from the proximal, mid, and distal portion along each stent. Sections were ground to a thickness of about 50 μm, optically polished, and stained with toluidine blue (paragon stain).

A computerized imaging system (ImagePro 4.0) was used for histomorphometric measurements of the following: (1) vessel size, defined as the area circumscribed by the external elastic lamina (EEL); (2) neointima, defined as the area between the lumen and the internal elastic lamina (IEL); (3) media, defined as the area between IEL and EEL; and (4) lumen, defined as the area circumscribed by the neointima-luminal border. Maximal neointima thickness was measured directly, and thickness for each of the 3 segments was averaged to obtain the mean thickness. An ordinal injury score, modified from that of Schwartz et al¹¹ was assigned where 0=IEL intact, 1=IEL fractured by strut, 2=strut lacerating the media, and 3=strut disruption of the EEL. Injury score for each of the 12 struts was averaged to obtain the mean injury score per segment. Lumen area, neointimal area, stenosis index, and neointimal thickness were plotted as a function of the injury score to adjust for any differences in degree of injury between control and treatment groups. The morphometric parameters were assessed separately by experienced observers both of whom were blinded as to treatment group. Interobserver variability was <5%.

Statistical Analysis

Data are expressed as mean±SD. Mean values of variables were compared by a two-sided unpaired *t* test. Linear regression models were used to assess differences in the outcome measures adjusting

QCA and Morphometry Variables

	Control	ETC-216	<i>P</i>
QCA variables			
Prestent injury diameter, mm	2.7±0.5	2.6±0.3	NS
Poststent injury diameter, mm	3.5±0.5	3.5±0.5	NS
Stent:artery ratio	1.3±0.2	1.3±0.2	NS
Mean luminal diameter, mm	1.9±0.5	2.6±0.5	0.01
Lumen loss index, %	43±13	21±22	0.01
Minimum luminal diameter, mm	1.5±0.5	1.9±0.6	0.1
Lumen loss index, %	48±18	38±17	0.2
Reference diameter, mm	3.0±0.8	2.9±0.6	NS
Morphometry variables			
Area measurements			
EEL area, mm ²	10.3±1.2	10.6±2.1	NS
IEL area, mm ²	8.8±1.0	9.0±1.9	NS
Media area, mm ²	1.4±0.4	1.6±0.3	NS
Adventitial area, mm ²	2.4±0.7	1.9±0.4	0.07
Stenosis index, mm ² /mm ²	0.76±0.15	0.59±0.15	0.01
Thickness measurements			
Neointimal thickness, mm	1.34±0.29	1.06±0.23	0.02
Normalized neointimal thickness, mm	0.15±0.03	0.12±0.03	0.04

Data are mean±SD; n=12.

Lumen loss index indicates (poststent injury diameter–Mean or minimum luminal diameter at 28 days/poststent injury diameter)×100; stenosis index, intimal area/IEL area; and normalized intimal thickness, intimal thickness/IEL area.

for injury score. A value of *P*<0.05 was considered statistically significant.

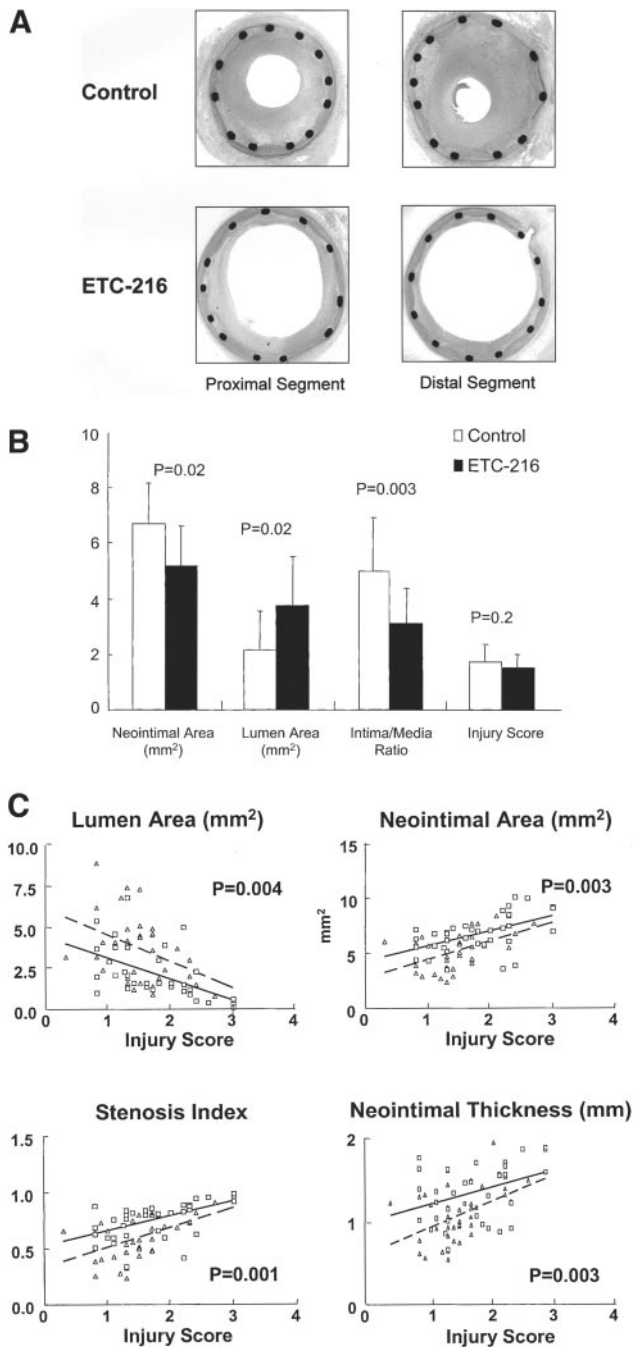
Results

QCA Data

The results of QCA analysis are shown in the Table. Mean luminal diameter was significantly larger, and the corresponding lumen loss index was significantly smaller in ETC-216–treated group. There was a nonsignificant trend in favor of ETC-216–treated group with respect to minimum luminal diameter and the corresponding lumen loss index.

Histomorphometric Data

Representative photomicrographs of coronary artery sections are shown in the Figure, A. The data are quantified in parts B and C and the Table. Treatment with ETC-216 significantly reduced the neointima area (–22%; *P*=0.02) and intimal-to-medial area ratio (–30%; *P*=0.003), and increased lumen area (+76%; *P*=0.02) compared with control (Figure, B). There were no significant differences in injury score (Figure, B), or the areas within the EEL, IEL, or medial area (Table). There was a nonsignificant reduction in adventitial area with ETC-216. Plots of lumen area, intimal area, stenosis index, and intimal thickness, each as a function of injury score are shown in the Figure, C. There were statistically significant differences in the intercept of the regression lines in the treatment group relative to the control group, indicating a true treatment effect with ETC-216.



A, Representative photomicrographs of coronary artery sections from control (top) and ETC-216-treated swine (bottom). Paragon stain, magnification $\times 20$. B, Bar graphs demonstrating the effect of ETC-216 on histomorphometric parameters. Values are mean \pm SD; $n=12$ observations in each group (average of 3 segments per artery). C, Individual values of lumen area, neointimal area, stenosis index, and neointimal thickness as a function of injury score; $n=36$ observations in each group. Injury score for each of the 12 struts was averaged to obtain the mean injury score per segment. Probability values are for differences between intercepts of the regression lines of control (solid lines) and ETC-216-treated swine (broken lines). Slopes of the regression lines did not differ in the 2 groups. Open squares indicate control; open triangles, ETC-216.

Discussion

The major finding of this study is that a single local intramural administration of ETC-216 produced a significant reduction in injury-induced luminal narrowing in the porcine coronary stent overstretch injury model through inhibition of neointimal hyperplasia. The efficacy of intramural treatment with ETC-216 in this porcine model offers encouragement that this agent may be effective in prevention of clinical restenosis.

In-stent restenosis is primarily caused by vascular smooth muscle cell (VSMC) migration and proliferation in response to mechanical injury imparted to the vessel by stenting.⁷ Adventitial constrictive remodeling, which is important in postangioplasty restenosis, does not play a major role in in-stent restenosis.⁷ Thus, it is likely that the target for treatment with ETC-216 is intimal hyperplasia. The exact mechanism by which ETC-216 reduces intimal hyperplasia is not known. Potential mechanisms include antiinflammatory (via suppression of macrophage infiltration),²⁻⁵ antiproliferative,³ and antioxidant effects^{1,4-6} that have been well characterized for recombinant apoA-I_m. Although we did not examine the effects of treatment with apolipoprotein or phospholipid alone, we and others have shown previously that the recombinant apoA-I_m phospholipid complex is essential for optimal biological effects.²⁻⁴ Other notable limitations include lack of tissue levels, drug delivery efficacy measurements, and dose response relationships.

The observation that intramural administration of a very low dose ETC-216 immediately before vascular injury inhibited neointimal hyperplasia is consistent with the notion that most of the critical steps in activation of VSMC proliferation and restenosis occur in the early hours after injury. However, treatment with ETC-216 did not entirely prevent neointima formation. This incomplete effect may have reflected either clearance of ETC-216 before the critical period for triggering restenosis had elapsed entirely or insufficient dose. The small delivery volume of the infiltrator catheter limited the quantity of ETC-216 that could be administered. Either higher dosages of drug (eg, through modifications in formulation that could increase the concentration of ETC-216 in the injectant) or exposure of injured tissue to drug for longer periods of time (as with drug-eluting stent) may further enhance the results of treatment with ETC-216 to prevent restenosis.

Acknowledgments

This study was funded in part by Esperion Therapeutics, Inc, Ann Arbor, Mich, the Eisner Foundation, the Heart Fund at Cedars Sinai, and the Entertainment Industry Foundation. We gratefully acknowledge Peter Barath, MD, and InterVentional Technology Inc, San Diego, Calif, for supplying the Infiltrator catheter.

References

- Franceschini G, Sirtori CR, Capurso A, et al. A-I Milano apoprotein: decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. *J Clin Invest.* 1980;66:892-900.

2. Ameli S, Hultgardh-Nilsson A, Cercek B, et al. Recombinant apolipoprotein A-I_{Milano} reduces intimal thickening after balloon injury in hypercholesterolemic rabbits. *Circulation*. 1994;90:1935–1941.
3. Soma MR, Donetti E, Parolini C, et al. Recombinant apolipoprotein A-I_{Milano} dimer inhibits carotid intimal thickening induced by perivascular manipulation in rabbits. *Circ Res*. 1995;76:405–411.
4. Shah PK, Nilsson J, Kaul S, et al. Effects of recombinant apolipoprotein A-I_{Milano} on aortic atherosclerosis in apolipoprotein E-deficient mice. *Circulation*. 1998;97:780–785.
5. Shah PK, MD. Yano J, Reyes O, et al. High-dose recombinant apolipoprotein A-I_{Milano} mobilizes tissue cholesterol and rapidly reduces plaque lipid and macrophage content in apolipoprotein E-deficient mice: potential implications for acute plaque stabilization. *Circulation*. 2001;103:3047–3050.
6. Bielicki JK, Oda MN. Apolipoprotein A-I_{Milano} and apolipoprotein A-I_{Paris} exhibit an antioxidant activity distinct from that of wild-type apolipoprotein A-I. *Biochemistry*. 2002;41:2089–2096.
7. Liu MW, Roubin GS, King SB III. Restenosis after coronary angioplasty: potential biologic determinants and role of intimal hyperplasia. *Circulation*. 1989;79:1374–1387.
8. Barath P, Popov A, Dillehay GL, et al. Infiltrator angioplasty balloon catheter: a device for combined angioplasty and intramural site-specific treatment. *Cathet Cardiovasc Diagn*. 1997;41:333–341.
9. Rosenthal EA, Bohlmeyer TJ, Monnet E, et al. An iron-binding exochelin prevents restenosis due to coronary artery balloon injury in a porcine model. *Circulation*. 2001;104:2222–2227.
10. Varenne O, Pislaru S, Gillijns H, et al. Local adenovirus-mediated transfer of human endothelial nitric oxide synthase reduces luminal narrowing after coronary angioplasty in pigs. *Circulation*. 1998;98:919–926.
11. Schwartz RS, Huber KC, Murphy JG, et al. Restenosis and the proportional neointimal response to coronary artery injury: results in a porcine model. *J Am Coll Cardiol*. 1992;19:267–274.