Intrapericardial Ethanol Delivery Inhibits Neointimal Proliferation after Porcine Coronary Overstretch

**Background.** Previous work has shown that ethanol dampens cell growth signals and inhibits smooth muscle proliferation in a restenosis model. Catheter-based approaches to intrapericardial (IPC) delivery of therapeutic agents have been recently demonstrated to be feasible. This study tested the effect of IPC instillation of ethanol on the injury response of overstretched porcine coronary arteries.

**Methods.** Ethanol, 30%, (E, 10 mL, n = 6) or saline, 0.9%, (C, 10 mL, n = 6) was administered IPC after overstretch injury of porcine coronary arteries. Animals were sacrificed 28 days after balloon dilation.

**Results.** The neointimal and adventitial area were significantly reduced in the E group (0.36 ± 0.05 mm²; 1.68 ± 0.09 mm²) as compared to the C group (0.61 ± 0.05 mm²; 2.61 ± 0.09 mm²; p < 0.001). The maximal intimal and adventitial thicknesses of the treated vessels were also significantly smaller than those of untreated vessels (0.44 ± 0.02, 0.38 ± 0.08 mm vs 0.57 ± 0.03, 0.54 ± 0.03 mm, respectively; p < 0.005). The calculated luminal stenosis decreased in the treated group, 16.1%, versus the control group, 25.3%.

**Conclusion.** Perivascular administration of a single-dose of ethanol significantly reduce neointimal proliferation in the porcine balloon-overstretch model. This data suggests that intrapericardial delivery of therapeutic agents may be useful and feasible in the coronary angioplasty setting for prevention of restenosis.

Although tremendous technical advances in coronary recanalization have been developed recently, the long-term patency is still seriously compromised by the development of restenosis. Catheter-based intrapericardial (IPC) space delivery of paclitaxel and nitric oxide donors has been reported to reduce neointimal proliferation in a porcine coronary overstretch model.

Previous studies have shown that ethanol can inhibit SMC proliferation. The present study was designed to examine the effects of catheter-based IPC space instillation of ethanol on neointimal proliferation induced by overstretching pig coronary arteries via balloon catheter.

**METHODS**

Animal care and handling followed the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* (Department of Health and Human Services, NIH publication No. 86-23, revised 1985). The Animal Care and Use Committee of the Indiana University School of Medicine approved the protocols. Twelve juvenile female domestic pigs weighing 23 to 25 kg were used for this study. The pigs were divided into 2 instillation groups; 30% ethanol (E, n = 6); and 0.9% saline as a control group (C, n = 6). All animals received a normal diet and were housed in similar runs.

Animals were fasted overnight and premedicated with aspirin (325 mg) 24 hours prior to sedation with intramuscular ketamine (20 mg/kg), xylazine (2 mg/kg), and atropine (0.05 mg/kg). Anesthesia was initiated with IV sodium pentothal (25 mg/kg). After intubation, the animals were ventilated using air mixed with oxygen (2 L/min) and isoflurane (2.5%). The ECG and blood pres-
sure were monitored.

Animals underwent coronary balloon dilation, as previously described. After systemic heparinization (200 U/kg) and lidocaine (30 mg), an 8F guiding catheter was used to engage the left coronary artery. After intracoronary nitroglycerin (200 μg), coronary angiography was performed. Diameters of the left anterior descending and left circumflex coronary arteries were measured using NIH Image (Bethesda, MA, USA), which allowed selection of target vessel sites that yielded balloon:artery ratios of 1.3:1 when a 20-mm length balloon dilatation catheter was used. Each artery was injured by 2 successive 30-second inflations at 10 atm with 60-second periods between inflations.

After ballooning, a pericardial access device (PerDUCER, Comedicus Inc., Columbia, MN, USA) was used for transthoracic insertion of a guidewire into the normal pericardial space as previously described (Fig. 1). The sheathed needle was inserted into the mediastinum through an introducer and positioned on the anterior surface of the pericardial sac, which was drawn into the hemispherical tip by suction, and pierced. Finally,

Fig. 1. Sequential fluorographic images obtained during the transthoracic access and delivery procedure. (A) A percutaneous tunnel is made below the xyphoid process using a 21-gauge needle. (B) The 0.038-inch guidewire and special introducer sheath is placed into the mediastinum over the anterior pericardium. (C) The perDUCER is inserted via the sheath. (D) A 0.018-inch guidewire is placed through the perDUCER. (E) A 0.018-inch guidewire in the pericardial space. (F) A 4F dilator catheter is placed and 3 cc of contrast mixed with saline is injected into the sac.
a 0.018” guidewire was placed through the needle and advanced several cm to confirm confinement within the pericardial space. A 4F hydrophilic-coated catheter was inserted, intrapericardial placement tested by contrast injection, and 10 mL of either 30% ethanol or 0.9% saline solution at room temperature was slowly delivered over ten minutes into the pericardial sac. The catheter was removed, the small puncture hole was sutured, and the animals were allowed to recover.

At 28 days after the procedure, animals were anesthetized, and final coronary angiography performed after heparin (200 U/kg) administration. The animals were euthanized by a lethal dose of pentobarbital (65 mg/kg), heart and pericardial tissues harvested, and coronary arteries perfusion-fixed with 10% buffered zinc formalin for 15 to 20 minutes at 80 mm Hg pressure.

To examine the entire LAD and LCx vessel lengths, the vessels were sectioned at 3- to 4-mm intervals from proximal to distal end, and embedded in paraffin with standard histological techniques. Paraffin sections were then cut at 6 μm and tissue sections were affixed to glass microscope slides. All histologic sections were stained with hematoxylin-eosin and Verheoff-Van Gieson’s elastic stain methods. As described previously,2 morphometric measurements were performed using a light microscope (Olympus) at low power (2.5 microscopic magnification) linked to a color video camera (Sony) and a computer-interfaced image analysis system with NIH Image software. This software allowed for the manual selection and delineation of artery areas. The endoluminal length (ELL), the circumference bounded by the internal and external elastic laminae (IEL and EEL) were traced manually, and luminal and intimal areas determined. Fracture length (FL) was defined as the arc length between the two fracture points of the IEL. Intimal area (IA) was measured directly. Maximal intimal thickness (MIT) was defined as the maximal distance between the lumen and EEL, while maximal adventitial thickness (MAT) was the analogous length between EEL and adventitia, normal to the arterial circumference. The percent stenosis (%) was described as the histologic lumen diameter at the site of maximal stenosis divided by the pre-angioplasty luminal diameter determined at the midpoint of the target segment.

Immunostaining was performed on selected segments using primary antibodies including anti-smooth muscle α-actin (1:1000, Dako) and von Willebrand factor (vWF, 1:600, Dako). Secondary antibody binding was revealed by avidin complex, with a staining reaction performed using 3,3’-diaminobenzidine (DAB) solution (Sigma, St. Louis, MO, USA). Nuclei were counterstained with hematoxylin or methyl green. Endogenous peroxidase activity was blocked with 3% H2O2 solution for 20 minutes. Negative controls were generated using nonimmune serum. To permit comparative qualitative analysis of the staining intensity of the study groups, staining of multiple segments from distinct study groups was conducted at the same time, using consistent development protocols for each antigen.

Results are presented as mean ± SEM. A nonparametric Mann-Whitney rank sum test was used to compare the morphometric measurement data. Differences are considered significant at p < 0.05. All statistical calculations used the SigmaStat™ (SPSS Inc., Chicago, IL, USA) software package.

RESULTS

Pericardial instillation was well tolerated by all animals. No complications developed, and no clinical evidence of ethanol-related toxicity was noted.

The artery diameters before balloon dilation (C, 2.56 ± 0.16 mm; E, 2.48 ± 0.18 mm; p = 0.19) and the balloon/artery ratios (C, 1.28 ± 0.05; E, 1.34 ± 0.06; p = 0.13) were not different between the control and ethanol groups.

A thin pericardial adhesion limited at the puncture site was found in 2 pigs in the saline group. In the treatment group, the pericardial adhesion was extended from the visceral to the parietal layer in all pigs.

Examination of hematoxylin-eosin and Verheoff-Van Gieson’s elastic stained slices, showed a variable degree of artery damage. The IEL rupture in most vessels was 20-40% of the circumferential coverage. There was no difference between the 2 groups for artery injury and the degree of IEL rupture. Microscopically, the neointimal growth was proportional with the size of IEL rupture. Fig. 2 (A and B) is micrographs characterizing the treated and untreated vessel segments. The neointimal area was
significantly decreased after ethanol treatment.

The Table 1 displays morphometric data for vessels examined from the 2 groups. In this model, the injury index (FL/FL+IEL) was similar between the 2 groups ($p = 0.21$). The neointimal and adventitial areas were significantly decreased, 41% and 36%, respectively, in the treated group as compared to the untreated group ($p = 0.001$) at 28 days after balloon injury. The ratio of intimal area to medial area was dramatically decreased from 0.47 to 0.36 for control to 0.36 to 0.07 for treated vessels ($p = 0.04$). The neointimal area normalized to FL was also significantly smaller in the treated group than in the untreated group ($p < 0.01$). Together, the degree of stenosis was reduced from 25.3% (control group) to 16.1% (treated group).

Immunohistochemical staining demonstrated that neointimal cells were predominantly immunoreactive for $\alpha$-actin in both groups. The neointima was composed of spindle-shaped cells and a large amount of loose extracellular matrix. At 28 days after balloon injury, complete vessel re-endothelialization had been achieved in most vessel segments between groups, as detected by vWF staining.

**DISCUSSION**

This study demonstrates the efficacy of a local intrapericardial delivery of ethanol in reducing restenosis up to 28 days after the balloon overstretch injury of the porcine coronary artery. Scott et al. have suggested that adventitial myofibroblasts play an important role in the process of vascular lesion repair by proliferation, synthe-

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**Table 1. Morphometry analysis of effect of intrapericardial ethanol on vascular healing 28 days after coronary balloon overstretch in pigs**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treated</th>
<th>$p$</th>
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<tbody>
<tr>
<td>FL/FL+IEL</td>
<td>0.20</td>
<td>0.18</td>
<td>0.03*</td>
</tr>
<tr>
<td>Intimal area (IA, mm$^2$)</td>
<td>0.61</td>
<td>0.36</td>
<td>0.05*</td>
</tr>
<tr>
<td>IA/FL</td>
<td>0.48</td>
<td>0.34</td>
<td>0.03*</td>
</tr>
<tr>
<td>Maximal intimal thickness (mm)</td>
<td>0.57</td>
<td>0.44</td>
<td>0.02*</td>
</tr>
<tr>
<td>Adventitial area (AA, mm$^2$)</td>
<td>2.61</td>
<td>1.68</td>
<td>0.09*</td>
</tr>
<tr>
<td>Maximal adventitial thickness (mm)</td>
<td>0.54</td>
<td>0.38</td>
<td>0.02*</td>
</tr>
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FL = fracture length; IEL = internal elastic laminae.

* $p$ relative to control; NS indicates not significant at the $p = 0.05$ level.
sizing of growth factors and smooth muscle actin, and migration into the neointima after angioplasty. Therefore, local perivascular delivery of therapeutic agents might be an effective method for prevention of restenosis due to their direct interference with adventitial cell proliferation and migration.

Alcohol has been utilized for prevention of post-angioplasty restenosis in several studies. The efficacy of low concentrations of alcohol consumption on the hypercholesterolemic rabbit after artery injury was demonstrated that moderate alcohol feeding reduces neointimal formation, the extent of lipid oxidation, and the number of foam cells in the neointimal area. A long-term effect of alcohol on the rabbit iliac and pig coronary arteries after balloon injury models has demonstrated that local delivery of a single-dose of 10% to 15% alcohol significantly decreased the smooth muscle cell hyperplasia and neointimal proliferation. These findings were consistent with the results of our studies.

Reducing thrombus formation by directly inhibiting platelet receptor (GP IIb/IIIa, P-selectin) expression and arresting cell hyperplasia may contribute to ethanol anti-restenosis effect. More recently, monocyte chemoattractant protein-1 (MCP-1)-mediated monocyte infiltration has been reported to play a critical role in vessel renarrowing after balloon injury in hypercholesterolemic rabbits. Anti-inflammatory properties via reduction in MCP-1 expression and macrophage accumulation can attenuate intimal hyperplasia. Long-term consumption of a moderate amount of wine has been shown suppress the expression of MCP-1.

IPC delivery of single-doses of ethanol does not cause damage to either the endothelial or medial layers. Endothelial regeneration was nearly complete at both groups. Likewise, medial cell densities and areas were no different among the groups.

This study identifies that administration of 30% ethanol into pericardial space may be above the tolerable threshold for pericardial tissue because the majority of pericardial adhesion was found in the treated pigs. An implicit limitation of these data resides in the absence of knowledge concerning less pericardial reaction using different ethanol concentration after delivery. Accordingly, it will be necessary to conduct studies which illustrate the most efficient dose and most diminished adhesion and/or toxic effects to support the safety of these approaches. Secondly, performing an in vivo pharmacokinetic study to address the local arterial wall drug level should be considered in the future.

In conclusion, our data demonstrated that catheter-based approaches to intrapericardial delivery of therapeutic agents are feasible. A single-dose intrapericardial administration of ethanol can reduces neointimal proliferation in the porcine balloon-overstretch model.

ACKNOWLEDGEMENTS

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REFERENCES

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