Intravascular Sonotherapy Decreases Neointimal Hyperplasia After Stent Implantation in Swine

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Background—Intimal hyperplasia and subsequent in-stent restenosis remain a major limitation after stent implantation. In vitro cell culture studies show that low-frequency, noncavitational ultrasound energy may impact smooth muscle cell proliferation. Accordingly, we assessed the efficacy of intravascular sonotherapy treatment on intimal hyperplasia in a swine stent model.

Methods and Results—After balloon injury, biliary stents (Johnson & Johnson) were implanted in the femoral arteries of 14 swine. A total of 48 stented sites were randomized to sonotherapy or sham treatment using a custom-built, 8-French catheter intravascular sonotherapy system (URX, PharmaSonics Inc). After stent deployment, ultrasound energy (700 KHz) was applied to the treatment group for up to 5 minutes. Smooth muscle cell proliferation was assessed using bromodeoxyuridine histology preparation (BrdU) at 7 days in 28 stented sites. At 28 days, the neointimal thickness and the ratio of neointimal/stent area (percent stenosis) was calculated by histomorphometric quantification in 20 stented sites. At 7 days, percent of BrdU staining was significantly reduced in the sonotherapy group compared with the sham group (24.1 ± 7.0% versus 31.2 ± 3.0%, P < 0.05). At 28 days, percent stenosis was significantly less in the sonotherapy group than in the sham group (36 ± 24% versus 44 ± 27%, P < 0.05), and the mean neointimal thickness in the sonotherapy group was less than in the sham group (417 ± 461 μm versus 643 ± 869 μm, P = 0.06).

Conclusions—In this swine peripheral model, intravascular sonotherapy seemed to decelerate cellular proliferation and decrease in-stent hyperplasia. Therefore, intravascular sonotherapy may be an effective form of nonionizing energy to reduce in-stent restenosis. (Circulation. 2001;103:1828-1831.)

Key Words: ultrasonics ■ stents ■ restenosis ■ hyperplasia

In-stent restenosis remains a significant limitation after both coronary and peripheral stent implantation.1,2 Clinical and histological studies have characterized the mechanism of in-stent restenosis as intimal hyperplasia initiated by the proliferation of smooth muscle cells (SMCs) in response to acute injury.3,4

Studies dating back to the mid-1970s have demonstrated that ultrasound can reduce mammalian cell viability.5 In the past decade, investigators have reported that high-energy cavitation/ultrasound is capable of inhibiting both in vitro SMC migration and adhesion.5,6 More recently, a study by Lawrie and colleagues8 showed that noncavitational ultrasound energy can directly reduce SMC proliferation in culture, suggesting the potential of ultrasound therapy in vivo to limit neointimal growth after stenting.

We report a newly developed, catheter-based intravascular sonotherapy (IST) system designed to deliver therapeutic ultrasound energy to a stented segment. This study represents the first in vivo testing of this system for reducing neointimal proliferation using a swine stent injury model.

Methods

Animal Preparation and Stent Implantation

Fourteen domestic swine were fed a normal diet. Aspirin (325 mg) was given 1 day before stent implantation, and it was continued through the follow-up period. Each animal was anesthetized with Telazol 8 mg/kg IM and maintained with isoflurane. Under fluoroscopic guidance, a 10-F sheath was inserted from the carotid artery into the iliac artery.

After the administration of heparin (200 U/kg) and nitroglycerin (200 μg), angiography was performed to identify the femoral arteries for stent implantation. Two target segments per femoral artery were identified as potential experimental stent sites. Of the possible 56 sites, 48 were chosen as suitable for stent therapy. Four segments contained multiple branches, 2 segments had severe spasm, and 2 dissected after wire placement. Intravascular ultrasound was performed on these experimental segments using a 3.2-F imaging system with a 30-MHz transducer (Ultra, BSC/CVIS). On the basis...
of the ultrasound measurements of vessel diameter, an oversized balloon (balloon/vessel ratio, 1.3 to 1.5) was selected. After balloon injury at 15 atm, a 20-mm biliary stent (Johnson & Johnson) was implanted. A total of 28 arterial sites were evaluated for the degree of cellular proliferation by histological assessment at 7 days. Another 20 arterial sites underwent histomorphometric analysis at 28 days. This study protocol was approved by the Institutional Laboratory Animal Committee at Stanford University Medical Center.

**IST**

An 8-F, over-the-wire catheter system (URX, PharmaSonics Inc) was developed. It incorporated a cylindrical ultrasonic transducer that was 8 mm in length and that operated in pulsed mode at a center frequency of 700 KHz (Figure 1A). This provides a mechanical index (ratio of peak rare fractional pressure to the square root of frequency: an indicator of the likelihood in producing cavitation) of 3.9

After additional nitroglycerin (200 μg), target segments were exposed to IST for 120 s for the cell proliferation study (n = 28), and for 120 s for the intimal growth study (n = 20). For the sham-treated group, the same catheter was placed within the target segments for the same dwell times, without transducer activation.

**Pathological Studies**

**Assessment of SMC Proliferation**

To assess the proportion of proliferating SMCs in the treated segments, 6 pigs were killed on day 7 after the intravenous administration of 30 mg/kg 5-bromo-2-deoxyuridine (BrdU; Sigma Chemical) 1 day before. A total of 28 treated vessels, 17 IST and 11 sham, were rapidly excised and fixed with formalin at a pressure of 100 cm H₂O for 15 minutes. Vessels were embedded and sectioned with a diamond saw and stained with hematoxylin and eosin. Using an antibody technique, cells incorporating BrdU were identified histologically. The percent of BrdU-labeled cells in the intima was determined by averaging the score of 3 cross-sections within the treated segment by a pathologist (R.V.) who was blinded to treatment category.

**Assessment of Intimal Growth**

At 28 days, 20 vessels, including 10 IST and 10 sham-treated segments, were excised and prepared in the manner described above. Three cross-sections per stent at the mid, distal, and proximal portions of the artery were analyzed using histomorphometric techniques by the pathologist, who was blinded to the treatment group. Histological images were digitized via a frame grabber with 8 bit resolution. Lumen area and stent area were measured. The neointimal/stent area (percent stenosis) was calculated as [(stent area–lumen area)/stent area]×100. The mean intimal thickness for each slice was determined by averaging 8 (45-degree interval) radial lines originating from the lumen center that were drawn to intersect the intimal and each stent border.

**Statistical Analysis**

Data are given as mean±SD. Student’s t test was used to compare parameters between the groups, and P<0.05 was considered statistically significant.

**Results**

**Baseline Characteristics**

There were no significant differences between the IST group and the sham-treated group with respect to procedural characteristics (Table). In both IST and sham groups, ultrasound delivery was performed without any significant vascular complications. One vessel in the IST group and one in the sham group had transient spasm associated with catheter passage, which was relieved by nitroglycerine.

**BrdU Analysis (7 Days)**

The BrdU preparation demonstrated significantly fewer proliferating cells in the intimal layer for the IST group than in the sham-treated group (24.1±7.0% versus 31.2±3.0%, P=0.02), as summarized in Figure 1B.

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**Baseline Characteristics for Swine Femoral Artery Vessel Wall Injury in Cell Proliferation Study at 7 days and in Intimal Hyperplasia Study at 28 days**

<table>
<thead>
<tr>
<th>Group</th>
<th>Vessel Diameter, mm</th>
<th>Balloon/Artery Ratio</th>
<th>Inflation Pressure, atm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell proliferation study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHT (n=11)</td>
<td>4.2±0.7</td>
<td>1.4±0.1</td>
<td>10.9±2.3</td>
</tr>
<tr>
<td>IST (n=17)</td>
<td>4.2±0.5</td>
<td>1.4±0.1</td>
<td>10.8±1.5</td>
</tr>
<tr>
<td><strong>Intimal hyperplasia study</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHT (n=10)</td>
<td>4.2±0.2</td>
<td>1.5±0.11</td>
<td>8.7±1.5</td>
</tr>
<tr>
<td>IST (n=10)</td>
<td>4.2±0.3</td>
<td>1.5±0.09</td>
<td>8.7±1.5</td>
</tr>
</tbody>
</table>

Values are mean±SD. SHT indicates sham-treated group.
Histomorphometric Analysis (28 Days)

All stent sites were examined morphometrically at 28 days follow-up. Cross-sections for both sham and IST groups are shown in Figure 2A. IST-treated arteries exhibited modest neointimal growth, whereas the sham-treated sites had a relative increase in overall neointimal growth within the target segment that impacted vessel patency (Figure 2B). The luminal area was significantly larger in the IST group compared with the sham group (11.5 ± 5.9 versus 9.4 ± 5.8 mm², \( P < 0.05 \)), resulting in significantly smaller percent stenosis in the IST group compared with the sham-treated group (36 ± 24% versus 44 ± 27%, \( P < 0.05 \)). Mean intimal thickness trended to be smaller in the IST group compared with the sham group (417 ± 461 versus 643 ± 869 μm, \( P = 0.06 \)). No incomplete stent apposition, excess intimal thickening at the stent edges, or intraluminal thrombus was observed in either group.

Discussion

In the present study, stents were deployed in an oversized manner to promote neointimal hyperplasia, resulting in significant vessel injury. In the IST arm, percent stenosis was reduced absolutely by 8% (relative reduction, 19%), and mean intimal thickness was reduced by 35%. These results are similar to the magnitude of changes observed with radiation studies in porcine models. Carter et al. demonstrated a 29% relative reduction in percent stenosis using high-dose radiation, and Waksman et al. demonstrated a 36% decrease in intimal thickness after brachytherapy compared with control.

The process of restenosis is initiated by tissue injury followed by thrombus formation and growth factor release. The second stage of this cascade is proliferation of SMCs or myofibroblasts in the media and/or adventitia, followed by their accumulation in the intima. Final maturation of neointima is accomplished by extracellular matrix deposition.\(^{12}\)

In the present study, the ultrasonic intensity applied to the vessel wall was below the intensity levels associated with mechanical cavitation. On the basis of the results of BrdU analysis, at these energy levels and contrary to prior cavitation levels used in vitro, there seemed to be a primary effect on cellular proliferation. These in vivo findings are consistent with the recent in vitro observations by Lawrie et al.\(^{8}\) in which inhibition of SMC mitosis was seen at this lower intensity ultrasound.

Intravascular ultrasound as an energy source may have several procedural advantages compared with ionizing sources. (1) The delivery of ultrasound does not require shielding in the catheterization laboratory. (2) Catheter-based therapeutic ultrasound used for clot dissolution and plaque ablation has been shown not to damage the normal vessel wall in both animal models and humans.\(^{13,15,16}\) (3) IST seems to cause transient vessel relaxation and may help limit ischemia during the dwell time of the catheter.\(^{17,18}\) (4) Ultrasound has been shown to enhance drug delivery as well as gene transfection,\(^{19,20}\) suggesting it has potential for further augmentation in conjunction with pharmacological approaches.

Limitations

The present study is limited by the relatively small number of stents in each treatment arm and the short-term (28 days) follow-up. It is possible that sonotherapy simply delays the restenotic process or even promotes late tissue growth. Neither of these processes would be detected in this experi-
mental design. The lack of dose-response analysis is another significant limitation of this study. There is no short-term evidence of side effects at the ultrasound doses used, leaving the possibility that higher doses may be more efficacious in reducing neointimal hyperplasia.

**Conclusions**
These preliminary findings indicate that IST favorably impacts neointimal hyperplasia in a swine stent-injury model. The exact mechanism of the effect of IST on the vessel wall, the optimal ultrasound power, and the duration of administration are not established at this point. Further research and clinical studies of IST are needed to elucidate the mode of action and to test the response in human vessels.

**References**
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