Brief Communication

Angiopeptin Inhibits Intimal Hyperplasia After Angioplasty in Porcine Coronary Arteries

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Background. Restenosis is mediated by uncontrolled neointimal growth at the site of coronary angioplasty. Angiopeptin is an octapeptide analogue of somatostatin that has been shown to decrease the experimental intimal hyperplasia associated with vascular injury in rats and rabbits. The study purpose was to determine if angiopeptin inhibits the development of intimal hyperplasia in normolipemic swine coronary arteries after overstretch-balloon injury.

Methods and Results. Overstretch-balloon injury was performed in normolipemic swine coronary arteries using a 3.5-mm angioplasty balloon. Treated animals received angiopeptin (50 μg/kg) 1 hour before and at the time of balloon injury. Angiopeptin was administered at 100 (μg/kg)/day SQ in two divided doses for 14 days. Animals were killed at 14 and 28 days (2 weeks after cessation of angiopeptin) after balloon injury. Treatment animals were compared with control animals receiving balloon injury alone. Angiopeptin significantly limited the experimental intimal hyperplasia estimated by the maximal intimal thickness and residual lumen (lumen area/lumen area + intimal area) compared with controls.

Conclusions. Angiopeptin inhibits the development of intimal hyperplasia in swine coronary arteries after balloon injury. The beneficial effect was detectable 2 weeks after cessation of angiopeptin therapy. (Circulation 1993;88:11-14)

Key Words • Brief Communications • angiopeptin • angioplasty

Restenosis after successful percutaneous transluminal coronary angioplasty is mediated in part by an uncontrolled proliferation of smooth muscle cells that migrate into the intima, proliferate, and encroach into the lumen.1 The development of the neointimal lesion is the end point of the natural healing process that has been initiated by vascular injury of the nonatheromatous segment of the dilated artery.1 Attempts to attenuate the development of intimal hyperplasia largely have been unsuccessful.

Angiopeptin, a synthetic cyclic octapeptide, is a somatostatin analogue that inhibits growth hormone release.2,3 Angiopeptin possesses significant antiproliferative effects in tissue cultures of neoplastic cells1 and inhibits myointimal hyperplasia after endothelial denudation injury in rats,4 rabbits,5,6 and nonhuman primates.7

We tested angiopeptin in the swine model of restenosis because of similarities of this model to human arteries with respect to laminar flow characteristics,8 atherogenesis,9 platelet-coagulation system,10 and response to vascular injury.11 We developed and reported a swine model of restenosis in which overstretch-balloon injury induces reproducible and measurable intimal hyperplasia 14 days after balloon injury.11,12 The acute injury to the media, the healing response, and the progression of intimal proliferation closely resemble the histological development and appearance of human neointima.11,12

The purpose of this study was to determine if daily subcutaneous administration of angiopeptin [100 (μg/kg)/day] for 14 days in two divided doses inhibits the development of intimal hyperplasia at 14 and 28 days after overstretch-balloon injury in normolipemic swine coronary arteries. The time interval and dose of angiopeptin treatment were tailored to be identical to the angiopeptin regimen in use in the human multicenter restenosis trial.7,13

Methods

All experiments and animal care conformed to National Institutes of Health and American Heart Association guidelines for the care and use of animals and were approved by the Emory University Animal Care and Use Committee.

Experimental Procedure

The experimental protocol including processing of the injured vessels, and morphometric analysis has been described previously.11 Twenty-four anesthetized female normolipemic swine (weight, 20 to 32 kg) were pretreated with oral aspirin (325 mg/day) 1 day before the beginning of the study and heparin (200 units/kg...
Table 1. Angiographic Analysis, Ratio of Balloon to Artery, and Injury-Grading Score

<table>
<thead>
<tr>
<th>Day 14</th>
<th>Total No. of vessels</th>
<th>LAD (No.)</th>
<th>LCx (No.)</th>
<th>Ratio of balloon to artery</th>
<th>Artery diameter before injury (mm)</th>
<th>Injury-grading system score (1 to 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td>1.31±0.06</td>
<td>2.69±0.13</td>
<td>2.6±0.2</td>
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<tr>
<td>Angiopeptin</td>
<td>10</td>
<td>6</td>
<td>4</td>
<td>1.27±0.07</td>
<td>2.76±0.14</td>
<td>2.4±0.3</td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>7</td>
<td>9</td>
<td>1.27±0.06</td>
<td>2.78±0.13</td>
<td>2.7±0.3</td>
</tr>
<tr>
<td>Angiopeptin</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>1.27±0.14</td>
<td>2.80±0.34</td>
<td>2.5±0.2</td>
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</table>

|                  | NS                  | NS        | NS        | NS                          | NS                                | NS                                  |

LAD, left anterior descending coronary artery; LCx, left circumflex artery.

IV) at the time of balloon injury. Overstretch-balloon injury was performed with a 3.5-mm angioplasty balloon inflated to 10 ATM three times for 30 seconds in the left anterior descending and left circumflex arteries.

**Study Groups**

Treatment animals received two doses of angiopeptin (50 µg/kg) administered subcutaneously 1 hour before and repeated at the time of balloon injury. Systemic angiopeptin [100 (µg/kg/day)] was administered in two divided doses subcutaneously for 14 days after balloon injury (n=10). Six of the angiopeptin-treated animals were killed 14 days after balloon injury, and the remaining (n=4) were killed at day 28 or 2 weeks after the completion of angiopeptin therapy. The control groups were composed of six animals that were killed at 14 days after balloon injury and eight that were killed at day 28. Vessels were harvested, perfusion-fixed in formalin for 15 minutes, and stored in buffered formalin for 2 to 4 weeks after obtaining follow-up angiograms on the day of death. The injured segments of the arteries were cut into serial 2-mm sections, embedded in paraffin, and stained with hematoxylin and eosin, Verhoeff–Van Gieson elastin, and Masson's trichrome.

**Data Analysis**

Before and after balloon injury the dilated sites were measured and compared with adjacent uninjured proximal and distal sites through the use of hand-held digital calipers.

The slides were interpreted by an experienced cardiovascular pathologist (M.B.G.) who was blinded to the treatment group. To ensure that control and treatment vessels were injured similarly, a previously published injury-grading system (from 1 to 4) was applied in evaluating the severity of the medial fracture, luminal encroachment, and adventitial reaction. Measurements of maximal intimal thickness (expressed in millimeters) and the residual lumen ratio ([lumen area/lumen area]+intima area) were measured on the most qualitatively reactive arterial segment (most stenotic section) using a computerized morphometry program.

**Statistical Analysis**

Data are presented as the mean±SD. All groups combined the left anterior descending and circumflex arteries from each animal. We previously demonstrated that there was no difference in the amount of neointimal development between the left anterior descending and left circumflex coronary arteries of the same animals. Data were analyzed using the unpaired Student's t test and ANOVA. Significance was established at the 95% confidence level.

**Results**

**Group Characteristics and Angiographic Analysis**

Table 1 depicts the baseline size of the vessels, ratio of balloon to artery diameter, and injury-grading score for all groups. No significant difference was noted among the control or treatment groups at 14 or 28 days.

**Histopathological Analysis**

Both the control (Fig 1A) and angiopeptin (Fig 1B to 1D) groups exhibited eccentric disruption of the media, creating an adventitial crater with an exposed external elastic lamina. In the angiopeptin group, the neointimal response at the fractured medial ends was reduced 14 days after balloon injury.

**Morphometric Analysis**

The maximal intimal thickness and the residual lumen are shown in Fig 2. The maximal intimal thickness was decreased significantly and the residual lumen was significantly greater at 14 and 28 days after balloon injury in the angiopeptin group.

**Discussion**

Angiopeptin significantly limits the development of intimal hyperplasia following overstretch-balloon injury in this swine model. These effects of angiopeptin appeared to be due to an inhibition of smooth muscle cell proliferation, migration from the media, or both and thus resulting in a reduced intimal mass. Although the beneficial effects of angiopeptin were detected 2 weeks after completion of angiopeptin treatment, the maximal intimal thickness of the neointima increased within 2 weeks after completion of therapy, suggesting the beginning of late neointimal growth.

The mechanism by which angiopeptin inhibits the development of neointima is unclear, although the ability of angiopeptin to attenuate smooth muscle cell proliferation in vitro, in the absence of systemic growth hormone, suggests that the beneficial effects of angiopeptin are unrelated to the somatostatin inhibitory effects on growth hormone. It is likely that angiopeptin directly inhibits mechanisms of smooth muscle cell replication at the subcellular level.
Angiopeptin in Porcine Coronary Arteries

FIG 1. Panel A: Control vessel 14 days after balloon injury. Note the neointima (N) between the fractured and separated medial (m) ends. ad, Adventitia. (Elastin, ×25.) Panel B: Angiopeptin-treated vessel 14 days after balloon injury. Note the reduction in the mass of the neointima (N). m, Media; IEL, internal elastic lamina. (Elastin, ×25.) Panel C: Angiopeptin-treated vessel 14 days after injury demonstrating neointima (N) limited to the right medial (m) end with a bare external elastic lamina (EEL). IEL, internal external elastic lamina. (Elastin, ×25.) Panel D: Angiopeptin-treated vessel 14 days after balloon injury. Note that only a few layers of smooth muscle cells are covering the external elastic lamina (EEL). (Elastin, ×25.)

been shown to activate a membrane-bound tyrosine phosphate that deactivates tyrosine kinase receptors common to known growth factors, such as epidermal growth factor, fibroblast growth factor, and platelet-derived growth factor.14

Lack of smooth muscle cell proliferation and/or migration with angiopeptin treatment (Fig 1) resulted in an unrepai red adventitial crater at the site of arterial injury in some animals. This nearly complete inhibition of the myointimal process raises the issue of the long-term vessel strength and vasoreactivity. Although it is desirable to contain the degree of the proliferative response, it may not be wise to completely block the natural healing response. Among the treated animals, there were no examples of loss of vascular integrity or adverse systemic reactions during or after the administration of angiopeptin.

Conclusions

Angiopeptin significantly reduced the development of experimental intimal hyperplasia after one overstretch-balloon injury. The histopathological examination revealed a significant clinical response to angiopeptin, and 2 weeks after completion of angiopeptin, the beneficial effects could still be demonstrated. In our model, as in humans, the interval during which proliferation must be inhibited after vascular injury is unknown and first must be determined to provide effective and lasting treatment. Longer treatment periods may be required to preclude potential late neointimal growth.

This is the first antiproliferative agent undergoing large-scale human trials that has been shown to be effective in all accepted animal models of restenosis. If angiopeptin proves efficacious, it may strengthen and validate our opinions as well those of others8 that testing
of potential inhibitors of restenosis should be proven effective in either swine or nonhuman primate models before initiating large-scale clinical trials.

Acknowledgment

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References

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