Trapidil in Preventing Restenosis After Balloon Angioplasty in the Atherosclerotic Rabbit

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Trapidil (triazolopyrimidine) possesses activity against platelet-derived growth factor–induced cellular proliferation in vitro and intimal proliferation in vivo. The objective of this study was to determine if trapidil could prevent restenosis in experimentally induced atherosclerotic rabbits. New Zealand White rabbits with preexisting iliac arterial lesions induced by balloon deendothelialization underwent balloon angioplasty. Arteriography was performed before, immediately after, and 4 weeks after the balloon dilatation. Tissue sections of the dilated arterial segment were also analyzed morphometrically. Seventeen rabbits were randomized to two groups: a control group (n=8) and a trapidil-treated group (n=9). The treatment group received 30 mg/kg s.c. trapidil twice daily. The angiographic luminal diameters before and after dilatation were similar. At the 4-week restudy, there was a significant preservation of luminal diameter in the trapidil group compared with the control group (1.27±0.20 vs. 0.94±0.48 mm, respectively; p<0.005). When luminal diameters immediately after dilatation were compared with diameters at the 4-week restudy (i.e., when the degree of restenosis was assessed), there was a greater luminal diameter reduction in the control group than in the trapidil group (0.70±0.44 vs. 0.30±0.25 mm, respectively; p=0.005). By morphometric analyses, the luminal areas were also greater in the trapidil group than the control group (0.80±0.25 vs. 0.57±0.33 mm², respectively; p=0.03). Intimal thickness was significantly less for the trapidil group than for the control group (0.33±0.15 vs. 0.44±0.15 mm, respectively; p=0.01), as well as medial thickness (0.09±0.03 vs. 0.11±0.03 mm, respectively; p=0.01). In this study, trapidil significantly increased the luminal area and reduced the intimal thickness in the atherosclerotic rabbit iliac artery after balloon angioplasty. (Circulation 1990;81:1089–1093)

Restenosis after successful percutaneous transluminal coronary angioplasty remains the major problem limiting the long-term efficacy of the procedure. Accumulated evidence has shown that restenosis occurring at 2–6 months after angioplasty is due to smooth muscle cell migration and proliferation as a response to balloon injury.

It is not clear how smooth muscle cell proliferation and migration are initiated by balloon injury. It has been postulated that smooth muscle cell proliferation is controlled by the balance between growth factors and growth inhibitors from the circulation and/or local tissue sites. Platelet-derived growth factor (PDGF) has been considered a major component, and recent studies in human atherosclerotic arteries have demonstrated its importance.4–6 If this hypothesis is correct, interference with growth factor–mediated cellular hyperplasia may be beneficial in inhibiting smooth muscle cell proliferation and, therefore, possibly reducing restenosis.

Trapidil (triazolopyrimidine) has been shown to reduce cellular proliferation induced by PDGF in cell culture and intimal thickening in carotid arteries treated with PDGF in the thrombocytopenic rat.7,8 Therefore, we conducted this study to determine whether this activity of trapidil might reduce the intimal proliferation and restenosis after balloon injury in the atherosclerotic rabbit.
Methods

Animal Preparation

New Zealand White rabbits (approximately 3 kg) of both sexes were used for this study. All experimentation and animal handling were conducted in such a manner as to minimize stress and discomfort to the animals and conformed to the guidelines of the American Physiological Society. The protocol was approved by the Institutional Animal Care and Use Committee of Emory University, Atlanta, Georgia. Five to 7 days after beginning a 2% cholesterol diet, bilateral focal endothelial denudation of the external iliac arteries was performed as follows: Anesthesia was achieved with intramuscular injection of ketamine hydrochloride and xylazine. Bilateral femoral arteriotomies were performed, and a 3F embolectomy catheter was advanced retrogradely under fluoroscopic guidance to an area within the external iliac artery, ascertained by anatomic landmarks. The balloon was inflated with 70% saline and 30% meglumine diatrizoate contrast medium and withdrawn antegrade approximately 10 mm and deflated. This procedure was repeated five times in the same segment in each artery to ensure complete endothelial removal. The catheter was then removed, the femoral arteries were ligated, and the incision was closed. The rabbits were then fed for 1 week with a 2% cholesterol diet alternating every other week with a normal diet. This continued for 7 weeks and resulted in the formation of focal bilateral stenoses in almost all the cases. If total occlusion of the artery resulted, the artery was not used for subsequent studies.

Instrumentation

Under ketamine and xylazine anesthesia and aseptic conditions, a right carotid arteriotomy was performed, and a 5F vascular sheath was introduced and anchored with ligatures. Heparin (500 units) was given intra-arterially, and a 4F Swan-Ganz catheter was advanced under fluoroscopic guidance to the abdominal aorta. With the balloon inflated, an aortoiliac angiogram was recorded on 35-mm cineradiographic film; a 3-ml injection of meglumine diatrizoate was used as contrast. A 3-mm grid was positioned beneath the animal to identify the stenotic segments and for subsequent arteriographic measurement. A 0.014-in. guide wire was then introduced through the catheter, the catheter was removed, and a balloon dilatation catheter 2.0 mm in diameter and 20 mm in length was advanced to the site of the stenoses. The balloon was inflated three times to 6, 7, and 8 atm or until a favorable angiographic result was achieved. The balloon catheter was pulled back to the origin of the external iliac arteries, and a satisfactory postdilatation angiogram was obtained by injecting contrast medium through the balloon catheter. The catheter was removed, and the carotid artery was repaired or ligated. The surgical incision was closed.

Restudy and Tissue Preparation

Four weeks after angioplasty, each rabbit was anesthetized with ketamine and xylazine, a left carotid arteriotomy was performed, and a 4F Swan-Ganz catheter was advanced to the abdominal aorta. A restudy arteriogram was obtained, and the animal was killed with an intravenous injection of 2 ml sodium pentobarbital (65 mg/ml). The treated arterial segment was perfusion-fixed for 15 minutes with 10% buffered formalin at 100 mm Hg of pressure through a cannula in the abdominal aorta with efflux through the inferior vena cava. The 10–12-mm treated arterial segments were excised and immersed in formalin for at least 48 hours. The segments were embedded in paraffin, and three sections were obtained from each segment. The sections were stained with hematoxylin and eosin.

Protocol and Administration of Medication

Seventeen rabbits were randomized to two groups: a control group (n=8) and a trapidil-treated group (n=9). The treatment group received 30 mg/kg s.c. trapidil twice daily. Trapidil in powder form was provided by Mochida Pharmaceutical, Tokyo, Japan, and was dissolved in sterile water as 50 mg/ml. The trapidil solution was sterilized by use of a 0.2-μm filter. Serum trapidil levels peaked at a mean of 8–9 μg/ml 1 hour after administration and fell to 3 μg/ml at 6 hours.

Trapidil was started 2 days before balloon angioplasty and continued until 1 day before death.

Arteriography and Morphometry

Predilatation, postdilatation, and restudy arteriograms of the dilated segments were measured on a Siemens projector by an experienced angiographer who was unaware of the treatment assignment. Measurements with digital electronic calipers were corrected to absolute luminal diameters by use of a 3-mm grid as an internal calibration standard. The previously dilated arteries were divided into 3-mm segments by using the underlying grid. Measurements of the most stenosed portion of each segment were made, and an overall mean minimal luminal diameter was calculated from all segments.

Morphometric analysis of arterial sections in the subcutaneous study was done by use of the Bioquant System IV Image Analysis System (R&M Biometrics, Nashville, Tennessee). Formalin-fixed iliac artery segments were cut in serial cross sections after paraffin embedding and processed routinely for light microscopy with hematoxylin and eosin staining. Luminal area was determined for each segment by tracing the luminal perimeter and internal and external elastic laminae of each section with a digitizing tablet. For intimal and medial thickness, the average of five equally spaced radial measurements for each section was used. The measurements were performed by a pathologist unaware of the treatment assignment.
**Statistical Analysis**

Comparison of predilatation, immediate postdilatation, and restudy luminal diameter of the control and trapidil-treated groups was performed by a two-factor repeated-measure ANOVA. When statistical significance was reached in the overall ANOVA test, the Bonferroni test was performed to determine the specific treatment effect for each time period. Because two lesions were analyzed from each animal (each iliac artery) and restenosis in these lesions may not be independent, additional ANOVAs were done with only one lesion from each animal in an effort to rigorously test the significance of the observation; the most severe lesion and the least severe lesion at baseline in each animal were analyzed separately. Analysis of angiographic luminal diameters between groups and between postdilatation and restudy was performed with an unpaired t test or a Mann-Whitney test, as appropriate. Data are presented as mean±1 SD; a value of \( p<0.05 \) was considered statistically significant.

**Results**

**Arteriography**

Luminal diameters before dilatation were similar (1.05±0.30 mm for the control group, 1.14±0.28 mm for the trapidil group). Diameters after dilatation were also similar (1.63±0.21 mm for the control group, 1.57±0.18 mm for the trapidil group). At the 4-week restudy, there was a greater luminal diameter in the trapidil group than in the control group (1.27±0.20 vs. 0.94±0.48 mm, respectively; \( p<0.005 \)) (Figure 1). When the most severe lesion from each rabbit was chosen for the same analysis, a significantly greater luminal diameter was found in the trapidil group. Similar results were obtained when the least severe lesion from each rabbit was analyzed (Table 1). When the changes in luminal diameter from postdilatation to restudy (i.e., the degree of restenosis) were compared, there was also a greater luminal reduction in the control group than in the trapidil group (0.70±0.44 vs. 0.30±0.25 mm, respectively; \( p<0.005 \)) (Figure 2).

**Histology**

**Morphometric analysis.** The luminal area measured from histologic sections was greater in the trapidil group than in the control group (0.80±0.25 vs. 0.57±0.33 mm\(^2\), respectively; \( p=0.03 \)). Intimal thickness was significantly less for the trapidil group than for the control group (0.33±0.15 vs. 0.44±0.15 mm, respectively; \( p=0.01 \)), as well as the medial thickness (0.09±0.03 vs. 0.11±0.03 mm, respectively; \( p=0.01 \)).

**Histopathologic findings.** Light microscopic examination demonstrated changes characteristic of experimentally induced atherosclerosis in the rabbit model. Marked intimal thickening was present, characterized by abundant foam cell infiltration, fibrocellular proliferation, and accumulation of extracellular material. The media and adventitia also were frequently infiltrated by foam cells. Focal intimal calcification was seen in 23% of segments. Disruption of elastic laminae was common. Luminal thrombosis was not observed. In addition to the morphometric measurements, a semiquantitative scoring system was devised (Table 2) to compare these histopathologic changes in the treated versus control groups. Scores for intimal and medial foam cells, elastic lamina fragmentation, and the percent of intimal calcification were not significantly different (Table 3).

**Discussion**

At present there is no ideal animal model for the study of restenosis after angioplasty. The restenotic lesion in this atherosclerotic rabbit model showed a substantial foam cell component, whereas human restenotic lesions consist mainly of smooth muscle cells and extracellular material. The variability of the response of atherosclerotic rabbit iliac arteries to balloon injury makes this model less than ideal. Despite this, trapidil was shown to significantly increase the luminal area angiographically and histo-
logically and decrease the intimal thickness, compared with control. One concern in interpretation of these results is that the increase in luminal area in the trapidil-treated group may be due to vasodilatation rather than to the decrease in intimal thickness. When the perimeter of the vessel wall was compared, there was no significant difference (6.10±0.65 mm in control and 5.84±1.02 mm in trapidil). In addition to the observed differences in intimal thickness, these findings support our conclusion that the increased lumen was due to a decrease in the proliferative response in the trapidil-treated group and not to vasodilatation of the vessel.

Although semiquantitative assessment of the foam cell component was not different between the two groups, it is difficult to be certain if the reduction of intimal thickness in the trapidil group was solely due to a decrease in smooth muscle cell proliferation and accumulation of extracellular material or other factors. It is possible, however, that there are quantitative differences in the amount of foam cells present that were not detectable with the relatively small sample size.

Although it is unknown how smooth muscle cell proliferation is initiated, substantial in vitro data, as well as indirect evidence from the study of human atherosclerotic arteries, support the concept that growth factors, particularly PDGF, play an important role in this process.\textsuperscript{5,6,9,10} The origin of PDGF is not limited to platelets. In vitro data have shown the possibility that PDGF may also be produced by smooth muscle cells and endothelial cells, as well as macrophages.\textsuperscript{11–13} Transforming growth factor beta, epidermal growth factor, and possibly other factors such as \(\gamma\)-interferon, interleukin-1, and angiotensin may also be involved in this process. If this is correct, complete removal of platelets from the circulation may not prevent smooth muscle cell proliferation or restenosis. This concept is supported by one animal study\textsuperscript{14} and the failure of antiplatelet agents to prevent restenosis in clinical trials.\textsuperscript{15,16} An alternative approach may be to inhibit the cellular proliferative response induced by growth factors. Unfortunately, detailed knowledge of the mechanism of cellular proliferation by their growth factors is lacking.

There are few clinically available compounds that have been shown to inhibit PDGF-induced cellular proliferation in vitro. Some agents that are thought to possess this effect include protamine sulfate, suramin, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, and trapidil.\textsuperscript{17–20} The mechanism of the inhibitory action of these agents is unclear. Heparin has been shown to be effective in reducing intimal proliferation,\textsuperscript{21} but the usefulness of heparin has been limited by its anticoagulant property and method of administration. A subfragment of heparin that retains antiproliferative activity, lacks the anticoagulant property, and can be given subcutaneously may be a promising choice and is currently undergoing investigation. Another group of commonly used cardiovascular drugs shown to have antiproliferative activity in animal models\textsuperscript{22} are the angiotensin converting enzyme inhibitors. These agents are also under investigation for their use in

### Table 1. Comparison of Luminal Diameters in Control and Trapidil-Treated Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Predilatation (diameter/mm)</th>
<th>Postdilatation (diameter/mm)</th>
<th>Restudy (diameter/mm)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Most severe lesion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.02±0.32</td>
<td>1.57±0.25</td>
<td>0.80±0.54</td>
<td>0.009</td>
</tr>
<tr>
<td>Trapidil</td>
<td>1.08±0.29</td>
<td>1.58±0.14</td>
<td>1.26±0.25</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>Least severe lesion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.13±0.28</td>
<td>1.74±0.12</td>
<td>1.15±0.28</td>
<td>0.013</td>
</tr>
<tr>
<td>Trapidil</td>
<td>1.21±0.27</td>
<td>1.57±0.23</td>
<td>1.29±0.14</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Values given are mean±1 SD.

### Table 2. Scoring System for Intimal and Medial Foam Cells and Elastic Lamina Fragmentation in the Rabbit Model

<table>
<thead>
<tr>
<th>Intimal foam cells</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0: None</td>
<td></td>
</tr>
<tr>
<td>1: Occasional</td>
<td></td>
</tr>
<tr>
<td>2: Groups of foam cells, less than (\frac{1}{2}) intimal area</td>
<td></td>
</tr>
<tr>
<td>3: Groups of foam cells, more than (\frac{1}{2}) intimal area</td>
<td></td>
</tr>
<tr>
<td><strong>Medial foam cells</strong></td>
<td></td>
</tr>
<tr>
<td>0: None</td>
<td></td>
</tr>
<tr>
<td>1: Occasional</td>
<td></td>
</tr>
<tr>
<td>2: Groups of foam cells, less than (\frac{1}{2}) medial area</td>
<td></td>
</tr>
<tr>
<td>3: Groups of foam cells, more than (\frac{1}{2}) medial area</td>
<td></td>
</tr>
<tr>
<td><strong>Internal elastic lamina fragmentation</strong></td>
<td></td>
</tr>
<tr>
<td>0: Intact</td>
<td></td>
</tr>
<tr>
<td>1: Occasional breaks</td>
<td></td>
</tr>
<tr>
<td>2: Discontinuous segments</td>
<td></td>
</tr>
<tr>
<td>3: Largely disrupted</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Scores Comparing Histopathologic Changes in Control and Trapidil-Treated Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Trapidil</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal foam cell score</td>
<td>2.47</td>
<td>2.13</td>
<td>NS</td>
</tr>
<tr>
<td>Medial foam cell score</td>
<td>2.80</td>
<td>2.63</td>
<td>NS</td>
</tr>
<tr>
<td>Elastic fragment score</td>
<td>2.13</td>
<td>1.69</td>
<td>NS</td>
</tr>
<tr>
<td>Intimal calcification (number of cases present)</td>
<td>3</td>
<td>4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Scores are based on scoring system presented in Table 2.
preventing restenosis after percutaneous transluminal coronary artery angioplasty (PTCA).

Trapidil is used as an antianginal agent in Japan and is pharmacologically related to dipyridamole. Its activity in inhibiting both PDGF-induced cellular proliferation and intimal proliferation has led to the interest in this agent as a possible therapy for restenosis prevention after PTCA. Trapidil is one of few agents shown to significantly reduce intimal thickness in the atherosclerotic rabbit model. Although its precise mechanism of action remains uncertain, we speculate that its activity against growth factor–induced cellular proliferation may be involved. One confounding factor in such speculation is that cholesterol plays an important role in this atherosclerotic model and agents that affect cholesterol metabolism might also influence the final result. Our initial observations on the serum cholesterol levels in this model were that there was a great variation, ranging from 300 to 1,600 mg%, and that the level of cholesterol was not related to the intimal thickness. Furthermore, in a pilot study in which rabbits were treated with trapidil orally once daily, cholesterol levels were not significantly different from those of a control group. It is difficult to exclude the possibility that trapidil may affect lipid metabolism and that this might account for some of the effect observed in our study. However, our observations and assessment of the foam cell component in this study suggest that inhibition of cellular proliferation may be a more important mechanism.

In summary, trapidil significantly increased the luminal area and reduced the intimal thickness in the atherosclerotic rabbit iliac artery after balloon angioplasty. Clinical trials with this type of agent may be warranted. Further investigations into growth factors and smooth muscle cell proliferation may help in finding a pharmacologic solution for restenosis after coronary angioplasty.

Acknowledgments

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