Matrix metalloproteinase inhibition limits arterial enlargement in a rodent arteriovenous fistula model

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Background. We administered a specific, nonselective matrix metalloproteinase (MMP) inhibitor (RS-113,456) to examine the effect of MMP inhibition on flow-mediated arterial enlargement in a rodent arteriovenous fistula (AVF) model.

Methods. Four groups of male Sprague-Dawley rats were created: sham (sham operated; n = 10), control (2.0 mm left common femoral AVF alone; n = 16), vehicle (AVF plus 0.5 mL vehicle orally twice a day; n = 20), and treatment (AVF plus 25 mg/kg RS-113,456 in 0.5 mL vehicle orally twice a day; n = 16). Heart rate, mean arterial pressure, and body weight were recorded on postoperative days 0, 7, 14, and 21. On day 21, AVF patency was confirmed, the infrarenal aorta and common iliac arteries were exposed, blood flow velocity and external diameter were measured, and wall shear stress (WSS) was calculated. Analysis was performed by paired, two-tailed Student t test, one-way analysis of variance, and the Bonferroni/Dunn procedure for post hoc testing.

Results. Heart rate, mean arterial pressure, and weight did not vary at any time between groups. Aortic and left iliac diameter was larger in the AVF groups than in sham groups (P < .001), and control and vehicle groups were larger than treatment groups (P < .0001). Changes in aortic and left iliac flow were also significant (AVF was more than sham and control, and vehicle was more than treatment). No difference in aortic and left iliac artery velocity and WSS or right iliac diameter, velocity, flow, or WSS was observed between groups.

Conclusions. MMP inhibition diminishes flow-mediated arterial enlargement in the rat AVF model.

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Supported in part by the American Heart Association, California affiliate grant 95-255, the Office of Technology and Licensing, Stanford University, and the Lifeline Foundation 1997 Student Fellowship Award.


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0039-6060/ 98/ $5.00 + 0 11/6/91338

Arteries enlarge proximally to arteriovenous fistulas (AVFs) as a result of increased blood flow. Flow mediates enlargement in part through blood/intimal interactions; diameter is regulated to maintain wall shear stress (WSS) within a narrow physiologic range. Acute medial and adventitial smooth muscle relaxation is mediated primarily through nitric oxide production and release. However, endothelium-dependent degradation of elastin and collagen occurs during chronic adaptation to increased flow, remodeling the extracellular matrix of the media and adventitia to achieve a new, steady-state diameter. Matrix metalloproteinases (MMPs) are a family of Zn²⁺-dependent endopeptidases produced by smooth muscle and endothelial cells, fibroblasts, and inflammatory cells. Extracellular matrix collagen and elastin remodeling is mediated through MMP activity. Increased MMP activity is present in arteries proximal to AVF during diameter enlargement as the result of increased flow or diameter reduction after decreased luminal flow. The role of MMP activity in regulating flow-responsive changes in arterial diameter has not been determined.

Many compounds may inhibit MMP activity in vitro and in vivo. RS-113,456 (Roche Bioscience, Palo Alto, Calif.) (Fig. 1) is a specific, nonselective, competitive MMP inhibitor with an in vitro inhibition profile against 8 human and 3 rat MMP subtypes (Table I). In vivo activity of RS-113,456 against rodent MMPs has been demon-
strated in a rodent cartilage sponge degradation assay after oral administration. We inhibited MMP activity in a rodent AVF model through oral administration of RS-113,456 to determine whether MMP activity is rate limiting during chronic, flow-mediated arterial enlargement.

MATERIAL AND METHODS

Adult male Sprague-Dawley rats (weight 350 to 450 g) were used for this experiment. Clean surgical technique was used for all procedures. Anesthetic (50 mg/kg sodium pentobarbital; Abbott Laboratories, Chicago, Ill.) was administered through intraperitoneal injection. Fistula construction was performed after intravenous injection of 100 units/kg beef lung heparin (The Upjohn Co, Kalamazoo, Mich.). Animals recovered from the operation in separate cages with free access to food and water. All experimental procedures were approved by the Animal Care and Use Committee of Stanford University and were in accordance with the “Guide for the Care and Use of Laboratory Animals,” Department of Health and Human Services publication no. 86-23, revised 1985.

Experimental groups and model preparation. Four experimental groups were created for survival surgical procedures (Table II). Sham rats (n = 10) underwent inguinal dissection followed by occlusion of the common femoral artery (CFA) and vein (CFV) for 45 minutes with Heifetz microvascular clips (Roboz Scientific, Rockville, Md.) (Fig. 2). The clips were then removed and the wound was closed. Diameter and blood flow measurements of the proximal CFA were recorded before and after occlusion. Rats in the control (n = 16), vehicle (n = 20), and treatment (n = 16) groups underwent AVF creation between the left CFA and CFV (Fig. 2) with running 8-0 nylon suture (Microsurgical Instruments Co, Belair, Texas) under 48× magnification as described previously. AVFs were created without knowledge of the randomization category of each rat. Diameter and blood flow were recorded from the proximal CFA before and after creation of the fistula. AVF patency was confirmed through observation of bright red, pulsatile CFV flow and a greater than 2× increase in CFA blood flow. Rats without patent AVFs were immediately killed by anesthetic overdose and excluded from further analysis.

Serial measurements. Baseline measurements of weight, blood pressure, and heart rate were obtained for the vehicle and treatment groups before the initial dosing (day -2) and were obtained and repeated for all 4 groups on the day of operation (day 0) and 7, 14, and 21 days after the operation. Ambulatory blood pressure and heart rate were measured by a noninvasive tail plethysmographic system (IITC Inc/Life Science Instruments, Woodland Hills, Calif.).

Vehicle/RS-113,456 administration. Rats in the vehicle group received vehicle solution consisting of 0.15 mol/L NaCl, 0.4% Tween 80 (wt/wt), 0.9% benzyl alcohol (vol/vol), and 0.5% carboxymethylcellulose (wt/wt), pH 7.4, 0.5 mL by gastric intubation twice a day for a total dose of 1.0 mL/day (Table II). Rats in the treatment group received 25 mg/kg RS-113,456 (Roche Bioscience) diluted in 0.5 mL vehicle twice a day for a total dose of 50 mg/kg/day.

Arterial diameter measurement. On day 21, rats were reanesthetized and AVF patency in the control, vehicle, and treatment groups was confirmed through direct dissection and inspection (Fig. 2). Rats with occluded AVFs were killed immediately by anesthetic overdose and excluded from further analysis. The left common carotid artery was cannulated with a transducer catheter (Millar Mikro-tip; Millar Instruments, Houston, Texas) in all groups and connected to a dual-pressure transducer amplifier (Crystal Biotech, Hopkinton, Mass.) for continuous monitoring of mean arterial pressure (Fig. 2). All 4 groups underwent midline exposure of the infrarenal aorta and iliac arteries. Aortic and left and right common iliac artery diameter was measured directly with

<table>
<thead>
<tr>
<th>MMP</th>
<th>Human K_i (nmol/L)</th>
<th>Rat K_i (nmol/L)</th>
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</thead>
<tbody>
<tr>
<td>Collagenase-1</td>
<td>70.0</td>
<td>—</td>
</tr>
<tr>
<td>Collagenase-2</td>
<td>0.13</td>
<td>—</td>
</tr>
<tr>
<td>Collagenase-3</td>
<td>0.17</td>
<td>0.34</td>
</tr>
<tr>
<td>Gelatinase A</td>
<td>0.054</td>
<td>0.099</td>
</tr>
<tr>
<td>Gelatinase B</td>
<td>0.065</td>
<td>—</td>
</tr>
<tr>
<td>Stomelysin-1</td>
<td>5.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Matrilysin</td>
<td>240.0</td>
<td>—</td>
</tr>
<tr>
<td>Metalloelastase</td>
<td>0.15</td>
<td>—</td>
</tr>
</tbody>
</table>

Fig. 1. Chemical structure of MMP inhibitor RS-113,456.
Microcalipers (L.S. Starrett, Athol, Mass.) and indirectly through still photography under 20× magnification with an internal scale for measurement ex vivo. Measurements from the photographic record were determined without knowledge of randomization category and were used for calculation of WSS and statistical analysis.

**Calculation of blood flow, pressure measurements, and WSS.** After arterial diameter measurements were performed, iliac and infrarenal aortic flow velocities were measured with a miniature Doppler flow transducer connected to a 20-MHz high-velocity pulsed Doppler velocimeter (Crystal Biotech) and were used to calculate blood flow and WSS. After all experimental measurements were obtained, animals were killed by anesthetic overdose. WSS, expressed in dynes per square centimeter, was calculated from the Hagen-Poiseuille formula: \( \text{WSS} = \frac{4 \mu Q}{\pi r^3} \), where \( \mu \) is the viscosity of blood (assumed to be 0.035 poise), \( Q \) is the blood flow (in milliliters per second), and \( r \) is one half of the external diameter.4

**Statistical analysis.** Paired measurements within groups were compared by the paired, two-tailed Student \( t \) test, with significance determined at the .05 level. Single time-point measurements were compared between groups by one-way analysis of variance, with significance determined at the .05 level. Serial measurements between groups were compared with repeated-measures analysis of variance, with significance determined at the .05 level. Pair-wise comparisons were performed according to the Bonferroni/Dunn procedure. This procedure prevented multiple-comparison error when looking for differences between groups; a \( P \) value \( \leq .0083 \) was necessary to achieve significance at the 95% confidence level. All analyses were performed on a personal computer with the StatView version 4.5 software package (Abacus Systems, Berkeley, Calif.).

**RESULTS**

All results are expressed as means ± SD. Three rats died during the initial surgical procedure, 3 were killed after unsuccessful AVF creation, 4 did not survive measurement of the aorta and iliac arteries, and 4 AVFs were found to be occluded during the final surgical procedure. Thus 48 rats were available for comparison at the final surgical procedure, including 10 in the sham, 10 in the control, 14 in the vehicle, and 14 in the treatment groups.

**Initial conditions.** AVF length was comparable between the three AVF groups (control = 2.76 ± 0.09 mm, vehicle = 2.73 ± 0.14 mm, and treatment = 2.74 ± 0.13 mm; difference not significant; Table III). Common femoral artery blood flow did not change after sham exposure of the common femoral artery (baseline, 2.90 ± 1.29 mL/min; after the procedure, 2.40 ± 0.70 mL/min; difference not significant; Table III). Creation of the AVF resulted in an immediate fourfold increase in blood flow in the proximal common femoral artery (baseline, control = 2.50 ± 0.97 mL/min, vehicle = 2.86 ± 0.86 mL/min, and treatment = 2.57 ± 0.65 mL/min; after AVF, 10.70 ± 6.22 mL/min, 13.00 ± 8.48 mL/min, and 11.36 ± 7.21 mL/min, respectively; \( P = .0029, .0005, \) and \(.0004, \) respectively) within all fistula groups (Table III). The increase was not different between fistula groups (Table III).

**Serial measurements.** Heart rate, mean arterial pressure, and the rate and extent of weight gain did not vary between groups at any time point during the experiment (Fig. 3).

**Outcome measurements.** Intra-arterial mean arterial pressure did not vary between groups during analysis of aortic and iliac diameter, blood flow, or velocity. External diameter of the infrarenal abdominal aorta was significantly larger in the control (2.63 ± 0.26 mm), vehicle (2.47 ± 0.20 mm), and treatment (2.06 ± 0.21 mm) groups than in the sham (1.66 ± 0.19 mm) group (\( P < .0001; \) Table IV; Fig. 4). The external diameter of the treatment group was significantly smaller than either the control or vehicle group (\( P < .0001 \)). Measured aortic blood flow velocity (in centimeters per second) did not vary among the 4 groups (Table IV). Despite this, as a result of measured diameter differences, aortic blood flow was greater in the control (74.40 ± 21.48 mL/min), vehicle (77.21 ± 18.34 mL/min), and

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**Table II. Experimental group design**

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedure</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>Sham operation</td>
<td>None</td>
</tr>
<tr>
<td>Control</td>
<td>AVF</td>
<td>None</td>
</tr>
<tr>
<td>Vehicle</td>
<td>AVF</td>
<td>0.5 mL vehicle twice a day</td>
</tr>
<tr>
<td>Treatment</td>
<td>AVF</td>
<td>25 mg/kg RS-113,456 in 0.5 mL vehicle twice a day</td>
</tr>
</tbody>
</table>

Sham operation refers to left inguinal dissection with exposure and occlusion of the CFA and CFV for 45 minutes with Heifetz microvascular clips followed by wound closure.
treatment (47.93 ± 22.25 mL/min) groups than in the sham (15.67 ± 6.06 mL/min) group (P < .0002; Table IV). Aortic blood flow was less in the treatment group than in either the control or vehicle groups (P < .0015; Table IV). Calculated WSS was not different between groups (Table IV).

Right common iliac artery diameter, blood flow, velocity, and WSS were equal among the 4 groups (difference not significant; Table IV; Fig. 4). Left common iliac artery diameter was significantly larger in the control (1.95 ± 0.13 mm), vehicle (1.91 ± 0.31 mm), and treatment (1.80 ± 0.26 mm) groups than in the sham (1.08 ± 0.18 mm) group (P < .0001, Table IV; Fig. 4). Although left iliac artery diameter in the treatment group was smaller than in the control or vehicle group, this difference did not reach significance. Left iliac artery blood flow was significantly greater in the control (43.90 ± 14.15 mL/min), vehicle (43.21 ± 14.69 mL/min), and treatment (25.86 ± 9.32 mL/min) groups than in the sham (15.67 ± 22.25 mL/min) group (P < .001; Table IV). Blood flow in the treatment group was lower than in the control or vehicle group (P < .0005). Neither left iliac flow velocity nor WSS varied between groups (difference not significant; Table IV).

DISCUSSION

Administration of RS-113,456 to rats with increased left CFA blood flow caused by an AVF prevented abdominal aortic enlargement compared with control AVF rats or AVF rats treated with vehicle alone. Treatment with RS-113,456 did not completely inhibit aortic enlargement; the average aortic diameter in the treatment group was significantly larger than in rats who did not have an AVF (sham group).

To our knowledge, prevention of flow-mediated arterial enlargement has not been attempted with other MMP inhibitors. As noted previously, de Kleijn et al.8 measured changes in diameter in rabbit carotid and femoral arteries after survival surgical procedures to increase (AVF) or decrease (partial ligature) luminal blood flow. After 21 days, arterial tissue from both groups was assayed by zymography for MMP-2 and MMP-9 activity. In the AVF animals, MMP-2 activity alone was increased, whereas the ligature group demonstrated increased MMP-2 and MMP-9 activities. As a method to measure total enzyme activity, zymography has significant limitations; it requires denaturing conditions that may lead to dissociation of MMP species and endogenous inhibitors such as tissue inhibitors of MMPs. Therefore zymography may not measure in vivo activity accurately. Because zymography may have dissociated any exogenous inhibitor/MMP complexes and thus confounded...
our results, the effect in our own series was measured based on the external diameter of the aorta and iliac arteries alone. Serum drug levels of RS-113,456 were not measured in the treatment group because the dosing protocol had previously been determined by Roche Bioscience to provide consistent MMP inhibition in the rat.

Because enzyme activity was not measured directly from tissues obtained in this experiment, it is possible that RS-113,456 limited arterial enlargement by some method other than MMP inhibition. Artifactual reduction in blood pressure (as reported for other MMP inhibitors), if caused by RS-113,456, may have influenced calculation of diameter. Ambulatory heart rate and blood pressure were measured throughout the experiment, and intra-arterial measurements were obtained during death; no effects attributable to RS-113,456 were noted. Also, similar weight gain profiles between groups decreased the likelihood that general toxic effects of RS-113,456 may have limited arterial enlargement. Although the possibility exists that RS-113,456 may have influenced our diameter measurements through a mechanism other than MMP inhibition, no such hemodynamic or metabolic effects were observed.

Flow velocities in the aorta and iliac arteries did not change despite marked changes in diameter in the face of stable and consistent blood pressure and heart rate. Consequently, calculations of blood flow demonstrated decreased blood flow in the smaller aortas of the treatment group compared with the control or vehicle groups. WSS, calculated as a function of radius and blood flow, also failed to demonstrate a difference between groups. However, as noted in Table IV, Doppler velocimetry as performed in this experiment did not provide consistent values. The standard deviation of the velocities obtained in these 1- to 2-mm arteries varied from 23% to 86% of the mean value. This variability was a direct result of problems we encountered obtaining good apposition between
the Doppler flow probe and the tortuous and elongated aortas and iliac arteries present in the groups with AVFs (Fig. 4). By comparison, the standard deviations of the diameter measurements by electronic calipers and still photography and the internal consistency present between the two methods suggested that this value was much more reliable and reproducible. It is likely that some differences in WSS existed between the treatment and control and vehicle aortas and left iliac arteries and, because of limitations in our method of measurement, we were simply unable to identify the differences in these vessels.

Table III. Initial experimental conditions of AVF construction including fistula length and preoperative and postoperative CFA blood flow

<table>
<thead>
<tr>
<th>Group</th>
<th>AVF length (mm)</th>
<th>CFA blood flow (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Preoperative</td>
</tr>
<tr>
<td>Sham</td>
<td>None</td>
<td>2.9 ± 1.3</td>
</tr>
<tr>
<td>Control</td>
<td>2.76 ± 0.09</td>
<td>2.5 ± 1.0</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.73 ± 0.14</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>Treatment</td>
<td>2.74 ± 0.13</td>
<td>2.6 ± 0.6</td>
</tr>
</tbody>
</table>

*Statistical difference from baseline measurement; \( P < .05 \) required for significance with a 95% confidence interval.

Table IV. Final measurements of external diameter, blood flow, flow velocity, and WSS in the infrarenal abdominal aorta, left common iliac artery, and right common iliac artery

<table>
<thead>
<tr>
<th>Group</th>
<th>External diameter (mm)</th>
<th>Blood flow (mL/min)</th>
<th>Velocity (cm/sec)</th>
<th>WSS (dynes/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infrarenal abdominal aorta</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham (n = 10)</td>
<td>1.66 ± 0.19</td>
<td>15.67 ± 6.06</td>
<td>27.59 ± 15.46</td>
<td>2.62 ± 1.62</td>
</tr>
<tr>
<td>Control (n = 10)</td>
<td>2.63 ± 0.26*</td>
<td>74.40 ± 21.48*</td>
<td>41.82 ± 19.53</td>
<td>3.20 ± 1.51</td>
</tr>
<tr>
<td>Vehicle (n = 14)</td>
<td>2.47 ± 0.20*</td>
<td>77.21 ± 18.34**</td>
<td>50.19 ± 12.45</td>
<td>3.93 ± 1.26</td>
</tr>
<tr>
<td>Treatment (n = 14)</td>
<td>2.06 ± 0.21**</td>
<td>47.93 ± 22.25**</td>
<td>43.14 ± 18.52</td>
<td>4.14 ± 2.23</td>
</tr>
</tbody>
</table>

*Differs from the sham group.

**Differs from all other groups; \( P < .0083 \) required for significance with a 95% confidence interval.

Although the clinical significance of flow-mediated arterial remodeling remains uncertain, chronic changes in flow at the aortic bifurcation appear to increase the likelihood that patients may have abdominal aortic aneurysms. Vollmar et al.\(^{11}\) reported a fivefold higher incidence of abdominal aortic aneurysms in patients 4 or more decades after unilateral above-knee amputation compared with a matched, biped control population. The shape and orientation of the aortic and iliac aneurysms present in the amputees suggested that years of asymmetric flow at the aortic bifurcation had influenced aneurysmal growth and development. More recently, McMillan et al.\(^{12}\) reported two patients with hemiparesis with aortic and iliac aneurysmal morphology strikingly similar to that first noted by Vollmar et al. Taken together with a recent report suggesting an increased risk of development of abdominal aortic aneurysms in patients with spinal cord injuries,\(^{13}\) these studies strongly support the hypothesis that changes in chronic flow at the aortic bifurcation are clinically significant and may influence the development of abdominal aortic aneurysms.

Strong evidence links MMP activity to the development of abdominal aortic aneurysms. Increased concentrations of MMP mRNA, protein, and activity are found in aneurysmal abdominal aortas compared with occlusive or normal aortas.\(^{14,15}\) Furthermore, McMillan et al.\(^{16}\) recently reported that increased concentrations...
of MMP-9 mRNA were present in the wall of successively larger abdominal aortic aneurysms up to 7 cm in diameter, after which the MMP-9 mRNA decreased despite increasing aortic size. The possibility that MMP inhibition may limit the size or rate of expansion of abdominal aortic aneurysms has been suggested by the work of Petrinec et al., and Holmes et al., who found that doxycycline and indomethacin inhibition of MMP activity in the Anidjar-Dobrin elastase-induced rat model of abdominal aortic aneurysms prevented aneurysmal degeneration of the abdominal aorta. This report confirms preliminary work from our laboratory suggesting inhibition as a promising therapy to prevent enlargement of abdominal aortic aneurysms. MMP inhibition clearly alters chronic adaptive responses involving structural modifications of the arterial wall caused by a variety of influences. Work is ongoing to identify the particular MMP species responsible for the enlargement observed in this model. Although the mechanisms responsible for initiation and progression of aneurysmal disease are poorly understood, the demonstrated efficacy of MMP inhibition in preventing arterial enlargement supports further study of MMP inhibition as a promising therapy to prevent enlargement of abdominal aortic aneurysms.

We thank Byron Brown, PhD, for reviewing the statistical analyses, Michelle Bendeck, PhD, for assistance with references and manuscript preparation, and Sarika C. Joshi and Joshua M. Hull for their technical assistance in animal husbandry and performance of procedures as described. Material support was provided by the Inflammatory Diseases Section, Roche Bioscience, Palo Alto, California.

REFERENCES


DISCUSSION

Dr Robert W. Thompson (St Louis, Mo.). I think you have devised a nice model to isolate the effects of flow with respect to arterial dilation and the potential mechanisms involved. One of the factors detracting from the results, as shown, is that the flow measurements in the treatment group were significantly less than those in the vehicle or control groups. One could come to the erroneous conclusion that the drug acted to decrease flow and therefore decrease diameter in that fashion, rather than vice versa, as you have concluded. I think you can reach that conclusion with an appropriate rigor because the flow measurements are actually derived from the velocity measurements, and the velocity measurements...
are not different. Therefore I think if the results are reported in terms of velocity, the study is valid, and I think the results are intriguing. They do indicate or suggest that metalloproteinase inhibition might reduce flow-mediated remodeling in the arterial wall. What MMPs are specifically involved in this process? We do know from the studies you have cited, and others, that in human abdominal aortic aneurysms, and other models of aneurysm, macrophage infiltration, metalloproteinase production, and elastin degradation are key components of this disease. Have you looked at the histologic and structural pathologic elements of these lesions, in the aorta particularly, to see whether there is any elastin degradation or mononuclear phagocyte inflammation? My other question relates to other potential inhibition of metalloproteinase-like activity in this system. Tumor necrosis factor, for example, has to be processed by metalloproteinase activity, called tumor necrosis-activating factor (TACE). This can be inhibited by many MMP inhibitors; does this particular drug have anti-TACE activity? Could some of the effects have been the result of an amelioration of any tumor necrosis factor-α-mediated effects induced by flow?

Mr Abbruzzese. Regarding which MMPs are involved, our work serves as a complementary study to the observational studies in human aneurysmal tissue showing involvement of MMPs 1, 2, 3, and 9 primarily. We have not looked at which MMP activity is involved ourselves. Recently, however, de Kleijn et al.8 from the Netherlands used an almost identical model of arteriovenous fistula causing arterial enlargement, reporting that MMP-2 and MMP-9 activity was increased at 21 days. Regarding histologic or pathologic elements, we have not done a complete histologic study of the arteries, primarily because of technical difficulties of fixing the tissue during pressure perfusion. This would have been particularly interesting for the left iliac artery. However, preliminary histologic analysis has shown that there is no elastin breakdown in the elastic lamina. Dr. Masuda6 from Akita University has performed elegant ultrastructural studies evaluating arteriovenous fistulas over time. He has demonstrated breakdown of the internal elastic lamina and cell migration through it, leading to enlargement. The appearance of the artery was described as a signet ring, because the elastin would be broken down allowing the ring of the artery to expand. However, we have not found any evidence of that in our model. Regarding anti-TACE activity, I do not know of any data for this particular compound against TACE.

Dr Julie Ann Freischlag (Milwaukee, Wis.). I am having a little trouble saying that this model really represents a model of aneurysm formation. Could you explain the connection to arteriovenous fistulas and why you think it is valid? Have you thought of any other way to look at aneurysm formation, such as the elastase infusion used in some rat models, to make sure this is indeed aneurysm formation and not just arterial enlargement?

Mr Abbruzzese. There are data in the literature to support a role for flow in the pathogenesis of abdominal aortic aneurysms. In one study by Vollmar et al.11 in 1989 with unilateral above-knee amputation, there was a 5 times higher incidence of abdominal aortic aneurysms, and actually the morphologic findings of the aneurysm suggested that the flow was playing a role in that process. Also, Pearce and McMillan12 reported 2 patients with hemiparesis who had abdominal aortic aneurysms with the same characteristic morphologic findings, suggesting that flow is involved. We have had some experience with the Anidjar/Dobrin model, the elastase model, of abdominal aortic aneurysms. We had some problems with variability in that model. Flow-mediated enlargement in this model is a generalized process in which arteries proximal to the fistula enlarge. In aneurysms, however, flow-mediated enlargement may also play a significant role.