Transluminal Angioplasty: Correlation of Morphologic and Angiographic Findings in an Experimental Model

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SUMMARY The morphologic consequences of transluminal angioplasty of stenotic atherosclerotic coronary arteries are unknown. This study describes the production of aortoiliac atherosclerosis in rabbits and reports the morphologic changes after transluminal angioplasty of stenotic arterial lesions. Atherosclerotic lesions were evaluated angiographically before and after transluminal angioplasty and were studied histologically and by electron microscopy after angioplasty. Moderately stenotic aortic segments showed demudation of endothelial cells and deposition of a carpet of platelets enmeshed in fibrin. Medial and intimal contractures were not seen. Intimal plaque disruption and splitting of atheromatous plaques were observed in more stenotic vessels where dilatation during angioplasty is relatively greater. Transluminal angioplasty, therefore, acutely causes desquamation of endothelial cells and superficial plaque elements, splitting of atheroma and subsequent deposition of platelets and fibrin in the area of angioplasty. This experimental model may be useful to evaluate the morphologic changes after angioplasty and might be used in further studies to determine the long-term pathophysiologic changes after transluminal angioplasty.

RECENT STUDIES by Gruntzig indicate that percutaneous coronary transluminal angioplasty with a balloon-tipped catheter is effective in the treatment of stenotic coronary artery disease in humans. In follow-up, coronary angiograms of patients treated by this technique show improved lumen diameter at the angioplasty site, thallium-201 perfusion images reveal fewer myocardial defects and patients are improved symptomatically.

Scanning electron microscopy after coronary transluminal angioplasty in normal dog coronary arteries has been studied. However, the morphologic basis of angiographically successful transluminal angioplasty of a stenotic atherosclerotic artery is virtually unknown. Studies performed on human hearts at autopsy show that angioplasty may lead to plaque rupture and medial dissection. However, at autopsy, the tissue is not viable and therefore is more susceptible to damage than vessels in vivo, and passage of shorter dilatation catheters may have caused dissection if forced through fixed stenotic segments.
This study was undertaken to develop an in vivo experimental model for studying the pathophysiologic consequences of transluminal angioplasty in stenotic atherosclerotic arteries, to describe the morphologic changes that occur in plaques after transluminal angioplasty and to correlate the angiographic and morphologic findings in successful dilatations.

Materials and Methods

Aortic atherosclerosis was initiated in 13 3-kg male New Zealand white rabbits by feeding a 2% cholesterol diet (ICN, Cleveland, Ohio). After 1 week on this diet, when serum cholesterol was 800–1000 mg%, all rabbits underwent endothelial debridement of the aorta using the Baumgartner technique. The rabbits were anesthetized with intravenous pentobarbital. A #4F or a #3F pediatric Fogarty catheter was passed retrogradely from a right femoral arteriotomy to the aortic arch. The balloon was inflated and the catheter withdrawn via the thoracic and abdominal aorta into the right iliac artery. This procedure was repeated three times and the catheter removed. The right femoral artery was ligated and the wound closed. All rabbits were maintained on the 2% cholesterol diet after aortic debridement.

Six to 12 weeks after endothelial debridement, femoral and right carotid arterial cutdowns and arteriotomies were performed. A #5 “bird’s-eye” (Goodale-Lubin) catheter was passed from the right carotid arteriotomy into the abdominal aorta and the tip of the catheter positioned just below the renal arteries by fluoroscopy. Five ml of a 50% Renografin/normal saline mixture were injected over 1 second using a hand syringe and 35-mm cineangiography was performed. Video images were evaluated for sites of abdominal aortic and right iliac stenoses, which were then marked by external markers under fluoroscopy. A 3.0-mm Grünzig transluminal angioplasty catheter (Schneider Co., Zurich, Switzerland) was then passed retrogradely under fluoroscopy through the left or right femoral arteriotomy. With the tip of the dilatation catheter positioned in a stenotic segment, the balloon was inflated twice over 5 seconds with a 50% Renografin/normal saline mixture to 5 atmospheres pressure. Inflation was recorded by cinefluoroscopy to document positioning. After the balloon was deflated and the catheter removed, repeat angiography was performed. Fifteen minutes later, all rabbits were killed with an overdose of pentobarbital.

In 10 rabbits, the abdominal aorta and the iliac arteries were fixed in situ by perfusion with 10% buffered formalin. Sections were taken from dilated and nondilated regions and routinely processed for light microscopy. Sections were stained with hematoxylin and eosin, Masson trichrome and elastic tissue stain.

After angioplasty in three additional rabbits, the aorta and iliac arteries were fixed by antegrade perfusion at a controlled pressure of 100 mm Hg through the angiography catheter in the abdominal aorta. After perfusion with 200 ml of cacodylate-buffered 2% glutaraldehyde for 15 minutes, the aorta and iliac arteries were dissected free and fixation of the intact vessel was continued for 24 hours. Preparation of specimens for electron microscopy included post-fixation in osmium tetroxide and dehydration in a graded series of alcohol/acetone. For transmission electron microscopy, cross sections from dilated and nondilated areas were cut into 2-mm² pieces and embedded in Epon-araldite. Blocks were sectioned with a diamond knife and sections viewed in a Phillips-201 electron microscope. For scanning electron microscopy, 5-mm segments of aorta were cut from dilated and nondilated areas, subjected to critical-point drying in liquid CO₂, longitudinally opened, mounted on stubs and gold coated in a sputter coater. The intimal surfaces were then viewed with an AMR 1400-scanning electron microscope.

Results

Angiographic and Gross Morphology

Nondilated Areas

By angiography and gross inspection, all of the abdominal aortas had prominent atherosclerotic lesions. Stenotic lesions were present in all areas of the abdominal aorta but maximal stenosis was most often measured just above the abdominal aortic lumen to a minimum of 1.2 mm (fig. 1). Regions of abdominal aortic ectasia were also seen in several rabbits. The severity of the atherosclerosis varied from rabbit to

Figure 1. Aortoiliac angiogram of a rabbit 8 weeks after debridement of the aorta and right iliac artery. Atherosclerotic narrowing is present above the aortic bifurcation and the right iliac artery (arrows). The left iliac artery, which had not undergone endothelial debridement, appears normal.
TABLE 1. Aortic and Iliac Artery Diameters from Angiogram, Before and After Angioplasty

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Aortic diameter (mm)</th>
<th>Difference (mm)</th>
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<td></td>
<td>Before</td>
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<td>2.1</td>
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<td>2</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>2.0</td>
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<td>6</td>
<td>3.2</td>
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<td>8</td>
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<tr>
<td>9</td>
<td>1.9</td>
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<tr>
<td>10</td>
<td>2.7</td>
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<tr>
<td></td>
<td>Right iliac artery diameter</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2.2</td>
<td>2.7</td>
</tr>
<tr>
<td>12</td>
<td>1.0</td>
<td>1.7</td>
</tr>
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rabbit but appeared maximal 6–8 weeks after endothelial debridement. Control areas that did not undergo angioplasty showed no change in lumen diameter after the Renografin injection.

Dilated Areas

Aortic and iliac artery lumen diameters of areas that underwent angioplasty were measured angiographically before and after angioplasty in 12 of the rabbits. An increase in aortic luminal diameter occurred in eight of 10 cases (table 1). Two rabbits had a small decrease in aortic diameter. Both iliac artery segments that underwent angioplasty showed an increase in lumen size after the procedure (fig. 2). All vessels were patent after angioplasty and there was neither angiographic nor gross evidence of intimal or medial dissection.

Light Microscopy

Sections of aorta from nondilated regions showed marked intimal thickening with large numbers of foam cells scattered throughout the intima and media (fig. 3). Focal medial calcification was also present in many of the aortas. Neither ulceration nor mural thrombus was evident in the nondilated control areas. All areas that had undergone transluminal angioplasty showed a loss of endothelial cells and a thin, overlying, platelet-fibrin mural thrombus (fig. 4). Medial and intimal compression were not seen. Considerable intimal plaque disruption was observed in one aortic specimen (fig. 5) in which the luminal half of the plaque appeared sheared off. There was no microscopic evidence of dissection in any of the dilated aortas. However, dilated iliac artery segments showed focal splitting of atheromatous plaques and in some cases extension of the splits along the internal elastic membrane (fig. 6).

Electron Microscopy

The nondilated upper abdominal aortic segments served as control areas (fig. 7). The intimal surface of
these segments was characterized by irregularly shaped and disoriented endothelial cells, large ruffled cells and numerous intercellular discontinuities often containing platelets adherent to the underlying matrix (fig. 8). In contrast, the dilated aortic segments showed marked denudation of endothelial cells, with only a few islands of normal endothelium remaining. The denuded areas were covered by a thin layer of platelets enmeshed in fibrin (fig. 9). The platelets were in various phases of metamorphosis and many elongated pseudopodia extended over and into the underlying matrix of the intimal plaque. Aortic branch vessels showed a relatively normal endothelial pattern and no platelet deposits. Transmission electron microscopy showed that surface endothelial cells in the area of balloon inflation were almost entirely absent and replaced by a layer of platelets and fibrin overlying the desquamated subendothelium (fig. 10).

**Discussion**

This study demonstrates the usefulness of a previously described animal model of atherosclerosis in attempts to elucidate the acute pathophysiologic mechanism of successful transluminal angioplasty. The rabbit model fulfills a number of important considerations. It is relatively inexpensive and it reliably produces stenotic atherosclerotic lesions within 6–8 weeks. Although only 40–50% stenoses were routinely produced in the abdominal aorta, more severe stenoses (up to 90%) approximating those seen in human atherosclerotic coronary arteries were generally present in deendothelialized right iliac arteries of rabbits.

Plaques produced in rabbits differ from human atherosclerosis in that there are abundant foam cells in the arterial lesions that are not often seen in human atherosclerotic plaques. The reaction of rabbit atherosclerotic plaques to angioplasty may, therefore, be different from that of human atherosclerotic lesions. However, the dissections after coronary angioplasty in human postmortem studies are similar to the splitting of plaques found in this study. This supports the concept that pathologic changes after angioplasty in rabbits and pathologic changes after angioplasty in human coronary arteries are analogous. Nevertheless, before our findings can be confidently extrapolated to
FIGURE 7. Scanning electron micrograph of nondilated upper abdominal aorta reveals severe luminal surface irregularities produced by cholesterol feeding and endothelial debridement. The rough areas correspond to the presence of numerous large ruffled cells (see figure 8) (original magnification \( \times 16 \)).

humans, they should be substantiated in other animal models and in studies of human arteries that have undergone angioplasty if they become available at post-mortem examination. Studies of angioplasty using a primate model for atherosclerosis (e.g., the baboon) may be useful in settling this issue.

Grüntzig has postulated that compression of atheroma by the angioplasty balloon may account for the increase in size of the arterial lumen seen angiographically after successful angioplasty.\(^{11}\) Though atheroma are compressible, such compression was not convincingly shown in this study. Instead, it appears that in areas of moderate stenosis, transluminal angioplasty produces endothelial desquamation and shearing off of superficial plaque elements. Further, in tightly stenosed vessels where the sizes of the constricted arterial lumen and the inflated angioplasty balloon are more disparate, splitting of the atheromatous plaque occurs. The improvement in lumen size seen angiographically immediately after successful angioplasty does not seem to be due to compression of plaques but rather to disruption and desquamation of plaque elements. The passage of

FIGURE 6. Sections of iliac arteries from dilated regions show splits in the intimal plaque. A) A short split extends from the luminal surface into the plaque. B) The split extends through the plaque and continues circumferentially at the internal elastic lamina. (A) Hematoxylin and eosin; magnification \( \times 45 \). B) Verhoff’s elastic tissue stain; magnification \( \times 55 \).
angiographic dye into the split areas probably accounts for the shaggy, irregular appearance of the arterial lesion often observed in angiograms performed immediately after angioplasty.

This study also shows that angioplasty initiates platelet deposition in the dilated arterial segments. This fact is not surprising, because less injurious procedures such as vibration of a small catheter and passage of a wire guide can damage endothelium and initiate platelet deposition and fibrin thrombus formation.\(^9\) Though transluminal angioplasty causes desquamation of most endothelial cells in the dilated area with exposure of subendothelial microfibrils and subsequent deposition of a carpet of platelets and fibrin, thrombus was not propagated in this model presumably due to brisk blood flow.\(^7\) It is possible that diminished blood flow through a distally stenosed vessel subjected to transluminal angioplasty might result in thrombotic occlusion caused by the initiation and propagation of fibrin and platelet thrombus. During a coronary angioplasty in patients, anticoagulant and antiplatelet suppressive regimens are used and continued on a long-term basis. These studies imply that suppression of platelet thrombus formation in the
FIGURE 10. Transmission electron micrographs from nondilated (A) and dilated (B) areas of the same rabbit aorta. A) A continuous layer of endothelial cells, containing large lipid vacuoles, is present on the luminal surface. B) Endothelial cells are absent and replaced by multiple layers of platelets undergoing metamorphosis (original magnifications ×7200).
area of angioplasty is beneficial. However, the efficacy of these regimens has not been substantiated. No anticoagulant or antiplatelet suppressive medications were given in this study.

How the healing process in the damaged atherosclerotic artery effects lumen size later after angioplasty cannot be answered from this study. Focal disruption of plaques might cause partial dissolution of the atheromatous material, which would account for the smooth, more normal appearance seen in areas of successful angioplasty in late follow-up angiograms. This interesting possibility will require evaluation by long-term studies using this model.

We speculate that there may be distal embolization of the desquamated endothelial cells and plaque elements secondary to angioplasty. Evidence of embolization has appeared in only 3% of patients who have undergone peripheral angioplasty of stenosed leg vessels. There has been no evidence of embolization in the initial reports of coronary angioplasty. Hence, at the present time embolization in human coronary angioplasty seems to be clinically unimportant. We did not attempt to collect desquamated segments of plaques distal to the site of angioplasty. Identification of plaque debris among the formed blood elements will be difficult, especially because the debris is probably composed of small particles. Labeling techniques to identify plaque elements may be needed to resolve this problem, which is an area for further investigation.

We hope this model will serve as a useful tool for evaluating the results of angioplasty on stenotic segments of arteries in vivo and help provide more information concerning other possible mechanisms of lumen enlargement that may occur after human coronary angioplasty. Further, it may serve as a method for studying the effects of antiplatelet and antifibrin therapy directed at minimizing the degree of platelet deposition and mural thrombus formation produced by transluminal angioplasty.

References
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