Transluminal Angioplasty in Experimental Atherosclerosis
Analysis for Embolization Using an In Vivo Perfusion System

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SUMMARY We used polarized light microscopy and thin-layer chromatography to determine whether embolization of atherosclerotic material occurs after transluminal angioplasty. The experimental model consisted of an in vivo perfusion system of the atherosclerotic rabbit left iliac artery. Of eight rabbits that underwent successful angioplasty, four had angiographic evidence of dissection and three showed aneurysm formation. Histologic studies demonstrated fracture of the intimal plaque, dissection, and stretching of the noninvolved portion of the vessel. Perfusate analysis revealed no detectable cholesterol by thin-layer chromatography in six of eight rabbits. In two rabbits, a very small amount of cholesterol was measured, which was totally accounted for by hemorrhage into the perfusate rather than from cholesterol in the plaque. No evidence of arterial wall embolic debris could be detected by polarized light microscopy in seven rabbits, but lipid debris from the plaque was found in the perfusate of one rabbit that had excessive arterial trauma.

We conclude that the major mechanism of successful transluminal angioplasty in this experimental model is intimal fracture combined with stretching of a noninvolved portion of the vessel. Furthermore, embolization of atheromatous lipid debris was an uncommon event related to arterial trauma during catheter placement rather than transluminal angioplasty itself.

TRANSLUMINAL ANGIOPLASTY is being increasingly as a nonsurgical alternative in the management of obstructive atherosclerotic disease. Using a balloon-tipped catheter developed by Gruentzig,1 successful reduction of stenosis has been reported in the peripheral,1 renal2, 3 and coronary arteries.4 How this technique works, however, is incompletely understood. Only two prior studies of the histologic effects of transluminal angioplasty using an experimental atherosclerosis model have been reported.5 6 Both studies demonstrated evidence of intimal plaque fracture, with dissection extending between the intima and media after transluminal angioplasty. Histologic evidence of stretching of the intima and media was also reported.

Angioplasty involves the enlargement of a narrowed arterial lumen by an intraluminal balloon catheter. Such a procedure could redistribute plaque contents throughout the arterial wall or actually cause embolization of arterial wall lipid debris. Reports of rare emboli after angioplasty in peripheral arteries1, 7 raise concern about this possibility. Distal embolization has not been reported in clinical coronary angioplasty. Furthermore, Gruentzig et al.8 reported that when transluminal angioplasty was performed during coronary artery bypass surgery in three patients, no evidence of embolic debris was found on millipore filters. However, detailed lipid analyses were not performed. Accordingly, we used the sensitive methods of polarized light microscopy and thin-layer chromatography to determine if there is any evidence of atheromatous lipid embolization after transluminal angioplasty in experimental atherosclerosis in an in vivo perfusion system.

Methods
Experimental Model and Design
Atherosclerosis was induced in the aorta and left iliac artery of 14 3-kg male New Zealand white rabbits (eight experimental and six control) using the Baumgartner technique,9 followed by feeding an atherogenic diet. A #3F Fogarty catheter was passed retrogradely through the left femoral artery to 20 cm, inflated to occlude the vessel, and slowly removed. Denudation of the aorta and left iliac endothelium was assured by passing the Fogarty balloon twice to 20 cm. The rabbits were then placed on a 2% cholesterol diet composed of rabbit chow supplemented with 2% cholesterol mixed with 10% peanut oil for 6 weeks. Previous studies have shown the development of significant atherosclerosis in 68% of animals placed on this atherogenic diet for this time period. At the end of 6 weeks, the eight experimental rabbits were reoperated under pentothal anesthesia to expose the aortic bifurcation and iliac arteries. Ligatures were placed in the upper aorta, right iliac artery, and side branches of the left iliac artery (fig. 1). A #5F Cordis sheath was inserted through an arteriotomy into the aorta, while a #3F Goodale-Lubin catheter was advanced retrogradely through the left femoral artery. Both catheters were secured with silk ties. The #3F catheter closely approximated the internal circumference of the femoral
artery and served to prevent collection of debris between the catheter and the vessel wall. The aortoiliac system was then perfused at 90 mm Hg with heparinized Ringer’s lactate solution (2000 U heparin/l).

Cineangiography was performed through the Cordis sheath to visualize the arterial lesions and confirm complete ligation of all side branches. This control ensured that all perfusate would be collected completely. Three milliliters of Renografin 76 were injected with a hand syringe over 3 seconds. Angiographic images recorded on 35-mm film using a Philips 6-inch image intensifier with a resolution of 3.8 line pairs/mm. Since the rabbit iliac artery approximates the size of the human coronary artery, a 2.5-cm Gruentzig intraoperative transluminal angioplasty catheter was found suitable and was advanced through the Cordis sheath to the site of greater iliac stenosis under fluoroscopic guidance. This position was confirmed by comparison with video recordings of the angiogram. Aliquots of the perfusate were collected before and after catheter insertion as a baseline determination before balloon dilation. Subsequently, the dilation catheter was inflated to 5 atmospheres for 30 seconds, then deflated and removed. The perfusate was again collected in three 1-minute intervals after transluminal angioplasty. Flow rates for all sampling areas were 2–5 ml/min and were a function of the resistance of the #3F catheter. Repeat angiography was then performed to document the results of angioplasty. Care was taken to position the image intensifier at the same height for both angiograms. A 1-cm grid was positioned on the film to permit calculation of the actual luminal diameter and to provide correction for any magnification errors between films. Immediately after angioplasty and the final perfusate collection, the aorta and iliac vessels were perfused with formalin at 80 mm Hg as previously described. The rabbits were then killed and the vessels were surgically removed and placed in formalin.

**Angiographic, Histologic and Biochemical Analysis**

Comparison of the pre- and postdilation cineangiograms was made on a Vanguard projector to determine change in luminal diameter. A change of 20% (0.4 mm when corrected for magnification) could easily be resolved using this technique and was considered significant. Angiographic dissection was defined as a linear density of contrast material extending beyond the luminal outline. Aneurysm formation was defined as a luminal diameter greater than that in proximal nondilated segments. Each angiogram was read independently by two angiographers and discrepancies were resolved by a subsequent simultaneous reading.

The surgically removed iliac vessels were examined histologically by preparing serial 1-cm segments of the left iliac artery and allowing for at least two segments through the dilated areas and the nondilated proximal segment. Sections stained with hematoxylin-eosin and Verhoff van Gieson-elastin were reviewed by at least two investigators and a consensus reading was made of the histologic findings. Intimal fracture was defined as a radial tear through the intima, dissection was defined as a circumferential tear along the internal elastic membrane, and stretching was defined as a thinning of the wall with loss of nuclear staining.

Both unspun and centrifuged (2000 rpm × 20 minute) samples of the five aliquots collected from each rabbit were examined by polarized light microscopy to determine birefringence, as previously described. Plasma cholesterol and lipid content on the perfusate samples were extracted overnight in 10 ml of chloroform:methanol (2:1 vol/vol), after which a Folch procedure was performed. Quantitative thin-layer chromatography was used to measure free cholesterol ester, and phospholipid in duplicate as previously described. Red cell lipid counts were also performed on the perfusate.

To document that the experimental model was highly atheromatous, and thus suitable for study of possible lipid embolization, we determined the lipid concentration in the arterial wall. Therefore, mean left iliac artery lipid composition was measured on a group of six control rabbits that underwent deendothelialization and cholesterol feeding as in the experimental group but not angioplasty and formalin fixation. After 6 weeks on the atherogenic diet, the rabbits were killed under pentotal anesthesia and the left iliac artery was removed, opened longitudinally, and rinsed free of any residual blood with cold saline. The intimal-medial portion of the vessel was then carefully stripped away from the adventitial layer according to the method of Wolinsky and Daily. The samples were minced with
Results

Experimental Atherosclerosis

After 6 weeks on the atherogenic diet, all eight experimental rabbits had angiographic and histologic evidence of marked atherosclerotic disease. The mean serum cholesterol was 1476 ± 467 mg/dl. An example of the type of lesion created is shown in figure 2, which is a cross section of the left iliac artery. It demonstrates marked intimal thickening that is highly cellular and contains accumulations of foam cells. The atheromatous nature of this lesion is dramatically visualized under polarized light microscopy. A considerable amount of lipid was deposited in the thickened intima and indicates the suitability of this experimental lesion for investigating the possibility of lipid embolization after balloon dilation.

The mean lipid composition (mg/g wet weight) of the left iliac artery of the six control animals that did not undergo angioplasty and formalin fixation was: cholesterol, 15.01 ± 11.1; cholesterol ester, 57.4 ± 38.2; and phospholipid, 7.13 ± 3.7. The mean value of four normal left iliac artery segments that did not have cholesterol feeding or endothelial denudation was: cholesterol, 0.8 ± 0.3; cholesterol ester, 1.23 ± 0.8; and phospholipid, 3.41 ± 0.8.

Angiography

Table 1 is a summary of the angiographic results before and after transluminal angioplasty. All eight rabbits showed an increase in luminal diameter of greater than 20%. Proximal segments that did not undergo angioplasty showed no change in luminal diameter. Dissection, defined as an angiographic linear density, was seen in four of eight animals. Three rabbits showed aneurysm formation.

No evidence of extravasation of dye, catheter perforation, or total occlusion of a vessel after angioplasty was noted. Positioning of the catheter at the site of greatest iliac stenosis was performed successfully and with little difficulty in all rabbits except one (no. 7), in which the angle between the Cordis sheath and the left iliac artery as well as the presence of a long concentric lesion made catheter placement difficult. Figure 3 is an example of the angiographic results of transluminal angioplasty.

Pathology

Three types of histologic results were seen in the dilated segments (table 2). In five of eight rabbits, there was fracture through the neointima, and a flap of

![Figure 2. A balloon/cholesterol fed lesion viewed with polarized light microscopy. The atheromatous lipid is seen as brightly illuminated birefringent material, accumulated primarily in the intima (I) and also in the media (M). Magnification × 200.](http://circ.ahajournals.org/Content/35/11/919/F2.large.jpg)
the thickened intima often folded back into the lumen. In addition, there was dissection along the internal elastic membrane, at times extending into the media, in six of eight rabbits (fig. 4). Two of eight rabbits showed an eccentric foam cell lesion. Angioplasty in these vessels resulted in thinning of the noninvolved portion of the vessel wall without intimal fracture. The stretched portion of the wall showed loss of nuclear staining and densely packed layers of extracellular matrix.

Perfusate Analysis

Despite the marked degree of fracture, dissection, and stretching of the vessel wall, no evidence of red cells, cholesterol, or debris was detected in the perfusate in six of eight rabbits that had successful angioplasty (table 3). As the perfusion system was maintained in a continuous infusion of heparinized Ringer’s lactate solution, no debris was found adherent to the arterial or catheter wall. As thin-layer chromatography can measure 1–2 μg of cholesterol, and lipid analysis of similar iliac vessels documented a great amount of cholesterol in the vessel wall, embolization of small fragments of arterial debris could be easily detected by this technique. In rabbits 1 and 7, minimal bleeding was detected in the perfusate. Since the aorta and iliac branches were ligated as confirmed by angiography, this bleeding was probably derived from capillaries that penetrated the diseased vascular wall. Since the ratio of perfusate red blood cells to whole blood and perfusate cholesterol to plasma cholesterol were both 1:100, this lipid in the perfusate could be totally accounted for by bleeding from the neovascularization rather than from the atheromatous arterial wall itself.

In rabbit 7, angioplasty was very difficult. Visually, the catheter penetrated out of the lumen and into the adventitia. Angioplasty was nevertheless performed, and in the first aliquot after dilation, a 5-mm piece of atheromatous debris was noted in the collection (fig. 5). The detection of cholesterol by thin-layer chromatography and debris by polarized light microscopy indicates that the perfusion system can detect lipid material when present.

Discussion

Since transluminal angioplasty caused intimal plaque fracture and dissection in experimental studies,⁵,⁶ embolization of atherosclerotic debris could be an undesirable consequence of successful transluminal angioplasty. Using an experimental model with significant lipid deposition in the neointima, the present study provides evidence that embolization of plaque material is rare and unlikely. With the highly sensitive

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**FIGURE 3.** The angiographic results of transluminal angioplasty. Arrows indicate two lesions of approximately 50% stenosis before transluminal angioplasty. They showed total resolution of luminal narrowing after dilation.
The technique of thin-layer chromatography, no cholesterol from the atheromatous plaque was found in the perfusate. Polarized light microscopy is useful for visualizing the atheromatous lipid (fig. 2). With this technique, subcellular lipid droplets as small as 0.5 μm have been identified, and as little as 10 ng of cholesterol were detected in the present study. Therefore, if embolization of arterial wall lipid debris were to occur after transluminal angioplasty, polarized light microscopy would be another sensitive method for detecting its presence. In the present study, no evidence of embolic atheromatous debris was noted in seven of eight rabbits. Rabbit 7 had marked arterial trauma due to difficult catheter placement in a diffusely diseased vessel. Therefore, the lipid material collected in the perfusate of this rabbit is attributed to the damage to the arterial wall during catheter placement rather than to angioplasty itself.

There have been a few clinical reports of rare embolization after transluminal angioplasty of peripheral arteries. Gruentzig found evidence of embolization in 3% of patients undergoing angioplasty of peripheral arterial stenosis; Zeitler et al. reported three of 161 clinically detected cases of emboli after peripheral angioplasty in which minor local ischemia was noted, which cleared spontaneously. In the only prior attempt to collect blood distal to the site of angioplasty, Gruentzig et al. found no evidence of embolic debris on millipore filters collected after transluminal angioplasty performed during coronary artery bypass surgery. Endothelial denudation was reported after transluminal angioplasty in normal canine coronary arteries. The fate of these superficial non-lipid-laden cells is unknown. No endothelial cells were noted in centrifuged samples of the perfusate examined by bright field or polarized light microscopy; however, staining was not performed. Certainly, there is no evidence that the majority of the atheromatous lesions embolized in the present study. We emphasize that care must be taken in catheter placement to prevent significant arterial trauma.

We used an experimental model of atherosclerosis to investigate the effects of transluminal angioplasty. Marked intimal thickening and highly cellular foam

<table>
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Abbreviations: + or 0 = presence or absence, respectively, of red cells detected with light microscopy or lipid debris seen by polarized light microscopy.

*The cholesterol reported is the total cholesterol (free cholesterol and cholesterol ester).

Figure 4. Effects of angioplasty on the balloon/cholesterol-induced lesion in the rabbit iliac artery. The thickened intima shows gross fracture that is separated from the thinned media along the internal elastic lamina. The noninvolved portion of the vessel wall is also thinned. Elastin stain; original magnification × 50.
cell lesions predominate in this model (fig. 2). In addition, the lesion created is highly vascular, with media and adventitia neovascularization. Thus, the model is not entirely analogous to advanced human atherosclerosis, which includes cell necrosis and calcification. However, Block et al. found histologic findings in three patients who died within 3 days of transluminal angioplasty that were similar to previous experimental findings. This is also found in the perfusion system used in the present study, as well as in prior in vivo rabbit experimental studies. It is thus likely that the findings of the present study reflect transluminal angioplasty in human atherosclerotic disease.

Experimental angioplasty studies have documented endothelial denudation and platelet adhesion, fracture of the intimal plaque with or without dissection along the elastic membrane, and stretching of the noninvolved portion of an eccentric lesion after transluminal angioplasty. Studies in coronary arteries of human cadaver hearts have shown endothelial disruption, and compression of atheroma has been suggested as a mechanism of dilation, although detailed histologic analysis was not performed. Morphometric studies measuring luminal and arterial wall size, and intimal and medial cross-sectioned areas are necessary to confirm compression and also to determine if aneurysm formation is a mechanism of successful angioplasty. Furthermore, although angioplasty is often successful in reopening a stenotic vessel, it still produces considerable arterial wall trauma. Thus, with respect to the response to injury hypothesis of atherogenesis, the long-term consequences of angioplasty remain to be determined.

References

Transluminal angioplasty in experimental atherosclerosis. Analysis for embolization using an in vivo perfusion system.

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