Restenosis Following Transluminal Angioplasty in Experimental Atherosclerosis


Percutaneous transluminal angioplasty has received considerable attention in the treatment of obstructive atherosclerotic lesions in humans. However, restenosis frequently occurs and has limited the long-term effectiveness of this procedure. To study restenosis, a model of atherosclerosis was developed in 16 New Zealand rabbits. Atherosclerosis was induced in one or both iliac vessels by balloon deendothelialization followed by a 2% cholesterol diet for 6 weeks. Angiographic lesions were demonstrable in all animals. Fourteen iliac vessels served as controls, and nine underwent successful angioplasty with an increase in luminal diameter from 1.0 ± 0.2 to 1.9 ± 0.4 mm (p < 0.01). After 4 weeks on a high cholesterol diet, all animals had another angiogram, which documented significant progression of disease in only six of 14 control iliac vessels, but in all nine dilated vessels. The average decrease in luminal diameter was 0.2 ± 0.3 mm for the control group compared with 1.6 ± 0.5 mm for the dilated group (p < 0.01). Histopathological correlates revealed further remodeling of the neointima, with the presence of additional loose fibrous tissue and thrombi at various stages of organization and recanalization.

In summary, this study demonstrates that restenosis occurs following transluminal angioplasty and is significantly more frequent than the natural progression of disease in a rabbit model of atherosclerosis. The mechanism of this restenosis appears to be related to intraluminal thrombosis and acceleration of atherosclerosis. Evaluation of antiplatelet drugs in the prevention of restenosis seems warranted.


One of the important central tenets in the hypothesis of atherosclerosis is injury of the vascular wall. Transluminal angioplasty, a technique that may cause endothelial denudation and vascular wall damage, might be expected to accelerate the atherosclerotic process.2-9 The clinical results of coronary angioplasty suggest that as many as 30% of patients who have undergone a successful procedure have a recrudescence of symptoms and significant restenosis at the site of initial successful coronary dilatation.10-11

We and others9-9 have previously shown that the rabbit is a suitable model for the study of transluminal angioplasty; the results of the immediate effects show a variety of histopathological findings including dramatic intimal splitting as well as dissection or stretching of the vessel wall. In addition, we have shown that the primary mechanism in angioplasty is stretching of the vessel wall and localized aneurysm formation.9 To examine the mechanism by which angioplasty may induce restenosis, we undertook this experimental animal study.

Methods

Aortic, left, and right iliac atherosclerosis was induced in 16 male New Zealand white rabbits whose average weight was 3.0 kg. Seven control animals had bilateral iliac atherosclerosis induced, thus providing 14 control vessels for analysis. Nine additional animals had unilateral left iliac atherosclerosis induced and subsequently underwent angioplasty. Ini-
ually, all rabbits were anesthetized with Pentothal after which aortic, left and/or right iliac endothelial debridement was performed via a distal femoral arteriotomy just above the knee, as described by Baumgartner. A 3-F Fogarty balloon catheter was passed retrograde to 30 cm and inflated until contact was made with the endothelium. Adequate deendothelialization was accomplished by gently withdrawing the catheter twice. All animals were placed on a 2% cholesterol diet composed of rabbit chow supplemented with 10% peanut oil. With this diet, serum cholesterol values ranged between 1000 to 2000 mg/dl.

Atherosclerosis was allowed to develop for 6 weeks; then angiography was performed. In those animals with unilateral diseases (n = 9), the right femoral artery was surgically exposed and a 4-F Goodale Lubin catheter was passed in a retrograde fashion via a right femoral arteriotomy positioned by fluoroscopy just above the aortic bifurcation. In all animals with bilateral disease, aortography was performed through a right carotid arteriotomy using a No. 5 Swan Ganz catheter placed just above the aortic bifurcation. Renografin 76 (3 cc) was injected over 3 seconds, and angiographic images were recorded on 35 mm film using a Philips 6-inch image intensifier (North American Philips Corporation, Shelton, Connecticut) with the resolution of 3.8 lines pairs/mm. In nine animals, the distal left femoral artery was surgically exposed just proximal to the previous ligation, and a 2.5 mm Gruentzig intraoperative transluminal angioplasty catheter (USCI, Billerica, Massachusetts) was positioned at the point of highest grade iliac stenosis as determined on initial angiography. The balloon was inflated three times to five atmospheres for 30 seconds. The size of the catheter was carefully chosen to closely approximate the size of the least stenotic proximal portion of the iliac vessel to prevent overdistention. After removal of the dilation catheter, a repeat angiogram was performed. The arteriotomy was then closed by ligation. During each angiogram, care was taken to position the image intensifier at the same height, and a 1 cm grid was positioned at the level of the spine to provide correction for magnification error. Cineangiograms were then viewed on a Vanguard projector (Vanguard Instrument Corporation, Melville, New York) with the diameter in millimeters recorded for the highest grade stenosis in each iliac vessel. Care was taken to measure the diameter at the same site in both the dilated and control segment at baseline and on all subsequent angiograms. A change in vessel diameter of 0.2 mm could be easily resolved using this technique and was felt to be significant. Each angiogram was independently read by two readers, and a discrepancy was resolved by a subsequent simultaneous reading.

All animals were maintained on a 2% cholesterol diet for the follow-up period. Four weeks after initial angiography, all rabbits were reanesthetized and underwent a repeat angiogram as described above. Within 24 hours of the repeat angiogram, the animals were sacrificed using Pentothal anesthesia, and a 10% formalin solution was infused via the aorta for 15 minutes at a pressure of 80 mm Hg. The aorta and iliac vessels were surgically removed and placed in formalin. Sections of the abdominal aorta and serial 1 cm sections of the left and/or right atherosclerotic iliac artery were obtained. This allowed at least two sections through the dilated area to be examined by light microscopy. Care was taken to examine sections that represented the highest grade stenosis in the nondilated sections. Standard hematoxylin and eosin as well as von Giesen elastin stains were prepared; stained sections were then reviewed by at least two investigators, and a consensus interpretation of the histological findings was made.

Morphometric analysis was performed by the following method. Stained histologic sections were projected onto a Zeiss MOP II digital image analyzer (Carl Zeiss, New York, New York) to allow calculation of the cross-sectional areas of the left and right iliac artery lumen and arterial wall (neointima plus media as demarcated by the external elastic lamina). The total vessel cross-sectional area was calculated by addition of the arterial wall and lumen areas. Nonpaired Student’s t test was used to determine statistical significance, and a p value of less than 0.05 was accepted as significant.

Results

Angiography

The iliac lesions were demonstrable by angiography in each experimental group, with a luminal diameter narrowing of 0.7 to 1.8 mm (Table 1). The control vessel and the dilated vessel before angioplasty showed similar degrees of luminal diameter narrowing. When the most stenotic portion of the vessel was compared with the proximal least diseased portion of the vessel, these lesions represented stenoses of 30% to 70%. Transluminal angioplasty increased the diameter of the iliac vessel by 0.3 mm or more in all dilated animals, with a mean increase in luminal diameter of 0.9 ± 0.4 mm (p < 0.01).

The follow-up 4-week angiogram showed significant narrowing of the luminal diameter in six of 14 control vessels and in all nine dilated vessels. Total occlusion of the vessel was angiographically demonstrated in one control vessel and in three dilated vessels. When the angiographic diameter at 4 weeks was compared to the diameter at the time of initial angiography, there was no significant change in the control group (mean change was −0.2 ± 0.3 mm). In contrast, however, the dilated vessels showed restenosis at 4 weeks, with an average decrease in luminal diameter of 1.6 ± 0.5 mm (p < 0.01). Figure 1 shows angiograms of an animal with progressive stenosis.
Table 1. Angiographic Results of Transluminal Angioplasty (TA): Lumen Diameter (mm)

<table>
<thead>
<tr>
<th>No.</th>
<th>Before</th>
<th>4 Weeks later</th>
<th>Change</th>
<th>No.</th>
<th>Before TA</th>
<th>Immediately after TA</th>
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<td>1.5</td>
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Mean ± SD: 1.2 ± 0.4, 1.0 ± 0.4, -0.2 ± 0.3, 1.0 ± 0.2, 1.9 ± 0.4, 0.7 ± 0.6, -1.6 ± 0.5

*p value: [----- NS -----]  [----- 0.01 -----]  [----- 0.01 -----]

A vs B: [-------------------- NS -------------------]  [-------------------- 0.01 -------------------]

Histopathology

At sacrifice, the control vessels demonstrated predominantly concentric foam cell lesions, with a markedly thickened neointima and fibrous cap, as we have previously shown. The media was also composed of foam cells with scattered areas of fibrosis. Rarely was calcification or necrosis evident.

Histopathologic examination of the dilated segment at 4 weeks disclosed several patterns. In Figure 2, an animal sacrificed 24 hours after successful transluminal angioplasty is contrasted with another animal sacrificed 4 weeks after effective angioplasty. As previously reported, the immediate result of angioplasty is a dramatic gross rupture through several layers of the intima with accompanying inflammation and stretching of the vessel. After 4 weeks, a pattern of fibrocellular tissue reminiscent of the original neointimal tears is seen, with the spaces filled by abundant loose connective tissue. This tissue is rich in lipid, is edematous, and shows a new fibrocellular cap creating a further occlusive process.

Other patterns observed included a multilaminated neointimal process with layers of loose connective tissue and lipid, alternating with denser fibrocellular tissue, as seen in Figure 3. Thrombus formation with various degrees of organization was noted in five animals. All but one occurred in totally occluded vessels. While only one thrombus occurred in the 14 control vessels, four of the nine vessels that underwent angioplasty demonstrated significant intraluminal thromboses (p < 0.01; Figure 4). Morphometric analysis revealed no
Figure 2.  

A. Acute effects of angioplasty on a rabbit iliac artery showing a concentric, mostly fibrocellular, intimal thickening. The intima is ruptured and extends as flaps into the lumen. The cleavage is partially along the internal elastic lamina and partially within the media. × 58  

B. Chronic effect 4 weeks after angioplasty. The flap-like extensions of thickened intima ruptured by the angioplasty are still visible (arrows). Most of the luminal space is filled with loose tissue containing foam cells and a loose extracellular matrix. Portions of this tissue have a myxoid appearance and contain only few cells. The internal elastic lamina in this section is intact. A new dense fibrocellular cap is formed around the very narrowed residual lumen. Note multilayered capillaries penetrating the media. × 67.
Figure 3. Detail of an occlusive lesion 4 weeks after angioplasty. The media (right) shows necrotic debris, foam cells, and penetrating capillaries. The extremely thickened intima shows multiple layers of varying densities of cells and extracellular matrix. Some loose portions of the intimal thickening have a myxoid appearance. × 200.

Figure 4. A well-organized thrombus is located on the predominantly fibrocellular intimal thickening of a rabbit iliac artery 4 weeks after angioplasty. Vascular lumina of varying sizes within the organized thrombus indicate recanalization. × 105.
significant differences between the nondilated control vessels and the dilated vessels (Table 2). However, the lack of significance appears to be related to the wide standard deviation with a trend toward a smaller lumen, larger intima and media, and larger total vessel area in the dilated vessels.

### Discussion

The development and clinical utilization of transluminal angioplasty has provided the clinician with an additional therapeutic tool in the treatment of obstructive atherosclerotic vascular disease. With the advent of coronary dilatation and Gruentzig's initial successful experiences, the procedure has received widespread publicity. Follow-up studies of patients who have undergone peripheral, as well as coronary, angioplasty have shown excellent short- and long-term results. However, in a significant number of patients (estimated at 20% to 30%), early restenosis following coronary angioplasty has been found; restenosis usually occurs within 6 months of the initial angioplasty. The factors responsible for this rapid restenosis are unknown.

We and others have previously demonstrated that angioplasty in this model of experimental atherosclerosis results in stretching of the vessel wall with or without intimal fracture or dissection, depending upon the arterial wall histopathology. Morphometric analyses of dilated vessels have verified that angioplasty works primarily through stretching of the vessel with localized aneurysm formation. No evidence for compression of the atheroma could be demonstrated in these studies. In addition, we have shown that acute embolization of lipid or debris does not occur in this model. These acute studies verify the pathology reports.

The results of the present study extend these observations and indicate that despite the initial angiographic improvement in luminal narrowing, restenosis occurs in all vessels after angioplasty. The lack of a change in the control vessels despite the continued stimulus of a high cholesterol diet suggests that it is the injury induced by transluminal angioplasty that stimulates restenosis, perhaps through further atherosclerotic progression. It is possible, however, that the double ligation of the left iliac vessel in our treatment group might have reduced blood flow in this vessel and accelerated atherosclerosis. This seems unlikely since the ligations were performed distally at the knee, a site with abundant collateral vessels. Additionally, angiography documented excellent flow through these vessels.

This study did not demonstrate a greater degree of atherosclerosis by morphometric analysis; however, a trend toward greater progression in the dilated area than in the control vessels is suggested. This is not surprising if one considers angioplasty in the context of what is commonly referred to as “the response to injury hypothesis” of atherogenesis. Ross and Glomset and others have suggested that injury to the vascular endothelium may result in a complex series of interactions between the vascular smooth muscle cells, platelets, and lipids. Following vascular injury, platelet adhesion, aggregation, and accumulation of fibrin on the vessel surface have been demonstrated. Pasternak et al. have shown that transluminal angioplasty can cause considerable endothelial debridement in dogs and that dense platelet adhesion to the denuded subendothelium occurs immediately. This interaction of platelets and subendothelium has been shown to be even more intense in the setting of reinjury of an atherosclerotic lesion. Repeated vascular injury even in the absence of hyperlipidemia has induced atherosclerosis. The importance of platelet adhesion for stimulating atherosclerosis has been emphasized by several recent studies. Metke and colleagues have shown that intimal hyperplasia in canine coronary bypass grafts can be successfully inhibited by antiplatelet drugs. Clinical studies have recently confirmed the usefulness of antiplatelet drugs in reducing coronary vein graft occlusion. While progression of atherosclerosis is suggested by our study, thrombosis appears to be a prominent component of restenosis following angioplasty, since four of nine animals had intraluminal clots and a marked degree of restenosis following angioplasty.

Attempts to extrapolate the results of this experimental study to human atherosclerosis can be validly criticized because of the differences in composition and complexity of atherosclerosis. Human lesions have significantly greater amounts of necrosis and calcification and, as such, these lesions might be less likely to show progression of disease. The type of atherosclerotic lesions in our study is analogous to the early “soft” atherosclerotic lesions commonly thought to be most suitable for angioplasty and also frequently found in bypass grafts. Despite this criticism, the results of animal experiments have closely paralleled the postmortem and histopathologic find-
ings in patients who died following angioplasty.\textsuperscript{2-4} In addition, the 30\% incidence of early restenosis with coronary angioplasty is consistent with the high incidence of restenosis found in this experimental model.

In conclusion, although transluminal angioplasty may have salutory effects in atherosclerotic narrowings, this study in experimental rabbit atherosclerosis documents a propensity for subsequent luminal narrowing. This restenosis may be partly explained by the apparent injurious nature of angioplasty in the face of continuing atherogenic stimulus. However, the importance of platelet adhesion needs further study. In addition, this study suggests that intraluminal thrombosis plays a prominent role in reocclusion of the vessel.

References
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