Acute Effects of Transluminal Angioplasty in Three Experimental Models of Atherosclerosis


Transluminal angioplasty has shown promise as a nonoperative treatment of atherosclerotic obstruction. Despite its increasing clinical use and potential importance, little is known of its mechanism and acute effects. To evaluate transluminal angioplasty, three rabbit models of experimental atherosclerosis were developed: Group 1 (n = 20) = high cholesterol diet plus balloon de-endothelialization; Group 2A (n = 12) = high cholesterol diet plus an Indwelling catheter; Group 2B (n = 10) = normal diet plus an indwelling catheter. After 6 weeks or 8 weeks, distinct angiographic and pathological lesions in the iliac artery were evident in all groups. Group 1 showed predominant foam cell lesions, while Group 2 showed eccentric mixed fibrous and foam cell or only fibrous lesions. Significant angiographic stenosis was present in 78% of the animals. Angioplasty of the highest grade iliac stenosis resulted in at least a 20% reduction in luminal diameter narrowing in 26 of 37 animals (70%). Histopathological examination 1 day following angioplasty in 17 animals showed two patterns. In Group 1 animals, neointimal fracture and dissection were evident, while in Group 2 animals thinning and stretching of the nonatherosclerotic portion of the vessel walls could be demonstrated. This study demonstrates that the New Zealand rabbit can be used to produce a spectrum of morphologically distinct atherosclerotic lesions that lend themselves to the study of transluminal angioplasty. The immediate consequences of angioplasty, which appear to depend upon the underlying histopathology and widening of the narrowed lumen, are frequently concurrent with intimal fracture, dissection, or thinning of the nonatherosclerotic portion of the vessel wall.

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The development and clinical utilization of transluminal angioplasty has provided a nonsurgical treatment of atherosclerotic obstruction. In 1964, Dotter and Judkins described a method for enlarging the lumen of stenotic peripheral vessels using a percutaneous catheter system. The development of a unique double balloon catheter system in 1973 has allowed successful nonsurgical treatment of peripheral, renal, coronary, and cerebral vascular disease in selected patients. The results of peripheral percutaneous dilatation have been encouraging with an initial success rate of 80% and a patency rate of 73% after 2 years. The use of transluminal angioplasty in the treatment of coronary artery obstruction has been most encouraging, since this disease comprises a major health problem worldwide. Initial reports from a national registry sponsored by the National Institutes of Health have indicated an initial success of 63% with a low mortality and excellent long term symptomatic relief of angina in the first 1500 patients reported.

Despite the early promise of this treatment, little is known about its mechanism of action, long-term consequences, and whether a lasting amelioration of obstructive atherosclerosis will be possible. It was initially postulated that the enlargement of the vascular lumen was due to compression of the atherosclerotic plaque. However, other possibilities include redistribution of the atheroma, embolization of atherosclerotic debris, stretching or aneurysm formation, and localized fracture or dissection of the plaque. This experimental animal study was undertaken to develop an animal model that would be suitable for the study of transluminal angioplasty and to evaluate the possible mechanism of its immediate effects.
Methods

Aortic and left iliac atherosclerosis was developed in 42 New Zealand white male rabbits each weighing 3 kg. Three distinct methods combining vascular injury with cholesterol feeding provided a spectrum of atherosclerotic disease. Group 1 rabbits (n = 20) were anesthetized with pentobarbital. After surgical exposure of the left femoral artery, aortic and left iliac endothelial debridement was performed by the technique described by Baumgartner. In this technique, a 3F Fogarty balloon catheter is passed to 30 cm and inflated until gentle contact with the vascular wall is achieved. De-endothelialization is accomplished by gradually pulling the catheter down the aorta and iliac vessels. To assure adequate de-endothelialization, the Fogarty balloon was passed twice to 30 cm. All animals were then placed on a 2% cholesterol diet composed of rabbit chow supplemented with 10% peanut oil for 6 weeks. On this diet, serum cholesterol levels were found to be in the range of 1000 to 1200 mg%.

Group 2 animals (n = 22) had atherosclerotic lesions produced by the surgical placement of a 20 cm P50 polyethylene catheter into the left femoral artery and advanced into the aorta to approximately the upper abdominal level as described by Moore. The end of the catheter was sutured in place and allowed to remain in the artery for 8 weeks. Previous pilot studies had indicated that an 8-week period was necessary to develop atherosclerosis with this method. Twelve animals were placed on a 2% cholesterol diet and the remainder on normal rabbit chow.

After 6 or 8 weeks, during which atherosclerosis was allowed to develop, all animals underwent abdominal arteriography in the following manner: surgical exposure of the right and left femoral artery was performed and the indwelling catheter in Group 2 animals removed. In no animal was this removal traumatic. A 4F or 5F Cournand or Goodale Lubin catheter was introduced into the right femoral artery and passed retrograde to a position 1 to 2 cm above the aortic bifurcation, just below the renal arteries as verified by fluoroscopy. Renograffin 76 (3 cc) was injected over 3 seconds, and angiographic images were recorded on 35 mm film using a Philips 6-inch image intensifier with a resolution of 3.8 line pairs/mm. A 2.5 or 3.0 mm Gruntzig intraoperative transluminal angioplasty catheter was then placed retrograde under fluoroscopic guidance through the surgically exposed left femoral artery. With use of a video recording of the angiogram, the dilatation catheter was positioned at the point of highest grade iliac stenosis and inflated three times to 5 atmospheres for 30 seconds. The catheter size and inflation pressure was chosen to carefully approximate clinical use. After removal of the catheter, a repeat angiogram was performed. 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 in order to provide correction for magnification error. The pre- and postdilatation cine-angiograms were then compared to estimate any change in the narrowest segment that underwent dilatation. Percent stenosis was estimated in each animal by using the proximal nondilated but diseased segment in the left iliac artery as the control segment. In addition, the size of the dilated segment and adjacent nondilated segment was calculated using the grid to correct for magnification.

A change in vessel size of 20% could readily be resolved using this technique and was felt to be significant. Each angiogram was read independently by two angiographers, and discrepancies resolved by a subsequent simultaneous reading.

One day following angiography, 17 animals (six in Group 1, and 11 in Group 2) were sacrificed by pentothal anesthesia during which a 10% solution of formalin was infused via the aorta at a pressure of 80 mm Hg for 15 minutes as previously described. The aorta and iliac vessels were surgically removed and placed in formalin. Sections of the abdominal aorta and control right iliac as well as serial 1 cm sections of the left iliac artery were obtained. This allowed examination by light microscopy of at least two sections through the dilated area. Sections stained with hematoxylin and eosin and Verhoff Von Giesen elastic were viewed by at least two investigators, and a consensus reading was made as to the histological findings. The remaining animals were followed to determine the longer term effects of angioplasty and were sacrificed at 2 or 4 weeks after angioplasty. Details of this longer term study are the subject of a later report.

Results

Atherosclerotic Model

Three morphologically distinct atherosclerotic lesions were produced by the combination of vascular injury and variations of cholesterol feedings, as shown in table 1. All animals, those sacrificed at 24 hours and at 2 or 4 weeks postangioplasty, were included in this analysis. Group 1 animals (n = 20) demonstrated concentric foam cell lesions characterized by marked intimal thickening and filled with lipid-laden cells and a fibrous cap. In addition, intracellular lipid was frequently noted in the media. Only rarely was significant fibrosis noted in this group of animals. A representative example of this type of lesion is seen in figure 1.

<table>
<thead>
<tr>
<th>Table 1. Histopathology of Experimental Models</th>
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<tr>
<td>Model</td>
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<tr>
<td>Group 1 (n = 20)</td>
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<td>Group 2A (n = 12)</td>
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<td>Group 2B (n = 10)</td>
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The animals in Group 2 that received a high cholesterol diet (Group 2A = 12) showed predominantly eccentric mixed lesions with a thick fibrous cap lying over a cellular lipid neointima. A representative example of the type of lesion is shown in figure 2.

Group 2 animals on a normal rabbit diet (Group 2B = 10) showed predominantly eccentric fibrous lesions with rare components of cellular lipid material in the neointima. A representative example is shown in figure 3. In both Group 2A and 2B animals, a small lumen could frequently be identified within the neointimal lesion that represented the site of the indwelling catheter.

Angiography

Angiography documented a range of left iliac stenoses. A significant focal lesion could be identified angiographically when the lesion exceeded 30% in diameter narrowing. Thus, a significant stenosis was present in 29 of 37 rabbits (78%) ranging from 30% to 100% stenosis (figure 4). Five animals did not have an adequate angiogram available for analysis. The most severe stenosis occurred in Group 1 animals, with eight of the 19 animals showing greater than 50% narrowing.

Angioplasty was possible in all three models, with a significant change in stenosis occurring in 26 of 37 animals (70%). The ability to dilate a lesion could not be related to the histopathological findings since a greater than 20% change in luminal diameter was noted in all three models. The greatest angiographic change, however, occurred in those animals with the highest degree of stenosis. One animal showed a 100% obstruction of the left iliac artery, and transluminal angioplasty was possible with a good angiographic result. A representative example of the angiographic change in a Group 1 animal is shown in figure 5.
Figure 2. Example of an eccentric, mixed lesion in the iliac artery of a rabbit of Group 2A. The intimal lesion consists of a fibrocellular cap of varying cell density and of a large accumulation of foam cells. The internal elastic lamina is fragmented. The media is thin and contains little lipid deposition. × 82

Figure 3. Example of an eccentric, predominantly fibrotic lesion in the iliac artery of a rabbit of Group 2B. Note the relatively well-preserved tunica media and the wavy internal elastica showing two small defects. The channel in the thickest portion of the lesion is caused by the indwelling catheter. × 75
Table 2. Immediate Results of Angioplasty in Group 1

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dilatation (%)</th>
<th>Fracture</th>
<th>Dissection</th>
<th>Thickening</th>
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**Histopathology**

In the 17 animals sacrificed within 24 hours of angioplasty, microscopic examination revealed two histopathological patterns that related to the morphology of the underlying lesion.

In Group 1 rabbits, the immediate consequence of transluminal angioplasty was the development of a marked neointimal fracture in four of six rabbits (table 2). This finding was most marked in animals showing the greatest degree of angiographic improvement. The neointimal fracture characteristically showed a dramatic rupture through several layers of neointima often extending into the media and splitting and fragmenting the internal elastic lamina (figure 6). On occasion, this fracture was associated with dissection, distortion, and thinning of the original media (figure 7). In addition, an acute inflammatory response was often noted adjacent to the intimal fracture (figure 8). In Group 2 animals with eccentric hard fibrocellular or mixed lesions, thinning and necrosis of the normal vessel wall was common (table 3). There were no differences in the histological findings between Groups 2A and 2B. Neointimal fracture was not seen in this group regardless of the degree of angiographic change in the lesion. It appeared that the major effect of angioplasty was thinning, possibly due to stretching of the normal media distant to the atherosclerotic plaque (figure 9). The stretched vessel wall frequently showed necrosis and loss of smooth muscle cells with moderate inflammatory response (figure 10). In no Group 1 or 2 animal was significant thrombosis present despite the lack of anticoagulation.

**Figure 4.** Angiographic results of transluminal angioplasty. **Left Panel:** Each bar represents the percent stenosis in each animal group. The numbers above each bar are the number of animals represented. **Right Panel:** The majority of animals had ≥ 20% reduction in stenosis after transluminal angioplasty.

**Figure 5.** An angiographic example of transluminal angioplasty in a Group 1 animal showing a high grade stenosis in the left iliac artery (arrow). The angioplasty balloon is placed at the lesion in the middle panel with improvement in the lesion immediately after. A small linear dissection is evident at the site of angioplasty.
Figure 6. Acute effect of the angioplasty on an iliac artery with a mixed intimal lesion. The thickened intima is ruptured and is separated from the media along the internal elastic lamina over a large portion of the circumference. × 60

Figure 7. Acute angioplasty effect on a rabbit iliac artery which was virtually occluded by a lesion. The angioplasty balloon has cleaved the thickened intima from the media, opening up a new lumen with aneurysmal dilations and overstretching of portions of the media. The intimal lesion containing many foam cells appears not to be compressed. × 40
Figure 8. Detail of the ruptured intima and the separation from the media 24 hours after angioplasty. Note the abundance of acute inflammatory cells infiltrating both damaged layers. × 196

Figure 9. Marked acute dilation by angioplasty of a rabbit iliac artery bearing an eccentric, relatively thin fibrocellular lesion in a Group 2B animal. The portion of the vessel wall with the intimal thickening seems to be less affected than the opposite portion, which shows extreme stretching and thinning of the media. × 60
Table 3. Immediate Results of Angioplasty in Groups 2A and 2B

<table>
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<th>Dilatation (%)</th>
<th>Fracture</th>
<th>Dissection</th>
<th>Thinning</th>
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Discussion

This study demonstrates that the New Zealand rabbit can be used to produce a spectrum of morphologically distinct lesions that lend themselves to the study of transluminal angioplasty. Experimental atherosclerosis created in this animal model varies from human atherosclerosis in that fibrosis, necrosis, extracellular lipid, and calcium are frequently absent. In contrast, the lesions created in the rabbit contain abundant lipid-laden foam cells with little extracellular cholesterol. However, Block et al.9 have suggested that the rabbit is a desirable animal model for the study of balloon angioplasty due to the rapid development of atherosclerosis, ease of handling, and relative low cost. In addition, the iliac vessels are similar in size and character to human coronary vessels and frequently develop concentric lesions of a high enough degree to be suitable for angioplasty. Recognizing the limitations of any one animal model of atherosclerosis, we have demonstrated that by using two methods of intimal injury, three histopathologically distinct types of atherosclerotic plaques can be created. This diversity provides a useful model for the study of angioplasty.

In this study, more than 75% of the animals developed significant angiographic obstruction and angioplasty could be accomplished irrespective of the histopathological findings. Clinical experience has suggested that fibrous lesions may be less amenable to angioplasty than soft lipid lesions.4 Although we are unable to comment on concentric fibrous lesions, the ability to dilate did not appear to relate to the degree of neointimal fibrosis. It is possible that human lesions with more necrosis, fibrosis, and calcium respond differently to transluminal angioplasty.

The mechanism by which transluminal angioplasty reduces luminal narrowing in humans remains largely undefined. Earlier clinical and pathological studies have suggested that redistribution or compression of the intramural lipid within the plaque might occur; however, there is little experimental evidence to support this hypothesis. Castaneda-Zuniga et al.10 suggested that aneurysmal dilatation of the atherosclerotic vessel occurs following balloon angioplasty. Studying human autopsy iliac and femoral arteries as well as normal dog peripheral vessels,

Figure 10. Detail of the overstretched nonatherosclerotic media opposite an eccentric intimal thickening 24 hours after angioplasty. Endothelial cells are absent, medial smooth muscle cells are necrotic, and the media is thin and contains polymorphonuclear leukocytes in its luminal portion. × 230
the authors showed that angioplasty results in a bulging at the site of dilatation, which could be prevented by placing the vessel within a constricting glass tube. The findings of the present study would support the concept that when eccentric, fibrotic lesions are present, balloon dilatation results in stretching of the nonatherosclerotic media and aneurysm formation. Pasternak et al. demonstrated that, in a normal canine coronary artery, angioplasty causes loss of endothelium with stimulation of platelet adhesion and thrombosis formation. Although heparin and aspirin were ineffective in reducing the platelet adhesion, dextran was effective. In subsequent experiments in rabbits, these authors demonstrated that angioplasty of the aorta and iliac vessels frequently caused desquamation of the endothelium and splitting of atheroma. The present study extends these preliminary observations and demonstrates that marked intimal damage with fracture through the neointima is common in the foam cell lesions typical of this model. This fracture frequently extends into the media with disruption of the internal elastic lamina. In addition, dissection, as well as thinning of the media, occurs with a significant acute inflammatory response often adjacent to the fracture plane. We have also demonstrated that in eccentric fibrocellular lesions thinning and stretching of the media occurs and neointimal fracture is distinctly absent. Thus, the histopathological lesion determines the immediate microscopic findings after angioplasty. It is noteworthy that no evidence of significant thrombosis occurred following angioplasty. Although we did not specifically examine the specimens for desquamation and platelet adhesion, it seems likely that this did occur. Perhaps the improvement in vessel flow and spontaneous thrombolysis prevented thrombotic complication.

Since no animal model of atherosclerosis exactly represents human atherosclerosis, direct extrapolation of these findings to patients is subject to criticism. However, the degree and type of histological findings in this study are consistent with human autopsy studies. Freudenberg et al. demonstrated that vascular dissection frequently occurred in autopsies of humans who died with 3 days of successful percutaneous transluminal angioplasty. However, Lee et al. reported that only minimal disruption of the arterial wall could be documented in 12 human cadaver hearts that underwent angioplasty. Pathological material obtained from these patients who died within 3 days of successful percutaneous transluminal angioplasty has shown significant fractures of the atheroma extending into the media. The histological findings in these patients are identical to that noted in our Group 1 animals.

The results of this study demonstrate that the New Zealand rabbit can be used to produce a spectrum of morphologically distinct atherosclerotic lesions that can be assessed by angiography and that lend themselves to the study of transluminal angioplasty. In addition to the appropriate size and degree of stenosis, angioplasty can be accomplished in the majority of animals. This study provides evidence that, depending on the nature of the stenosing lesion, either marked intimal fracture and dissection, or thinning of the vessel wall, can occur as a consequence of successful angioplasty. Although these findings document the injurious nature of angioplasty, it is possible that these acute changes could result in long-term patency. In addition, despite marked histological findings, angiography documented no instance of perforation or sudden occlusion of the vessel. Further investigation of the long-term consequence of transluminal angioplasty and methods of improving the success of this procedure in this animal model should provide valuable information applicable to human angioplasty.

References

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