Atherosclerosis in Rabbit Vein Grafts

Robert M. Zwolak, Thomas R. Kirkman, and Alexander W. Clowes

Human coronary saphenous vein bypass grafts develop atherosclerosis more readily than do grafts made of internal mammary artery. The reasons for this increased susceptibility, particularly in the presence of hyperlipidemia, are not known. In this study in rabbits, we investigated the possibility that the increased susceptibility might be attributed to increased smooth muscle proliferation and foam cell accumulation in vein grafts compared to native artery. Hypercholesterolemic and control rabbits underwent placement of jugular vein grafts in the carotid artery. Dietary cholesterol content was adjusted to maintain serum cholesterol levels of 200 to 600 mg/dl in the fat-fed rabbits. The vein graft intimal thickness in hypercholesterolemic rabbits was greater than in normolipemic rabbits at 3 and 6 months after implant. The increased thickness in the hypercholesterolemic group was largely accounted for by an accumulation of lipid-laden macrophages. Medial thicknesses increased during the first month, remained constant at later times, and were similar in control and hypercholesterolemic animals. In both groups, endothelial and smooth muscle cell proliferation (thymidine labeling) increased immediately after graft implantation and declined at 3 and 6 months. No incremental mitogenic stimulus could be attributed to the hypercholesterolemia. In immunohistochemical preparations, the large foam cells were noted to be macrophages, and the intimal proliferating cells, to be smooth muscle. Proximal and distal carotid artery segments adjacent to the vein grafts in hypercholesterolemic rabbits had virtually no accumulation of lipid-laden macrophages. These results suggest that the endothelial and smooth muscle cell response in vein grafts adapting to the arterial circulation is not altered by hyperlipidemia, but that the wall becomes thick because of an accumulation of lipid-laden macrophages. (Arteriosclerosis 9:374–379, May/June 1989)

A utogenous saphenous vein grafts undergo early intimal thickening and accelerated atherosclerosis,1–13 both of which may contribute to eventual graft failure. While estimates of late vein graft stenosis and failure vary, in a 10-year angiographic follow-up study, Campeau et al.1 demonstrated that 30% of aorto coronary vein grafts patent after the first postoperative year were occluded at 10 years, and 32% were significantly stenosed. The mechanism of late failure appears to be atherosclerosis.13 Atherosclerosis commonly develops in vein grafts and appears to progress more rapidly than in native arteries. Hyperlipidemia is not only the most common risk factor associated with native artery atherosclerosis in Western society, but also is related closely to the accelerated failure of coronary vein grafts.1

Several studies have characterized the effects of hyperlipidemia on intimal thickening in experimental vein graft models. Rabbit femoral vein grafts subjected to intimal injury in the presence of hyperlipidemia develop a greater surface coverage by atherosclerotic plaque than do native arteries or native veins in the same animals or vein grafts in normolipemic rabbits.14 Similar findings have been described in hyperlipidemic dogs and rhesus monkeys.2,5,15,16 Although these studies all provide a morphological description of atherosclerosis in vein grafts, none has examined in detail the contribution of cell proliferation and accumulation in the process. In addition, it is not clear why vein grafts are so susceptible or how graft atherosclerosis differs from that in native artery.

Our earlier studies and those of other laboratories17,18,19 have shown that normocholesterolemic vein grafts become thicker largely because of smooth muscle proliferation and accumulation in the intima. The current study was designed to determine the effect of cholesterol feeding on wall thickening, cell accumulation, and the endothelial cell and smooth muscle cell proliferative response in rabbit vein grafts. We have addressed the possibility that accelerated vein graft atherosclerosis is really due to an augmented smooth muscle proliferative response to hyperlipidemia, as well as to an accumulation of lipid-laden macrophages.

Methods

Animal Model

Male New Zealand White rabbits weighing approximately 3 kg each were begun on an atherogenic diet containing 0.2% cholesterol.20 Venous blood samples were analyzed monthly for serum cholesterol and triglycerides. The concentration of cholesterol in the diet was adjusted to maintain the serum cholesterol level between 200 and 600 mg/dl. Control rabbits were fed commercial rabbit chow and underwent monthly phlebotomy for serum lipid analysis.
After 1 month of the atherogenic diet, the rabbits underwent surgical placement of an autologous jugular vein graft into the left common carotid artery.\textsuperscript{17} Anesthesia was induced with xylazine (7 mg/kg) and ketamine (35 mg/kg), and the left carotid artery and jugular vein were exposed through a vertical midline neck incision. After intravenous heparinization, a 3-cm segment of jugular vein was dissected free and sutured into the divided common carotid artery with interrupted 7-0 polypropylene sutures as described previously. Topical papaverine (15 mg) diluted in lactated Ringer’s solution was administered to the vein during the procedure. Animal care complied with the “Principles of Laboratory Animal Care” and the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 80-23, revised 1978).

**Morphology**

Groups of fat-fed and control rabbits were sacrificed at 4, 12, and 24 weeks after implantation of the vein grafts. One hour before sacrifice, the rabbits were anesthetized with ketamine and xylazine and were given intravenous doses of tritiated thymidine (0.5 mCi/kg; 6.7 Ci/mmol/l; New England Nuclear, Incorporated, Billerica, MA), Evans blue dye (25 mg/kg), and heparin (1000 units).

A 22-gauge angiocath was inserted in the brachial artery for constant blood pressure monitoring, and just before sacrifice, 25 mg/kg of intravenous sodium pentobarbital was administered. Systolic blood pressure was kept over 90 mm Hg with an infusion of intravenous lactated Ringer’s solution while a midline laparotomy was performed for placement of an abdominal aortic perfusion catheter and an inferior vena caval drainage catheter. The vascular system was flushed with lactated Ringer’s solution. Glutaraldehyde (2.5% in phosphate buffered saline, pH 7.4) was used for perfusion fixation with regulation of the inflow pressure and outflow rates to keep the intravascular pressure to 90 to 100 mm Hg. Vessels were excised at 1 hour and were fixed by immersion for 24 hours. Samples of graft were then prepared for light, transmission, and scanning electron microscopy, morphometric analysis of intima and media, cross-section smooth muscle autoradiography, and endothelial autoradiography.\textsuperscript{17}

Tissue samples to be used for immunohistochemical analysis were immersion-fixed in methyl Carnoy’s solution. Samples were processed as previously described and stained with antibodies specific for smooth muscle cells (HHF-35) or rabbit macrophages (RAM-11).\textsuperscript{21-23}

**Results**

The hyperlipidemic diet was well tolerated, and the fat-fed animals gained weight at the same rate as the controls. In general, serum cholesterol levels rose over 2 months and then remained stable. Response to the diet was variable among rabbits. Some required continuation of the 0.2% cholesterol regimen to maintain moderately elevated serum cholesterol, while others had markedly elevated serum cholesterol levels despite being adjusted down to 0.05% cholesterol. The cholesterol levels were maintained between 200 and 600 mg/dl. Triglycerides were not significantly elevated.

The fat-fed and control rabbits tolerated the surgical procedure equally well with no perioperative mortality and no difference in patency of the grafts (90%).

**Morphology and Morphometry**

By 1 month, in vein grafts of control rabbits, the patchy endothelial loss produced by the surgical manipulations\textsuperscript{17} was fully repaired, and thickening of the intima and media was evident. Most of the thickening was due to the accumulation of smooth muscle cells as identified by immunohistology.

At 1 month, the light microscopic appearance of the control and fat-fed groups was similar except that foci of lipid-laden macrophages (as identified by the macrophage antibody RAM-11) were located near the luminal surface in the hyperlipidemic rabbits (Figure 1A). Most of the graft wall stained positive for smooth muscle (HHF-35) (Figure 1B). At 3 and 6 months, a distinct inner layer or band of foam cells of grafts was present in the intima of fat-fed but not in the control animals (Figure 2). These cells were identified as macrophages based on their immunostaining characteristics and appearance in transmission electron micrographs (Figure 3). Because of this lipid-rich inner layer of intima, the total intimal thickness was greater in the fat-fed grafts at 3 and 6 months (Figures 2 and 4). The foam cell thickness did not change between the third and sixth months (Figure 4). In both groups, the medial thickness increased dramatically in the first month and then remained constant at the later time points (normal vein: 12±2 μm; 1-month grafts: control, 57±2 μm; fat-fed, 61±5 μm). In general, the cross-sectional area increased in parallel with intimal thickness up to a maximum at 3 months.

No accumulation of foam cells was seen at any time point in the adjacent carotid artery or in nongrafted veins in the fat-fed rabbits.

**Endothelial and Smooth Muscle Proliferation**

The endothelial cell thymidine labeling was increased at 1 month, was reduced at 3 and 6 months (Table 1), and was the same in the grafts of control and cholesterol-fed animals at all times. The pattern of smooth muscle proliferation was similar to what was observed for endothelial proliferation. Smooth muscle cell thymidine labeling was increased at 1 month and declined thereafter (Table 2). The values were similar in both dietary groups at each time point.

**Discussion**

In this study, the experiments were designed to examine cellular events in a model system of vein graft atherosclerosis. We found significant increases in the thickness of the vein graft wall in hyperlipidemic animals, with little direct effect on the underlying response of the venous endothelial cell reparative response or the smooth muscle accumulation after transplantation. The increased thickness could be attributed entirely to an influx of macrophages.

Perhaps the most striking feature of these data is the similarity in smooth muscle cell and endothelial cell labeling indices in vein grafts of normal and hyperlipidemic animals.
rabbits, but a marked difference in intimal thickness. Although vein grafts placed in the carotid artery were highly susceptible to early lesion formation, the effect of hyperlipidemia appeared to be on the addition of an inner layer of foamy macrophages, rather than an additional or superimposed stimulation of intimal smooth muscle cell proliferation. Thus, the data suggest that, at least in the short term, the mechanism stimulating smooth muscle cell proliferation in the adapting vein graft is not altered by cholesterol feeding. Since macrophages secrete growth factors for smooth muscle cell proliferation in vitro, we expected to see increased proliferation of smooth muscle cells adjacent to the foam cell layer. That we did not might be because of the absence of a sufficient concentration of growth factor or because of our inability to detect low, but significantly increased, levels of proliferation.

In an earlier series of experiments, we studied the effects of cholesterol feeding on injury-induced intimal thickening in rat carotids. We found that lipid accumulated preferentially in the injured segment in comparison to the rest of the artery. The cholesterol feeding did not produce an increase in smooth muscle hyperplasia above that seen in the damaged vessels of serum cholesterolemic rats even as late as 1 year. In ballooned rabbit arteries, others have demonstrated a marked increase in lipid accumulation and intimal thickening in regions covered by regenerating endothelium. As in the vein grafts, the increase in thickness could be attributed largely to an increase in foamy macrophages and not to smooth muscle hyperplasia.

Taken together, these observations suggest that the hyperlipidemia induced by cholesterol feeding does not have an additive or synergistic effect with injury on smooth muscle growth, at least in the short term (0 to 12 months). It is possible that over the long term, smooth muscle cells...
Figure 3. Transmission electron micrographs of vein graft from cholesterol-fed animal at 6 months. A. Region just below endothelium (EC) contains lipid-containing macrophages (M). B. Region below layer of foam cells contains smooth muscle cells (SMC), some of which contain small lipid vacuoles. A. × 11 000, B. × 16 000

might proliferate in response to some secondary phenomenon related to the hyperlipidemia such as focal injury and thrombosis.

In contrast to human vein graft atherosclerotic plaque, the rabbit lesions showed no evidence of a smooth muscle cell cap over the macrophage layer and thus were
more similar to the human fatty streak lesion. This may not be surprising since the changes noted in human grafts at less than 1 year appear to be more intimal myoproliferative in nature, while typical atherosclerotic plaques are not seen before 1 year.

In summary, vein grafts in cholesterol-fed rabbits display increased wall thickness that is almost entirely due to subendothelial accumulations of foamy macrophages. No influence of the hyperlipidemia or of the macrophages present in the intima could be detected on the proliferative index or the accumulation of intimal or medial smooth muscle cells.

Acknowledgment

The assistance of Patricia Bartnick in the preparation of the manuscript is gratefully acknowledged.

References

16. Halmovtd H, Maller N. Histologic examination of the intimal layer of large foamy macrophages (see Figure 2B) accounts for the difference in intimal thickness between control and fat-fed rabbit grafts. The differences in thickness were significant at 3 and 6 months (p<0.05, t test).

Table 1. Endothelial Thymidine Labeling Indices

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<td>Control</td>
<td>0.69±0.67 (5)*</td>
<td>0.04±0.03 (4)</td>
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<td>Fat-fed</td>
<td>0.28±0.06 (3)</td>
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*Thymidine labeling index (%)±SD; numbers in parentheses are the number of grafts analyzed.

Table 2. Smooth Muscle Thymidine Labeling Indices

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<td>Fat-fed</td>
<td>0.53±0.42 (3)</td>
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Index Terms: atherosclerosis • vein graft • smooth muscle cell • macrophage • hypercholesterolemia
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