Protection From Atherosclerosis in Vein Grafts by a Rigid External Support

Jean Batellier, Michel Wassef, Régine Merval, Micheline Duriez, and Alain Tedgui

Atherosclerosis is a common feature of autogenous vein bypass grafts resulting in their long-term failure. Arterial pressure–induced distension is thought to play a major role in the wall thickening of vein grafts, which may in turn favor atherosclerotic complications. In this study, we evaluated the influence of vein distension on the development of atherosclerotic lesions in jugular vein grafts interposed into the common carotid arteries of rabbits. The proximal half of each vein graft was wrapped with a 4-mm-diameter polytetrafluoroethylene graft that reduced the vein graft diameter by 46±5%. Fourteen animals were fed a 1% cholesterol-rich diet for 8 weeks, and five animals were fed a normal diet. In normocholesterolemic and hypercholesterolemic animals, the wall thickness and the total cross-sectional area were significantly reduced in wrapped compared with unwrapped segments. Foam cells were never observed in normocholesterolemic animals. In hypercholesterolemic rabbits, the sudanophilic lesions covered 62±4% of the luminal surface in unwrapped segments and 31±7% in wrapped segments (p<0.0001). In transverse sections, the surface areas of foam cells were also markedly reduced in wrapped compared with unwrapped segments. Reduction of the wall distension using a rigid external support protected the vein grafts from atherosclerosis, possibly as a result of the decrease in wall thickening that occurred in response to arterialization. (Arteriosclerosis and Thrombosis 1993;13:379–384)

Key Words • atherosclerosis • jugular vein • common carotid artery • vein grafts • rabbits

Several forms of arterial reconstruction can be used, but the autologous saphenous vein graft is the mainstay for both the lower-limb and coronary artery bypass graft (CABG). However, vein graft occlusion remains a major short- and long-term problem. In the first year after CABG, 15% of vein grafts are occluded.1 In the first weeks after grafting, the occurrence of arterial pressure in a venous graft leads to thickening of the graft wall as a result of intimal and medial hyperplasia.2 Thickening of the vein graft in the arterial circulation can be considered as an adaptive process in response to elevated wall stresses.3 Yet these structural modifications might increase the vein graft affinity for plasma low density lipoprotein,4 leading to the accumulation of lipids in the vessel wall and development of atherosclerotic plaques. Walton et al.,5 studying the natural history of lesions in vein grafts, have shown that lipid deposits are late changes that occur in the already thickened wall. Therefore, we hypothesized that maintaining as much of the normal wall structure as possible to the arterialized vein would prevent the graft from developing atherosclerosis.

Taking advantage of the fact that reduction of vein graft distension by a restrictive external support diminishes the hyperplastic response of the vein wall,6,7 we investigated the effect of reduced wall distension on the development of atherosclerosis in the rabbit jugular vein positioned in the transected carotid artery of cholesterol-fed animals.

Methods

Animal Model

New Zealand White rabbits (2.2–2.5 kg) were pre-anesthetized with sodium pentobarbital (0.5 mg/kg body wt i.v.), and then anesthesia was performed with an intravenous injection of ketamine hydrochloride (0.5 mg/kg body wt) every 15 minutes. Each rabbit received 500 units of heparin intravenously at the beginning of the dissection. Through a median longitudinal cervical incision, the left jugular vein and common carotid artery were dissected free and flooded with topical papaverin to prevent vessel spasm during dissection. Flow through the common carotid artery was interrupted with microvascular clamps; the vein was proximally ligated to prevent distension. A 3-cm segment of jugular vein distal to the main branch point and lacking valves was dissected and then transected, and an end-to-side anastomosis was performed proximally on the artery by using two uninterrupted 7/0 polypropylene sutures. After the first anastomosis was completed, the free end of the vein graft was passed through a 10-mm-long tube of nonreinforced polytetrafluoroethylene (PTFE), with an internal diameter of 4 mm and a 60-μm interenal distance (Gore-Tex, Gore & Associates Inc., Elkton, Md.), which was positioned in the proximal half of the vein. The diameter of the wrap was chosen to be smaller than that of the distended, unwrapped vein graft to prevent full distension of the graft. The second distal end-to-side anastomosis was then completed. The carotid artery was ligated between the two anastomoses.
and the flow reestablished through the jugular vein graft. The skin was closed with a 3/0 polyglycolic acid suture. The mean length of the vein grafts was 2.9±0.1 mm. The animals were observed until they were awake and ambulatory. Animals were cared for in accordance with the European Community Standards on the Care and Use of Laboratory Animals (No. 00577).

Five animals were fed a normal diet, and these constituted the normocholesterolemic (NC) group. Fourteen animals were fed a 1% cholesterol diet for 8 weeks, beginning 1 day after surgery, and these constituted the hypercholesterolemic (HC) group. At the end of the feeding period, the rabbits were anesthetized by the same procedure as described above, and 500 units of heparin was given intravenously. The proximal carotid artery was ligated and cannulated. Blood was sampled for determination of plasma cholesterol concentration with an enzymatic colorimetric test (Boehringer Mannheim). Animals were given a fatal dose of pentobarbital (5 mL/kg body wt). Blood was washed out by perfusion with Krebs-Ringer solution, and the vein grafts were perfused with 5% glutaraldehyde and 2.5% paraformaldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, from a height of 100 cm H₂O. The distal common carotid artery was ligated and the fixation under pressure continued for 15 minutes. The vein graft was then excised and further fixed by immersion in 10% buffered formalin solution.

Morphology

The wraps were carefully removed from the vein grafts to facilitate histological studies. This was easily done, and no trace of tissue was present on the PTFE wraps.

The vein grafts and a segment of the contralateral normal jugular vein were opened longitudinally and stained with Sudan IV. The opened vein graft was pinned, endothelial side up, to a cork board and photographed. The percent intimal involvement by atherosclerosis was determined by the distribution of Sudan dye staining using computer-assisted planimetry (Nachet 1500).

For histological studies, samples were embedded in paraffin. Five-micron transverse cross sections were stained with hematoxylin-eosin, orcein, Weigert-van Gieson's, and Masson's trichrome stains. Slides were processed by an automatic image-analysis processor (Nachet 1500). The thickness of the intima plus media was measured in seven locations spaced evenly around the perimeter of the vessel, and a mean value was calculated for each vessel. Measurements were made by defining the intima plus media as the region between the vessel lumen and the external elastic lamina. Independent measurement of intima and media was not performed, as there was no clear morphological difference between these two layers. The cross-sectional wall area and the foam cell area were measured by planimetry. Repetitive measurements were performed by two observers. Interobserver variation was less than 10%. All measurements were averaged for each vein graft segment.

Statistical Analysis

Results are expressed as mean±SEM. A two-factor repeated-measures analysis of variance (ANOVA) was constructed with the wall thicknesses and cross-sectional area data to assess the effects of the presence of the wrap and the cholesterol diet. Comparisons between control (unwrapped) and wrapped segments in the two groups were performed using a paired t test. Differences were considered significant at p<0.05.

Results

Two deaths occurred in the HC group because of technical failure. After 8 weeks, 17 of 19 vein grafts (89.5%) were patent. Total plasma cholesterol markedly increased in cholesterol-fed (HC) animals (13.0±0.9 g/L, n = 12) compared with NC rabbits (0.66±0.05 g/L, n = 5).

The diameter of the unwrapped segment of vein graft increased from 6.3±0.2 mm just after the vein transplantation to 7.6±0.1 mm after 8 weeks. The PTFE external support reduced the diameter of the vein graft by 46±5% (n = 17) similarly in both NC and HC groups.

The total thickness of the vein graft wall (intima plus media) was markedly increased compared with normal vein, regardless of the diet fed. However, the wrapped segments were thinner than the unwrapped control segments (Figures 1 and 2). A heterogeneous distribution of smooth muscle cells was observed in the intima/media. Smooth muscle cells seemed to be oriented both transversely and longitudinally (Figures 3A and 3B).

Values of total thickness and cross-sectional area are given in Table 1. An ANOVA constructed for the total

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**Figure 1.** Photomicrographs showing a cross section of the normal vein (panel A) and of unwrapped (panel B) and wrapped (panel C) segments of the vein graft in a normocholesterolemic rabbit. Hematoxylin-eosin-safran staining, ×388.
FIGURE 2. Photomicrographs showing a cross section of the unwrapped (panel A) and wrapped (panel B) segments of the vein graft in a hypercholesterolemic rabbit. Hematoxylin-eosin-safran staining, ×485.

wall thickness and cross-sectional area values showed that both the wrap and the diet had a significant effect (p<0.0001 and p<0.0004, respectively). The total thickness and cross-sectional area values were markedly lower in wrapped than in unwrapped segments. The 8-week cholesterol diet increased these values in both wrapped and unwrapped segments. Furthermore, a significant interaction between the two factors was seen (p<0.025 for thickness data and p<0.004 for cross-sectional area data), indicating that the effect of the wrap was different in the HC group compared with that in the NC group. Indeed, the protective effect of the wrap was more pronounced in HC than in NC animals. The total thickness of wrapped segments was decreased by 45% compared with that of unwrapped segments in the HC group and by only 36% in the NC group. The cross-sectional area was decreased by 68.5% in the HC group and 63% in the NC group.

No macroscopic accumulation of sudanophilic foam cells was seen in nongrafted veins in normal as well as cholesterol-fed rabbits. Vein grafts in the NC group were also macroscopically and histologically free of atherosclerosis. In the HC group, sudanophilic lesions were markedly reduced in wrapped compared with unwrapped segments (31±7% and 62±4%, respectively, n=12, p<0.0001).

In histological sections, significant infiltration of the vein wall with foam cells was observed in unwrapped segments: a thick continuous layer of foam cells covered with endothelial cells developed on the luminal surface (Figure 3A). Lipid accumulation was also seen in individual cells deeper within the vein wall. In the wrapped segments, the layer of foam cells was reduced and discontinuous or absent (Figure 3B). A patchy distribution of foam cells was seen in the inner wall. Cholesterol crystals were observed in the unwrapped vein graft of one HC animal (Figure 4).

Total foam cell areas were significantly reduced in wrapped segments from HC rabbits (0.043±0.021 mm² versus 0.51±0.13 mm² in control segments, p<0.004). When the foam cell area was subtracted from the total cross-sectional area of HC animals, a significant difference still existed compared with the total cross-sectional area for unwrapped segments of NC rabbits (p<0.02), whereas the difference was no longer significant for wrapped segments.

Discussion

Veins rarely develop atheromatous lesions unless arterialized, even though veins can be exposed to high plasma cholesterol levels. Conversely, such lesions are frequent in failed vein bypass grafts. By 10 years, arteriographic examination of CABGs that were functional at 1 year after grafting shows that 32% present significant atherosclerotic stenosis and another 30% are occluded. Atherosclerosis of the arterialized vein graft seems to be secondary to the hyperplastic response of the vein wall. Walton et al reported that in humans, the
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vein graft first adapts its structure by increasing its thickness, cell mass, and extracellular matrix. This vein remodeling was seen as early as a few months after surgery, whereas lipid deposits occurred at a later stage and were observed only after 1 year. Inasmuch as the wall hypertrophy of the arterialized vein graft is believed to be an adaptive reaction to a high-pressure environment triggered by pressure-induced stretching of the wall, we attempted to prevent the hyperplastic/hypertrophic response of the vein wall by avoiding extensive distension of the vein by using a rigid external support. As a result, we observed a marked reduction of atheromatous involvement in the wrapped arterialized segment.

Supporting the vein with an external rigid wrap diminished the wall distension and relieved the hoop stresses, but concomitantly, the shear stress at the endothelial surface was increased. Both mechanisms might account for the reduced thickness observed in wrapped segments.

Earlier studies showed that elevated circumferential stresses act as a stimulus for enhancing wall thickness until an ideal circumferential stress has been achieved. Zwolack et al showed that the thickness of the rabbit jugular vein transplanted into the carotid arterial circulation increased in such a way that the circumferential stress, which is proportional to the luminal radius to wall thickness ratio, equaled the value in the normal carotid artery. These authors investigated the time course of vein graft thickening and reported values for total thickness (116 μm) and cross-sectional area (2.14 mm²) after 12 weeks that were very similar to

Table 1. Thickness and Cross-sectional Areas of Unwrapped and Wrapped Vein Graft Segments in Normocholesterolemic and Hypercholesterolemic Rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Normocholesterolemic</th>
<th>Hypercholesterolemic</th>
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<tbody>
<tr>
<td></td>
<td>Unwrapped</td>
<td>Wrapped</td>
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<tr>
<td>Thickness (μm)</td>
<td>105.5±3.9 (p&lt;0.0001)</td>
<td>67.5±2.7</td>
</tr>
<tr>
<td>Cross-sectional area (mm²)</td>
<td>2.28±0.07 (p&lt;0.0003)</td>
<td>0.84±0.03</td>
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Values are mean±SEM.
FIGURE 4. Photomicrograph of a cross section of the unwrapped segment of the vein graft in a hypercholesterolemic rabbit, showing crystals of cholesterol. Masson's trichrome staining, ×188.

those found in the present work in unwrapped segments in NC animals (105.5 μm and 2.28 mm², respectively).

To prevent distension of the vein, we used the procedure described by Kohler et al.7 Our results are in agreement with those reported by these authors, who found a reduction of the adaptive response to arterIALIZation in wrapped segments of normal-diet-fed rabbits, with a significant decrease in the cross-sectional wall areas, smooth muscle cell volume, and matrix deposition. However, a significant reduction in thickness was observed in the present study, whereas in the work of Kohler et al, there existed a tendency for diminished thickness, although the level of statistical significance was not reached. Barra et al10 used a restrictive mesh to reduce by 50% the diameter of jugular vein grafts in sheep and reported a marked decrease of graft wall thickening ranging between 31% and 53%, depending on whether the maximal or minimal thickness was considered, results that agree with our findings.

Another mechanism that could contribute to a reduction in graft thickening is the increase in shear stress. The blood flow velocity was expected to be much higher in the wrapped than in the unwrapped segment. In arteries, it has been shown that caliber and structure can be regulated by shear stress.12,13 Recently, Kohler et al14 reported that high blood flow can inhibit neointimal hyperplasia in endothelialized PTFE vascular grafts. Dobrin et al,2 studying the effect of flow velocity on the remodeling of canine femoral veins that were used to bypass femoral arteries, found that the intimal hyperplasia occurring in vein grafts was diminished when the velocity was high. Accordingly, the decreased wall thickening observed in wrapped segments might be due to increased velocity in these segments, in addition to decreased circumferential stresses.

Zarins et al15 observed that in monkeys with thoracic coarctations, protection from atherosclerosis was associated with a decreased thickness distal to the coarctation. Bell et al,16 using a similar constricted-aorta model in cholesterol-fed rabbits, also found a marked reduction of lipid deposition within the stenosis. These authors attributed this effect to the high blood velocity within the stenosis, as well as to the reduction in artery wall motion.

The development of atherosclerosis in vein grafts in cholesterol-fed rabbits has been previously reported by Zwolack et al.11 Our findings in unwrapped segments are in agreement with theirs. However, when foam cell area was subtracted from the total cross-sectional area for cholesterol-fed rabbits, these authors found no significant difference in comparison with the total cross-sectional area of control rabbits. In the present work, a significant difference was found. It has been shown that cholesterol might stimulate smooth muscle cell proliferation,17 and this could account for our results. Nevertheless, it cannot be ruled out that in the present work the foam cell area values were slightly underestimated because of the fact that some sparse foam cells were not considered.

To the best of our knowledge, no previous study has been conducted to investigate the effect of a wrap on the development of atherosclerosis in arterialized veins. However, it has been suggested that a reduction in circumferential stress in arteries inhibits atherosclerosis.18 The reduction of arterial intramural stress by a rigid cast placed on the renal arterial branch and the aortic bifurcation prevented the development of atherosclerotic lesions in cholesterol-fed rabbits.18

The mechanism for decreased lipid accumulation in wrapped segments might be related in part to macromolecule transport in the vessel wall. It has been shown that veins have a very high permeability to macromolecules compared with that of arteries, and this property is believed to be associated with the vein's lack of atherosclerosis.19 In this laboratory, we have found that when vascular macromolecular clearance was abolished in the rabbit inferior vena cava by wrapping the vein with a semipermeable polyacrylonitrile membrane, lipid deposits were formed in the veins of cholesterol-fed animals.20 Increased thickness and fibrosis possibly enhance the resistance to the transport of plasma low density lipoprotein across the grafted venous wall and favor atherosclerosis.4 Such a mechanism might be relevant in atherosclerosis of the arterialized vein graft. By preventing the thickening of the vein wall, favorable
transport properties have been preserved to the vein graft, thus protecting it from atherosclerosis.

In conclusion, protecting the vein graft from extensive distension with a rigid wrap prevented development of atherosclerosis. This might be of interest in clinical use to improve vein bypass graft patency.

References

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