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Prevention of Lipid Accumulation in Experimental Vein Bypass Grafts by Antiplatelet Therapy

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SUMMARY The ameliorative effect of antiplatelet therapy on atherogenesis of vein grafts was assessed in autologous cephalic veins grafted into femoral arteries of 16 normolipemic and 11 hyperlipemic stump-tailed macaque monkeys. Before grafting, only half of each vein was distended at high pressure (700 mm Hg) and the other half at low pressure (350 mm Hg). Eight normolipemic monkeys were treated with aspirin, 80 mg/day, and dipyridamole, 50 mg/day, and eight were controls. When grafts were harvested at 12 weeks, tissue cholesterol and β-apolipoprotein content in grafts from untreated monkeys were significantly higher than in ungrafted, uninjured veins. Antiplatelet therapy eliminated the increase in lipid content of vein segments distended at low pressure, and significantly lowered lipid content of segments distended at high pressure, though not to the control levels of ungrafted veins. Seven of the 11 hyperlipemic monkeys received antiplatelet drugs and four did not. The lipid content of all graft segments was significantly higher than in grafted or ungrafted veins from normolipemic monkeys. Antiplatelet therapy again significantly reduced the lipid content in vein segments distended at both levels of pressure, and also reduced the elevated cholesterol content in ungrafted veins. Although this animal preparation differs in many ways from human coronary bypass operations, these observations may be pertinent to the prevention of atherosclerosis in human vein bypass grafts.

SAPHENOUS VEIN aortocoronary bypass grafts can develop atherosclerotic changes that lead to graft stenosis or occlusion. The pathogenesis of these atherosclerotic changes in vein grafts is probably similar to that of atheroma in native arteries, although the process is accelerated. According to the most prevalent hypotheses about atherogenesis, endothelial injury exposes the subendothelial connective tissue to platelets and other blood constituents. Platelets adhere to the subendothelial collagen, aggregate, and release the contents of their granules. The massive infiltration of platelet contents, plasma lipoproteins, and possibly other blood components leads to focal proliferation of smooth muscle cells in the vessel wall stimulated by a mitogenic factor released by platelets, to formation of connective tissue matrix, and to deposition of lipids.

Since some degree of trauma to vein grafts during harvesting and preparation for coronary revasculariza-
tion is unavoidable, the development of graft atherosclerosis might be accelerated by the above mechanisms. We developed a model of vein graft atherogenesis in macaque monkeys in which we demonstrated that the usual surgical practice of distending vein grafts before implantation causes endothelial injury and a significant increase in graft uptake of cholesterol.4 Vein grafts examined 7 months after distention and subsequent implantation as femoral artery bypass grafts had two to four times as much cholesterol as undistended, ungrafted veins. The amount of cholesterol was directly proportional to the pressure at which grafts had been distended before implantation.

Studies in humans and animals5,6 indicate that vein grafts develop platelet-fibrin intimal thrombi early after implantation that may be the precursors of atherosclerotic lesions. Studies in animals have also shown that antiplatelet therapy reduces mural platelet deposition in vein grafts and reduces intimal thickening.7,8 We therefore hypothesized that antiplatelet therapy might inhibit the process of atherosclerotic change in vein grafts, as manifested in our model by the uptake of tissue lipids. In this study, we performed vein grafts in peripheral arteries of normolipemic and hyperlipemic macaque monkeys and examined graft lipid uptake both with and without platelet inhibition.

Methods

We studied 27 male stump-tailed macaque monkeys (Macaca arctoides) that weighed 8–12 kg. Anesthesia was induced with i.m. ketamine, 25 mg, and maintained with supplemental doses of i.v. thiamylal sodium. A segment of cephalic vein was removed from the foreleg, and a flexible polyethylene cannula with a side connection for pressure measurements was inserted into the distal end of the vessel. The entire segment was distended for 1 minute with autologous heparinized blood at a hydrostatic pressure of 350 mm Hg; an occlusive clamp was placed across the middle of the vein, and the distal half was distended for 1 minute at a pressure of 700 mm Hg. The clamp site was marked with a suture. The monkeys were given i.v. heparin, 1 mg/kg, and the common, superficial and profunda femoral arteries, which had been previously isolated through a longitudinal groin incision, were occluded with vascular clamps. The femoral veins were not mobilized. Longitudinal arteriotomies were made in the common and superficial femoral arteries, and anastomoses were constructed between the ends of the vein and the arteriotomies using optical magnification, microsurgical technique, and 7–0 polypropylene monofilament suture. The femoral artery was ligated just distal to the origin of the profunda femoral artery and just proximal to the distal graft anastomosis, but was not divided. This maneuver provided maximal flow through the vein graft without undue tension on the graft that might have caused trauma in an unstandardized manner. When the graft was subsequently removed, blood supply to the leg was maintained through the profunda femoral artery.

Normolipemic Monkeys

The 16 normolipemic monkeys were fed a standard laboratory diet (Purina 5045) and were normolipemic throughout the study (table 1). Eight monkeys were treated with dipyridamole, 50 mg/day, beginning 1 week before operation, and aspirin, 80 mg/day, beginning intraoperatively through a gastric tube and continuing orally thereafter. The drugs were given in two divided doses; the monkeys took the medicines readily when the pills were embedded in fruit. Eight control monkeys received no medication. Grafts were harvested 12 weeks after insertion. The reference suture facilitated division of each specimen into halves that had been distended at high pressure or low pressure. Tissue adjacent to the anastomoses or the reference suture was discarded and the remaining material was divided into sections for biochemical analysis. A segment of normal femoral vein outside the surgical field was removed simultaneously for analysis.

Tissue specimens were finely divided and homogenized with 0.15 M sodium chloride. The cholesterol content was determined in an aliquot of the homogenate using a gas-liquid chromatography procedure.4,9 Extraction of β apoprotein from the tissue homogenate was allowed to continue overnight. Gentle centrifugation was used to separate the tissue debris, and the β-apoprotein content of the supernatant was determined using a specific radioimmunoassay technique.4,10 Plasma cholesterol was determined by a semiautomated technique.11

Hyperlipemic Monkeys

Eleven monkeys were rendered and maintained hyperlipemic with an atherogenic diet (Teklad 170050) that was begun 2 weeks before operation. Seven mon-

Table 1. Normal Diet: Graft Lipid Content and Plasma Cholesterol Level

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (mg/100 mg)</th>
<th>β apoprotein (μg/100 mg)</th>
<th>Plasma cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NV</td>
<td>LP</td>
<td>HP</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.08 ± 0.01</td>
<td>0.19 ± 0.04*</td>
<td>0.33 ± 0.06†</td>
</tr>
<tr>
<td>Platelet inhibition</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.22 ± 0.04†</td>
</tr>
<tr>
<td>p</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± sd.

* p <0.05 low-pressure distention vs normal vein.
† p <0.05 high-pressure distention vs low pressure distention.

Abbreviations: NV = normal vein; LP = low-pressure distention; HP = high-pressure distention.
keys received dipyridamole and aspirin according to the same protocol described earlier. Four control monkeys received no medication. The grafts were harvested and processed as described for the normolipemic monkeys.

Statistical Analysis

Differences between the untreated monkeys and those receiving antiplatelet therapy were tested for significance using the unpaired t test. An analysis of variance was used to compare normal ungrafted veins, low-pressure graft segments and high-pressure graft segments within each group. A probability level of 0.05 was considered significant. Values are expressed as mean ± SD.

Results

All grafts were widely patent at harvesting, with no evidence of stenosis, kinking, or other obstruction to flow.

Normolipemic Monkeys

There was no difference in plasma cholesterol between the eight monkeys who received antiplatelet therapy and the eight who did not (table 1). In control monkeys (no antiplatelet therapy), segments distended at high pressure had significantly greater cholesterol and apo-protein content than segments distended at low pressure, and both had significantly more cholesterol and apo-protein than normal ungrafted veins. Antiplatelet therapy eliminated the increase in cholesterol and apo-protein content in vein segments distended at low pressure, and significantly decreased the cholesterol and apo-protein content of vein segments distended at high pressure, though not to control levels of ungrafted veins. The cholesterol and apo-protein content of ungrafted veins from treated normolipemic monkeys was similar to that of the untreated group.

Hyperlipemic Monkeys

All monkeys that received the atherogenic diet developed sustained hyperlipemia. There was no significant difference between plasma cholesterol in the seven monkeys that received antiplatelet therapy and in the four that did not (table 2). In control monkeys, segments distended at high pressure had significantly greater cholesterol and apo-protein content than segments distended at low pressure, and both had significantly more cholesterol and apo-protein than normal ungrafted veins. This was true even though normal, ungrafted veins in hyperlipemic monkeys had significantly more cholesterol and apo-protein than the ungrafted veins in the normolipemic monkeys. Antiplatelet therapy significantly reduced cholesterol and apo-protein content in vein segments distended at high or low pressure, though both remained significantly elevated compared with ungrafted veins. Antiplatelet therapy also significantly reduced cholesterol content in ungrafted veins, but did not significantly reduce apo-protein content.

Discussion

The results of our study indicate that antiplatelet therapy reduces lipid uptake in experimental vein grafts placed in the peripheral arteries of normolipemic and hyperlipemic monkeys. Even in hyperlipemic monkeys, antiplatelet therapy significantly lowered both cholesterol and apo-protein content in high- and low-pressure segments of grafted veins, though not to the levels in normolipemic monkeys. The diminished benefit in hyperlipemic monkeys is not surprising, as the atherogenic process is dramatically accelerated in monkeys fed a diet artificially high in fat. We previously showed that in this hyperlipemic monkey model, vein grafts manifest grossly visible atherosclerotic lesions 32 weeks after insertion. (In that study grafts were not examined earlier.) Antiplatelet therapy alone could not be expected to completely prevent this artificially accelerated process, which has little parallel in man. Pick et al. showed that antiplatelet drugs can inhibit the development of atherosclerosis in the native arteries of monkeys fed an atherogenic diet.

Choice of Experimental Design

Long-term patency of the bypass grafts is the sine qua non of successful coronary bypass operations. Atherosclerotic changes in vein bypass grafts are now being recognized often, and between 5 and 10 years postoperatively, vein graft atherosclerosis is likely to be the principal cause of reoperation.

The implications of our findings for the management of patients after coronary bypass depend on the relevance of our experimental preparation to the clinical setting. Although the distending pressures applied to the veins in this study may seem high, they are

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Table 2. Atherogenic Diet: Graft Lipid Content and Plasma Cholesterol Level

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (mg/100 mg)</th>
<th>apo-protein (μg/100 mg)</th>
<th>Plasma cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NV</td>
<td>LP</td>
<td>HP</td>
</tr>
<tr>
<td>Untreated</td>
<td>0.13 ± 0.03</td>
<td>0.56 ± 0.07*</td>
<td>1.05 ± 0.05†</td>
</tr>
<tr>
<td>Platelet inhibition</td>
<td>0.08 ± 0.01</td>
<td>0.24 ± 0.05*</td>
<td>0.69 ± 0.08†</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*p <0.05 low-pressure distention vs normal vein.

†p <0.05 high-pressure distention vs low pressure distention.

Abbreviations: See table 1.
frequently achieved clinically, and many surgeons now use a commercial balloon device to limit the distending pressure that can be applied to veins.13 Nonetheless, the lower distending pressure used in our study (350 mm Hg) is near the lowest practical pressure for clinical work. It is therefore noteworthy that antiplatelet therapy completely eliminated the increase in graft lipid content at this pressure in normolipemic monkeys.

We studied the stump-tailed macaque monkey because its lipid metabolism and the appearance of its atherosclerotic lesions closely resemble those of humans.4 Coronary bypass grafts in monkeys are impractical because the small size of monkey coronary arteries poses forbidding technical obstacles to achieving high rates of graft patency, and the high cost of monkeys makes frequent failures unacceptable. Short peripheral vascular grafts are a sensible alternative. The stump-tailed macaque’s femoral artery is approximately 2.5 mm in diameter, the size of the human right coronary artery. The macaque cephalic vein is approximately 3.5–4 mm in diameter, and resembles the human saphenous vein in wall thickness and composition, unlike the delicate jugular and iliac veins used in canine studies.4

The regimen of two antiplatelet drugs, each of which acts by a different mechanism,6 was selected because it inhibits platelet function and prolongs platelet survival in humans17 and animals.7,8,13 We divided the 11 hyperlipemic monkeys unequally so that seven received antiplatelet therapy and four were untreated controls; our previous study showed a marked increase in the lipid uptake of grafts in untreated hyperlipemic monkeys.

It is also important to consider whether the graft lipid uptake in our monkeys is related to mature atherosclerotic lesions or is a transient postoperative phenomenon. We demonstrated that the cholesterol in the vein grafts is derived from plasma cholesterol, and grafts harvested 32 weeks postoperatively from hyperlipemic monkeys had visible atherosclerosis, including plaque formation.4 Lipid content in the grafts from normolipemic monkeys was almost identical at 32 weeks to values in the present study at 12 weeks, indicating that the increased lipid content in grafted veins does not decline with time in monkeys that do not receive antiplatelet drugs. Monkeys have little propensity to develop spontaneous atherosclerosis on their regular diet, so it is not surprising that within 32 weeks the normolipemic monkeys failed to accumulate even more lipid or develop gross atherosclerotic lesions in the grafts.

In these experimental grafts, intraoperative trauma to vein grafts enhances graft lipid uptake by damaging graft endothelium so that subintimal tissue is exposed to the blood,3 intimal fibrinolytic activity is reduced,18 platelet and fibrin deposition is enhanced.7,8 a chronic injury-repair process is initiated that is often followed by fibrous and myoepithelial proliferation,3 and endothelial permeability to plasma lipid is increased.4 If this proposed sequence of events can lead to lipid accumulation in human grafts as well, and if the increase in graft lipid content presages the development of arteriosclerotic plaques, effective measures to interrupt this sequence would include surgical techniques to minimize intraoperative trauma to vein grafts, postoperative measures to control dietary and plasma lipids, and the administration of antiplatelet drugs.

References
Prevention of lipid accumulation in experimental vein bypass grafts by antiplatelet therapy.
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