Accelerated Cholesterol Accumulation in Homologous Arterial Transplants in Cholesterol-Fed Rabbits

A Surgical Model to Study Transplantation Atherosclerosis

Erik Hjelms and Steen Stender

Accelerated coronary artery disease has become a major complication to heart transplantation in humans. Therefore, we have developed a surgical model in the rabbit, with transplantation of the thoracic aorta as a bypass graft onto the abdominal aorta of another rabbit. The model permits the study of cholesterol metabolism in transplanted arteries. The graft did not accumulate cholesterol for as long as 298 days, provided that the rabbits were normocholesterolemic, i.e., with plasma cholesterol levels of 0.3–0.7 mmol/l. However, after a few weeks of cholesterol feeding resulting in plasma cholesterol levels of 2–5 mmol/l, the homologous graft accumulated cholesterol compared with intact aortic tissue in the rabbits and also compared with autologous aortic grafts. The intimal clearance of plasma cholesteryl ester, mainly high density lipoprotein cholesteryl ester, in the luminal layer of the aortic graft was 60–150 nl/cm²/hr⁻¹ 1–2 hours after transplantation. The intimal clearance in the corresponding intact thoracic aorta of the recipient animal was 5–20 nl/cm²/hr⁻¹. The values were 1,500–3,000 nl/cm²/hr⁻¹ 51–298 days after transplantation, while the intimal clearance of the rabbit's own aorta remained unchanged. A pronounced increase in plasma lipoprotein permeability is thus an early event in transplanted arteries. It results in a higher cholesteryl ester influx that leads to cholesterol accumulation in the artery, but only if the rabbits are fed a cholesterol-enriched diet. This rabbit model may be useful in the search for interventional measures to prevent or diminish the accelerated coronary artery disease in transplanted hearts in humans. (Arteriosclerosis and Thrombosis 1992;12:771–779)

KEY WORDS • arterial wall • atherosclerosis • cholesterol • transplantation

One of the major complications of otherwise successful human heart transplantation is accelerated coronary vascular disease. This complication can be demonstrated as early as 1 year after transplantation and is found in approximately 40% of patients after 5 years. The microscopic picture of a coronary artery disease in transplant patients represents a mixture of typical atheromatous lesions and unique transplant-related, progressive, distal obliterative disease. It has been suggested, based on clinical as well as experimental data, that immunological reactions play a major role in the development of this type of atherosclerosis. Previous experimental models for studying this vessel disease consist of rabbits and rats undergoing heterotopic heart transplantation. Studies in these models have primarily focused on the morphology and ultrastructure of the arterial walls.

Thus, arterial lesions in cardiac allografts placed in the neck of recipient rabbits developed within a few weeks. The arterial lesions were described as proliferative when the recipient rabbits were fed a lipid-poor diet and fatty proliferative when the recipient rabbits were fed a cholesterol-enriched diet. Some of the latter lesions bore a close resemblance to lesions in human coronary atherosclerosis. The authors suggest, based on ultrastructural studies, that immunological endothelial injury due to rejection is an early event and acts in synergy with hypercholesterolemia, leading to the rapid development of transplantation atherosclerosis.

Quantification of cholesterol in transplanted arteries and of endothelial permeability and cholesterol transfer from plasma into the grafted vascular tissue has previously not been done. A new surgical model in the rabbit has been developed to study these aspects of cholesterol metabolism in transplanted arteries.

Methods

Animals and Anesthesia

We used male white rabbits of the Danish Country strain from Statens Seruminstitut, Copenhagen. The body weight was 2.5–3.5 kg. Each pair of donor and recipient rabbits were siblings from the same litter. Experimental protocols were in accordance with Danish regulations for experiments on animals.
Anesthesia was induced and maintained with repeated small doses of intravenous sodium thiopental. A total dose of about 50 mg/kg body wt was given on average to each rabbit.

Surgical Technique

The donor rabbit was anesthetized, and the thoracic aorta was removed under sterile conditions, flushed with heparin saline solution, and immediately thereafter implanted in the recipient rabbit after suturing of any side branches with polypropylene 7-0. Approximately 3 cm of the cranial descending thoracic aorta was used (Figure 1A).

The abdominal aorta of the recipient animal was exposed through a midline incision. A segment just beneath the origin of the renal arteries was dissected free, and the animal was heparinized with 200 IU heparin/kg body wt. The aorta was cross-clamped over a small segment and incised longitudinally, and the graft was anastomosed to the segment end to side with running atraumatic 7-0 polypropylene stitches. The graft was then cross-clamped, and the cross-clamps of the recipient aorta were released for 5 minutes to reestablish caudal blood supply.

The cross-clamps were then reapplied 2.5-4 cm more caudally on the recipient abdominal aorta to carry out distal anastomosis. This anastomosis was performed in the same manner as the cranial one. Finally, the recipient abdominal aorta was ligated immediately cranial to the caudal anastomosis to direct the entire aortic blood flow through the graft. The heparin was not neutralized. The duration of the operation was usually about 1 hour. The rearrangement of the vessels is shown in Figure 1A. Postoperatively, the animals were allowed free access to food and water for a period of at least 14 days before they were allocated to the various experimental groups.

Aortic Cholesterol Accumulation and Plasma Cholesterol Level

To investigate the relations between cholesterol level in plasma and duration of hypercholesterolemia on the accumulation of cholesterol in the aortic graft, the following groups of rabbits were used. One group of seven rabbits with transplanted aortas was maintained on standard rabbit pellets (Altromin, 2113 Lage) without the addition of cholesterol (group E; Tables 1 and 2). The animals were killed and the aortas removed 51-298 days after transplantation. Eight other rabbits with transplanted aortas were placed on a 1% cholesterol-enriched diet (CH-USP cholesterol, Sigma Chemical Co., St. Louis, Mo.) 14 days after the operation.

Each rabbit received daily 90 g rabbit pellets enriched with 1 g cholesterol dissolved in 9 g corn oil (oleum maides BP 80, Mecobenzon, Copenhagen). Four of the rabbits (group A; Table 2) were killed 29 days after the initiation of the cholesterol feeding, and the remaining four rabbits (group B; Table 2) were killed after 14-16 days of cholesterol feeding.

Seven other transplanted rabbits (group C; Table 2) were fed a cholesterol-enriched diet for a period of 10 days. The amount of cholesterol added to the diet of each rabbit was adjusted according to daily blood cholesterol determinations. The aim was to obtain a mean cholesterol level in plasma of about 5 mmol/1 in each rabbit. The same procedure was followed in another group consisting of six transplanted rabbits (group D; Table 2). In this group, the aim was to maintain a mean cholesterol level of 2 mmol/1 for 10 days before removal of the aorta.

Comparison of Homologous and Autologous Aortic Transplants

Two animals survived a modified operation to determine to what extent cholesterol accumulation in the transplanted artery was affected by traumatic lesions suffered by the grafted vessel during the surgical procedure. A segment of the abdominal aorta from the donor animal was transplanted as a bypass on the abdominal aorta of the recipient animal, as described for the thoracic grafts. The segment of the recipient abdominal aorta between the two anastomoses was then removed, handled in a manner similar to that of the homologous transplant, and reinserted as a graft from the homologous transplant to the recipient abdominal aorta distal to the homograft (Figure 1B).
Fourteen days after transplantation the animals were placed on a cholesterol-enriched diet for 1 month, after which time the aorta was removed.

**Measurements of Intimal Clearance of Plasma Cholesteryl Ester**

Autologous normocholesterolemic plasma was incubated at 37°C for 48 hours after addition of 20–50 μCi (1α,2αO-[3H]-cholesterol in 20–50 μl ethanol to 2–5 ml plasma or 5–20 μCi [4-14C]cholesteryl ester in 40–160 μl ethanol to 10–20 ml plasma. Labeled cholesterol was obtained from Amersham (Birkerød, Denmark).

Labeled free cholesterol that was still nonesterified after the incubation with plasma was partly removed by exchange with unlabeled free cholesterol in autologous erythrocytes for a 4–6-hour period. Typically, the final preparation contained <5% of the radioactivity in free cholesterol. The labeled lipoprotein preparation was filtered through a Millipore filter (0.22 μm) and then injected intravenously into the rabbit, and blood samples were drawn at 5, 30, and 60 minutes and hourly thereafter until the aorta was removed after 1–3 hours of in vivo exposure to the labeled lipoproteins.

Four recipient animals were injected intravenously with [4-14C]cholesterol–labeled plasma preparations 5–10 minutes after transplantation while the animals were still under anesthesia. The aorta was removed from these animals 2–3 hours after the injection, corresponding to 0.13 day after transplantation (Figure 6).

Seven other recipient rabbits were injected intravenously with cholesterol–labeled plasma preparations 5–10 minutes after transplantation while the animals were still under anesthesia. The aorta was removed from these animals 2–3 hours after the injection, corresponding to 0.13 day after transplantation (Table 5). The latter preparation was injected to obtain an estimate of the quantitative importance of plasma cholesterol that adhered to the transplanted aortic tissue.

The intimal clearance was calculated by dividing the amounts of labeled cholesteryl ester in the inner aortic layer (dpm/cm²) by the area below the plasma cholesteryl ester radioactivity concentration versus time curve (dpm/cm²·hr)⁸. The plasma contamination in the tissue was calculated by dividing the amounts of radioactivity in the aortic tissue by the corresponding plasma concentration of radioactivity for the labeled preparation that had been injected just before removal of the aorta.

**Preparation of Aortic Tissue**

After the entire aorta had been removed from the recipient rabbit, a small cross-sectional ring was removed from the graft and the corresponding piece of the thoracic aorta of the host. The small tissue specimens were placed in 10% phosphate-buffered formalin for fixation and subsequent microscopic evaluation.

The remaining two pieces of aortic tissue, i.e., the graft and the corresponding host tissue, were cleansed of adventitial tissue, cut open, and placed on a cork board. The luminal surface area was outlined, and the tissue was divided into an outer and an inner layer using two forceps and stored at −20°C until further processing. The inner layer comprised the intima and inner media, and the outer layer comprised the remaining part of the media and residues of adventitial tissue.

**Analytical Procedures**

Extraction of lipids; separation of free and esterified cholesterol by thin-layer chromatography; and determination of the radioactivity and mass of total, free, and esterified cholesterol were performed as previously described. The amount of protein in tissue was determined by the method of Lowry et al⁹ after extraction of the lipids and digestion of the residue for 24 hours with 5 M NaOH. The reference serum of animal origin (Seronorm, Nygaard and Co., Oslo, Norway) was treated in a similar way and used to calibrate the protein determination. Very low density lipoprotein plus intermediate density lipoprotein (VLDL+IDL; d<1.019 g/ml), low density lipoprotein (LDL; 1.019<d<1.063 g/ml), and high density lipoprotein (HDL; d>1.063 g/ml) in plasma samples were separated by ultracentrifugation at 4°C at 1.63×10⁶g·min in a TFF 45.6 Beckman rotor and subsequently tube sliced. Cholesterol in the top and bottom fractions was determined enzymatically (Boehringer Mannheim). Recovery of cholesterol from the ultracentrifuge tubes was 85–105%.

**Statistics**

Results are given as mean±SEM.

**Results**

**Surgery**

After a period of learning, the operative mortality was reduced to <2% and the incidence of immediate postoperative ischemic damage to the spinal cord was reduced to almost 25%. If the animals survived the first day after the operation without signs of ischemic damage, they thrived well, gained body weight, and had no visible side effects of the transplantation.

**Aortic Grafts in Normocholesterolemic Rabbits**

Macroscopically, the graft was embedded in fatty tissue from the host when the animal was killed 14 or more days after transplantation. The adventitial fat on the graft was not as easily removed as that on the host aorta. When the aorta was opened, the inner surface of the graft was macroscopically indistinguishable from the inner surface of the adjacent aorta in the host animal (Figure 2A). The wall of the graft was, however, considerably thicker than the wall of the corresponding piece of thoracic aorta in the host.

Microscopically, there was cellular thickening of the intima but without fatty changes (Figure 2C). In none of the sections from the graft was it possible to identify endothelial cells. Media had signs of necrosis with loss of smooth muscle cells. Adventitia showed an inflammatory reaction with infiltration of lymphocytes and granulocytes.

The amounts of cholesterol in the inner layer of the graft expressed per milligram wet weight of the tissues were similar to those found in the corresponding part of the recipient’s own aorta, even as long as 298 days after transplantation (Table 1). This was also the case when the cholesterol concentration in the tissue was expressed per milligram protein (data not shown).
FIGURE 2. Panel A: Macroscopic photograph of host aorta and aortic graft in a normocholesterolemic rabbit. The graft was transplanted onto the abdominal aorta of the recipient rabbit 125 days before the entire aorta was removed. Note centimeter rule for scale. Panel B: Photomicrograph of the thoracic aorta of the host. ×16. Panel C: Photomicrograph of the transplanted thoracic aorta. ×16.
TABLE 1. Cholesterol in Plasma and in the Inner Layer of Intact Thoracic Aortas From the Host Rabbits and in Transplanted Thoracic Aortas (Graft) in Normocholesterolemic Rabbits (Group E)

<table>
<thead>
<tr>
<th>Rabbit identification No.</th>
<th>Rabbit thoracic aorta</th>
<th>Days after transplantation</th>
<th>Plasma cholesterol (mmol/l)</th>
<th>Luminal surface area (cm²)</th>
<th>Wet weight (mg)</th>
<th>Cholesterol (nmol/mg)</th>
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<tr>
<td>E-1</td>
<td>Host</td>
<td>51</td>
<td>0.67</td>
<td>7.6</td>
<td>140</td>
<td>4.8</td>
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<tr>
<td></td>
<td>Graft</td>
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<td>5.6</td>
<td>140</td>
<td></td>
<td>4.1</td>
</tr>
<tr>
<td>E-2</td>
<td>Host</td>
<td>51</td>
<td>0.28</td>
<td>9.3</td>
<td>220</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Graft</td>
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<td>1.8</td>
<td>40</td>
<td></td>
<td>5.5</td>
</tr>
<tr>
<td>E-3</td>
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<td>6.7</td>
<td>170</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>Graft</td>
<td></td>
<td>1.3</td>
<td>40</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>E-4</td>
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<td>240</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Graft</td>
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<td>1.4</td>
<td>30</td>
<td></td>
<td>3.6</td>
</tr>
<tr>
<td>E-5</td>
<td>Host</td>
<td>155</td>
<td>0.51</td>
<td>5.4</td>
<td>260</td>
<td>2.4</td>
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<tr>
<td></td>
<td>Graft</td>
<td></td>
<td>4.2</td>
<td>100</td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>E-6</td>
<td>Host</td>
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<td>0.47</td>
<td>6.4</td>
<td>230</td>
<td>1.8</td>
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<td>Graft</td>
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<td>2.3</td>
<td>90</td>
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<td>4.2</td>
</tr>
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<td>E-7</td>
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<td>190</td>
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</tr>
<tr>
<td></td>
<td>Graft</td>
<td></td>
<td>3.2</td>
<td>130</td>
<td></td>
<td>5.1</td>
</tr>
</tbody>
</table>

Aortic Grafts in Hypercholesterolemic Rabbits

Macroscopically, the luminal surface of the homologous aortic transplant in rabbits that had been hypercholesterolemic for 29 days was obviously abnormal, with several complicated lesions (Figure 3A). The changes were less pronounced after 16 and 10 days with hypercholesterolemia. Compared with the host aorta, there was microscopically marked intimal thickening with numerous lipid-filled foam cells, the media appeared necrotic, and there were inflammatory cells in the adventitia, which also contained lipid-filled foam cells (Figure 3B).

The cholesterol content in the inner as well as in the outer layer (not shown) was much higher than that in the corresponding part of the host animal’s aorta (Table 2). The highest cholesterol concentration values were always observed in the inner layer of the wall. This was the case when tissue cholesterol concentrations were expressed in relation to both the wet weight of the tissue and the protein content of the tissue. The inner layer of the graft accumulated cholesterol even after 10 days of cholesterol feeding, which resulted in only moderately elevated cholesterol levels in plasma (Table 2).

Before the addition of cholesterol to the diet, about 80% of the plasma cholesterol was in the HDL fraction and the remaining part was in the VLDL+IDL and LDL fractions. After 10 days of a 0.2% cholesterol-enriched diet, the plasma concentration of VLDL+IDL and LDL increased dramatically, whereas the increase in HDL concentration was less pronounced (Table 3).

The relation between cholesterol concentration in the graft and the area below the plasma cholesterol concentration versus time curve for all of the transplanted and cholesterol-fed rabbits demonstrates that the higher the area, the higher the cholesterol content in the graft (Figure 4). In the combined homologous/autologous graft, the cholesterol content in the autograft was higher than in intact aortic tissue. The cholesterol content of the homologous graft was much higher than in the autologous graft (Table 4). A representative aortic graft from one of the animals is shown in Figure 5.

Intimal Clearances of Plasma Cholesteryl Ester in Aortic Grafts of Normocholesterolemic Host Rabbits

The intimal clearance of plasma cholesteryl ester in the intact thoracic aorta of the animals that had received an aortic graft was 5-20 nl x cm⁻² x hr⁻¹ (Figure 6). These values are very similar to the mean values of 4.8-9.7 nl x cm⁻² x hr⁻¹ for the same aortic locations measured by a similar technique in rabbits that had not been transplanted. The intimal clearance of plasma cholesteryl ester in the graft was four- to 30-fold higher than in the analogous piece of intact aortic tissue in the recipient animal (H₁ in Figure 1), even during the first hours after transplantation (Figure 6). The intimal clearance of cholesteryl ester in the graft increased further during the following weeks and reached values as high as several hundred times those found in the analogous parts of the rabbit's own aorta. There was no evidence of a further increase from day 51 to day 298 (Figure 6).

The amounts of adhering plasma on the surface of the graft may be larger than those reported previously from autologous aortic surfaces of cholesterol-fed rabbits, i.e., 10 nl x cm⁻². However, the intimal clearance of cholesterol in the graft was so high that adhering plasma was of minor quantitative importance for the validity of the cholesterol influx calculation (Table 5).
FIGURE 3. Panel A: Macroscopic photograph of host aorta and an aortic graft in a cholesterol-fed hypercholesterolemic rabbit. The graft was transplanted onto the abdominal aorta of the recipient rabbit 43 days before the entire aorta was removed. Cholesterol feeding was commenced 14 days after transplantation. Note centimeter rule for scale. Panel B: Photomicrograph of the transplanted artery. ×16.

Discussion

Immune and Physical Damage to the Vessel

In the present study we have focused only on the quantitative aspects of the interaction between plasma cholesterol and transplanted arteries. In the cholesterol-fed rabbits we observed a pronounced accumulation of cholesterol in the transplanted arteries compared with the accumulation in nontransplanted arteries exposed to exactly the same elevated level of plasma cholesterol. The accumulation of cholesterol in the artery was preceded by an increase in lipoprotein permeability of the luminal surface of the transplanted artery. The increase in lipoprotein permeability was considerable even a few hours after transplantation and increased further during the following weeks (Figure 6).

Schwenke and Zilversmit11-13 have studied permeability changes after mild and more severe aortic injury produced by balloon catheterization in cholesterol-fed rabbits. They observed that lipoprotein permeability (mainly β-VLDL permeability) remained unchanged 0-2 days after a mild injury affecting only the endothelial layer.11 The permeability increased with time and was higher 30-31 days later compared with uninjured aorta.12 With a more severe injury also affecting the

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rabbits</th>
<th>Duration from operation to initiation of cholesterol feeding (days)</th>
<th>Addition of cholesterol to diet (g/day)</th>
<th>Duration of hypercholesterolemia (days)</th>
<th>Plasma cholesterol (mmol/l)</th>
<th>Inner layer of aorta (nmol/mg wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>14</td>
<td>1.0</td>
<td>29</td>
<td>46±8</td>
<td>158±6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22±3</td>
<td>3.7±0.4</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>14</td>
<td>1.0</td>
<td>14-16</td>
<td>33±4</td>
<td>65±16</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20±4</td>
<td>2.8±0.4</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>22-125</td>
<td>0.8-2.0</td>
<td>10</td>
<td>9.0±0.7</td>
<td>34±6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5±0.5</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>51-92</td>
<td>0.05-0.2</td>
<td>10</td>
<td>2.9±0.3</td>
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</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>2.2±0.1</td>
<td>2.4±0.2</td>
</tr>
<tr>
<td>E*</td>
<td>7</td>
<td>...</td>
<td>0.00</td>
<td>0</td>
<td>0.5±0.1</td>
<td>4.1±0.4</td>
</tr>
</tbody>
</table>

*Individual values for this group are shown in Table 1.
TABLE 3. Total Cholesterol and Distribution of Cholesterol Between Plasma Lipoproteins in Rabbits Before and After a Moderate Cholesterol Feeding

<table>
<thead>
<tr>
<th>Time relative to cholesterol feeding</th>
<th>Total</th>
<th>VLDL+IDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before (n=5)</td>
<td>0.48±0.08</td>
<td>0.06±0.02</td>
<td>0.04±0.00</td>
<td>0.38±0.09</td>
</tr>
<tr>
<td>After* (n=6)</td>
<td>2.19±0.10</td>
<td>0.35±0.06</td>
<td>1.24±0.11</td>
<td>0.60±0.07</td>
</tr>
</tbody>
</table>

VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein. Values are mean±SEM and are in millimoles per liter.

*Rabbits were fed a 0.2% cholesterol- and 10% corn oil-enriched diet for 10 days.

The medial layer of the artery, these investigators observed a 30–60-fold increase in permeability to cholesteryl ester, primarily reflecting β-VLDL permeability immediately after injury. If we assume that the HDL permeability of the artery as measured in the present study also reflects the permeability of the much larger β-VLDL, then the permeability increase immediately after transplantation as observed in our study suggests that transplantation changes the surface of the artery in a manner similar to a more severe physical injury to the artery. The assumption about the proportionality between aortic permeability to HDL and aortic permeability to VLDL is based on findings in nontransplanted aortas of cholesterol-fed rabbits.

The events preceding the change in lipoprotein permeability of the luminal surface are not known. They may consist of acute damage due to the surgical trauma in addition to a more long-acting and more severe damage due to host-versus-graft reactions. It has been suggested, based on studies of humans with transplanted hearts, that development of circulating donor-specific cytotoxic antibodies induces or aggravates endothelial injury. It is possible to envisage a considerable number of cellular and humoral immune mechanisms of importance for the development of the atherosclerotic plaque. Several of these mechanisms may be exacerbated in the transplanted arteries, leading to their increased lipoprotein permeability and cholesteryl ester accumulation.

We did not measure permeability in the homologous and autologous grafts placed in the same host. However, based on the comparison of cholesterol accumulation in the two types of tissue, it is reasonable to assume that the damage to the autologous intima is not as pronounced as that to the homologous intima. The additional damage to the homologous artery may, therefore, be ascribed more to host-versus-graft reactions than to the physical damage to the vessel. It is noteworthy, however, that removal and reinsertion per se of an aortic segment subsequently caused an increased accumulation of cholesterol.

Lipoprotein Influx and Accumulation in Transplanted Arteries

Cholesterol does not accumulate in the graft during normocholesterolemia despite a persistent and more than 100-fold higher cholesteryl ester influx compared with that in autologous nontransplanted aortic tissue. The turnover time of cholesterol in the inner layer of the graft in the normocholesterolemic rabbit is, therefore, relatively short (3–12 days) compared with the
FIGURE 5. Macroscopic photograph of the upper part of the graft (from 1.0 cm to 4.0 cm; DA in Figure 1B), the homologous aortic transplant. Lower part of the graft (from 0.5 cm to 1.0 cm) is the autologous aortic transplant (HA in Figure 1B). The rabbit was placed on a cholesterol-enriched diet 14 days after transplantation, and the entire aorta was removed after 30 days of cholesterol feeding. Cholesterol concentrations in the two segments are given in Table 4 as the first and third entries.

The turnover time in intact aortic tissue (200–1,500 days). The turnover time was calculated by dividing the amounts of cholesterol in the inner layer (nmol×cm⁻²; Table 2) by the cholesteryl ester influx. The cholesteryl ester influx was 2.08–10.9 nl×cm⁻²×hr⁻¹ times the plasma concentration of 0.5 nmol×L⁻¹ (Table 5).

The observation that normocholesterolemic rabbits do not accumulate cholesterol in the arteries even after severe damage to the vessel wall is in accordance with the findings of other groups. The latter group observed that complete deendothelialization of the aorta in normocholesterolemic rabbits did not lead to aortic cholesterol accumulation even after 2 years.

It is relatively easy to observe conditions with prolonged elevations of plasma concentrations of LDL or VLDL in humans and in some experimental animals, especially rabbits. It has proved to be much more difficult to study the effects of increased HDL concentrations in plasma of humans and animals.

We increased the influx of plasma cholesteryl ester into the aortic wall by a factor of 100 in the present study. This was accomplished not by raising the plasma cholesteryl ester concentration in plasma by a factor of 100 but by increasing the permeability of the aortic luminal surface by that factor. Because the major portion of cholesterol in plasma in these rabbits was HDL cholesterol (Table 3), the flux of cholesteryl ester into the graft consists primarily of HDL cholesteryl ester. This notion is based on the assumption that the distribution of labeled cholesteryl ester in plasma mimics the distribution of unlabeled cholesteryl ester in plasma because of the high activity of cholesteryl ester exchange protein in rabbits. The observed lack of cholesterol accumulation in the wall indicates that HDL leaves the wall as fast as it enters, even when the influx is increased several hundred times compared with the influx of HDL into the intact aorta. The much lower amounts of VLDL, IDL, and LDL that also enter the wall, assuming a size- and concentration-dependent influx, are apparently also able to leave the wall again.

When plasma cholesterol concentration increases in response to cholesterol feeding, the increase occurs primarily in the concentrations of LDL, IDL, and VLDL (Table 3). An increase from 0.06 to 0.4 mmol/L for VLDL+IDL and from 0.04 to 1.24 mmol/L for LDL results in significant cholesterol accumulation in the graft. The accumulation continues when the cholesterol concentration increases further (Figure 4). When plasma total cholesterol concentration exceeds 20 mmol/L, the increase in concentration occurs almost exclusively in the VLDL fraction.

The macroscopic photograph of the upper part of the graft (from 1.0 cm to 4.0 cm; DA in Figure 1B), the homologous aortic transplant. Lower part of the graft (from 0.5 cm to 1.0 cm) is the autologous aortic transplant (HA in Figure 1B). The rabbit was placed on a cholesterol-enriched diet 14 days after transplantation, and the entire aorta was removed after 30 days of cholesterol feeding. Cholesterol concentrations in the two segments are given in Table 4 as the first and third entries.

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The observation that normocholesterolemic rabbits do not accumulate cholesterol in the arteries even after severe damage to the vessel wall is in accordance with the findings of other groups. The latter group observed that complete deendothelialization of the aorta in normocholesterolemic rabbits did not lead to aortic cholesterol accumulation even after 2 years.

It is relatively easy to observe conditions with prolonged elevations of plasma concentrations of LDL or VLDL in humans and in some experimental animals, especially rabbits. It has proved to be much more difficult to study the effects of increased HDL concentrations in plasma of humans and animals.

We increased the influx of plasma cholesteryl ester into the aortic wall by a factor of 100 in the present study. This was accomplished not by raising the plasma cholesteryl ester concentration in plasma by a factor of 100 but by increasing the permeability of the aortic luminal surface by that factor. Because the major portion of cholesterol in plasma in these rabbits was HDL cholesterol (Table 3), the flux of cholesteryl ester into the graft consists primarily of HDL cholesteryl ester. This notion is based on the assumption that the distribution of labeled cholesteryl ester in plasma mimics the distribution of unlabeled cholesteryl ester in plasma because of the high activity of cholesteryl ester exchange protein in rabbits. The observed lack of cholesterol accumulation in the wall indicates that HDL leaves the wall as fast as it enters, even when the influx is increased several hundred times compared with the influx of HDL into the intact aorta. The much lower amounts of VLDL, IDL, and LDL that also enter the wall, assuming a size- and concentration-dependent influx, are apparently also able to leave the wall again.

When plasma cholesterol concentration increases in response to cholesterol feeding, the increase occurs primarily in the concentrations of LDL, IDL, and VLDL (Table 3). An increase from 0.06 to 0.4 mmol/L for VLDL+IDL and from 0.04 to 1.24 mmol/L for LDL results in significant cholesterol accumulation in the graft. The accumulation continues when the cholesterol concentration increases further (Figure 4). When plasma total cholesterol concentration exceeds 20 mmol/L, the increase in concentration occurs almost exclusively in the VLDL fraction.

The aortic accumulation of non-HDL cholesterol during cholesterol feeding and the absence of HDL cholesterol accumulation despite a high aortic influx may be ascribed to a difference in interaction between...
the two classes of lipoproteins and the proteoglycans within the extracellular matrix of the arterial wall.\textsuperscript{20,21}

In conclusion, we developed a surgical model in the rabbit for studying quantitative aspects of cholesterol metabolism in transplanted arteries. We observed an early increase in HDL permeability of the luminal surface of the transplanted artery. There was no cholesterol accumulation in the graft when the influx into the graft consisted primarily of HDL. However, cholesterol accumulated in the wall in response to a moderate increase in plasma VLDL, IDL, and LDL levels after a few weeks of cholesterol feeding.

The present rabbit model may thus be useful for further studies concerning the pathogenesis of graft atherosclerosis and its retardation. Such studies may have more general interest, as immune mechanisms may also be of importance for the development of the atherosclerotic plaque in nontransplanted arteries.\textsuperscript{15}

Acknowledgments

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References


<table>
<thead>
<tr>
<th>Rabbit identification No.</th>
<th>Days after transplantation</th>
<th>Uptake time for labeled cholesferyl ester (hours)</th>
<th>Mean value of labeled cholesterol ester in plasma (dpm/μl)</th>
<th>Labeled cholesterol ester in inner layer of aorta (dpm/cm²)</th>
<th>Intimal clearance of cholesterol ester* (μl/cm²/hr)</th>
<th>Contaminating plasma* (μl/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-7</td>
<td>298</td>
<td>1.6 0.17</td>
<td>67.0 (40.2) 674</td>
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<td>3.83 0.16</td>
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<td>E-8</td>
<td>112</td>
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<td>52.2 (34.9) 361</td>
<td>181 135</td>
<td>2.08 0.37</td>
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<tr>
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<td>155</td>
<td>1.5 0.08</td>
<td>64.5 (38.6) 681</td>
<td>267 43</td>
<td>2.76 0.06</td>
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</tr>
<tr>
<td>E-9</td>
<td>105</td>
<td>1.2 0.08</td>
<td>44.0 (25.2) 305</td>
<td>573 233</td>
<td>10.9 0.76</td>
<td></td>
</tr>
</tbody>
</table>

Values in parentheses are those in the final plasma sample, i.e., 5–10 minutes before removal of the aorta.

*The intimal clearance is calculated without taking plasma contamination into account, and the contamination is calculated without taking influx during the short exposure time into account. The contamination values are thus maximum values.
Accelerated cholesterol accumulation in homologous arterial transplants in cholesterol-fed rabbits. A surgical model to study transplantation atherosclerosis.

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