Electron Microscopic Findings of Experimental Atheromatous Lesions in Rats

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Abstract. Electron microscopic observation was carried out on the abdominal aorta and coronary artery of rats fed an atherogenic diet (containing 3% cholesterol, 1% cholic acid, 0.3% thioracil and 21% hydrogenated fat) for 28 days combined with oral administration of vitamin D₃ (320,000 IU/kg/day) for the initial 4 days. Although the changes found in these two different types of artery were essentially similar to each other and characterized by the presence of lipid containing foam cells especially in the intima, some differences were detected between these arteries. Namely, deposition of fine fibrillar and granular materials, appearance of extracellular lipid crystals and increase in number of modified smooth muscle cells were apparent in the coronary artery, while increase in number of lipid-laden smooth muscle cells was conspicuous in the abdominal aorta. In addition, the present study demonstrated the possibility of transformation of both the smooth muscle cell and monocyte into foam cell.

Altman [1] reported on rapid induction of the atherosclerosis by short-term loading of vitamin D₃ (VD₃) and cholesterol in rats. Okawa et al. [9, 10] and Yasoshima et al. [15], who performed experiments according to a method similar to Altman’s, failed to produce atherosclerotic lesions but arteriosclerotic ones. Recently, Ishihara et al. [8] succeeded in provocation of atheromatous changes by long-term high-fat diet feeding combined with short-term VD₃ loading in rats and described light microscopic findings. The present study was undertaken to confirm the results of Ishihara et al. [8] and to elucidate ultrastructural events occurring in the abdominal aorta and coronary artery in rats. In this connection, there was very little reports up to the present time in which the two different type of artery, i.e. aorta and coronary artery, were comparatively examined by electron microscopy.

Materials and Methods

A total of 20 male rats of Sprague-Dawley strain weighing about 200 g were used. Half of the animals was fed a semi-synthetic atherogenic diet containing 3% cholesterol, 1% cholic acid, 0.3% thioracil and 21% hydrogenated fat [14] for 28 days. In addition, they were orally administered with 320,000 IU of VD₃ (Dai-ichi Pure Chemicals, Co., Ltd.) in 2 ml of olive oil (Nakarai Chemicals, Co., Ltd.) per kg of body weight per day for the first 4 days. The remaining half served as controls was fed a basal diet (CE-2; CLEA Japan, Inc.). All animals were kept in an air-conditioned room and allowed to have diet and water ad libitum.

The outline of clinicopathological findings was shown in Table 1. Six survivors of atherogenic diet group and all
controls (Table 1) were sacrificed and subjected to pathological examination on the 28th day of experiment. At the time of sacrifice, 3 animals each of the 2 groups were perfused with 2.5% glutaraldehyde in phosphate buffer solution under ether anesthesia as previously described [15]. Samples of abdominal aorta obtained from just below the renal bifurcation and of the left descending branch of coronary artery were then immersed in the same cold fixative for 2.5 hrs, and were dissected transversely into small pieces. They were post-fixed in 1% osmium tetroxide in phosphate buffer solution, dehydrated in graded alcohol, and embedded in epoxy resin. Ultra-thin sections were doubly stained with uranyl acetate and lead citrate, and observed by an electron microscope JEOL's model JEM-100 C.

**RESULTS**

**Abdominal aorta:** The endothelial cells swelled with increasing electron opacity in their cytoplasm. They contained lipid droplets and numerous profiles of rough-surfaced endoplasmic reticulum (ER) which exhibited often dilation (Figs. 1 and 2). Occasionally, mononuclear cells were found adherent to the luminal surface of endothelium.

The intima was thickened by accumulation of smooth muscle cells with variable number of intracytoplasmic lipid droplets (hereinafter referred to as lipid-laden SMCs) (Fig. 2) and foam cells (FCs) (Fig. 3). The FCs, which markedly enlarged with numerous lipid droplets and vacuoles containing myelin figures and exhibited typical foamy appearance, were usually seen beneath the endothelial cells (Fig. 3). In lipid-laden SMCs, myofilament bundles were located to the periphery along the cellular membrane (Fig. 4).

Inner or middle layer of the media underwent disorganization with marked deformation, disruption and calcific deposition of elastic membrane. In these areas, deposition of lipid and calcium salts was noticed in and around necrotic SMCs (Fig. 5). Occasionally, small clusters of lipid-laden SMCs, accompanied with one or two SMCs which showed prominent proliferation of rough-surfaced ER and distinct decrease in organized myofilaments (hereinafter referred to as modified SMCs), were observed in the inner media. The outer media and adventitia showed no significant changes.

**Coronary artery:** The endothelial cells exhibited changes similar to those of aorta, and the intima was widened with a mass of fine fibrillar and granular materials which contained several lipid-laden and modified SMCs (Fig. 6). The FCs were generally observed along the partially calcified and disrupted inner elastic membrane (Fig. 6). In some parts of the

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Body weight (g)</th>
<th>Food intake (g/head/day)</th>
<th>Serum calcium content (mEq/l)</th>
<th>Serum total cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>23.6</td>
<td>5.54±0.03</td>
</tr>
<tr>
<td>Fed a basal diet</td>
<td>10</td>
<td>200</td>
<td>393</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed an atherogenetic diet and loaded with VD3</td>
<td>6</td>
<td>205</td>
<td>218</td>
<td>11.6</td>
<td>5.82±0.14</td>
</tr>
</tbody>
</table>

Remarks: 1) OCPC method, 2) Enzyme method.
intima, especially around the destructed FCs, needle- or stick-like extracellular lipid crystals were conspicuous (Fig. 7). In addition, mononuclear cells were occasionally detected just below the endothelial cells (Fig. 6). Their cytoplasm contained numerous free ribosomes, some large mitochondria, a variable number of lysosomal granules and several lipid droplets, and had no myofilaments (Fig. 8).

Similar to the intima, extracellular lipid and calcium deposits and accumulation of cellular debris were also seen in a mass of fine fibrillar and granular materials in the media. Virtually, normal SMCs were rare and most of SMCs in the media were transformed into characteristic modified SMCs which accompanied small amount of connective tissue fibers around them (Fig. 9). In contrast to those in aorta, changes covered almost all over the media and even the adventitia was involved locally in lesions.

DISCUSSION

Electron microscopic observation was carried out on the abdominal aorta and coronary artery of rats fed an atherogenic diet and loaded with VDs. In consistent with the light microscopic findings reported by Ishihara et al. [8], atheromatous changes characterized by the presence of lipid containing FCs were also detected in the present study. The authors previously pointed out the intimal edema and medial degeneration with calcific deposition as the early arterial lesions in rats briefly loaded with VDs and cholesterol [10, 15]. Therefore, it seems reasonable to conclude that the present atheromatous changes might be brought about by deposition of lipids due to marked hypercholesterolemia (Table 1) in the foregoing lesions resulting from hypercalcemia in the early phase [8, 15].

The outline of ultrastructural changes found in the abdominal aorta and coronary artery was similar to each other in the present materials. It is, however, worthy to note some differences between either types of artery. Namely, deposition of fine fibrillar and granular materials probably derived from blood plasma [9], appearance of extracellular lipid crystals possibly liberated from destructed FCs [4] and increase in number of so-called modified SMCs [2] were more conspicuous in the coronary artery, while increase in number of lipid-laden SMCs was more prominent in the abdominal aorta. These peculiarity may reflect the difference in architecture of vascular wall, mode of response of SMCs and rheological environment of the respective site.

As to the candidate for FCs found in the intimal atheroma, the followings have been proposed up to the present time: lipophages in circulating blood [3], blood-born monocytes [4, 8, 11] and SMCs migrating from the media [2, 6, 7, 12, 13]. Particularly in rats, Bálint et al. [3] and Clowes et al. [4] suggested that SMCs did not participate in producing atheroma. On the other hand, in the present cases, lipid-laden SMCs very similar to “transitional forms between smooth muscle cells and foam cells” [12] were noticed especially in the intima of abdominal aorta and mononuclear cells bearing the same ultrastructural characteristics with those of “lipid-laden macrophages” [4] or “hypertrophied monocytes, i.e. transitional forms between monocytes and foam cells” [5] were detected beneath the endothelial cells of coronary artery. In addition, Gerrity [5], who studied experimental atherosclerosis in pigs, described that SMC-derived FCs were more prominent than monocyte-derived FCs in the advanced abdo-
minal aortic lesions while monocyte-derived FCs were dominant in any of the stage of lesions in the aortic arch. Accordingly, it is quite logical to postulate that both SMC and monocyte could transform into FC and that the role of either cells varied by the type of artery, stage of lesion and species of animals.

References
Description of Figures

Figs. 1 to 5 are abdominal aorta and Figs. 6 to 9 coronary artery.

Fig. 1. Endothelium. Cistern-like dilation of interendothelial space (arrow). Lipid droplets are seen in the right endothelial cell. ×13,000.

Fig. 2. Intima showing marked thickening with accumulation of lipid-laden SMCs. ×4,000.

Fig. 3. Intima containing FCs. IEM exhibits calcification, deformation and partial disruption. ×3,000.

Fig. 4. Part of lipid-laden SMC. Myofilament bundles (arrow) along the cellular membrane. ×20,000.

Fig. 5. Media exhibiting deposition of calcium salts and lipids (arrow). ×4,000.

Fig. 6. Intima. Lipid-laden SMCs, FCs and mononuclear cells (arrow) in a large mass of fine fibrillar and granular materials. ×2,000.

Fig. 7. Intima. Extracellular LCs near DFC. ×3,000.

Fig. 8. MNC with several lipid droplets (arrow) just below endothelial cells. ×10,000.

Fig. 9. Typical view of modified SMC. ×4,000.

Abstract

The experimentally induced atherosclerosis in rabbits by daily administration of cholesterol (1% cholesterol + 0.3% cholic acid + 21% pig’s liver oil) for 28 days led to atherosclerotic lesions in the aorta and coronary arteries. The atheromatous plaques were characterized by the accumulation of lipid-laden SMCs, mononuclear cells, and extracellular collagenous matrix. The intima of the atherosclerotic lesions showed marked thickening with calcification, deformation, and partial disruption. The media exhibited deposition of calcium salts and lipids. The modified SMCs were observed with typical myofilament bundles. The results of this study suggest that atherosclerosis in rabbits can serve as a model for studying the pathogenesis and treatment of human atherosclerosis.