Early Ultrastructural Changes of Aorta in Rats Loaded with Vitamine D₂ and Cholesterol

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ABSTRACT. Ultrastructural events in the early stage of the changes of the abdominal aorta in rats loaded with vitamin D₂ and cholesterol were studied. The first appearing noticeable change was vesiculation in the intima which was attributable to the infiltration of blood plasma from the surface of endothelial cells. Then, intimal edema and deformation of the endothelial cells occurred. The subsequent course was characterized by degeneration of the inner and middle layers of the media accompanied with deposit of crystalline calcium salt. In the early stage of medial degeneration, crystallines of calcium salt first appeared within mitochondria of the smooth muscle cells. It was suggested that hypercalcemia due to vitamin D₂ loading might play an important role in producing the early aortic lesions.

Experimental vascular lesions induced by vitamin D [1-9] have generally been regarded as an animal model of arteriosclerosis and it has been known that early aortic lesions in this experimental disease are characterized by necrosis of the media accompanying calcification. Previously, Okawa et al. [11] described the histopathological findings of acute phase in the aorta and other organs in rats loaded with vitamin D₂ and cholesterol by a simple method similar to Altman's [1]. The present study was undertaken to elucidate ultrastructural events principally occurring in the aorta in the early phase following the treatment with massive dose of vitamin D₂ and cholesterol.

MATERIALS AND METHODS

The materials used were the same with those described in the previous report [11]. Namely, male Sprague-Dawley rats, nine and a half weeks of age and weighing about 320 g, were administered with a mixture of 8 mg of vitamin D₂, 40 mg of

cholesterol, and 1.5 ml of olive oil per kg of body weight consecutively from days 1 to 4 of experiment. Samples were collected from two animals each sacrificed on days 2 to 5 and 11 of experiment. Specimens were also harvested from two rats each of untreated control group in the schedule as mentioned above. Under ether anesthesia, the animals were injected into the left ventricle with 2.5% glutaraldehyde in phosphate buffer solution. At the same time, femoral arteries were cut for outflow. After 3 min of perfusion the abdominal aorta about 1 cm in length was removed from just below the renal bifurcation. Specimens were then immersed in cold fixative which is the same as above-mentioned perfusion for 2.5 hr, and were dissected transversally into 1 mm long rings. 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 double-stained with uranyl acetate and lead citrate, and observed by an electron microscope JEOL's model JEM-100 C.

RESULTS

As shown in Fig. 1, characteristic architecture of the aortic wall preserved in the specimens of aorta from control animals.

In the treated group, rounding and in-

creasing in number of the smooth muscle cells occurred on day 2 focally in the media just below the internal elastic membrane (Fig. 2). Many vesicles with various sizes occurred between the endothelial cells and the internal elastic membrane, resulting slight edema of the intima (Fig. 3). The change was seen scatteringly only in limited areas.

On day 3, the intimal change, similar to but more prominent than that seen on day 2, was observed extensively (Fig. 4). The vesicles, often coalesced into large ones, contained amorphous proteinaceous precipitates in them. Mononuclear cells having pseudopod-like processes were occasionally found to be attached on the surface of the endothelial cells. Many of the smooth muscle cells exhibited rounding and the architecture of the inner media was slightly disorganized.

On day 4, intimal edema, that is, vesiculation in the subendothelial space was remarkable and the endothelial cells exhibited deformation and extreme thinning in places. In addition, partial dilation of interstices was seen in the intima. Inner or middle layer of the media underwent marked disorganization with deformation and disruption of the elastic membranes which exhibited occasional increase in electron density (Fig. 5). In these areas, edematous thickening was noticed in the interstitium, and smooth muscle cells were irregular in their contour and separated from each other. Mitochondria of these deformed smooth muscle cells often swelled and contained electron-dense needle-like crystallites (Fig. 6). A small amount of such crystallites were also recognized in the interstitium in proximity to the elastic membrane (Fig. 7). They were believed to be crystals of calcium salt from their mode of occurrence.

On day 5, deformation of the endothelial cells and partial dilation of the interstices among them were seen at times, but vesiculation was seldom observed in the intima. Crystallites of calcium salt were present everywhere in the inner and middle layers of the media, where fragmentation of the elastic membranes and smooth muscle cells was noticed. In most severely affected areas, the basic architecture of the media was no longer discernible. Crystallites of calcium salt found in the necrotic smooth muscle cells and interstitium were relatively large, while those occurred in the elastic membranes were fine (Fig. 8). In contrast with the inner and middle layers of the media, the outer layer exhibited essentially normal appearance except for elongation of smooth muscle cells.

On day 11, slight thickening of the intima with the increase of fine fibrillar and granular materials between the endothelial cells and internal elastic membrane was seen. Mononuclear cells containing many lysosomal granules occasionally occurred beneath the endothelial cells. Most of the smooth muscle cells of the inner and middle layers of the media deformed into rounded contour, and the elastic membranes occasionally ruptured and disappeared. In addition, cellular debris was found here and there in the lesion. Abundant electron-dense needle-like or granular materials presumably corresponded to the calcium salt deposited on and along the elastic membranes (Fig. 9).

DISCUSSION

Electron microscopic observation was carried out on the abdominal aorta of rats loaded with vitamin D₂ and cholesterol. Intimal edema was pointed out as the first detectable and noticeable changes following the treatment with vitamin D₂ and cho-

lesterol. Then severe degenerative changes occurred in the inner and middle layers of the media and they were accompanied by calcium deposit. Namely, the aortic changes began focally in the intima and thereafter extended all over the inner and middle layers of the aorta. Above-mentioned sequence of events seemed to indicate that hypercalcemia induced by loading with vitamin D₂ resulted in infiltration of blood plasma into the intima.

In the present observation, the appearance of fine needle-like crystallites, believed to be those of calcium salt, was noticed first within mitochondria in the necrotic smooth muscle cells. It is said that the mitochondria possess a high-affinity with calcium ion and yet correlate to biological calcification processes [10]. Concerning the pathogenesis of necrosis of muscle cells in various muscle diseases, Wroegeman et al. [12] suggested the process that excessive amounts of calcium ion influxed into cells accumulate in the mitochondria, resulting defect of oxidative phosphorylation, and thus this may cause cell necrosis. Judging from the results of present observation, it is possible that the similar mechanism also involved in the pathogenesis of the aortic lesions in rats loaded with vitamin D₂. Mode of occurrence of crystallites of calcium salt in the advanced lesions was essentially similar to those in the aorta of rabbits loaded with vitamin D₂, as described by Eisenstein et al. [5]. In their communication, however, they did not mention concerning the appearance of calcium salt within the mitochondria of the smooth muscle cells in the early period after loading with vitamin D₂.

The present results principally coincided with the light microscopic findings on the same materials [11]. In the histopathological examination, however, lipid infiltration

was noticed in the media of the aorta in addition to calcification. As described in the present paper, no findings corresponding to the light microscopic lipid infiltration were obtained by the ultrastructural observation. With regard to the mode of presence of lipid in the affected aorta, it is possible that the lipid may be diffusely distributed in a form of complex with such substances as calcium salt, mucopolysaccharide and elastin on and along the degenerate elastic membranes.

Further noticeable finding was the appearance of mononuclear cells containing many lysosomes beneath the endothelial cells. It seems that the cells, as scavenger cells, immigrated from the blood stream into necrotic areas.

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EXPLANATION OF FIGURES

Abbreviation

E: Endothelial cell.
 EM: Elastic membrane.
 IEM: Internal elastic membrane.

L: Lumen.
 M: Mitochondrion.
 SMC: Smooth muscle cell.

Fig. 1. Abdominal aorta from a control rat. The endothelial cells of the intima are thin and regular array of elastic membranes and smooth muscle cells are conspicuous. $\times 2,500$.

Fig. 2-9 are abdominal aorta from treated rats.

Fig. 2. On day 2. Several rounded atypical smooth muscle cells are seen protruding the intima up to the lumen. $\times 2,500$.

Fig. 3. On day 2. Typical picture of intimal edema. Many vesicles appeared between the endothelial cells and internal elastic membrane. Rounding of the smooth muscle cell is also seen. $\times 3,300$.

Fig. 4. On day 4. The intima shows marked edema, and endothelial cells appear to protrude into the lumen. $\times 3,300$.

Fig. 5. On day 4. Severe edema and necrotic changes result in disorganization of the intima and inner and middle layers of the aorta. $\times 2,500$.

Fig. 6. On day 4. A part of deformed and necrotic smooth muscle cell. Myofilaments are

irregularly arranged and became loose. Mitochondria are mostly swollen. $\times 10,000$. Insert: Higher magnification of a mitochondrion. The mitochondrion contains electron-dense and needle-like crystallites which seem to be crystals of calcium salt, and fine structure of the mitochondrion is obscure. $\times 48,000$.

Fig. 7. On day 4. High-power magnification of a part of the media. A few fine needle-like crystallites (arrows) are seen in the interstitium adjacent to the elastic membrane. $\times 40,000$.

Fig. 8. On day 5. High-power magnification of a part of the media. Large crystals (arrows) in the interstitium, and abundant fine crystallites in the elastic membrane are shown. $\times 30,000$.

Fig. 9. On day 11. Most of the smooth muscle cells of the inner and middle layers of the media become rounded. Large amount of electron-dense deposits of crystallites (arrow heads) are seen along the elastic membrane. $\times 2,500$.

要 約

ビタミン D₂ およびコレステロール負荷ラット大動脈の初期病変の電顕所見: 八十島昭・大川 仁・土井邦雄・岡庭 梓 (田辺製薬株式会社薬理研究所) ——大量のビタミン D₂ およびコレステロールを負荷したラットの腹大動脈の初期病変を電顕で検索した。早期には血漿成分の浸透に関連して内膜に小水疱が出現し、ついで内皮細胞の変形および内膜水腫が目立った。これらの変化にひきつづいて、中膜の内層および中層に、カルシウム塩結晶の沈着をともなった変性が観察された。中膜の変性初期には、最初に平滑筋細胞のミトコンドリア内にカルシウム塩の結晶がみとめられた。以上の成績から、大量のビタミン D₂ 投与により誘発された高カルシウム血症が大動脈初期病変を惹起する上で重要な役割を果していることが強く示唆された。