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Micro- and Ultrastructure of the Pial Arteries in Experimental Hypercholesterolemia

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Morphological changes induced by experimental cholesterolemia in the walls of pial arteries of Chinchilla rabbits are studied by light and electron microscopy. It is found that these changes are typical of small artery atherosclerosis.

Key Words: *hypercholesterolemia; atherosclerosis; pial arteries*

Atherosclerosis is the major cause of chronic cerebral circulation disorders [5,7]. Information regarding the atherosclerosis-related morphology of cerebral and pial blood vessels is scarce [1,3,11]. Our objective was to examine morphological changes occurring in pial arteries in experimental hypercholesterolemia (HC).

MATERIALS AND METHODS

Hypercholesterolemia was routinely induced in 3-year-old male Chinchilla rabbits weighing 3 to 3.5 kg by daily injections of 10% oil suspension of cholesterol in a dose of 0.2 g/kg for 3 months [9]. Group 1 (control) included intact rabbits (n=17) maintained on standard vivarium diet, and group 2 (n=18) consisted of cholesterol-treated animals. Fragments of the brain with pia mater at the level of vertical cortex were fixed routinely for light and electron microscopy [8,10]. For light microscopy paraffin sections were stained with hematoxylin and eosin; semithin sections were stained with methylene blue. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a TESLA BS-500 electron microscope.

RESULTS

Light microscopy revealed no changes in the pial arteries of control rabbits. Endothelial cells (EC) with moderately basophilic round-oval nuclei and thin internal elastic membrane (IEM) were seen in the tunica intima; the tunica media consisted of two or three layers of smooth muscle cells arranged in a circular fashion and a poorly developed tunica adventitia gradually transforming to the connective tissue of the pia mater. In HC, the walls of pial arteries were thickened, loose, edematous, with light spaces under IEM and signs of perivascular edema. The EC nuclei were pyknotic, thickened, sometimes swollen, light, vertically oriented towards the vessel lumen; desquamation of EC was noted in some areas. Marked vacuolization of the brain indicated its edema.

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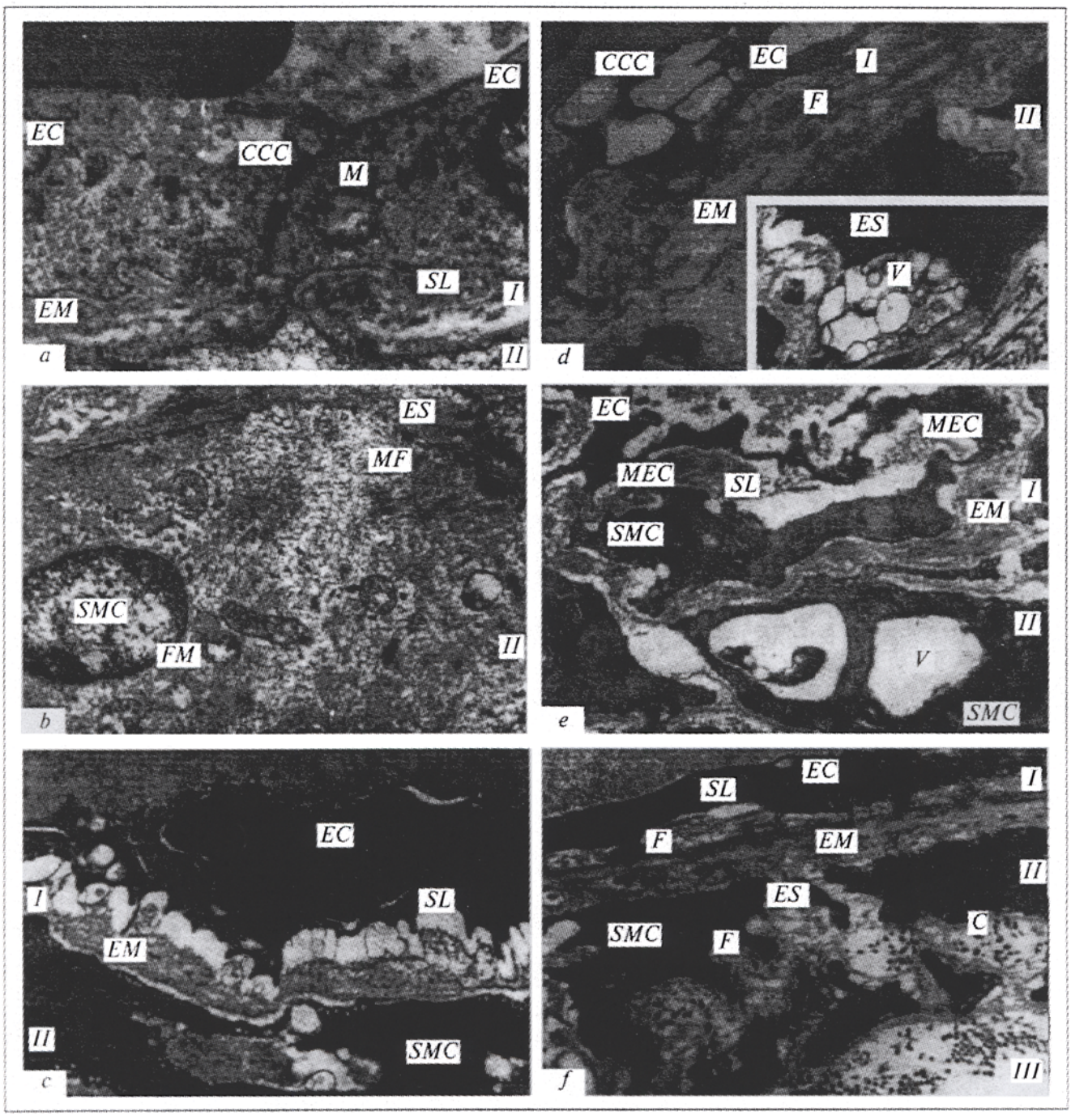

Fig. 1. Pial arteries of Chinchilla rabbits. *a*, *b*) control animals; *c-f*) hypercholesterolemic animal. Tunica intima (*I*), media (*II*), and adventitia (*III*); *EC*: endothelial cell; *M*: mitochondria; *SMC*: smooth muscle cell; *CCC*: cell-to-cell contact; *SL*: subendothelial layer; *EM*: elastic membrane; *ES*: extracellular space; *MEC*: myoendothelial contact: *FM*: fragmentation of mitochondria; *MF*: myofilaments; *F*: fibrin; *V*: vacuoles; *C*: collagen. *a*) ×22,000; *b*, *e*) ×15,000; *c*, *d*, and *f*) ×17,500.

Electron microscopy revealed EC with moderately developed cytoplasm containing typical organelles and electron-transparent roundish nuclei with peripheral rings of heterochromatin. It should be noted that the number of microvilli on the luminal surface was decreased, while the number of microprocesses varying in length on the basal surface was increased. These processes were oriented towards the subendothelial layer packed with microfibrills. The cytoplasm contained small numbers of micropinocytic vesicles and ribosomes and numerous microfilaments (Fig. 1, *a*).

Depending on the density of cytoplasmic organelles, EC were classified as low, medium, and

high electron density cells. Low electron density EC were rare. Their cytoplasm contained occasional osmiophilic microcorpuscles and large electron-transparent vacuoles. These vacuoles may result from destruction of large swollen mitochondria seen along with small and medium-sized ones, which looked intact. Cell-to-cell contacts with solitary transparent vesicles and gap junctions of high electron density EC were readily identified.

An electron-transparent IEM with occasional microfibrils was disrupted, particularly at the level of EC microprocesses, and looked "ulcerated" from the side of the endothelium. The layer under IEM was narrower than the subendothelium and had microfibrils (Fig. 1, *a*).

Medial smooth muscle cells were large, had a low electron density cytoplasm and electron transparent nuclei with heterochromatin located at the periphery (Fig. 1, *b*). The cells contained numerous micropinocytic vesicles (under the plasma membrane) and myofilaments. The mitochondria displayed the signs of heterogeneity and fragmentation. The spaces between the myocytes were narrow. The poorly developed tunica adventitia contained collagen fibrils and few fibroblasts with long thin processes sometimes layered onto each other and forming a capsule-like structure together with the cell bodies.

Hypercholesterolemia caused ultrastructural changes in all membranes of the pial arterial wall. Endothelial cells were electron dense (the nucleus was hardly discernible from the cytoplasm), very thinned, and elongated (Fig 1, *c-f*). No organelles or other cytoplasmic structures were seen, except solitary micropinocytic vesicles and electron transparent vacuoles, which were sometimes rather large. The accumulations of electron-dense microcorpuscles were present in some cells. The longest of the numerous microprocesses on the basal surface of EC penetrated the IEM rather deeply. There were few microprocesses on apical surface, similarly to the control. Cell-to-cell contacts were not seen. Gap junctions were not seen either because of the high electron density of EC. However, cell-to-cell contacts sometimes were widened and formed large cavities (Fig. 1, *d*). The vacuoles penetrated into the subendothelium, which was widened due to edema and filled with vacuoles (Fig. 1, *c*) or fibrin filaments (Fig. 1, *d, f*).

In some cases, the IEM was thickened, loose, and markedly "ulcerated" from the side of the endothelium (Fig. 1, *c*), thinned, compact, and fragmented in other cases (Fig. 1, *e*), and sometimes was not seen (Fig. 1, *c-e*). Fibrinoid edema developed under the IEM (Fig. 1, *d, f*).

Smooth muscle cells in the tunica media were electron dense, flattened, and had numerous microprocesses and thin processes (Fig. 1, *c, e, f*). Some of them, penetrating via the ruptures in IEM, contact with EC (Fig. 1, *e*); others were oriented parallel to the vessel lumen. Electron transparent micropinocytic vesicles and large vacuoles were distinguished in the smooth muscle cell cytoplasm, microcorpuscles being hardly discernible, while myofilaments and other organelles were not seen. Spaces between the cells were edematous: they were dilated and often filled with fibrin filaments and vacuoles (Fig. 1, *c, d*). The content of extracellular matrix was markedly increased, particularly that of collagen fibrils (Fig. 1, *f*). Smooth muscle cells in the media were compressed by exudate, fibrin, or collagen fibrils (Fig. 1, *c-f*). These ultrastructural changes (similarly to those observed in EC) are indicative of destructive processes.

Smooth muscle cells with destructive changes with fragments of IEM coating them from the outside were also seen in the intima (Fig. 1, *e*). Migration of smooth muscle cells from the media into the intima can be traced from the microphotographs: first, thin processes appear in the intima, then large microprocesses, and then the cell body. The adventitia was loose and edematous.

Thus, HC causes morphological changes in the entire wall of pial arteries. Pronounced destructive changes in EC and the presence of large vacuoles in their cytoplasm and substantial local dilatations in cell-to-cell contacts indicate that the barrier and transport functions of the endothelium are impaired and endothelial permeability for blood plasma is increased. Accumulation of fluid and fibrin in the subendothelium and deeper layers of the vascular lead to edema. Accumulation of the fluid in the main substance of the connective tissue and collagen outgrowth widened the extracellular space, which caused compression of EC and smooth muscle cells followed by development of marked destructive changes in them.

It was reported that the increase in the permeability of all blood vessels, including the aorta, is typical of HC [6]. The vascular wall edema was observed in the aorta and large and medium cerebral arteries in experimental HC [1] and in human pial arteries at the early stages of atherosclerosis [11]. Focal edema of large arteries has been regarded as a prelipid change occurring in atherosclerosis [2]. Ultrastructural destructive changes of EC, fragmentation and focal lysis of the IEM, and migration of smooth muscle cells into the aortic intima were found in hypercholesterolemic and old animals [1,4]. An increase in the number of

collagen fibers is one of the atherosclerosis markers. Taken together, our observations indicate that experimental HC induces pronounced atherosclerotic changes in pial arteries.

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Smooth Muscle Cell Heterogeneity in Intimal Thickenings of Various Genesis and in Organized Thrombi

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A population of smooth muscle cells heterogeneous by the expression of myosin, h-caldesmon, and calponin is identified by indirect immunofluorescence in the intima of normal aorta and femoral artery, in the subendothelium in atherosclerosis and Takayasu's disease, and in organized thrombi. The similarity of the extracellular matrix in various forms of arteriosclerosis implies that its hyperproduction is a typical response of smooth muscle cells.

Key Words: *smooth muscle cells; intimal thickenings; thrombi*

Smooth muscle cells (SMC) are the major cell type responsible for the synthesis of the extracellular matrix (ECM) in the subendothelial layer. They contribute to the formation of diffuse intimal thickening (DIT) in elastic and muscle-elastic arteries which starts in childhood and propagates with age [9]. Increased synthesis of ECM has been observed in atherosclerosis and other types of arteriosclerosis and in organized mural thrombi. The attention of researchers has been focused on the behavior of SMC in atherosclerotic lesions. It has been hypothesized that in an atherosclerotic plaque SMC appear as a result of migration from the media into the subendothelial layer followed by their proliferation [12, 16]. However, this hypothesis was only indirectly confirmed by experiments with endothelial damage and by a wide variety of phenotypic modifications of SMC cultured *in vitro* [3,18]. So far, it remains unclear how SMC populate the subendothelial layer of the intima during the DIT development, how they appear in atherosclerotic plaques, and whether phenotypic modifications of SMC are specific for athe-

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