ULTRASTRUCTURAL CHANGES IN BLOOD CAPILLARIES AFTER IMPLANTATION OF A DMBA PELLET

G. V. Mardanova

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Implantation of a pellet of 9,10-dimethyl-1,2-benzanthracene (DMBA) in the CNS induces the formation of a capsule of special type around the carcinogen [2-4]. The ultrastructure of the hairy cells forming the inner layer, i.e., that in direct contact with the carcinogen, of this capsule was studied in detail in previous investigations and the role of these cells in phagocytosis of DMBA was established [6, 7]. At the same time, in the early stages of chemical carcinogenesis the vascular layer of this capsule is known to play an important role, for alongside the wall of the changed blood vessels, namely in the substance of the fibrinoid and fibrinous masses, the first atypical cells giving rise to tumor growth are subsequently found [2, 3]. Accordingly it was decided to determine changes in the walls of the blood capillaries in the zone of implantation of DMBA.

EXPERIMENTAL METHOD

Experiments were carried out on female albino rats of the SHK line, into the right cerebral hemisphere of which a DMBA pellet weighing 1.5-2 mg was implanted. A paraffin wax pellet of the same volume was implanted in the same region of control animals. Pieces of brain containing the pellet were excised after 1, 14, 90, and 270 days. The material was processed by the usual electron-microscopic method, full details of which were given previously [6, 7].

EXPERIMENTAL RESULTS

In the perifocal zone 24 h after implantation of DMBA into the brain blood capillaries with two types of endothelial cells were observed. The cytoplasm of the cells of one type was electron-optically transparent. The nuclear chromatin was distributed uniformly. The number of mitochondria was average. Mitochondria with a festooned outline and also juvenile forms of mitochondria could be seen. Cisterns of the smooth cytoplasmic reticulum were dilated and formed large and small vesicles. The rough cytoplasmic reticulum was represented by single flattened cisterns, the membranes of which were degranulated in places. The tubules of the lamellar complex were dilated and surrounded by many vesicles. Besides these cells, the walls of the blood capillaries surrounding the DMBA pellet contained endothelial cells in whose cytoplasm, besides the ordinary unchanged organelles, distinctive lipid-like structures, more frequently polygonal in shape, and separated from the cell cytoplasm by a membrane, could be seen (Fig. 1). In their structure and shape these lipid-like structures were similar to those observed in the cytoplasm of hair cells surrounding the DMBA pellet [6, 7]. Just as in the hair cells, so also in the endothelial cells the characteristic contact between the membrane of the lipid-like structure and the mitochondrial membrane could be observed (Fig. 1). The basal layer of the remaining blood capillaries showed only slight changes. Areas of loosening of structure and also small areas with disturbance of the fibrillary structure were found. The picture of the changes in the wall of the blood capillaries 24 h after implantation of the paraffin wax pellet differed only a little from that in the experimental animals, but no lipid-like structures were present in the cytoplasm of the endothelial cells.

Electron-microscopic investigation of the blood capillaries located near to the carcinogen 14 days after its implantation showed that a few projections and indentations were formed on the surface of the endothelial cells facing the lumen. The chromatin of the nucleus was distributed uniformly in the center, but at the same time it formed a narrow compact band near the inner membrane of the nucleus. The matrix of the mitochondria

Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 7, pp. 118-121, July, 1980. Original article submitted December 6, 1978.

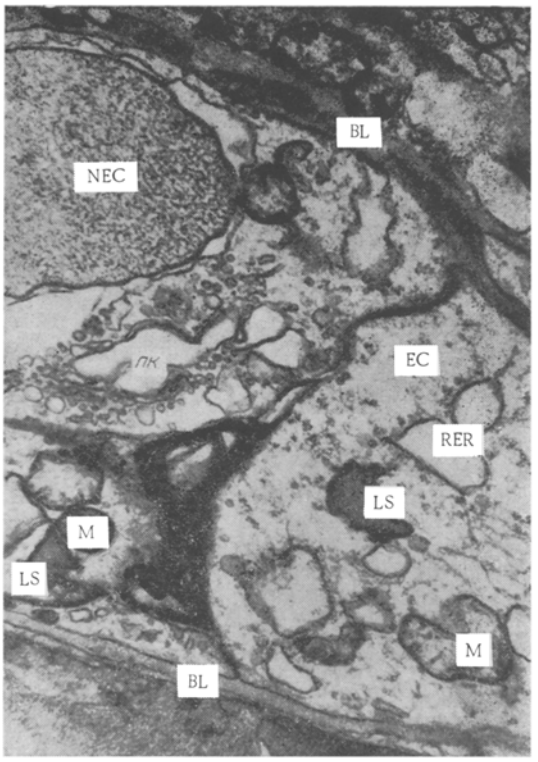

Fig. 1

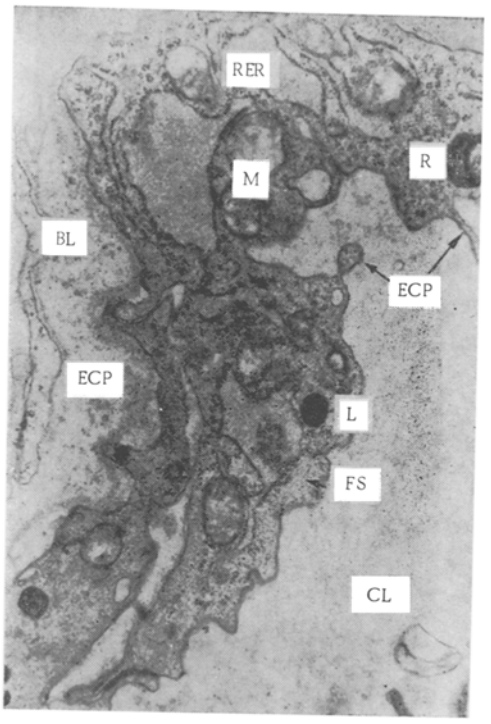

Fig. 2

Fig. 1. Fragment of blood capillary in zone of implantation of pellet, 24 h after implantation of carcinogen. NEC) Nucleus of endothelial cell; M) mitochondria; RER) rough endoplasmic reticulum; EC) endothelial cell; LS) lipid-like structures; BL) basal layer. 25,000×.

Fig. 2. Fragment of blood capillary wall in zone of implantation of pellet: 90 days after implantation of carcinogen. RER) Rough endoplasmic reticulum, M) mitochondria, L) lysosome-like structure; R) ribosomes; FS) fibrillary structures; ECP) endothelial cell processes; CL) capillary lumen; BL) basal layer. 20,000×.

was more frequently so intensively stained with osmium that the cristae were not always clearly distinguishable. The rough and smooth cytoplasmic reticulum showed little change compared with the previous period. The lamellar complex consisted of a system of short dilated tubules and vesicles. In the cytoplasm of the endothelial cells the number of free ribosomes and polysomes and also the number of micropinocytotic vesicles were somewhat increased. The basal layer was loose in structure and thin in places. In the brain tissue next to the basal layer cells similar in the structure of their nucleus and cytoplasm both to endothelial cells and to hair cells could be seen. The cytoplasm of these cells contained polygonal lipid-like structures. The endothelial cells of the blood capillaries 14 days after implantation of a paraffin wax pellet into the brain had a few short processes on their surface facing the lumen. The nuclear chromatin was distributed unevenly: in the center it was finely dispersed, but at the periphery it formed small masses. Mitochondria intensively stained with osmium, solitary canals of the rough and smooth endoplasmic reticulum, a moderate number of free ribosomes, and numerous micropinocytotic vesicles could be seen. In places the basal layer was very thin. Small areas of loosening of the structure of the basal layer were observed.

The edge of the cytoplasm of the endothelial cells facing the lumen 90 days after implantation of DMBA formed a few processes and deep indentations (Fig. 2). The nuclear chromatin was distributed in the form of small electron-dense masses. Marked polymorphism of the mitochondria was observed. Some mitochondria had a matrix which stained intensively with osmium, but the cristae were hardly distinguishable, other mitochondria were pale, and some were widened at one pole. Cisterns of the rough endoplasmic reticulum were greatly dilated and their lumen was filled with finely granular structures. Compared with the previous times, the lamellar complex showed hypertrophy and hyperplasia. Numerous fibrillary structures running in different directions appeared in the cytoplasm. There was a marked increase in the number of free ribosomes. The basal layer was loose in structure, widened, and separated into layers. It contained areas of concentration of fibrillary structures (Fig. 2).

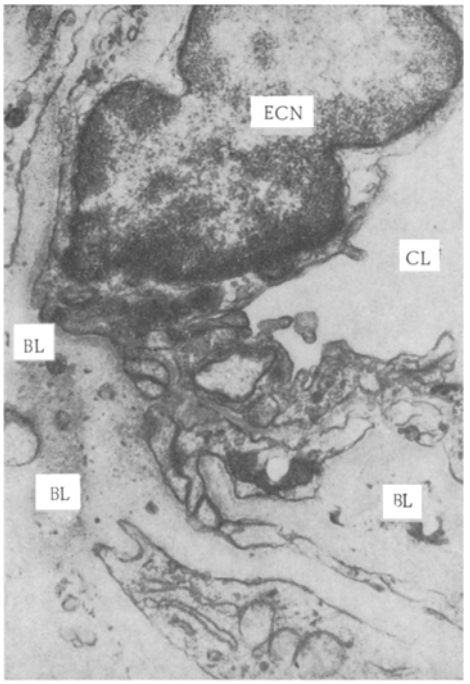

Fig. 3. Fragment of blood capillary wall in zone of implantation of pellet: 270 days after implantation of carcinogen. ECN) Endothelial cell nucleus; CL) capillary lumen; BL) basal layer. 25,000×.

In most animals tumors developed around the DMBA pellet 270 days after implantation. The cytoplasm of the endothelial cells of the blood capillaries, as before, contained numerous processes from the surface facing the lumen. It was usually thin and surrounded the nucleus as a narrow rim (Fig. 3). The outlines of the nucleus were uneven. The nuclear chromatin was distributed irregularly, forming small masses in the center of the nucleus but distributed in the form of a wide, uneven, electron-dense band near the inner nuclear membrane. Such endothelial cells contained single mitochondria with a few widened intracristal spaces. The cisterns of the rough endoplasmic reticulum were variously dilated and were free from ribosomes over wide areas. In the lumen of the cisterns micellary and homogeneously granular structures could be seen. The lamellar complex was located in the paranuclear zone, and consisted of single separate elements composed of dilated tubules and vesicles. A moderate number of ribosomes, mainly forming focal concentrations, were found in the cytoplasm. The basal layer was homogenized (Fig. 3) and had lost its fibrillary character over considerable areas. In some places the basal layer was widened. Homogenized areas joined together to form more extensive areas in the pericapillary zone. Zones of cytoplasm of different cells, as it were immured in the homogeneous-fibrillary substance, could be seen in such areas.

In the animals of the control group the ultrastructure of the blood capillary walls corresponded to that observed in the normal rat brain.

The pattern of changes observed in the walls of the blood capillaries around the pellet of carcinogen in the CNS is evidence of their active participation in the pretumor process. For instance, starting 24 h after injection of DMBA, besides nonspecific changes in the wall of the blood capillaries caused by trauma and inflammation, changes in the vessel wall that could be associated with the direct action of the carcinogen could be distinguished. These changes affected primarily the endothelial cells, in whose cytoplasm lipid-like structures were found. Such structures also were found at the same time in nearby undifferentiated cells, but in the later stages of the experiment they were confined to the hair cells. The appearance of such lipid-like structures can be attributed to the ability of DMBA to interact with lipoprotein complexes of the cell membrane [7] and to its solubility in lipids. After implantation of DMBA, i.e., as a result of trauma, depolymerization of the protein–mucopolysaccharide complexes of the basal layer took place, and this may perhaps facilitate the penetration of DMBA particles through it, to interact with the membrane of the endothelial cell. It was

evidently at this stage that lipids were formed in the cell and the DMBA dissolved in them with the formation of endogenous carcinogens, subsequently giving rise to malignant transformation of the glial cells. The possibility likewise cannot be ruled out that endothelial cells, which under certain conditions possess phagocytic activity [5, 8-15], could leave the bloodstream. That may perhaps be why similar cells to them were found after 24 h (and even after 14 days) in the perivascular tissue. Later (after 90 days) the endothelial cells underwent considerable structural changes, evidence of changes in the process of protein synthesis. Structural changes of this kind could cause the endothelial cells to lose their ability to utilize the newly formed proteins. Accumulation of pathological proteins in the cytoplasm of the endothelial cells led to their degeneration and death (270 days).

The basal layer of the blood capillaries underwent the most severe changes. Whereas in the early stages of the experiment (12 h and 14 days) loosening of the structure of the fibrillary components of the basal layer and disturbance of its permeability were observed, later there was a gradual destruction of the basal layer (90 days) followed by considerable changes in the form of thickening and homogenization. Probably it is in these areas that secondary endogenous [1] carcinogenic metabolites, whose action is associated with the appearance of the first atypical glial cells, may accumulate.

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