INTERACTION BETWEEN 9,10-DIMETHYL-1,2-BENZANTHRACENE AND "HAIRY" CELLS IN THE CNS

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After injection of the carcinogen 9,10-dimethyl-1,2-benzanthracene (DMBA) into the right cerebral hemisphere of rats a capsule forms around it, the first layer of which is formed by "hairy" cells. Two fundamentally distinct mechanisms have been distinguished in these hairy cells: simple phagocytosis and biochemical interaction between the carcinogen and membrane. It is suggested that in the latter case secondary carcinogenic products consisting of steroid hormones are formed; morphologically these can be identified with lipid-like structures of the hairy cell.

KEY WORDS: carcinogenesis; CNS; membrane; steroid hormones.

The problem of carcinogenesis is currently being studied from various aspects: biological, biochemical, and biophysical [4, 5, 8, 10, 13, 14]. In this connection it seemed interesting to study the morphological substrate of the carcinogenic action of the compound 9,10-dimethyl-1,2-benzanthracene (DMBA) on the cell. Experimental models with implantation of a pellet of the carcinogen into various organs can not only shed light on the stages of malignant change, but also on the mechanism of contact between the cell and the noxious agent. In experiments by Avtsyn and Yablonovskaya a pellet of carcinogen was implanted into one of the cerebral hemispheres or into the cerebellum [2, 3]. Around the pellet a three-layered capsule formed, the first layer consisting of what were described as "hairy" cells, making direct contact with the carcinogen.

In the previous paper [6] the writer described the ultrastructure of these cells and postulated a possible role for them in the phagocytosis and metabolism of particles of carcinogen. In the present investigation morphological aspects of interaction between the carcinogen and membrane of the hairy cell, the external appearance, and the localization of the carcinogen in the cytoplasm, and the possible mechanisms of penetration of the carcinogen into the cytoplasm of the hairy cells were examined.

EXPERIMENTAL METHOD

A pellet of chemically pure DMBA weighing 1.5-2.0 mg was implanted into the region of the nuclei of the right cerebral hemisphere of female SHK albino rats weighing 60-80 g. A pellet of paraffin wax of the same weight and volume was implanted in the control animals. Altogether 25 experimental and 5 control rats were used. Pieces of brain tissue for electron-microscopic investigation were removed intravitally under ether anesthesia, and the animals were decapitated 14, 33, and 90 days later. Material was examined in the IEM-100B electron microscope.

EXPERIMENTAL RESULTS

Precursors of hairy cells were found close to the blood vessels 12 h after the beginning of the experiment and a cellular layer immediately around the pellet of carcinogen was formed after 48 h. Mature hairy cells had an eccentric nucleus with long processes pointing toward the carcinogen. The most characteristic structures of the hairy cell were the lipid-like formations found in the cytoplasm as early as 24 h after the

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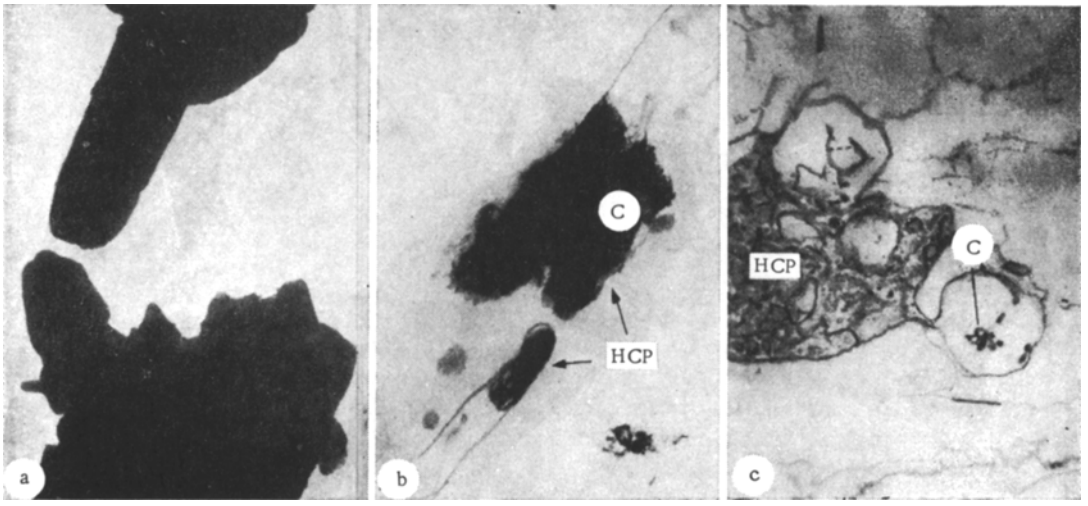

Fig. 1. Phagocytosis of native carcinogen by membranes of processes of hairy cell: a) native carcinogen DMBA, 10,000×; b) phagocytosis of large fragments of carcinogen (C) by membranes of hairy cell process (HCP) in region of apical part, 90 days after beginning of experiment, 50,000×; c) phagocytosis of small particles of carcinogen (C) by membranes of hairy cell process (HCP) in region of apical part, 90 days after beginning of experiment, 10,000×.

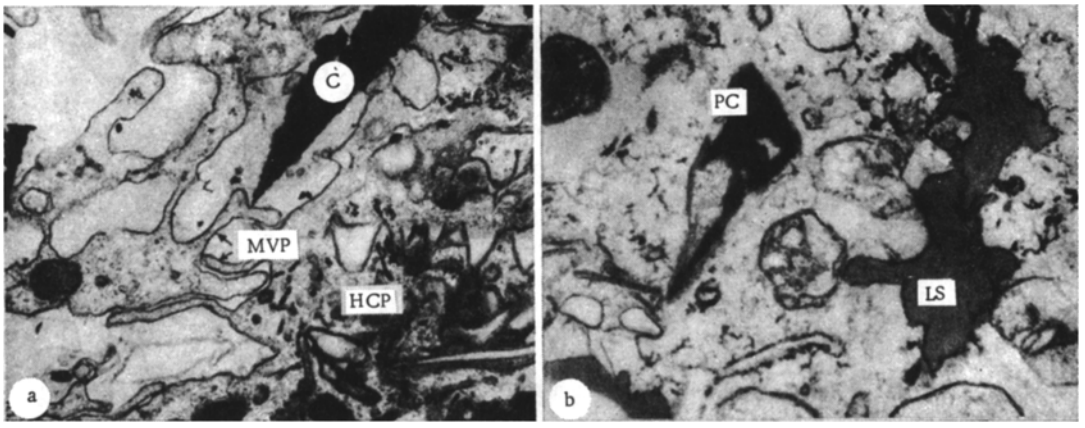

Fig. 2. Phagocytosis of native carcinogen by hairy cell processes and its localization in cytoplasm: a) phagocytosis of large fragments of carcinogen (C) by processes (HCP) and microvilli of processes (MVP) of hairy cell, 90 days after beginning of experiment, 20,000×; b) phagocytized carcinogen (PC) in cytoplasm of hairy cell. Alongside – lipid-like structures (LS); 33 days after beginning of experiment, 20,000×.

beginning of the experiment. The hairy cells formed the first layer of the capsule, nearest to the carcinogen, and were in direct contact with the DMBA. Different forms of interaction between cell and carcinogen were identified. The most active role in this interaction was played by the cell processes. The membrane of the processes throughout their extent was surrounded by a veil-like structure. In the apical part, nearest to the carcinogen, the processes resembled thin veil-like structures composed of two three-layered membranes. These membranes separated to surround lumps or granules of carcinogen and closed around it. The carcinogen was thus surrounded by the processes in the form of particles of varied diameter (Fig. 1a-c). The mechanism of uptake of carcinogenic particles by the microvilli of the processes (Fig. 2a) and by the cell body were similar. In one case granules of carcinogen were surrounded by microvilli of the processes of the hairy cell, whereas in the other case the carcinogen appeared to invaginate the cell membrane, and later regions of the plasmalemma closed together above it. In both these cases the carcinogen appeared to be in the cytoplasm of the cell, surrounded by plasmalemma, as particles of geometrical shape (triangles, rods with rough or even indented outlines; Fig. 2b). The carcinogen could thus be ingested by the hairy cell by phagocytosis.

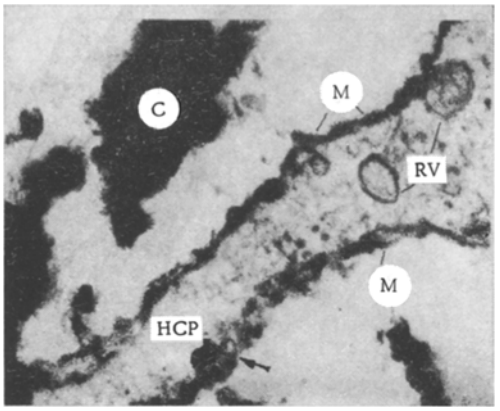

Fig. 3. Contact between carcinogen (C) and membrane of hairy cell process. Preservation of three-layered structure of membrane (M) and simultaneous homogenization of inner and outer layers. Ropheocytotic vesicles (RV); 14 days after beginning of experiment, 50,000×.

DMBA could also penetrate into the cytoplasm in another, more complex way. In some cases where the carcinogen was in contact with the plasmalemma both in the zone of the cell body and in the zone of the base of the processes, the outer and inner electron-dense layers of the membrane were grossly and irregularly thickened and homogenized. The membrane retained its three-layered structure in this case. Very often different stages of ropheocytosis (Fig. 3) could be seen in these areas; electron-dense particles of DMBA (and its metabolites?) apparently encrusted the plasmalemma.

The carcinogen can thus penetrate into the hairy cells, which are a special type of foreign body giant cells [3], in at least two ways: 1) by simple phagocytosis of particles of carcinogen mainly by the processes and their microvilli, and also phagocytosis by the cytoplasm of the cell body; 2) by the transmembrane route, through contact and subsequent biochemical interaction between the carcinogen and cell membrane with a local change in its properties in the zone of contact with DMBA. In both cases cell membranes participate in this process. It is in them that the carcinogen is metabolized, and its products subsequently act on the genetic apparatus of the cells.

The polycyclic hydrocarbons, the group to which DMBA belongs, are known to bind sulfhydryl groups and oxidize them, thereby causing marked changes in lipoprotein complexes [7, 9]. As a result, a protomer with a different structure is incorporated into the membrane and causes conformation of the membrane [12]. A decisive role in this change in the molecular properties of the membrane belongs to cholesterol, which begins to be converted into steroid hormone [11]. Steroid hormones, which are similar in structure to polycyclic hydrocarbons, themselves possess potential carcinogenic activity. It can accordingly be postulated that the malignant transforming action is exerted not by the carcinogen itself, but by the products of its interaction with the plasmalemma of the hairy cell. The formation of this product can be expressed visually by the appearance of lipid-like structures in the cell, which in the present experiments were found in the hairy cell 24 h after the beginning of the experiment. It is difficult as yet to predict the pathogenetic significance of these formations. If it can be shown biochemically that they are precursors of steroid hormones, this will confirm to some degree the views of some workers that carcinogenic agents, including DMBA, on entering the body, initiates the formation of endogenous carcinogens which are the direct cause of the malignant change [1, 15].

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POSSIBILITY OF USING GUINEA PIGS FOR STUDYING THE CARCINOGENIC ACTIVITY OF SOME ENDOGENOUS SUBSTANCES

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The tryptophan metabolites 3-indolylacrylic acid and 2-aminoacetophenone, whose carcinogenic activity has been demonstrated by experiments on inbred mice, were injected into noninbred guinea pigs. Both substances induced tumors in the animals of the experimental groups earlier than in the control and the tumors differed significantly in their morphology from those in animals of the control groups, evidence that both compounds have a carcinogenic effect. The results indicate that guinea pigs can be used to study the carcinogenic activity of weak carcinogens of the endogenous class.

KEY WORDS: guinea pigs; tryptophan metabolites; carcinogenic activity; endogenous carcinogens.

The extremely low frequency of spontaneous neoplasms in guinea pigs [6, 11] and the fact that their blood serum has an inhibitory effect on certain transplantable mouse tumors [8, 13] has led to the view that guinea pigs are resistant to induction of tumors and, as a result, the term "carcinoresistance" has become firmly attached in the specialized literature to the animals of this species [5, 10, 12].

Shabad [2], however, has shown that guinea pigs are not absolutely refractory to the carcinogenic activity of dibenzanthracene, and later work has revealed the high sensitivity of guinea pigs as a species to nitroso compounds, aflatoxins, cycasin, hormones, etc. [4, 5, 9, 12, 14, 15].

The object of this investigation was to use guinea pigs in an attempt to study the carcinogenic activity of tryptophan metabolites, which are found in an increased concentration in the urine of patients with leukemia and have a carcinogenic effect in experiments on inbred mice [1].

EXPERIMENTAL METHOD

Experiments were carried out on 90 noninbred guinea pigs of both sexes obtained from the Kryukovo nursery at the age of 2 months and kept on a standard diet in individual cages. The animals were divided into

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