Effect of 3,4-Benzopyrene on Ultrastructure of Sinusoidal Cells of the Liver in Adult Male Rats

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Reorganization of sinusoidal cells (endotheliocytes, Kupffer cells, and Ito cells) in the liver microregion of male rat liver exposed to 3,4-benzopyrene was studied. Synchronous activation of the lysosome-vacuole systems in Kupffer cells and endotheliocyte indicates cooperation of these cells in detoxification of benzopyrene and its metabolites. Ito cells lose lipid inclusions and actively proliferate; the appearance of intermediate forms between lipocytes and fibroblasts attests to activation of fibrogenesis in the liver.

Key Words: *liver; sinusoidal cells; endotheliocytes; Kupffer cells; lipocytes; 3,4-benzopyrene*

Morphogenesis of adaptive and compensatory processes in the liver in response to destabilizing environmental factors is largely determined by interactions between parenchymatous and sinusoidal cells (SC) [3]. Sinusoidal cells arranged along sinusoidal capillaries play a role in xenobiotic detoxification by hepatocytes. In the hepatic lobule SC are presented by endotheliocytes (EC), Kupffer cells (KC), Ito cells, and pit cells [2,5,13].

Unique structure of branching and anastomosing sinusoids (intermittent basal membrane, fenestrated endothelium, liver macrophages closely contacting with processes of adjacent EC, and low blood flow velocity) ensures maximum contact of the blood with hepatocytes and realization of specialized protective effects. Numerous signals, regulatory molecules produced by SC (cytokines, prostaglandins, *etc.*) modulate hepatocyte function and increase their resistance to injuries and tumor growth [6].

We investigated ultrastructural parameters of metabolic changes in liver SC in adult rats postnatally exposed to 3,4-benzopyrene.

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MATERIALS AND METHODS

Benzopyrene (20 mg/kg) was intraperitoneally injected to adult male Wistar rats for 3 days (total dose of 60 mg/kg). Controls received olive oil.

Liver samples for ultrastructural analysis were collected 24 h after the last injection, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), postfixed in buffered osmium fixative, dehydrated, and embedded in Epon-812. Sections were made on an LKB-8800 ultratome. Semithin sections (1 μ) were stained with toluidine blue. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under JEM-7A and JEM-100S/SEC electron microscopes. Each group consisted of 10 rats, 5 tissue blocks were obtained from each animal.

Morphometry of hepatic nonparenchymatous cells was carried out using open test systems of squares with 0.252 μ distance between points, the final magnification during projection of the negative image on the test system ×59,500. Volume and surface densities of endoplasmic reticulum, Golgi complex elements, surface density of external and internal mitochondrial membranes, and volume and surface densities of lysosomes were measured. In Ito cells, the volume and numerical densities of lipid droplets were analyzed [9]. Areas of SC profiles were evaluated using an open

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test system at ×35,000 final magnification of the negative, the distance between points being 1.28 μ (with consideration for the final magnification).

At each stage, 15-30 profiles of EC, KC, and Ito cell were measured [10], the same numbers of all cells types from each animal were examined. SC types were differentiated as recommended elsewhere [12]. Numerical density of cytoplasmic organelles was estimated as described elsewhere [11].

The results were statistically processed using Student's *t* test, differences between the experimental and control groups were considered significant at $p<0.05$. Stereological parameters, terms, symbols, and sizes are given as recommended by the International Stereological Society [11].

RESULTS

Liver EC exposed to benzopyrene are round and edematous. Their perinuclear spaces are extended, often contain polysomes and enlarged Weibel—Palade granules. Mitochondria form fusing blocks. This is apparently due to changed properties of biomembranes, which is in line with the data on rapid penetration of carcinogen into liver cells, inactivation and rapid aging of mitochondria [1].

Morphometric analysis revealed activation of lysosomal system and particularly of secondary lysosomes in EC. The volume and numerical density of secondary lysosomes in EC significantly increased ($p<0.05$, Table 1), while the surface and volume density of primary lysosomes remained at the baseline level and only their numerical density increased significantly ($p<0.05$, Table 1).

This was paralleled by activation of endocytosis: the surface, volume, and numerical density of micropinocytosis vesicles significantly increased ($p<0.05$, Table 1).

Liver KC often had irregular shape and formed long processes tightly contacting with glycogen-saturated hepatocytes. The cytoplasm of KC often looked foamy and contained large secondary lysosomes with hemosiderin. Phagocytosis of glycogen particles, cell detritus, and sometimes of erythrocytes from extracel-

TABLE 1. Effect of 3,4-Benzopyrene on Structures of EC, KC, and Ito Cells of Male Rat Liver Tissue (*M*±*m*)

Structure, parameter	EC		KC		Ito cells	
	control	experiment	control	experiment	control	experiment
Granular endoplasmic reticulum Sv	2.88±0.81	2.35±0.19	4.403±0.666	3.540±0.273	3.463±0.373	4.944±0.876
Vv	0.12±0.03	0.06±0.01	0.129±0.019	0.098±0.007	0.171±0.048	0.274±0.049
Mitochondrial membranes, Sv						
outer	0.90±0.25	0.81±0.07	1.094±0.166	0.929±0.071	1.257±0.353	0.842±0.149
inner	2.58±0.72	1.58±0.13	3.894±0.589	2.880±0.222	2.513±0.706	3.262±0.578
Golgi complex, Sv	1.79±0.50	1.27±0.10	0.167±0.025	1.109±0.085	0.304±0.085	3.032±0.537
Lysosomes						
first Sv	0.12±0.03	0.09±0.01	0.210±0.032	0.350±0.027*	0.152±0.430	—
Vv	0.008±0.002	0.010±0.001	0.012±0.002	0.063±0.005*	0.018±0.005	—
N	0.58±0.16	2.086±0.170*	23.60±3.57	68.376±5.268*	0.697±0.196	—
second Sv	0.484±0.136	0.507±0.041	0.357±0.054	1.149±0.088*	—	—
Vv	0.026±0.007	0.061±0.005*	0.036±0.005	0.135±0.010*	—	—
N	4.882±1.371	16.572±1.351*	17.38±2.63	73.294±5.647*	—	—
Micropinocytosis vesicles Sv	0.402±0.043	0.944±0.077*	—	—	—	—
Vv	0.051±0.005	0.090±0.007*	—	—	—	—
N	4.147±0.449	12.500±1.019*	—	—	—	—
Lipid inclusions						
Sv	—	—	—	—	0.177±0.049	—
Vv	—	—	—	—	0.042±0.011	—
N	—	—	—	—	0.455±0.128	—

Note. *$p<0.05$ *vs.* the control.

lular spaces and regurgitation of myelin-like formations was seen. After exposure to benzopyrene these stellate reticuloendotheliocytes acquired signs of actively phagocytosing cells, which was confirmed by morphometric findings. Thus, the surface density of membranes and volume density of secondary lysosomes increased significantly ($p<0.05$, Table 1) and their numerical density sharply increased. Acute exposure to benzopyrene increased the surface and volume density of primary lysosome membranes, as well as of their numerical density ($p<0.05$, Table 1).

Presumably, the heterogeneity of liver macrophage population is responsible for the presence of KC whose ultrastructural features indicated high secretory activity, along with actively phagocytosing cells. It should be noted that these KC were characterized by the presence of vast Golgi zone, dilated channels of the endoplasmic reticulum, and numerous polysomes. Liver macrophage mitochondria united in blocks and membranes of granular endoplasmic reticulum tightly adhered to mitochondria. KC nuclei were enlarged, often irregularly shaped, phagocytic activity was low.

Fat-accumulating cells with long processes penetrating deep between hepatocytes, were seen in the perisinusoidal spaces. Characteristically, after postnatal injections of benzopyrene, Ito cells completely lose fatty droplets, vitamin A stores (Table 1). Ito cells are characterized by well developed granular endoplasmic reticulum, occupying up to half of the cytoplasm volume, with moderately or well dilated cisterns with small granules or fine filaments.

The lysosome-vacuole system of KC, participating in endo-, exo- and diacytosis, mediates the relationship between xenobiotic metabolism and the environment [8]. Our data indicate an important role of the lysosome-vacuole system of liver macrophages in benzopyrene detoxification, which confirms the concept on the possibility of uniting the hydrolytic potential of lysosomes and xenobiotic detoxification system of somatic cells with the still higher potential of lysosomal hydrolases of nonparenchymatous cells [7].

Hepatocytes and KC synthesize and lyze fibrillar proteins [4], which reflects the antagonistic regulation of this function. Normally the stroma-parenchyma relationships are strictly regulated, the synthesis of fibrillar proteins being inhibited by parenchymatous cells, KC, and Ito cells. The disappearance of lipid droplets in Ito cell cytoplasm and changes in endoplasmic reticulum indicate activation of fibrogenic potential of lipocytes under the effect of postnatal exposure to the toxicant.

Hence, under conditions of acute exposure to benzopyrene, ultrastructural changes in SC create specific microenvironment of the liver tissue microregion in male rats, which determines the realization of injuries.

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