MORPHOLOGY AND PATHOMORPHOLOGY

Benzo[a]pyrene Induction of Cytochrome P450 1A1/1A2 in the Lymph Nodes of Rats

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Studies of mesenteric lymph nodes of rats by indirect immunoperoxidase method using monoclonal antibodies to cytochrome P450 1A1/1A2 after oral dose of benzo[a]pyrene showed the presence of these cytochrome forms in monocytes, macrophages, reticular and litoral cells, cell detritus, and liquid contents of the paracortical zone and medullary substance sinuses. Oxidation of various exo- and endogenous toxins in the lymph nodes was revealed.

Key Words: *cytochrome P450; benzo[a]pyrene; lymph nodes; macrophages; endotheliocytes*

The monooxygenase enzyme system located in membranes of the endoplasmatic reticulum is one of the first systems of the body facing environmental xenobiotics and responsible for biotransformation [14]. Wide substrate specificity of monooxygenases is due to functioning of numerous forms of cytochrome P450 (CYP) 1A1.

Cytochromes P450, subfamily 1A (CYP1A1 and CYP1A2) play an important role in chemical carcinogenesis by catalyzing the detoxification and toxication reactions of prevalent carcinogens (polycyclic aromatic carbohydrates and heterocyclic amines). Cytochromes P450 1A are inducible, *i.e.* their content and activity depend on the effects of some chemicals.

The liver monooxygenase system is now best studied. CYP1A1 can be induced not only in the liver, but also in the prostate and seminal vesicles [10], CNS [4,7], epidermis [13], Bowman glands, and olfactory and respiratory nasal epithelium [15]. CYP1A1 mRNA was detected in mammary tissue [9], gastrointestinal epithelium [5,10], renal glomeruli, ovary [5], and vascular endothelium [5,12]. Expression and activity of CYP1A1 mRNA can be induced in the thymus, spleen, and thymocyte and splenocyte cultures. CYP1A1 activity was not detected in normal human lymphocytes [11], but CYP1A1 mRNA expression can be induced in culture of human peripheral blood lymphocytes [6,14]. The system of cytochrome P450 coenzymes, including CYP1A1, plays the key role in the degradation of toxins and minor molecules in human monocytes and macrophages, considerable induction of this enzyme is possible in monocytes [3]. Hence, metabolism of xenobiotics can take place in different cells of various organs.

The lymphatic system is involved in pathological processes: shock of different etiology, inflammation, allergic and adaptation reaction of the organism. Functional and morphological signs of damage, defense, and adaptation manifest in the lymph system at the earliest stages. The lymph nodes are the initial filters of the lymph system restraining foreign substances [1].

A. F. Safina *et al.* [2] using highly specific substrate for CYP1A1 (7-ethoxyresorufine) detected induction of this cytochrome in mesenteric and mediastinal lymph nodes of rats after oral administration of benzo[a]pyrene.

Despite numerous reports on CYP1A1 in various organs, tissues, and systems, there are no data on the presence and location of this enzyme in lymph nodes,

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though their detoxifying functions are well known; this prompted the present study.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 150-200 g (Tomsk Breeding Center). Benzo[a]pyrene dissolved in mineral oil was administered orally (through a tube) in a dose of 30 mg/kg for 3 days. On day 5 of the experiment the animals were decapitated. Control rats received mineral oil (solvent) [2]. Three independent experimental series were carried out.

For light microscopy the mesenteric lymph nodes were fixed in 4% paraformaldehyde in phosphate buffer, dehydrated in ascending ethanols, clarified in xylol, and embedded in paraffin. The sections (4-5-μ) were deparaffinized in xylol, rehydrated in ethanol gradient, and washed in 1% Triton X-100 in phosphate buffer for 1 h, after which the sections were incubated with 3% H_2O_2 for 10 min for suppressing the endoperoxidase activity. After washing in phosphate buffer the sections were incubated with 10% fetal serum in this buffer and then incubated with rat antibodies to CYP1A1/1A2 diluted 1:50 (monoclonal antibodies were obtained using the hybrydoma technology, clone 14H5 [8]) at 4°C for 24 h. The resultant complexes were treated with peroxidase-labeled antimurine antibodies at 37°C for 2 h. After washing in phosphate buffer the reaction products on sections were visualized with 3,3-diamidobenzidine (substrate for peroxidase) [12]. The preparations were examined under an oil immersion microscope. For more accurate identification of cell types the sections were poststained with hematoxylin, embedded in polystyrene, and examined under a light microscope at ×2500.

RESULTS

Induction of CYP1A1/1A2 was not detected in the cortical plateau and lymphoid follicles, but was present in the paracortical zone and medulla (Figs. 1-3). A clearly discernible interface between the cortical plateau, follicles, and paracortex was seen due to the presence or absence of the enzyme (Fig. 1).

In the paracortical zone induction of CYP1A1/1A2 was found in the cytoplasm of macrophages, reticular cells, and endothelium of intermediate sinuses (litoral cells; Fig. 2). No CYP1A1/1A2 was found in vascular endothelium. In macrophages CYP1A1/1A2 was present either diffusely in the cytoplasm, or only in lysosomes. In addition, it was induced not only in cells, but in the contents of intermediate sinuses. It is noteworthy that CYP1A1/1A2 in high concentration was present in the parenchyma near the sinuses.

In the medullary substance induction of monooxygenase enzymes was detected in monocytes and macrophages of pulp cords, in the endothelium, macrophages (diffusely in lysosomes and on cytoplasmic membrane; Fig. 3), cell detritus, and medullary sinus fluid.

Thus, it can be hypothesized that benzo[a]pyrene induced CYP1A1/1A2 in the intestinal wall cells directly at the site of its contact. Then, some cells die (from benzo[a]pyrene or its toxic metabolites) and CYP1A1/1A2 is found in the interstitium. Macrophages loaded with toxins and microsomal enzymes from the intestinal wall, microsomes and cytochromes from

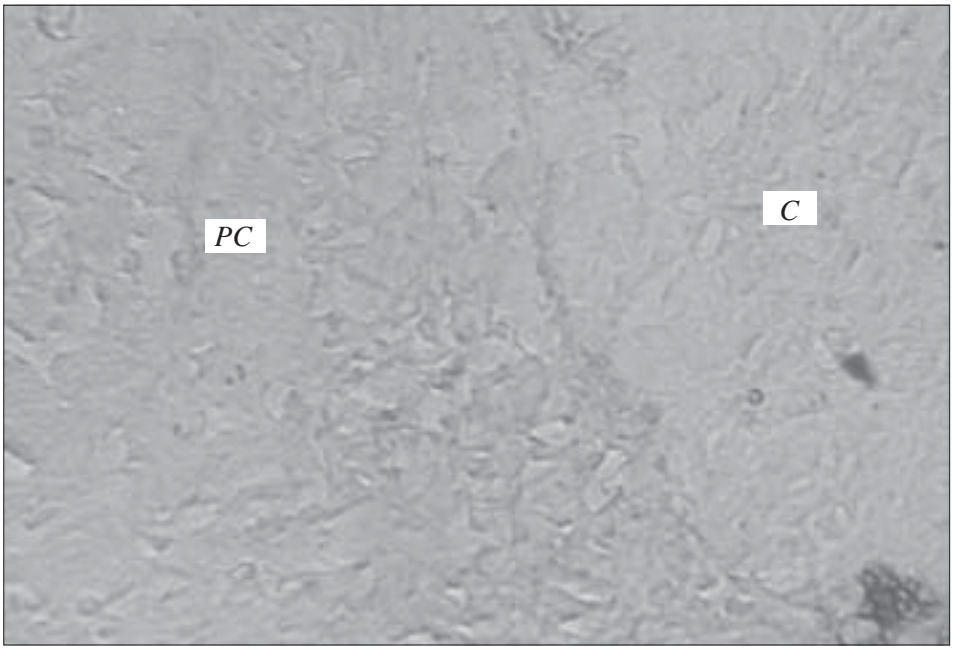

Fig. 1. Rat mesenteric lymph node after oral treatment with benzo[a]pyrene: CYP1A1/1A2 induction in the paracortical zone (*PC*) and no enzyme activation in the cortical plateau (*C*). Diamidobenzidine staining, ×350.

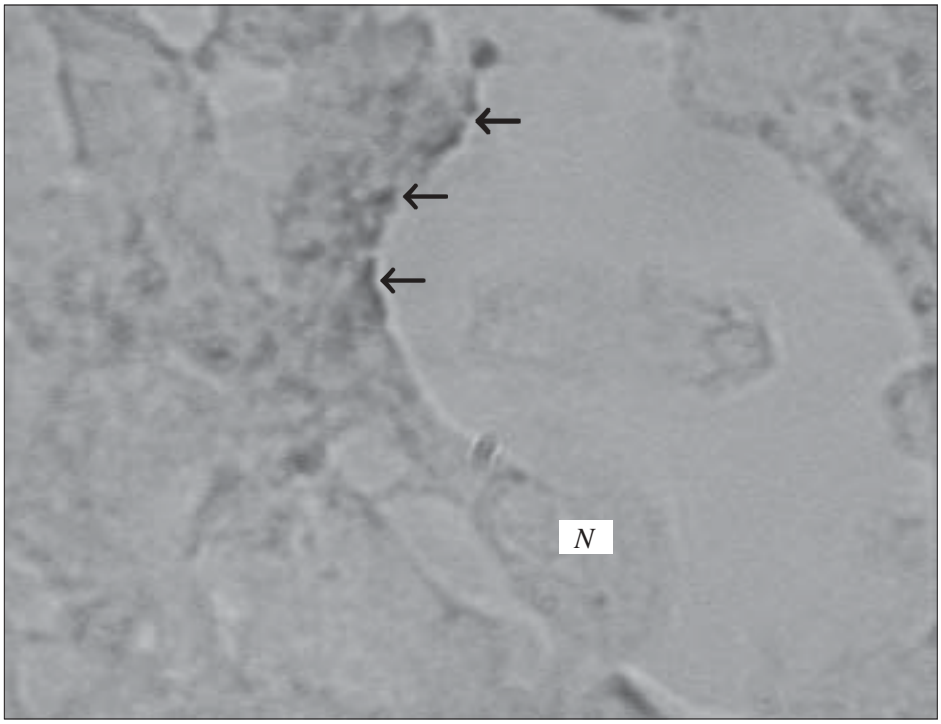

Fig. 2. Induction of CYP1A1/1A2 in the cytoplasm of litoral cell (arrows) of intermediate sinus in the paracortical zone of rat mesenteric lymph node after oral administration of benzo[a]pyrene: negative reaction to cytochrome in cell nucleus (*N*). Diaminobenzidine staining, ×2500.

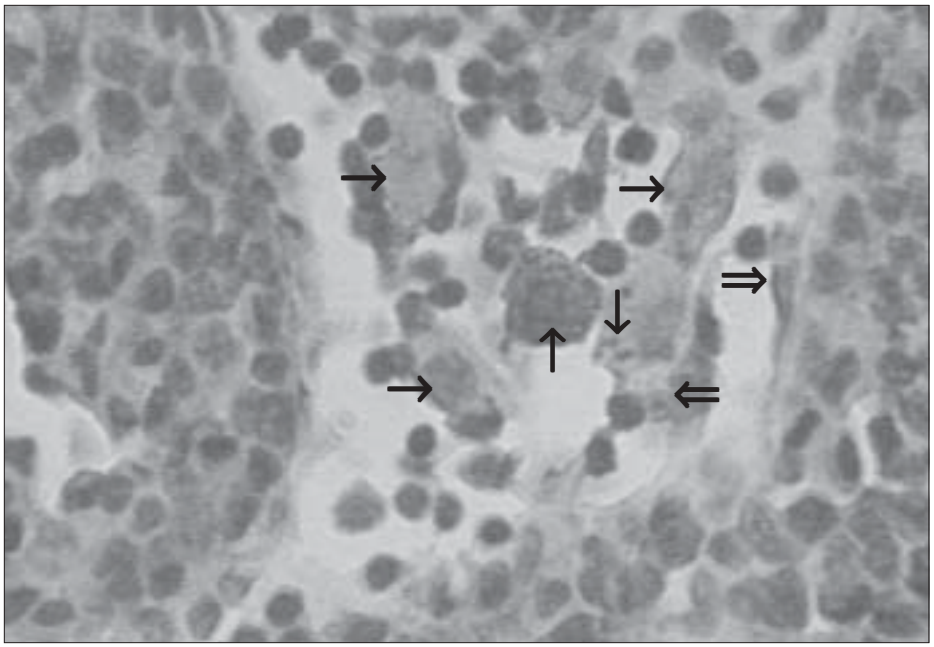

Fig. 3. Medullary sinus of rat mesenteric lymph node after oral administration of benzo[a]pyrene: induction of CYP1A1/1A2 in macrophages (arrows) and litoral cells (double arrows). Diamidobenzidine and hematoxylin staining, ×960.

destroyed cells appear in the lymph and are transported to the mesenteric lymph nodes.

During transportation the enzymes in and, presumably, outside the cells proceed with benzo[a]pyrene oxidation. The lymph in the lymph nodes is filtered in the intermediate sinus network, cytochromes are induced in the litoral cells of these sinuses (direct contact with benzo[a]pyrene), the toxin penetrates through sinus endothelium into the paracortical parenchyma and is absorbed by macrophages. Moreover, macrophages absorb detritus with activated cytochromes from processed lymph and lymphoid parenchyma. Toxin not absorbed by macrophages, macrophages proper (from the intestine and lymph node), and microsomal enzymes from destroyed cells enter the medullary sinuses, where benzo[a]pyrene processing continues.

We found that benzo[a]pyrene administered orally induced CYP1A1/1A2 in monocytes, macrophages, reticular and litoral cells, cell detritus, and liquid contents of the paracortical zone and medullary sinuses of rat mesenteric lymph nodes. We therefore conclude that lymph node cells participate in oxidation of exo-

and endogenous toxins. Foreign substances entering the lymph nodes undergo degradation in the macrophagal cells and sinus endothelium. Biotransformation of toxins outside the cells (in intermediate and medullary sinuses) is also possible.

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