MORPHOLOGY AND PATHOMORPHOLOGY

The Possibility of Cytochrome P450 1A1/1A2 Induction in Cells of Distant Lymph Nodes of Rats after Enteral Treatment with Benzo[a]pyrene

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The mesenteric, mediastinal, and popliteal lymph nodes of rats were studied by indirect immunoperoxidase method using monoclonal antibodies to cytochrome P450 1A1/1A2 after oral treatment with benzo[a]pyrene. These cytochrome forms were detected in monocytes, macrophages, reticular and littoral cells, cell detritus, and liquid contents of the paracortical and medullary sinuses of all studied lymph nodes. The results indicate that exo- and endogenous toxic substances are oxidized not only in the liver, but also in lymph nodes.

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

The lymph system is involved in many pathological processes: shock of different etiology, inflammation, allergic and adaptation restructuring of the organism. Functional and morphological signs of injury, defense, and adaptation are early detected in the lymph system. The lymph nodes are the initial filters of the lymph system entrapping foreign substances.

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

Xenobiotic metabolism can take place in cells of different organs. The liver monooxygenase system is best studied. CYP1A1 induction is possible not only in the liver, but in the prostate and seminal vesicles [10], CNS (brain and spinal cord) [4,7], epidermis [13], Bowman glands, olfactory and respiratory epithelium of the nasal cavity [15]. CYP1A1 mRNA was detected in mammary gland tissues [9], gastrointestinal epithelium [5,10], renal glomeruli, ovaries [5], vascular endothelium [5,12]. CYP1A1 mRNA expression and CYP1A1 activity can be induced in the thymus, spleen, thymocyte and splenocyte cultures. CYP1A1 activity was not detected in normal human lymphocytes [11], but the expression of CYP1A1 mRNA can be induced in human peripheral blood lymphocyte culture [6,14]. The key role in degradation of toxins and minor molecules in human monocytes and macrophages is played by CYP coenzyme system, including CYP1A1; significant induction of this enzyme in monocytes being possible [3].

Induction of this cytochrome in the mesenteric and mediastinal lymph nodes of rats after oral treat-

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ment with benzo[a]pyrene was detected by biochemical methods using a highly specific substrate for CYP1A1 (7-ethoxyresorufin) [2]. Benzo[a]pyrene administered into the gastrointestinal tract causes CYP1A1/1A2 induction in monocytes, macrophages, reticular and littoral cells, cell detritus, and liquid contents of the paracortical and medullary sinuses of the rat mesenteric lymph nodes [1]. Presumably, regional lymph node cells actively participate in oxidation of toxins entering the body via the gastrointestinal tract.

The possibility of induction of this enzyme in distant lymph nodes was not studied, though it is known that the entire lymph system reacts to any violation of hemostasis. This prompted us to undertake this study.

MATERIALS AND METHODS

Benzo[a]pyrene solution in mineral oil was administered to male Wistar rats (150-200 g; Tomsk Breeding Center) orally through a tube (30 mg/kg) for 3 days for CYP1A1 induction. On day 5 after the start of the experiment the animals were decapitated. Control rats received mineral oil (solvent) [2]. The results of three independent experiments are presented.

Mesenteric, mediastinal, and popliteal lymph nodes were fixed in 4% paraformaldehyde in phosphate buffer, dehydrated in ascending ethanols, clarified in xylene, and embedded in paraffin. The sections (4-5 μ) were deparaffinized in xylene, rehydrated in ethanol gradient, and washed in 1% Triton X-100 in phosphate buffer for 1 h. The sections were then incubated with 3% hydrogen peroxide for 10 min in order to suppress endoperoxidase activity. After washout in phosphate buffer the sections were preincubated with 10% fetal serum in the same buffer and then incubated with antibodies to rat CYP1A1/1A2 (1:50) for 24 h at 4°C (monoclonal antibodies were obtained using hybrydoma technology, clone 14H5 [8]). The resultant complexes were treated with peroxidase-labeled murine antibodies for 2 h at 37°C. After washout in phosphate buffer the reaction products on the sections were visualized using 3,3-diamidobenzidine (substrate for peroxidase) [1,12]. The preparations were examined under an oil immersion microscope. For more accurate identification of the cell type, the sections were post-stained with hematoxylin, embedded in polystyrene, and examined under a light microscope at magnification of up to 2500.

RESULTS

Induction of CYP1A1/1A2 in the paracortical zone and medulla of the mesenteric, mediastinal, and popliteal lymph nodes was detected after treatment with benzo[a]pyrene (Figs. 1-3). CYP1A1/1A2 was detected in macrophages, littoral cells lining the intermediate and medullary sinuses, reticular cells, and fibroblasts (Figs. 2, 3). No microsomal enzymes were detected in the cortical plateau (Fig. 1), lymphoid follicles (Fig. 1), and vascular endothelium. A clear-cut border between the cortical pla-

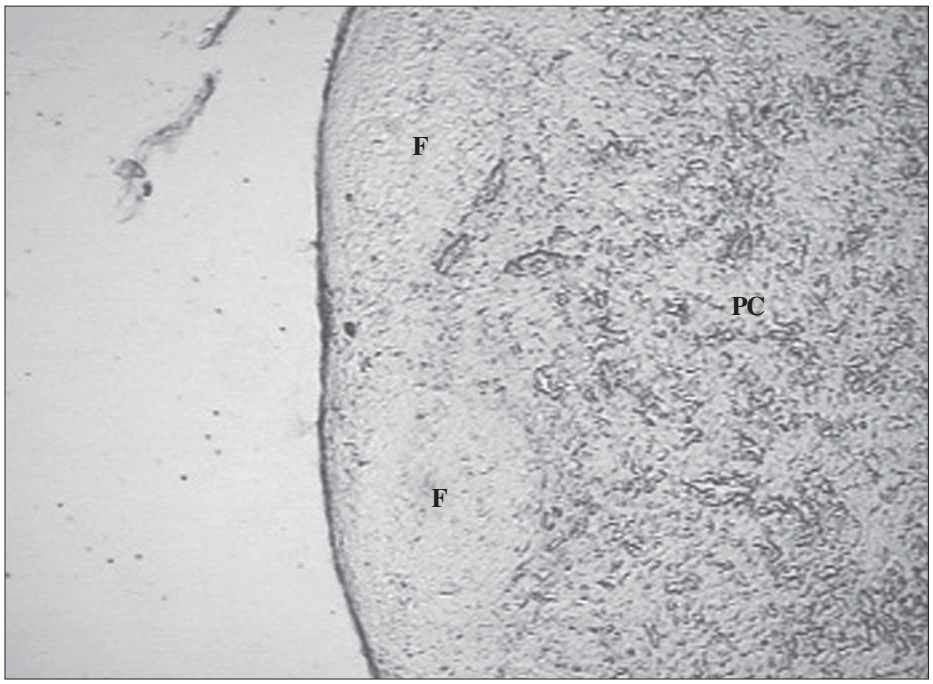

Fig. 1. Rat mesenteric lymph node after benzo[a]pyrene treatment. CYP1A1/1A2 induction in the paracortex (PC). No reaction with antibodies in the cortical plateau and lymphoid follicles (F). Diaminobenzidine staining, ×75.

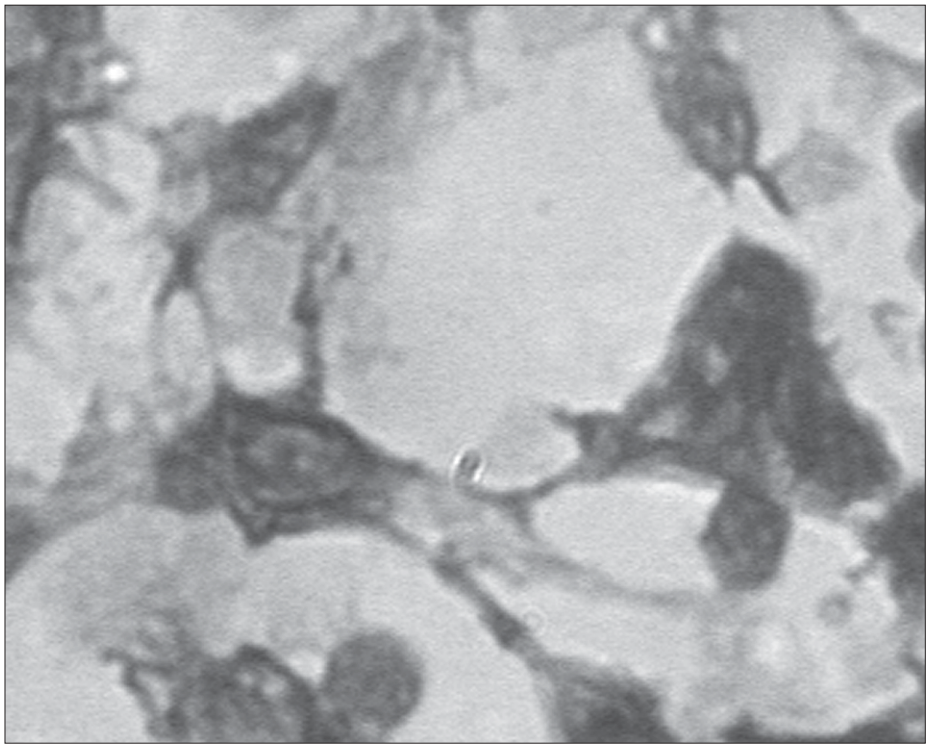

Fig. 2. Induction of CYP1A1/1A2 in reticular cells of the medullary sinuses of rat mediastinal lymph node after treatment with benzo[a]pyrene. Diamidobenzidine and hematoxylin staining, ×1800.

teau, follicles, and paracortex was due to the presence/absence of the enzyme (Fig. 1). Induction of CYP1A1/1A2 in the mediastinal and popliteal lymph nodes was less pronounced than in mesenteric ones (Figs. 1-3).

In macrophages CYP1A1/1A2 was diffusely spread in the cytoplasm or was present only in lysosomes. Moreover, CYP1A1/1A2 was induced not only in cells, but also in cell detritus (Fig. 3) and sinus contents. High concentrations of CYP1A1/1A2 were detected in the parenchyma adjacent to the sinuses.

Hence, not only the liver, but also regional (mesenteric) and distant (mediastinal and popliteal) lymph nodes were involved in oxidation of aromatic hydrocarbons entering the body through the gastrointestinal tract.

After entering the gastrointestinal tract, benzo[a]pyrene penetrated from the small intestine into blood and lymph vessels. During absorption the toxin induced CYP1A1/1A2 in macrophages [1,3] and capillary endotheliocytes [1,5,12] of the lamina propria of the small intestinal mucosa.

From blood capillaries the hydrocarbon through the portal system gets into the liver, where microsomal enzymes are induced in hepatocytes and Kupffer cells [3]. Benzo[a]pyrene passed through the liver is transported with the blood in the body and can induce monooxygenases in organs and tissues.

Apart from the liver, benzo[a]pyrene can penetrate (from the small intestine) into regional lymph nodes, where CYP1A1/1A2 is induced in macrophages, reticular and littoral cells lining the intermediate and medullary sinuses [1].

Presumably, benzo[a]pyrene is transported into mesenteric lymph nodes not only with the lymph flow, but also in macrophages from the lamina propria of intestinal mucosa. Migration of macrophages loaded with foreign substances is amply described. Unoxidized hydrocarbon from these nodes is transported into the next-order lymph nodes, after which benzo[a]pyrene can penetrate into the blood together with the lymph through the lymph-venous anastomosis. In addition, benzo[a]pyrene can be released into the blood system from the lymph (or together with the lymph) at the level of the first-order mesenteric nodes.

Benzo[a]pyrene is distributed in the entire body with the blood and appears in the mediastinal and popliteal lymph nodes. Two routes of the toxin entry in these organs are possible: directly from blood vessels through intranodular blood vessels; first from blood to tissues and then from tissues with the lymph flow into lymph nodes. Benzo[a]pyrene reaches these organs in a far lower concentration than mesenteric lymph nodes, and hence, induction of microsomal enzymes in the mediastinal and popliteal lymph nodes is less pronounced than in the mesenteric ones.

Hence, oral benzo[a]pyrene causes induction of CYP1A1/1A2 in monocytes, macrophages, reticular and littoral cells, cell detritus, and liquid con-

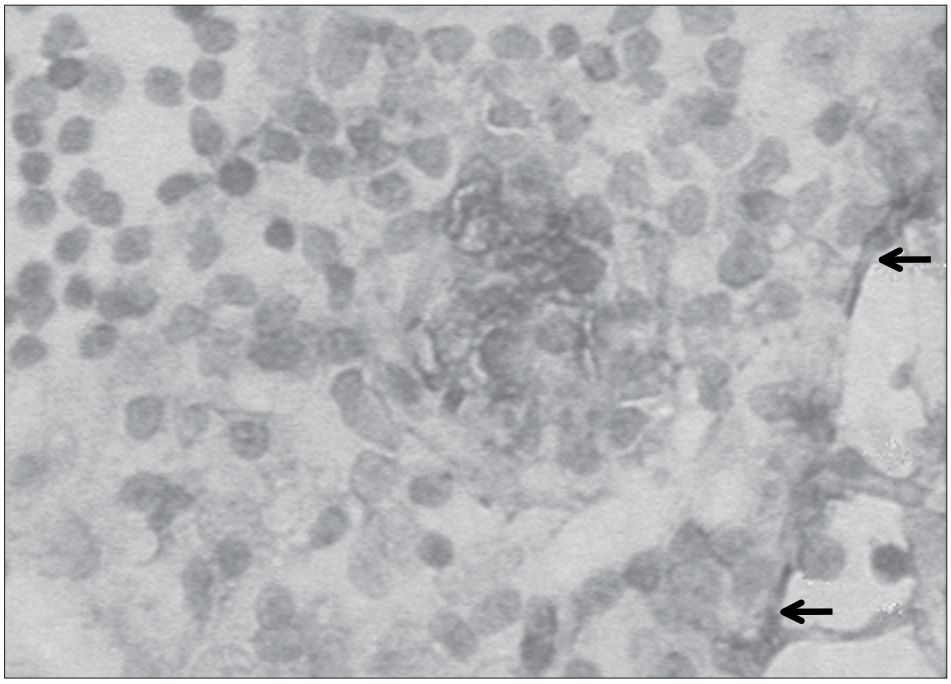

Fig. 3. Rat popliteal lymph node after treatment with benzo[a]pyrene. CYP1A1/1A2 induction in littoral cells of medullary sinuses (arrows) and cell detritus in medullary cords. Diamidobenzidine and hematoxylin staining, ×750.

tents of paracortical and medullary sinuses of the mesenteric, mediastinal and popliteal lymph nodes of rats. We therefore conclude that exo- and endogenous toxins are oxidized not only in the liver, but also in the lymph nodes.

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