Basal Induction of Cytochrome P-450 1A1/1A2 in Cells of the Liver, Small Intestinal Villi, and Lymph Nodes in Rats

Yu. I. Borodin, I. V. Maiborodin, A. F. Safina, and D. N. Strunkin

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Rat liver, small intestinal wall, mesenteric, mediastinal, and popliteal lymph nodes of rats were studied by indirect immunoperoxidase method with monoclonal antibodies to cytochrome P-450 1A1/1A2. These cytochrome forms were detected in low concentrations in hepatocytes, macrophages of the small intestinal villi and mesenteric and mediastinal lymph nodes of intact animals. Basal induction of monooxygenase enzymes can be caused by cytochrome inductors entering with air (environmental pollution) or food.

Key Words: *cytochrome P-450; liver; lymph nodes; rats; environmental pollution*

The monooxygenase system located in membranes of the endoplasmic reticulum, is one of the first systems of the body, facing environmental xenobiotics and biotransforming them [14].

The aryl-hydrocarbon receptor binds numerous prevalent environmental pollutants, including polycyclic aromatic carbohydrates, and mediates their carcinogenic effect. The ligand-bound receptor activates transcription of cytochrome P-450 (CYP) 1A1 gene through interaction with a specific DNA sequence [13].

CYP 1A is often present in certain amounts in the liver and lungs of intact animals, which is most likely a result of induction with environmental pollution. The initial induction was detected even in polar bears under natural conditions [4] and in fish liver [3]. Activity of this CYP can serve for pollution monitoring and for screening the duration of contact with aromatic carbohydrates [3]. Some authors did not detect activity of this CYP in intact liver [11].

An attempt at detecting precisely the location of CYP 1A1/1A2 in the liver and lymph nodes [1,2] revealed basal induction of this enzyme in hepatocytes, macrophages of the lamina propria of the small intestinal mucosa, mesenteric and mediastinal lymph nodes of intact rats in a breeding center situated in a large industrial city. Basal induction of this CYP in the liver was described [3,4], but the possibility of basal induction of CYP 1A1/1A2 in the small intestinal wall and lymph nodes was never reported.

MATERIALS AND METHODS

For light microscopy the liver, small intestinal fragments and mesenteric, mediastinal, and popliteal lymph nodes of intact male Wistar rats (150-200 g) from Tomsk Breeding Center were fixed in 4% paraformaldehyde in phosphate buffer, dehydrated in ascending ethanols, clarified in xylene, and embedded in paraffin. Sections (4-5 μ) were deparaffinated 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% H_2O_2 for 10 min in order to suppress endoperoxidase activity. After washing in phosphate buffer the sections were preincubated in 10% fetal serum in this buffer and then incubated with antibodies to

Institute of Clinical and Experimental Lymphology, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** imai@mail.ru. I. V. Maiborodin

rat CYP 1A1/1A2 (monoclonal antibodies were obtained by the hybridoma technology, clone 14H5 [9]) in 1:50 dilution for 24 h at 4°C. The resultant complexes were treated with peroxidase-labeled antimurine antibodies for 2 h at 37°C. After washing in phosphate buffer the reaction products on sections were visualized with 3,3-diamidobenzidine (peroxidase substrate) [1,2]. The preparations were examined under an oil immersion microscope; for more accurate identification of cell types the sections were post-stained with hematoxylin, embedded in polystyrene and examined under a light microscope at a final magnification of up to 2500.

RESULTS

Weak positive reaction with antibodies to CYP 1A1/1A2 was detected in hepatocytes of some liver lobules (Fig. 1). Examination of rat liver sections at higher magnification showed no induced CYP 1A1/1A2 in endothelial and Kupffer cells. Induction of these enzymes in Kupffer cells [8] and endotheliocytes is possible [7,12]. The presence of CYP 1A1/1A2 activity in the liver of intact animals was previously detected [3,4], but other authors [11] denied the possibility of the presence of this CYP activity in the liver of intact animals.

Induced CYP 1A1/1A2 was found in some cells of the lamina propria of the small intestinal mucosa (Fig. 2). Minor basal induction of CYP 1A1/1A2 was detected in the mesenteric and mediastinal lymph nodes, in macrophages of the paracortex and medullary sinuses, in the contents of intermediate sinuses (Fig. 3). Induction of CYP 1A1/1A2 was not detected only in the popliteal lymph nodes (in none of the sections from intact animals).

CYP 1A1/1A2 is an inducible enzyme; these cytochromes are not activated under conditions of normal cell functioning. Presumably, minor induction of CYP 1A1/1A2 in the liver is caused by environmental pollution. The rats grew in a breeding center in a city, breathed air containing car exhaust, electric station discharge, and admixtures of this kind with high concentrations of aromatic carbohydrates [6].

Presumably, chemical compounds responsible for activation of CYP 1A1/1A2 in the liver and intestine enter the body with food [10] or were formed in the gastrointestinal tract as a result of vital activity of some bacteria.

CYP inductors are then transported from the small intestine into the blood and lymph system. During absorption these substances induce CYP 1A1/1A2 in macrophages [1,5] and capillary endo-

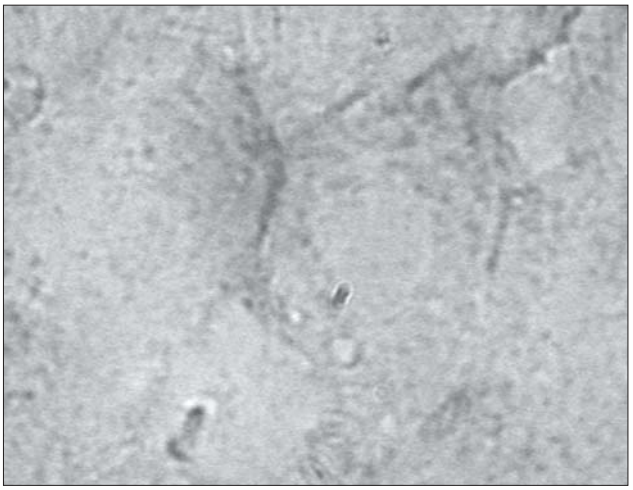

Fig. 1. Basal induction of CYP 1A1/1A2 in intact rat hepatocytes. Diamidobenzidine staining; ×1800.

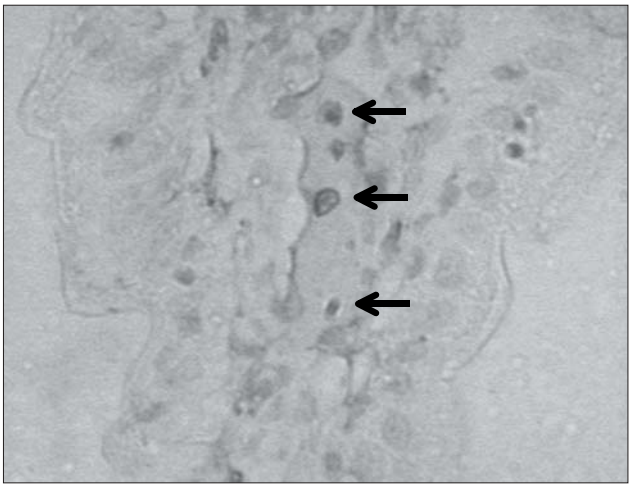

Fig. 2. Intact rat small intestine. Slight basal induction of CYP 1A1/1A2 in the villous mucosal lamina propria (arrows). Diamidobenzidine and hematoxylin staining; ×470.

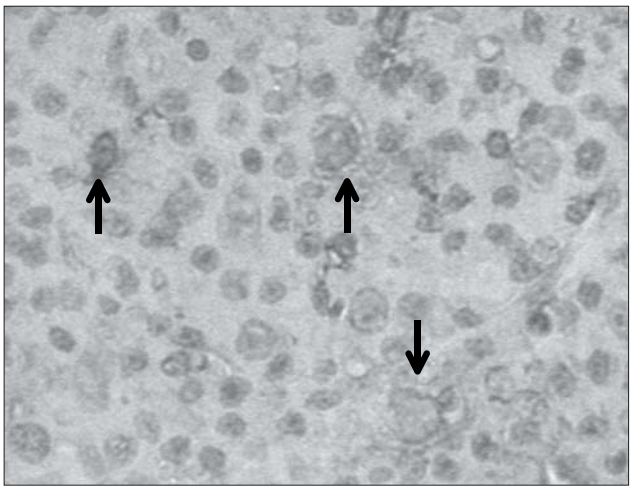

Fig. 3. Basal induction of CYP 1A1/1A2 in macrophages (arrows) in intact rat mediastinal lymph node medullary sinuses. Diamidobenzidine and hematoxylin staining; ×750.

theliocytes [7,12] of the lamina propria of the small intestine.

Activators of microsomal enzymes can be released into the mesenteric lymph nodes of intact rats from the intestinal lumen. CYP 1A1/1A2 inductors can get into the mediastinal lymph nodes from the lungs, after passing through pulmonary, bronchial, and tracheal nodes, as even in ecologically clean regions the air contains car exhaust aromatic carbohydrates and other CYP inductors [4,6].

Presumably, CYP inductors are transported into the mesenteric lymph nodes not only with lymph flow, but also in macrophages from the lamina propria of the small intestine mucosa. Migration of macrophages loaded with foreign substances is sufficiently well described in relevant publications.

We conclude that basal activation of the monooxygenase system enzymes in the detoxication system cells (liver and lymph system) is possible.

Hence, basal induction of CYP 1A1/1A2 is present in the liver, small intestinal lamina propria, mesenteric and mediastinal lymph node macrophages of intact rats bred under conditions of a city breeding center; this induction seems to be caused by environmental pollution — entry of CYP inductors with food or formation of toxins in the gastrointestinal organs.

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