EFFECT OF REPEATED PERCUTANEOUS APPLICATIONS OF HEAVY PYROLYSIS RESIN ON THE CYTOCHROME P-450 LEVEL AND GLUTATHIONE TRANSFERASE ACTIVITY IN RAT LIVER MICROSOMES AND CYTOSOL: CORRELATION WITH TOXIC ACTION OF PYROLYSIS RESIN ON INTERNAL ORGANS

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UDC 616.36-008.931:[577.152.13+577.152.2]-02:/615.917:665.944].032.77-092.9

KEY WORDS: cytochrome P-450, glutathione transferase, heavy pyrolysis resin, skin, liver.

Repeated applications of industrial mixtures containing polycyclic aromatic hydrocarbons (PAH) to the skin of animals caused the appearance of malignant neoplasms in the skin and other organs [1, 7]. This effect is associated with oxidation of the PAH on cytochrome P-450 (P-450) of the endoplasmic reticulum of the liver, during which highly toxic intermediate metabolites (epoxides of PAH) are formed [16]. These form covalent bonds with proteins and DNA [2] and thus disturb the normal functioning of cells by creating the conditions for their malignant degeneration [15]. Detoxication of epoxide metabolites of PAH take place with the involvement of epoxide hydrolase and glutathione transferase, which are found in the microsomal and cytosol fractions of the liver [3]. Epoxide hydrolase converts epoxide metabolites of PAH into nontoxic diols. Under certain conditions, however, diols may again become substrates for oxidation on P-450 and be converted into even more cytotoxic diol-epoxides of PAH [2]. Microsomal glutathione transferase (GT_m) and cytosol glutathione transferase (GT_c) convert epoxide derivatives of PAH into nontoxic glutathione conjugates. They do not undergo any further metabolic activation with the formation of cytotoxic intermediates, and they are therefore true detoxication products of PAH epoxides [6]. Consequently GT_m and GT_c play highly important roles in protection of cells against the damaging action of PAH metabolites [4]. It has been shown that repeated applications of PAH-containing industrial mixtures to the skin of animals caused a considerable rise of the P-450 level and increased by many times the activity of P-450-dependent aryl-hydrocarbon hydroxylase, forming epoxides [10]. This inductive effect also is observed in the skin at sites of contact with PAH-components, and also in the liver of animals [11]. Changes in activity of detoxication enzymes have received far less study. The aim of this investigation was to study the effect of repeated percutaneous applications of a heavy pyrolysis resin (HPR) with a high content of PAH (up to 30%) on the P-450 level and GT_m and GT_c activity in the liver, and also on correlation between the P-450/GT_m and P-450/GT_c levels with the toxic action of HPR on the internal organs of rats.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 400-450 g, in November. The animals received a balanced protein diet and water ad libitum. HPR was applied to the skin of the tail of rats of the experimental group by immersing it for two-thirds of its length in HPR mass. The results of chemical analysis (by N. A. Garyachev) showed that the sample contained 3,4-benzpyrene and 1,2-benzpyrene in amounts of 19 and 25 μg/g respectively. A single exposure of HPR lasted 4 h on 1 day, and during this period the animals were kept in individual cages. After the end of exposure, remaining traces of HPR were removed from the skin with ethanol. The applications were made daily for 5 days per week for 4 weeks. The experimental and control rats were decapitated simultaneously 2 days after the last application of HPR. Microsomal and cytosol fractions of liver were obtained by differential

Institute of Work Hygiene and Occupational Diseases, Academy of Medical Sciences of the USSR. Central Research Institute of Gastroenterology, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 365-367, October, 1990. Original article submitted December 28, 1989.

TABLE 1. Effect of Repeated Percutaneous Applications of HPR on P-450 Level, GT_m and GT_c Activity, and Parameters of Oxidative Processes in Rat Liver Cells (M ± m)

Parameter	Group of animals		Percent of control
	control (n = 8)	experimental	
P-450, nmoles/mg protein	0,535±0,139	0,955±0,210**	178,5
GT_m, mmoles/min/mg protein	107,9±18,3	157,4±22,8*	145,9
GT_c, mmoles/min/mg protein	860,0±49,5	1592±241***	185,1
GSH, μmoles/g tissue	6,7±0,38	7,33±0,34	109,4
TBA-reactive products, mmoles/g tissue	208,0±10,8	230,7±20,3	110,9

Legend. *p < 0.05, **p < 0.01, ***p < 0.001 Compared with control.

TABLE 2. Parameters of Toxic Action of Repeated Percutaneous Applications of HPR in Rats (M ± m)

Parameter	Group of animals		Percent of control
	control	experimental	
Weight coefficient, relative units; liver (n = 8)	2,86±0,185	3,44±0,1689**	120,3
Thyroid gland (n = 8)	0,0057±0,0004	0,0070±0,0007**	122,8
Thymus (n = 8)	0,0295±0,0079	0,0197±0,0013*	66,8
Number of monocytes in lymph nodes, per 1000 cells	1 (2/9)	2—6 (5/7)	
Number of monocytes in blood, %	1—2 (2/10)	1—3 (5/10)	

Legend. *p < 0.05, **p < 0.01, ***p < 0.001 Compared with control. Frequency of recording monocytes in animals in group given in parentheses.

centrifugation of the course homogenate [8] on a Beckman L-8-70 ultracentrifuge (USA) in the SW55Ti rotor. The residue of microsomes thus obtained was washed to remove adsorbed proteins by recentrifugation under the same conditions. All procedures for the isolation of subcellular fractions were carried out at 4°C. The P-450 content in the microsomes was determined by the method in [14]. Activity of GT_m and GT_c was determined by the method in [9] with 1-chloro-2,4-dinitrobenzene as substrate at 36°C. The reduced glutathione (GSH) level was determined in deproteinized coarse liver homogenate by the method in [5] and the level of lipid peroxidation (LPO) in coarse liver homogenate with 2-thiobarbituric acid (TBA) by the method in [13]. Protein in microsomes, cytosol, and coarse liver homogenate was determined by the method in [12]. All spectral measurements were made on a "Specord M-40" recording spectrophotometer (East Germany), with thermostated cuvette. The results were subjected to statistical analysis by the Fisher—Student parametric test. Differences were considered to be statistically significant at the $p < 0.05$ level.

EXPERIMENTAL RESULTS

After 20 applications of HPR to the skin of the tail, there was a marked increase in the rate of microsomal oxidation of foreign compounds in the liver, linked with a rise of the P-450 level on average by 79% (Table 1). Meanwhile considerable induction of GT_m and GT_c activity was observed. The GT_c level rose by 85% and the level of induction of GT_m was much lower, namely 46%. The LPO level, estimated from the quantity of TBA-reacting products, and the intracellular GSH concentration were chosen as indicators of the intensity of oxidative processes in the liver cells. Repeated applications of HPR to the skin had no effect on the LPO level but caused a small but significant increase (by 9%) in the GSH concentration. With anormal LPO level, such an increase in the intracellular GSH concentration could be the result of a general increase in the intensity of biosynthetic processes under the influence of HPR applications.

To assess the levels of toxic action of percutaneous HPR applications, synthetizing and immunotoxic effects and also changes in the mass coefficients of the liver and thyroid gland (Table 2) were studied. No sensitizing action of HPR could be established. An immunotoxic effect took the form of reduction of the mass coefficient of the thymus (by 33%) and an increase in the number of monocytes in the peripheral blood and lymph nodes. On macroscopic examination of the lymph nodes, in the experimental animals they had the appearance of bunches of grapes, and were less eslatic than the single, regularly circular form of the lymph nodes of rats of the control group. Microscopic examination of the lymph nodes revealed disturbances of their morphological structure and, in particular, the absence of reactive centers. Percutaneous applications of HPR also induced a marked increase in the mass coefficient of the liver (by 20%) and thyroid gland (by 23%).

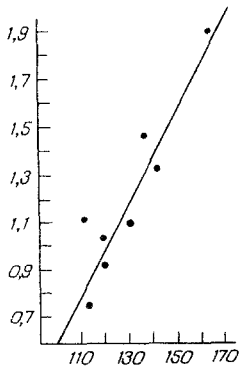

Fig. 1

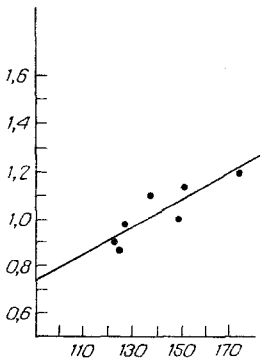

Fig. 2

Fig. 1. Correlation between relative levels of induction P-450 GT_m (ordinate, relative units) and relative changes in mass coefficient of thyroid gland in rats (abscissa, %).

Fig. 2. Correlation between relative levels of induction P-450/GT_c (ordinate, relative units) and relative changes in mass coefficient of thyroid gland (abscissa, %).

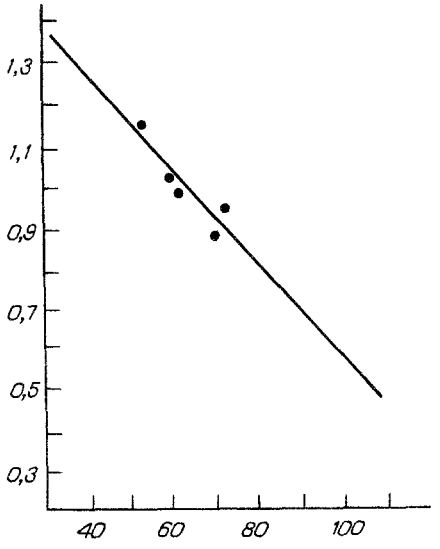

Fig. 3. Correlation between relative levels of induction P-450/GT_c (ordinate, relative units) and relative changes in mass coefficient of thymus (abscissa, %).

Analysis of the results showed that the relative increase in the mass coefficient of the thyroid gland in all rats of the experimental group, expressed as a percentage of the control, correlated closely with the P-450/GT_m ratio ($r = 0.9355$, $p < 0.001$), and that in seven animals of this group close correlation was observed between the relative increase in the mass coefficient of the thyroid gland and the P-450/GT_c ratio ($r = 0.8911$, $p < 0.01$; Figs. 1 and 2).

The levels of P-450, GT_c, and GT_m, for which values of the ratios P-450/GT_m and P-450/GT_c were established, were expressed relative to the control values of these parameters. However, the relative increase in mass coefficient of the thyroid gland did not correlate with the level of induction by P-450, GT_c, or GT_m, if these were taken separately. It was

also shown that the relative decrease in the mass coefficient of the thymus gland in five rats of the experimental group, expressed as a percentage of the control level, correlated closely with the P-450/GT_c ratio ($r = 0.971$, $p < 0.01$) but did not correlate with the P-450/GT_m ratio or levels of induction by P-450, GT_m, or GT_c, if these were taken separately (Fig. 3).

Thus percutaneous application of HPR, repeated 20 times, causes significant induction of the P-450 level and GT_m and GT_c activity, and has a marked toxic action on the immune and endocrine systems. Individual differences between rats of the experimental group as regards the toxic action of HPR on the thyroid gland and thumus did not correlate with levels of induction of P-450, GT_c, and GT_m, but did correlate closely with the ratio between levels of induction of these parameters in the liver.

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COMPARISON OF THE PROTECTIVE ACTION OF CERULOPLASMIN FROM HEALTHY HUMAN BLOOD AND PATIENTS WITH HEPATOCEREBRAL DEGENERATION ON ERYTHROCYTES

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UDC 615.366.153.1:577.152.1].015.4:[616.155.1-02:616.36+616.88]-007.17-092.4

KEY WORDS: ceruloplasmin, erythrocytes, copper- and iron-induced lysis, hepatocerebral degeneration.

Ceruloplasmin (CP) is a highly important antioxidant in human blood [5]. The protective action of CP on erythrocytes (ER), whose membranes contain large quantities of polyunsaturated fatty acids, and which are therefore very sensitive to destruction by radicals, is particularly important.

The aim of this investigation was to compare the protective action of CP from healthy human blood and ceruloplasmin-like protein (p-CP), isolated from the blood of patients with

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 10, pp. 368-370, October, 1990. Original article submitted November 21, 1989.

0007-4888/90/0010-1340$12.50