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EFFECT OF PROPHYLACTIC BENZONAL ON HEPATIC CYTOCHROME P-450 LEVEL IN IRRADIATED RATS

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KEY WORDS: benzonal; irradiation; liver; cytochrome P-450

Besides disturbance of the functions of such vitally important systems as the hematopoietic, endocrine, and immune systems, the irradiated organism develops a "hepatic syndrome" [16], which, in turn, makes a substantial contribution to the aggravation of postradiation changes in these systems. In the investigation described below, from a wide spectrum of liver functions we chose its detoxicating function, which is effected by monooxygenases, located in membranes of the endoplasmic reticulum; their terminal constituent is cytochrome P-450, which is marked by high inducibility and broad substrate specificity. However, in postradiation toxicosis the inducibility of the hemoprotein is blocked and its activity considerably reduced, a condition associated with qualitative and quantitative changes in cytochrome P-450 [1, 2, 9, 14, 17, 19]. On the basis of modern views regarding the development of the hepatic syndrome, aggravating the course and complicating the treatment of radiation sickness, the search for preparations with a marked hepatoprotective effect is fully justified. Some anticonvulsants, including benzonal, are known to be inducers of microsomal oxidation, and to have a beneficial effect on the general functional state of the liver [4, 7, 12].

The aim of this investigation was to study the effect of prophylactic benzonal on the content of the hepatic microsomal hemoprotein P-450 and, on the rat liver as a whole, during the 1st, 2nd, and 4th days of development of acute radiation sickness.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 160-190 g, kept on the standard animal house diet. The rats were deprived of food for 20-24 h before the experiment but were given water ad lib. The animals were divided into three groups. Rats of one group received a suspension of benzonal in starch gel per os in a dose of 100 mg/kg body weight daily for 3 days before irradiation. Rats of the second group received starch gel alone before irradiation, by the same schedule. The induced and noninduced rats were irradiated in a dose of 12 Gy

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TABLE 1. Content of Cytochrome P-450 in Microsomal Fraction of Liver of Irradiated Rats and after Preliminary Induction by Benzonal

Time, h	Control	Irradiation			Benzonal + irradiation		
		1st day	2nd day	4th day	1st day	2nd day	4th day
10	0.327±0,02 $p<0,001$	0,249±0,06 $p_1<0,05$	0,199±0,03 $p_1<0,001$		0,866±0,061 $p_2<0,001$	0,508±0,048 $p_2<0,001$	
12	0,480±0,038 $p<0,001$	0,224±0,03 $p_1<0,001$	0,162±0,02 $p_1<0,001$	0,111±0,02 $p_1<0,001$	0,573±0,043 $p_2<0,001$	0,580±0,033 $p_2<0,001$	0,394±0,02 $p_2<0,001$
15	0,470±0,045 $p<0,001$	0,308±0,05 $p_1<0,05$	0,260±0,04 $p_1<0,02$		0,851±0,05 $p_2<0,001$	0,509±0,029 $p_2<0,001$	
21	0,189±0,019	0,175±0,03	0,269±0,02 $p_1<0,02$		0,441±0,054 $p_2<0,001$	0,573±0,09 $p_2<0,001$	

Legend. p) Significance of value within control group compared with minimal value at 9 p.m.; p_1) level of significance compared with control; p_2) level of significance compared with irradiated, uninduced animals.

TABLE 2. Content of Cytochrome P-420 in Microsomal Fraction of Liver of Intact and Irradiated Rats

Time, h	Control	Irradiation		
		1st day	2nd day	4th day
10	0,100±0,02 $p>0,05$	0,143±0,06 $p_1>0,05$	0,220±0,035 $p_1<0,01$	
12	0,086±0,02	0,104±0,01 $p_1>0,05$	0,222±0,04 $p_1<0,001$	Traces
15	0,185±0,019 $p<0,01$	0,197±0,056 $p_1>0,05$	0,161±0,02 $p_1>0,05$	
21	0,159±0,03 $p<0,01$	0,090±0,016 $p_1<0,05$	0,150±0,025 $p_1>0,05$	

Legend. p) Significance of values within group compared with minimal value at noon; p_1) significance compared with control.

on the day after the last injection of the substances. Irradiation was given on the RUM-17 apparatus, under the following conditions: filter 0.5 mm Cu + 1 mm Al, tube voltage 20 kV, focal distance 60 cm, dose rate $2.15 \cdot 10^{-4}$ A/kg. Irradiation lasted about 25 min and ended at 9 a.m. The rapidity of development of radiation sickness was determined by the times of the experiment: the animals were killed by decapitation at 10 a.m., 12 noon, and 3 and 9 p.m. during the 1st and 2nd days after irradiation, and also on the day of the death en masse (12 noon, 4th day). Intact rats served as the control (group 3): they were killed at the same times of day, 6-8 rats being used in each group at each time. The liver was quickly removed and weighed, and a weighed sample of liver tissue was minced and washed in 0.154 M KCl solution. The microsomal fraction was isolated by differential centrifugation in 0.154 M KCl (pH 7.4) [3]. The molarity of cytochromes P-450 and P-420 was determined by dividing the height of the peaks in optical density units by the molar extinction coefficient (MEC), and was $91 \cdot 10^3$ and $111 \cdot 10^3$ $cm^{-1} \cdot M^{-1}$ respectively [18]. Concentrations of cytochromes were given in nanomoles per milligram microsomal protein [13]. The results were subjected to statistical analysis by Student's test [5].

EXPERIMENTAL RESULTS

Irradiation in a dose of 12 Gy led to a decrease in the cytochrome P-450 content in the microsomal fraction of rat liver 1, 3, and 6 h after exposure (corresponding to 10 a.m., noon, and 3 p.m. on the 1st day) compared with the intact level; the greatest fall, by 1.9 times, was observed 3 h after irradiation (Table 1). On the 2nd day of development of radiation sickness the cytochrome P-450 content remained low, but the greatest fall now amounted to 300% (12 noon). At the same time, 10 a.m. and noon, 2nd day, a considerable (by 2-2.5 times) increase in the concentration of the functionally inactive form of the hemoprotein P-450, namely cytochrome P-420, was observed

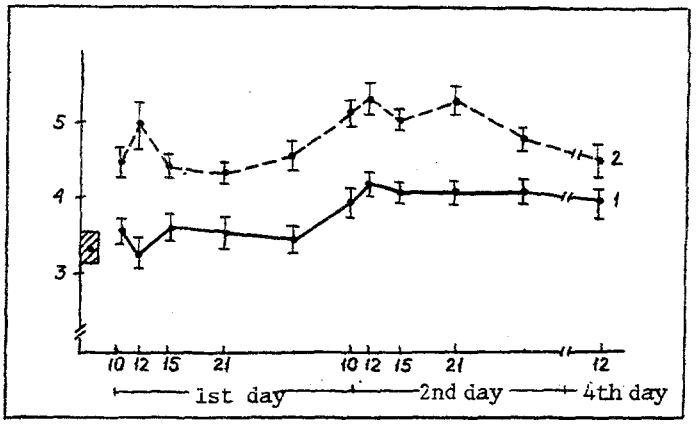

Fig. 1. Changes in mass index of liver (MIL = mass of organ/body of mass ·100) in animals uninduced (1) and induced + irradiated (2). Abscissa, time (in h). Shaded region denotes MIL of intact rats.

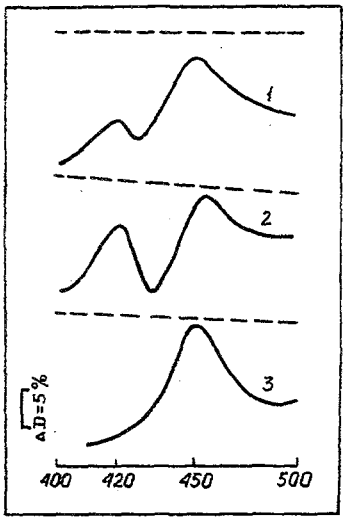

Fig. 2. Differential spectra of cytochromes P-450 and P-420 in microsomal fraction of liver of intact (1), irradiated (2), and benzonal-induced, irradiated (3) rats at noon on 2nd day. Broken line is zero line.

(Table 2) in the microsomal fraction of the liver of the irradiated rats. On the day of death of the irradiated animals en masse (4th day) the content of cytochrome P-450 fell to its minimum, only 23% of the intact level, and virtually no cytochrome P-420 could be detected. Thus irradiation in a dose of 12 Gy, during the first few hours after exposure, and later thereafter at subsequent times of development of the acute form of radiation sickness, the content of the CO-binding hemoprotein underwent a significant change, leading to a lasting disturbance of its time course, evidence of weakening of compensatory reactions forming a basis for an adequate change in the cytochrome P-450 content in EPR membranes in the course of the 24-h period. Modern views on the mechanisms of the postradiation disturbance of regulation of the cytochrome P-450 content were discussed by the writers previously [1], but the leading mechanism is evidently a marked increase in the rate of generation of highly active superoxide ($O_2^{\cdot}$) and hydroxyl ($OH^{\cdot}$) radicals, causing spontaneous induction of lipid peroxidation (LPO) reactions [15]. The decrease in the total pool of multiple forms of cytochrome P-450, which we observed during the first few hours after irradiation, may be due to predominance of processes of destruction of the most sensitive forms of this hemoprotein to LPO

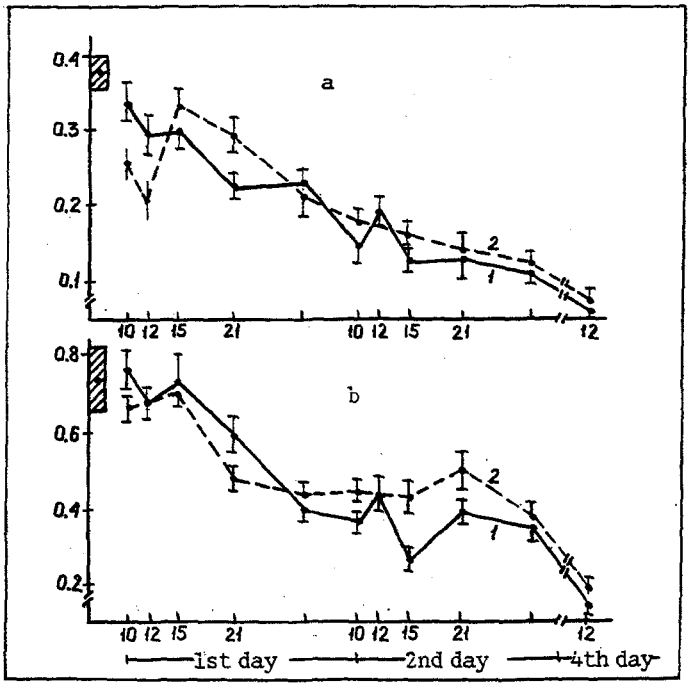

Fig. 3. Changes in mass of thymus (a) and spleen (b) of uninduced (1) and induced, irradiated (2) rats. Shaded region indicates mass of organs in intact rats. Abscissa, time (in h); ordinate, mass of organs (in g).

products [9, 15]. An increase in the quantity of functionally inactive hemoprotein P-420 in the liver of the irradiated rats (our own data) and the possible induction of hemooxygenase, one substrate of which is this cytochrome [2], suggest that a process of inactivation (conversion) of the most radiosensitive forms of this hemoprotein may perhaps be the mechanism of the postradiation depression of the cytochrome P-450 content. The absence of any clear stoichiometry between the fall in the content of cytochrome P-450 and the rise in P-420 can be taken as evidence that the point of application of the converting action of irradiation is the lipid component of the EPR membrane [10].

To correct the microsomal detoxicating system in the liver, the substance benzonal – a cyclic derivative of urea (5-ethyl-5-phenyl-1-benzoylbarbituric acid) – was chosen. Widely and successfully used in the treatment of epilepsy, benzonal also has a well marked hepatoprotective action. Hypertrophy of the liver observed after administration of benzonal is accompanied by proliferation of EPR-membranes, and an increase in the content of RNA, glycogen, and protein (including hemoprotein P-450), evidence of its beneficial effect on synthetic processes in the liver and also of the strengthening of its antitoxic function [4, 7, 12]. As the results of our investigations showed irradiation, even in such a massive dose, does not abolish the inducing effect of benzonal, but in the process of development of acute radiation sickness, it is manifested less clearly. Thus a high mass index of the liver can be traced during the first 2 days after irradiation, after which, on the 4th day, the difference compared with the irradiated animals disappears (Fig. 1). Simultaneously with an increase in mass of the liver in the induced irradiated animals the content of cytochrome P-450 also increased (Table 1), and it thereafter remained quite high (up to 200-300%) relative to the change in uninduced, irradiated rats throughout the period of observation. On the 2nd day its content stabilized at a level reaching on average 60% of the initial value, but later, on the 4th day, it fell to 40%, but this was more than three times higher than in uninduced irradiated animals. Injection of benzonal also stabilizes cytochrome P-450 in the functionally active form (Fig. 2), and cytochrome P-420 appeared in trace amounts only on the 4th day after irradiation. On the other hand, injection of benzonal did not prevent involution of the radiosensitive immunocompetent organs, although it did not worsen their state: reduction of the mass of the thymus and spleen was equally characteristic both of the irradiated rats and rats irradiated after preliminary induction (Fig. 3).

Analysis of an abundance of experimental data and preliminary studies of the metabolism and pharmacokinetics of benzonal, and the pharmacologic effect of this compound as an anticonvulsant for peroral administration, is dependent on its principal metabolite, phenobarbital (PB) [8]. It is quite possible that the inducing effect of benzonal on the cytochrome P-450-dependent system is effected through phenobarbital, although some workers do not rule out the inducing activity of the benzonal molecules themselves, although in this case in experiments in vitro [7]. The stabilizing effect of benzonal on cytochrome P-450 which we observed during irradiation is evidently due to the formation of PB-stable forms of this hemoprotein, less sensitive to inactivation and destruction, when intensified after irradiation [6, 15]. Besides, malonic dialdehyde formation is significantly depressed in PB-induced rats, evidence of suppression of LPO activity [6] and, consequently, its possibly lower "starting" level in benzonal-induced animals before irradiation.

Thus benzonal, when used as a radioprotector, has a stabilizing action on the functional state of cytochrome P-450, and on the liver as a whole; this provides a basis for further research in which inducers of microsomal oxidation may be used as one component of the complex formula used in the treatment of radiation sickness.

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