Stimulation of Immunotoxicity of Chemicals Metabolizing *In Vivo* into Highly Toxic Compounds by the Monooxygenase System Inductors

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Oral treatment of experimental random-bred albino rats with inductors of the monooxygenase system phenobarbital (50 mg/kg) and benzenal (70 mg/kg) for 3 days until acute poisoning with toxins (methanol, ethylene glycol, and dichloroethane in doses of 1.0 LD_{50}) metabolizing in the body to compounds with higher toxicity (phenomenon of lethal synthesis) increased immunotoxicity of these inductors.

Key Words: *inductors of liver monooxygenase system; benzenal; phenobarbital; toxic chemicals; immunotoxicity*

The system of cytochrome P-450-dependent monooxygenases (monooxygenase system; MS) discovered in the beginning of the 1950s as a result of many-year studies of xenobiotic metabolism is closely related to immunological mechanisms involved in the maintenance of chemical homeostasis [3,10]. Cytochrome P-450 is present mainly in the liver, but other tissues (lymphoid organs, kidneys, skin) also contain MS. An important characteristics of MS is its capacity to enzyme induction, as a result of which xenobiotic-stimulated capacity of P-450-dependent monooxygenases to realize their biotransformation drastically increases [3,8,10].

In the lymphoid tissues of humans and animals the following monooxygenase enzymes were detected: benz(a)pyrene hydroxylase, ethoxyresorufin-O-deethylase, and aminopyrin-n-demethylase. In addition, some forms of cytochrome P-450 (P-450PB-1, P450PB-4, P-450MC-1α, P450MC-1β) were identified [8].

The data on immunotropic characteristics of MS inductors are contradictory: MS inductors (barbiturates and other compounds) possess both immunosuppressive and immunostimulating effects [3,11]. Presumably, many xenobiotics (specifically, barbiturates) via induction of MS can stimulate the realization of immunotoxic effects of toxic chemicals (TC) forming highly toxic biotransformation products ("lethal synthesis"), *e.g*. methanol, ethylene glycol, dichloroethane, tetrachloromethane, parathione, *etc.* [3,10].

The effects of monooxygenase system inductors on the immunotoxic characteristics of TC metabolized in the body to highly toxic compounds have not received proper attention, which determined the aim of our study.

MATERIALS AND METHODS

Experiments were carried out on random-bred albino rats of both sexes (180-250 g). TC metabolized by the type of lethal synthesis were used. Their metabolism can be stimulated by MS inductors [3,6,8,10]. The animals were orally treated with liver MS inductors phenobarbital or benzenal in doses of 50 and 70 mg/kg, respectively, for 3 days until acute poisoning with TC. Treatment with these barbiturates in daily doses of 40-70 mg/kg according to this protocol ensures induction of microsomal enzymes in mice and rats [6,12]. TC often causing acute poisoning with high mortality rate [3-5,7,9] (methanol, ethylene glycol, and dichloroetha-

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ne) were given orally in a dose of 1.0 LD_{50}. The mean lethal doses of methanol, ethylene glycol, and dichloroethane for rats are 10.2±2.7, 11.7±2.9, and 0.93±0.10 g/kg, respectively.

The immunity parameters were evaluated by the methods routinely used in experimental immunotoxicology [3]. Humoral immune reaction to T-dependent (sheep erythrocytes) and T-independent (typhoid fever Vi antigen; Vi-Ag) antigens was evaluated after 5 days by the number of antibody-producing cells in the spleen after acute poisoning with TC and simultaneous intraperitoneal immunization of rats with these antigens in doses of 2×10^8 cells and 8 μg/kg, respectively. Humoral immune reaction to sheep erythrocytes in this test characterized the capacity of type 1 T-helpers (Th1) to contribute to IgM production by B-lymphocytes (plasma cells), while humoral immune response to Vi-Ag reflected the intensity of IgM production by these cells without participation of Th1 [3]. Activity of natural killer cells was evaluated spectrophotometrically by the natural cytotoxicity index 48 h after acute poisoning with TC. Antibody-dependent cell cytotoxicity was evaluated spectrophotometrically 5 days after immunization of rats with 10^8 sheep erythrocytes (splenocytes). The formation of delayed type hypersensitivity (DTH) was evaluated by the increase in the weight of the hind paw pad and expressed in percent. The rats were intraperitoneally immunized with 10^8 sheep erythrocytes 30 min after TC administration. The resolving dose of sheep erythrocytes (5×10^8) was injected under the hind paw aponeurosis after 4 days and DTH was evaluated after 24 h.

Protein content and cytochromes P-450 and b5 in liver microsomal fraction were measured 3 days after phenobarbital and benzenal treatment.

The data were statistically processed using Student's *t* test.

RESULTS

Humoral immune response to T-dependent and T-independent antigens, antibody-dependent cell cytotoxicity, and DTH reaction slightly increased after treatment with phenobarbital (1.19, 1.12, 1.28, and 1.21 times, respectively, $p>0.05$) and benzenal (1.24, 1.18, 1.23, and 1.26 times, respectively, $p>0.05$) (Table 1). Treatment with phenobarbital and benzenal increased activity of natural killer cells (1.26 and 1.32 times, respectively, $p<0.05$). The immunotropic effects of both chemicals were virtually the same.

Acute poisoning with methanol inhibited T-dependent and T-independent humoral immune responses, natural cytotoxicity, antibody-dependent cell cytotoxicity, and DTH reaction by 1.45, 1.40, 1.43, 1.41, and 1.35 times, respectively ($p<0.05$), ethylene glycol poisoning decreased these parameters by 1.63, 1.33, 1.69, 1.51, and 1.30 times ($p<0.05$), and intoxication with dichloroethane decreased them by 1.71, 1.54, 1.91, 1.64, and 1.63 times ($p<0.05$), respectively. The mean decrease in the immunity parameters under the effects of these substances was 1.41, 1.50, and 1.69 times, respectively.

Acute poisoning with methanol, ethylene glycol, and dichloroethane after 3-day treatment with pheno-

TABLE 1. Effect of Acute Poisoning with TC Metabolizing with Formation of More Toxic Compounds on Immunity Parameters of Rats Treated with MS Inductors (*M*±*m*, *n*=6-10)

Experimental series		Cells producing antibodies to sheep erythrocytes, 10^3	Cells producing antibodies to Vi-Ag, 10^3	Natural cytotoxicity, %	Antibody-dependent cellular cytotoxicity, %	DTH, %
Control		33.4±3.2	25.4±2.5	28.2±1.8	12.1±1.2	31.3±2.7
Phenobarbital		39.6±3.8	28.6±2.8	35.7±2.7*	15.5±1.4	37.8±2.9
Benzenal		41.3±4.4	30.1±3.0	37.1±2.5*	14.9±1.5	39.4±3.0
Methanol		23.1±2.2*	18.1±1.9*	19.7±1.9*	8.6±1.1*	23.2±2.5*
Ethylene glycol		20.4±2.3*	19.0±1.7*	16.9±2.0*	8.0±1.0*	24.0±2.1*
Dichloroethane		19.5±2.0*	16.5±2.0*	14.8±1.5*	7.4±0.8*	19.2±2.3*
Phenobarbital	+methanol	15.2±1.4*+	10.2±1.6*+	10.5±1.2*+	4.7±0.9*+	16.7±1.7*+
	+ethylene glycol	11.9±1.3*+	12.4±1.7*+	15.0±1.1*+	4.9±0.8*+	15.0±1.6*+
	+dichloroethane	9.8±1.1*+	10.5±1.8*+	10.3±1.4*+	4.1±0.7*+	12.3±1.3*+
Benzenal	+methanol	13.0±1.5*+	8.1±1.4*+	14.0±1.7*+	5.0±0.9*+	15.1±1.8*+
	+ethylene glycol	10.7±1.9*+	13.5±1.5*+	12.7±1.6*	5.4±1.0*+	16.5±1.6*+
	+dichloroethane	12.4±1.6*+	9.5±1.3*+	9.5±1.3*	4.0±1.1*+	14.3±1.4*+

Note. $p<0.05$ compared to *control, + values after intoxication with TC without MS inductors.

TABLE 2. Effects of Phenobarbital and Benzenal on Protein and Cytochrome Content in Rat Liver Microsomes ($M \pm m$, n=7-9)

Parameter	Control	Phenobarbital	Benzenal
Protein, mg/organ	60.5±6.7	155.3±16.3*	143.4±9.1*
Cytochrome P-450, nmol/g protein	0.80±0.04*	3.58±0.40*	2.84±0.25*
Cytochrome b5, nmol/g protein	0.35±0.03	0.56±0.05*	0.65±0.06*

Note. *p<0.05 compared to the control.

barbital and benzenal appreciably suppressed the immunity parameters in comparison with the control and parameters of the immune status after intoxication with TC without treatment with MS inductors (p<0.05); in general, the most severe poisoning was caused by benzenal in combination with dichloroethane. Antibody production in response to T-dependent and T-independent antigens, natural cytotoxicity, antibody-dependent cell cytotoxicity, and DTH reaction after acute intoxication with benzenal were 1.57, 1.74, 1.55, 1.85, and 1.34 times lower, respectively, than after acute dichloroethane intoxication (p<0.05).

Treatment with phenobarbital and benzenal significantly increased the content of protein and cytochromes P-460 and b5 in the liver (Table 2), which was presumably responsible for the increase in natural cytotoxicity and a trend to an increase in other immunity parameters. For example, it is proven that vitamin A, levamisole, phenobarbital, and other MS-inducing substances stimulate activities of T-lymphocytes and natural killer cells as a result of induction of cytochrome P-450-dependent monooxygenases in immunocytes [11].

Due to intensive stimulation of methanol, ethylene glycol, and dichloroethane biotransformation, MS in the liver and lymphoid tissue cause the formation of compounds more toxic than the substances received orally (phenomenon of lethal synthesis) [4-7,10]. It seems that, in addition to TC, immunocompetent cells and natural killer cells are exposed to their highly toxic metabolites whose concentrations during the toxicogenic phase of intoxication are much higher than those of toxic biotransformation products forming without treatment with MS inductors. This leads to an appreciable increase in immunotoxicity of TC metabolized with the formation of more toxic substances than the initial compound.

More intensive suppression of humoral and cell immune reactions after treatment with MS inductors under the effects of highly toxic metabolites of methanol (formaldehyde and formic acid), ethylene glycol (glycolic aldehyde, glycolic, glyoxylic, and oxalic acids) [5,7], and dichloroethane (2-chloroethanol, chloroacetic aldehyde, chloroacetic acid) [4,9] seem to be caused by dysfunction of immunocompetent cells as a result of their reactions with sulfhydryl and amino groups of immunocyte enzymes and by inhibition of tissue respiration and oxidative phosphorylation in lymphocyte and macrophage mitochondria [7,10, 13,14].

Hence, oral treatment with MS inductors phenobarbital (50 mg/kg) and benzenal (70 mg/kg) for 3 days until acute poisoning of animals with TC (methanol, ethylene glycol, and dichloroethane in doses of 1.0 LD_{50}), metabolizing in the body into highly toxic compounds (the lethal synthesis phenomenon) causes an increase of their immunotoxicity.

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