Effects of Dimephosphone, Xydiphone, and Ionol on the Content and Activities of Rat Liver Cytochromes P-450 during Long-Term Treatment with Phenobarbital

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Effects of dimephosphone, xydiphone, and ionol administered in parallel with phenobarbital on the content of cytochromes P-450 in rat liver and on the rate of C-hydroxylation of diazepam, haloperidol, and prednisolone by rat liver microsomal enzymes were studied *in vitro*. Dimephosphone, xydiphone, and ionol exhibited similar inductive effects on C-hydroxylation reactions in the CYP P-450 system during treatment with phenobarbital. Xydiphone and ionol in a dose of 1 mmol/kg canceled phenobarbital-induced increase in P-450 cytochrome content in rat liver. Sex-dependent cytochromes P-450 are involved in the prednisolone and haloperidol C-hydroxylation reactions in rats.

Key Words: *cytochromes P-450; dimephosphone; xydiphone; ionol; phenobarbital*

Enzymes of the cytochrome P-450 system (CYP P-450) play an important role in drug metabolism in humans and animals. Hundreds of CYP P-450 isoenzymes were identified, new ones are discovered, and their physiological role is intensivelystudied. Drug interactions at the level of biotransformation lead to untoward reactions. For example, cisapride, astemizole, and mibefradil were canceled for clinical use because of untoward reactions resultant from their interactions with other drugs at the level of cytochromes P-450 [7]. Therefore, the study of drug biotransformation is a priority task.

Dimephosphone is an original Russian drug belonging to monophosphonates. It is widely used as an antiinflammatory drug and regeneration stimulant. Antioxidant activity underlies numerous biological effects of dimephosphone [1]. R. S. Garaev demonstrated shortening of hexenal-induced sleep in rats treated with dimephosphone for a long time, which was regarded as an evidence of dimephosphone induction of rat liver monooxygenase activity. Phenobarbital (PB) is a classical inductor of cytochromes P-450. It is widely used in clinical, specifically, psychiatric practice. Among the main types of CYP P-450-dependent reactions are C-hydroxylation reactions. Numerous drugs are metabolized in this pathway. Haloperidol, diazepam, and prednisolone used in psychiatry were selected as substrates for the studies of hydroxylation activity.

We studied the effects of dimephosphone on the content and activities of rat liver cytochromes P-450 in comparison with xydiphone (biphosphonate) and ionol (antioxidant) during long-term PB treatment.

MATERIALS AND METHODS

Random-bred albino rats were divided into 6 groups. All animals received the test drugs daily for 14 days through a metal tube (1 ml/100 g). Group 1 (intact control; n=10, 5 males and 5 females) received saline; group 2 (PB control; n=10, males) received PB in a

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dose of 50 mg/kg (0.23 mmol/kg). Animals of four experimental groups received PB in the same dose as in group 2 and the following drugs: group 3 (PB+dimephosphone; *n*=8, males) dimephosphone in a dose of 1 mmol/kg (208 mg/kg); group 4 (PB+xydiphone; *n*=7, males) xydiphone in a dose of 0.23 mmol/kg (45 mg/kg); group 5 (PB+xydiphone; *n*=7, males) received xydiphone in a dose of 1 mmol/kg (198 mg/kg); and group 6 (PB+ionol; *n*=7, males) received ionol in a dose of 1 mmol/kg (220 mg/kg). The animals were decapitated on day 15 of the experiment under light ether narcosis and the liver was taken for analyses. Liver tissue was washed from the blood in 0.15 M potassium chloride and homogenized in manual homogenizer on cold. Liver microsomes were isolated in sodium phosphate buffer (0.05 M, pH 7.0). Supernatants I were centrifuged at 14,000*g* for 10 min, the resultant supernatants II containing the microsomal fraction were used as the enzyme preparation [4,10]. The concentrations of cytochromes P-450 in supernatants II were measured by spectrometry [9]. Substrate hydroxylation activities were evaluated in a reaction mixture containing 10 μmol substrate, 0.1 M phosphate buffer (pH 6.8), and 0.5 μmol NADPH. The reaction was started by adding NADPH and was carried out for 30 min at 37°C. Spectrophotometry was carried out at λ=340 nm ($\varepsilon=6.2\times10^6$ M^{-1} cm^{-1}). Activity was expressed in nmol NADPH/nmol P-450/h. The results were statistically processed using Wilcoxon—Mann—Whitney test [3].

RESULTS

Sex-specific differences in the intensity of C-hydroxylation reactions were detected in the intact control group: these reactions were more intensive in males than in females. Sex-dependent CYP P-450 isoenzymes participated in diazepam, prednisolone, and haloperidol hydroxylation in rats, which was previously shown only for diazepam [12] (Table 1). Considering these differences, further studies of dimephosphone and the reference drugs on the content of cytochromes and activity of C-hydroxylation were carried out only on male rats.

TABLE 1. Sex-Specific Differences in Diazepam, Prednisolone, and Haloperidol C-Hydroxylation (nmol NADPH/nmol P-450) by Liver Tissue in Control Rats (*M*±*m*; *n*=5)

Substrate	Males	Females
Diazepam	28.0±5.2	1.5±0.3*
Prednisolone	48.0±14.5	3.3±0.6*
Haloperidol	152.6±17.7	14.8±0.9*

Note. *$p<0.0001$ compared to males.

After 2-week treatment with PB the concentration of cytochrome P-450 in rat liver increased 2.5-fold in comparison with intact control (Table 2), which agrees with published data [6,8]. PB induced isoenzymes CYP2C6, CYP2C7, CYP2C11 (specific for males), CYP3A2, CYP3A1 [6] and CYP1A1, CYP2B1 [8], and CYP2B2 [5] in rats. In our experiments PB 8-fold increased intensity of diazepam and prednisolone hydroxylation and 4-fold stimulated haloperidol hydroxylation compared to intact control (Table 3). Dimephosphone and xydiphone in a dose of 0.23 mmol/kg virtually did not modify liver concentration of cytochrome P-450 during PB treatment (*vs.* PB control; Table 2). Increasing the dose of xydiphone to 1 mmol/kg led to a 2-fold decrease in liver content of cytochromes P-450 compared to PB control, the parameters not differing from intact control (Table 1). Ionol produced similar effect (Table 1). Hence, xydiphone and ionol in a dose of 1 mmol/kg abolished PB induction of cytochromes P-450. Presumably, xydiphone and ionol in this dose suppress *de novo* synthesis of cytochromes P-450 induced by PB. Dimephosphone increased the intensity of diazepam C-hydroxylation by 52% and of prednisolone C-hydroxylation by 172% *vs.* PB control, without modifying haloperidol C-hydroxylation (Table 3). Xydiphone in a dose of 0.23 mmol/kg increased the intensity of haloperidol C-hydroxylation by 202%

TABLE 2. Content of Cytochromes P-450 (nmol/mg protein) in the Liver of Male Rats Treated with Dimephosphone, Xydiphone, or Ionol in Parallel with PB in a Dose of 50 mg/kg for 14 Days (*M*±*m*)

Group	P-450	p^*	p^+
Intact control	0.28±0.09		0.02
PB control	0.72±0.11	0.02	
PB+dimephosphone, 1 mmol/kg	0.67±0.25	0.08	0.29
PB+xydiphone, 0.23 mmol/kg	0.66±0.16	0.05	0.78
PB+xydiphone, 1 mmol/kg	0.3±0.1	0.94	0.03
PB+ionol, 1 mmol/kg	0.37±0.05	0.30	0.03

Note. Compared to *intact control, +PB control.

TABLE 3. Activity of Diazepam, Prednisolone, and Haloperidol (nmol NADPH/nmol P-450) C-Hydroxylation in Liver of Male Rats Treated with Dimephosphone, Xydiphone, or Ionol in Parallel with PB (*M*±*m*)

Group, substrate		Activity	p^*	p^+	IC %	PB-C %
IC	diazepam	28.0±5.2	—	<0.0001	100	—
	prednisolone	48.0±14.5	—	<0.0001	100	—
	haloperidol	152.6±17.7	—	0.021	100	—
PB-C	diazepam	168.1±23.2	<0.0001	—	786	100
	prednisolone	363.4±61.2	<0,0001	—	756	100
	haloperidol	636.2±123.9	0,021	—	418	100
PB+dimephosphone, 1 mmol/kg						
	diazepam	256.6±14.6	<0.0001	0.025	914	152
	prednisolone	9878±173.3	<0.0001	0.001	2056	272
	haloperidol	651.2±141.6	<0.0001	0.554	428	102
PB+xydiphone, 0.23 mmol/kg						
	diazepam	384.7±154.3	<0.0001	0.54	1371	175
	prednisolone	826.5±236.6	<0.0001	0.084	1720	228
	haloperidol	1923.2±597.2	<0.0001	0.012	1265	302
PB+xydiphone, 1 mmol/kg						
	diazepam	409.3±88.5	<0.0001	0.049	1460	186
	prednisolone	2030.5±401.7	<0.0001	<0.0001	4229	560
	haloperidol	2657.4±514.9	<0.0001	<0.0001	1748	418
PB+ionol, 1 mmol/kg						
	diazepam	360.1±52.0	<0.0001	0.014	1286	164
	prednisolone	680.9±99.9	<0.0001	0.014	1417	188
	haloperidol	782.3±94.7	<0.0001	0.095	515	123

Note. In comparison with *intact control, +PB control. IC: intact control; PB-C: PB control.

in comparison with PB control, but did not modulate diazepam and prednisolone hydroxylation. Xydiphone in a dose of 1 mmol/kg increased diazepam C-hydroxylation by 86%, prednisolone hydroxylation by 460%, and haloperidol hydroxylation by 318% *vs.* PB control (Table 3). The concentration of cytochrome P-450 in this subgroup (1 mmol/kg xydiphone) was lower than in PB control and corresponded to the level of intact control. Prednisolone is used as a marker of CYP3A4 isoenzyme activity in humans [13], while CYP3A [12] and CYP2C11 [11] participate in diazepam C-hydroxylation in rats. Therefore, it can be hypothesized that dimephosphone and xydiphone in a dose of 1 mmol/kg produce a stimulating (and parallel with PB) effect on one (CYP3A) or both isoenzymes. Against the background of decreased total content of cytochrome P-450, xydiphone in a dose of 1 mmol/kg stimulated C-hydroxylation for all substrates in rat liver. This is possible due to changed quantitative ratio of CYP450 isoenzymes involved in oxidation of these substrates (diazepam, prednisolone, haloperidol). Activation of individual CYP P-450 isoenzymes under the effect of xydiphone is also possible. Ionol stimulated C-hydroxylation of diazepam by 64% and that of prednisolone by 87% (but not of haloperidol) in comparison with PB control (Table 3), and presumably, like dimephosphone, induced activity of CYP3A and, probably, CYP2C11, similarly as PB.

Thus, we revealed some similarities and differences in the effects of dimephosphone, xydiphone, and ionol on the liver monooxygenase system in male rats. Similarly as PB, the drugs stimulated C-hydroxylation reactions, while the differences were qualitative and quantitative. For example, only xydiphone stimulated C-hydroxylation of haloperidol. The stimulatory effects of the drug combinations with PB on rat liver monooxygenase system can also manifest in humans determining a wide spectrum of drug interactions. The intrinsic inductive effect of dimephosphone, xydiphone, and ionol cannot be ruled out. Our findings suggest desirability of further studies of the effects of these Russian drugs on drug biotransformation, including marker substrates of CYP P-450 isoenzymes in humans and the mechanism of realization of these effects.

Hence, treatment with dimephosphone, xydiphone, and ionol in parallel with PB had a universally

directed inductive effect on C-hydroxylation reactions in the CYP-450 system. Xydiphone and ionol in a dose of 1 mmol/kg abolish the PB-induced increase in cytochrome P-450 content in the liver of rats, while sex-dependent cytochrome P-450 isoenzymes participate in prednisolone and haloperidol C-hydroxylation reactions in rats.

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