BIOPHYSICS AND BIOCHEMISTRY

Effect of Triphenyldioxane on Phase I Xenobiotic Metabolism Enzymes in the Liver of Rats and Rabbits

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We studied the effect of triphenyldioxane on phase I xenobiotic metabolism enzymes in the liver of rats and rabbits. Total cytochrome P450 content, protein concentration, and catalytic activity of CYP2B, CYP3A, and CYP2C isoforms were measured. Triphenyldioxane significantly increases specific activity of CYP2B and CYP2C in the liver of rats and rabbits, respectively. Immunoblotting analysis of microsomal enzymes in the liver of animals showed that the increase in specific activity of CYP is related to high content of apoenzymes. We showed for the first time that rats and rabbits are characterized by interspecies differences in the induction of cytochrome P450 isoforms under the influence of triphenyldioxane.

Key Words: *CYP2B; CYP2C; triphenyldioxane; induction*

Cytochrome P450 (CYP) presented by a multigenic superfamily serves as a key enzyme of the microsomal monooxygenase system. This system is responsible for phase I metabolism of xenobiotics and endogenous compounds in living organisms. Inducibility, *i.e.* increase in enzyme activity after treatment with the corresponding xenobiotic, is an important property of various CYP forms.

The phenomenon of species-specific induction is important for the study metabolic transformations and mechanism of drug action. Strong evidence exists that animals of different species exhibit different reactions to the same xenobiotic. The inductive effects of a highly effective compound triphenyldioxane (TPD) were extensively studied in our laboratory. TPD is a potent inductor of liver CYP2B in rats, but not in mice [1]. On the other hand, phenobarbital induces CYP2B in animals of both species. The species-specific induction is typical not only of the CYB2B subfamily. For example, antituberculous drug rifampicin induces CYP3A in rabbits, but not in rats [3]. It remains unclear why the same chemical compound has different effects on CYP induction in the liver of experimental animals.

Here we studied the effect of TPD on phase I xenobiotic metabolism enzymes and induction of CYP isoforms in the liver of rats and rabbits.

MATERIALS AND METHODS

Experiments were performed on male Spraque-Dawley rats (100-120 g) and New Zealand rabbits (3.0-3.5 kg). The animals fed a standard laboratory diet and were deprived of food 1 day before euthanasia. TPD was dissolved in vegetable oil and injected

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intraperitoneally (10 mg/kg). The animals were killed 3 days after administration of the inductor. Liver microsomal fraction was isolated routinely by differential centrifugation at 4°C. Protein content in microsomes was measured by the method of Lowry using bovine serum albumin as a standard. Total CYP content was estimated by the method of Omura and Sato. The rate of 7-pentoxyresorufin O-dealkylation (PROD) was measured fluorometrically by the formation of resorufin. The rate of erythromycin N-demethylation (EDM) was measured spectrophotometrically by the formation of formaldehyde. 7-Pentoxyresorufin and erythromycin are the substrates highly specific for CYB2B and CYP3A, respectively. The rate of testosterone hydroxylation was estimated by the formation of hydroxyl metabolites [4]. These compounds were separated by high-performance liquid chromatography. We used a Waters 1525 HPLC system equipped with a Supelco LC-18 column (250×16 mm). CYP2B, CYP3A, and CYP2C oxidize testosterone in the 16β- (16β-OHT), 6β- (6β-OHT), and 16α- and 17-position (16α-OHT and 17-OT), respectively. For immunochemical detection of CYP proteins, the microsomal protein (15 mg) was separated by gel electrophoresis, put on a nitrocellulose membrane, and incubated with polyclonal antibodies against rat CYP2B (1:100), CYP3A (1:500), and CYP2C (1:1000, Sigma) and peroxidase-conjugated secondary antibodies (1:1000, Sigma). Immunoreactive bands were visualized using 4-chloro-1-naphthol. The results were analyzed by Student's *t* test.

RESULTS

TPD increased total CYP content and PROD (CYP-specific reaction) in rats by 2 and 12 times, respectively. However, TPD had no effect on these parameters in rabbits (Table 1). TPD did not modify the rate of EDM (CYP3-specific reaction) in the liver of rats and rabbits. 16β-Hydroxylase activity specific for the CYP2 isoform significantly increased in TPD-treated rats. We revealed high basal activity of 6β-, 16α-, and 17-hydroxylases. Therefore, the constitutive level of CYP3A and CYP2C proteins was high in rat liver. Injection of TPD to rats had little effect on the rate of 6β-, 16α-, and 17-hydroxylation of testosterone, while in rabbits induction with TPD was followed by a significant increase in 16α- and 17-hydroxylase activities (by 15.5 and 5 times, respectively) and less pronounced increase in 16β- and 6β-hydroxylase activities (less than by 2 times).

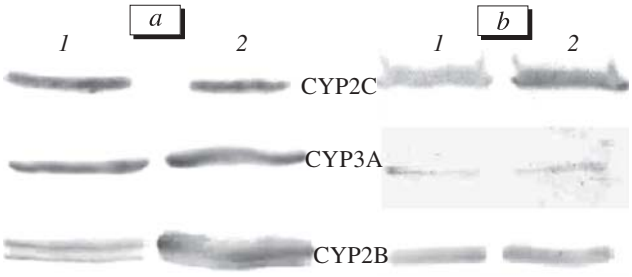

Fig. 1. Immunoblotting analysis of CYP proteins in liver microsomes of rats (*a*) and rabbits (*b*). Control (*1*) and TPD-induced animals (*2*).

TPD significantly increased CYP2B protein content in rat liver (Fig. 1, *a*), but had little effect on the concentration of immunoreactive proteins CYP2C and CYP3A. The induction with TPD was followed by a significant increase in CYP2C apoenzyme content in the liver of rabbits (Fig. 1, *b*). Our results suggest that the induction with TPD produces an increase in CYP2B- (PROD rate and 16β-hydroxylase activity) and CYP2C-specific activities (activities of 16α-hydroxylase and 17-hydroxylase) in liver microsomes of rats and rabbits, respectively. These changes are related to the increase in apoenzyme concentration. CYP2C induction was not accompanied by the increase in total CYP content, which probably resulted from suppression of other isoforms of the enzyme.

We conclude that TPD is a species-specific inductor of CYP. These data are consistent with the results of our previous experiments on rats and

TABLE 1. Effect of TPD on Phase I Xenobiotic Metabolism Enzymes in the Liver of Rats and Rabbits (*M*±*m*)

Experimental object		CYP, nmol/mg protein	PROD, nmol/mg protein/min	EDM, nmol/mg protein/min	Testosterone hydroxylation rate, nmol/mg protein/min			
					16β-OHT	16α-OHT	6β-OHT	17-OT
Rats	control	0.6±0.2	11±6	2.3±0.2	N.a.	0.53±0.11	0.95±0.03	0.18±0.05
	TPD	1.2±0.4*	133±32**	3.8±0.9	0.18±0.09	0.43±0.03	1.0±0.1	0.14±0.07
Rabbits	control	0.9±0.4	5±4	1.3±0.4	0.08±0.01	0.06±0.01	0.35±0.06	0.14±0.03
	TPD	1.0±0.3	8.2±5.3	0.8±0.4	0.15±0.04*	0.94±0.15**	0.68±0.07*	0.68±0.1**

Note. N.a., not assayed. *$p<0.05$ and **$p<0.001$ compared to the control.

mice [1]. A comparative study of CYP-specific activities and protein content in the liver of control and TPD-induced animals showed that TPD does not have a pleiotropic effect on xenobiotic metabolism enzymes. This compound is a highly specific inductor of CYP2B and CYP2C isoforms in the liver of rats and rabbits, respectively.

REFERENCES

1. V. M. Mishin, N. I. Gutkina, V. V. Lyakhovich, *et al*., *Biokhimiya*, **55**, 29-36 (1990).
2. O. U. Lowry, N. I. Rosebrough, A. L. Farr, and R. I. Randall, *J. Biol. Chem.*, **153**, 265-275 (1951).
3. S. A. Wrighton, E. G. Shuetz, P. B. Watkins, *et al*., *Mol. Pharmacol*., **28**, 312-321 (1985).