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COMPARATIVE STUDY OF ZIXORYN AND PHENOBARBITAL AS INDUCERS OF ENZYMES OF THE LIVER MONO-OXYGENASE SYSTEM

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Enzymic induction is nowadays regarded as an adaptive response associated with an increase in the number of molecules of a specific enzyme, to the inducing substance [7]. Cytochrome P-450-dependent enzymes of microsomal oxidation, which are responsible for detoxication of many substrates and for homeostasis of the chemical environment of the organism [1, 6], are induced by various compounds. Common properties of inducers of enzymes of the mono-oxygenase system of the hepatocyte are their lipophilicity, ability to bind with cytochrome P-450, and a high half-elimination period. The most widespread and best studied compound of this group is phenobarbital, whose effect is connected with selective proliferation of the smooth endoplasmic reticulum and hypertrophy of the liver. It considerably accelerates the biotransformation of several drugs and thereby reduces their therapeutic activity, and it also stimulates metabolism of endogenous compounds [9]. From this point of view of matter of clinical interest is an increase in bilirubin clearance in unconjugated hyperbilirubinemia, in the Gilbert, Crigler–Najjar, and Dubin–Johnson syndromes and also, probably, in intrahepatic cholestasis. However, the use of phenobarbital in liver pathology is restricted because of its central action, its low therapeutic index, and the presence of several side effects. There is no doubt that drugs with a selective action on biosynthesis (and, or activity) of enzymes of the mono-oxygenase system of the hepatocyte endoplasmic reticulum are needed for clinical practice for the pharmacological regulation and correction of biotransformation of physiologically active substances.

TABLE 1. Effect of Phenobarbital in a Dose of 80 mg/kg (5-day course) and of Z in Doses of 80 and 40 mg/kg (5- and 2-day courses) on Concentrations of Cytochromes P-450 and b_5 and on Aniline Hydroxylase Activity (AHA) of Rat Liver (M ± m)

Group of animals	Experimental conditions	number of animals	Cytochrome P-450		Cytochrome b_5		AHA, nanomoles/mg protein
			nanomoles/g tissue	nanomoles/mg protein	nanomoles/g tissue	nanomoles/mg protein	
1	Control	7	20,2±1,9	0,390±0,061	12,40±1,46	0,260±0,023	0,177±0,021
2	Phenobarbital, 80 mg/kg (5-day course)	6	66,3±6.8	1,45±0,15	17,80±1,95	0,388±0,039	—
3	Z, 80 mg/kg (5-day course)	5	22,8±1,4	0,522±0,046	25,10±2,64	0,579±0,080	0,228±0,021
4	Z, 80 mg/kg (4-day course)	6	15,4±1,4	0,654±0,048	10,60±1,55	0,224±0,030	—
5	Z, 20 mg/kg (4-day course)	6	23,9±2,9	0,370±0,040	12,4±1,06	0,273±0,017	—
	P_{1-2}		<0,01	<0,001	<0,05	<0,05	
	P_{1-3}				<0,01	<0,01	
	P_{1-4}			<0,05			

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TABLE 2. Protein Concentration of Liver and Its Microsomal-Cytosol Fraction (in mg/g tissue), Weight of Liver (in g), Weight Ratio (weight of liver/body weight × 100), and Body Weight of Rats (in g) after Administration of Phenobarbital in a Dose of 80 mg/kg (5-day course) and of Z in Doses of 80 and 40 mg/kg (5- and 2-day courses) (M ± m)

Group of animals	Experimental conditions	Number of animals	Protein concentration		Liver		Body weight
			liver	microsomal-cytosol fraction	weight	weight ratio	
1	control	7	186,0±3,6	102,0±3,5	5,07±0,31	3,13±0,17	163,0±7,0
2	Phenobarbital, 80 mg/kg (5-day course)	6	180,0±1,6	100,0±4,3	7,65±0,72	3,50±0,11	217,0±13,7
3	Z, 80 mg/kg (5-day course)	5	189,0±7,8	96,0±3,6	6,22±0,23	3,93±0,13	159,0±4,8
4	Z, 80 mg/kg (2-day course)	6	195,0±3,9	103,0±2,2	5,96±0,22	3,29±0,10	182,0±6,4
5	Z, 40 mg/kg (2-day course)	6	179,0±7,1	99,0±5,0	5,01±0,27	3,27±0,22	156,0±11,3
	P_{1-2}				<0,05	—	0,02
	P_{1-3}				<0,02	<0,01	—
	P_{1-4}				<0,02	—	—

Hungarian researchers [10] have suggested the drug Zixoryn (m-trifluoromethyl-α-ethylbenhydrol) which, according to their observations, has a selective inducing action on cytochrome P-450-dependent hydroxylation enzymes.

The aim of this investigation was to assess the inducing effect of zixoryn (Z) and to compare it with the action of phenobarbital.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 156-217 g, divided into five groups: 1) control, intact rats; 2) peroral administration of phenobarbital in a dose of 80 mg/kg once a day for 5 days; 3) administration of Z by a similar scheme in a dose of 80 mg/kg; 4 and 5) administration of Z in doses of 80 and 40 mg/kg respectively for 2 days. Concentrations of cytochromes P-450 and b_5 [2, 8] and also aniline hydroxylase activity [4] were determined in the microsomal-cytosol fraction of the liver, obtained as described previously [3]. In experiments *in vitro* after incubation of the test fraction for 30 min at 37°C with Z and phenobarbital in a final concentration of 10^{-3} M hydroxylation of aniline was studied by measuring accumulation of p-aminophenol [4].

EXPERIMENTAL RESULTS

The cytochrome P-450 concentration was unchanged 144 h after administration of Z in a dose of 80 mg/kg (group 3), although a definite tendency was observed for it to increase when expressed per milligram protein (Table 1). After two injections of Z in this dose (group 4) the cytochrome P-450 level was increased by 68%, i.e., the effect of induction by Z after 144 h was weaker after a 5-day course than after a 48-h course; this can be interpreted as a form of extinction of the inducing action of the drug when administered repeatedly. Unlike Z, phenobarbital led to a sharp increase in the content of cytochrome P-450 by 3.3 and 3.7 times calculated per gram of tissue and per milligram of protein respectively.

A comparative study of the cytochrome b_5 content (Table 1) showed a marked increase (almost twofold) under the influence of Z (group 3) and a more moderate increase under the influence of phenobarbital (an increase of 50%). Consequently, the inducing action of Z is probably realized to a greater degree in the NADH-dependent redox-chain of hydroxylation, whereas with phenobarbital it is realized through the electron-transport system of NADPH-dependent reactions.

The results of determination of the hydroxylation product p-aminophenol after a 5-day course of Z in a dose of 80 mg/kg (group 3) showed an increase in aniline-hydroxylase activity (AHA) of the liver (29%). In experiments *in vitro* with the microsomal-cytosol fraction, Z in a final concentration of 1 mM did not affect the aniline-hydroxylase reaction (AHA in the control was 0.177 ± 0.021 nanomole/mg protein, and in the experiment 0.134 ± 0.006 nanomole/mg protein; $P < 0.1$). On the basis of these results, Z can be regarded as an inducer of microsomal oxidation enzymes, the mechanism of whose action is evidently connected with accelerated synthesis (an increase in quantity) of the corresponding enzymes.

Determination of the liver protein and its microsomal-cytosol fraction after administration of Z and phenobarbital revealed no significant differences compared with the control (Table 2). However, the weight of the animals' liver was increased by 23 and 17% after 5- and 2-day courses of Z in a dose of 80 mg/kg (groups 3 and 4 respectively). Phenobarbital (group 2) caused a greater increase in weight of the liver (51%). The weight ratio of the liver (weight of liver × 100/body weight) also increased significantly under the influence of Z (group 3). Consequently, the total protein content, calculated per weight of the liver increased considerably under the influence of both Z and phenobarbital.

It can be postulated on the basis of these results that Z is similar to some extent to phenobarbital in the character of its inducing action: It increases the weight of the liver and the concentrations of cytochromes P-450 and b_5, and in that way activates hydroxylation reactions of endogenous substrates and xenobiotics, thus leading to their inactivation and subsequent elimination from the body. Meanwhile Z probably cannot be classed unreservedly among inducers of the phenobarbital type. First, this substance is much weaker than phenobarbital in its activity. Second, the character of the change in content of microsomal oxidation enzymes in the case of phenobarbital induction [1] differs appreciably from that under the influence of Z. Attention is drawn to the untypically sharp increase in the level of cytochrome b_5, which is known to be an intermediate electron carrier primarily from NADH to cytochrome P-450 in microsomal oxidation chains. One result of this may be the formation of hemoprotein complexes of cytochromes P-450 and b_5, leading to stabilization of cytochrome P-450 in a catalytically active conformational state and to an increase in the velocity of hydroxylation reactions [5]. Third, the effectiveness of the inducing action of Z when given in a 5-day course is less than when given for 2 days, possibly on account of self-induction of the compound.

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