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Influence of Ethanol on the Interaction Between Polychlorinated Biphenyls and Drug Metabolism

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Investigations described previously (KRAMPL and KONTŠEKOVÁ 1978) showed that a single dose as well as short-term administration of Delor 103, a polychlorinated biphenyl, increased the elimination rate of antipyrine from plasma of rats and that plasma half-life of antipyrine in workers occupationally exposed to a commercial mixture of polychlorinated biphenyls (PCBs) was significantly shorter than in control subjects. These results strongly suggested that the rate of metabolism of antipyrine is accelerated by PCBs.

It has also been shown (KALANT et al.1976, KHANNA et al.1976, SELLERS and HALLOWAY 1978) that long-term ingestion of ethanol, one of the most widespread environmental chemical of human intake, stimulates the hepatic microsomal mixed-function oxidative (MFO) system and accelerates drug metabolism. It was interesting therefore, to study the influence of ethanol on the interaction between PCBs and drug metabolism.

The purpose of these studies was to: 1) examine the effects of repeated doses of ethanol, polychlorinated biphenyl and their combination on the hepatic microsomal MFO system and on the elimination rate of antipyrine from plasma of rats, 2) determine the kinetics of antipyrine in plasma of subjects with alcohol dependence syndrome, 3) compare the results of antipyrine kinetics in alcoholic subjects with previous data of drug study in workers occupationally exposed to PCBs.

MATERIALS AND METHODS

H e p a t i c m i c r o s o m a l M F O s y s t e m s t u d y . Male Wistar rats, 135-150 g maintained on Larsen diet were used in these experiments and were fasted overnight prior to killing. There were three experimental groups. One group received 30 % ethanol (3.3 ml/kg), the second polychlorinated biphenyl Delor 103 alone (1.25 mg/kg) and the third a combination of

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both ethanol and Delor 103 in the same doses. These substances were administered in pure vegetable oil by means of oral tube. Control animals received only oil.

The enzyme activity of O-demethylase of p-nitroanisole and oxidative detoxification of O-ethyl-O-(4-nitrophenyl)phenyl phosphonothioate (EPN)[1] system in whole homogenates of the livers was assayed by the method of KINOSHITA et al.(1966). Cytochrome P-450 content in microsomal fraction of liver homogenates was assayed by the method of OMURA and SATO (1964).

D r u g s t u d y i n a n i m a l s . Experimental design was the same as in microsomal hepatic MFO system study. Both experimental and control animals received orally a single dose of antipyrine (50 mg/kg) 72 hr after the last treatment. The animals were sacrificed by decapitation at 0.5, 1, 2 and 4 hr after administration of antipyrine. Plasma concentrations of antipyrine were assayed by precipitation procedure according to method of BRODIE et al.(1949).

D r u g s t u d y i n a l c o h o l i c s u b j e c t s . Two groups of subjects were investigated. A group of 16 male out-patients with alcohol dependence syndrome, ranging in age from 21 to 50 years. A control group of 30 healthy non alcoholic subjects from other geographical area. All control subjects were men between 21 and 57 years. No subject investigated received medication during or for one month preceding the investigation.

After an overnight fast, 1 g of antipyrine was administered orally. Venous blood was collected in heparinized tube at 3, 6, 9, 12, and in some cases 24 hr after administration. Plasma antipyrine half-life (t 1/2) was read from the linear part of the time-concentration curve on a semilog graph. The apparent volume of distribution (aVd) was calculated from the dose and the drug concentration in plasma at time 0. The apparent metabolic clearance rate (MCR) was calculated using the formula, $MCR = \frac{0.693 \cdot aVd}{t\ 1/2}$.

RESULTS AND DISSCUSION

As shown in Table 1 short-term administration of both ethanol and Delor 103 induced significant increase in p-nitroanisole O-demethylase and EPN detoxification

[1] O-ethyl O-(4-nitrophenyl)phenyl phosphonothioate (EPN) was supplied gratis by the Du Pont de Nemours and Co., Wilmington, Delaware, USA

activity and cytochrome P-450 content. However, an increase of these microsomal components was more pronounced after administration of Delor 103 than after administration of ethanol. Treatment of rats with a combination of ethanol and Delor 103 also significantly increased the activities of these microsomal enzymes and cytochrome P-450 content. This increase after administration a combination of ethanol and Delor 103 was significantly greater than that in the ethanol-treated rats and non significantly lower than that in the Delor 103-treated rats.

TABLE 1

The mean values and S.D. of hepatic microsomal enzymes and cytochrome P-450 content 24 hr after the last administration of treatment. Rats received ethanol, Delor 103 and their combination respectively, daily for 21 days.

Treatment	O-demethylase (µg p-nitrophenol formed per 50 mg/60 min)	EPN detoxification	Cytochrome P-450 (nmol/mg microsomal protein)
Controls	4.0 ± 0.4	3.3 ± 1.3	0.93 ± 0.12
30 % Ethanol (3.3 ml/kg/day)	5.3 ± 0.8[a]	4.9 ± 1.1[a]	1.22 ± 0.17[a]
Delor 103 (1.25 mg/kg/day)	7.8 ± 1.4[ab]	7.0 ± 1.2[ab]	1.97 ± 0.40[ab]
30 % Ethanol + Delor 103 (3.3 ml + 1.25 mg/kg/day)	6.9 ± 1.1[ab]	6.8 ± 0.9[ab]	1.74 ± 0.34[ab]

[a] Significantly different from control group ($P < 0.05 - 0.001$)

[b] Significantly different from group receiving ethanol ($P < 0.05 - 0.001$)

Data of the present hepatic microsomal MFO system study are consistent with previous studies, in which increases in liver microsomal cytochrome P-450 and associated enzymic activities were found after repeated administration of ethanol to rats (RUBIN and LIEBER 1968, RUBIN et al. 1970, KALANT et al.1976, JOLY et al.1977).

On the other hand, present study showed that ethanol in a combination with Delor 103 did not influence significantly the increases of microsomal components induced by Delor 103 alone. It was also found in this study that ethanol is a much less potent inducer in rats than polychlorinated biphenyl.

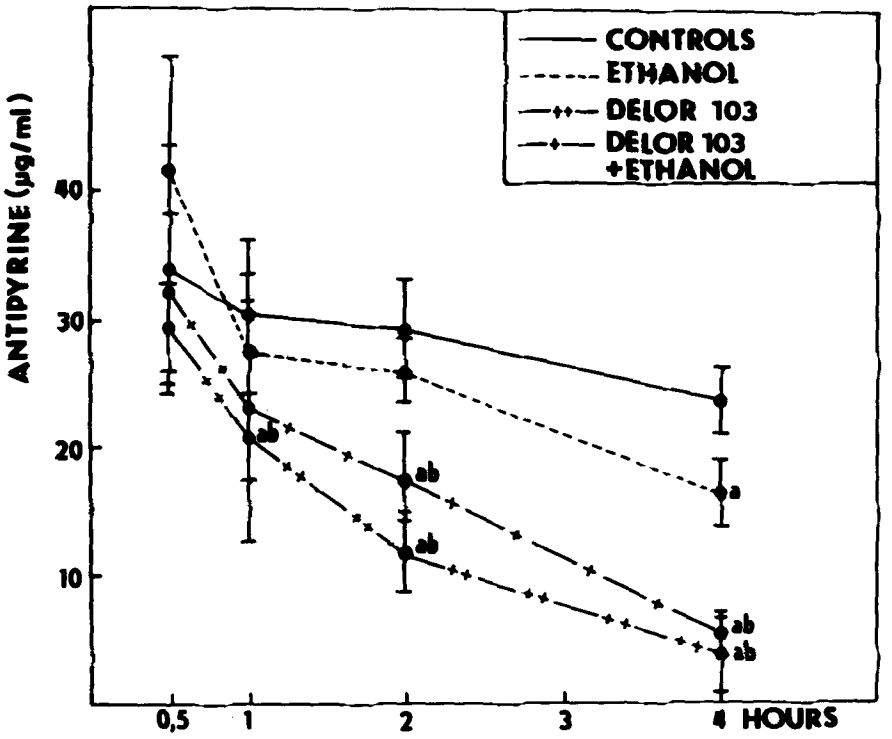

Figure 1. The elimination rate of antipyrine from plasma of rats treated with the repeated daily doses of 30 % ethanol alone (3.3 ml/kg), Delor 103 alone (1.25 mg/kg) and a combination of 30 % ethanol and Delor 103 (3.3 ml + 1.25 mg/kg) respectively and in the control group. Antipyrine was administered orally 72 hr after treatment. Each point represents the mean value and S.D. of 6 animals.

[a]Significantly different from control $P<0.05 - 0.001$)

[b]Significantly different from group receiving ethanol ($P<0.05 - 0.02$)

The plasma elimination rate of antipyrine in rats was increased by short-term administration of both ethanol, Delor 103 and their combination respectively. Increase in elimination rate was greater after administration of both Delor 103 alone and its combination with ethanol than that after administration of ethanol alone. The elimination rate of antipyrine in plasma of rats treated with a combination of ethanol and Delor 103 was nonsignificantly lower than that in the Delor 103-treated rats (Figure 1).

In the present antipyrine kinetics study the increased rate of elimination of antipyrine from plasma was accompanied by increases in microsomal enzymic activity and cytochrome P-450 content. Higly significant statistical correlations were found between antipyrine concentrations in plasma and indices of drug metabolism studied in liver at 4 hr after antipyrine was given to rats treated by ethanol, Delor 103 and their combination (Table 2). The relationship observed in this study suggests that the plasma elimination rate of antipyrine reflects the activity of hepatic microsomal MFO system in rats.

TABLE 2

Correlations between plasma antipyrine concentrations and indices of hepatic microsomal MFO system observed at 4 hr after a single oral dose of antipyrine (50 mg/kg). Antipyrine was administered at 72 hr after last administration of treatment with ethanol, Delor 103 and their combination.

Antipyrine vs. Cytochrome P-450	- 0.81 $^{+}$
Antipyrine vs. O-demethylase	- 0.80 $^{+}$
Antipyrine vs. EPN detoxification	- 0.71 $^{+}$

Values of correlation coefficient (r) are given.

P - values for significance of correlation coefficient:

$^{+}$ = 0.001

Subjects with alcohol dependence syndrome showed that long-term ingestion of ethanol accelerated the rate of antipyrine metabolism. The plasma half-life of antipyrine in alcoholics was lower than that in non alcoholic subjects. These differences in half-life were accompanied by increased metabolic clearance rates in alcoholics

(Table 3). This was in agreement with previous studies, in which the half-life of antipyrine was shortened in 6 healthy subjects after 21 days of ethanol administration (VESELL et al.1971) and in 7 alcoholic subjects with enlarged liver of normal histology. The enhancement of antipyrine metabolism and enlarged liver in alcoholics were associated with increase of the total amount of hepatic cytochrome P-450 (PIRTTIAHO et al.1978).

TABLE 3

Plasma antipyrine half-life, apparent volume of distribution and metabolic clearance rate in alcoholics and controls.

	Antipyrine		
	t 1/2 (hrs)	a V d (L)	M C R (ml/min)
Controls (n=30)			
Mean	17.8	47.9	21,5
S.D.	5.9	12.3	6.6
Alcoholics (n=16)			
Mean	14.1[a]	52.0	44.8[a]
S.D.	6.4	12.3	15.9

[a]Significantly different from control ($P<0.01$ resp. 0.001)

Data of present pharmacokinetic study in alcoholic subjects were then compared with those in workers occupationally exposed to PCBs described previously (KRAMPL 1978). The comparison showed that in alcoholics the mean half-life was significantly longer and the mean metabolic clearance rate of antipyrine nonsignificantly lower than in workers occupationally exposed to PCBs. Alcoholic subjects had practically the same mean half-life but significantly greater mean clearance rate than control subjects from the factory, occupationally not exposed to PCBs (Table 4). However, it was found that PCBs concentrations in blood samples of control subjects from the factory were higher than those in normal subjects from other geographical area (HLADKÁ 1979). The increased PCBs concentrations of control subjects from factory may be due to exposure of PCBs residue levels in the general environment. Further chemical

factors of both industrial and general environment may also play a role.

TABLE 4

Data of antipyrine kinetics in alcoholics compared with pharmacokinetic data of workers occupationally exposed to PCB. The mean values and S.D. are presented.

	Antipyrine	
	t 1/2 (hr)	M C R (ml/min)
Controls (n=30)	17.8 ± 5.9	21.5 ± 6.6
Not exposed to PCBs (n=18) (Subjects from the same factory)	14.6 ± 1.7[a]	33.4 ± 5.9[a]
Alcoholics (n=16)	14.1 ± 6.4[a]	44.8 ± 15.9[ab]
Exposed to PCBs (n=26) (Workers in the production of PCBs)	10.2 ± 4.1[abc]	53.2 ± 20.7[ab]

[a]Significantly different from control ($P<0.02 - 0.001$)

[b]Significantly different from not exposed to PCBs ($P<0.02 - 0.001$)

[c]Significantly different from alcoholics ($P<0.05$)

On the basis of these observations, influence of ethanol on the interaction between PCBs and drug metabolism seems to be not significant. Thus, accelerated antipyrine metabolism observed previously in workers occupationally exposed to PCBs may probably be attributed to effect of PCBs alone.

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