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EFFECT OF REPEATED ORAL ADMINISTRATION OF PHENOBARBITONE OR DDT ON HEPATIC GLUTATHIONE S-TRANSFERASE ACTIVITY IN NON-HUMAN PRIMATES: COMPARISON WITH THE RAT

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Abstract—Hepatic GSH S-transferase activity towards four different substrates was induced significantly ($P < 0.001$) by DDT pretreatment of Sprague–Dawley rats (15 mg/kg/day orally for 21 days). Similar pretreatment of baboons or rhesus monkeys did not induce the corresponding hepatic GSH S-transferase activities. Phenobarbitone pretreatment of rats (15 mg/kg/day orally for 21 days) significantly induced hepatic GSH S-transferase activity towards two of the four substrates studied. No induction of the corresponding hepatic GSH S-transferase activities occurred in similarly pretreated non-human primates except in activity towards 1,2-dichloro-4-nitrobenzene ($P < 0.05$). The results obtained suggest that the inducibility of the GSH S-transferases is probably much lower in non-human primates than in rodents.

The glutathione S-transferases (EC 2.5.1.18) are a group of soluble enzymes that catalyse the conjugation of GSH with a wide range of electrophilic agents (for recent reviews, see [1, 2]). The inducibility of these enzymes was probably first discovered indirectly when it was demonstrated that Y protein or ligandin (GSH S-transferase B) was induced by phenobarbitone pretreatment of rats [3]. This was later confirmed [4, 5], and the list of known inducers of GSH S-transferase activity in rodents has since been extended to include polycyclic aromatic hydrocarbons [6, 7] and organochlorine compounds [8, 9].

Induction of the GSH S-transferases has been studied mainly in rodents. The effect of enzyme inducers on GSH S-transferase activity in a species closer phylogenetically to man is reported in this paper. In these studies, DDT was employed as an enzyme inducer, and a well-tolerated dose level in non-human primates was 15 mg/kg. Consequently a similar dose level of phenobarbitone was used so that the inducibility of GSH S-transferases by the two classes of compounds could be better compared.

MATERIALS AND METHODS

Phenobarbitone (sodium salt) and diethyl maleate were obtained from British Drug Houses Ltd., Poole, Dorset, U.K., 1,2-dichloro-4-nitrobenzene and *trans*-benzylideneacetone from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K., and cyclohex-2-en-1-one and DDT (>99 per cent pure) from Aldrich Chemical Co. Ltd., Wembley, Middlesex, U.K.

Animal experiments. Adult male baboons (*Papio anubis*) and male rhesus monkeys (*Macaca mulatta*) of a bodyweight range of 3.5–5.5 kg, and originally obtained from their natural habitat, were maintained on a complete dry diet (250 g/day). Drinking water was available *ad lib.*, and supplemented at weekly intervals with blackcurrant juice and vitamin B_{12}. The animals were housed separately in metabolism cages and allocated randomly into a control group and two test groups. The former were dosed with the dose vehicle (corn oil or water, 2 ml/animal/day, administered to similar numbers of animals) for 21 days. One of the test groups was similarly dosed with phenobarbitone (15 mg/kg/day) in water (2 ml) and the other test group with DDT (15 mg/kg/day) in corn oil (2 ml).

Male CD (Sprague–Dawley origin) rats of bodyweight *ca.* 200 g (Charles River, Manston, Kent, U.K.) were maintained on a standard laboratory diet and drinking water *ad lib.* [10] and were dosed as described above except that water or corn oil (0.5 ml/rat/day) was administered.

Eighteen hours after the final dose of dose vehicle, phenobarbitone or DDT, the animals were sacrificed [rats by cervical dislocation, non-human primates by exsanguination after sedation with phencyclidine (Sernylan, Parke-Davis, Pontypool, U.K.) and their livers removed for preparation of a dialysed 105,000 g supernatant by standard procedures [10].

Enzyme activity. GSH S-transferase activity towards 1,2-dichloro-4-nitrobenzene [11], diethyl maleate [12], cyclohex-2-en-1-one [13], and *trans*-benzylideneacetone [13], was measured in the dialysed 105,000 *g* supernatants prepared from rat, baboon and rhesus monkey livers. Protein concentrations were determined by the method of Lowry *et al.* [14] using bovine serum albumin as a standard.

Cytochrome P-450 levels were measured by the procedure of Omura and Sato [15] using a molar extinction coefficient of 91 $mM^{-1}cm^{-1}$.

RESULTS AND DISCUSSION

Liver weights

Results from corn oil- or water-treated control animals were pooled. Repeated oral administration of phenobarbitone to baboons and rhesus monkeys

significantly increased ($P < 0.01$) the liverweight/bodyweight ratios, 3.5 ± 0.2 S.E.M. in each species compared to controls, 2.3 ± 0.2 S.E.M. and 2.6 ± 0.2 S.E.M., respectively. Lower but significant ratio increases ($P < 0.05$) also occurred after treatment with DDT (3.0 ± 0.2 S.E.M. and 3.0 ± 0.1 S.E.M. in baboons and rhesus monkeys respectively). Liverweight/bodyweight ratios in rats were significantly increased ($P < 0.01$) albeit to a lesser extent than in non-human primates, after phenobarbitone treatment (4.5 ± 0.4 S.E.M.) compared to controls (4.1 ± 0.02 S.E.M.), or to a similar extent after DDT treatment (5.4 ± 0.1 S.E.M., $P < 0.01$).

Cytosol protein concentrations

Soluble hepatic protein concentrations in baboons (mean 52.3 mg/g liver ± 5.0 S.E.M.) and rhesus monkeys (mean 64.6 mg/g liver ± 6.3 S.E.M.) were not significantly increased ($P > 0.05$) by pretreatment of the animals with phenobarbitone (mean control levels, 49.8 ± 2.7 S.E.M. and 57.4 mg/g liver ± 4.9 S.E.M., respectively). Similar results were obtained after DDT treatment (51.4 ± 2.5 S.E.M. and 55.3 mg/g liver ± 2.7 S.E.M. in baboons and rhesus monkeys respectively). However, soluble hepatic protein concentrations in rats were significantly increased ($P < 0.05$) (mean 58.0 mg/g liver ± 0.5 S.E.M.) after phenobarbitone treatment compared to controls (51.9 mg/g liver ± 2.0 S.E.M.) but not ($P > 0.05$) after DDT treatment (49.0 mg/g liver ± 2.0 S.E.M.).

Enzyme activity

Control GSH S-transferase activity was several-fold greater in dialysed rat liver supernatant than in the corresponding supernatants from non-human primates (Table 1). The latter activities are similar to those reported for human liver preparations assayed with the same substrates under almost identical conditions [13, 16, 17]. Notable was the almost undetectable activity towards cyclohex-2-en-1-one in non-human primate liver (Table 1). Relatively low GSH S-transferase activity towards this substrate has also been reported for human liver preparations [13].

Many workers [2, 6, 11, 18–20] have measured GSH S-transferase activity in rat liver preparations towards 1,2-dichloro-4-nitrobenzene. Their results are similar to those shown in Table 1 provided due allowance is made for the different conditions used, such as substrate concentrations and incubation temperature.

There appear to be few measurements of GSH S-transferase activity in non-human primates [19, 21]. In one comparative study, the ratio of activities (rat : rhesus monkey) was 3.5 [19] which is similar to the ratio of activities, 3.8, shown in Table 1.

GSH S-transferase activity towards two of the four substrates studied was induced by phenobarbitone pretreatment of rats (Table 1). Activity towards diethyl maleate was also induced in Wistar rats administered a higher oral dose (50 mg/kg/day for 21 days) [2]. After treatment of Sprague–Dawley rats for 14 days with dietary phenobarbitone (40 mg/kg/day), hepatic and even some intestinal GSH S-transferase activity towards five different substrates (including 1,2-dichloro-4-nitrobenzene) was induced [22]. Thus it appears that repeated oral doses of phenobarbitone of about 50 mg/kg are probably sufficient to induce GSH S-transferase activities in rat liver towards most of their substrates. Many of the reported studies of induction of GSH S-transferase activity by phenobarbitone pretreatment have used intraperitoneal doses such as 80 mg/kg/day for 7 days [6].

The results in Table 1 suggest that hepatic GSH S-transferase activity was more readily induced by phenobarbitone pretreatment in the rat than in the non-human primate. In the latter species, only GSH S-transferase activity towards 1,2-dichloro-4-nitrobenzene was significantly induced in rhesus monkeys at the dose of phenobarbitone employed. It is probable that the inducibility of human GSH S-transferases is more likely to resemble that of the non-human primate than that of the rat. Nevertheless, Fleischner *et al.* [23] have reported that ligandin levels (ligandin = GSH S-transferase B in the rat liver [5]) in humans were induced twofold by pretreatment with phenobarbitone, from a level of 17.9 μg/mg ± 3.6 S.E.M. in untreated subjects ($n = 13$) to 40.6 μg/mg ± 4.7 S.E.M., in "induced" subjects ($n = 5$).

Induction of rat hepatic GSH S-transferase activity towards different substrates by DDT was highly significant ($P < 0.001$), but no significant induction ($P > 0.05$) of such activity in the dialysed liver supernatants of non-human primates treated with DDT was detected compared to controls (Table 1). Furthermore, there was no apparent trend towards induction of GSH S-transferase activity in the livers of those non-human primates treated with DDT; this suggests that higher but still tolerated doses of DDT would be unlikely to produce statistically significant increases in enzyme activity compared to controls.

Parkki *et al.* [9] have reported that a two-fold induction of rat liver GSH S-transferase activity towards styrene oxide can be achieved even with a single intraperitoneal dose of DDT (160 mg/kg) or a chlorinated biphenyl (100 mg/kg). Due to uptake of these organochlorines into adipose tissue and their subsequent slow release, GSH S-transferase activity remained induced for at least 30 days after the single dose. GSH S-transferase activity towards sulphobromophthalein was apparently induced about six-fold by repeated intraperitoneal doses of DDT (50 mg/kg/day for 3 days) to rats [24]. The results in Table 1 show that marked induction of hepatic GSH S-transferase activity in rats may be achieved with lower oral doses of DDT.

The failure of known inducers of rodent GSH S-transferases to exert similar effects in non-human primates studied is apparently not due to pharmacokinetic reasons. Cytochrome P-450 levels were significantly induced in baboons or rhesus monkeys by the doses of phenobarbitone or DDT employed (Table 1). More surprising, however, was the lack of induction of rat liver cytochrome P-450 after phenobarbitone treatment at the dose level used.

Thus, on the basis of the data obtained in this study (Table 1) using phenobarbitone or DDT as enzyme inducers, and the present knowledge of the

Table 1. Effect of repeated oral administration of DDT (15 mg/kg/day) or phenobarbitone (15 mg/kg/day) on hepatic GSH S-transferase activity and cytochrome P-450 levels in rats and non-human primates

Substrate	Rat			Baboon			Rhesus monkey		
	Control (n=16)	DDT-treated (n=6)	Pheno-barbitone-treated (n=6)	Control (n=5)	DDT-treated (n=4)	Pheno-barbitone-treated (n=4)	Control (n=6)	DDT-treated (n=4)	Pheno-barbitone treated (n=4)
Diethyl maleate†	6.42 ± 0.35	10.02 ± 0.62***	7.29 ± 0.42	1.57 ± 0.37	1.87 ± 0.57	1.94 ± 0.79	2.52 ± 0.16	2.59 ± 0.26	2.59 ± 0.20
1,2-Dichloro-4-nitro-benzene‡	492 ± 27	722 ± 31***	695 ± 63*	63.5 ± 16.4	56.7 ± 6.1	73.8 ± 8.2	129 ± 15	133 ± 2	189 ± 15*
Cyclohex-2-en-1-one†	23.0 ± 2.5	46.5 ± 5.5***	36.5 ± 2.5**	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Benzylidene-acetone†	2.73 ± 0.27	4.46 ± 0.41***	2.63 ± 0.21	0.23 ± 0.07	0.29 ± 0.17	0.24 ± 0.15	0.41 ± 0.12	0.61 ± 0.14	0.44 ± 0.10
Cytochrome§ P-450	0.30 ± 0.01	0.54 ± 0.03***	0.28 ± 0.01	0.30 ± 0.03	0.58 ± 0.06**	0.87 ± 0.10***	0.36 ± 0.02	0.64 ± 0.03***	0.67 ± 0.07***

Significance level, ('t'-test) test vs. control, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

† Expressed as μmoles GSH reacted/hr/mg soluble protein ± S.E.M.

‡ Expressed as nmoles conjugate formed/hr/mg soluble protein ± S.E.M.

§ Expressed as nmoles cytochrome P-450 present/mg microsomal protein ± S.E.M.

levels of the GSH S-transferases in human liver preparations [2], it appears unlikely that induction of GSH S-transferases is of general practical importance in humans. Confirmation of this opinion requires the appropriate studies in man. It also appears that induction of GSH S-transferases is species-dependent such that they are more readily induced in rodents. This is somewhat in contrast to microsomal drug-metabolizing enzyme activity (e.g. cytochrome P-450, see Table 1) which was examined in other studies and shown to be induced in baboon liver as well as in rat liver by pretreatment of the animals with DDT at this dose level [25].

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