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The Effect of DDT on Hepatic Microsomal Drug-Metabolising Enzymes in the Baboon: Comparison with the Rat

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Sufficiently high doses of certain foreign compounds cause increases in microsomal drug-metabolising enzyme activity in animals (REMMER, 1962; CONNEY, 1967). This phenomenon is regarded as enzyme induction. The organochlorine pesticide, p,p'-DDT is an enzyme inducing agent (HART & FOUTS, 1965), in numerous species, such as the rat (KINOSHITA et al, 1966; HOFFMAN et al, 1970; BICKERS et al, 1974; VAINIO, 1974) and squirrel monkey (JUCHAU et al, 1966; CRANMER et al, 1972). Accidental (POLAND et al. 1970; KAY, 1974) or even intentional (THOMPSON et al, 1969) exposure of humans has resulted in increased drug-metabolising enzyme activity (HUNTER & CHASSEAUD, 1976). The present paper reports hepatic microsomal drug metabolism and induction by DDT in the baboon compared with the rat, a popular laboratory animal. p,p'-DDT was administered to both species at different dose levels, for periods of up to 21 days.

MATERIALS AND METHODS

Chemicals: p,p'-DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane], was purchased from the Aldrich Chemical Co. Inc., Milwaukee, Wis, U.S.A. and was more than 99% pure. Antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) and phenylbutazone (4-butyl-1,2-diphenyl-3,5-pyrazolidine-dione) were obtained from Sigma Chemical Co. Ltd., Kingston, Surrey, U.K. DDT and phenylbutazone were administered orally as solutions in corn oil, and antipyrine in aqueous solution.

Studies with Baboons: Eight adult baboons (Papio anubis) of bodyweight range 3.5 - 5.5 kg were maintained on complete dry diet (250 g/day). Drinking water was available ad libitum, to which blackcurrant juice and vitamin B_{12} supplement were added once weekly. The baboons were housed singly in metabolism cages. At weekly intervals, 24-hour urine samples were collected from each baboon, after which plasma half-lives of antipyrine and phenylbutazone were determined for each animal. The baboons were then allocated randomly into four groups. The control group received corn oil (2 ml/baboon/day) for 21 days and the other groups received DDT

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by oral intubation for 21 days, at dose levels of 5, 15 or 50 mg/kg/day respectively. After 4, 11 and 18 days administration of DDT, the plasma half-lives of antipyrine or phenylbutazone were determined for each baboon, and a 24-hour urine sample was collected from each animal for measurement of 6-β-hydroxy-cortisol and D-glucaric acid. Eighteen hours after the final dose of DDT, the baboons were sacrificed and their livers removed for preparation of hepatic microsomes.

Studies with rats: Male CD rats (Sprague-Dawley strain) of bodyweight ca. 200 g (Charles River, Manston, Kent, U.K.) were maintained on standard laboratory diet and drinking water ad libitum. At weekly intervals, 24-hour urine samples were collected from each rat, and the plasma half-life of phenylbutazone was determined for each animal. The rats were then allocated randomly into four groups. The control group received corn oil (0.5 ml/rat/day) for 21 days and the other groups received DDT for 21 days at dose levels of 5, 15 or 50 mg/kg/day respectively. After 4, 11 and 18 days administration of DDT, the plasma half-life of phenylbutazone was determined in each rat, and 24-hour urine samples were collected for measurement of urinary ascorbic acid. Eighteen hours after the final dose, the rats were killed and their livers removed for preparation of hepatic microsomes.

Measurement of plasma drug half-life: Each rat or baboon was starved overnight for 16 hours before and for 4 hours after administration of either drug. Antipyrine (100 mg/kg) or phenylbutazone (10 mg/kg), were administered to baboons and rats by oral intubation. Blood samples were taken, centrifuged and the plasma removed for measurement of antipyrine by the method of WELCH et al, (1967) or phenylbutazone by the method of HERRMAN, (1966).

Measurement of urinary metabolites: 6-β-Hydroxycortisol in baboon urine was oxidised to androst-4-ene-3,6,11,17-tetrone, which was measured by gas-liquid chromatography using electron capture detection, (CHAMBERLAIN, 1971). D-Glucaric acid in baboon urine was measured by the method of MARSH, (1963); boiling the urine at pH 2 converts D-glucaric acid to D-glucaro-1,4-lactone, which can be measured by its specific inhibition of β-glucuronidase, using phenolphthalein β-glucuronide as substrate. β-Glucuronidase was prepared from rat liver and stored at -20°C until used. Ascorbic acid was measured in rat urine by the method of ROE, (1954), using 2,6-dichlorophenol-indophenol as an indicator.

Preparation of hepatic microsomes: Samples of baboon liver (ca. 20 g) were homogenised in 4 volumes ice-cold orthophosphate buffer containing 1.15% (w/v) KCl, pH 7.4, using a tissue mill. The coarse homogenate was subjected to four passes in a glass Potter-Elvehjem-type homogeniser. Rat livers were homogenised with 4 volumes of ice-cold orthophosphate buffered-KCl, pH 7.4, using only the Potter-Elvehjem homogeniser. The homogenates were centrifuged at 10,000 g for 20 minutes at 4°C, and the supernatant decanted off and centrifuged at 105,000 g for 1 hour at 4°C. The gelatinous pellet was resuspended in ice-cold buffered-KCl solution, so that the protein concentration of the suspension was approximately 10 mg/ml (equivalent to ca. 300 mg liver/ml). Enzyme assays were performed only on freshly prepared microsomes.

Measurement of hepatic microsomal drug-metabolising enzyme activity and cytochrome P_{450} concentrations: p-Nitroanisole-O-demethylase was determined using a modification of the method of NETTER & SEIDEL (1964), the reaction product, p-nitrophenol being measured at 420nm. Ethylmorphine-N-demethylase was measured by the method cited by MAZEL (1971), the formaldehyde produced was assayed by the method of NASH (1953), using double strength reagent (COCHIN & AXELROD, 1959). Aniline hydroxylase was measured by a modification of the method of WILLS (1969). p-Nitrobenzoic acid reductase was assayed anaerobically (MAZEL, 1971). Glucuronyltransferase was assayed by the method of POGELL & KRISMAN (1960). Enzyme activity was expressed as nanomoles product formed/hour/mg microsomal protein. Hepatic microsomal cytochrome P_{450} concentrations were measured by the method of OMURA & SATO (1967) using an apparent molar extinction coefficient of the reduced cytochrome P_{450}-CO complex of $91 mM^{-1} cm^{-1}$. The protein concentration of microsomal suspensions was measured by the method of LOWRY et al, (1951), using crystalline bovine serum albumin as a standard.

RESULTS

During 21 days oral administration of DDT to rats and baboons at dose levels of 5, 15 and 50 mg/kg/day (equivalent to an approximate dietary intake of 100, 300 and 1000 ppm respectively), no adverse effects were observed on food consumption, bodyweight gain, general health or behaviour.

Phenylbutazone half-life was shorter in baboons than in rats (Table 1), and was further reduced in some cases after treatment with DDT. Antipyrine half-life in baboons was shorter after 4 days exposure to DDT, but appeared to become increasingly longer after 11 and 18 days (Table 1).

TABLE 1

Plasma drug half-lives (hours) in baboons and rats during repeated oral administration of DDT

DDT dose level (mg/kg/day)	Dosing (days)	Antipyrine half-life	Phenylbutazone half-life	
		Baboon[a]	Baboon[a]	Rat[b]
Control	4	2.2	3.7	7.7 ± 1.8
	11	2.6	4.1	8.1 ± 1.2
	18	2.4	4.3	6.0 ± 0.4
5	4	2.0	4.8	6.4 ± 0.2
	11	2.1	4.6	6.2 ± 1.7
	18	2.8	4.8	4.9 ± 1.5
15	4	1.6	3.2	3.9 ± 0.1
	11	3.4	3.8	5.8 ± 0.8
	18	3.7	3.2	5.7 ± 0.4
50	4	1.9	4.3	3.2 ± 0.3
	11	3.0	3.5	5.7 ± 1.5
	18	5.1	3.4	4.8 ± 0.9

[a]Data are the Mean of results from two baboons in each group

[b]Data are the Mean ± SEM of results from three rats in each group

6-β-Hydroxycortisol excretion by baboons was neither increased by DDT at any dose level, nor by prolonged administration (Table 2). An increase in D-glucaric acid excretion, however, was apparently dose-related, maximal excretion occurring in the 50 mg/kg/day group. Ascorbic acid excretion by rats was increased after DDT treatment, and the maximal effect was detected in rats receiving 15 mg/kg/day (Table 2).

TABLE 2

Urinary excretion[a] of 6-β-hydroxycortisol, D-glucaric acid and ascorbic acid during daily oral administration of DDT

DDT dose level (mg/kg/day)	Dosing (days)	Baboons[b]		Rats[c]
		6-β-hydroxycortisol excretion	D-glucaric acid excretion	Ascorbic acid excretion
Control	4	0.20	3.1	17.0 ± 1.9
	11	0.18	1.8	24.9 ± 3.8
	18	0.16	2.7	24.0 ± 3.9
5	4	0.07	3.6	32.7* ± 8.1
	11	0.04	3.4	43.4* ± 6.3
	18	0.10	3.7	52.7* ± 13.4
15	4	0.22	2.6	61.0* ± 4.5
	11	0.14	4.0	71.5* ± 11.0
	18	0.18	4.0	46.0* ± 7.6
50	4	0.09	8.7	44.7* ± 7.4
	11	0.31	6.0	55.2* ± 8.4
	18	0.15	10.1	42.4* ± 12.7

[a] Results expressed as μmoles excreted in 24 hours

[b] Data are the Mean of results from two baboons in each group

[c] Data are the Mean ± SEM of results from six rats in each group

Significance level, control vs. test *$P < 0.001$ (t-test)

Increases in the liverweight (as a function of bodyweight) after treatment with DDT was of similar magnitude in both species, and maximal increases were achieved after administration of 15 and 50 mg/kg/day of p,p'-DDT to baboons and rats respectively (Table 3)

TABLE 3

Hepatic microsomal drug-metabolising enzyme activities in baboons[a] and rats[b] after oral administration of DDT for 21 days

DDT dose level (mg/kg/day)	Liverweight/bodyweight ratio[c]		Cytochrome P_{450}[e]		Glucuronyl-transferase[d]	
	Baboon	Rat	Baboon	Rat	Baboon	Rat
Control	1.88	4.14_{+}^{-} 0.10^{-}	0.33	0.29_{+}^{-} 0.02^{-}	37.7	10.4_{+}^{-} 4.2^{-}
5	1.99	4.35_{+}^{-} 0.04^{-}	0.34	0.64_{+}^{***-} 0.05^{-}	34.0	9.5_{+}^{-} 1.7^{-}
15	2.67	5.30_{+}^{***-} 0.14^{-}	0.60	0.47_{+}^{***-} 0.03^{-}	23.6	9.2_{+}^{-} 2.5^{-}
50	2.56	5.56_{+}^{***-} 0.16^{-}	0.66	0.61_{+}^{***-} 0.02^{-}	12.6	8.7_{+}^{-} 0.7^{-}

[a] Data are the Mean of results from two baboons in each group

[b] Data are the Mean $\pm$ SEM of results from six rats in each group

[c] Expressed as % bodyweight [d] Expressed as nmoles/hour/mg microsomal protein. [e] Expressed as nmoles/mg microsomal protein.
Significance level, control vs. test *** $P<0.001$

Hepatic cytochrome P_{450} concentrations in rats were increased by similar amounts at all dose levels whereas those in baboons were dose-related. Glucuronyltransferase activity in both species was apparently not changed after DDT administration at dose levels of 5 and 15 mg/kg/day, and was partly inhibited at 50 mg/kg/day (Table 3). The increase in activities of O-demethylase and N-demethylase in baboons was generally dose-related, wherease in rats, O-demethylase activity was increased relative to controls, but this increase was not dose-related. In baboons and rats, hydroxylase and reductase activities were both increased relative to controls, but this increase was not dose-related (Table 4).

TABLE 4

Hepatic microsomal drug-metabolising enzyme activities in baboons[a] and rats[b] after oral administration of DDT for 21 days

DDT dose level (mg/kg/day)	p-Nitroanisole-O-demethylase		Ethylmorphine-N-demethylase		Aniline hydroxylase		p-Nitrobenzoic acid reductase	
	Baboon	Rat	Baboon	Rat	Baboon	Rat	Baboon	Rat
Control	36.1	15.7± 2.8	58.8	195.7± 21.0	22.5	16.5± 2.2	14.9	15.0± 1.2
5	132.4	***42.2± 3.0	139.5	***282.0± 21.0	29.1	***32.3± 0.9	23.8	***28.4± 2.4
15	202.2	***40.2± 3.7	179.2	206.3± 14.0	38.0	*22.5± 3.2	23.3	*18.8± 0.7
50	159.4	**28.0± 3.4	210.8	***369.0± 25.7	37.4	***33.8± 1.7	27.9	***35.2± 1.8

[a] Data are the Mean of results from two baboons in each group

[b] Data are the Mean ± SEM of results from six rats in each group

Enzyme activities are expressed as nmoles/hour/mg microsomal protein

Significance level, control vs. test * $P<0.05$
** $P<0.01$
*** $P<0.001$

DISCUSSION

DDT has been administered frequently to rats at different dose levels to produce changes in hepatic mixed function oxidase (MFO) activity.

After dietary intake of DDT at levels of 50 ppm for periods of up to 13 weeks, it was shown that maximal increases (induction) of enzyme activity occurred after 3 weeks administration, and then remained constant after this time (KINOSHITA, et al, 1966). Dosage at higher levels (500-750 ppm) for periods of between 2 and 4 weeks (HOFFMAN et al, 1970) caused increases of MFO activity of between 150 and 250% of control levels. Twenty-one days dietary administration at 150 ppm induced MFO activity in rats to an extent more closely related to tissue concentrations of DDT than to the actual dietary intake (BUNYAN et al, 1972), although changes in drug metabolism rates in humans exposed accidentally to DDT did not correlate with the serum concentrations of DDT (POLAND et al, 1970). Oral administration of DDT to squirrel monkeys at 50 mg/kg/day for periods of 14 weeks proved fatal; administration at 5 mg/kg/day caused increases in MFO activity after 8 weeks, though no further increases were detected during periods of up to 6 months (CRANMER et al, 1972). Intraperitoneal administration of DDT to squirrel monkeys at 5 and 10 mg/kg/day for 7 days produced increases in MFO activity which was greatest in animals receiving 5 mg/kg/day; intraperitoneal administration at 20 mg/kg/day for 7 days caused toxicity, as indicated by reduction in bodyweight (JUCHAU et al, 1966).

In the present studies, DDT was administered orally to rats and baboons at 5, 15 or 50 mg/kg/day, and MFO activity was assayed at 7-day intervals during 21 days. No toxic effects were observed in any animal during the dosing period. The rate of drug metabolism (as shown by plasma drug half-lives) was increased after 4 days DDT administration, but this was decreased in some cases after 11 and 18 days. Indeed, excessive administration of foreign compounds may disrupt induction of MFO activity in the liver, and hence prolong toxic effects (KAY, 1974). Increase in glucuronidation was dose-related and maximal after 18 days dosing with DDT. The data obtained indicated that induction of MFO activity after 21 days treatment with DDT was dose-related, and was of a similar magnitude in rats and baboons. This evidence may endorse the value of the baboon as a laboratory model.

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