INDUCTION OF ETHOXYRESORUFIN O-DEETHYLASE BY TCDD IN LIVER MICROSOMES OF MARMOSET MONKEYS (Callithrix jacchus) AND RATS

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ABSTRACT

Adult marmoset monkeys (*Callithrix jacchus*) were induced by subcutaneous administration of a single dose of 167 or 300 ng TCDD/kg body wt. After 4 to 8 weeks the induction of EROD was determined in liver microsomes and the TCDD concentration was determined in the liver. The EROD activity was compared with that in hepatic microsomes of adult male rats treated with 32, 107 and 644 ng TCDD/kg body wt.

1. In marmosets the EROD activity was increased five-times by the lower dose of 167 ng TCDD/kg body wt and ten-times by the higher dose of 300 ng TCDD/kg body wt.
2. The EROD induction correlated very well with the TCDD content in the liver.
3. In rats the EROD induction and the TCDD content were also in good correlation.
4. The EROD activity in vehicle-treated rats and marmosets differed considerably, the values of the marmosets were 3.5-times higher than in rats.
5. The effect of low doses of TCDD seems to be higher in marmosets calculated on the levels of TCDD tissue concentration.

KEYWORDS

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin = TCDD; Marmoset monkeys; *Callithrix jacchus*; Non-human primates; Enzyme induction; Ethoxyresorufin O-deethylase = EROD; Wistar rats

ABBREVIATIONS

DMSO = Dimethylsulfoxide; EROD = Ethoxyresorufin O-deethylase;
TCDD = 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

INTRODUCTION

The inductive potency of TCDD for monooxygenases in rodents is well known (KITCHIN & WOODS, 1978; ABRAHAM et al., 1988), but data on the TCDD induction in non-human primates are still lacking. The pharmacokinetics of TCDD is different in rats and primates. First attempts to estimate the persistence in man suggested an elimination half-life of several years (POIGER & SCHLATTER, 1986). In contrast to observations in man, much shorter elimination half-lives (in the range of weeks) were observed for TCDD in rodents (ROSE et al., 1976; ABRAHAM et al., 1988). A recent study showed a half-life of about eight weeks in the liver of *Callithrix jacchus* (NEUBERT et al., 1990). Marmosets are new world monkeys, and have been shown before to be very susceptible to the teratogenic action of thalidomide (MERKER et al., 1988). Furthermore, as in man, some monooxygenase activities are also demonstrable prenatally in *Callithrix jacchus* (NEUBERT et al., 1978; SCHULZ & NEUBERT, 1988).

In order to reveal whether the induction of EROD differs in non-human primates from that in rodents we performed studies in marmoset monkeys and rats. The animals were treated with a single dose of TCDD. Enzyme activity and liver concentration in marmosets were measured after two different doses and at two different times after administration. EROD activity and TCDD concentration in rats were determined seven days after application.

MATERIAL AND METHODS

Chemicals
TCDD (Lot No. 851:142-26) was supplied by Dow Chemical Co., Midland, Michigan, USA. ^{14}C-TCDD, supplied by Cambridge Isotope Laboratories (Woburn, USA) had a radiochemical purity of 97% and a specific activity of 33 mCi/mmol (according to the manufacturer). Ethoxyresorufin was synthesized according to the method of KLOTZ et al. (1984).

Animal maintenance and treatment
Ten female and two male adult marmosets between one and eight years old weighing 300 - 385 g were housed in the colony at the Institute of Toxicology and Embryopharmacology, Berlin at a constant day/night cycle, at 25 ± 1°C and 50 ± 5% relative humidity. The marmosets were fed an Altromin^{R} marmoset pellet diet and given access to tap water ad libitum. Three times a week boiled egg, carrots, apples and bananas were given. Every two weeks the body weight was controlled and the animals were supplemented with a multivitamine mixture and calcium.

The maintenance of rats is described elsewhere (SCHULZ-SCHALGE et al., 1990).

TCDD was dissolved in toluene and 2 volumes of DMSO were added in order to prepare a solution suitable for subcutaneous injection (ABRAHAM et al., 1989). The solution was injected into the back of the animals at a volume of 0.2 ml/kg body wt using a 100 µl-Hamilton^{R}-syringe. Rats were treated with ^{14}C-TCDD (32.2, 107 and 644 ng/kg body wt).

The marmosets were sacrificed four weeks (300 ng/kg body wt) and eight weeks (167 ng/kg body wt) after application of the TCDD solution. The rats were sacrificed seven days after administration.

The animals were starved overnight. The livers were removed and weighed, and after removal of the gall bladder an aliquot was immediately frozen for the determination of TCDD concentrations - in the case of marmosets - or solubilised. Following solubilisation of tissue samples in 3 ml TS1^{R} (Zinsser, Frankfurt, FRG), sonication for 30 min two days later and addition of about 15 ml scintillation cocktail (Hionic-Fluor^{R}, Packard, USA), the radioactivity was measured by scintillation counting.

Preparation of microsomes
The remainder was homogenized in a Potter-Elvejhem homogenizer using 4 ml 0.25 M sucrose per g liver. The homogenate was centrifugated at 10.500 for marmoset or 9.500 for rats x g for 20 min, the supernatant was filtered through gauze and centrifuged at 100.000 x g for 60 min. The pellet was washed in 100 mM Tris HCl buffer, pH=7.4, containing 150 mM KCl and centrifuged again at 100.000 x g for 60 min. The microsomal pellet was resuspended in 100 mM potassium phosphate buffer, pH = 7.4, containing 20% glycerol and 1 mM EDTA. All enzymatic assays were performed on the day of microsome preparation.

Measurement of EROD activity
EROD was measured continuously using a modification of the method by BURKE et al. (1985). The reaction was carried out in fluorimeter cuvettes at 37°C, the reaction mixture, containing microsomes, substrate (10 µl of a stock solution in DMSO) and 0.1 M potassium phosphate buffer (pH 7.8) was equilibrated for 2 min at 37°C. The substrate concentration used was 0.5 µM (marmosets) or 5 µM (rats). The reaction was started by the addition of 50 µl NADPH-regenerating system (yielding a final concentration of 0.25 mM NADP, 5 mM glucose-6-phosphate, 2.5 mM magnesium chloride and 0.5 units per ml glucose-6-phosphate-dehydrogenase). The final reaction volume was 2 ml and the reaction was monitored for 2 min. The fluorimeter (RF 540, Shimadzu, Kyoto, Japan) settings were: excitation and emission slits 5 nm; excitation and emission wave-lengths 550 and 585, respectively. The calibration was performed with resorufin.

TCDD-Analyses
Details of cleanup and GC/MS analyses have been described elsewhere (HAGENMAIER et al., 1990).

RESULTS

Marmosets: The TCDD concentration was considerably higher in the liver samples of the higher dose group which was sacrificed four weeks after application (Table 1). The liver samples of the lower dose group had rather different TCDD concentrations, but the EROD induction was in good agreement with the TCDD concentration (Fig. 1). The control (vehicle-treated) marmosets showed a 3.5-times higher EROD activity than the control (vehicle-treated) rats, but the values of the marmosets were in the same range as the results for untreated marmosets (SCHULZ-SCHALGE & WEBB, 1989).

Rats: A dose of 32 ng/kg body wt led to a 3-fold induction of EROD (Table 2). Hepatic concentration of TCDD was comparable to the results of the 167 ng/kg body wt group of marmosets, but the EROD activity of the marmoset microsomes was 5-times higher. At a level of about 1 to 2 ng TCDD/g liver the inductive potency was comparable in both species.

DISCUSSION

For the first time an induction of a monooxygenase by TCDD could be demonstrated in non-human primates. The results of the induction of EROD activity and the corresponding hepatic concentrations of TCDD suggested a similiar sensitivity of marmosets and rats to TCDD, but the effect of low doses of TCDD seems to be higher in the marmoset. Further investigations are necessary to elucidate the significance of the different slopes of the tissue concentration/effect curves for rats and marmosets (Fig. 1).

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Figure 1:

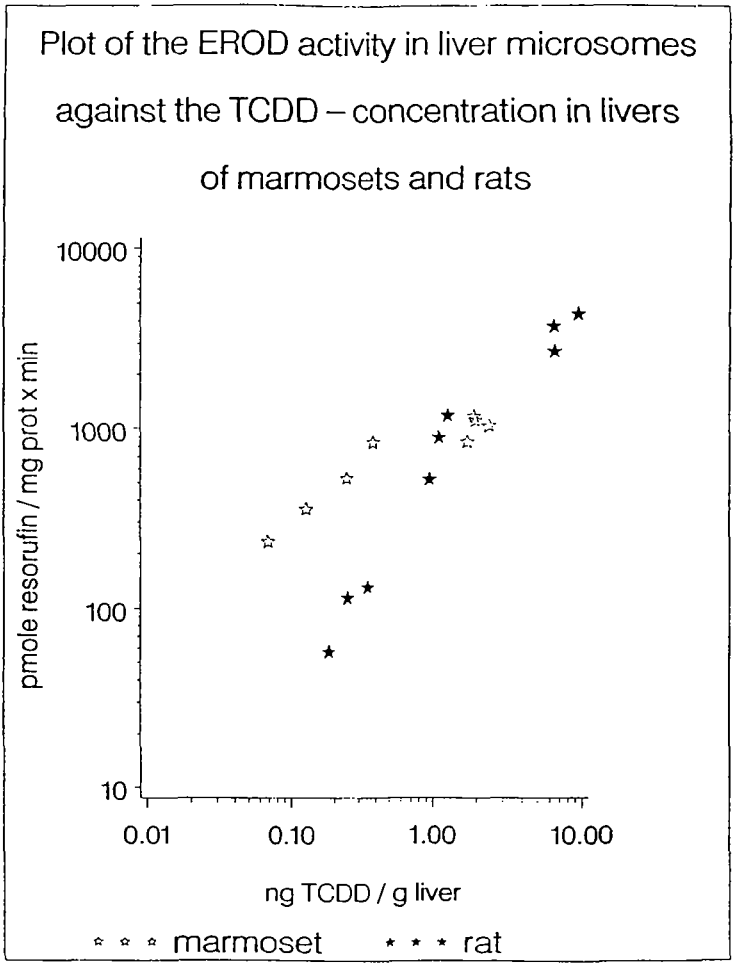

Table 1: **Concentration of TCDD in Liver and Induction of EROD by TCDD in Hepatic Microsomes of Marmosets.**

Animal No.	Sex	Dose	TCDD concentration	EROD
		a)	b)	c)
188	f	-	-	121
189	f	-	-	168
192	f	-	-	94
193	f	-	-	84
Mean ± SD				116 ± 37
190	f	167	130	363
191	f	167	70	237
194	f	167	250	540
195	f	167	390	854
Mean ± SD			210 ± 140	499 ± 268
182	m	300	2500	1060
183	f	300	2020	1140
184	m	300	1770	861
185	f	300	1980	1200
Mean ± SD			2070 ± 310	1065 ± 148

a) ng TCDD x kg body wt^{-1} c) pmole resorufin x mg $protein^{-1}$ x min^{-1}

b) pg TCDD x g $liver^{-1}$

Table 2: **Concentration of TCDD in Liver and Induction of EROD by TCDD in Hepatic Microsomes of Male Rats.**

	n	Dose	TCDD concentration	EROD
		a)	b)	c)
Mean ± SD	14	Control		33 ± 10
Mean ± SD	3	32	266 ± 80	102 ± 39
Mean ± SD	3	107	1140 ± 160	887 ± 340
Mean ± SD	3	644	7640 ± 1600	3700 ± 860

a) ng TCDD x kg body wt^{-1} c) pmole resorufin x mg $protein^{-1}$ x min^{-1}

b) pg TCDD x g $liver^{-1}$