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Comparative Ability of TCDD to Induce Lipid Peroxidation in Rats, Guinea Pigs, and Syrian Golden Hamsters

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is one of the most toxic and stable chemicals known (Schwetz et al., 1973). It has received much attention because it occurs as an unwanted contaminant in the production of chlorophenols and pesticide products derived therefrom (McConnell et al., 1978a).

Extensive interspecies variability exists in the sensitivity towards TCDD. For example, the following single-dose oral LD_{50} values for major laboratory species have been reported: guinea pigs, 0.6-2.1 μg/kg; male rats, 22 μg/kg; female rats, 45 μg/kg; rabbits, 115 μg/kg (Schwetz et al., 1973); and mouse, 114 μg/kg (Vos et al., 1974). Evaluation of acute oral toxicity data in dogs indicates that the LD_{50} is between 300 and 3000 μg/kg for this species (Schwetz et al., 1973). The acute oral LD_{50} for female rhesus monkeys has been estimated as 70 μg/kg (McConnell et al., 1978b). The 50-day single-dose LD_{50} for male hamsters was determined to be 1157 μg/kg by Olson et al. (1980a).

Numerous investigations have not revealed either the target tissues of TCDD or the biochemical lesions induced by TCDD (Poland and Knutson, 1982; Kociba and Schwetz, 1982). The histopathology of TCDD is similar in various species and has been reviewed by Poland and Knutson (1982). Gross necropsy has shown that the primary target organs affected in the hamster are the same as in the rat and other laboratory species, and include the liver, testes, and thymus (Henck et al., 1981). However, no apparent hepatic damage occurs in TCDD-treated guinea pigs (Gupta et al., 1973).

The differences in LD_{50} values between various species may be based in part on relative rates of metabolism and clearance from the body. The whole body half-life of TCDD was estimated to be from 24 to 31 days in the rat (Rose et al., 1976), between 22 and 43 days in the guinea pig (Nolan et al., 1979; Gasiewicz and Neal, 1979), and 10.8 to 12.0 days in the hamster (Olson et al., 1980b). The similarities in half-lives between these species argues against rate of clearance from the body as a major determinant of toxicity.

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We have previously proposed that the toxicity of TCDD may involve membrane lipid peroxidation, and have shown that lipid peroxidation occurs as a result of TCDD administration to rats (Stohs et al., 1983). If lipid peroxidation is involved in TCDD toxicity as we have hypothesized, then a dose of TCDD which produces hepatic lipid peroxidation in the guinea pig should have no effect on the rat or hamster. Furthermore, a dose of TCDD which induces lipid peroxidation in the rat should have no effect in the hamster. In this study lipid peroxidation and other biochemical parameters are compared in a highly sensitive animal (guinea pig), a moderately sensitive animal (rat) and a highly resistant animal (hamster) with respect to TCDD toxicity.

MATERIALS AND METHODS

Female Sprague-Dawley rats, guinea pigs and Syrian golden hamsters were obtained from Sasco Co., Inc., Omaha, NE. The animals were caged and allowed free access to water and lab chow. They were maintained at a temperature of 21°C, with lighting from 6:00 a.m. to 6:00 p.m. daily.

Rats were treated with 40 μg TCDD/kg in corn oil for three days. Guinea pigs were given 1 μg TCDD/kg body weight for three days. Hamsters were treated with 200 μg TCDD/kg in corn oil for three days. Control animals from each species received the corn oil vehicle. At six days post-TCDD treatment all animals were killed between 4:00-5:00 a.m. to eliminate effects due to diurnal variation.

Livers were homogenized in an ice cold 0.05 M Tris buffer, pH 7.4, containing 1.15% KCl, and microsomes were prepared by differential centrifugation as previously described (Stohs et al., 1971). For the determination of lipid peroxidation, isolated microsomes were suspended in 0.10 M phosphate buffer, pH 7.4, and incubated with NADPH for 10 min. Malondialdehyde, which was formed as a result of lipid peroxidation, was coupled with thiobarbituric acid as described by Miles et al., (1980), and measured spectrophotometrically. Hepatic reduced glutathione (sulfhydryl) content was measured by the fluorometric method of Hissin and Hilf (1976). For the determination of glutathione peroxidase, livers were homogenized on ice in 0.25 M sucrose and the 105,000 g supernatant fraction was used. Glutathione peroxidase activity were measured by the procedure of Paglia and Valentine (1967) as modified by Lee et al., (1981). Hydrogen peroxide (0.25 mM) was used as the substrate for the selenium dependent glutathione peroxidase and cumene hydroperoxide (1.5 mM) was used to determine total activity of glutathione peroxidase.

Cytosol fractions from liver were prepared by the method of Benson et al., (1979) for the determination of glutathione S-transferase activity. The spectrophotometric method of Habig et al., (1974) was employed to measure glutathione S-transferase activity, using 1.0 mM 1-chloro-2,4-dinitrobenzene as a substrate. Glutathione reductase activity was assayed on 10,000 g supernatant fractions of

liver which were prepared by the method of Benson et al., (1979). The activity of this enzyme was determined by measuring the decrease in absorbance of NADPH as reported by Calberg and Mannervik (1975).

Hepatic aryl hydrocarbon hydroxylase activity was measured on microsomes isolated in tris buffer (Stohs et al., 1971) using the fluorometric method of Dehnen et al., (1973). Protein concentrations in the different cellular fractions were determined by the standard method of Lowry et al., (1951). All data are presented as the means with the standard deviations. Significance between mean values was determined by Student's t test.

RESULTS AND DISCUSSION

The administration of 40 μg TCDD/kg/day for three days to female rats resulted in approximately a 7-fold increase in hepatic microsomal lipid peroxidation (Table 1), agreeing with previous observations (Stohs et al., 1983). TCDD is a well known inducer of aryl hydrocarbon hydroxylase (AHH) activity, and a 7-fold increase in the activity of this enzyme in the liver was observed. Hepatic reduced glutathione content was decreased by approximately 26%, while total and selenium dependent glutathione peroxidase activities were decreased by 42% and 66%, respectively. In the rat, the activities of glutathione reductase and glutathione S-transferase activities were increased by 1.6 and 2.3-fold, respectively. Six days following the TCDD treatment, the rats had lost an average of 8.8% of their body weight, while an 8.1% decrease in liver weight was observed (Table 1). However, on a per kg basis, no difference existed between the two groups.

Syrian golden hamsters received 200 μg TCDD/kg in corn oil for three days and were sacrificed six days later. Hamsters are highly resistant to TCDD, and this dose is 5-times the dose given to the rats. As can be seen in Table 2, no affect on hepatic microsomal lipid peroxidation was observed. Furthermore, this dose of TCDD did not significantly alter AHH activity, hepatic glutathione content, or glutathione peroxidase activities. TCDD treatment of hamsters did induce small but significant increases in hepatic glutathione reductase and glutathione S-transferase activities. A small but insignificant decrease in body weight was observed, while an 18% increase in liver weight was noted (Table 2).

Guinea pigs are highly sensitive to TCDD (Schwetz et al., 1973). As such, guinea pigs were treated with 1 μg TCDD/kg/day for three days. These results are presented in Table 3. A significant (1.6-fold) increase in hepatic microsomal lipid peroxidation was induced by TCDD administration to guinea pigs. TCDD treatment had no effect on total glutathione peroxidase, glutathione reductase, or glutathione S-transferase activities. No selenium-dependent glutathione peroxidase activity could be detected in either control or treated animals. A 1.8-fold increase in AHH activity was observed following TCDD administration. No change in hepatic glutathione content occurred. In control animals a 14.7% increase

in body weight occurred, while a 9.3% decrease in body weight was noted following TCDD administration. A small but insignificant decrease in liver weights occurred as a result of TCDD treatment (Table 3).

Table 1. Effect of TCDD on Various Parameters in Rats

Parameter	Control	TCDD Treated
Lipid peroxidation (nmol/min/mg protein)	69.1 ± 11.1	500.3 ± 70.8*
Aryl hydrocarbon hydroxylase (nmol/min/mg protein)	0.14 ± 0.01	1.34 ± 0.13*
Glutathione, reduced (µg/mg protein)	9.12 ± 1.50	6.17 ± 0.69*
Total Glutathione Peroxidase (µmol/min/mg protein)	0.42 ± 0.01	0.28 ± 0.03*
Selenium Dependent Glutathione Peroxidase (µmol/min/mg protein)	0.27 ± 0.01	0.10 ± 0.01*
Glutathione Reductase (µmol/min/mg protein)	207.4 ± 11.4	399.2 ± 28.2*
Glutathione S-Transferase (µmol/min/mg protein)	21.3 ± 0.5	68.3 ± 5.0*
Body Weight (gm)		
Initial	149.2 ± 5.6	150.7 ± 8.2
Final	170.5 ± 2.1	135.2 ± 8.5*
Liver Weight		
gm/animal	7.14 ± 0.32	5.65 ± 1.18*
gm/kg	41.9 ± 2.0	41.6 ± 7.4

Female rats received 40 µg/kg TCDD in corn oil for 3 days. Control animals received the vehicle only. All animals were killed 6 days post-TCDD treatment. Each value is the mean ± S.D. from 5-8 animals. * $P < 0.05$ with respect to control group.

Table 2. Effect of TCDD on Various Parameters in Syrian Golden Hamsters

Parameter	Control	TCDD Treated
Lipid Peroxidation (nmol/min/mg protein)	58.3 ± 9.6	51.5 ± 13.4
Aryl hydrocarbon hydroxylase (nmol/min/mg protein)	1.08 ± 0.18	0.83 ± 0.09
Glutathione, reduced (μg/mg protein)	10.93 ± 0.94	9.00 ± 1.08
Total Glutathione Peroxidase (μmol/min/mg protein)	0.68 ± 0.07	0.70 ± 0.12
Selenium Dependent Peroxidase (μmol/min/mg protein)	0.40 ± 0.05	0.37 ± 0.04
Glutathione Reductase (μmol/min/mg protein)	179.8 ±11.2	226.9 ± 16.3*
Glutathione S-Transferase (μmol/min/mg protein)	37.5 ± 2.9	65.0 ± 5.6*
Body Weight (gm)		
Initial	151.0 ± 18.0	152.4 ±11.0
Final	148.2 ± 19.5	143.4 ±11.9
Liver Weight		
gm/animal	6.86 ± 0.75	8.09 ± 1.01
gm/kg	46.1 ± 5.0	56.2 ± 6.8

Syrian golden hamsters received 200 μg/kg TCDD in corn oil for 3 days. Control animals received the vehicle only. All animals were killed 6 days post-TCDD treatment. Each value is the mean ± S.D. from 5-8 animals. * $P < 0.05$ with respect to control group.

The results indicate that lipid peroxidation is induced by TCDD in rats and guinea pigs at doses of xenobiotic that are known to be toxic (Tables 1 & 3). Lipid peroxidation is not induced in hamsters (Table 2) at a dose which is 13-fold greater than the LD_{50} for rats and approximately 600 times the LD_{50} for guinea pigs. The extensive lipid peroxidation shown in rat liver supports the existing morphologic and biochemical evidence of liver damage in this species (Fowler et al., 1973; Lucier et al., 1973; Gupta et al., 1973; Vos et al., 1974). Although TCDD reportedly produces little if any liver damage in guinea pigs, atrophy and necrosis of

Table 3. Effect of TCDD on Various Parameters in Guinea Pigs

Parameter	Control	TCDD Treated
Lipid Peroxidation (nmol/min/mg protein)	42.1 ± 8.13	69.5 ± 17.4*
Aryl hydrocarbon hydroxylase (nmol/min/mg protein)	0.40 ± 0.10	0.74 ± 0.16*
Glutathione, reduced (μg/mg protein)	8.24 ± 1.62	7.67 ± 0.59
Total Glutathione Peroxidase (μmol/min/mg protein)	0.23 ± 0.04	0.19 ± 0.03
Selenium Dependent Glutathione Peroxidase (μmol/min/mg protein)	not detected	not detected
Glutathione Reductase (μmol/min/mg protein)	532.9 ± 64.8	622.5 ± 79.2
Glutathione S-Transferase (μmol/min/mg protein)	76.56 ± 9.2	69.6 ± 18.5
Body Weight (gm)		
Initial	220.4 ± 24.4	223.6 ± 17.4
Final	252.9 ± 27.7	202.8 ± 40.9
Liver Weight		
gm/animal	10.23 ± 1.67	9.02 ± 1.42
gm/kg	40.5 ± 5.7	44.4 ± 6.9

Female guinea pigs received 1 μg/kg TCDD in corn oil for 3 days. Control animals received the vehicle only. All animals were killed 6 days post-TCDD treatment. Each value is the mean ± S.D. from 5-8 animals. * $P < 0.05$ with respect to control group.

testes, thymus, and bone marrow occur (Gupta et al., 1973; McConnell et al., 1978a; Gasiewicz and Neal, 1979). Furthermore, mobilization of adipose tissue fatty acids with extensive hyperlipidemia is induced in guinea pigs by TCDD (Swift et al., 1981). The difference in tissue specificity does not preclude the possibility that lipid peroxidation plays a central role in TCDD-induced cellular damage and the general toxicity of TCDD.

A decrease in liver and body weights is characteristic of TCDD toxicity (Poland and Knutson, 1982). Previous studies have shown that TCDD may result in an increase in liver weight in hamsters (Olson et al., 1980a) which has been attributed to hypertrophy of

hepatocytes as a result of marked proliferation of smooth endoplasmic reticulum. Our results in Table 2 agree with this observation.

Inhibition of hepatic glutathione peroxidase activity in the liver by TCDD may constitute one mechanism by which lipid peroxidation is effected. In the rat, TCDD extensively inhibits selenium dependent glutathione peroxidase (Table 1). As a consequence, H_2O_2 and lipid hydroperoxides may accumulate, resulting in cellular damage. If inhibition of selenium-dependent glutathione peroxidase and possibly other peroxidases constitutes a mechanism by which TCDD-induced lipid peroxidation occurs, then one would expect to observe no effect of TCDD on this enzyme in hamsters, as is observed in Table 2. The low activity of selenium-dependent glutathione peroxidase found in this study in guinea pigs (Table 3) is in agreement with other investigators (Lawrence & Burk, 1978; Burk et al., 1980). Furthermore, Lawrence and Burke (1978) have shown wide variations in total and selenium-dependent glutathione peroxidase activities among various animal species and tissues. The variations in the levels of this enzyme in various animal species may in part explain variations in toxicity to TCDD, and may partially account for the sensitivity of the guinea pig to this xenobiotic.

Enzyme induction is the most extensively studied response to TCDD, and AHH activity is the most frequently assayed enzyme. For TCDD and its congeners, there is an excellent correlation between their potency to induce AHH activity and their toxic potency (Poland and Knutson, 1982). In our studies, an excellent correlation exists between the ability to induce AHH activity and the extent of hepatic lipid peroxidation in the three species. Greatest induction of AHH activity occurred in rats which also showed the most extensive degree of lipid peroxidation (Table 1). In the hamster neither induction of AHH activity or lipid peroxidation occurred (Table 2), while moderate enzyme induction as well as lipid peroxidation was observed in the guinea pig (Table 3). TCDD induction of mixed function oxidases may result in metabolic auto-oxidation or the metabolic activation of endogenous molecules resulting in free radical formation which subsequently give rise to lipid peroxidation. Thus, the data suggest that TCDD-induced lipid peroxidation may involve free radical formation either through metabolic activation or through accumulation of H_2O_2 resulting from glutathione peroxidase inhibition. Depletion of reduced glutathione (Table 1) may also contribute to the enhanced lipid peroxidation.

The relationship between glutathione reductase and glutathione S-transferase activities and TCDD toxicity is not known. However, our studies do indicate that TCDD is an inducer of both enzymes in rats and hamsters but not guinea pigs. Therefore, the induction of glutathione reductase and glutathione S-transferase may not be related to the toxic manifestations of TCDD. Mukhtar et al., (1981) have also shown that TCDD is an inducer of glutathione S-transferase activity.

In summary, microsomal membrane lipid peroxidation may serve as an index of TCDD-induced liver toxicity. Further studies must be conducted to determine whether this relationship also exists for other organs.

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