Author   Gasiewicz, Thomas A.

Corporate Author

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TEMPORAL- AND DOSE-RELATED CHARACTERISTICS OF BIOCHEMICAL AND MORPHOLOGICAL ALTERATIONS IN THE HAMSTER INDUCED BY 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

Thomas A. Gasiewicz[1*], Ellen C. Henry[1], Raymond B. Baggs[2], George Rucci[1], and Arnold Schecter[3]

[1]Division of Toxicology, Department of Radiation Biology and Biophysics
and [2]Division of Laboratory Animal Medicine
University of Rochester, Rochester, NY 14642 USA

[3]Department of Preventative Medicine
Uptstate Medical Center
State University of New York
Binghamton, NY 13901

ABSTRACT: The temporal- and dose-related characteristics of hepatic enzymes induced and toxic responses elicited in the hamster by 2,3,7,8-tetrachlorodibenzo-p-dioxin were examined. The results demonstrate a dissociation of the induction of these hepatic enzymes from TCDD-elicited lethality, but not necessarily hepatic damage, in this species. Furthermore, the hyperthyroidism observed is suggestive of a role of these hormones in the relatively insensitive nature of this species to TCDD treatment.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most potent member of a large family of halogenated aromatic and polycyclic aromatic hydrocarbons including other halogenated dibenzo-p-dioxins and dibenzofurans and polyhalogenated biphenyls. These compounds have been shown to be potent inducers of a number of enzymes, and elicit a variety of toxic responses which are tissue- and species-specific (1). Structure-activity studies, as well as those utilizing inbred strains of mice, have implicated a role of the Ah receptor, a soluble intracellular gene regulatory protein, in these responses (1). Although the hamster is relatively insensitive to the toxic effects of TCDD (LD50 > 3000 µg/kg) (2), hamster tissues contain an Ah receptor molecule with similar concentrations and biochemical properties as observed in other, more sensitive, species (3). Since both the enzyme inductive and toxicological responses to TCDD appear to be mediated by its interaction with the Ah receptor, it was the goal of this study to examine and compare the characteristics of both types of responses in the hamster, and compare these results to those which have been observed in other species. We have chosen hepatic cytochrome P-450 (P-450) concentrations, and the activities of microsomal 7-ethoxycoumarin-0-deethylase (ECOD) and soluble reduced NAD(P):menadione oridoreductase (NMOR) to be representative of the pleotrophic biochemical response elicited by TCDD.

MATERIALS AND METHODS

A stock solution containing 200 µg TCDD/ml of olive oil was prepared as described previously (4). All subsequent dosing solutions were prepared from this stock such that 0.25 ml of olive oil was administered per 100 g body weight. Male Syrian golden hamsters weighing 100-115 g were purchased from the Charles River Breeding Laboratories (Wilmington, MA). For

the time course study, animals were administered intraperitoneal doses of 0, 10, or 500 μg/kg body weight. To examine the time-dependent alteration of serum thyroid hormones, 100 μg TCDD/kg was used. For the dose-response study, animals were administrered doses of TCDD ranging from 0 to 500 μg/kg. These animals were sacrificed at day 7 after treatment.

Livers were separately homogenized in four volumes of buffer containing 50 mM potassium phosphate, pH 7.6, and 1 mM EDTA. The activity of ECOD was determined by the method of Greenlee and Poland (5) using the 10,000 x g supernate. The activity of NMOR was determined using the method described by Kumaki et al. (6). The method of Omura and Sato (7) was used to determine the concentration of cytochrome P-450 in the microsomal pellet. Protein concentrations were determined by the method of Markwell et al. (8) using bovine serum albumin as a standard. Thyroxine (T_4) and triiodothyronine (T_3) were determined using Coat-A-Count T_3 and T_4 kits (Diagnostic Products Corp., Los Angeles, CA).

RESULTS

A dose-dependent decrease in body weight gain was observed. However, even at the highest dose used (500 μg/kg) hamsters maintained their original body weight throughout the 35-day period, and no lethality was observed. Relative liver weights were increased in a time- and dose-dependent manner. At day 35, the mean relative liver weight of the 500 μg/kg group (6.6 ± 0.5 g/100 g body weight) was approximately 60 percent greater than that of the control group (4.1 ± 0.2 g/100 g body weight). At day 7 the ED50 for this effect was approximately 15 μg/kg (Table I). No significant morphological alterations in the liver were observed by light microscopy in the 10 μg/kg treatment group at day 35 as compared to the vehicle-treated group. However, livers from the 500 μg/kg group demonstrated alterations characterized by bile duct hyperplasia and the presence of numerous inflammatory cells. Clusters of hypertrophic hepatocytes appeared near the hyperplastic, disorganized bile ducts.

The administration of 500 μg TCDD/kg produced a decrease in thymus weight that was nearly maximal by day 7. This value fell from a control level of approximately 47 to 7 mg/100 g body weight by day 34. At day 7, the approximate ED50 for this response was 100 μg/kg (Table I).

Hepatic concentrations of P-450 were increased within two days following TCDD treatment. At a dose of 10 μg/kg, P-450 concentrations reached a maximal level (3.94 ± 0.09 vs. 2.24 ± 0.05 nmoles/mg microsomal protein) by day 7 and remained at this level for up to 35 days. Hamsters treated with 500 μg/kg demonstrated an increased concentration of P-450 up to day 4, followed by a return to near control levels by day 14. This decreased concentration (relative to the maximal level at day 4) persisted up to day 35 following treatment. The temporal pattern observed for the P-450 associated ECOD activity was similar. Maximal levels of ECOD activity for 10 and 500 μg TCDD/kg were 8.6 ± 0.6 and 8.9 ± 0.3 nmol product/min/mg protein, respectively, as compared to 2.1 ± 0.2 for the control. In contrast, the activity of cytosolic NMOR reached respective maximal levels of induction (2430 ± 150 and 3130 ± 190 EU/mg protein for 10 and 500 μg/kg, respectively, vs. 510 ± 20 for the control) by day 14 and remained at or slightly below these levels for 35 days following treatment. At day 7, the ED50 values for the induction of P-450, ECOD and NMOR were approximately 0.5, 1.0, and 2.0 μg TCDD/kg, respectively (Table I).

At a dose of 100 μg TCDD/kg, both serum T_4 and T_3 concentrations were increased as compared to controls. For T_4, maximal concentrations (4.5 ± 0.3 vs. 1.9 ± 0.4 for the

TABLE I

Summary of ED50 Values for the Hamster at Day 7 Following TCDD Treatment

Approximate ED50 (µg TCDD/kg)	Response
Lethality	>3000 (2)
Increased liver weight	15
Decreased thymus weight	100
Increased hepatic P-450	0.5
Increased hepatic ECOD	1.0
Increased hepatic NMOR	2.0
Increased serum T_4	15
Increased serum T_3	20

controls) were obtained by day 7 and persisted up to day 35. Serum T_3 values showed a similar pattern, being increased from approximately 83 ± 12 ng/dl in the control to 120 ± 11 ng/dl for the treated animals. At day 7, the ED50 values for increased serum T_4 and T_3 concentrations were approximately 15 and 20 µg TCDD/kg, respectively (Table I).

DISCUSSION

The ED50 values observed for the induction of P-450 and the activities of ECOD and NMOR (Table I; 1.6, 3.2, and 6.4 nM, respectively; calculated assuming uniform distribution of TCDD _in vivo_) are similar to the determined apparent equilibrium dissociation constant (K_D) (0.3 nM) of TCDD binding to the hamster hepatic Ah receptor (3). Furthermore, these values are similar to the relative ED50 values for the induction of aryl hydrocarbon hydroxylase (AHH) and ECOD activity in the C57BL/6J mouse (1.8 and 1.7 nM, respectively (5)), and AHH activity in the rat (0.9 nM (9)). These data are consistent with the role of the Ah receptor in the induction of these enzymes (1). Furthermore, the duration of sustained induction of these enzymes in the hamster was similar to that observed previously in both rats (9) and mice (6). However, since these relative ED50 values for enzyme induction are approximately three orders of magnitude less than the reported LD50 value (Table I), it appears that the induction of these hepatic enzymes cannot be directly associated with TCDD-induced lethality in the hamster.

Despite these considerations, significant toxicity and lethality occurs in other mammalian species at doses of TCDD which are well below the reported LD50 dose for the hamster (1). Thus, additional factors other than the ability of the TCDD-receptor complex in the hamster to invoke a prolonged modulation of gene expression are responsible, in part, for these differences. The tissue-specific control over the battery of genes expressed (or repressed) may be one determining factor. Also, there may be species-specific biochemical differences which may modulate the animal's response to the genes expressed following TCDD treatment. It has recently been postulated that the greater regulatory role of brown adipose

tissue in the hamster (a hibernating animal) for the control of energy metabolism via nonshivering thermogenesis contributes to the relative insensitivity of this species (10). It is of further interest that thyroid hormones, which are known to play a role in brown adipose tissue regulation of thermogenesis (11), are elevated in the hamster following treatment with TCDD. In contrast, decreased serum concentrations of thyroid hormones have been observed in TCDD-treated rats which are more sensitive to the toxicity of this compound (12).

Although induced hepatic enzymes may not play a direct role in determining lethality in the TCDD-treated hamster, they may be associated with biochemical, morphologic, and functional changes in this tissue. Limited examination of liver sections by light microscopy revealed significant morphologic changes which appeared only in the day 35, 500 ug/kg treatment group and were concomitant with a lowering in the hepatic concentration of P-450 as well as one of its associated activities, ECOD. An association of reduction in P-450 concentrations with liver injury produced by a variety of hepatotoxins has been documented. This may be due to a variety of mechanisms including enzyme inhibition, decreased enzyme synthesis, increased enzyme degradation, or enzyme-catalyzed peroxidation of associated microsomal membranes. These factors may be highly dependent upon the species-specific pattern of P-450 isozymes induced as well as their relative substrate specificities.

Finally, since a maximal, sustained modulation of gene expression elicited by TCDD occurred in the hamster liver at doses well below that which produces a generalized toxicity, this species may be a useful _in vivo_ model to further examine the role of the _Ah_ receptor in these processes, as well as the relationship of these processes to the specific lesion produced in this tissue.

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