USING TISSUE DOSE OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) AS A PREDICTIVE RESPONSE FOR REVERSIBLE BIOCHEMICAL CHANGES

Janet J. Diliberto, Michael J. DeVito, and Linda S. Birnbaum

Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, United States Environmental Protection Agency, Research Triangle Park, North Carolina, 27711, USA

Introduction

Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin) and dioxin-like compounds causes a wide range of adverse effects in both genders of experimental animals. These effects occur at various life stages in a diversity of cells, tissues, and organs and range from biochemical alterations through overtly toxic responses. They can be produced regardless of exposure route and time and increase in severity with increasing exposures. Extrapolation of these animal data to humans is problematic due to the uncertainty in dose extrapolation. Default procedures such as allometric scaling or uncertainty factor approaches do not provide adequate estimates of human equivalent exposures due to the more than 100-fold difference in half-life between humans and rodents.

When body burden is used as the measure of dose, humans are as sensitive as experimental animals for such end points as cancer, chloracne, and induction of CYP1A1 [1]. CYP1A induction, a sensitive Ah-receptor-mediated TCDD-inducible response, has been shown to be reversible in rodents and is associated with the pharmacokinetics of the administered compound [2,3,4]. Review of these data suggests that tissue CYP1A activities reflect tissue TCDD concentration, which is directly related to body burden. Thus, to study the appropriate dose metric in rodents, female B6C3F1 mice were acutely and subchronically exposed to TCDD. The dependency of reversible biochemical responses on these dose metrics is described below. Therefore, estimating appropriate dose metrics derived from animal data can be useful in facilitating the risk characterization of adverse health effects in humans to dioxin and dioxin-like compounds.

Study Objective

The objective of this study was to test the hypothesis that tissue concentration of TCDD is a predictive response for reversible biochemical changes. Tissue dosimetry and response data from studies in female B6C3F1 mice exposed to TCDD using different dosing regimens (acute and subchronic exposures) were analyzed using the Hill model. In these analyses, tissue and blood concentrations and body burdens were used as the dose metric and enzyme induction was used as the response.

Materials and Methods

In the acute study [2], female B6C3F1 mice received a single oral dose (0, 0.1, 1, or 10 µg [^{3}H]TCDD/kg) and were killed 1, 2 3, or 4 wks post-treatment.

In the subchronic study [3], female B6C3F1mice were dosed by gavage with corn oil solution of the test chemical 5 da/wk (Mon-Fri) for 4, 8, 13, or 17 wks (0, 1.5, and 150 ng [^{3}H]TCDD/ kg/da).

Additional doses were used (0.15, 0.45, 4.5, 15, 45, and 450 ng [^{3}H]TCDD/kg/da) for the 13 wk time point. In addition, some animals were exposed for 13 wks to either 0, 1.5, or 150 ng [^{3}H]TCDD/kg/da followed by 4 wks without dosing.

In both the acute and subchronic studies, TCDD concentrations in tissues and hepatic ethoxyresorufin *O*-deethylase activity (EROD) and acetanilide-4-hydroxylase activity (ACOH) (markers for CYP1A1 and 1A2, respectively) were determined. The dose-response and time-course data from the acute and subchronic studies were used to determine the appropriate dose metric for CYP1A. For curve fitting the data, calculations were performed using Sigma Stat (Jandel Scientific, San Rafael, CA) according to the Hill equation. The Hill model was fit to enzymatic data using either administered dose, daily dose, or tissue concentration (liver, blood, and body burden) as dose metric. Individual data points from each animal from both the acute and subchronic studies were used in the curve fittings.

Hill Equation: $E = E_0 + [(Emax * X^n)/(b^n + X^n)]$

E = enzyme activity at dose X
E_0 = enzyme activity at X = 0
Emax = maximum response
X = dose (either administered dose, tissue or body burden)
b = ED50
n = Hill shape parameter

Results

The Hill model was used to curve fit dose-response and time-course data sets from the acute [2] and subchronic [3] studies to determine the appropriate dose metric for CYP1A. The model was fit to enzymatic data using either administered dose, daily dose, or tissue concentration as the dose metric. When acute or subchronic EROD or ACOH data sets were analyzed separately, using total administered or daily dose showed adequate fits to the data ($R>0.75$). However, when acute and subchronic EROD or ACOH data sets were analyzed together, using either total administered dose or daily dose as dose metric did not provide an adequate description of the dose response relationship for either endpoint examined.

Using TCDD tissue concentrations (liver, blood, and body burden) as dose metric resulted in similar dose response relationships when acute or subchronic EROD and ACOH data were analyzed separately or combined. In addition, when tissue concentrations were used as dose metric, the fits resulted in $R>0.88$ and $p<0.001$ for all data sets analyzed.

When the Hill model was fit to the EROD enzymatic activity data using liver concentration as dose metric, the Hill shape parameters were estimated at 0.77 ± 0.07, 0.97 ± 0.11, and 0.86 ± 0.06 for acute, subchronic, and combined data sets, respectively. The ED50s were estimated at 3.81 ± 0.48, 4.85 ± 0.54 and 4.33 ± 0.37 ng TCDD/g tissue for acute, subchronic, and combined data sets, respectively. When the Hill model was fit to the ACOH enzymatic activity data using liver concentration as dose metric, estimates of the Hill coefficient were 0.40 ± 0.03, 0.61 ± 0.06, 0.51 ± 0.04 for acute, subchronic, and combined data sets, respectively. The ED50s were estimated at 1.06 ± 0.18, 3.09 ± 0.56, and 2.00 ± 0.27 ng TCDD/g tissue for acute, subchronic, and combined data sets, respectively.

When blood concentration was used as dose metric, the Hill model was fit to the EROD data and Hill shape parameters were very similar (~1-1.3) for acute, subchronic, and combined data sets. The ED50s were also similar (0.011 ± 0.001 ng TCDD/g tissue) for the acute, subchronic, and combined data sets. Again, using blood concentration as dose metric, the Hill model was fit to the ACOH data,

estimates of the Hill coefficient were 0.53 « 0.05, 0.84 « 0.097, 0.72 « 0.056 for the acute, subchronic, and combined data sets, respectively. The ED50s were estimated at 0.004 « 0.0006, 0.008 « 0.0012, and 0.006 « 0.0007 ng TCDD/g tissue for the acute, subchronic, and combined data sets, respectively.

When body burden was used as dose metric, the Hill model was fit to the EROD data and Hill shape parameters were very similar (~1-1.2) for acute, subchronic, and combined data sets. The ED50s were also similar (~530-540 ng TCDD/kg body wt) for acute, subchronic, and combined data sets. Again, using body burden as dose metric, the Hill model was fit to the ACOH data, estimates of the Hill coefficient were 0.52 « 0.04, 0.78 « 0.09, 0.67 « 0.05 for acute, subchronic, and combined data sets, respectively. The ED50s were estimated at 202 « 25, 471 « 63, and 312 « 35 ng TCDD/g tissue for acute, subchronic, and combined data sets, respectively.

Discussion

The Hill shape parameter indicates that when tissue dose is used as dose metric, dose response curves are supralinear. The Hill shape parameter >1.5 suggests a non-linear or threshold-like relationship while shape parameter <1.5 suggests a low-dose linear relationship. For EROD activity, Hill shape parameters were consistent with linear dose response relationships except for the subchronic dose-response data which resulted in shape parameter estimates of 2 (data not shown), which suggests non-linearities. Using either TCDD tissue concentration or body burden as the dose metric resulted in similar dose response relationships and similar estimates of the ED50 when acute or subchronic EROD and ACOH data were analyzed separately or combined. While estimates of ED50s were similar for EROD data, the ED50s were slightly higher (2-3 fold) for ACOH following subchronic exposure compared to acute exposure. Despite different exposure regimens (acute or subchronic), tissue CYP1A activities reflected tissue TCDD concentrations and body burdens. These studies suggest tissue concentrations and body burdens are useful dose metrics for describing dose response relationships for reversible biochemical responses to dioxin and related compounds.

Implications

Use of body burden as a dose metric will decrease the uncertainty in risk assessment for dioxin and related compounds and allow for better estimates of potential adverse human health effects. In addition, it is clearly better than using daily dose. Furthermore, use of body burden allows for interspecies comparison—with no need of safety factors for pharmacokinetics.

References

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