Physiological Modeling of Effects of TCDD on Thyroid Hormones in the Rat

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The extreme potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as a liver carcinogen in female rats has been used to estimate human cancer risks from exposure to this chemical.[1] However, the incidence of thyroid tumors in male rats and female mice[1] may be an even more sensitive end point. Quantitative relationships for biochemical responses to TCDD exposure that correlate with that end point would permit extrapolation of the incidence of thyroid tumors at doses used in laboratory experiments to the incidence predicted to result from environmental exposure.

Administration of TCDD to rats leads to decreased serum thyroxine (T_4) concentrations[2,3] and increased serum concentrations of 3,5,3′-triiodothyronine (T_3)[2,4] and thyrotropin (thyroid stimulating hormone, TSH).[2] Continuous elevation of serum TSH levels results in hyperplasia and increased thyroid weight.[5] Prolonged stimulation by TSH may ultimately lead to thyroid neoplasia.[6]

Thyroxine is metabolized by UDP-glucuronosyltransferase (UGT) isoform 1 to a glucuronide which is secreted in the bile.[2] TCDD increases the hepatic activity of this enzyme[2,4] by an aryl hydrocarbon (Ah) receptor-dependent mechanism.[7] As serum T_4 inhibits secretion of TSH from the pituitary, induction of UGT might be responsible for the decrease in serum T_4 and the increase in serum TSH postulated to lead to thyroid hyperplasia and cancer risk consequent to TCDD treatment. In order to determine if this mechanism is compatible with current knowledge about the regulation of these hormones, a physiologically based model of the above processes was constructed and its predictions compared with experimental data.

1. Methods

Female Sprague–Dawley rats were given biweekly oral doses of 0.35–125 ng TCDD/kg body weight/day in corn oil for 31 weeks.[8] After this time, the rats were killed and the blood levels of T_4, T_3, and TSH were measured. *UGT1* mRNA was quantitated by reverse transcriptase–PCR. Thyroid morphology was quantified by image analysis of photomicrographs.

The physiological dosimetric model of Kohn *et al.*[9] for the distribution of TCDD in the rat and its effects on hepatic enzymes and receptor proteins included compartments for blood, fat, liver, other rapidly perfused tissues, and slowly perfused tissues. This model was extended by inclusion of compartments for the GI tract, kidney, pituitary, and thyroid. Brown and white fat were treated as separate compartments. Blood was distributed among an arterial plus venous compartment and compartments for capillary blood in the various tissues. Hepatic metabolism of TCDD was modeled with Hill kinetics, and the parameter values were estimated by formal optimization to reproduce the time courses of TCDD in the liver and fat following a subcutaneous injection.[10]

TSH-stimulated release of T_4 from the thyroid was modeled with hyperbolic kinetics. TSH release from the pituitary was modeled as stimulated by the hypothalamic peptide thyrotropin releasing hormone (TRH) and inhibited by the hypothalamic peptide somatostatin (SS).[11] Secretion of TRH was assumed to be inhibited by blood T_4, and secretion of SS was assumed to be stimulated by blood T_4. Kinetics for receptor binding and deiodination of thyroid hormones were obtained from the literature.

The original model included binding of TCDD to the Ah receptor, and this liganded receptor was treated as stimulating transcription of the *UGT1* gene with Michaelis–Menten kinetics. Parameters were estimated by formal optimization to fit the induction data of Vanden Heuvel *et al.*[12] Hyperbolic kinetics were also used for synthesis of UGT protein on the mRNA template. Where possible, constants in the model were obtained from data in the literature. Nine constants were adjusted to reproduce the distribution and enzyme induction data of Tritscher *et al.*[8]; eight constants were adjusted to reproduce the dose–responses of thyroid hormones and TSH reported here.

2. Results and Discussion

The model's computed dose–responses of T_4, T_3, and TSH are given in Table 1 and reproduce the observed concentrations very well. The computed decrease in blood T_4 was dependent on induction of UGT. When the amount of this enzyme was not allowed to

Table 1: Blood hormone concentrations after 31 weeks of biweekly oral dosing with TCDD[a]

Dose, ng/kg/day	T_4, nM	T_3, nM	TSH, pM
0.0	28.6 (26–32)	0.753 (0.61–0.85)	77.3 (36.1–162)
0.35	28.3	0.752	78.2 (23.2–93.2)
1.0	27.9	0.750	79.0 (22.5–77.5)
3.5	26.6 (23–35)	0.744	80.3 (32.1–117)
10.7	24.4 (18–32)	0.737	82.9
35.7	20.4 (12–21)	0.751	88.2
125.0	15.3 (12.5–20)	0.908 (0.55–1.10)	97.9 (31.8–189)

a. Range of observed values in parentheses.

increase, the calculated blood T_4 concentration remained high over all dose groups. The computed increase in T_3 at higher doses of TCDD was dependent on the specification of the deiodinase kinetics in the model. For example, when competition by T_4 for that enzyme's binding site was neglected, the model predicted a decrease in blood T_3 at the highest dose of TCDD compared to controls.

The model was used to simulate several other experiments, and the predicted induction of

UGT was compared with that observed (Table 2). The model reproduces the observed induction at TCDD doses in the range that was used to construct this model (< 2 μg/kg), but there is a tendency to underpredict the induction at higher doses. Owing to the lack of data, many processes in this model (e.g., production of binding proteins) were represented by greatly simplified empirical equations whose constants were adjusted to yield a good fit to the hormone dose–responses. These parameter values may not be applicable outside the range of doses used to estimate these quantities, and this may be the origin of the poorer fits to UGT induction data for high doses.

Table 2: Computed induction of UGT following oral doses of TCDD

Dose, μg/kg	Latency[a]	UGT, nmole/g	Calculated fold induction	Observed fold induction
1.0	1	0.411	1.87	1.53[13]
0.2	3	0.698	1.81	1.38–2.57[14]
1.0	3	0.981	2.54	1.67–5.06[14]
5.0	3	1.110	2.88	3.85–4.87[14]
0.05	6	0.541	1.24	1.02[15]
0.25	6	0.855	1.97	1.96[15]
1.0	6	1.226	2.82	3.10[15]
5.0	6	1.422	3.27	5.88[15]
10.0	10	1.496	3.39	3.71[16]

a.Days between dosing and sacrifice

The ability of the model to reproduce the data is consistent with the hypothesis that TCDD increases serum TSH by reducing T_4 inhibition of TSH secretion consequent to induction of UGT. Furthermore, in this study it was found that TCDD induced morphological alterations of the thyroid characterized by increased hyperplasia, increased follicular cell size, and decreased colloidal follicle size. These changes are consistent with prolonged stimulation of the thyroid by TSH and may represent an early stage in the induction of thyroid tumors identified in previous two-year bioassays. Thus, increases in UGT activity could be used as a biomarker for TCDD exposure that may predict thyroid tumor incidence at low exposures.

3. References

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