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2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN AS AN ANTIESTROGEN: EFFECT ON RAT UTERINE PEROXIDASE ACTIVITY

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Abstract—Treatment of 25-day-old female Sprague–Dawley rats with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) significantly lowered constitutive uterine peroxidase activity and decreased uterine wet weights in a dose–response fashion. In cotreatment studies with 17β-estradiol, 2,3,7,8-TCDD antagonized the increase in uterine peroxidase activity and uterine wet weights, and these effects persisted for up to 156 hr. In the rat uterus, the antiestrogenic affects of two potent *Ah* receptor agonists, 2,3,7,8-TCDD and 2,3,4,7,8-pentachlorodibenzofuran, were comparable at a dose of 80 μg/kg, whereas the weaker *Ah* receptor agonist, 1,2,4,7,8-pentachlorodibenzo-*p*-dioxin, was relatively inactive at this dose. These results show that 2,3,7,8-TCDD antagonizes a well-characterized estrogen-induced response (uterine peroxidase activity), and the structure–activity data suggest that the *Ah* receptor is involved in mediating the antiestrogenic responses in target cells/organs.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) has been used extensively as a prototype for investigating the mechanism of action of the toxic halogenated aryl hydrocarbons which include the polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls [1–4]. 2,3,7,8-TCDD elicits a broad spectrum of species, age, strain and tissue-specific responses, and the results of most studies support a mechanism which involves the initial binding of the toxin to an intracellular protein designated the 2,3,7,8-TCDD or *a*ryl *h*ydrocarbon (*Ah*) receptor. The molecular biology of one 2,3,7,8-TCDD-mediated response, namely the induction of cytochrome P-4501A1 gene expression [3–5], has been investigated thoroughly, and the data indicate that the induction responses are dependent, in part, on the interaction of nuclear *Ah* receptor complexes with specific genomic regulatory elements.

Kociba and coworkers [6] reported that long-term feeding of 2,3,7,8-TCDD to female Sprague–Dawley rats results in a significant increase in hepatocellular carcinomas. However, it was also noted that the levels of mammary and uterine tumors are lower in the 2,3,7,8-TCDD-treated rats than in the control animals. Since the growth of many mammary and uterine tumors is estrogen-dependent, these data suggest that 2,3,7,8-TCDD can act as an antiestrogen. Several reports have confirmed the activity of 2,3,7,8-TCDD as an antiestrogen. For example, 2,3,7,8-TCDD antagonizes estradiol-mediated increases in uterine wet weight and uterine estrogen and progesterone receptor levels [7, 8]. 2,3,7,8-TCDD also inhibits the estradiol-induced excretion of tissue plasminogen activator activity in MCF-7 cells [9] and antagonizes the 17β-estradiol-induced increases in uterine wet weights in mice [10].

Several studies have characterized uterine peroxidase activity as an estrogen-regulated uterine response [11–17], and this papef reports the effects of 2,3,7,8-TCDD and related compounds on this enzyme in the female Sprague–Dawley rat uterus.

MATERIALS AND METHODS

Chemicals and biochemicals. 2,3,7,8-TCDD, 1,2,4,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD), and 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) were synthesized in our laboratory to greater than 98% purity as determined by gas–liquid chromatography. Estradiol, hydrogen peroxide, and guaiacol were obtained from the Sigma Chemical Co. (St Louis, MO).

Animal treatment. Immature, 21-day-old female Sprague–Dawley rats were purchased from Harlan Laboratories (Houston, TX), and housed in a controlled environment with a 12-hr light/dark cycle. Animals were 25 days old when treated and at least four rats were used in each treatment group. Animals in the 2,3,7,8-TCDD dose–response study were injected i.p. with 5, 20, 40 or 80 μg/kg (administered in corn oil at 10 mL/kg) and terminated 48 hr later. Animals in the time–course study were treated with either estradiol (5 μg/rat) alone or with TCDD (80 μg/kg). All animals received subsequent injections of estradiol every 24 hr thereafter until termination. Animals in the structure-activity study were injected i.p. with either 80 μg/kg 2,3,7,8-TCDD, 1,2,4,7,8-PeCDD or 2,3,4,7,8-PeCDF alone, or coadministered with estradiol (5 μg/rat, s.i.d. $\times$2), and terminated 48 hr later.

Uterine wet weight and peroxidase activities. At the appropriate time, animals were killed by cervical dislocation, and their uteri were dissected free of fat and connective tissue, excised, nicked, blotted on

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Table 1. Dose-response effects of 2,3,7,8-TCDD on uterine peroxidase activity and uterine wet weights

Treatment (dose)	Uterine peroxidase enzyme (units/g/tissue)	Uterine wet weight (% of body weight)
Corn oil	16.4 ± 3.14	0.21 ± 0.02
2,3,7,8-TCDD (5 μg/kg)	17.4 ± 4.63	0.25 ± 0.04
2,3,7,8-TCDD (20 μg/kg)	12.2 ± 2.31*	0.22 ± 0.03
2,3,7,8-TCDD (40 μg/kg)	10.1 ± 2.36*	0.21 ± 0.03
2,3,7,8-TCDD (80 μg/kg)	3.14 ± 1.44*	0.11 ± 0.01*

Values are means ± SD with at least four animals per treatment group.
* Significantly lower than control ($P < 0.05$).

filter paper, and weighed. Uterine wet weights are herein expressed as percent total body weight. Uteri were homogenized in 12 mL polycarbonate tubes using a Brinkmann homogenizer at setting 6 for 15 sec. Tissues were homogenized in 10 mM Tris–HCl, pH 7.2, buffer and centrifuged for 45 min at 39,000 *g* at 2°. The resultant pellet was washed and resuspended in 10 mM Tris–HCl buffer containing 0.5 M $CaCl_2$ using a Wheaton homogenizer. The extract was collected by centrifugation of the sample for 45 min at 39,000 *g* at 20°. The final pellet was washed and resuspended in Tris–HCl + $CaCl_2$ (50 mg tissue/mL). Uterine peroxidase activity was determined as described by Lyttle and DeSombre [11, 12]. Briefly, the assay mixture (3.0 mL total volume) contained 13 mM guaiacol and 0.3 mM H_2O_2 in the extraction buffer. The reaction was started by the addition of 1.0 mL of the uterus extract. The initial rate (60 sec) of guaiacol oxidation was monitored on a Beckman spectrophotometer using a time drive program at 470 nm. An enzyme unit was defined as the amount of enzyme required to produce an increase of 1 absorbance unit/min under the assay conditions described. Enzyme activity is expressed per gram tissue. The data presented in the tables and figures are means ± SD with at least four animals per treatment group. Significant differences were determined by ANOVA.

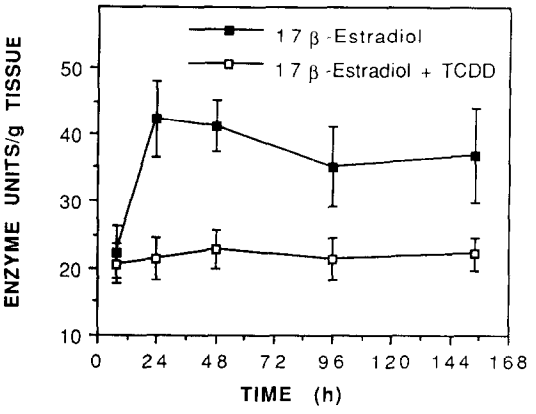

Fig. 1. Time–course effects of 17β-estradiol and 2,3,7,8-TCDD plus 17β-estradiol on uterine peroxidase activity in the 25-day-old female Sprague–Dawley rat. The treatment protocols are outlined in Materials and Methods and at least four animals were utilized for each data point. The results are expressed as means ± SD and there were significant differences ($P < 0.05$) between the two treatment groups at all time points between 24 and 156 hr inclusive.

RESULTS

The results in Table 1 show the dose-response effects of 2,3,7,8-TCDD on constitutive uterine peroxidase activity and uterine wet weights. Figures 1 and 2 summarize the effects of 2,3,7,8-TCDD on 17β-estradiol-induced uterine peroxidase activity and uterine wet weight increase over a period of 156 hr after the first dose of 17β-estradiol. The peak of uterine peroxidase activity was observed after 24 hr and the enzyme activity remained significantly elevated over the entire experiment. In the 17β-estradiol plus 2,3,7,8-TCDD treated animals, significant antagonism of the induction of uterine peroxidase by 17β-estradiol was observed after 24 hr, and the enzyme activity was decreased for up to 156 hr. A comparison of the differences in uterine wet weights in the 17β-estradiol and 17β-estradiol plus 2,3,7,8-TCDD treated animals showed that the weights of the latter group of animals were significantly lower than those observed in the former group for the duration of the experiment. The effects of structure on the activity of selected halogenated aryl hydrocarbons as antiestrogens in the female rat uterus are summarized in Table 2. At a dose of 80 μg/kg, both 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF decreased constitutive uterine peroxidase and uterine wet weights within 48 hr after treatment. In contrast, 1,2,4,7,8-PeCDD treatment did not alter significantly these uterine parameters compared to the control (corn oil-treated) animals. Cotreatment of the rats with 17β-estradiol and either 2,3,7,8-TCDD or 2,3,4,7,8-PeCDF resulted in inhibition of the 17β-estradiol-induced uterine wet weight increase and uterine peroxidase activity whereas, at

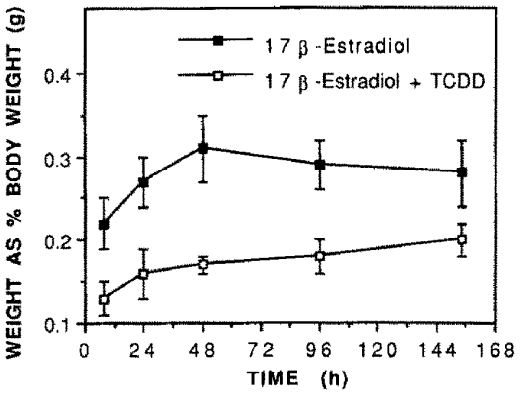

Fig. 2. Time–course effects of 17β-estradiol and 2,3,7,8-TCDD plus 17β-estradiol on uterine wet weights in the 25-day-old female Sprague–Dawley rat. The data were obtained from the same animals used to obtain the results summarized in Fig. 1. There were significant differences ($P < 0.05$) between the two treatment groups at all time points between 8 and 156 hr inclusive.

induce significantly uterine peroxidase activity in female Sprague–Dawley rats; however, cotreatment of rats with 17β-estradiol plus progesterone or related progestins (e.g. R5020 or norethindrone) markedly inhibits estrogen-induced uterine peroxidase activity [17]. In contrast, the non-steroidal antiestrogen, tamoxifen, is an estrogen receptor agonist for the induction of rat uterine peroxidase activity [16]. However, tamoxifen can act as both an estrogen receptor agonist or as a partial antagonist and these actions are response, tissue and species dependent [18]. For example, like 17β-estradiol, administration of tamoxifen also increases rat uterine wet weights, whereas progesterone treatment only slightly elevates this response. Moreover, progesterone also inhibits the tamoxifen-induced increase in uterine wet weight in female rats [16].

Previous reports have characterized 2,3,7,8-TCDD as an antiestrogen [7–10], and the effects of progesterone and 2,3,7,8-TCDD on estrogen-induced progesterone and estrogen receptor levels in rat uteri and uterine strips (*in vitro*) are compar-

Table 2. Effects of 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF and 1,2,4,7,8-PeCDD on 17β-estradiol-induced uterine peroxidase activity and uterine wet weights in female Sprague–Dawley rats

Treatment (dose)	Uterine peroxidase enzyme (units/g tissue)	Uterine wet weight (% of body weight)
Corn oil	17.1 ± 3.33	0.25 ± 0.02
17β-Estradiol (2 × 5 μg/rat)	35.3 ± 4.31	0.31 ± 0.04
2,3,7,8-TCDD (80 μg/kg)	2.18 ± 0.71*	0.11 ± 0.01*
2,3,7,8-TCDD + 17β-estradiol	22.9 ± 3.86†	0.24 ± 0.04†
2,3,4,7,8-PeCDF (80 μg/kg)	4.56 ± 0.04*	0.12 ± 0.01*
2,3,4,7,8-PeCDF + 17β-estradiol	12.5 ± 1.82†	0.16 ± 0.08†
1,2,4,7,8-PeCDD (80 μg/kg)	23.8 ± 1.82	0.02 ± 0.05
1,2,4,7,8-PeCDD + 17β-estradiol	30.2 ± 2.75	0.26 ± 0.02

Values are means ± SD with at least four animals per treatment group.
* Significantly lower than control ($P < 0.01$).
† Significantly different from 17β-estradiol-treated rats ($P < 0.01$).

a dose of 80 μg/kg, 1,2,4,7,8-PeCDD was inactive as a uterine antiestrogen.

DISCUSSION

Peroxidase enzyme activity is readily induced in rat uteri by 17β-estradiol and has been characterized extensively as an estrogenic response [11–17]. The effects of antiestrogens on constitutive uterine peroxidase activity and their interaction with 17β-estradiol have been reported, and the results are highly variable and dependent on the structure and mode of action of the antiestrogen. For example, the steroidal antiestrogen, progesterone, does not

able, i.e. both compounds decrease cytosolic and nuclear estrogen and progesterone receptor levels (*in vivo*) and decrease nuclear estrogen levels (*in vitro*). The results reported in this study clearly extend the parallel activities of 2,3,7,8-TCDD and progesterone as antiestrogens. 2,3,7,8-TCDD significantly reduced constitutive uterine peroxidase activities and inhibited 17β-estradiol-induced uterine peroxidase activity for up to 156 hr. Comparable effects were observed for uterine wet weight increases in the rats treated with 2,3,7,8-TCDD and 2,3,7,8-TCDD plus 17β-estradiol. The persistence of the 2,3,7,8-TCDD-mediated antiestrogenic effects is unique for antiestrogenic compounds but not sur-

prising in light of the well-known tissue persistence of this toxin [19].

The mechanisms of action of steroidal and non-steroidal antiestrogens are complex and have not been unambiguously delineated [18, 20]; however, their actions are initiated by binding to the progesterone (for progestins) or estrogen (for tamoxifen-like compounds) receptors. The results in Table 2 summarize the comparative antiestrogenic effects of two potent *Ah* receptor agonists, 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF and a weaker agonist, 1,2,4,7,8-PeCDD [2]. It is apparent from the results that at comparable doses, 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF exhibited potent antiestrogenic activity, whereas the 1,2,4,7,8-PeCDD congener was relatively inactive. In most studies 2,3,7,8-TCDD is more toxic than 2,3,4,7,8-PeCDF [2]; however, the results in Table 2 indicate that the latter compound was more active as an inhibitor of 17β-estradiol induction of uterine peroxidase activity. The reason for this potency difference was not investigated further. The structure–activity relationships observed in this study were similar to those previously reported for the effects of 2,3,7,8-TCDD and related compounds on estrogen receptor levels [7], and these data support the role of the *Ah* receptor in mediating the antiestrogenic effects of toxic halogenated aryl hydrocarbons. Moreover, previous studies have shown that 2,3,7,8-TCDD does not bind to the steroid hormone receptors nor do the steroid hormones bind with the *Ah* receptor [7, 8, 21].

The results from this study clearly showed that 2,3,7,8-TCDD antagonized a well-characterized estrogen-induced response (i.e. uterine peroxidase activity) and suggest that this compound can be further utilized as a probe for unraveling the complex cellular and molecular mechanisms associated with antiestrogenicity and the role of the *Ah* receptor in these processes.

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