Characterization of the Deviant Structure-Activity Relationship for Chlorinated Dibenzo-*p*-Dioxins (CDDs) in the Resistant Han/Wistar Rats

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Introduction

An animal model for mechanistic studies on dioxin toxicity has been established in our laboratory. It is based on >1000-fold sensitivity difference in acute lethality to TCDD between two rat strains (1). Long-Evans (*Turku A/B*; L-E) rats are very sensitive having a LD50 value of 10 μg/kg. Han/Wistar (*Kuopio*; H/W) rats, on the other hand, are the most TCDD resistant mammals with the LD50 of >9600 μg/kg. The AH receptor (AHR) of H/W rats has been recently shown to be structurally abnormal harboring a point mutation in an intron sequence, which results in altered C-terminal structure and altered physicochemical properties of the receptor protein (2, 3). Another interesting feature is that while L-E rats exhibit the normal rank order of sensitivity to CDDs (TCDD > PeCDD > HxCDD > HpCDD), H/W rats are exceptionally sensitive to the higher chlorinated congeners (HxCDD > HpCDD ≥ PeCDD > TCDD) in terms of acute lethality (4, 5). This study was conducted to find out if this deviant structure-activity relationship can be extended to other endpoints of CDD toxicity in H/W rats, and to compare the dose-responses for these endpoints in H/W and L-E rats.

Materials and Methods

Chemicals. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD), 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) and 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) were purchased from Ufa Oil Institute (Ufa, Russia), and they were found to be >99% pure as confirmed by GC-MS. The CDDs were dissolved in corn oil (Sigma, St. Louis, MO, USA).

Animals. Adult (13-15-week-old) female H/W and L-E rats were bred in the SPF-barrier of the National Public Health Institute (Kuopio, Finland). They were housed in stainless steel wire-bottom cages 5-6 rats per cage.

Experimental design. Rats were randomly divided into experimental groups of 5-6 animals and given a single oral dose of one of the CDDs by oral gavage (4 ml/kg). There were 7-9 dose levels of each CDD covering the whole range of responses from submaximal EROD induction to doses potentially causing lethality. Control animals received corn oil. On day 8 the rats were decapitated, trunk blood collected and serum separated. Liver and thymus were quickly removed and weighed. Liver samples were frozen in liquid nitrogen and stored at -80°C until analyses.

Biochemical analyses. Ethoxyresorufin O-deethylase (EROD) activity in liver S9 fraction was measured according to Kennedy and Jones (6) with slight modifications. Gel retardation assay was used to compare the inducibility of AHR/ARNT-DRE complexes by the different CDDs in H/W and L-E rats as described by Pohjanvirta *et al.* (2).

Results and Discussion

L-E rats were slightly more sensitive to body weight loss, and they lost more weight (maximally about 25%) than H/W rats (about 8%) during the 8-day study period (Fig. 1 top). However, body weight loss strictly followed the normal rank order of sensitivity of CDDs (TCDD > PeCDD > HxCDD > HpCDD) also in H/W rats. Body weight related thymus weights showed a comparable dose-dependent decrease in both strains indicating that H/W and L-E rats are similarly sensitive to this effect (Fig. 1 middle), as shown before (7). In addition, thymus atrophy complied the normal rank order of potency of CDDs likewise in both strains. Induction of liver EROD activity was nearly similar in H/W and L-E rats (Fig. 1 bottom). At high dose levels the activity was decreased from its maximal value more clearly in L-E than in H/W rats. Also sensitivity to EROD induction followed the normal congener potency of CDDs both in L-E and in H/W rats. Gel retardation assay showed that the ability of the four CDDs to transform AHR to the DNA-binding state did not differ in L-E and H/W rats, and that the congener potencies were similar in these strains.

These results demonstrate that the highly deviant sensitivity of H/W rats to higher chlorinated CDDs is limited to acute lethality. Other typical endpoints of toxicity closely followed the expected rank order of sensitivity to CDDs, which has been established for a variety of experimental models, and which forms the basis of the currently used toxic equivalency factor (TEF) concept (8-10). The results also indicate that TEFs derived from lethality data are not necessarily valid for sublethal endpoints of toxicity. Moreover, it is likely that endpoints with different congener sensitivities have differences in their mechanisms of toxicity.

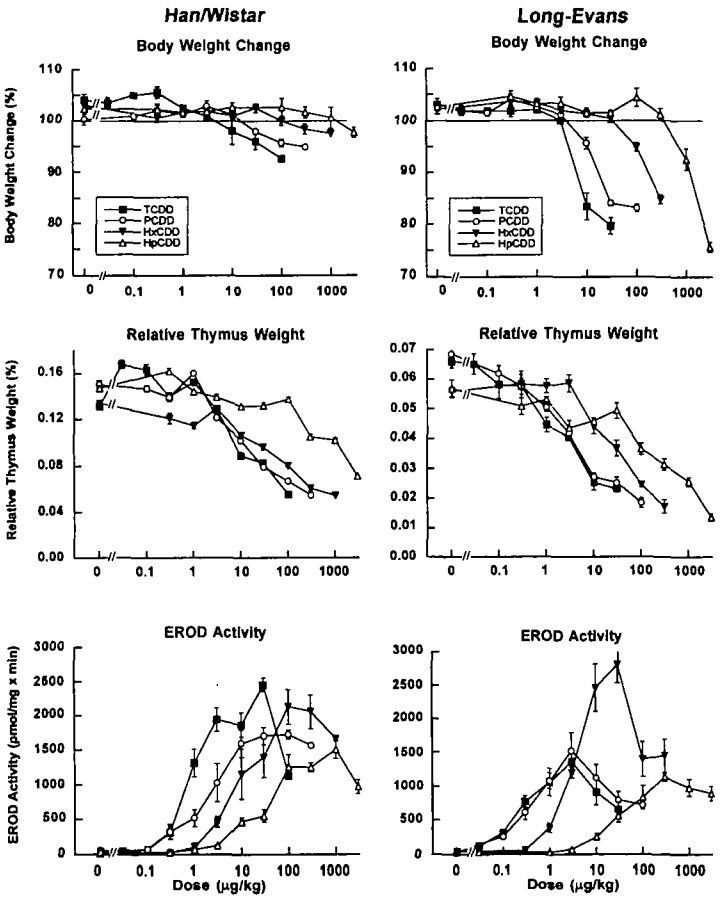

Fig. 1. Dose-responses of body weight change, relative thymus weight and liver EROD activity in Han/Wistar (left panel) and Long-Evans (right panel) rats 8 days after a single oral dose of TCDD, PeCDD, HxCDD or HpCDD. Group means ± SE, n = 4-6.

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