IMMUNOTOXICITIES OF BROMINATED DIOXINS IN MICE

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Abstract

Previous data have suggested that brominated dioxins, such as 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD), are more toxic to the immune system than their chlorinated congeners. We previously established the IL-5 production by the splenocytes from ovalbumin (OVA)-immunized mice as one of the most sensitive endpoints that reflect immnotoxicites of dioxins. In the present study we compared immunotoxic potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PeCDD), and their brominated congeners TBDD and 1,2,3,7,8-pentabromodibenzo-*p*-dioxin (PeBDD), using IL-5 production as the index. TCDD, PeCDD, and TBDD at 1.0-10 μg/kg dose-dependently inhibited IL-5 production in a similar manner and extent one another. The inhibition of IL-5 by these three compounds was greater than 90% at 10 μg/kg. On the other hand, PeBDD inhibited IL-5 production in a dose-independent manner. PeBDD at 1.0 μg/kg inhibited IL-5 production more strongly than the other 3 compounds, while 10 μg PeBDD/kg was less toxic than the others. Even at 50 μg/kg, PeBDD inhibited IL-5 production by about 70%. These results do not support the hypothesis that brominated dioxins may be more toxic to the immune system than their chlorinated congeners. They also suggest that PeBDD has a unique mode of action among the four compounds.

Introduction

Dioxins, including polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, are widespread and persistent environmental pollutants. In addition, polybrominated dibenzo-*p*-dioxins/furans have been detected in the environment and have gained considerable concern in recent years[1,2]. The brominated dioxins are shown to exert their toxic effects through the same mechanism as their chlorinated congeners, that is, through activation of a ligand-dependent transcription factor, the arylhydrocarbon receptor (AhR). However, potency of each brominated congener is largely unknown. Previous studies reported that the potency relative to TCDD (relative potency) of TBDD and PeBDD to induce arylhydrocarbon hydroxylase (AHH) activity in rat hepatoma cells *in vitro* were 14% and 10%, respectively[2,3]. On the other hand, the relative potency of TBDD and PeBDD to induce AHH activity in rat liver *in vivo* were reported to be about 1600% and 50%, respectively[2,3]. In terms of thymus atrophy, one of the well-known measures of dioxins toxicity, TBDD was reported to be three times as potent as TCDD[2,3]. These results suggest that brominated dioxins may be more toxic to the immune system than their chlorinated congeners.

As indicated in the thymus atrophy, the immune system is one of the most sensitive targets of TCDD toxicity. In our recent research studying the effects of TCDD exposure on the humoral immune reactions in mice immunized with OVA, we found that the production of Th2 type cytokines, especially IL-5, by splenocytes is greatly and sensitively suppressed by TCDD exposure[4]. The deficit in cytokine production was implicated in the immune suppression by TCDD.

In the present study, we investigated whether the immune system is a vulnerable target of brominated dioxins, by comparing the effects of TCDD, PeCDD, and their brominated congeners TBDD and PeBDD on the IL-5 production in addition to the thymus atrophy in mice.

Materials and Methods[4,5]

TCDD administration and immunization: TCDD was purchased from Cambridge Isotope Laboratory (Andover, MA). PeCDD, TBDD, and PeBDD were obtained from Wellington Laboratories Inc. (Ontario, Canada). Female C57BL/6J mice (6 weeks old, 5 mice/group) were administered a single dose of TCDD, PeCDD, TBDD, PeBDD (1.0, 3.0, or 10 μg/kg) or vehicle (corn oil containing 2% nonane and 2% toluene) by gavage. In another experiment, mice were given PeBDD (1.0, 3.0, 10, or 50 μg/kg) or vehicle. They were also subsequently immunized intraperitoneally with 100 μg of OVA using alum as an adjuvant. On day 7 after

the treatments, these animals were sacrificed by cervical dislocation under a diethyl ether anesthesia, and their thymuses and spleens were examined. These mice were handled in a humane manner following the NIES guidelines for animal experiments.

Thymocyte and splenocyte preparation: Single cell suspensions of thymuses and spleens were prepared by expressing cells in RPMI1640 medium-10 % FCS (complete medium) through a stainless steel mesh. Cell numbers were counted with a hemocytometer following staining with trypan blue. Their cellular populations were examined using a FACSCalibur (BD Biosciences, San Diego, CA).

IL-5 measurement: The splenocytes were cultured at a 1×10^6 cells/200 µl complete medium with or without OVA (100 µg/ml) for 3 days. The culture medium was then separated, and the IL-5 in the supernatant was measured by ELISA.

Statistical analysis: Significant differences among the vehicle-control group and dioxins-treated groups were analyzed by one-way ANOVA, followed by Dunnett's test, a *post hoc* comparison using StatView statistical software (version 5.0, SAS Institute, Cary, NC). A p value less than 0.05 was considered significant.

Results and Discussion

IL-5 suppression by TCDD and PeCDD: First, we examined the relative potency of PeCDD, to which TEF 1.0 is assigned, in terms of suppressive effect on IL-5 production by splenocytes. C57BL/6 mice were orally administered TCDD, PeCDD (1.0, 3.0, or 10 µg/kg) or vehicle, and were subsequently immunized with OVA/alum. Seven days later, the splenocytes were prepared from the spleens and cultured in the presence of OVA to re-stimulate the effector T cells for 3 days. The IL-5 in the supernatants was examined by ELISA. The results showed that both TCDD and PeCDD suppressed the IL-5 production in a dose-dependent manner with significant suppression at 3.0 and 10 µg/kg in two independent experiments. The two compounds showed similar extent of inhibition and inhibited the IL-5 production by more than 90% at 10 µg/kg. Thus, the TEF 1.0 of PeCDD was confirmed to be applicable for the immune suppression.

IL-5 suppression by TBDD and PeBDD: Suppressive effects of TBDD (1.0, 3.0, 10 µg/kg) on IL-5 production were examined in a similar manner as described above. TBDD also showed similar effects as TCDD and PeCDD, inhibiting IL-5 production in a dose-dependent manner with more than 90% inhibition at 10 µg/kg.

On the other hand, PeBDD (1.0, 3.0, 10 µg/kg) did not show a dose-dependent inhibition on the IL-5 production. PeBDD at 1.0 µg/kg inhibited IL-5 production by about 70% and the suppressive effect was larger than the suppression (30 - 55%) by 1.0 µg/kg of TCDD, PeCDD, or TBDD. On the other hand, PeBDD at 10 µg/kg inhibited IL-5 production by 50 - 65% and the effect was weaker than the effects of 10 µg/kg of TCDD, PeCDD, or TBDD, which caused more than 90% inhibition. We further examined the effects of PeBDD at 50 µg/kg. Even at 50 µg/kg, PeBDD inhibited IL-5 production by about 70%.

Thymus atrophy: Thymus weight and thymocyte number were reduced by exposure to TCDD, PeCDD and TBDD in a dose-dependent manner in the range of 1.0 - 10 µg/kg. All the three compounds significantly reduced thymus weight and thymocyte number at 10 µg/kg. On the other hand, PeBDD up to 10 µg/kg did not significantly suppress the weight and cell number. PeBDD at 50 µg/kg was found to significantly suppress thymus weight and cell number to levels similar to those observed by exposure to 10 µg/kg of the other compounds.

The results of the present study clarified that TCDD, PeCDD and TBDD show a very similar immunotoxic effects when evaluated with regard to IL-5 suppression and thymus atrophy. On the other hand, higher doses of PeBDD was less toxic than the other compounds. Thus, the hypothesis that brominated dioxins are more toxic than their chlorinated congeners was not supported. However, a lower dose of PeBDD suppressed IL-5 production more effectively than the other three compounds, suggesting that PeBDD acts in a way different from the other three compounds. in terms of metabolism, AhR-activation and so on. Further studies on the toxicity

of lower doses of PeBDD may be required.

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