DIFFERENCES BETWEEN GENDERS IN SUSCEPTIBILITY TO ANTIOXIDANT ENZYMES IN THE RATS FOLLOWING SINGLE EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent member of a large family of dioxin-like compounds that are ubiquitous environmental contaminants. One of the most characteristic toxic manifestations of an acute lethal dose of TCDD in mammals is the wasting syndrome[1]. Other remarkable aspects of the toxicity of TCDD are the great differences in sensitivity among species and between genders within the same species[2,3]. Most of the biological adverse effects of TCDD are thought to be mediated by the AhR.[4,5] The activation of the AhR leads to an induction of drug-metabolizing enzymes, such as CYP1A subfamily.[5] Although induction of CYP1A does not directly manifest the toxicological endpoint of TCDD, it is an extremely sensitive marker for exposure and tissue responsiveness to TCDD.[5,6] Therefore, the activity of ethoxyresorufin *O*-deethylase (EROD), CYP1A mediated monooxygenase, has been measured as a marker enzyme activity in the previous studies on dioxin-related toxicity. The present study was designed to investigate differences between genders in susceptibility to antioxidant enzymes in the rat serum after single exposure to TCDD.

Materials and Methods

Both male and female Sp.D rats were administered with a single intraperitoneal dose of 25 or 125 μg TCDD/kg body weight. These two doses of TCDD are sublethal and lethal doses in Sp.D

rat, respectively[7]. Control animals received an equal volume of corn oil. Body weight was recorded every day prior to sacrifice. At 5 days after dosing, the animals were sacrificed and sera were isolated from whole blood. Livers also rapidly removed, quickly frozen in liquid nitrogen and stored at –70 °C until further processing. The activity of EROD and induction of CYP1A1 in the microsomes of each of the liver were measured. Antioxidant enzyme activities in the serum were measured. Glutathione S-transferase (GST) was assayed using the method of Habig *et al*. by measuring the formation of GSH-CDNB conjugate using extinction coefficient 9.6 $mM^{-1}cm^{-1}$ at 340 nm[8]. Glutathione reductase (GR) was assayed in 100 mM Tris-HCl buffer, pH 8.0 by measuring the oxidation of NADPH at 340 nm with GSSG as the substrate9. Cu/Zn-SOD activity was determined according to the method of McCord and Fridovich[10].

Results and Discussion

A single injection of TCDD at 25 and 125 μg/kg body weight led to a significant wasting syndrome, indicating that TCDD had a toxic effect in both male and female Sp.D rats. Body weight gain was significantly decreased in TCDD-treated rats when compared with the corresponding group of control rats over time (Fig. 1). At 5 days after the single administration of TCDD, 25 μg/kg TCDD dosage group in female rat was less body weight gain compared to the corresponding group of male rat. At 125 μg/kg TCDD dosage group, female group had much body weight loss compared to the corresponding male group. The difference in body weight gain in control, 25 and 125 μg/kg TCDD-treated male rats was 13.8, 1.1, and –3.7% at 5 days post-injection of TCDD, respectively. The difference in body weight gain in control, 25 and 125 μg/kg TCDD-treated female rats was 7.1, 1.2, and –9.2% at 5 days post-injection of TCDD, respectively. Body weight loss in female rat exposed to 125 μg/kg TCDD was severer than in corresponding male rat. The differences in body weight gain between both these groups were statistically significant at all days measured ($p<0.05$). As markers on dioxin toxicity, we measured the activity of EROD and induction of CYP1A1/2 in the hepatic microsomes of both male and female rats exposed to 25 and 125 μg TCDD/kg b.w (Fig. 2). Results of both EROD assay and Western blot analysis show that TCDD strongly induces EROD activity and CYP1A1 in the liver of both male and female rats. Interestingly, Western blot analysis shows that CYP1A2 was specifically induced in the female rat liver, but not in the male rat liver by the exposure to the 125 μg/kg b.w/day (Fig. 2). These data suggest that the induction of CYP1A2 by acute exposure of rat to TCDD was dose-

dependent and gender-specific. The effects of TCDD on GST, Cu/Zn-SOD, and GR were measured in male and female rat serum (Fig. 3). TCDD at 25 and 125 μg/kg body weight showed 1.4 and 2.3-fold induction in the activity of GST towards 1-chloro-2,4-dinitrobenzene, phase II drug-metabolizing enzyme, in the female rat serum compared to that in the control rat, respectively. However, there was only a small increase of the GST activity in the male rat serum by dose of TCDD at 25 and 125 μg/kg body weight compared to that in the female rat serum. Responses of GR and Cu/Zn-SOD activities to TCDD also had the same trends as that of GST activity (Fig. 3). These alterations of these antioxidant enzymes in rat serum may be characteristic of gender-specific against TCDD toxicity. The current data show the differences in the susceptibility of males and females to TCDD toxicity. Our *in vivo* findings are consistent with a very recent report in which they also found that *in vitro* treatment of human liver cells with TCDD resulted in increased Cu/Zn-SOD activity[11,12]. Our data from males and females suggest that many of the gender-specific differences in the actions of TCDD can be possibly resulted from differential sensitivity of antioxidant enzymes between genders.

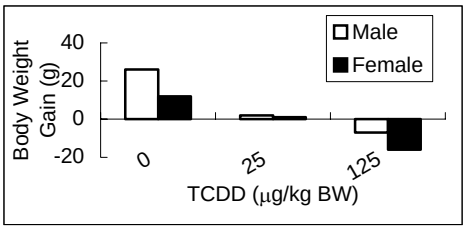

Fig.1. Effect of acute exposure to TCDD on body weight gain in the male or female Sp.D rat. Animals were treated with 25 and 125 μg TCDD/kg or corn oil for control group. Body weight gain was measured at 5 day after TCDD exposure.

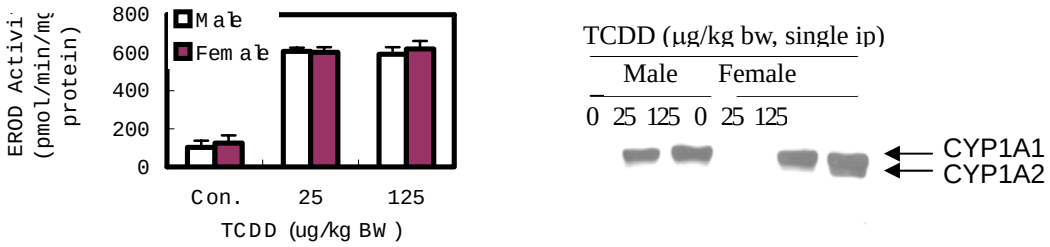

Fig. 2. (Left) EROD activity in the liver of both male and female Sp.D rat after exposure to TCDD. (Right) Western blot analysis for the measurement of CYP1A inducibility by single dose of TCDD in Sp.D rat liver microsomes.

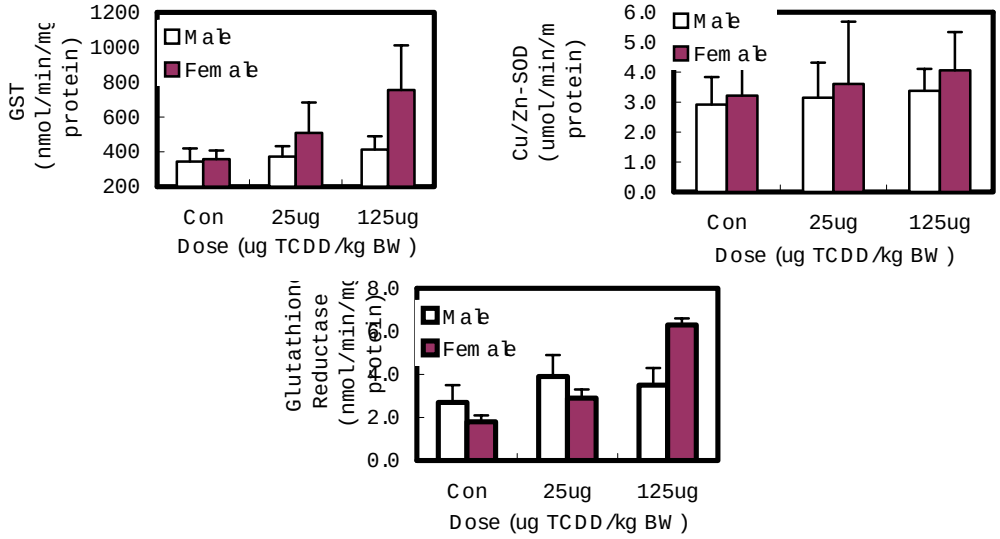

Fig. 3. GST (A), Cu/Zn-SOD (B), and GR(C) activities in the serum of both male and female Sp.D rat after exposure to TCDD.

Acknowledgments

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