Letter to the Editor

Subacute Effect of Decabromodiphenyl Ethane on Hepatotoxicity and Hepatic Enzyme Activity in Rats*

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Information regarding decabromodiphenyl ethane (DBDPE) effects on hepatotoxicity and metabolism is limited. In the present study, Wistar rats were given oral DBDPE at different doses. DBDPE induced oxidative stress, elevated blood glucose levels, increased CYP2B2 mRNA, CYP2B1/2 protein, 7-pentoxyresorufin *O*-dependentylase (PROD) activity, and induced CYP3A2 mRNA, CYP3A2 protein, and luciferin benzylether debenzylase (LBD) activity. UDPGT activity increased with its increasing exposure levels, suggesting that oral DBDPE exposure induces drug-metabolizing enzymes in rats via the CAR/PXR signaling pathway. The induction of CYPs and co-regulated enzymes of phase II biotransformation may affect the homeostasis of endogenous substrates, including thyroid hormones, which may, in turn, alter glucose metabolism.

Decabromodiphenyl ethane (DBDPE) has become an important commercial flame retardant. For a long time, DBDPE was thought to be released minimally into the environment during all phases of its use and is not available biologically due to its large molecular size and low aqueous solubility.

In 2003, DBDPE was discovered in environment and was identified in sewage sludge, sediment, indoor air, birds, captive pandas and benthic food web, demonstrating that DBDPE is leaking out of the technosphere and present in environment.

It has been shown that liver is the primary target organ of brominated flame retardants (BFRs) which also induce hepatic detoxification enzymes, including phase I CYP450 and phase II conjugation enzymes (e.g., UDPGT), suggesting that these enzymes can also metabolize BFRs. Some BFRs are suspected to act as endocrine disruptors and/or affect fetal development. In particular, the induction of drug-metabolizing enzymes via the aryl hydrocarbon receptor (AhR), pregnane X receptor (PXR), or constitutive androstane receptor (CAR) can interfere with the homeostasis of thyroid hormones and steroids via interactions that initiate a cellular response[1]. It is believed that oral exposure to hexabromocyclododecane (HBCD) induces drug-metabolizing enzymes in rats via the CAR/PXR signaling pathway[1]. It was reported that BDE-71 and BDE-79 induce hepatic enzyme activities of ethoxyresorufin-O-deethylase (EROD), 7-pentoxy-resorufin *O*-dependentylase (PROD), and UDPGT[2]. Despite this fact, databases on the toxicity of DBDPE in animals are lacking.

In the present study, Wistar rats administrated oral DBDPE for 28 days. To investigate the hepatotoxicity of DBDPE, 14 clinical chemistry parameters associated with hepatotoxicity were assayed. To characterize the hepatic metabolism of DBDPE, expression of mRNA and protein in CYP enzymes was detected. UDPGT activity was assayed by spectrofluorometry. Catalytic activities of PROD in isolated microsomes were measured with (P450-PROD(CYP2B2) assay kit (Genmed Scientifics Inc., USA) after adjustment for total protein, according to its manufacturer's instructions. Benzylether debenzylase (LBD) activity was analyzed using the P450-Glo™ CYP3A4 assay kit (Promega, Germany) according to its manufacturer's instructions.

In this study, approximately 7-weeks old rats were randomly divided into control group and 5 DBDPE treatment groups (4 male rats and 4 female rats in each group). Rats in control group administered corn oil only, and those in DBDPE treatment groups administered DBDPE at the dose of 50, 100, 250, 500, and 1 000 mg/kg·d as previously described[3-4]. The rats administered oral DBDPE daily for 28 consecutive days. Animals were weighed weekly and their signs of toxicity were observed daily. Blood sample was taken for

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laboratory test. Liver was harvested, washed in ice-cold physiological saline, and weighed. Portions of the livers were collected, immediately frozen in liquid nitrogen, and then stored at -80 °C until further processing.

Clinical chemistry parameters were determined on an auto-analyzer using standard kits. Expression of mRNA and protein in CYP enzymes was detected by RT-PCR and Western blot, respectively. Hepatic enzyme activities, including UDPGT, PROD, and LBD, were also assayed.

Means between DBDPE treatment groups and control group were compared by one-way ANOVA. RT-PCR data were analyzed using iQ5 software (Bio-Rad). Ct data were normalized to the internal control gene. GAPDH and the fold change in target gene mRNA abundance between DBDPE treatment groups and control group was calculated using $2^{-\triangle\triangle Ct}$. The fold change data were normally distributed, and passed the equal variance test for all assays. $P<0.05$ was considered statistically significant.

No significant changes were observed in liver and relative liver weights following exposure to DBDPE, indicating that DBDPE at the dose of 50-1 000 mg/kg·d does not result in overt toxicity. The Glu, TBA, ALT, and AST levels following exposure to various doses of DBDPE were markedly higher in DBDPE treatment groups than in control group (Table 1, $P<0.05$), indicating that oxidative stress increases due to the accumulation of DBDPE and/or its metabolites[4]. The Glu level was higher after exposure to DBDPE than before exposure to DBDPE. Although the biological mechanism underlying this association between DBDPE and diabetes is still unclear, the potential of xenobiotics in disrupting glucose metabolism in mammals has been well documented in the field of toxicology. It was reported that pollutants lead to glucosuria and gluconeogenesis[5]. No significant difference was found in other parameters between DBDPE treatment groups and control group in this study.

The expression level of mRNA and protein in CYP2B2 was significantly higher in DBDPE treatment groups than in control group following exposure to DBDPE at the doses of 100, 250, 500, and 1 000 mg/kg·d (Figure 1A, Figure 1B). The hepatic microsomal activities of UDPGT increased with the DBDPE exposure in a dose-dependent manner (Figure 2A). DBDPE resulted in a significant induction of PROD activity at the doses of 250 and 500 mg/kg·d (Figure 2B). CYP-catalyzed LBD activities in microsomes increased with the DBDPE exposure (Figure 2C).

The effects of BFRs in the liver were associated with the induction of hepatic cytochrome P450 drug-metabolizing enzymes, especially CYP1A, CYP2B, and CYP3A. The hallmark of PXR activation is the induction of CYP3A[6]. Other signaling pathways that regulate the CYP level include the CAR-mediated induction of CYP2B, and AhR-mediated induction of CYP 1A[1]. The present study showed that DBDPE, at relatively high doses, could induce expression of mRNA and protein but not CYP1A1/2 mRNA and protein in CYP2B and CYP3A2, suggesting that DBDPE is an activator for xenobiotic nuclear receptors, namely PXR and CAR. CAR is a member of the orphan receptor subfamily, and functions as a sensor for endobiotic and xenobiotic substances with PXR. The presence of these substances results in a CAR-mediated upregulation of enzymes responsible for their metabolism and excretion. PXR and CAR are activated by xenobiotics, and their activation results in their regulation of phase I through III enzymes involved in the detoxification and elimination of steroids, bile acids, and xenobiotics[7]. In addition to phase I CYPs, other detoxification genes are also induced, such as UDPGT[7]. The CYPs and UDPGT were upregulated following DBDPE exposure in this study. Xenobiotics affects circulation levels of thyroid hormones by inducing UDPGT, which conjugates thyroxin (T4), and thereby increases its renal

Table 1. Effects of DBDPE on Serum Glu (A), TBA(B), ALT(C), and AST(D) Levels in Different Groups

Group	Control Group	50 mg/kg·d	100 mg/kg·d	250 mg/kg·d	500 mg/kg·d	1000 mg/kg·d
Glu (mmol/L)	5.65±0.28	6.06±1.30	6.03±1.16	7.96±1.53*	8.71±1.14*	9.40±2.30*
TBA (μmol/L)	134.63±32.20	157.87±37.51	161.53±17.22	207.73±23.55*	247.84±55.47*	267.44±44.81*
ALT (U/L)	41.02±8.64	63.25±11.04*	64.13±12.17*	64.37±15.51*	67.38±18.36*	73.03±19.98*
AST (U/L)	119.28±18.11	130.05±26.58	134.28±11.72	159.15±40.19*	174.01±47.74*	187.62±35.55*

Note. Data are expressed as mean±SD, statistically significant differences were determined by one-way ANOVA ($n=8$), $^*P<0.05$ *vs.* control group.

excretion[8]. It has been shown that there is a 'cross-talk' between CAR and PXR, which results in overlapping functions[7]. A PXR-mediated induction of UDPGT, coupled with the biliary conjugate export pump Mrp2 in rats, can lead to a massive decrease in circulating thyroxin levels[9]. A similar mechanism has been suggested for the CAR-regulated expression of conjugating enzymes[9]. It was reported that thyroid function is related with cardiovascular risk factors, such as blood glucose[10]. Thus, we believe that DBDPE is able to obstruct thyroid function via CAR

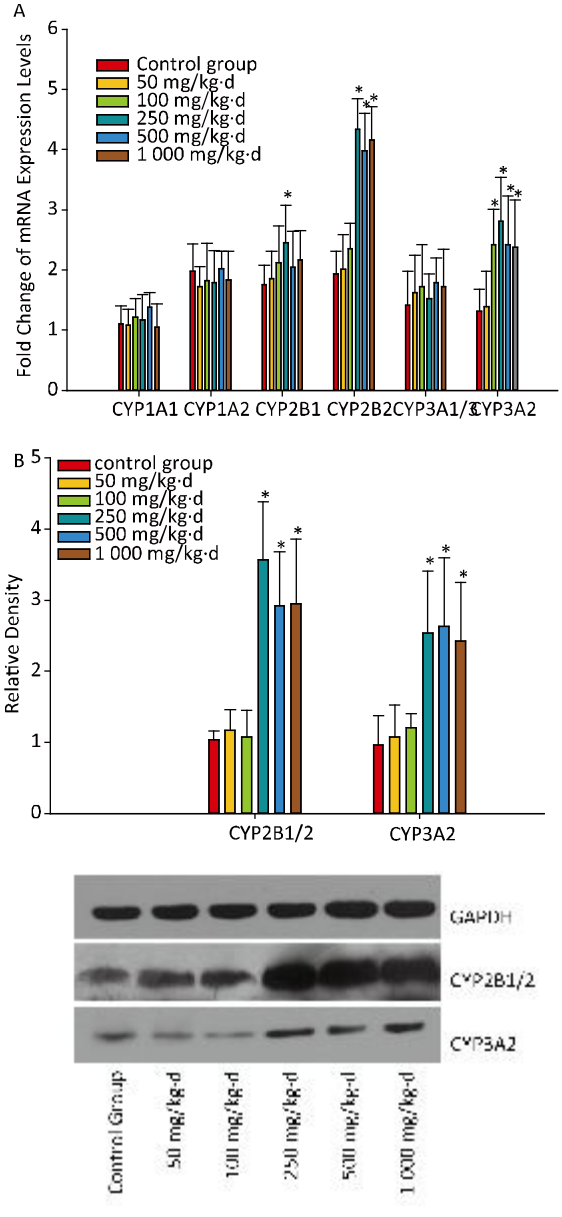

Figure 1. Dose-dependent effects of oral DBDPE over 28 days on expression level of CYP2B2 mRNA (A) and protein (B) in rats. Data are expressed as mean±SD, statistically significant differences were determined by one-way ANOVA (n=8), $^{*}P$<0.05 *vs.* control group.

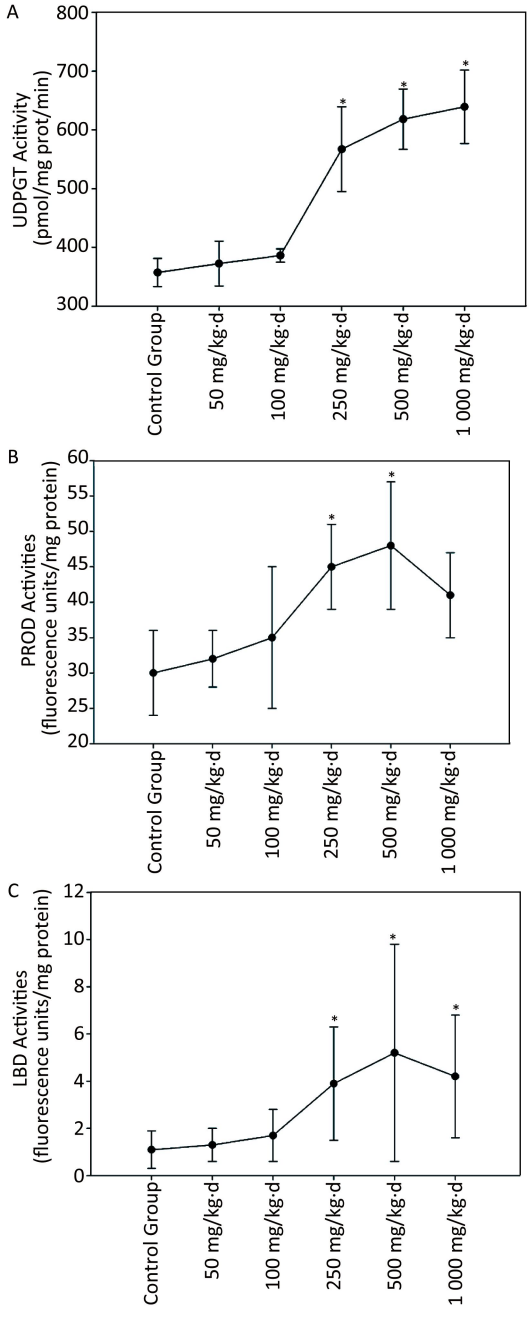

Figure 2. Dose-dependent effects of oral DBDPE over 28 days on hepatic UDPGT activities (A), CYP-catalyzed PROD activities (B), and LBD activities (C). Data are expressed as mean±SD, statistically significant differences were determined by one-way ANOVA (n=8), $^{*}P$<0.05 *vs.* control group.

and PXR, and thereby results in adverse health effects in both animals and humans (e.g., altering blood glucose levels).

In conclusion, DBDPE at relatively high doses, can damage rat liver. Furthermore, DBDPE may increase blood glucose levels in rats. The CAR/PXR signaling pathway is associated with the induction of drug-metabolizing enzymes, including CYPs and UDPGT, which further lead to adverse health effects. Further studies are warranted to characterize the entire metabolic pathway, including the signaling pathways, involved in DBDPE-induced increases in blood glucose levels.

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