Induction of hepatic microsomal UDP-glucuronosyltransferase by methylsulfonyl metabolites of polychlorinated biphenyl congeners in rats

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Introduction

In the previous papers [1-3], we reported that nine 3-methylsulfonyl (3-$MeSO_2$) metabolites of polychlorinated biphenyls (PCBs) were potent inducers of hepatic microsomal drug-metabolizing enzymes, CYP2B1/2 at levels several hundred fold lower than required for equivalent induction by parent PCBs, while their isomeric 4-$MeSO_2$ metabolites were not. Additionally we showed that the 3- and 4-$MeSO_2$ metabolites of tetra-, penta- and hexaCBs reduced serum thyroxine (T_4) level and increased serum thyroid stimulating hormone (TSH) level in rats [4, 5].

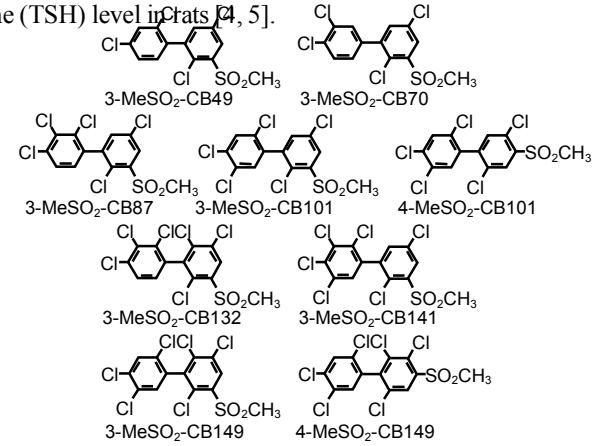


Fig. 1. Chemical structures of methyl sulfone derivatives of PCB congeners

A number of compounds known to induce microsomal drug-metabolizing enzymes in rat liver have been demonstrated to induce also UDP-glucuronosyltransferase (UDP-GT), an enzyme typical of those catalyzing phase II reactions [6, 7]. In the present study, therefore, we have investigated the effects of 3- and 4-$MeSO_2$ metabolites of nine PCB congeners on the UDP-GT activity and the relationship between the alterations of thyroid hormone levels and the induction of UDP-GT. These metabolites are major $MeSO_2$ metabolites accumulated in human liver and adipose tissue and the tissues of several mammalian species [8-10]. Fig. 1 shows the chemical structures of $MeSO_2$ derivatives of PCB congeners used in this study.

Materials and Methods

Chemicals. The $MeSO_2$-PCBs were prepared as described elsewhere [11]. The purity of these compounds was >99% when analyzed by gas chromatography. All other chemicals were obtained commercially in appropriate grades of purity.

Animal treatments. Male Sprague-Dawley rats, weighing 180-200 g (Charles River Japan Inc.), were housed three or four per cage in the laboratory with free access to commercial chow and tap water, and maintained on a 12-hr dark/light cycle (8:00 a.m.-8:00 p.m. light) in a room with controlled temperature (24.5 ± 1℃) and humidity (55 ± 5%). Rats received four consecutive intraperitoneal injections of 20 μmol/kg $MeSO_2$-tetra-, penta- and hexaCBs dissolved in Panacete 810 (5 ml/kg). Control animals received an equivalent volume of corresponding vehicle. The rats were killed seven days after final administration.

Microsomal preparation and enzyme assays. Microsomes were prepared according to the procedure described previously [1]. The protein content was determined by the method of Lowry *et al*. [12] with bovine serum albumin as a standard. Cytochrome P450 content was estimated according to the method of Omura and Sato [13]. 7-Ethoxyresorufin *O*-dealkylase and 7-pentoxyresorufin *O*-dealkylase activities in microsomes were determined by the method of Burke *et al*. [14]. UDP-GT activities toward 4-nitrophenol and 4-methylumbelliferone were assayed spectrophotometrically [15] and fluorometrically [16], respectively. UDP-GT activities toward chloramphenicol and T_4 were as

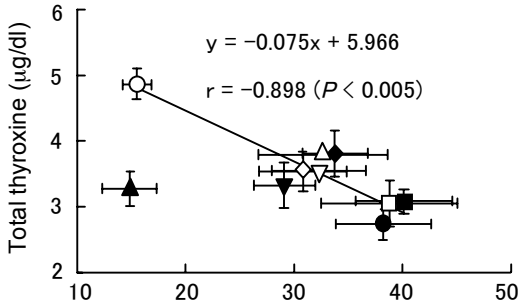
described by Ishii *et al*. [17] and Barter and Klaassen [18], respectively.

Results and Discussion

All seven 3-$MeSO_2$-PCBs significantly increased the cytochrome P450 content and the activity of 7-pentoxyresorufin *O*-dealkylase (CYP2B1/2). 3-$MeSO_2$-CB49, 3-$MeSO_2$-CB87, 3-$MeSO_2$-CB101 and 3-$MeSO_2$-CB132 also increased the activity of 7-ethoxyresorufin *O*-dealkylase (CYP1A1/2). On the other hand, two 4-$MeSO_2$ derivatives showed no significant effect on cytochrome P450 content and the activities of the enzymes.

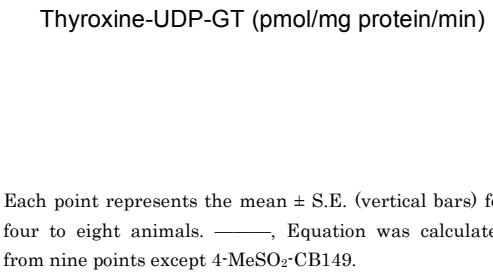
UDP-GT activity toward chloramphenicol (UGT2B1) was increased by all $MeSO_2$-PCBs. The extent of induction of UDP-GT activities by all seven 3-$MeSO_2$-PCBs was dramatically high. UDP-GT activities toward 4-nitrophenol (UGT1A1) and 4-methylumbelliferone (UGT1A1) were increased upon treatments with all seven 3-$MeSO_2$-PCBs and 4-$MeSO_2$-CB101. The UDP-GT activities were not significantly altered with 4-$MeSO_2$-CB149 treatment. UDP-GT activity toward T_4 was significantly increased by treatments with all seven 3-$MeSO_2$-PCBs and 4-$MeSO_2$-CB101 (87-157% increases). Namely, seven 3-$MeSO_2$-PCBs and 4-$MeSO_2$-CB101 induce both UGT2B1 and UGT1A1, and 4-$MeSO_2$-CB149 induces UGT 2B1.

The relationship between thyroid hormone concentrations and both phase I and phase II enzyme activities was studied. Serum TSH concentrations on day 3 correlated significantly with both 7-pentoxyresorufin *O*-dealkylase activity (r=0.796, $p<0.01$) and UDP-GT activity toward chloramphenicol (r=0.833, $p<0.005$) on day 7 after the administration of all nine $MeSO_2$ derivatives. Therefore, the induction of CYP2B1/2 and UGT2B1 may be responsible for increase in serum TSH level. A significant correlation existed between serum total T_4 concentration on day 2 and UDP-GT activity toward 4-nitrophenol (r=-0.831, $p<0.01$) and between serum total T_4 concentration on day 2 and UDP-GT activity toward 4-methyl-umbelliferone (r=-0.871, $p<0.005$) on day 7 after the administration of all $MeSO_2$ derivatives except 4-$MeSO_2$-CB149.



The negative correlation between UDP-GT activity toward T_4 on day 7 and serum total T_4 concentration on day 2 was presented ($r=-0.898$, $p<0.005$) after the administration of all $MeSO_2$ derivatives except 4-$MeSO_2$-CB149 (Fig. 2).

In conclusion, the results from the present study indicate that the reduction of serum T_4 levels produced by seven 3-$MeSO_2$-PCBs and 4-$MeSO_2$-CB101 are caused by a mechanism in which increased hepatic T_4 glucuronidation by induction of UGT1A1 plays an important role.



Each point represents the mean ± S.E. (vertical bars) for four to eight animals. ———, Equation was calculated from nine points except 4-$MeSO_2$-CB149.

Fig. 2. Correlation between hepatic microsomal UDP-glucuronosyltransferase activity toward thyroxine and serum total thyroxine concentration in $MeSO_2$-PCB derivatives-administered rats

○, Control; ▼, 3-$MeSO_2$-CB49; ▽, 3-$MeSO_2$-CB70;
◆, 3-$MeSO_2$-CB87; □, 3-$MeSO_2$-CB101; ■, 4-$MeSO_2$-CB101; ●, 3-$MeSO_2$-CB132; ◇, 3-$MeSO_2$-CB141;
△, 3-$MeSO_2$-CB149; ▲, 4-$MeSO_2$-CB149.

Acknowledgements

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