Reduction of Thyroid Hormone Levels by Methylsulfonyl Metabolites of Polychlorinated Biphenyl Congeners in Rats

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Introduction

Recently, methylsulfonyl ($MeSO_2$) metabolites of polychlorinated biphenyls (PCBs) were detected in Swedish mother's milk [1], in human blood, liver and adipose tissue [2, 3]. The main $MeSO_2$-PCBs in human milk and tissues of human and mammals have been shown to be 3- and 4-$MeSO_2$ derivatives of PCBs with chlorine atoms in the 2,5- or 2,5,6-position, e.g. 3-$MeSO_2$- and 4-$MeSO_2$-2,2',4',5,5'-pentachlorobiphenyls (3-$MeSO_2$-CB101 and 4-$MeSO_2$-CB101), 3-$MeSO_2$- and 4-$MeSO_2$-2,2',4',5,5',6-hexachlorobiphenyls (3-$MeSO_2$-CB149 and 4-$MeSO_2$-CB149) [1, 3-7]. The biological activities and toxicological significances of $MeSO_2$ metabolites have not yet been clarified.

In our preceding papers [8-10], we reported that nine 3-$MeSO_2$ metabolites such as 3-$MeSO_2$-2,3',4',5-tetrachlorobiphenyl (3-$MeSO_2$-CB70), 3-$MeSO_2$-CB101 and 3-$MeSO_2$-2,2',3',4',5,5'-hexachlorobiphenyl (3-$MeSO_2$-CB141) were strong inducers of hepatic microsomal drug-metabolizing enzymes, while their isomeric 4-$MeSO_2$ metabolites were not. We also showed that 3-$MeSO_2$-2,2',4',5-tetrachlorobiphenyl (3-$MeSO_2$-CB49) and 3-$MeSO_2$-CB101 have highly potent CYP2B1 and CYP2B2 inducing activities, in fact with a potency up to several hundred fold of those of parent PCBs [9, 10]. Additionally we suggested that some 3- and 4-$MeSO_2$ metabolites may act as liver tumor promoters [11].

In this study, we have investigated the potential ability to reduce thyroid hormone levels of 3- and 4-$MeSO_2$ metabolites of PCB congeners which were major $MeSO_2$-PCB determined in human liver, milk and the tissues of several mammalian species [1, 3-7] since the thyroid is a common target organ of toxicity. This is the first evaluation of the acute endocrine-disrupting effects by the $MeSO_2$ metabolites of PCB congeners. Fig. 1 shows the chemical structures of $MeSO_2$ derivatives of PCB congeners used in this paper.

Materials and Methods

Chemicals. The $MeSO_2$-PCBs were prepared as described elsewhere [12]. The purity of these compounds was >99% when analyzed by gas chromatography. All other chemicals were

obtained commercially in appropriate grades of purify.

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 (8AM-8PM light) in a room with controlled temperature (24.5 ± 1℃) and humidity (55 ± 5%). Rats received four consecutive intraperitoneal injections of $MeSO_2$-PCBs dissolved in Panacete 810 (5 ml/kg) or phenobarbital (PB) sodium dissolved in 0.9% saline (5 ml/kg). Control animals received an equivalent volume of corresponding vehicle. On days 1, 2, 3, 4 and 7 after last dosing, 0.2 ml of blood was drawn from the tail vein. Blood was collected from animals between 10:30 and 11:30 AM. After clotting at room temperature, serum was separated by centrifugation and stored at -50℃ prior to determination of total thyroxine (T_4) and total triiodothyronine (T_3) levels by radioimmunoassay using Amerlex-MT4 and Amerlex-MT3 purchased from Ortho-Clinical Diagnostics Co. (Amersham, UK). All rats were killed by decapitation on day 7, and the thyroid glands and liver were removed and weighed.

Biochemical analyses. Microsomes were prepared according to the procedure described previously [8]. The protein content was determined by the method of Lowry *et al.* [13] with bovine serum albumin as a standard. Total cytochrome P450 content was estimated according to the method of Omura and Sato [14].

Determination of $MeSO_2$-PCBs in tissues. The clean-up and determination of $MeSO_2$-PCB from liver and thyroid glands were done as described previously [15].

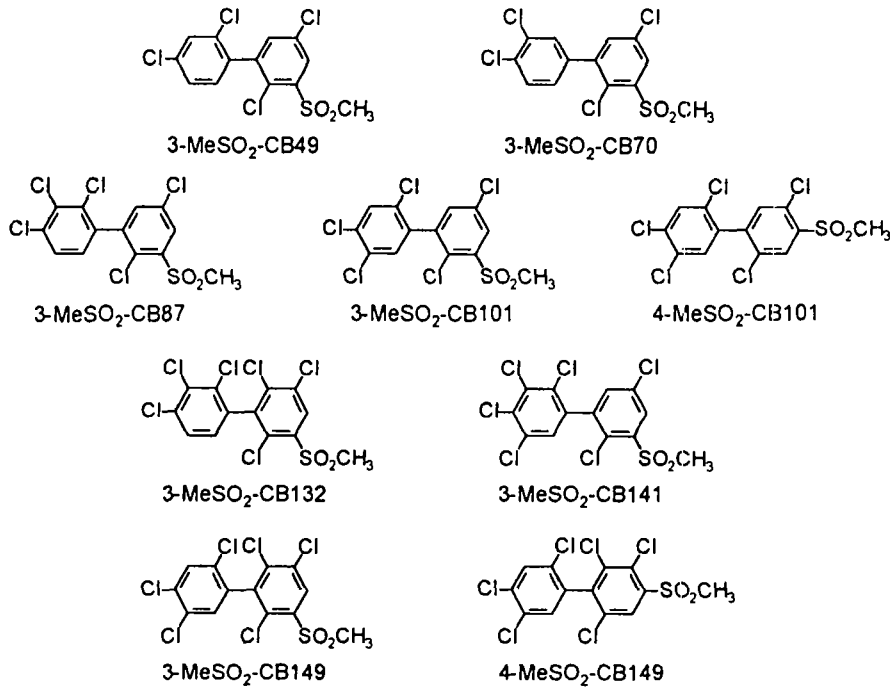

Fig. 1. Chemical structures of methyl sulfone derivatives of PCB congeners

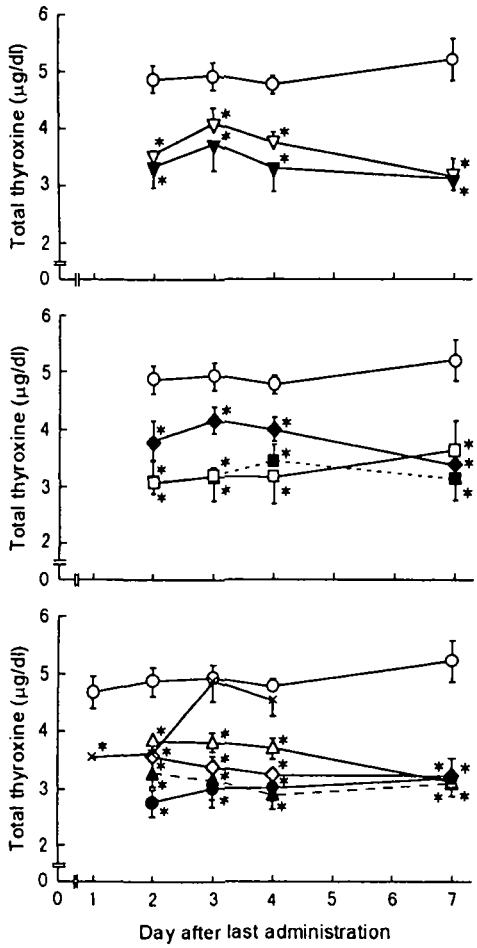

Fig. 2. Effects of $MeSO_2$ derivatives of PCBs and phenobarbital on serum total thyroxine concentration in rats

$MeSO_2$-PCBs (20 μmol/kg) and phenobarbital (431 μmol/kg) were given i.p. to rats once daily for four days.
○—, 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;
×- -, phenobarbital.
Each point represents the mean ± S.E. (vertical bars) for 4-6 rats.
*P<0.05, significantly different from the control.

Results and Discussion

Serum concentrations of total T_4 (Fig. 2) were reduced by all treatments, but varied in the extent of the reduction. PB (four consecutive daily doses of 431 μmol/kg) reduced serum total T_4 concentrations only at days 1 and 2 (25% decrease), whereas 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 and 4-$MeSO_2$-CB149 at much lower dose (four consecutive daily doses of 20 μmol/kg) than PB significantly reduced total T_4 levels 16-44% at days 2, 3, 4 and 7. The reason might be due to remaining of the metabolites in the liver and adipose tissue over long time periods.

Serum concentration of total T_3 was reduced by 3-$MeSO_2$-CB49 and 4-$MeSO_2$-CB149 at day 7 (37% decrease). A significant increase in thyroid weight was observed with 3-$MeSO_2$-CB101 and 3-$MeSO_2$-CB141 treatment. This suggests that 3-$MeSO_2$-CB101 and 3-$MeSO_2$-CB141 produces hyperplasia in the thyroid glands.

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 and 3-$MeSO_2$-CB149 significantly increased total cytochrome P450 content, while 4-$MeSO_2$-CB149 and PB did not.

It is reported that some PCB congeners such as 3,3',4,4'-tetrachlorobiphenyl (CB77), 2,3',4,4',5- and 3,3',4,4',5-pentachlorobiphenyls (CB118 and CB126), and 2,2',4,4',5,5'- and 2,3,3',4,4',5-hexachlorobiphenyls (CB153 and CB156) reduce thyroid hormone levels [16-18]. Though the effects of corresponding parental PCB congeners of 3- and 4-$MeSO_2$ metabolites tested in this study on thyroid hormone levels have not been reported, 3-$MeSO_2$-CB132, which is by far predominant $MeSO_2$ metabolite accumulated in human liver [3], 3-$MeSO_2$-CB101, 4-$MeSO_2$-CB101, 3-$MeSO_2$-CB141, 3-$MeSO_2$-CB149 and 4-$MeSO_2$-CB149, which are major $MeSO_2$-PCB determined in human liver and milk [1, 3], produced continuous reductions of total T_4 at each time point examined.

In conclusion, tested 3- and 4-$MeSO_2$ metabolites of PCB congeners possess the ability to

reduce serum thyroid hormone levels in rats, indicating that the $MeSO_2$ metabolites may play an important role in the alterations in the hormone levels following exposure to the PCB congeners. The results suggest an endocrine-disrupting potential of these compounds, and that the risk assessment should be made for the toxicity of $MeSO_2$ metabolites of PCB congeners. Further studies are needed to reveal the details of their mechanism of reduction.

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