REDUCTION OF SERUM THYROXINE LEVEL BY METHYLSULFONYL METABOLITES OF CHLORINATED BENZENES IN MALE SPRAGUE-DAWLEY RATS

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Introduction

The chlorinated benzenes are important environmental contaminants that are used for both private and industrial applications [1]. A number of studies on the toxicity of chlorinated benzenes indicate that the liver and kidneys are their principal target sites [2,3]. However, several chlorinated benzenes, e.g., 1,2-dichlorobenzene (1,2-DCB) and 1,2,4-trichlorobenzene (1,2,4-TCB), have also been reported to cause a reduction in plasma thyroxine (T_4) levels [4,5].

In the preceding papers [6,7], we reported that the corresponding dichlorophenyl methyl sulfones ($DCPSO_2Mes$) were detected in the several tissues of rats dosed with 1,3-DCB, and the trichlorophenyl methyl sulfones ($TCPSO_2Mes$) were detected in the several tissues of rats administered 1,2,4-TCB. The administration of these methylsulfone compounds resulted in the effective induction of hepatic microsomal drug-metabolizing enzymes in rats [7-10]. We showed that the effect of 1,3-DCB and 1,2,4-TCB in inducing hepatic microsomal drug-metabolizing enzymes is not attributable to the actions of 1,3-DCB and 1,2,4-TCB per se but to those of their methylsulfonyl ($MeSO_2$) metabolites, 3,5-$DCPSO_2Me$ and 2,3,5-$TCPSO_2Me$, respectively [7,11]. However, the effect of $MeSO_2$ metabolites of chlorinated benzenes on serum thyroid hormone levels have not yet been clarified.

In this study, therefore we have investigated the potential ability of the $MeSO_2$ metabolites of chlorinated benzenes to reduce thyroid hormone levels in rats.

Materials and Methods

Chemicals. The $MeSO_2$ derivatives of chlorinated benzenes were prepared as described elsewhere [6]. All other chemicals used in the present experiments were commercially obtained.

Animal treatments. Male Sprague-Dawley rats, weighing about 250 g, were housed three or four per cage 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°C) and humidity (55 ± 5%). Rats received an intraperitoneal injection of the $MeSO_2$ derivatives of chlorinated benzenes dissolved in Panacete 810 (5 ml/kg). Control animals received an equivalent volume of vehicle. The rats were killed by decapitation on designated time, and the thyroid glands and liver were removed and weighed. Blood was collected from animals between 10:30 and 11:30 a.m. After clotting at room temperature, serum was separated by centrifugation and stored at -50°C prior to determination of total thyroxine (T_4) and thyroid stimulating hormone (TSH) levels by radioimmunoassay using Amerlex-MT4 (Ortho-Clinical Diagnostics Co.; Amersham, UK) and Biotrak rTSH [^{125}I] assay system (Amersham Life Science Ltd.; Little Chalfont, UK), respectively.

Preparation of hepatic microsomes and enzyme assays. Microsomes were prepared according to

the procedure described previously [8]. The protein content was determined by the method of Lowry *et al.* [12] with bovine serum albumin as a standard. The microsomal activities of UDP-glucuronosyltransferase (UDP-GT) toward 4-nitrophenol and chloramphenicol were determined as described by Isselbacher *et al.* [13] and Ishii *et al.* [14], respectively.

Determination of $MeSO_2$ derivative of 1,3-DCB in the liver. The concentration of 3,5-DCPSO_2Me present in the liver were determined with HPLC as described previously [7] with some modification.

Results and Discussion

3,5-DCPSO_2Me and 2,3,5-TCPSO_2Me (two consecutive daily doses of 50 μmol/kg) significantly reduced the serum concentrations of total T_4 at days 1 and 2 after last dosing, while 4-monochlorophenyl methyl sulfone (4-MCPSO_2Me), 2,3-, 2,4-, 2,5- and 3,4-DCPSO_2Mes, and 2,4,5-TCPSO_2Me did not (Fig. 1). On the other hand, no change was observed in the serum concentration of TSH after the administration of 3,5-DCPSO_2Me and 2,3,5-TCPSO_2Me. A significant increase in thyroid weight was observed with 2,3,5-TCPSO_2Me treatment.

When 3,5-DCPSO_2Me (50 μmol/kg) was administered once daily for 1, 2, 3 and 4 days, the negative correlation between the serum concentration of total T_4 and the hepatic concentration of 3,5-DCPSO_2Me on days 1 and 2 was observed (r = -0.766, P<0.05) after the administration.

The hepatic concentration of 3,5-DCPSO_2Me increased in proportion to dosages of 3,5-DCPSO_2Me (two consecutive daily doses of 5-50 μmol/kg). Two consecutive daily doses of 5 μmol/kg of 3,5-DCPSO_2Me significantly reduced the serum concentrations of total T_4. The methyl sulfone caused dose-dependent reductions in the concentration of total T_4 (Fig. 2), and dose-dependent

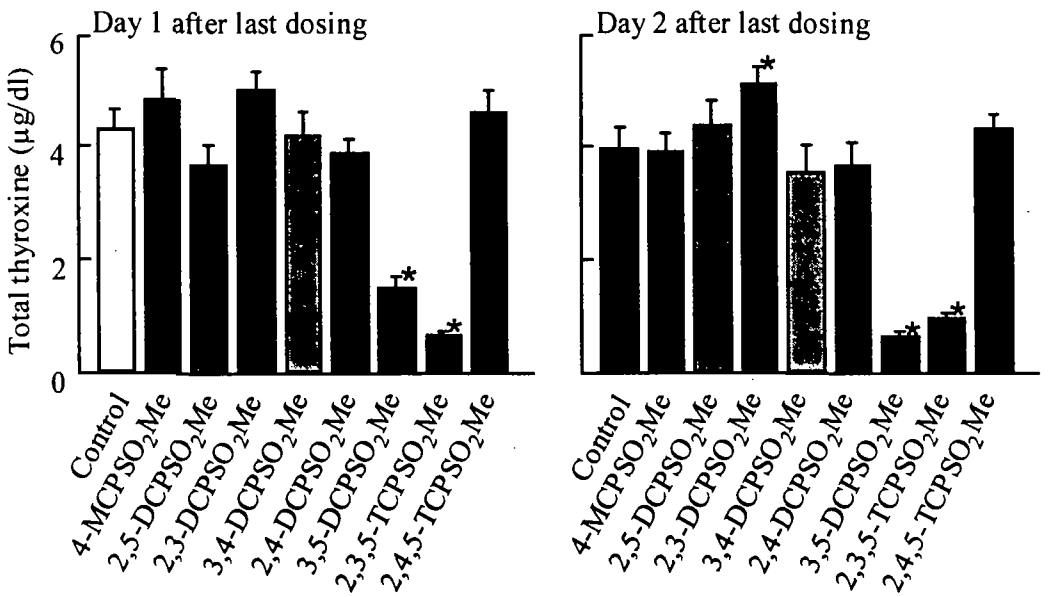

Fig. 1. Effects of $MeSO_2$ derivatives of chlorinated benzenes on serum total thyroxine concentration in rats. $MeSO_2$ derivatives of chlorinated benzenes (50 μmol/kg each) were given i.p. to rats once daily for two days. Results are expressed as the mean ± S.E. for five to eight animals. *P<0.05, significantly different from the control.

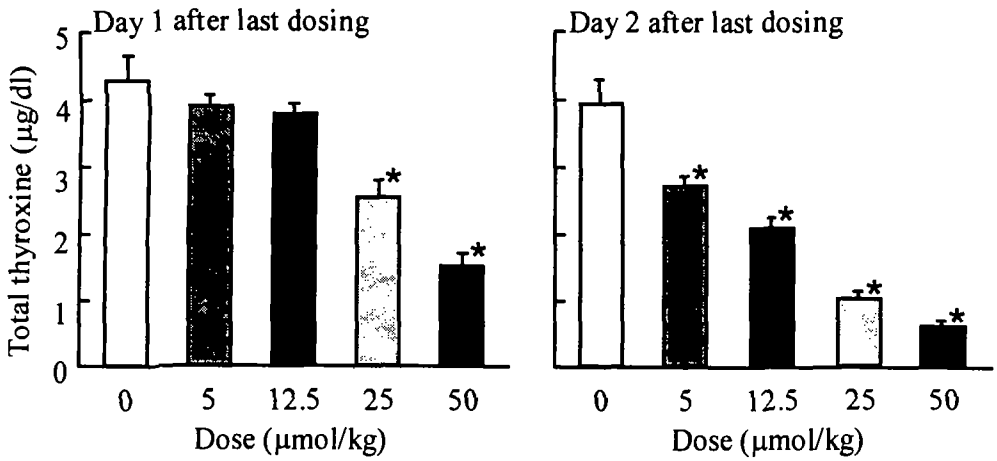

Fig. 2. Effects of graded doses of 3,5-DCPSO$_2$Me on serum total thyroxine concentration in rats. Rats were given i.p. 3,5-DCPSO$_2$Me twice with a 24 hr interval at the various doses indicated. Results are expressed as the mean ± S.E. for five to eight animals. *P<0.05, significantly different from the control.

Increases in the activity of UDP-GT (UGT1A6) toward 4-nitrophenol in the range of 5-50 μmol/kg. Furthermore, 3,5-DCPSO$_2$Me and 2,3,5-TCPSO$_2$Me significantly increased the activity of UDP-GT toward T_4.

In conclusion, 3,5-DCPSO$_2$Me and 2,3,5-TCPSO$_2$Me possess the ability to reduce serum T_4 levels in rats. The results of the present study indicate that the reduction of serum T_4 levels produced by 3,5-DCPSO$_2$Me and 2,3,5-TCPSO$_2$Me are caused by a mechanism in which increased hepatic T_4 glucuronidation by induction of UGT1A1 and UGT1A6 plays an important role.

Acknowledgements

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