RELATION BETWEEN THE INCREASE IN HEPATIC UDP-GLUCURONOSYLTRANSFERASE AND THE DECREASE IN SERUM THYROXINE LEVEL IN KANECHLOR-500-TREATED RATS

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Introduction

Some PCB congeners, such as 2,3',4,4',5- and 3,3',4,4',5-pentachlorobiphenyls, and 2,2',4,4',5,5'- and 2,3,3',4,4',5-hexachlorobiphenyls, alter the levels of serum thyroid hormone and hepatic drug-metabolizing enzymes [1,2]. Several reports have suggested that the induction of UDP-glucuronosyltransferase (UDP-GT) contributed to the decrease in T_4 levels by such inducers as 3,3',4,4',5-pentachlorobiphenyl, Aroclor 1254 and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [2-4].

We previously reported that Kanechlor-500 (KC500), a technical PCB mixture, possess the ability to reduce serum total thyroxine (T_4) level in rats and mice, and further suggested that increase in the hepatic T_4 glucuronidation by KC500-mediated induction of UGTs, UGT1A1 and UGT1A6, caused the reduction of serum T_4 levels in rats [5].

In the present study, therefore, we examined to clarify whether the reduction of serum total T_4 level in rats is dependent on increase in the hepatic T_4 glucuronidation by KC500.

Materials and Methods

Chemicals

PCBs were synthesized by using the Cadogan coupling reactions [6].

Animal treatments

Male Wistar rats (160-200 g) and homozygous Gunn rats (190-260 g), which are hereditarily UGT1s deficient, were obtained from Japan SLC., Inc. (Shizuoka, Japan). Male Wistar and Gunn rats 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 at a controlled temperature (24.5 ± 1º) and humidity (55 ± 5%). The rats received an intraperitoneal injection of KC500 (100 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals received an equivalent volume of vehicle. All rats were killed by decapitation on day 4 after the dosing, and the liver was 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 -50º prior to determination of total T_4, total triiodothyronine (T_3) and thyroid stimulating hormone (TSH) levels by radioimmunoassay using T-4·RIABEAD, T-3·RIABEAD (DAINABOT Co., Ltd, Tokyo, Japan) and Biotrak rTSH [^{125}I] assay system (Amersham Life Science Ltd.; Little Chalfont, UK).

Table 1. Effects of KC500 on serum total T_4, total T_3 and TSH concentrations in Wistar and Gunn rats.

	Total T_4 (μg/dl)		Total T_3 (ng/ml)		TSH (ng/ml)	
Treatment	Wistar	Gunn	Wistar	Gunn	Wistar	Gunn
Control	2.95 ± 0.15	8.59 ± 0.21†	0.34 ± 0.03	0.96 ± 0.05†	7.43 ± 1.74	20.9 ± 1.79†
KC500	0.51 ± 0.04*	1.66 ± 0.02*	0.30 ± 0.04	0.86 ± 0.03	5.48 ± 0.85	17.2 ± 2.61

Animals were given 100 mg/kg of KC500 i.p. and killed at 4 days after the administration. Results are expressed as the mean ± S.E. for three to six animals.
*P<0.01, significantly different from control.
†P<0.01, significantly different from control of Wistar rats

Preparation of hepatic microsomes and the microsomal enzyme assays

Hepatic microsomes were prepared according to the procedure described previously [7]. The protein content was determined by the method of Lowry *et al.* [8] with bovine serum albumin as a standard. The activities of 7-ethoxyresorufin, 7-pentoxyresorufin and 7-benzyloxyresorufin *O*-dealkylase in hepatic microsomes were determined by the method of Burke *et al.* [9]. The microsomal activities of UDP-GT toward chloramphenicol and T_4 were determined as described by Ishii *et al.* [10] and Barter and Klaassen [11], respectively.

Western blot analysis

Polyclonal anti-peptide antibodies against the common region of UGT1A isoforms and a specific antibody against UGT2B1 isoform were used in Western blot studies. Western analyses were performed with microsomal preparations as described by Luquita *et al.* [12].

Determination of PCBs in the liver

The concentration of PCBs present in the liver was determined with GC/MS as described by Mimura *et al.* [13].

Results and Discussion

A significant increase in liver weight was observed with KC500 treatment in Wistar rats but not in Gunn rats. The hepatic concentration of total PCBs was 1.2 times higher in Gunn rats than in Wistar rats. KC500 administration resulted in significant increase in hepatic microsomal enzyme activity; benzyloxyresorufin *O*-dealkylase activity: 84- and 12-fold, pentoxyresorufin *O*-dealkylase activity (CYP2B1/2): 27- and 7-fold, and ethoxyresorufin *O*-dealkylase activity (CYP1A1/2): 45- and 9-fold in Wistar and Gunn rats, respectively, 4 days later.

KC500 treatment resulted in significant increases in activity of UDP-GT (UGT2B1) toward chloramphenicol and content of the UGT2B1 isoform in both Wistar and Gunn rats. The extent of both increases in Wistar rats was higher than that in Gunn rats. The activities of UDP-GT (UGT1A1 and

UGT1A6) toward T_4 and content of the UGT1A isoforms were significantly increased by KC500 in Wistar rats but not in Gunn rats. On the other hand, serum total T_4 level was significantly decreased by KC500 treatment in both Wistar and Gunn rats (Table 1). The extent of the decrease in Wistar rats was the same as that in Gunn rats. No significant change in the levels of serum total T_3 and TSH was observed in both rats (Table 1).

In conclusion, the present findings demonstrate that KC500 possess the ability to reduce serum total T_4 level in both Wistar and Gunn rats. Since KC500 influences hardly the activity and content of UGT1A responsible for the glucuronidation of T_4 in Gunn rats, the reduction of serum T_4 levels was not necessarily correlate with increase in hepatic T_4 glucuronidation activity. The results suggest that in addition to increase in the hepatic T_4 glucuronidation through KC500-mediated induction of UGT1A1 and UGT1A6, another mechanism might be concerned with the reduction of serum total T_4 level in Wistar rats.

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References

1. Ness, D.K., Schantz, S.L., Moshtaghian, J. and Hansen, L.G. (1993). *Toxicol. Lett.* 68, 311-323.
2. Van Birgelen, A.P.J.M., Smit, E.A., Kampen, I.M., Groeneveld, C.N., Fase, K.M., Van der Kolk, J., Poiger, H., Van den Berg, M., Koeman, J.H. and Brouwer, A. (1995). *Eur. J. Pharmacol.* 293, 77-85.
3. Barter, R.A. and Klaassen, C.D. (1994) *Toxicol. Appl. Pharmacol.* 128, 9-17.
4. Schuur, A.G., Boekhorst, F.M., Brouwer, A. and Visser, T.J. (1997) *Endocrinology* 138, 3727-3734.
5. Kato, Y., Yamazaki, T., Haraguchi, K., Nemoto, K., Masuda, Y., Degawa M. and Kimura, R. (2001) *Organohalogen Compounds*, 53, 47-49.
6. Cadogan, J.I.G. (1962) *J. Chem. Soc.* 4257-4258.
7. Kato, Y., Haraguchi, K., Kawashima, M., Yamada, S., Masuda, Y. and Kimura, R. (1995) *Chem.-Biol. Interact.* 95, 257-268.
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) *J. Biol. Chem.* 193, 265-275.
9. Burke, M.D., Thompson, S., Elcombe, C.R., Halpert, J., Haaparanta, T. and Mayer, R.T. (1985) *Biochem. Pharmacol.* 34, 3337-3345.
10. Ishii, Y., Tsuruda, K., Tanaka, M. and Oguri, K. (1994) *Arch. Biochem. Biophys.* 315, 345-351.
11. Barter, R.A. and Klaassen, C.D. (1992) *Toxicol. Appl. Pharmacol.* 115, 261-267.
12. Luquita, M.G., Catania, V.A., Sánchez Pozzi, E.J., Veggi, L.M., Hoffman, T., Pellegrino, J.M., Ikushiro, S., Emi, Y., Iyanagi, T., Vore, M. and Mottino, A.D. (1999) *J. Pharmacol. Exp. Ther.* 298, 49-56.
13. Mimura, K., Tamura, M., Haraguchi, K. and Masuda, Y. (1999) *Fukuoka Acta Med.* 90, 192-201.