Cod: 1.1010

INDUCTION OF HEPATIC T4 TRANSPORTERS BY POLYCHLORINATED BIPHENYL IN RATS

Y. Kato[1], K. Haraguchi[2], A. Fujii[3], Y. Fujii[2], O. Kimura[4], C. Ohta[5], T. Endo[4], N. Koga[5], S. Yamada[3], M. Degawa[3]

[1]*Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Kagawa, Japan*

[2]*Daiichi College of Pharmaceutical Sciences, Fukuoka, Japan*

[3]*School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan*

[4]*Faculty of Pharmaceutical Sciences, Health Science University of Hokkaido, Hokkaido, Japan*

[5]*Faculty of Nutritional Sciences, Nakamura Gakuen University, Fukuoka, Japan*

Introduction

In general, polychlorinated biphenyls (PCBs), including 2,2',4,4',5,5'-hexachlorobiphenyl (CB153), 3,3',4,4',5-pentachlorobiphenyl (CB126) and Aroclor 1254, have abilities to decrease serum thyroid hormone levels in rats and mice, and the decreases are thought to occur through the induction of thyroxine (T_4)-UDP-glucuronosyltransferases (UDPGTs)[1-3], especially UGT1A1 and UGT1A6[4]. However, we have previously found that the PCB-mediated reduction of the serum T_4 level in rats and mice is not necessarily correlated with an increase in hepatic T_4 glucuronidation activity[5], that Kanechlor-500 (KC500)-treatment results in a significant decrease in the level of serum total T_4 not only in Wistar rats but also in Gunn rats (UGT1A-deficient Wistar rats)[6,7], and that the KC500-mediated decrease in rats occurs through an increase in accumulation level of T_4 in the liver rather than an increase in hepatic T_4-UDPGT activity[7]. Furthermore, we have demonstrated that in mice, the 3,3',4,4'-tetrachlorobiphenyl (CB77)-, 2,3',4,4',5-pentachlorobiphenyl (CB118)-, CB126- and 2,2',4,4',5,5'-hexachlorobiphenyl (CB153)-mediated decrease in serum T_4 level occurred mainly through increased accumulation of T_4 in the liver and partially through increased excretion of biliary [^{125}I]T_4 metabolite(s) and/or development of liver hypertrophy[8-11].

It has been reported that T_4 homeostasis are regulated by several transporters responsible for the excretion of the T_4 and T_4-glucuronide to the bile duct[12-17]. The several transporters, including apical and basolateral T_4-transporters, are reported to exist in the liver cells[15,16,18]. In the present study, therefore, we studied on PCB-mediated changes in the expression and activity of hepatic T_4 transporter(s) to understand the liver-selective accumulation of T_4, and a relationship between the decrease in serum total T_4 level and the increased expression of hepatic T_4 transporters by PCB using Wistar rats.

Materials and methods

Animal Treatments. Male Wistar rats (163-263 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). They were housed three or four per cage with free access to commercial chow and tap water, maintained on a 12-h dark/light cycle (8:00 AM-8:00 PM light) in an air-controlled room (temperature, 24.5 ± 1°C; humidity, 55 ± 5%), and handled with human care under the guidelines of the University of Shizuoka (Shizuoka, Japan). Rats received an intraperitoneal injection of KC500 (100 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals were treated with vehicle alone (5 ml/kg).

In Vivo Study. Rats were killed by decapitation at 3, 6, 12, 24, 48 and 96 hrs after the intraperitoneal administration of KC500, and the liver was removed and weighed. Blood was collected from each animal between 10:30 and 11:30 AM. After clotting at room temperature, serum was separated by centrifugation and stored at –50 °C until used.

Analysis of serum hormones. Serum levels of total T_4 and free T_4 were measured by radioimmunoassay using Total T4 and Free T4 kit (Diagnostic Products Corporation; Los Angels, CA), respectively.

RT-PCR analysis for gene expression of hepatic transporter. Total hepatic RNAs were prepared with ISOGEN (NipponGene, Japan) and used for the determination of the gene expression of eight T_4 transporters such as organic anion transport polypeptide 1 (Oatp1), Oatp2, Oatp3, Oatp4, sodium-dependent taurocholate cotransporting polypeptide (Ntcp), L-type amino acid transporter 1 (LAT1), LAT2 and multidrug resistance associated protein2 (Mrp2). A portion of (4 μg) of total RNA was converted to cDNA by use of poly d(N)6 primer (Pharmacia Biotech) and Moloney murine leukemia virus reverse transcriptase (GIBCO, BRL) in an RT-reaction mixture (20 μl). PCR was performed in a total reaction mixture (25 μl) containing 0.8 μl of the RT-reaction mixture, 0.5 μl of each primer set

and AmpliTaq Gold DNA polymerase (Perkin Elmer). The primer sets used were as follows: Oatp1, 5'-CAT GAG TGT ACT TCT CTC TTG G-3' (forward) and 5'-ATT CTG CTG GGT CTT GCG TTG G-3' (reverse); Oatp2, 5'-TGC ACA CTT AGC ATT CTG GC-3' (forward) and 5'-TGC ATG TAA CCC AAC TCC AA-3' (reverse); Oatp3, 5'-AGG AAA TTC ATC TGC AGT CCT GGG GT-3' (forward) and 5'-TTG GTT CTG GCC TAC CAT GTT GG-3' (reverse); Oatp4, 5'-GCA CCT AGG TAC TCT GCA TAC TAT AGC AAT GAT TGG-3' (forward) and 5'-GGT ACA TCT ATG TGA GAG TCC ACT GGG TTC A-3' (reverse); Ntcp, 5'-ATG CCC TTC TCT GGC TTT CT-3' (forward) and 5'-GCT CCA TGG TTC TGA TGG TT-3' (reverse); LAT1, 5'-GCT GTG GAT TTT GGG AAC TAC C-3' (forward) and 5'-CCA CAC ACA GCC AGT TGA AGA A-3' (reverse); LAT2, 5'-GCC TGT GGT ATC ATT GTT GTA GG-3' (forward) and 5'-AGT TGA CCC ATG TGA GCA GC-3' (reverse); Mrp2, 5'-ACC TTC CAC GTA GTG ATC CT-3' (forward) and 5'-GAT TTC CCA GAG CCT ACA GT-3' (reverse). The PCR program used for the analyses of hepatic transporter in rats and the separation of each PCR-product were determined by the methods previously described[19-26]. In the present experiments, β-actin mRNA was used as an internal control.

Ex Vivo Study. Four days after intraperitoneal administration of KC500, the rats were anesthetized with saline solution (2 ml/kg) containing sodium pentobarbital (25 mg/ml) and potassium iodide (1 mg/ml). The femoral artery was cannulated (polyethylene tube SP31, Natsume Inc., Tokyo, Japan) and primed with heparinized saline (33 units/ml), and then animal's body was warmed to 37°C. Fifteen minutes later, the rats were given i.v. 1 ml of [^{125}I]T_4 (15 μCi /ml) dissolved in the saline containing 10 mM NaOH and 1 % normal rat serum.

Tissue distribution of [^{125}I]T_4. The study on the tissue distribution of [^{125}I]T_4 was performed according to the modified method of Oppenheimer et al.[26]. Briefly, at 5 min after administration of [^{125}I]T_4 to KC500-pretreated rats, blood was sampled from abdominal aorta. Then, the tissues, such as cerebrum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, liver, kidney, adreanal gland, spleen, pancreas, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, lleum, caecum, brown fat, skeletal muscle, bone marrow skin, spinal cord and fat, were removed and weighted. Radioactivities in serum and the tissues were determined by a γ-counter (COBRATM#AUTO-GAMMA®5002, Packard Co., Meriden, USA), and amounts of [^{125}I]T_4 in the tissues were shown as ratios of tissue-to-serum.

Isolation of liver parenchymal cells and uptake experiments. The amount of [^{125}I]T_4 uptake in the rat liver parenchymal cells was measured in accordance with the modified method previously described by Nakamura et al.[27].

Statistics. The data obtained were statistically analyzed according to Student's t test or Dunnett's test after the analysis of variance. In addition, the amounts of uptake of [^{125}I]T_4 in the rat liver parenchymal cells were statistically analyzed according to Newman-Keuls' test after analysis of variance.

Results and discussion:

Effects of KC500 on levels of serum thyroid hormones were examined in Wistar rats (Table 1). KC500 treatment resulted in decreases of the serum total T_4 and free T_4 at 24 hr, and the decreases in serum total T_4 and free T_4 level were minimum 72 h after the dosing. The tissue-to-serum concentration ratios (Kp values) of the thyroid gland and liver were the greatest among all the tissues examined in Wistar rats, and the Kp values in thyroid gland and liver were 3.9 and 3.7 times, respectively, as compared to those in the corresponding control rats. The increases of Kp values in other tissues, such as cerebrum, sublingual gland, mandibular gland, heart, lung, kidney, adrenal gland, spleen, pancreas, prostate gland, seminal vesicle, caecum, brown fat, and fat, were also observed in KC500-pretreated rats.

In control (KC500-untreated) rats, accumulation level of [^{125}I]T_4 was the highest (31% of the [^{125}I]T_4 dosed) in the liver among the tissues examined, and the liver accumulation level was increased to more than 58% by KC500-pretreatment.

The amounts of [^{125}I]T_4 uptake in the liver parenchymal cells were significantly increased by KC500-treatment. Furthermore, expression level of mRNA of hepatic LAT1, a T_4-transporter, increased in a time-dependent manner at least up to 4 days after KC500-treatment. Concerning other hepatic T_4-transporters, the amount of Oatp2 mRNA increased at 72 hr after the dosing, while no KC500-mediated increases in the amounts of Oatp1, Oatp3, Oatp4, Ntcp, LAT2 and Mrp2 mRNAs were observed at any periods examined.

In conclusion, in the present study, we verified our hypothesis[8-11] that the decrease in serum T_4 level caused by exposure to PCBs occurs mainly through increased accumulation of T_4 in the liver and proposed that hepatic T_4-transporters, such as LAT1 and Oatp2, might partially contribute to the PCB-mediated increase of the liver-selective accumulation of T_4. Further studies on the effects of PCBs on hepatic T_4-transporters would be necessary to understand the exact mechanism for the PCB-induced decrease in serum T_4 level.

Acknowledgements:
This work was supported in part by the Grant-in-Aid for Scientific Research (C) (no. 26340043, Y.K.) from Japan Society for the Promotion of Science.

References:
1. Barter R.A., Klaassen C.D. (1994) Toxicol. Appl. Pharmacol. 128, 9-17.
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., Brouwer A. (1995) Eur. J. Pharmacol. 293, 77-85.
3. Craft E.S., DeVito M.J., Crofton K.M. (2002) Toxicol. Sci. 68, 372-380.
4. Visser T.J. (1996) Acta Med. Austriaca 23, 10-16.
5. Kato Y., Haraguchi K., Yamazaki T., Ito Y., Miyajima S., Nemoto K., Koga N., Kimura R., Degawa M. (2003) Toxicol. Sci. 72, 235-241.
6. Kato Y., Ikushiro S., Haraguchi K., Yamazaki T., Ito Y., Suzuki H., Kimura R., Yamada S., Inoue T., Degawa M. (2004) Toxicol. Sci. 81, 309-315.
7. Kato Y., Ikushiro S., Takiguchi R., Haraguchi K., Koga N., Uchida S., Sakaki T., Yamada S., Kanno J., Degawa M. (2007) Drug Metab. Dispos. 35, 1949-1955.
8. Kato Y., Haraguchi K., Kubota M., Seto Y., Okura T., Ikushiro S., Koga N., Yamada S., Degawa M. (2010) Drug Metab. Dispos. 38, 150-156.
9. Kato Y., Onishi M., Haraguchi K., Ikushiro S., Ohta C., Koga N., Endo T., Yamada S., Degawa M. (2011) Toxicol. Appl. Pharmacol., 254, 48-55.
10. Kato Y., Onishi M., Haraguchi K., Ikushiro S., Ohta C., Koga N., Endo T., Yamada S., Degawa M. (2013) Biol. Pharm. Bull., 36, 1594-1601.
11. Kato Y, Haraguchi K, Onishi M, Ikushiro S, Endo T, Ohta C, Koga N, Yamada S, Degawa M. (2014). Biol. Pharm. Bull., 37, 504-509.
12. Abe T., Kakyo M., Tokui T., Nakagomi R., Nishio T., Nakai D., Nomura H., Unno M., Suzuki M., Naitoh T., Matsuno S., Yawo H. (1999) J. Biol. Chem. 274, 17159-17163.
13. Friesema E.C.H., Docter R., Moerings E.P.C.M., Stieger B., Hagenbuch B., Meier P.J., Krenning E.P., Hennemann G., Visser T.J. (1999) Biochem. Biophys. Res. Comm. 254, 497-501.
14. Friesema E.C.H., Docter R., Moerings E.P.C.M., Verrey F., Krenning E.P., Hennemann G., Visser T.J. (2001) Endocrinology 142, 4339-4348.
15. Friesema E.C.H., Jansen J., Visser T.J. (2005) Biochem. Soc. Trans. 33, 228-232.
16. Lecureux L., Dieter M.Z., Nelson D.M., Watson L., Wong H., Gemzik B., Klaassen C.D., Lehman-McKeeman L.D. (2009) Toxicol. Sci. 108, 482-491.
17. Pizzagalli F., Hagenbuch B., Stieger B., Klenk U., Folkers G., Meier P.J. (2002) Mol. Endocrinol. 16, 2283-2296.
18. Visser W.E., Friesema E.C.H., Visser T.J. (2011) Mol. Endocrinol. 25, 1-14.
19. Kudo N., Katakura M., Sato Y., Kawashima Y. (2002) Chem. Biol. Interact., 139, 301-316.
20. Vos T. A., Ros J. E., Havinga R., Moshage H., Kuipers F., Jansen P.L M., Müller M. (1999) Hepatology, 29, 1833-1839.
21. Walters H.C., Craddock A.L., Fusrgawa H., Willingham M.C., Dawson P.A. (2000) Am. J. Physiol. Gastrointest. Liver. Physiol., 279, G1188-G1200.
22. Choudhuri S., Ogura K., Klaassen C.D. (2000) Biochem. Biophys. Res. Commun., 274, 79-86.
23. Boado R.J., Li J.Y., Nagaya M., Zhang C., Pardridge W.M. (1999) Proc. Natl. Acad. Sci. U.S.A., 96, 12079-12084.
24. Kudo Y., Tamai I., Uchino H., Suzuki F., Sai Y., Tsuji A. (2001) J. Pharm. Pharmacol., 53, 497-503.
25. Vos T.A., Hooiveld G.J.E.J., Koning H., Childs S., Meijer D.K.F., Moshage H., Jansen P.L.M., Müller M. (1998) Hepatology, 28, 1637-1644.
26. Oppenheimer J.H., Bernstein G., Surks M.I. (1968) J. Clin. Invest. 47, 1399-1406.
27. Nakamura T., Yuasa H., Inoue K., Hayashi Y, Watanab J. (2002) Biol. Pharm. Bull. 25, 356-360.

Table 1. Effect of KC500 on serum total T_4 level (μg/dl) in rats

	Time after the administration (hr)					
	3	6	12	24	48	96
Control	4.13 ± 0.43	3.98 ± 0.20	3.53 ± 0.39	2.98 ± 0.30	3.19 ± 0.49	3.44 ± 0.34
KC500	4.80 ± 0.61	3.61 ± 0.44	2.45 ± 0.29	1.26 ± 0.28*	0.68 ± 0.12*	0.27 ± 0.10*

Animals were given KC500 (100 mg/kg) i.p., and killed at the appropriate times after the administration.
Results are expressed as the mean ± S.E. for three to five animals.
*P<0.05, significantly different from each control.