Possible mechanism for the polychlorinated biphenyl-induced liver-selective accumulation of thyroxine in rats

Yoshihisa Kato¹, Aki Fujii², Koichi Haraguchi³, Yukiko Fujii³, Kazutaka Atobe¹, Tetsuya Endo⁴, Osamu Kimura⁴, Nobuyuki Koga⁵, Chiho Ohta⁵, Shizuo Yamada² and Masakuni Degawa²

¹Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, 1314-1, Shido, Sanuki, Kagawa 769-2193, Japan
²School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
³Daiichi College of Pharmaceutical Sciences, Fukuoka 815-8511, Japan
⁴Faculty of Pharmaceutical Sciences, Health Science University of Hokkaido, Hokkaido 061-0293, Japan
⁵Faculty of Nutritional Sciences, Nakamura Gakuen University, Fukuoka 814-0198, Japan

(Received March 29, 2017; Accepted August 1, 2017)

ABSTRACT — We have previously reported that decrease in level of serum thyroxine T₄ by Kanechlor 500 (KC500) in rats would occur through the increase in hepatic T₄ accumulation rather than the increase in hepatic T₄-glucuronyl transferase activity. In the present study, to understand the mechanism underlying the KC500-mediated increase in hepatic T₄ accumulation, we examined the relationship between the KC500-mediated changes in hepatic T₄ accumulation and the expression levels of mRNAs of hepatic transporters including T₄ transporters. [¹²⁵I]T₄ was intravenously injected into KC500-pretreated and control (KC500-untreated) Wistar rats, and [¹²⁵I]T₄ uptake levels of liver parenchymal cells were comparatively examined. The amount of [¹²⁵I]T₄ uptake by hepatic cells increased in a time-dependent manner up to 96 hr after KC500 treatment. Following KC500 treatment, a time-dependent increase in the mRNA level of hepatic T₄ influx transporter LAT1 was observed up to 96 hr later, while a significant increase in hepatic T₄ influx transporter Oatp2 mRNA occurred only at 96 hr later. No KC500-mediated increases in the mRNAs of other hepatic transporters (Oatp1, Oatp3, Oatp4, Ntcp, LAT2, and Mrp2) were observed at any timepoints, although the mRNA expression of the T₄ conjugate(s) efflux transporter Mrp3 significantly increased in a time-dependent manner 24-96 hr following KC500 treatment. The present findings suggest that KC500-mediated increase in hepatic T₄ accumulation occurs, at least in part, through the increase in the expression of hepatic T₄-transporters, such as LAT1 and Oatp2.

Key words: Kanechlor-500, Polychlorinated biphenyl, Thyroxine, Liver, L-type amino acid transporter 1

INTRODUCTION

Polychlorinated biphenyls (PCBs), including 2,2′,4,4′,5,5′-hexachlorobiphenyl, 3,3′,4,4′,5-pentachlorobiphenyl, and Aroclor 1254, can decrease serum thyroid hormone levels in rats and mice. This is thought to occur through the induction of thyroxine (T₄)-UDP-glucuronosyltransferases (UDP-GTs) (Barter and Klaassen, 1994; Van Birgelen et al., 1995; Craft et al., 2002), especially UGT1A1 and UGT1A6 (Visser, 1996). However, it has been reported that the magnitude of decrease in serum total T₄ level was not necessarily correlated with that of increase in T₄-UDP-GT activity (Craft et al., 2002; Hood et al., 2003). We have previously found that the Kanechlor-500 (KC500)-mediated reduction of serum T₄ levels in rats and mice was not necessarily correlated with the increase in hepatic T₄ glucuronidation activity (Kato et al., 2003), and further indicated that KC500-mediated decrease in serum total T₄ level occurred in rats, mice, hamsters, and guinea pigs, although significant KC500-mediated increase in the excretion level of biliary T₄-glucuronide occurred only in rats, but not in other rodents (Kato et al., 2010a). Furthermore, KC500-mediated decrease in serum T₄ level occurs not only in Wistar rats but also in UGT1A-deficient Wistar (Gunn) rats (Kato et al., 2004, 2007).

Correspondence: Yoshihisa Kato (E-mail: kato@kph.bunri-u.ac.jp)
We have recently found the PCB-mediated increases in hepatic T4 accumulation in experimental rodents including rats (Kato et al., 2004, 2007, 2010a), mice (Kato et al., 2010a, 2010b, 2011, 2013, 2014), and hamsters (Kato et al., 2010a), and guinea pigs (Kato et al., 2010a). These findings propose a hypothesis that the PCB-mediated decrease in serum T4 level in the rodents occurs through the increase in hepatic T4 accumulation rather than the increase in hepatic T4-UDPGT activity. Incidentally, hepatic T4 level is primarily controlled by several transporters responsible for the influx of T4 to the liver and the excretion of T4-glucuronide from the liver (Friesema et al., 2005; Lecureux et al., 2009; Visser et al., 2011).

In the present study, therefore, we examined the relationship between the PCB-mediated changes in the [125I]T4 uptake in liver parenchymal cells and the mRNA expression of hepatic T4 transporter(s) to understand the mechanism for hepatic accumulation of T4 using Wistar rats.

**MATERIALS AND METHODS**

**Chemicals**

Panacect 810 (medium-chain triglycerides) was purchased from Nippon Oils and Fats Co., Ltd. (Tokyo, Japan). [125I]T4, radiolabelled at the 5'-position of the outer ring, was obtained from PerkinElmer Japan Co., Ltd. (Kanagawa, Japan). KC500 used in the present experiments contains 2,2′,2′,3,3′,3′,4,4′,4′,4′,4′,5,5′-hexachlorobiphenyl (5.6% of total PCBs), 2,2′,3,3′,6-pentachlorobiphenyl (6.5%), 2,2′,4,5,5′-pentachlorobiphenyl (10%), 2,3,3′,4,4′,5-pentachlorobiphenyl (7.4%), 2,3′,4,4′,5-pentachlorobiphenyl (7.4%), 2,2′,3,4,4′,5′-hexachlorobiphenyl (5.6%), and 2,2′,4,4′,5,5′-hexachlorobiphenyl (5.4%) as major PCB congeners (Haraguchi et al., 2005). All other chemicals were obtained commercially at appropriate grades of purity.

**Animal treatment**

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-hr dark/light cycle (08:00-20:00 light) in an air-controlled room (temperature, 24.5 ± 1°C; humidity, 55 ± 5%). Treatments of rats with KC500 were performed by the method previously described (Kato et al., 2003). Briefly, rats received an intraperitoneal injection of KC500 (100 mg/kg) dissolved in Panacect 810 (5 mL/kg). Control animals were treated with vehicle alone (5 mL/kg). The present animal experiments were approved by the Institutional Animal Care and Experiment Committee of the University of Shizuoka (Shizuoka, Japan).

**Analysis of serum T4**

All rats were starved for about 18 hr before being killed. KC500-treated rats were killed by decapitation at 12, 24, 48, and 96 hr later, and their livers were removed and weighed. Blood was collected from each animal between 10:30 and 11:30. After clotting at room temperature, serum was separated by centrifugation (1,400 xg, 10 min) and stored at –50°C until used. Serum levels of total T4 and free T4 were measured by radioimmunoassay using Total T4 and Free T4 kits (Diagnostic Products Corporation, Los Angeles, CA, USA), respectively.

**Tissue distribution of [125I]T4**

The tissue distribution of [125I]T4 and the tissue-to-serum concentration ratio (Kp value) were assessed by the method previously described (Kato et al., 2010a). Briefly, four days after the treatments with KC500 and vehicle alone (control), rats were intravenously administered 1 mL of [125I]T4 (15 μCi/mL) dissolved in saline containing 10 mM NaOH and 1% normal rat serum, and 5 min after the [125I]T4 treatment, accumulation levels of [125I]T4 in the liver and other tissues (cerebrum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, kidney, adrenal gland, spleen, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, ileum, and cecum) were measured.

**Isolation of liver parenchymal cells and [129I]T4 uptake experiment**

The preparation of liver parenchymal cells and the measurement of [129I]T4 uptake were performed according to a modified method of Nakamura et al. (2002). Briefly, 4 days after the intraperitoneal administration of KC500 to rats, liver parenchymal cells were prepared and used for [129I]T4 uptake experiments. The viability of liver parenchymal cells assessed by the trypan blue exclusion test was greater than 90% in all preparations. The parameters of maximum uptake rate (Vmax) and Michaelis-Menten constant (Km) were calculated using the Michaelis-Menten equation using conventional methods (Nakamura et al., 2002).

**Reverse transcription PCR analysis of hepatic transporter gene expression**

Rats were killed by decapitation at 12, 24, 48, and 96 hr after treatment with KC500, and their livers were removed and weighed. Total hepatic RNA was extracted with ISOGEN (NipponGene Co., Ltd., Toyama, Japan) and used to determine the gene expression of transport-
ers organic anion transport polypeptide 1 (Oatp1), Oatp2, Oatp3, Oatp4, sodium-dependent taurocholate cotransporting polypeptide (Ntcp), L-type amino acid transporter 1 (LAT1), LAT2, multidrug resistance-associated protein 2 (Mrp2), and Mrp3. A portion (4 μg) of total RNA was converted into cDNA using the poly d(N)6 primer (GE Healthcare UK Ltd., Pollards Wood, UK) and Moloney murine leukemia virus reverse transcriptase (Life Technologies Japan Co, Tokyo, Japan) in a 20 μL reaction. PCR was performed in a total volume of 25 μL containing 0.8 μL of cDNA, 0.5 μL of each primer set, and AmpliTaq Gold DNA polymerase (PerkinElmer Japan Co., Ltd.). The primer sets were as follows: Oatp1, 5′-CATGAGTGTACTTCTCTTTGG-3′ (forward) and 5′-ATTCTGCTGGGTCTTGCGTTGG-3′ (reverse) (Kudo et al., 2002); Oatp2, 5′-TGCACACTTAGCATTCTGGC-3′ (forward) and 5′-TGCATGTAACCCAACTCCAA-3′ (reverse) (Vos et al., 1999); Oatp3, 5′-AGGAAAATCTCTGCAGTCCTGGGGT-3′ (forward) and 5′-TTGGTTCTGGCCTACCATGTTGG-3′ (reverse) (Walters et al., 2000); Oatp4, 5′-GCACCTAGGTACTCTGCATACTATAGCAATGATTGG-3′ (forward) and 5′-GGTACATCTATGTGAGAGTCCACTGGAATCA-3′ (reverse) (Choudhuri et al., 2000); Ntcp, 5′-ATGCCCTTCTCTGGCTTTCT-3′ (forward) and 5′-GCTCCATGGTTCTGATGGTT-3′ (reverse) (Vos et al., 1999); LAT1, 5′-GCTGTGGATTTTGGGAACTACC-3′ (forward) and 5′-CCACACACAGCCAGTTGAAGAA-3′ (reverse) (Boado et al., 1999); LAT2, 5′-GCCTGTGGTATCATTGTTGTAGG-3′ (forward) and 5′-AGTTGACCCATGTGAGCAGC-3′ (reverse) (Kudo et al., 2001); Mrp2, 5′-ACCTTCCAAGCCTAGTCTCCAT-3′ (forward) and 5′-GGCTAGGCACACGAGCT-3′ (reverse) (Rost et al., 2002).

cDNA amplifications were performed using the GeneAmp PCR System 9700 (Thermo Fisher Scientific Inc., Waltham, MA, USA) by the methods previously described for the analyses of hepatic transporter mRNAs (Boado et al., 1999; Choudhuri et al., 2000; Kudo et al., 2001, 2002; Rost et al., 2002; Vos et al., 1998, 1999; Walters et al., 2000). β-actin mRNA was used as an internal control (Vos et al., 1999), and was amplified with the primer pair 5′-CCTAAGGGCAACCGTGGAAAAAG-3′ (forward) and 5′-TCTTCTAAGGCTGCTAGGAGCA-3′ (reverse).

Statistical analysis

Data were analyzed according to the Student’s t test or Dunnett’s test after the analysis of variance. The amount of [125I]T4 uptake by rat liver parenchymal cells was analyzed according to the Newman-Keuls test after the analysis of variance.

RESULTS

Serum T4 level

The effect of KC500 on serum T4 level was first examined in Wistar rats. Total T4 and free T4 in sera were decreased in a time-dependent manner at least up to 96 hr following treatment with KC500 (Fig. 1). Their amounts 96 hr later were below 10% of the corresponding control levels (KC500-untreated rats).

Tissue distribution of [125I]T4

In control rats, the administered [125I]T4 was distributed especially in the liver, and its accumulation level

![Fig. 1. Effects of KC500 on total T4 and free T4 levels in the serum. Rats were killed at appropriate times after the administration of KC500 (100 mg/kg), and levels of serum thyroid hormones were measured. Each point represents the mean ± S.E. (vertical bar) for four to five rats. *P < 0.05, compared with control.](image-url)
(％ of dose) was about 30％ (Table 1). On the other hand, the accumulation levels of the kidney and other tissues (cerebrum, cerebellum, pituitary gland, thyroid gland, sublingual gland, submandibular gland, thymus, heart, lung, kidney, adrenal gland, spleen, testis, prostate gland, seminal vesicle, stomach, duodenum, jejunum, ileum, and cecum) were only about 4％ and below 1％, respectively. Furthermore, relative liver weight (125％ of control) and hepatic [125I]T4 accumulation level (60％ of dose) were significantly increased by KC500 pretreatment (Table 1), but its pretreatment showed no significant effect on body weight of rats (data not shown). In addition, the accumulation levels in the kidney and other tissues were 4.5％ and below 1％, respectively, even after pretreatment with KC500. Tissue (liver)-to-serum concentration ratios (Kp value) in control and KC500-pretreated rats were 0.6 and 3.7, respectively (Table 1).

Effect of KC500-pretreatment on [125I]T4 uptake in hepatic parenchymal cells

[125I]T4 uptake in hepatic parenchymal cells was examined at various doses of [125I]T4 (Fig. 2). In both cells from control and KC500-pretreated rats, [125I]T4 uptake increased in a dose-dependent manner up to a maximum (saturation) of 7.5 μM (Fig. 2). [125I]T4 uptake in KC500-pretreated cells was higher than in control cells at all the concentrations of [125I]T4 used.

Kinetic parameters for [125I]T4 uptake by hepatic parenchymal cells are summarized in Table 2. The maximum reaction rate (Vmax) and Michaelis constant (Km) of [125I]T4 uptake in KC500-pretreated cells were 2.4 and 1.8 times of the corresponding controls, respectively (Table 2).

Hepatic T4-transporter gene expression

Effects of KC500 treatment on mRNA expression levels of hepatic influx T4-transporters, such as LAT1 and Oatp2, were first examined. Following KC500 treatment, hepatic LAT1 mRNA expression increased in a time-de-
pendent manner up to 96 hr (Fig. 3), while Oatp2 mRNA expression was significantly increased at 96 hr compared with the controls, but not at any other timepoints (Fig. 4). The mRNA expression of hepatic efflux T4 conjugate-transporter Mrp3 increased in a time-dependent manner 24-96 hr after KC500 treatment (Fig. 5). In addition, no significant KC500-mediated increases in the mRNA levels of Oatp1, Oatp3, Oatp4, Ntcp, LAT2 and Mrp2 were observed at any timepoints (data not shown).

**DISCUSSION**

In the present study, we first confirmed the decrease in serum T4 level and the liver-selective increase in T4 level by a single intraperitoneal injection of KC500 (100 mg/kg). These effects of KC500 were previously observed in the rats treated with repeated injections (10 mg/kg, i.p., once daily for 10 days) of KC500 (Kato et al., 2007). These findings indicate that the study on biological effects of single treatment with PCB at a high dose is effective for predicting those of its consecutive exposure at a low dose.

There was a clear tissue-difference in distribution of the administered [125I]T4. In control rats, [125I]T4 accumulation level was especially high in the liver (30% of dose) and relatively high in the kidney (4% of dose), as compared with other tissues (below 1% of dose). Such tissue-difference would be primarily dependent on the differences in the tissue volume and the blood flow in a tissue. In the present study, we demonstrated the KC500-mediated increases in hepatic T4 influx transporters, such as LAT1 and Oatp2 (Friesema et al., 2005; Lecureux et al., 2009;
Visser et al., 2011). Furthermore, the increase in relative liver weight (% of body weight) was observed in KC500-treated rats, and this agreed with our previous findings (Kato et al., 2010a). Accordingly, the increase in $[^{125}]$T$_4$ accumulation in the liver by KC500 treatment would be dependent on, at least in part, the increases in hepatic T$_4$ influx transporters, such as LAT1 and Oatp2 (Friesema et al., 2005; Le cureux et al., 2009; Visser et al., 2011), and in the tissue volume. Incidentally, $V_{max}$ value of $[^{125}]$T$_4$ uptake increased, as expected, in hepatic parenchymal cells prepared from KC500-treated rats, although an exact reason for the increase in $K_{m}$ value remains unclear. In addition, T$_4$ influx transporters, LAT1 and Oatp2, are not expressed in the kidney (Kakyo et al., 1999; Kanai et al., 1998).

Since the amount of T$_4$ glucuronide formed in the liver is increased through the KC500-mediated induction of T$_4$-UDP-GT (Kato et al., 2010a), KC500-mediated increase in the expression of an efflux T$_4$ conjugate transporter Mrp3 (Szabo et al., 2009) would promote the excretion of T$_4$ glucuronide from the liver. Under such situation, the amount of hepatic T$_4$ increased in the KC500-treated rats, suggesting that KC500-mediated increase in T$_4$ influx activity is greater than that in the efflux activity for T$_4$ glucuronide.

In conclusion, in the present study, we suggest that KC500-mediated liver-selective accumulation of T$_4$ occurs, at least in part, through the increase in the expression of T$_4$ influx transporters, such as LAT1 and Oatp2, and further suggest that KC500-mediated decreases in serum T$_4$ level in experimental rodents would occur through enhancement of hepatic T$_4$ accumulation, induction of T$_4$-UDP-GT (increase in T$_4$ conjugate formation), and/or increase in Mrp3-mediated T$_4$ conjugate excretion. To understand an exact mechanism for the PCB-induced decrease in serum T$_4$ level, further studies on the PCB-mediated alteration of hepatic activities for the absorption, distribution, metabolism and excretion of T$_4$ would be necessary.

ACKNOWLEDGMENTS

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

Conflict of interest—The authors declare that there is no conflict of interest.

REFERENCES


Induction of hepatic T₄ transporters by PCB


