SPECIES DIFFERENCE AMONG MICE, HAMSTERS, RATS AND GUINEA PIGS IN KANECHLOR-500-INDUCED ALTERATION OF SERUM THYROID HORMONE LEVEL

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Introduction

In general, the decrease[1-4] in serum thyroxine (T_4) level by PCBs such as 3,3',4,4'-tetrachlorobiphenyl, 2,3',4,4',5'-pentachlorobiphenyl, 2,3,3',4,4',5- and 2,2',4,4',5,5'-hexachlorobiphenyls and Aroclor 1254 has been thought to occur through the induction of T_4-UDP-glucuronosyltransferases (T_4-UDP-GTs)[4-6]. However, we previously found that Kanechlor-500 (KC500), a commercial PCB mixture, treatment resulted in a significant decrease in serum T_4 level in both rats and mice, whereas a significant increase in activity of the UDP-GT responsible for glucuronidation of T_4 (T_4-UDP-GT) was observed in rats but not in mice[7]. More recently, we indicated that the activity of T_4-UDP-GT was significantly increased in Wistar rats, but not in UGT1A-deficient Gunn rats, by KC500-treatment, although serum total T_4 levels in the both strains of rats were significantly reduced by the treatment[8]. These findings suggest strongly that the decrease in serum total T_4 level in not only mice but also rats by PCBs would not occur only through increase in hepatic T_4 glucuronidation.

In the present study, we further examined the species differences among mice, hamsters, rats and guinea pigs in the induction of drug-metabolizing enzymes, in the *in vivo* metabolism of KC500 and in the alteration of serum thyroid hormone level by KC500.

Materials and Methods

Animal treatments. Male ddy mice weighing 28-36 g, male Syrian hamsters weighing 95-120 g, male Wistar rats weighing 160-200 g, and male Hartley guinea pigs weighing 400-540 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) and humidity (55 ± 5%). The animals received an intraperitoneal injection of KC500 (37.5 mg/kg) dissolved in Panacete 810 (5 ml/kg). Control animals received an equivalent volume of vehicle. All animals were killed by decapitation on day 4 after the dosing, and the tissues 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 prior to determination of the levels of total T_4,

total triiodothyronine (T_3) and thyroid stimulating hormone (TSH) by radioimmunoassay using Amerlex-MT4, Amerlex-MT3 (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 the microsomal enzyme assays. Hepatic microsomes were prepared according to the procedure described previously[9]. The protein content was determined by the method of Lowry *et al.*[10] with bovine serum albumin as a standard. The activities of 7-ethoxyresorufin, 7-pentoxyresorufin and 7-benzyloxyresorufin *O*-dealkylases in hepatic microsomes were determined by the method of Burke *et al.*[11] The microsomal activities of UDP-GT toward chloramphenicol and T_4 were determined as described by Ishii *et al.*[12] and Barter and Klaassen[13], 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 for the UGTs were performed with microsomal preparations as described by Luquita *et al.*[14]

Determination of PCBs in the liver. The concentration of PCBs in the liver was determined with GC/MS as described by Mimura *et al.*[15] The quantification of hydroxyl (OH) and methylsulfonyl ($MeSO_2$) metabolites was performed on GC/ECD (GC-14A, Shimadzu) by comparison with internal standards of 2,2',3,4',5,5',6-heptachloro-4-[^{13}C]-biphenylol for OH metabolites, and 4-methyl-3-$MeSO_2$-2,2',3',4',5'-pentachlorobiphenyl for $MeSO_2$ metabolites.

Results and Discussion

Serum total T_4 level was significantly decreased by KC500 treatment in mice, hamsters, rats and guinea pigs (Fig. 1). The activity of UDP-GT (UGT1A1 and UGT1A6) toward T_4 was significantly increased by KC500 in only guinea pigs (Fig. 2). The content of UGT1A isoforms was significantly increased by KC500 in rats and guinea pigs. Serum total T_3 level was significantly decreased by KC500 treatment in guinea pigs. No significant change in the level of serum TSH was observed in any species of animals used. KC500 administration resulted in significant increases in hepatic microsomal enzyme activities: benzyloxyresorufin *O*-dealkylase activity, 3-, 3.7- and 34.4-fold in mice, hamsters and rats, respectively; pentoxyresorufin *O*-dealkylase activity (CYP2B1/2), 2-, 1.7- and 19.2-fold in mice, hamsters and rats, respectively; ethoxyresorufin *O*-dealkylase activity (CYP1A1/2), 1.6-, 27.6- and 3.7-fold in hamsters, rats and guinea pigs, respectively. KC500 treatment resulted in significant increases in the activity of UDP-GT (UGT2B1) toward chloramphenicol in rats and guinea pigs and the content of the UGT2B1 isoform in rats and hamsters. The total hepatic concentrations of 3- and 4-$MeSO_2$ metabolites were higher in the order as followed: mice > guinea pigs > rats > hamsters. The total hepatic concentrations of OH metabolites were higher in the order as followed: guinea pigs > hamsters > rats > mice.

In conclusion, there are marked differences in the decrease of serum total T_4 and T_3 levels and in the induction of UGT1A1, UGT1A6 and UGT2B1 by KC500 among mice, hamsters, rats and guinea pigs. Thus species difference in the reduction of serum total T_4 level is not necessarily correlated with that in the metabolism of KC500 and with that in alteration of the activities of microsomal hepatic UGT1A1 and UGT1A6. The present findings suggest that in guinea pigs, the reduction of serum total T_4 level by KC500 would occur at least in part by an increase in T_4 glucuronidation through the induction of hepatic T_4-UDP-GT, while in mice, hamsters and rats, the decrease of serum total T_4 level may occur through alternative mechanisms.

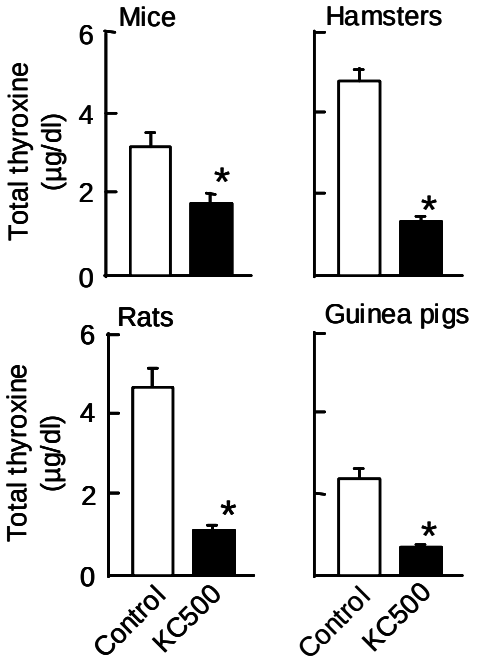

Fig. 1. Effects of KC500 on serum total thyroxine concentration in mice, hamsters, rats and guinea pigs. Animals were given KC500 (37.5 mg/kg) i.p. and killed at 4 days after the administration. Each column represents the mean } S.E. (vertical bars) for six animals. *P <0.05, significantly different from each control.

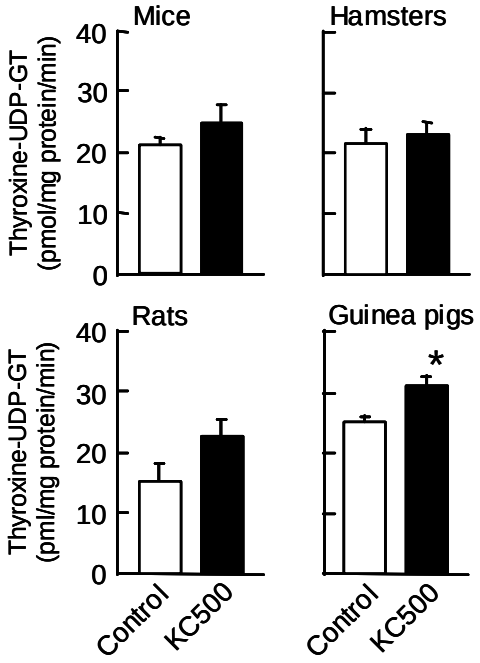

Fig. 2. Effects of KC500 on UDP-glucuronyltransferase activity toward thyroxine of liver microsomes in mice, hamsters, rats and guinea pigs. The experimental conditions were the same as described in the note to Fig. 1. Each column represents the mean } S.E.(vertical bars) for six animals. *P<0.05, significantly different from each control.

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