IDENTIFICATION AND TISSUE DISTRIBUTION OF CATECHOL METABOLITES OF POLYCHLORINATED BIPHENYLS IN RODENTS DOSED WITH KANECHLOR 500

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Introduction

The enzyme-mediated biotransformation of polychlorinated biphenyls (PCBs) depends on both the congener structure and the metabolic capacity of the species. *In vivo* metabolism of PCBs proceeds via cytochrome P450-mediated formation of arene oxide intermediate, which results in both hydroxylated (OH) and methylsulfonyl ($MeSO_2$) metabolites. Some of these metabolites are persistent and shown to have a specific retention in blood or tissues of animals exposed to PCBs[1], which would be due to binding to transthyretin[2] and cause alteration in the thyroid hormone metabolism[3]. These PCB metabolites have been detected in the blood from wildlife[4] as well as humans,[5, 6] in which the residual metabolite profiles have been shown to vary among species. Recently, we detected catechol metabolites in tissues of rodents dosed with 2,4,5,2',5'-pentaCB and 2,3,4,2',3',6'-hexaCB, which were markedly retained in blood of hamsters[7].

The present study has focused on determining the chemical structures, residue patterns and levels of catechol PCB metabolites in the liver and blood of rats, hamsters and guinea pigs exposed to Kanechor 500 (KC500), a technical PCB mixture.

Material and Methods

Chemicals

The catechol (veratrole) metabolites of PCBs were synthesized by diazo-coupling of 4-amino-veratrole with chlorinated benzene, followed by chlorination with sodium chlorate. All solvents were of pesticide grade.

Animals.

KC500 was injected i.p. to male Wistar rats, male Hartley guinea pigs and male Syrian hamsters at a single dose of 37.5 mg/kg. In addition, male Wistar rats (160-200 g) and homozygous Gunn rats (190-260 g), which are hereditarily UDP-glucuronosyltransferase- deficient, received an i.p. injection of KC500 (100 mg/kg). Prior to sacrifice, animals were starved for 12 h. After the dosing, these animals were killed by decapitation on day 4 and several tissues and blood were removed and stored at –20°C prior to analysis.

Isolation and determination of metabolites

Sample cleanup and quantification were carried out according to our previous methods[8] using three internal standards, 2,3,4,5,3',4',5'-heptaCB, ^{13}C-labelled 4-OH-2,3,5,6,2',4',5'-heptaCB and 4'-methyl-3'-$MeSO_2$-2,3,4,5,5'-pentaCB. After GPC purification, the metabolites were partitioned between neutral and acidic fractions. The acidic metabolites were methylated by diazomethane and identified on GC/MS system (EI, SIM mode, AOC-17, GC-17A, QP-5000, Shimadzu) with a DB-5 capillary column (60 m x 0.25 mm, i.d.) by comparison with authentic synthesized metabolites (as methoxy or veratrole derivatives).

Results and Discussion

Residual PCB pattern in liver

The original composition of individual PCBs in KC500 was CB52 (5.6% of total PCBs), CB95 (6.6%), CB101 (10.0%), CB110 (7.4%), CB118 (7.7%), CB138 (5.3%) and CB153 (5.4%). After exposure of animals to KC500, the dominating PCB residues in liver were CB138 (13.7%) for rats, CB153 (17.0%) for hamsters and CB118 (32.2%) for guinea pigs. The non-planar PCBs (e.g. CB85, CB99, CB128) were the highest in rats, whereas the coplanar PCBs (e.g. CB37, CB77, CB126) were the highest in guinea pigs. PCBs with 2,5- or 2,3,6-chlorine substitution (e.g. CB52, CB95, CB101, CB110) were eliminated rapidly in rats and guinea pigs, although they were slightly retained in hamsters 4 days after exposure.

$MeSO_2$ metabolites in liver and lung

Rat liver retained preferably 3- and 4-$MeSO_2$ metabolites derived from CB70, CB87, CB101 and CB149, with the similar concentration ratios of 3- and 4-substituents. The hamster liver retained only slightly 3- and 4-$MeSO_2$ metabolites of CB70 and CB101. In contrast, guinea pig liver retained selectively 3-$MeSO_2$ metabolites derived from CB64, CB87, CB95, CB101, CB132, CB141 and CB149 (the ratio of total 3-/ 4-$MeSO_2$-PCBs was 9.2). Sum of $MeSO_2$-PCBs formed were the highest in guinea pigs, followed by in rats. In lung of rats, 4-$MeSO_2$-2,5,2',4',5'-pentaCB was strongly retained (4-/3-$MeSO_2$-CB101 ratio was >11.1), but not specific for lungs of hamsters and guinea pigs.

Phenolic metabolites in liver and blood

A specific retention of 4-OH-2,3,5,3',4'-pentaCB (4-OH-CB107, >85% contribution of total OH-PCBs) was observed for liver and blood of rats, whereas 3-OH-2,4,5,2',4'-pentaCB (3-OH-CB99) and 3-OH-2,4,5,2',3',4'-hexaCB (3-OH-CB138) were distributed in tissues of hamsters and guinea pigs. In liver of hamsters, both 3- and 4-hydroxy metabolites from CB138, CB146, CB153 and CB187 were detected. Sum of OH-PCBs were the highest in hamsters, followed by in rats. The ratio of 3-OH-CB99/parentCB99 was the highest (>1.0) in serum of hamsters and guinea pigs, whereas the ratio of 4-OH-CB107/CB118 was the highest in serum of rats.

Catechol metabolites in liver and blood

After exposure to KC500, hamster produced several dihydroxy PCB metabolites, which were identified as catechol PCB metabolites derived from CB70, CB87, CB101, CB110, CB132 and CB149 by GC/MS comparison with authentic compounds. Rats and guinea pigs also produced these catechols at trace amounts. The chemical structures of catechol metabolites and their levels in blood of rats, hamsters and guinea pigs 4 days after the dosing are shown in Table 1. In serum of hamster, about 45% of the total persistent PCB metabolites consisted of catechol metabolites, the major metabolites being diOH-CB101, diOH-CB110 and diOH-CB149.

In contrast to male Wistar rats, homozygous Gunn rats, which are hereditarily UGT1s deficient, highly produced catechol metabolites, the major congeners being diOH-CB101 and diOH-CB110

in serum. Table 2 shows the levels of total PCBs and their metabolites in liver of Wistar and Gunn rats. The catechol PCB metabolites were highly distributed in other tissues such as kidney, lung and brain and adipose tissues in Gunn rats. The comparative study between both rats suggests that formation and retention of catechol PCB metabolites are strongly related to the deficiency of UDP-GT, leading to poor formation of $MeSO_2$-PCBs in Gunn rats.

These catechol PCB metabolites are considered to be formed by further oxidation of phenolic PCBs or by hydrolysis of epoxide intermediates. It remains unclear whether high concentrations of catechol PCB metabolites exert toxic effects on humans. However, they can be further metabolized to highly reactive quinones, which in turn may react with endogenous nucleophiles[9]. In addition, hydroxylated PCB metabolites are known to exhibit the estrogenic effects. Kester *et al*[10] demonstrated that various environmentally relevant PCB metabolites are extremely potent inhibitors of human enstrogen sulfotransferase. Garner *et al*[11] demonstrated the in vitro estrogenicity of catechol PCB metabolites, which would not necessarily result in lowering the total estrogen burden of a PCB exposed organism.

Table 1. Concentration of PCB metabolites in serum of rats, hamsters and guinea pigs dosed with Kanechlor 500 (37.5 mg/kg, i.p.)

		Concentration (ng/g, wet weight)		
Congener		Rat	Hamster	Guinea pig
PCB				
2,4,5,2',4',5'-hexachlorobiphenyl	CB153	109 ± 35	58.0 ± 22	43.5 ± 16
Phenolic PCBs				
2,4,5,2',4'-pentachloro-3-biphneylol	3-OH-CB99	8.1 ± 4.8	79.5 ± 27	51.2 ± 15
2,3,5,3',4'-pentachloro-4-biphneylol	4-OH-CB107	339 ± 111	32.2 ± 14	3.5 ± 1.0
2,5,4,2',3',4'-hexachloro-3-biphenylol	3-OH-CB138	15.6 ± 7.5	18.8 ± 8.2	35.7 ± 16
Catechol PCBs				
2,5,3',4'-tetrachloro-3,4-biphenyldiol	diOH-CB70	5.0 ± 2.5	40.0 ± 11	1.0 ± 1.3
2,5,2',3',4'-pentachloro-3,4-biphenyldiol	diOH-CB87	4.5 ± 3.2	13.1 ± 8.4	1.5 ± 0.3
2,5,2',4',5'-pentachloro-3,4-biphenyldiol	diOH-CB101	12.4 ± 5.1	62.7 ± 15	8.5 ± 2.1
2,3,6,3',4'-pentachloro-4,5-biphenyldiol	diOH-CB110	10.1 ± 6.4	75.4 ± 15	2.1 ± 1.0
2,3,6,2',3',4'-hexachloro-4,5-biphenyldiol	diOH-CB132	5.0 ± 4.1	5.0 ± 2.3	2.1 ± 1.0
2,3,6,2',4',5'-hexachloro-4,5-biphenyldiol	diOH-CB149	8.5 ± 3.0	85.1 ± 25	5.2 ± 1.5

Each value represents the mean ± S.D. for six animals

Table 2. The levels of PCBs and their metabolites in liver of Wistar and Gunn rats exposed to Kanechlor 500 (100 mg/kg, i.p.)

	Levels (ng/g wet weight)	
Congeners	Wister rat	Gunn rat
∑ PCB	8715 ± 566	3964 ± 1796
∑ OH-PCBs	451 ± 62.7	144 ± 77
∑ $(OH)_2$-PCBs	29.1 ± 7.5	290 ± 129
∑ $MeSO_2$-PCBs	566 ± 80.1	139 ± 77.8
Ratio		
∑ PCB metabolites / ∑ PCB	0.12	0.15

Each value represents the mean ± S.D. for four animals.

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