Comparative metabolism of polychlorinated biphenyls with 2,4,5-trichloro substitution in rats: Regioselective formation of hydroxy metabolites with high blood affinity

Koichi Haraguchi, Yoshihisa Kato*, Ryohei Kimura* and Yoshito Masuda

Daiichi College of Pharmaceutical Sciences, 22-1 Tamagawa-cho, Minami-ku, Fukuoka 815-8511, Japan
*School of Phamaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan.

Introduction

Polychlorinated biphenyls (PCBs) are in general biotransformed to hydroxylated or methylthio/methylsulfonyl metabolites, depending on the chlorine substitution pattern. Some of the hydroxylated PCB metabolites have been shown to be retained in blood, at the comparable levels to the unchanged PCBs, of grey seal and human in the Swedish environment [1], as well as in experimental rat [1], mink and mouse [2]. These metabolites have been shown to have an effect on the endocrine system by disrupting thyroid hormone transport [3-5]. Hydroxy metabolites with high blood affinity are known to have a structural similarity with chlorine atoms on the adjacent positions to the hydroxy group, and therefore such products may be originated from PCBs with 3,4-dichloro, 2,3,4- or 2,4,5-trichloro substitution.

In the present study, we compared the metabolism of four 2,4,5-trichloro-substituted PCBs (Fig. 1) in rats, with regard to the hydroxylation products in feces and their concentrations in blood.

Materials and method

Male Wistar rats (200g) received a single i.p. injection of 2,4,5,3',4'-pentaCB[1] (80 μmol/kg), 2,4,5,2',3',4'-hexaCB, 2,4,5,2',4',5'-hexaCB and 2,4,5,3',4',5'-hexaCB (each of 40 μmol/kg). Feces were collected daily for 4 days. Rats were killed 96 hours after administration, and blood was removed and analyzed for metabolites. Clean-up and determination of metabolites were performed as described previously [6]. For identification of metabolites, methoxy derivatives of PCBs were synthesized according to a method by Bergman *et al.* [7]. Other chemicals were obtained as commercial sources.

[1] The numbering of the chlorines is not according to the IUPAC rules but has been chosen to facilitate the reading of the structures.

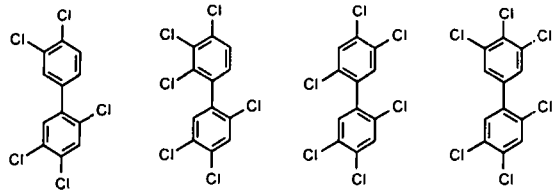

Fig. 1 Chemical structures of PCB congeners investigated

Results and discussion

2,4,5,3',4'-pentaCB (CB118[2]): Major fecal metabolites were identified as 4-OH-2,5,3',4'-tetraCB, 4-OH-2,3,5,3',4'-pentaCB, 4'-OH-2,4,5,3',5'-pentaCB and 5'-OH-2,4,5,3',4'-pentaCB which were excreted in about a 12:4:1:1 ratio, respectively. Among them, only 4-OH-2,3,5,3',4'-pentaCB and 4'-OH-2,4,5,3',5'-pentaCB were selectively retained in blood in an about 4:1 ratio. Both metabolites have been also observed in seal and human plasma [1]. The 4-OH-2,3,5,3',4'-pentaCB has been also originated from 2,3,4,3',4'-pentaCB (CB105) in mink and mouse [2]. Recently we found that the fecal amounts and blood concentration of 4-OH-2,3,5,3',4'-pentaCB from CB105 were similar to those from CB118 treated rats [9].

2,4,5,2',3',4'-hexaCB (CB138): Two hydroxy metabolites were isolated from CB138 treated rats and identified as 3-OH-2,4,5,2',3',4'-hexaCB and 5'-OH-2,4,5,2',3',4'-hexaCB. The fecal excretion ratio was about 10:1. NIH-shifted or dechlorinated hydroxy product was not identified in feces and blood. The 3-OH-2,4,5,2',3',4'-hexaCB was selectively retained in blood.

2,4,5,2',4',5'-hexaCB (CB153): Only one metabolite, 3-OH-2,4,5,2',4',5'-hexaCB was isolated from feces and blood of rats dosed with CB153. The fecal metabolite level from CB153 treatment was less than one-tenth as compared to that from CB138.

2,4,5,3',4',5'-hexaCB (CB167): Rats dosed with CB167 excreted an about 5:1 ratio of 4-OH-2,5,3',4',5'-pentaCB and 4-OH-2,3,5,3',4',5'-hexaCB in feces. Both metabolites were detected in blood in an about 1:3 ratio, indicating the higher blood affinity of 4-OH-2,3,5,3',4',5'-hexaCB. In fact, this metabolite has been identified in human plasma [10].

Comparing the metabolism of four PCB congeners in rats, we found that 2,4,5-trichlorophenyl nucleus could undergo hydroxylation more preferably than 3,4-di-, 2,3,4-tri- or 3,4,5-trichlorophenyl nucleus. In addition, hydroxylation mechanism of CB118 and CB167 was clearly different from that of CB138 and CB153. Metabolism of CB118 and CB167 involved selectively 4-hydroxylation via a 3,4-epoxide, followed by NIH-shift or dechlorination of 4-chlorine, whereas metabolism of CB138 and CB153 involved selectively 3-hydroxylation via direct insertion of a hydroxy group into the 3-position. These findings

[2] Numbering of CB's according to Ballschmiter *et al.* [8]

suggest that the regioselectivity of hydroxylation at the 2,4,5-trichlorinated ring is closely related to the planarity of the molecule, i.e., the number of *ortho*-chlorine on the opposite ring.

Table 1 shows the concentrations of major hydroxy metabolite and unchanged PCB in blood of rats 96 hours after PCB treatment. All rats dosed with each PCB congener exhibited higher concentration ratios (2.6-27) of hydroxy metabolites as compared to the unchanged PCB in blood, although the metabolite level from CB153 was rather low. Dechlorinated hydroxy products, which were major metabolites in feces of rats dosed with CB118 and CB167, showed much less or no affinity to blood. Thus, hydroxy metabolites for blood affinity had a structural similarity with chlorine atoms on the adjacent position to the hydroxy group, as shown in previous study [1,5]. Most of metabolites identified in this study have been also detected in rats exposed to Aroclor 1254, as well as in seal and human blood [1,10]. The retention has been shown to be due to binding to transthyretin concomitant with a reduction in levels of circulating thyroid hormone and vitamin A in the blood [3-5].

Table 1. Structures of hydroxy metabolites with high affinity to blood and their concentrations in rats dosed with each PCB congener.

PCB congener	Dose (μmol/kg, i.p.)	Structure of major hydroxy metabolites in blood	Concentration (nmol/g) Unchanged PCB	Concentration (nmol/g) Hydroxy metabolite	Ratio (Hydroxy/ Unchanged)
CB118	80	4-OH-2,3,5,3',4'-pentaCB	0.21 ± 0.10	1.55 ± 0.14	7.4
CB138	40	3-OH-2,4,5,2',3',4'-hexaCB	0.018 ± 0.009	0.49 ± 0.16	27.2
CB153	40	3-OH-2,4,5,2',4',5'-hexaCB	0.025 ± 0.006	0.065 ± 0.035	2.6
CB167	40	4-OH-2,3,5,3',4',5'-hexaCB	0.023 ± 0.006	0.43 ± 0.17	18.7

Values are means ± SD obtained from 4 rats.

In conclusion, PCBs with 2,4,5-trichloro substitution can be extensively metabolized to 3- or 4-hydroxy metabolite in rats by two hydroxylation processes which may be mediated by different cytochrome P450 isozymes induced by PCB congeners. Since PCBs and related compounds having 2,4,5-trichloro substitution are abundant in environemntal extracts, hydroxy metabolites derived from them may contaminate the blood of various species including human.

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research (C) (no. 09680531) from the Ministry of Education, Science, Sports and Culture of Japan.

References

[1] Bergman, Å., Klasson-Wehler, E. and Kuroki, H.; *Environm. Health Perspect.*, **1994**, 102, 464.

[2] Klasson-Wehler, E., Lindberg, L., Jonsson, C.-J. and Bergman, Å.; *Chemosphere*, **1993**, 27, 2397.

[3] Brouwer, A., Lans, M.C., de Haan, L.H.J., Murk, A.J. and Morse, D.C.; *Organohalogen compounds*, **1994**, 20, 465.

[4] Darnerud, P.O., Morse, D., Klasson-Wehler, E. and Brouwer, A.; *Toxicology*, **1996**, 106, 105.

[5] Lans, M.C., Klasson-Wehler, E., Willemsen, M., Meussen, E., Safe, S. and Brouwer A.; *Chem.-Biol. Interact.*, **1993**, 88, 7.

[6] Haraguchi, K., Kato, Y., Kimura, R. and Masuda, Y.; *Drug Metab. Dispos.*, **1997**, 25, 845.

[7] Bergman, Å., Klasson-Wehler, E., Kuroki, H. and Nilsson, A.; *Chemosphere*, **1995**, 30, 1921.

[8] Ballschmiter, K.H., Mennel, A. and Buyten, J.; *Fresenius J. Anal. Chem.* **1992**, 346, 396.

[9] Haraguchi, K., Kato, Y., Kimura, R. and Masuda, Y.; *Jpn. J. Toxicol. Environ. Health*, **1998**, 44, P20.

[10] Klasson-Wehler, E., Hovander, L. and Bergman, Å.; *Organohalogen Compounds*, **1997**, 33, 420.