SELECTIVE RETENTION OF HYDROXYLATED PCB METABOLITES

Eva Klasson Wehler, Environmental Chemistry, Wallenberg Laboratory, Stockholm University, S-106 91 Stockholm, Sweden.

Polychlorinated biphenyls (PCB) are lipophilic compounds resistant to chemical reactions in the physical environment and they are therefore accumulated in the foodwebs. By enzymatically catalyzed metabolic reactions, in at least higher animals, PCB congeners are chemically transformed. Metabolically, the parent compounds are generally oxidized to more polar products such as hydroxylated metabolites. These semi-polar chlorobiphenyl (CB) metabolites can be conjugated to glucuronic acid or to sulphate which increases the hydrophilicity and facilitates subsequent excretion via bile and urine. However, metabolites are not always excreted but may instead be retained selectively in tissues, as shown for several methyl sulphonyl metabolites of PCB (reviewed by prof Bergman and therefore not included here). In the present work, the selective retention of hydroxylated CB metabolites (OH-CBs) will be reviewed.

Irrespective of species, the rate of metabolism depends on the number and positions of chlorine atoms. Thus, highly chlorinated biphenyls (CBs) without vicinal unsubstituted positions are slowly metabolized, e.g. 2,4,5,2',4',5'-hexaCB (CB-153[1]) whereas CBs with vicinal hydrogens in the *meta-/para*-positions are more rapidly metabolised[2]. An example is 2,5,2',5'-tetraCB, with vicinal unsubstituted positions in the *meta-/para*-positions, which is rapidly metabolized both to hydroxylated metabolites and products from the mercapturic acid pathway, e.g. mercapturic acid conjugates and methylsulphonyl-CBs[3]. CBs with chlorine atoms in 3,4- or 2,3,4-position are also metabolized to hydroxylated metabolites, with, or without, rearrangement via a 1,2-shift[4,5]. Thus, e.g. 3,4,3',4'-tetraCB (CB-77) is metabolized to 5-OH-3,4,3',4'-tetraCB and to the rearranged metabolite 4-OH-3,5,3',4'-tetraCB[4].

To observe selective retention of compounds, metabolized or not, the use of radiolabelled material and whole-body autoradiography, greatly facilitates the study. The radiolabel shows if and where selective tissue retention occurs, and analysis shows the identity of the retained compound. One example of this is the autoradiographic study of 2,5,4'-triCB (CB-31) in pregnant mice[6]. The autoradiography showed strong accumulation of radiolabelled compound(s) in the bronchi, the uterine fluid and the yolk sac epithelium. The radiolabel in the bronchi was earlier shown to consist of 4-methylsulphonyl-CB-31[7]. Methylsulphonyl metabolites (0.3 nmol/animal) were present in he uterine luminal fluid as well, but in this case the dominant metabolites OH-triCBs (0.8 nmol/animal). Conjugated OH-triCBs (0.2

[1]Numbering of CBs according to Ballschmiter *et al*[1]

nmol/animal) were also found, but no unmetabolised triCB was present. The hydrophilic uterine fluid contained much higher concentration of these lipophilic compounds than did the surrounding tissue which may point to the presence of carrier proteins[6].

CB-77 has also given rise to a similar high concentration of radiolabelled material in uterine fluid in pregnant mice as reported by Darnerud and co-workers[8]. There was also a particularly strong accumulation in the blood and the soft tissues of fetuses in late pregnancy (dosing day 13 and analysis day 17)[8]. Analysis of the whole fetuses showed that the radioactivity corresponded mainly to hydroxylated metabolites, the major metabolite being identified as 2-OH-CB-77. However, this was later shown to be wrong and 4-OH-3,5,3',4'-tetraCB was instead determined as the major metabolite (unpublished data). The accumulation of the *para*-substituted CB-77 metabolite in blood was confirmed by analysis of plasma from virgin mice, where the hydroxy-metabolite was present in 15 times higher concentration than the parent CB five days after an oral dose of CB-77[9], and in fetal and maternal mouse blood, where the 4-OH-3,5,3',4'-tetraCB to CB-77 ratio was similar (unpublished data). The concentration of the metabolite was 4.7 times higher in fetal blood than in the maternal (unpublished data). In an experiment with rats, exposure *in utero* to CB-77 caused a similar accumulation of 4-OH-3,5,3',4'-tetraCB in rat fetuses[10].

2,3,4,3',4'-PentaCB (CB-105) also gives rise to a similar high concentration of a 1,2-shifted metabolite - 4-OH-2,3,5,3',4'-pentaCB (15 times higher than the parent CB) - in mouse blood[5]. The selective retention in blood of the OH-metabolites of CB-77 and CB-105 may be due to their ability to bind to transthyretin (TTR), a thyroxin transporting protein. All three have halogen on each *meta*-position in the same ring as the *para*-substituted hydroxy-group; this allows the OH-CBs to compete with thyroxin for the TTR binding site[11,12,13]. Brouwer has shown that 4-OH-3,5,3',4'-tetraCB, the CB-77 metabolite, binds to TTR and thereby interferes not only with the thyroxin-transportation but also with the transportation of vitamin A[14]. *In vitro* binding studies of synthesized OH-CBs has also shown that *para*-hydroxylated metabolites of CB-77 and CB-105 have a higher affinity (2.5 and 6 times, respectively) than thyroxine for the binding site on TTR[11,12].

In order to investigate whether other CBs are metabolized to OH-CBs that are selectively retained in blood, rats were dosed with a technical PCB mixture, Aroclor 1254, and 24h, 7 days and 14 days after dosing the rats were killed and the blood analyzed. The GC/MS analysis showed one dominant OH-CB and another 12 minor OH-CBs in the blood[15]. The dominant OH-CB was 4-OH-2,3,5,3',4'-pentaCB - a CB-105 metabolite The levels of the 4-OH-2,3,5,3',4'-pentaCB was 10 times higher than that of the most persistent CB, CB-153, one week after dosing. To check whether OH-CBs also could be determined in an environmentally exposed species, Baltic grey seal blood was analyzed and shown to also contain high concentrations of OH-CBs . The level of the CB-105 metabolite was approximately 20% compared to that of CB-153 (7.4 ng/g f.w., range 4.4-45 ng/g). The OH-CBs were generally the same as in the rat blood but the relative amounts of the total 14 OH-CBs that were indicated by GC/MS, differed compared to the rat. Also in human plasma samples, randomly selected from a blood donor bank in Stockholm, OH-CBs were determined in concentration in the same range as PCB (total PCB 3.6 ± 1.6 ng/g f.w.), a ratio of 0.08-0.35 (mean 0.18) of the OH-metabolite of CB-105 over CB-153. Again the same OH-CBs as in the other two species were identified but again, the relative amounts differed and in the human plasma samples a dominant OH-CB was identified as 4-OH-2,3,5,6,2',4',5'-heptaCB, a possible metabolite of CB-187 and/or CB-183.

The OH-CBs retained in blood from humans, seals and rats, are structurally similar, with

chlorine atoms on the adjacent positions to the hydroxy-group, which was either in a *meta*- or a *para*-position. Considering that the number of potential hydroxy-metabolites (at least 2 metabolites/CB) that may be formed from the PCB congeners that e.g. rats (Aroclor 1254; ca 80 CBs[16]) are exposed to, the selective retention is striking.

The analyses of OH-CBs in plasma were performed by partitioning the extracts between an organic solvent (hexane) and potassium hydroxide (1 M KOH in 50% ethanol), in order to isolate the phenolic metabolites from the neutral CBs. The isolated OH-CBs were re-extracted into an organic solvent, after acidification of the aqueous phase, and derivatized with diazomethane. Methylation as derivatization method was chosen since the products, MeO-CBs, give characteristic fragmentation patterns as determined by electron ionization mass spectrometry (MS(EI))[17]. Thus *ortho*-substituted MeO-CBs give abundant (M-50) fragments, *para*-substituted MeO-CBs give abundant (M-15) fragments whereas the *meta*-MeO-CBs gives (M-43) as abundant fragments.

In the analysis of plasma and blood samples, GC/MS was used for structural identification of OH-CBs, analyzed as the methylated derivatives, to determine the position of the MeO-group and the number of chlorine atoms. However, to identify the structure of the OH-CBs, synthesized standards are needed. A large number of MeO-CBs were therefore synthesized in order to identify at least the major OH-CBs in the plasma samples. To date, 51 different MeO-CBs have been synthesized and characterized by MS(EI) and negative ion chemical ionization mass spectrometry (MS(NICI))[18]. Either the Cadogan diaryl coupling reaction between a chloroaniline and a chloroanisole, or the Ullman diaryl coupling reaction between an iodoanisol and a chloroiodobenzene, and subsequent chlorination of the positions *ortho* to the MeO-group of the obtained product were used for synthesis of the MeO-CBs.

Even with standards, the unambiguous identification of metabolites may not be straightforward. Many of the relevant MeO-CBs co-elute also with capillary GC, e.g. on a DB- 5 column. It was necessary to analyse the samples on two different columns, a DB-5 fused silica capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific Inc) and a cyano derivatized column (SP-2331, 0.25 mm i.d., Supelco Inc), in order to identify the retained OH-CBs. For example the CB-105 is metabolized to two *para*-substituted metabolites, one by an 1,2-shift in the dichlorinated phenyl ring - 4-OH-3,5,2',3',4'-pentaCB, and one by a 1,2,-shift in the trichlorinated phenyl ring -4-OH-2,3,5,3',4'-pentaCB[5]. The methyl derivatives of these two isomers have identical GC retention times on a DB-5 column, and identical fragmentation patterns in MS(EI), but different retention times on the SP-2331 column. On the other hand, some MeO-CBs that separate on the DB-5 column co-elute on the SP-2331 column, thus two columns were needed for identification. By using both columns, it was shown that 4-OH-2,3,5,3',4'-pentaCB was retained in rat and mouse blood, whereas both isomers were present in seal and human blood[14].

In conclusion, metabolism does not necessarily lead to excretion of the metabolites. Even semi-polar metabolites such as OH-CBs may be retained, when a sufficient structural similarity with endogenous compounds is at hand. The toxicological significance of selective tissue localization of compounds must be considered in risk assessment.

References

1) Ballschmiter, K.H., A. Mennel and J. Buyten. Long chain alkyl-polysiloxanes as non-polar stationary phases in capillary gas chromatography. Fresenius J. Anal. Chem. 346 (1992) 396-402.

2) Bergman, Å., M. Athanasiadou, S. Bergek, K. Haraguchi, S. Jensen and E. Klasson Wehler. PCB and PCB methyl sulphones in mink treated with PCB and various PCB fractions. Ambio, 21 (1992) 570-576.
3) Bakke, J.E., Feil, V.J. and Bergman, Å.: Metabolites of 2,4',5-trichlorobiphenyl in rats. Xenobiotica 13 (1983) 555-564.
4) Klasson Wehler, E., Bergman, Å., Darnerud, P.O., Wachtmeister, C.A.: 3,3',4,4'-Tetrachlorobiphenyl: Excretion and tissue retention of hydroxylated metabolites in the mouse. Drug. Metab. Dispos, 17 (1989) 441-448.
5) Klasson Wehler, E., Lindberg, L., Jönsson, C.J. and Bergman Å. Tissue retention and metabolism of 2,3,3',4,4'-pentachlorobiphenyl in mink and mouse. Chemosphere, 27 (1993) 2397-2412.
6) Brandt, I., Darnerud, P.O., Bergman, Å. & Larsson, Y. Metabolism of 2,4',5-trichlorobiphenyl: enrichment of hydroxylated and methyl sulphone metbaolites in the uterine luminal fluid of pregnant mice. Chem.-Biol. Interact. 40 (1982) 45-60.
7) Bergman, Å., Brandt, I. and Jansson, B. Accumulation of methyl-sulfonyl derivatives of some bronchial-seeking polychlorinated biphenyls in the respiratory tract of mice. Toxicol. Appl. Pharmacol. 48 (1979) 213.
8) Darnerud, P.O., Brandt, I., Klasson-Wehler, E., Bergman, Å., d'Argy, R., Dencker, L. and Spoerber, G.O.: 3,3',4,4'-Tetrachloro-[^{14}C]-biphenyl in pregnant mice: Enrichment of phenol and methyl sulphone metabolites in late gestational fetuses. Xenobiotica 16 (1986) 295-306.
9) Klasson Wehler, E., *Synthesis of some radiolabelled organochlorines and metabolism studies in vivo of two PCBs. Thesis (ISBN: 91-7146-729-7) 1989.*
10) Morse, D., Klasson Wehler, E., van de Pas, M., de Bie, A.Th.H.J., van Bladeren, P.J. and Brouwer, A. metabolism and biochemical effects of 3,3',4,4'-tetrachlorobiphenyl in pregnant and fetal rats. Chem.-Biol. Interact., *in press.*
11) Brouwer, A., Klasson-Wehler, E., Bokdam, M., Morse, D. & Traag, W.A. Competitive inhibition of thyroxine binding to transthuretin by monohydroxy metabolites of 3,4,3',4'-tetrachlorobiphenyl. Chemosphere, 20 (1990) 1257-1262.
12) Lans, M.C., Klasson Wehler, E., Willemsen, M., Meussen, E., Safe, S. and Brouwer, A.: Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-*p*-dioxins and - dibenzofurans with human transthyretin. Chem.-Biol. Interact, 88 (1993) 7-21.
13) A. Brouwer. Inhibition of thyroid hormone transport in plasma of rats by polychlorinated biphenyls. In: "Biological monitoring of exposure and the repsponse at the subcellular level of toxic substances". Arch Toxicol. Suppl. 13:440-445. Springer-Verlag 1989.
14) Brouwer, A., Blaner, W., Kukler, A. & van den Berg, K. Study on the mechansim of interference of 3,4,3',4'-tetrachlorobiphenyl with the plasma retinol-binding proteins in rodents. Chem.-Biol. Interact 68 (1988) 203-217.
15) Bergman, Å., Klasson Wehler, E. and Kuroki, H.: Selective retention of hydroxylated PCB metabolites in blood from mammals. Environ. Health Perspec., 102 (1994) 464-469.
16) D.E. Schultz, G. Petrick, J.C. Duincker, Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography electron capture detection. Environ. Sci. Technol. 23: 852-859 (1989).
17) Jansson, B. & Sundström, G. Mass spectrometry of the methyl ethers of isomeric hydroxybiphenyls - potential metabolites of chlorobiphenyls. Biomed. Mass spectrom. I (1974) 386-392.
18) Bergman, Å., Klasson Wehler, E., Kuroki, H. and Nilsson, A. Synthesis and mass spectrometry of some methoxylated PCBs. Manuscript intended for Chemosphere.