PLACENTAL TRANSFER OF COPLANAR PCB'S, FETAL METABOLITES AND MICROSOMAL ENZYME ACTIVITIES IN MICE

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The presence of polychlorinated biphenyls (PCBs) in the environment is, in spite of their decreased usage, still a major health hazard. Of special concern is the exposure of fetuses and neonatal individuals, because of the vulnerable, developmental processes that could be disturbed. In the Japan and Taiwan PCB intoxications, children to women who ingested the contaminated rice oil expressed a wide range of disorders, including those affecting CNS[1,2].

Within the broad spectrum of PCBs, a number of congeners are of special concern regarding toxic effects: They include the coplanar, Ah-receptor-binding forms 3,3',4,4'-tetrachlorobiphenyl (I-77), 3,3',4,4',5-pentachlorobiphenyl (I-126) and 3,3',4,4',5,5'-hexachlorobiphenyl (I-169), which exert dioxin-like effects on the organism[3]. Furthermore, I-77 metabolites have binding affinity to a thyroxin-binding protein, TTR, in rodents[4]. Effects on serum thyroxin levels, and indirectly also on the retinoid levels, have been seen after PCB exposure[5,6], and could therefore be a possible explanation to some of the observed effects seen after PCB exposure, including those of CNS. Interestingly, behavioural changes and morphological CNS disorders have been seen in mice after exposure to I-77 during pregnancy[7].

In an earlier study we have shown that I-77 was markedly accumulated in late fetuses of C57BL mice, and that the fetal uptake consisted mainly of phenolic I-77 metabolites[8]. Further studies on these fetal metabolites, on the fetal microsomal enzyme activities and on the comparative fetal accumulation of coplanar PCB congeners in C57BL and NMRI mice are presented herein. These studies will aim at elucidating the mechanism of fetal accumulation of PCB congeners, specifically I-77.

Methods

C57BL and NMRI mice were purchased from Alab AB, Sollentuna, Sweden. The [^{14}C-U]labelled congeners I-77, I-126 and I-169 were synthesized, with a spec. act. of 24.4, 12.2, and 30.0 Ci/mol and >99% pure. To follow the fetal uptake of these compounds at late pregnancy, the congeners were given in equimolar doses (2.05 µmol (=0.6-0.74 mg)/kg body wt.) to pregnant mice, which were killed on day 17 of gestation (4 days after injection). Fetal and maternal plasma and tissues were excised and whole-fetus homogenates were made, for subsequent liquid scintillation measurements and, after homogenate extraction, GC analysis. The microsomal enzyme induction was followed by measuring 7-ethoxyresorufin-O-deethylase (EROD) activity in fetal and maternal microsomes after in vivo treatment with I-77, I-126 or I-169. The EROD activity was determined fluorometrically essentially as described by Pohl and Fouts[9].

Results and Discussion

The marked fetal accumulation of I-77 equivalents in blood and soft tissues consisted mainly of several phenolic metabolites, the major one being 4-OH-3,5,3',4'-tetrachlorobiphenyl (4-OH-tetraCB). As the EROD activities are believed to mirror the capacity of microsomal I-77 metabolism, the lack of these enzyme activities in non-induced and moderately I--77 induced fetuses (Fig. 1), indicates a low rate or absence of I-77 metabolism in this compartment. The metabolites are instead suggested to be formed in the

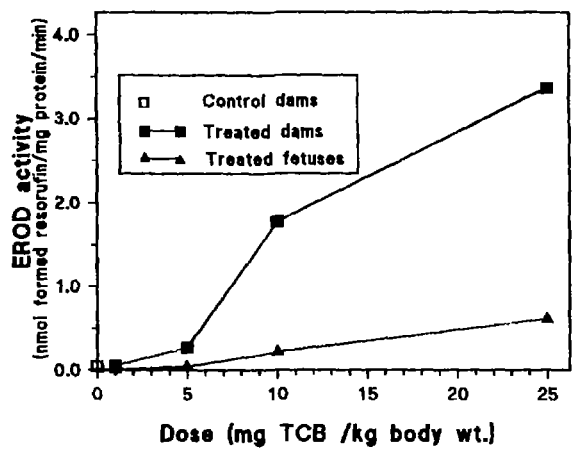

Fig. 1. Effect of I-77 (=TCB) dose on EROD activities in hepatic microsomes of C57BL mice fetuses and dams at day 17 of pregnancy, two days after treatment (treated dams/fetal litters: n=3-4; control dams: n=5).

maternal compartment and transplacentally transferred to the fetus. The fetal accumulation of the other two studied congeners, I-126 and I-169, was considerably lower (except I-126 in fetal liver) (Table 1), and preliminary TLC results show that the fetal radioactivity in these cases, especially I-169, contained mainly unmetabolized substance. The fetal EROD induction capacity of I-126 was high above those of I-77 and I-169, roughly reflecting the situation in the adult animal (Table 2). Fetal I-126 induction of its own metabolism is therefore not excluded. Regarding strain differences, it was seen that I-77 equivalents were accumulating more strongly in fetuses of C57BL mice than of NMRI (Table 1). Also, whereas EROD activites were lacking in non-induced fetuses of both strains, the activities in NMRI dams were twice as high as in C57BL. This indicates that a too fast metabolism of I-77 could favour excretion out of the body instead of transfer of metabolites to the fetus. The GC analysis showed differences in metabolic pattens between the two strains that in some way could correlate to the observed strain differences.

Table 1. ^{14}C-Accumulation in fetal and maternal tissues, and in whole-fetus homogenate ("fetal body"), in pmoles/mg wet wt. (µl serum), of C57BL mice at day 17 of pregnancy four days after i.v. injection of labelled PCB congener (n=4; ±S.D.).

	I-77	I-126	I-169
fetal serum	0.76±0.13	0.13±0.02	0.04±<0.01
" liver	0.13±0.02	0.55±0.22	0.18±0.03
" body	0.28±0.03	0.10±0.03	0.06±0.01
maternal serum	0.12±0.05	0.06±0.01	0.05±0.01
" liver	0.18±0.07	15.86±4.03	2.80±0.24
" fat	1.52±0.27	5.55±2.23	8.53±0.94

Table 2. EROD activities (in nmol formed resorufin/mg protein x min) in fetal and maternal hepatic microsomes of C57BL mice at day 17 of pregnancy, two days after i.p. injection of 5 mg/kg of PCB congener to the dam.

PCB congener	compartment	formed resorufin	range (n=2-3)
I-77	maternal	0.44	0.34-0.59
	fetal	0.04	0.03-0.06
I-126	maternal	2.10	1.5-2.6
	fetal	0.84	0.70-1.4
I-69	maternal	1.40	1.1-1.8
	fetal	0.03	0.02-0.03

The recent studies by Brouwer and coworkers (cited above) showing that certain PCB congeners, after metabolism, could bind to a thyroxin-binding protein in serum, transthyretin (TTR), could have implications for the present studies: Thus, the above described fetal binding of I-77 could be an effect of such a serum protein binding. If so, effects on the fetus or offspring as a consequence of altered thyroxin and retinoid levels could not be excluded. As it has been shown that many xenobiotics bind to TTR in vitro, some with considerably higher affinity than has 4-OH-tetraCB[10], this mechanism for hormone disturbance could have a wider toxicological significance.

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