Perinatal Accumulation of Hydroxylated Polychlorobiphenyl Metabolites in Rats Following Maternal Aroclor 1254 Exposure.

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1. Introduction

The selective retention of hydroxylated metabolites of polychlorinated biphenyls (PCBs) in blood plasma has been demonstrated in experimentally exposed rats as well as evironmentally exposed humans and wildlife species[1]. The occurence of these hydroxylated PCB metabolites in plasma may be relevant to the developmental toxicity of these compounds for two reasons: firstly it has been demonstrated that the prenatal exposure of pregnant mice and rats to individual PCB congeners (4-chlorobiphenyl and 3,3',4,4'-tetrachlorobiphenyl) results in the accumulation of phenolic PCB metabolites in the fetal compartment[2,3,4]; secondly in fetal and neonatal rats these compounds interfere with the transport and metabolism of thyroid hormone, which plays a major role in fetal and neonatal development[4,5]. There is however, no information on to which extent hydroxylated PCB congeners accumulate in the fetal compartment when animals are exposed to a complex mixture of PCBs. The recent identification of the environmentally most relevant hydroxylated PCB congeners and the synthesis of the authentic reference compounds has provided a powerfull tool to investigate this matter[1].

We therefore conducted a study to identify and quantify the major hydroxylated PCBs present in the plasma and brains of fetal, neonatal and adult offspring of pregnant rats following exposure to the technical PCB mixture Aroclor 1254, and to compare the accumulation of hydroxylated PCBs to thyroid hormone status.

2. Materials and methods:

Animals: Pregnant Wistar rats were administered an oral dose of 0, 5 or 25 mg Aroclor 1254/kg bodyweight in 2 ml cornoil/kg on day 10 to 16 of gestation. Pregnant animals were sacrificed on day 20 of gestation, maternal and fetal blood as well as fetal forebrains were collected. Male and female neonates were sacrificed on day 4 and 21 postpartum and adult offspring were sacrificed 90 days postpartum. Trunk blood and forebrains were collected from the offspring after decapitation. Plasma and brain tissue were pooled by sex and treatment group for each timepoint examined and freeze-dried prior to chemical analysis. Plasma samples for thyroid

hormone analysis were collected individually for male and female animals from each nest.

Analysis of PCBs and hydroxy-PCBs:

Freeze-dried plasma (ca. 1 g dry wt., corresponding to ca. 20 ml.) was dissolved in 5 ml water, then 5 ml methanol was added and the samples were extracted and analysed according to a previously described method[1]. The freeze-dried brains (ca. 7 g dry weight) were soaked in water (5 ml), and extracted by the same procedure as the plasma samples.

Thyroid hormones: Plasma total thyroxine (TT4) levels were analysed using a immunochemiluminesence kit, obtained from Amersham, Amersham, UK.

3. Results

The major hydroxylated PCB metabolite, determined as its methylated derivative, found in all plasma samples and the fetal brain samples, was 4-MeO-2,3,5,3',4'-pentachlorobiphenyl (4-MeO-pentaCB). 4-MeO-pentaCB constituted more than 73% of all phenolic metabolites (mass basis) present in the samples until day 90 postpartum.

There was a substantial accumulation of 4-MeO-pentaCB in the fetal compartment, with a fetal/maternal plasma ratio of 3.5 in the 5 mg/kg dose group and of 2.1 in the 25 mg/kg dose group. In the fetal plasma the concentration of 4-MeO-pentaCB (0.6 and 1.6 ppm, fresh wt. basis in 5 and 25 mg/kg dose groups, respectively) exceeded the concentration of the persistent PCB congener 2,4,5,2',4',5'-hexachlorobiphenyl (CB 153) by a factor of 15. Although the concentration of 4-MeO-pentaCB was approximately 10-fold lower in the fetal brains than plasma (fresh wt. basis), it was still 4.2 and 2.4 fold greater than the concentration of CB-153 in the brain in the 5 and 25 mg/kg dose groups, resepectively. On day 20 of gestation maternal plasma also exhibited a high ratio of 4-MeO-pentaCB to CB 153 (3.8 and 4.4 in the low and high exposure groups, respectively).
The highest plasma concentrations of CB 153 were observed in neonatal plasma from day 4 postpartum (approximately 0.13 and 0.63 ppm, fresh weight basis, 5 and 25 mg/kg dose group, respectively). Plasma concentrations of 4-MeO-pentaCB were similar in 4 day old pups and fetuses from day 20 of gestation.

Both plasma CB 153 and 4-MeO-pentaCB concentrations decreased 10-fold between day 4 and day 21 postpartum in neonates from the high exposure group and decreased 5-fold in neonates from the low exposure group (plasma 4-MeO-pentaCB concentrations: 0.28 and 0.14 ppm fresh wt. in the 5 and 25 mg/kg dose groups, respectively). However, control plasma CB 153 and 4-MeO-pentaCB levels increased over the same period, and were similar to concentrations observed in PCB-exposed animals from the 5 mg/kg exposure group. Furthermore, 4-MeO-pentaCB plasma levels were a factor 2 higher in the low dose group than in the high exposure group. Neonatal levels of CB 153 (1.1 and 4.0 ppm lipid wt basis, 5 mg/kg and 25 mg/kg dose group, respectively) on day 21 postpartum were similar to fetal brain levels. 4-MeO-pentaCB was below the limit of detection (< 2 ppb) in neonatal brains 21 days after birth from all treatment groups.

A 10-fold decrease in plasma CB 153 and 4-MeO-pentaCB concentrations in both Aroclor exposed groups was observed between postnatal day 21 and 90, resulting in concentrations in the low ppb range (2 and 4 ppb, fresh wt. in the 5 and 25 mg/kg dose groups, respectively). Brain levels of CB 153 remained elevated in the Aroclor 1254 exposed groups, averaging 140 ppb and

300 ppb on a lipid wt. basis in the 5 mg/kg and 25 mg/kg dose groups, respectively.

Total thyroxine (TT4) levels were severely decreased in fetal plasma by 52% and 74% relative to controls in the low and high PCB exposure group, respectively. Significant reductions in neonatal plasma TT4 levels of 31% and 64% (low and high exposure group, respectively) were observed on day 4 postpartum. By day 21 postpartum plasma TT4 levels had recoverd in the low dose group, and a reduction of 32% was observed in the high dose group relative to controls. No effects of PCB exposure were observed on plasma TT4 levels 90 days after birth.

4. Discussion

The results demonstrate that the exposure of pregnant rats to the technical PCB mixture Aroclor 1254, results in the highly selective accumulation of one hydroxylated PCB metabolite, 4-OH-2,3,5,3',4'-pentachlorobiphenyl (4-OH-pentaCB), in maternal, fetal and neonatal plasma. The placenta does not form a barrier for this compound, for the concentrations were higher in fetal plasma than in maternal plasma on day 20 of gestation. Compared to the levels of CB 153, 4-OH-pentaCB readily enters the brain of fetal rats, however, this metabolite could not be detected in the brains of Aroclor 1254-exposed neonatal or adult offspring. This observation indicates that after birth the blood-brain barrier effectively excludes the accumulation of 4-OH-pentaCB in the brain and that 4-OH-pentaCB that had entered the fetal brain is highly diluted in neonates due to rapid postnatal brain growth and/or is mobilized from brain tissue.

The severe depression of plasma thyroid hormone levels was observed in the fetal and early neonatal period, when plasma 4-OH-pentaCB concentrations were at their highest. The metabolite 4-OH-pentaCB meets the structural requirements to compete with T4 for binding to transthyretin, the major thyroid hormone transport protein in rat plasma[7]. Previous research has shown that the binding of 4-OH-3,5,3',4'-tetrachlorobiphenyl to transthyretin following the exposure of rats to 3,4,3',4'-tetrachlorobiphenyl results in decreased plasma T4 levels[8,9]. The high levels of 4-OH-pentaCB in the plasma relative to the total PCB concentration suggest that 4-OH-pentaCB also has a high affinity binding site in the plasma. This is supported by the observation that a structural analogue of 4-OH-pentaCB (namely 4-OH-3,5,2',3',4'-pentachlorobiphenyl) has a higher binding affinity for TTR than the natural ligand T4[7]. We hypothesize that 4-OH-pentaCB is transported over the placenta to the fetus, and that the fetus is then not able to eliminate the compound from its circulation, resulting in the accumulation in the fetal plasma and subsequent reductions of fetal plasma T4 levels. The reductions in fetal plasma T4 concentrations are of sigificance for the fetal brain, which by day 20 of gestation is mainly dependent on the local deiodination of T4 to provide the biologically active hormone triiodothyronine[9].

In conclusion, maternal exposure to the PCB mixture Aroclor 1254 results in the substantial accumulation of mainly one hydroxylated metabolite (4-OH-2,3,5,3',4'-pentachlorobiphenyl) in the fetal plasma, and thereby probably contributes to the decreases observed in fetal plasma thyroxine levels. The metabolite-induced decreases in plasma T4 levels during brain development may be an indirect mechanism by which PCBs may alter neurological developement. Also the selective accumulation of 4-OH-pentaCB in the fetal brain warrants toxicological investigation.

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5. References

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