EFFECTS ON THYROID HORMONE AND RETINOID METABOLISM IN TRANSTHYRETIN-NULL MICE BY POLYCHLORINATED BIPHENYL ISOMERS 118 and 114

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Introduction

Some congeners/isomers of polychlorinated biphenyls (PCBs) and their metabolites are known to disturb thyroid and retinoid metabolism in laboratory animals and humans. Among 209 isomers of PCBs, 12 PCB isomers termed as the coplanar PCBs are grouped into dioxin-like chemicals based on their resemblance of toxic effects and mechanism(s) to dioxins. Mechanism(s) of toxicity by the dioxin-like chemicals have been established to be mediated through aryl hydrocarbon receptor (AhR). The toxic potency of each congener/isomer of dioxin-like chemicals has been evaluated by relative potency to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as the toxic equivalent factor (TEF)[1]. Because dioxins in environments are generally distributed as mixtures, the toxic equivalency (TEQ) concept has been adopted to evaluate the health risk of exposure to complex environmental mixtures. TEQ values are calculated by multiplication of the sum of the chemical concentrations by the corresponding TEF values. The present study was undertaken to investigate the effects of two coplanar PCBs, PCB118, highly detected in human tissues and milk (TEF value: 0.0001), and PCB114 (TEF value: 0.0005) on thyroid hormone and retinoid metabolism. Possible involvement of transthyretin (TTR), the principal carrier of thyroid hormone and retinol-binding protein in the rodent, in PCBs-induced disruption of thyroid and retinoid homeostasis was investigated.

Materials and Methods

Chemicals: 2,3',4,4',5-pentachlorobiphenyl (PCB118: 10 mg/ml), 2,3,4,4',5-pentachlorobiphenyl (PCB114: 10 mg/ml), were diluted in corn oil.

Animals and Treatment: TTR (+/-) mice were kindly provided by Dr. S. Maeda (Yamanashi Univ.), back-crossed with C57BL/6J and bred at NIES. Animals were treated with humane care according to the guideline of animal experiment at NIES.

Male and Female TTR-null mice and their wild-type (C57BL/6J) mice, 12-weeks old, were administered with PCB118 (50 mg/kg b.w.) or PCB114 (50 mg/kg b.w.) at a single oral dose as shown in the parentheses. Control mice received corn oil as vehicle.

Sample collection and processing: Blood and the liver were collected 7 days post-administration under light diethylether anesthesia. Liver were fixed in Zamboni's fixative and embedded in paraffin. The absence of TTR in the serum was confirmed by SDS-PAGE western blotting analysis.

Analysis of PCBs:

PCB concentrations in the liver were analyzed by high resolution GC/MS[2].

Thyroid hormone and retinoid analyses: Serum total thyroid hormone (TT4) was determined by radioimmunoassay. Retinoids in the serum were determined by HPLC after chemical extraction.

Immunohistochemical detection of CYP1A1: CYP1A1 in the liver was visualized according to the method described in our previous paper[3].

Statistical analysis: Values were expressed as mean and standard deviation, and differences in means were analyzed by Student's t-test.

Results and Discussion

Concentrations of PCB 118 and PCB 114 in the liver: The concentrations of PCB 118 in the liver of wild-type mice and TTR-null mice were 13.8, and 10.5, respectively, and those of PCB 114 in the liver of these two strains of mice were 8.8 and 14.1 μg/g, respectively. The results strongly suggest that difference in the manifestations of toxicity of PCBs used here is a consequence of characteristic response to each congener rather than difference in amounts of PCBs incorporated into tissues (Fig. 1).

Serum concentrations of total T4 (TT4) and retinol: Concentrations of total T4 in the serum from wild-type mice and TTR-null mice were 2.4 and 1.4 μg/ml, respectively, which suggests that approximately 50 to 60 % of TT4 in the serum was eliminated when TTR is not present in the serum. While serum TT4 concentration was not affected by PCB 114 treatment both in TTR-null and wild-type mice, PCB 118 exposure significantly decreased serum TT4 concentration in wild-type mice, but not in TTR-null mice, compared to their corresponding vehicle-treated mice (Fig. 2).

Serum retinol concentrations in wild-type and TTR-null mice were 1.30 and 0.11 (unit/ml), respectively. In TTR-null mice, the serum retinol concentration was decreased to approximately 10% of those in wild-type mice. A significant decrease in serum retinol by PCB 118 was produced in the wild-type mice compared with their corresponding vehicle-treated mice, whereas PCB 114 did not affect serum retinol levels.

These results support the hypothesis that TTR plays a role in maintaining concentrations of thyroid hormones and retinol at the appropriate levels in the blood circulation.

PCB 118 has been suggested to be converted into hydroxylated form[4] that can inhibit not only thyroid, but also retinol to bind with TTR[5]. Therefore, the most plausible explanation for lowering serum thyroid hormone levels and retinol concentrations following PCB 118 treatment is a consequence of an accelerated excretion of retinal by a competitive binding of the hydroxylated PCB with TTR.

Induction of CYP1A1 in the liver: Immunohistochemical examination revealed a marked induction of CYP1A1 in hepatocytes in the periportal region following PCB 114 treatment, but not by PCB

118 (Fig. 3). Immunostaining for CYP1A strongly suggested that mechanisms involved in the toxicity of PCB114 and PCB118 could be mediated by AhR-dependent and AhR-independent manner, respectively.

In our previous study, PCB153, non-planar PCB with having no TEF value but highly detected in human tissues and milk, decreased serum TT4 level both in TTR-null mice and wild-type mice[5]. Since PCB153 do not induce CYP1A1 in the liver and is not readily metabolized to form hydroxylated metabolite, PCB153 suggested to reduce serum TT4 by an AhR-independent, TTR-independent, but a yet known mechanism.

PCB isomers used in the present study differ in chemical properties. While the mechanisms involved in the toxicity of PCB 118, readily metabolized into hydroxylated PCB in the body could be AhR-independent, and PCB 114 is thought to be AhR-dependent. On the other hand, the toxicity mechanism of PCB 153, relatively low in toxicity, is believed to be different from those of PCB 118 or PCB 114.

Recently, we performed DNA microarray analysis to delineate the toxicity mechanisms of these PCB isomers, and found that there are differences in profile of mRNA expressions induced by different type of PCB isomers. These results suggested that the reduced serum TT4 concentrations induced by each PCB isomer could be mediated by the different mechanisms.

Risk assessment of the dioxin-like chemicals hitherto has been evaluated by TEF mainly based on an affinity for AhR or inducible capability of AhR-mediated enzymes. In the present study we provide evidence that certain coplanar PCB isomers affect thyroid homeostasis and retinoid metabolism through TTR, but not by AhR-dependent mechanism or via unknown mechanisms other than via TTR or AhR. Establishment of alternative risk assessment scheme might be needed for non-AhR-mediated toxicity produced by certain coplanar PCBs.

Conclusion

The present results suggest that PCB114 exerted their disrupting effects on thyroid hormone and retinoids mainly via AhR-dependent mechanism whereas PCB118 could disrupt thyroid hormone and retinoid homeostasis via AhR-independent, and possibly TTR-dependent mechanisms. Further research is needed to seek the possibility to establish toxicity equivalent factor for non-AhR-mediated toxicity of certain coplanar PCBs.

Acknowledgement

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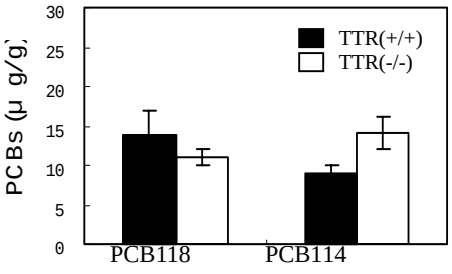

Figure 1. PCB118 or PCB114 concentrations in the liver of TTR-null or wild-type mice (Mean ± SD, n=4-5).

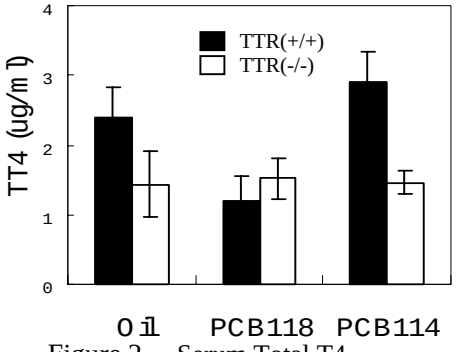

Figure 2. Serum Total T4 concentrations in TTR-null or wild type mice administered with PCB118 or PCB114 (Mean ± SD, n=6-7).
* Significant difference from vehicle-treated control mice (P<0.05).

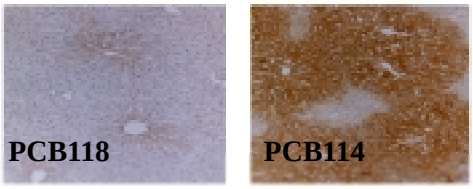

Figure 3. Immunostaining for the CYP1A1 in the liver of the wild type mice administered with PCB118 or PCB114.