DIFFERENTIAL DISRUPTING EFFECTS OF POLYCHLORINATED BIPHENYL ISOMERS ON HOMEOSTATIS OF THYORID HORMONE AND RETINOID IN MICE

C. Tohyama [1, 2, 3], Noriko Nishimura[2,3] and J. Yonemoto[2, 3]

[1]Environmental Health Sciences Division and [2]Endocrine Disruptors/Dioxin Research Project, National Institute for Environmental Studies, Tsukuba 305-8506, Japan. [3]CREST, Japan Science/Technology Corp., Kawaguchi 332-0012, Japan.

Introduction

A number of dioxin and polychlorinated biphenyl (PCB) isomers are often found in the environment and biological specimens including humans, and have been reported to affect reproductive, brain and immune functions in laboratory animals and perhaps in humans. For comparative and integrative purposes, the toxicity of these compounds is designed as toxic equivalence factor (TEF), that is based upon the toxicity of 2,3,7,8-tetrachloro-*p*-dibenzodioxin (TCDD)[1]. However, it has not been clarified whether possible health effects of TCDD-like PCB or non-TCDD like PCB could be covered by the TEF-based scheme. Since recent epidemiological studies suggest that there may be a subtle change in cognitive functions of offspring according to the function of TCDD/PCB exposed level[2], and TCDD and PCB have been known to affect thyroid and retinoid metabolism[3, 4], we tried to clarify differential effects of each of these compounds on the homeostasis of thyroid and retinoid metabolism in rodents. In the present study, we used three different PCB isomers, PCB126, PCB77 and PCB153, which were assigned by WHO expert committee for TEQ values of 0.1, 0.0001 and no value, respectively, and selected the dose of the above-described each isomer, each of which suppressed a circulating serum T4 level at approximately 60-70% of the control level.

Materials and Methods

Chemicals: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD: 4 μg/ml), 3,3',4,4'-tetrachloro-biphenyl (PCB77: 5 mg/ml), 3,3',4,4',5-pentachlorobiphenyl (PCB126: 100 μg/ml), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153: 10 mg/ml) were diluted in corn oil.

Animals and Treatment: AhR (+/-) mice and TTR (+/-) mice were kindly provided by Dr. Y. Fujii-Kuriyama and Dr. S. Maeda, respectively, and back-crossed with C57Bl/6J and bred at NIES. Animals were treated with humane care according to the guideline of animal experiment at NIES.

AhR (+/-) mice were mated each other, and pregnant mice were given a single oral dose of TCDD (10 μg/kg b.w.) on gestation day (GD) 12.5. On postnatal day (PND) 21, male and female pups, having either AhR (+/-) or AhR(-/-), were sacrificed for the collection of

blood and liver.

Female TTR-null mice and its wild-type (C57Bl/6J) mice, 13-weeks old, were administered TCDD (10 μg/kg b.w.), PCB77 (50.0 mg/kg b.w.), PCB126 (1.0 mg/kg b.w.) and PCB153 (200 mg/kg b.w.) at a single oral dose as shown in the parentheses. Control mice received corn oil as vehicle. Blood and the liver were collected 7 days post-administration. The absence of TTR in the serum was confirmed by SDS-PAGE western blotting analysis.

Thyroid hormone and retinoid analyses: Serum total thyroxine (TT4) was determined by radioimmunoassay. Retinoid in the serum or liver was determined by HPLC analysis after chemical extraction.

RT-PCR: Total RNA was extracted from the liver by the use of Qiagen RNeasy mini kit, and mRNAs of cytochrome P450 1A1, 1A2, UDP-glucuronosyltransferase 1A6 (UGT1A6) and AhR were analyzed by RT-PCR. The intensity of band on agarose gel electrophoresis was detected by EDAS120 system. Beta-actin was used as internal standard.

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

Results and Discussion

Effects of TCDD and PCB isomers on thyroid hormone homeostasis: When TCDD and PCB126 were administered to 13-week-old female C57Bl/6J mice, amounts of CYP1A1, CYP1A2 and UGT1A6 mRNA in the liver were significantly increased compared to their corresponding vehicle-treated control mice. On the other hand, PCB77 and PCB153 administration did not show any alterations. Since perinatal exposure to TCDD on GD12.5 caused a significant decrease in serum TT4 concentration in the offspring of AhR (+/-) mice on PND21 compared to their corresponding vehicle-treated mice, but did not cause any difference in the serum TT4 concentration of AhR-null mice from their corresponding vehicle-treated mice (Fig.1). Similarly, TCDD exposure caused significant elevation of hepatic UGT1A6, CYP1A1 and CYP1A2 mRNA levels in the offspring of AhR (+/-) mice on PND21 compared to that of vehicle-treated mice, and no such significant changes were observed for AhR-null mice.

In the adult female C57Bl/6J mice, treated with either, TCDD, PCB126, PCB77 or PCB153, a significant decrease in serum TT4 concentration was observed. TTR-null mice had 50% of serum TT4 concentration of the wild-type (C57Bl/6J) mice, which is consistent with the earlier observation[5], suggesting that TTR is responsible for regulating the T4 concentration in the blood circulation. It is very interesting to note that the response of serum TT4 concentration to TCDD exposure differed considerably from wild-type mice from TTR-null mice: that is serum TT4 level of wild-type mice decreased to 70% of the corresponding vehicle controls whereas that of TTR-null mice decreased to 10%, suggesting that T4 bound to TTR cannot be easily subject to degradation by UGT1A6. In both TTR-null mice and their wild-type control mice that were treated with TCDD or PCB126, the induction

of CYP1A1, CYP1A2 and UGT1A6 mRNAs were observed but not in those treated with PCB77 or PCB153. These observations suggest that the decrease in TT4 levels in the blood circulation found in both TTR-null mice and wild-type mice administered TCDD and PCB126 were mediated by AhR-dependent mechanism, and the both strain mice administered PCB77 or PCB153 were thought to be AhR-independent, but a yet unknown mechanism.

PCB153, a non-TCDD-like PCB, has been reported to induce phase I and II drug-metabolizing enzymes possibly via phenobarbital-response unit[6], and has been considered as a prototype for phenobarbital-type chemical to induce UGT enzymes to decrease serum TT4 concentration when total UGT1A isozymes were determined[7, 8]. Although induction of UGT1A6 was not found in the liver from PCB153-exposed mice in the present study, PCB153 might be able to induce other isoforms other than UGT1A6.

When the suppression of serum TT4 concentrations was used as a biomarker of effect of TCDD or PCB isomers, TCDD (10 µg/kg b.w.), PCB126 (1 mg/kg b.w.) and PCB153 (200 mg/kg b.w.) were found to have a similar degree of intensity to one another, and PCB77 (50 mg/kg b.w.) had slightly higher intensity. When the relative biological intensity in terms of the suppression of serum TT4 level is adopted on the basis of TCDD, PCB126, PCB 153 and PCB 77 will be assigned to have TEQ values of 0.02, 0.0001 and 0.0004, respectively, which is very different from the current TEF values for these compounds, 0.1, 0.0001 and no value. It may be necessary to introduce a new indicator for thyroid hormone homeostasis.

Next, we studied possible effects of TCDD and PCB isomers on retinoid metabolism and found that TCDD administration deceased retinoid concentration in the liver of female homo- and heterozygous AhR knockout mice compared to vehicle-treated wild-type mice. This observation was supported in male wild-type mice and TTR-knockout mice in which either TCDD or PCB126 administration decreased hepatic retinoid levels, suggesting that this decrease is caused by AhR-mediated mechanism. On the other hand, factors other than AhR and TTR are thought to be involved in the decrease in hepatic retinoid level found in the TTR-knockout mice treated with either PCB77 or PCB153.

Conclusion

The present results suggest that TCDD and PCB126 exerted their disrupting effects on thyroid hormone and retinoid homeostasis mainly via AhR-dependent mechanism whereas PCB77 and PCB153 did via AhR-independent, and possibly TTR-independent mechanisms. Further research is needed to seek the possibility to establish toxicity equivalence factor for non-AhR-mediated toxicity of some PCB isomers.

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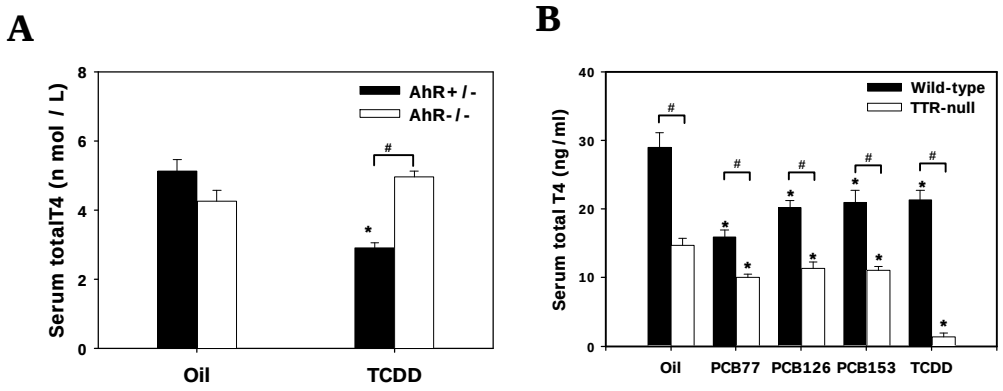

Figure 1. Serum total T4 concentrations in AhR-null mice (A) and TTR-null mice (B), administered with TCDD or PCB isomers. * Significant difference from vehicle-treated control mice ($P<0.05$). # Significant difference from AhR(+/-) mice or TTR wild-type mice.