EFFECTS OF PERINATAL EXPOSURE TO METHYLMERCURY AND/OR POLYCHLORINATED BIPHENYLS ON MOUSE NEUROBEHAVIORAL DEVELOPMENT AND ANALYSIS OF GENE EXPRESSION IN THE BRAIN

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Abstract

Methylmercury (MeHg) is a neurotoxicant and occurs as a contaminant in fish together with polychlorinated biphenyls (PCBs). A developing nervous system is considered to be particularly sensitive to these two chemicals. In this experimental study, we investigated the effect of perinatal co-exposure to MeHg and PCBs on neurobehavioral development of mouse (C57BL/6Cr) offspring and analysis of gene expression in the brain. The experimental groups were as follows: MeHg (5 ppm as Hg in diet) alone, PCBs (Aroclor 1254, 18 mg/kg body weight/3days) alone, PCBs+MeHg, or Control. In several neurobehavioral tests, significant effects of exposure to MeHg alone or PCBs alone, and interaction of co-exposure were observed. Microarray analysis of gene expression on postnatal day 21 revealed that a large number of genes were down-regulated by MeHg exposure in the cerebellum and hippocampus, and by co-exposure in the hippocampus. However, the PCBs exposure group showed many up-regulated genes. Several highly up-regulated genes in the MeHg, or PCBs group, were down-regulated in the co-exposure group. In conclusion, gene expression affected by co-exposure differed from that affected by exposure to MeHg alone or PCBs alone, and did not show a simple additive or synergistic alternation by co-exposure.

Introduction

Methylmercury (MeHg) is an environmental pollutant that is known to be highly neurotoxic, particularly to a developing nervous system. The main route of exposure to MeHg is food, particularly through the consumption of fish and fish products[1]. It is readily distributed throughout the body, easily penetrating the blood-brain and placenta barriers. Polychlorinated biphenyls (PCBs) are also neurotoxic and occur as contaminants in fish together with MeHg. Developing fetuses and infants are at high risk of PCBs exposure because PCBs can cross the human placenta and are also present in human milk[2,3].

Neurodevelopmental alternations in children prenatally exposed to MeHg have been reported. In the Faroe Islands, prenatal exposure to low MeHg levels via maternal consumption of fish was associated with neurodevelopmental deficits in children[4]. In contrast to the Faroe Islands study, children in the Seychelles Islands did not show any neurobehavioral deficit associated with prenatal mercury exposure[5,6,7]. Chemical analysis of the umbilical cord in the Faroe Islands revealed PCB-associated deficits[8]. It has been speculated that the effects observed in the Faroe Islands could be due to the additive or synergistic effects of MeHg and PCBs.

In experimental animals, some studies on the effects of co-exposure to MeHg and PCBs were reported. Roegge et al. reported a motor deficit in the rotating rod task in rats co-exposed to MeHg and PCBs[9]. Widholm *et al*. examined spatial alteration, and they concluded that co-exposure to MeHg and PCBs does not exacerbate the impairments induced by MeHg or PCBs in spatial alteration tasks[10]. In vitro studies also support the hypothesis that the two chemicals may have additive or synergistic effects on the nervous system function. In the striatal tissue punches from the rat brain, co-exposure to MeHg and PCBs resulted in greater decreases in tissue DA concentrations and elevations in media DA than exposure to PCBs alone[11]. Bemis *et al*. also found that low concentrations of MeHg and PCBs synergistically increased intracellular calcium concentrations measured by flow cytometry[12]. These finding provide evidence for the synergistic and antagonistic interactions between PCBs and MeHg at the level of Ca^{2+} ion concentration regulation that may ultimately lead to alterations in cellular function, including changes in dopamine regulation.

In previous studies, regarding the effects of co-exposure to MeHg and PCBs, no consistent results were obtained. In this study, we aimed to examine the possible interaction between MeHg and PCBs in the perinatal period in terms of the effects of co-exposure to these chemicals on neurobehavioral development in mice and

gene expression analysis in the brain using the DNA microarray technique, which covers the whole range of changes in gene expression focusing on behavior, development, and calcium homeostasis.

Materials and Methods

In this experiment, the following four groups were studied: MeHg alone, PCBs alone, co-exposure to PCBs+MeHg, or Control. Female mice (C57BL/6Cr) were exposed to MeHg (5 ppm as Hg in diet), 4 weeks prior to mating with an unexposed male from gestation to weaning, and/or PCBs (Aroclor 1254, 18 mg/kg body weight/3days) by gavage starting from Day5 of gestation to weaning. Aroclor 1254 contains several dioxin-like PCBs. The doses of PCBs were chosen considering on the basis of our previous results indicating a clear neurobehavioral alteration following the perinatal administration of PCBs[13]. Before the weaning of offspring, physical and neurobehavioral developments were observed on postnatal days (PNDs) 4, 7, 10, 12, 14, and 16 for the mice of both sexes. For the assessment of physical development, several observations were carried out, such as, eye opening, pinna detachment, hair growth, and incisor eruption. For the assessment of neurobehavioral development, pups were tested for the following reflexes and responses: grasp reflex, righting reflex, walking, negative geotaxis, and cliff avoidance. Results of these tests were evaluated by logistic regression for each time point. A value of $p<0.05$ was considered significant. At 8 weeks old, they were tested in the open field and the water maze. At 9 weeks old, spontaneous locomotion activity was observed. Male offspring were used in these experiments. Two-way ANOVA was performed to determine the significant effects in the open-filed, water maze, and locomotion activity tests. To evaluate the effects of two-chemical exposures on gene expression in the brain, microarray experiments were carried out as follows. Total RNAs were extracted from mouse cerebral cortical, cerebellar, and hippocampal tissues on PND21 (n=3, each group). The qualities of RNAs used in microarray experiments were evaluated with Bioanalyser 2100 (Agilent). The experimental groups in the microarray experiments were MeHg group (MeHg/Control), PCB group (PCBs/Control), and M + P group (MeHg+ PCBs /Control). A Gene Pix laser scanner was used to detect hybridization signals in microarray slides (Agilent Technologies, 22K, 2-color method). Gene Spring GX was used for normalization (LOWESS) and further data analysis. Genes demonstrating a fold change of 0.77 (0.67) < or >1.3 (1.5) were considered to be significantly altered. To consider biological functions, genes were categorized on the basis of their functions derived from "Gene Ontology (GO) " categories. This study was carried out in accordance with the Guide of Animal Experimentation of Tohoku University Graduate School of Medicine.

Results and Discussion

Several tests showed significant effects of MeHg alone or PCBs alone. The effects of interaction of co-exposure to MeHg and PCBs were also observed in several behavioral tests. Before weaning, the assessment of eye opening revealed the interactive effect between MeHg and PCBs on PND12. We also observed the delays of grasp reflex on PND12 and PND14 owing to exposure to MeHg alone. When the offspring were at 8 weeks olds, the group exposed to PCBs alone showed increased numbers of defecations and spots of urinations in the open field test. Analysis of the latency of the open field test revealed the interaction between the two chemicals exposures. Exposure to MeHg decreased the walking distance and interacted with PCBs exposure. The water maze test showed that exposure to MeHg prolonged the time to reach the platform, but this effect did not interact with PCBs exposure. Spontaneous locomotion activity at 9 weeks old was not affected by co-exposure to MeHg and PCBs. These results indicate that perinatal co-exposure to MeHg and PCBs do not produce a simple additive or synergistic effects.

The results of gene expression analysis using DNA microarrays are shown in Table 1, Figs 1 and 2. The genes, which were passed through the quality control, were used for further analysis. The numbers of expressed genes are shown in Table 1. In the cerebellum and hippocampus, the numbers of down-regulated genes were larger than those of up-regulated genes in the MeHg group compared with the other groups. The number of down-regulated gene was larger than that of up-regulated genes of the M+P group in the hippocampus. On the other hand, the numbers of up-regulated genes in the PCB groups were larger than those of down-regulated and up-regulated genes in the other groups. In the hippocampus of the M+P group, the number of up-regulated genes was much smaller than that of the PCB group. The cluster analysis of gene expression profiles with exposure differences revealed that the gene expression patterns in the cerebellum and hippocampus of the MeHg and M+P groups were similar, whereas the expression in the PCB group showed a different pattern (Fig.1). Several highly up-regulated genes in the MeHg group, as well as in the PCBs group, were found to be down-regulated in the

M+P group and this was considered to be characteristic response to co-exposure. Gene expressions patterns in cerebral cortex were similar in the three exposure groups. The GO terms on the basis of GO categories of up-regulated or down-regulated genes associated with behavior and development were examined. The GO terms of significantly down-regulated genes associated with behavior and development in the cerebellum of the MeHg group were locomotory behavior, learning, neuron development, and positive regulation of development. Concerning behavior and development, gene expression was affected strongly in cerebellum by MeHg exposure. As for the hippocampus, many genes for GO categories of behavior such as locomotory behavior, learning and/or memory, regulation of behavior, behavioral fear response, and chemosensory behavior were significantly up-regulated in the M+P group (Fig.2). In the previous studies, several mechanisms and molecular targets have been proposed to be involved in MeHg neurotoxicity, including alteration in calcium homeostasis[14], binding to SH groups, apoptosis/necrosis, formation of reactive oxygen species, and disruption of mitochondrial function. Among the neurotoxic manifestations of PCBs, disturbances in brain development and cognition are known. The cellular and molecular basis for PCB-induced developmental neurotoxicity remains unclarified; however, a series of in vitro studies and some in vivo studies have revealed that the disruptions of Ca^{2+} homeostasis and Ca^{2+} mediated signal transduction play a significant role[15]. In this study, we also found that calcium ion binding in GO term was significantly down-regulated in the hippocampus of the M+P group. This might indicate that MeHg and PCBs co-exposure led to alteration in calcium homeostasis associated with disturbances in brain development. In conclusion, gene expression affected by co-exposure differed from that affected by exposure to MeHg alone or PCBs alone, and did not show a simple additive or synergistic alternation by co-exposure.

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Table. 1 Numbers of expressed genes in cerebral cortex, cerebellum, and hippocampus exposed to MeHg and/or PCBs

	Cerebral Cortex		Cerebellum		Hippocampus	
	up-regulated fold change > 1.5	down-regulated fold change < 0.67	up-regulated fold change > 1.5	down-regulated fold change < 0.67	up-regulated fold change > 1.5	down-regulated fold change < 0.67
Methylmercury	30	75	47	147	16	53
PCBs	19	13	155	64	122	73
Methylmercury + PCBs	15	19	98	117	39	90

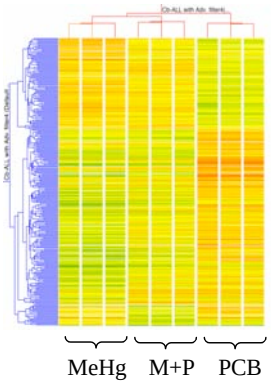

Fig. 1 Cluster analysis of gene expression profiles in the cerebellum

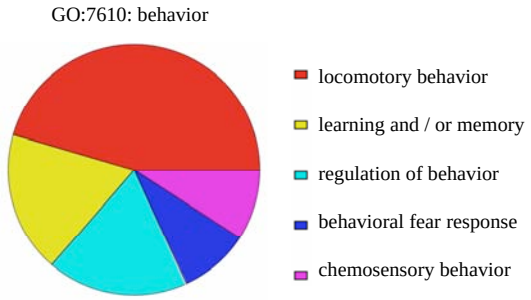

Fig. 2 GO terms of up-regulated genes associated with behavior in the hippocampus of the M+P group

References

1. National Research Council. In: *Toxicological Effects of Methylmercury*, National Academy Press, Washington, DC, 2000: 31
2. Ando M, Saito H, Wakisaka I. *Arch Environ Contam Toxicol* 1985; 14:51.
3. Bush B, Snow J, Connor S, Koblintz R. *Arch Environ Contam Toxicol* 1985; 14:443.
4. Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R, Jorgensen PJ. *Neurotoxicol Teratol* 1997; 19: 417.
5. Myers GJ, Davidson PW, Shamlaye CF, Axtell CD, Cernichiari E, Choisy O, Choi A, Cox C, Clarkson TW. *Neurotoxicology* 1997; 18: 819.
6. Davidson PW, Myers GJ, Cox C, Axtell C, Shamlaye C, Sloane-Reeves J, Cernichiari E, Needham L, Choi A, Wang Y, Berlin M, Clarkson TW. *JAMA* 1998; 280:701.
7. Myers GJ; Davidson PW; Cox C; Shamlaye CF; Palumbo D; Cernichiari E; Sloane-Reeves J; Wilding GE; Kost J; Huang L-S; Clarkson TW. *Lancet* 2003; 361:1686.
8. Grandjean P, Weihe P, Burse VW, Needham LL, Storr-Hansen E, Heinzow B, Debes F, Murata K, Simonsen H, Ellefsen P, Budtz-Jorgensen E, Keiding N, White RF. *Neurotoxicol Teratol* 2001; 23:305.
9. Roegge CS, Wang VC, Powers BE, Klintsova AY, Villareal S, Greenough WT, Schantz SL. *Toxicol Sci* 2004; 77:315.
10. Widholm JJ, Villareal S, Seegal RF, Schantz SL. *Toxicol Sci* 2004; 82:577.
11. Bemis JC, Seegal RF. *Environ Health Perspect* 1999; 107:879.
12. Bemis JC, Seegal RF. *Neurotoxicology* 2000; 21:1123.
13. Sugawara N, Nakai K, Nakamura T, Ohba T, Suzuki K, Kameo S, Satoh C, Satoh H. *Arch Toxicol* 2006; 80: 286.
14. Marty MS, Atchison WD. *Toxicol. Appl. Pharmacol.* 1997; 147:319.
15. Sharma R, Derr-Yelin EC, House DE, Kodavanti PRS, *Toxicology* 2000; 156:13.