Correlation between the concentration of serum polychlorinated biphenyls (PCBs) in pregnant cynomolgus monkeys and their offspring's behavioral scores in eye-contact test and finger maze learning test

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Introduction

A recent review suggested that pre- or perinatal exposure of developing fetuses to dioxins, the widespread environmental contaminants, such as polychrorinated biphenlys (PCBs), induce the irreversible abnormalities in the functions of central nervous system (CNS) in human[7]. These chemicals can be transferred to each fetus and naonate transplacentally and lactationally in rhesus monkey[3]. Several studies also reported the adverse effect of PCB on CNS development in rodents[1] and monkeys[8, 9] as well as on behavior in rodents[4, 10] and monkeys[2, 5].

In the present study, we show a preliminary data about the correlation between the serum concentrations of PCBs in pregnant cynomolgus monkeys (*Macaca fascicularis*) and the scores of two behavioral tests, eye-contact test and four-step finger maze test, which evaluate consciousness against human observer and learning ability, respectively, in their offspring.

This experimental surveillance system using non-human primates would be useful to predict the risk of PCBs exposure in human fetuses because of the similarities of cynomolgus monkey to human with regard to reproduction, developmental parameter, and others.

Methods and Materials

Collection of blood sample:

Six pregnant cynomolgus monkeys (around four years old) in a bleeding farm in Japan were subjected to this study. Blood samples were collected at last stage of gestation (gestational day 131; typical pregnancy period of cynomolgus monkey is 150 – 160 days). Serum samples were harvested by centrifugation at 3,000 g for 15 min and were kept at –20 ^{o}C until analysis.

Analysis:

After adding 22 species of $^{13}C_{12}$-labeled PCBs to the serum samples as internal standards, identification and quantification of PCBs were performed using Hewlett Packard 6890 Series high-

resolution gas chromatography interfaced with a Micromass Autospec – Ultima high-resolution mass spectrometer.

Eye-contact test:

At 8 – 9 months old, five male and one female offspring were subjected to the eye-contact test. The apparatus for the eye-contact test was a testing cage (69 cm x 61 cm x 75 cm) (Fig.1A). Three highly disciplined observers (26, 38, and 55 years old, respectively) attended to the eye-contact test. They had no contact with the experimental subject until the test. In a trial, randomly assigned observer was sitting 85 cm away from the eye-contact test apparatus and counted the frequency of his meeting subject's gaze for 1 min, and three trials were performed consecutively. Thus, each subject took three trials consecutively by three unfamiliar observers in random order.

Four step finger maze test:

The 4-step non-correction-method-type finger maze test (4FM) was carried out against four male and one female offspring at 16 – 18 months old according to a previous report[11] with some modifications. Briefly, the apparatus was made of acrylic plastic and comprises 4 steps (Fig.1C). One direction of each step was connected to the error box. The other direction was connected to the lower step or the feeding box. Before testing, the subjects could insert only their fingers into the 4FM apparatus. A piece of apple (2.5 g) was the reward in each trial. The monkeys performed 15 trials in a morning session and 15 trials in an afternoon session 5 days per week. The subjects were habituated to an experimenter and apparatus in two sessions of the first day. In task 1, the reward was put on step 1 of the 4FM at the start of a trial. The trial with 10 sec of inter-trial interval ended when the monkey retrieved the reward (correct trial) or dropped it into the error box (error trial). The learning criterion only applied when the subject performed ≥14 trials correctly in a series of two sessions. When a monkey attained this criterion, it proceeded to the task 2. In the task 2, a reward was on step 2. The monkey had to move the reward to the feeding box by passing through step 1 and to retrieve it. The rest of the procedure and the learning criterion were the same as those of the task 1. In the task 3, the reward was on step 3 and the procedures were the same as those of previous tasks. At the start of the trial for the task 4, a reward was on step 4. We recorded the number of sessions required to fully accomplish these tasks.

Statistical analysis:

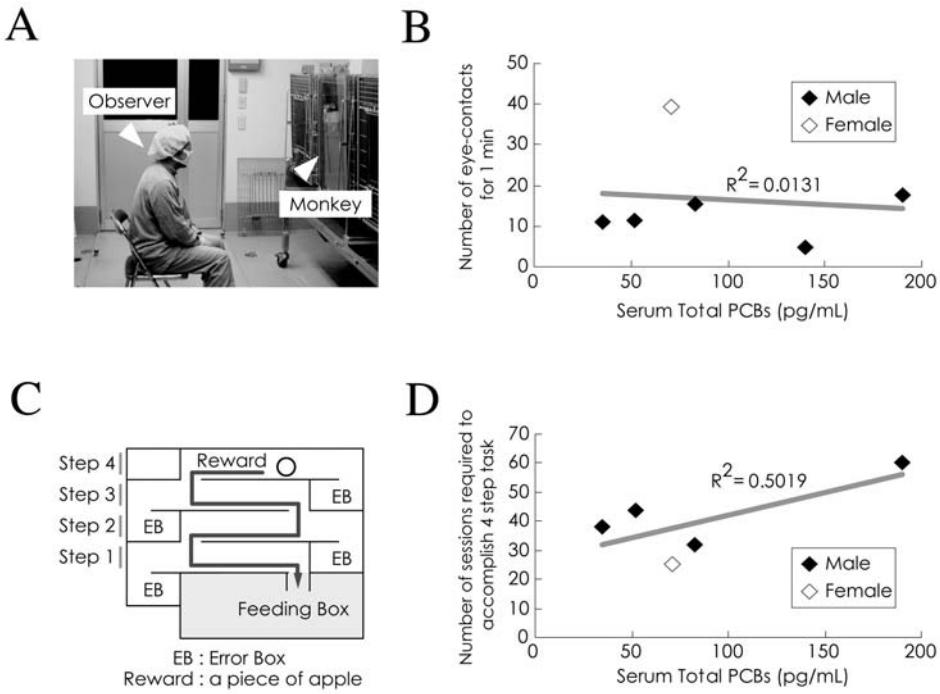
Correlation between the maternal serum PCBs and behavioral scores were analyzed by regression analysis and analysis of variance. The significance level was set at $p<0.05$.

Results and Discussion

Although all of six serum samples from pregnant monkeys at gestational day 131 used in this study contained some detectable congeners of PCBs (Table 1), the mothers successfully delivered five male and one female offspring and no offspring showed morphological abnormalities such as malformations. There is no correlation between the maternal serum concentrations of total PCBs and the number of eye-contacts for 1 min in eye-contact test (Fig.1B; $R^2=0.0131$, $p>0.8$). Further analysis on each congener of PCB also showed no correlation (data not shown). On the other hand, a slight positive correlation between maternal serum concentrations of total PCBs and the number of sessions required to accomplish the 4FM was observed (Fig.1D), although this correlation was not statistically significant ($R^2=0.5019$, $p=0.180$). Two congeners of PCBs, #28-TrCB ($R^2=0.6564$,

$p<0.1$) and #74-TeCB ($R^2=0.7085$, $p<0.1$), correlated a little with the number of sessions in the 4FM.

Figure 1: Manner of eye-contact test (A). There is no correlation between the concentration of serum total PCBs and the number of eye contacts for 1 min (B). Apparatus of the four-step finger maze (C). There is a slight correlation between the serum total PCBs and the number of sessions required to accomplish 4 step tasks (D).



In the present preliminary study, we tried to detect the correlation between the maternal contamination of PCBs that would also be transferred to her fetus, and the offspring's behavioral scores. A slight negative correlation between maternal PCBs contaminations and offspring's learning ability was observed, although this was not statistically significant. A previous study using monkeys[6] reported the intricate alteration, including facilitative effects, in learning ability using discrimination-reversal test by perinatal PCB exposure, while the present result shows the tendency of negative correlation between PCBs contamination and learning ability using maze learning test. We think that the direct comparison of these two results is impossible, because behavioral task for learning in each study was quite different. In addition, while monkeys in the previous study were experimentally exposed to PCBs, we focused on the degrees of background maternal PCB contamination in monkeys that were not intended to experimental exposure. We think that the present surveillance strategy is highly useful to predict a human risk of PCB exposure on the

developing CNS because of similarities of monkey to human, and it is acceptable in view of animal welfare, because of needlessness of experimental chemical administration, that is non-invasive.

Table 1: Summary of behavioral scores of monkey offspring and concentration of PCBs (total and mainly contaminating species) of each mother.

	Offspring used in this study; ID and sex					
	101 Female	103 Male	104 Male	105 Male	106 Male	107 Male
Behavioral scores						
Eye-contact test[1)]	39.3	11.0	15.3	11.3	4.7	17.7
4 step finger maze test[2)]	25	38	32	44	N.A.[3)]	60
Concentrations in serum samples from each mother at GD 131 (pg/mL)						
Total PCBs	71	35	83	52	140	190
#28-TrCB	4.1	2.4	3.7	4.7	7.8	9.2
#49-TeCB	1.6	<1	2.1	1.0	4.5	6.6
#52/69-TeCB	1.5	0.2	2.4	1.2	5.2	7.3
#66-TeCB	2.1	1.3	3.2	3.0	8.1	17.0
#70-TeCB	1.8	<1	2.5	1.1	5.8	13.0
#74-TeCB	1.2	<1	1.6	2.1	3.9	8.0
#101-PeCB	1.4	<1	2.1	<1	5.4	5.9
#118-PeCB	2.4	1.7	3.9	2.0	6.0	12.0
#153-HxCB	3.0	1.7	5.1	2.3	4.6	9.7

[1)] Number of eye-contacts for 1 min

[2)] Number of sessions required to complete 4 step finger maze learning test

[3)] Not applicated

However, there are two problems to be cleared by all means in the present study in the future. The detection limit of each congener of PCB was 1 pg/mL in this study, and the concentrations of some congeners were under the detection limit in most of pregnant monkeys, which make it impossible to completely discuss congener-specific correlations. There is a possibility that these minor congeners specifically damage CNS development of fetuses[7], because many studies reported the congener-specific activities such as anti-estrogen and anti-thyroid hormone. More precision technique is preferred. Next, more large-scale examination is required. Indeed, one pregnant monkey (No. 107) showed higher concentrations of PCBs compared with others and her offspring needed the largest number of trials in the finger maze test. This combination mainly determined the

correlation between the concentrations of some PCB congeners as well as that of total PCBs in maternal plasma and offspring's scores of maze learning.

We certainly think that more pregnant monkeys are essential for more reliable prediction about the correlation of maternal PCBs contamination and the behavioral trait of offspring.

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