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分担研究報告書

Prenatal Exposure to Perfluorinated Chemicals and Neurodevelopment in Early Infancy

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研究要旨

We assessed the effects of perfluorooctane sulfonate (PFOS)/perfluorooctanoate (PFOA) concentrations, as most well detected PFCs in humans, on neurodevelopment of infants in early infancy. Mother-child pairs were analyzed in this birth cohort between 2002 and 2005 in Japan. The prenatal PFOS and PFOA levels were measured in maternal serum samples by liquid chromatography-tandem mass spectrometry. Neurodevelopment of infants at 6 and 18 months of age were assessed by Bayley Scales of Infant Development. Associations with log10-transformed PFC concentrations were estimated using linear regression models adjusted for potential confounders. In the fully adjusted model, PFOA, not PFOS, had a negative association with mental developmental index (MDI) among female infants. In addition, we observed negative association between PFOA and MDI/PDI at 18 months of age but it did not meet significant p-value. We observed no association between concentrations of PFOS and neurodevelopmental scores in infants. Our data suggest an inverse association between low-dose PFOA exposure and MDI in early life. In future studies, assessment of the effects of PFCs with longer carbon-chain on neurodevelopment of infants and children with bigger sample size and different battery tests is in need.

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A. 研究目的

Perfluorinated chemicals (PFCs) are ubiquitous and stable chemicals widely detected in humans and environment. The most widely studied and detected PFCs are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). They are slowly eliminated from the human body and are resistant to metabolism with mean half-lives of 5.4 and 3.8 years for PFOS and PFOA, respectively (Olsen et al. 2007). Both of these compounds showed neurodevelopmental toxicity in animal studies; maternal or neonatal exposure to PFOS and PFOA caused neurodelopmental delay, increased motor activity and reduced habituation that were accompanied by hypothyroxinemia (Lau et al. 2003; Butenhoff et al. 2009). A strong correlation of these compounds has been demonstrated between maternal and cord blood samples in

humans, indicating that neonates are exposed to PFCs via the placental passage (Inoue et al. 2004). Exposure of pregnant women and infants to PFCs had raised concerns, and they may have permanent neurodevelopmental toxic effects in humans.

However, the effects of prenatal PFCs exposure on neurodevelopment of next generation, especially in early infancy is not well understood. In the present analyses, we explored the relationship of prenatal PFOS/PFOA exposure with Mental and Psychomotor Developmental Indices (MDI, PDI) at 6 and 18 months of age assessed by Bayley Scales of Infant Development, 2nd edition (BSID II).

B. 研究方法

This study was a part of the Hokkaido Study on Environment and Children's Health performed between July 2002 and October 2005, and the details have been previously described (Kishi et al. 2011 and 2013). Of 1,796 approached Japanese pregnant women, 514 (28.6%) pregnant women agreed to participate in this study. A self-administered questionnaire survey was completed after the second trimester containing information related to smoking, economic status, educational levels, and alcohol and caffeine intake during pregnancy. Medical information including maternal age, maternal body mass index (BMI) before pregnancy, parity, gestational age, type of delivery, infant sex, and birth size were obtained from participant medical records.

A 40-mL blood sample was taken from the maternal peripheral vein after the second trimester of pregnancy, and PFOS and PFOA levels in maternal serum were measured by column-switching liquid chromatography -tandem mass spectrometry (LC/MS/MS). The PFOS values of all samples were detected, and for samples with PFOA levels below the detection limit (0.50 ng/mL), we used a value of half the detection limit (0.25 ng/mL). BSIDII were used to assess MDI and PDI in infants at 6 and 18 months of age. The environmental conditions of the subjects were assessed by using the questionnaire of home environment devised by Anme et al. (1997).

For the analysis of associations between maternal PFCs and BSID II, the following subjects were excluded: women with pregnancy-induced hypertension (n=11), women with diabetes mellitus (n=1), mother-infant pairs with fetal heart failure (n=1), and twins (n=7). After the exclusion of these subjects, 428 mother–infant pairs had available PFOS and PFOA concentrations. In addition, eligibility criteria for analysis of subjects were: babies born at term (37–42 weeks' gestation), Apgar score of > 7 at 1 min, infants without congenital anomalies or diseases, and BSID-II completed.

We analyzed correlations between PFC concentrations and the characteristics of the mothers and infants (also BSID II scores and characteristics) using the Spearman correlation test, the Mann–Whitney *U*-test, and the Kruskal–Wallis test. We performed multiple-regression analysis to examine the association between BSID-II scores and the levels of PFCs in maternal blood. The levels of PFCs in maternal blood were $\log_{10}$ transformed, and the analysis was adjusted for maternal age (year), parity ($0/\geq 1$), maternal educational levels (categorical), alcohol consumption and smoking during pregnancy (yes/no), caffeine intake during pregnancy (milligrams per day), blood sampling period (before and after delivery),

breast feeding (less or more than 3 months), Index of Child Care Environment, and total dioxin levels (TEQ, WHO 2005). We performed all of the statistical analyses using JMP pro 10 (SAS Institute Inc., NC, USA). Results were considered significant if $p < 0.05$.

（倫理面への配慮）

This study was conducted with the written informed consent of all participants and the study protocol was approved by the institutional ethical board for epidemiological studies at the Graduate School of Medicine and Center for Environmental and Health Sciences, Hokkaido University.

B. 研究結果

The basic characteristics of the study population are presented in Table 1. The mean scores were 90.5 (SD = 5.7) for MDI and 90.2 (SD = 10.3) for PDI at 6 months of age. Whereas, the mean scores for MDI and PDI were 84.2 (SD = 12.0) and 86.4 (SD = 10.9) at 18 months of age. The mean values of maternal PFOS and PFOA levels were 6.2 ng/mL and 1.3 ng/mL, respectively. In crude and adjusted model, PFOS and PFOA did not show significant association with MDI nor PDI at 6 months of age (n=174). After sex stratification, we found a negative significant association between prenatal PFOA and MDI score (but not PDI) only in female infants (β= -0.317; CI, -12.5 to -0.95; *p*-value= 0.023) in adjusted model. We did not find any significant association between PFOS levels with BSID II scores before and after sex stratification in adjusted model. Regarding the association between PFCs and neurodevelopment at 18 months (n=134), although there was a negative association between PFOA and MDI (β= -0.155; CI, -16.5 to 3.8; *p*-value= 0.219) and PDI (β= -0.135; CI, -14.2 to 4.3; *p*-value= 0.295) in adjusted model, but it was not significant. PFOS did not show any significant association with neurodevelopmental outcomes at 18 months of age. Sex stratification was applied to find sex differences of PFCs exposure on neurodevelopment but we did not find any significant association in either sexes at 18 months of age.

D. 考察

To our knowledge, this study is one of few epidemiological reports regarding the association between PFC levels and neurodevelopment, and the first study to examine correlation of PFCs and Bayley's test. We did observe no association between low prenatal PFOS exposure and BSID II scores at 6 and 18 months of age. We found negative correlation between prenatal PFOA exposure and MDI at 6 months of age only among female infants. We observed an inverse association between PFOA and MDI/PDI at 18 months of age but p-value was not significant. It may be due to small sample size in our study at 18 months of age (n=134).

There are few reports regarding the association of PFCs and neuro-development in infants and children. Fei et al. (2008) reported no convincing associations between prenatal PFCs (PFOS and PFOA) and developmental milestones at 6 and 18 months of age reported by mothers. In this report, PFOS and PFOA levels were higher than those in our study with a large sample size (6-months, n=1336; 18-months, n=1,255). In another recent study, there was no association between prenatal PFCs exposure and behavioral/affective disorders

such as ADHD in offspring (n=965) at 20 years of age (Strom et al. 2014). In contrast to our result, a Taiwanese report indicated PFOS but not PFOA levels in cord blood plasma were adversely associated with neurodevelopment among 2-year children using a national questionnaire (Chen et al. 2013). In our study, exposure levels of PFCs were lower than all of mentioned studies. Comparison of above results with our results is difficult due to difference of exposure levels, biomedia, societies, age of children and applied battery test. Our results suggest negative association between in utero PFOA exposure and neurodevelopment in early infancy especially among female infants.

E. 結論

Our result suggest in utero PFOA exposure is associated with lower neurodevelopmental indexes in early infancy especially among girls. Previously, our group reported time trends of 11 types of PFCs between 2003 and 2011 in plasma samples of pregnant women in Hokkaido (Okada et al. 2013). The results indicate that PFOS and PFOA concentrations declined, whereas long-chain PFCs (including PFNA and PFDA) levels increased. In future studies, assessment of the effects of newly emergent PFCs (such as PFNA and PFDA) on neurodevelopment of infants and children with bigger sample size and different battery tests is necessary.

F. 研究発表

1. 論文発表

In preparation

2. 学会発表

Houman Goudarzi, Tamiko Ikeno, Sachiko Kobayashi, Atsuko Araki, Chihiro Miyashita, Seiko Sasaki, Sonomi Nakajima, Hiroyuki Nakazawa, Reiko Kishi: Exposure to perfluoroalkyl chemicals and neurodevelopment at 6 months of age. International Society for Environmental Epidemiology (August 24-28, Seattle, USA, poster presentation)

G. 知的財産権の出願・登録状況（予定を含む。）

1.特許取得

なし

2.実用新案登録

なし

3.その他

なし

参考文献

1) Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, et al. 2007. Half-life of serum elimination of perfluorooctanesulfonate perfluoro-hexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect. 115:1298-305.

2) Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME,et al. 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicol Sci.74:382-92.

3) Butenhoff JL, Ehresman DJ, Chang SC, Parker GA, Stump DG. 2009. Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: developmental neurotoxicity. Reprod Toxicol. 27:319-30.

4) Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, et al. 2004. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect.

112:1204-7.
5) Kishi R, Sasaki S, Yoshioka E, Yuasa M, Sata F, Saijo Y, et al. 2011. Cohort profile: the Hokkaido study on environment and children's health in Japan. Int J Epidemiol. 40:611-8.
6) Kishi R, Kobayashi S, Ikeno T, Araki A, Miyashita C, Itoh S, et al. 2013. Ten years of progress in the Hokkaido birth cohort study on environment and children's health: cohort profile--updated 2013. Environ Health Prev Med. 18:429-50.
7) Anme T, Shimada C, Katayama H. 1997. Evaluation of environmental stimulation for 18 months and the related factors. Nihon Koshu Eisei Zasshi. 44:346-52.
8) Fei C, McLaughlin JK, Lipworth L, Olsen J. 2008. Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environ Health Perspect. 116:1391-5.
9) Strøm M, Hansen S, Olsen SF, Haug LS, Rantakokko P, Kiviranta H. et al. 2014. Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomes--a prospective study with long-term follow-up. Environ Int. 68:41-8.
10) Chen MH, Ha EH, Liao HF, Jeng SF, Su YN, Wen TW. et al. 2013. Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiology. 24:800-8.
11) Okada E, Kashino I, Matsuura H, Sasaki S, Miyashita C, Yamamoto J. et al. 2013. Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003-2011. Environ Int. 60:89-96.

Table 1. Characteristics of mothers and infants

Characteristics	At 6 months No. (%)	At 18 month No. (%)
Maternal characteristics		
Age (years) [a]	30.8±4.6	31.1±4.4
Prepregnancy BMI (kg/m2) [a]	21.2±2.9	21.2±2.9
Parity (times)		
0	83 (48.0)	65 (48.9)
≥1	90 (52.0)	68 (51.1)
Smoking during pregnancy		
Yes	53 (30.5)	39 (29.1)
No	121 (69.5)	95 (70.9)
Alcohol intake during pregnancy		
Yes	51 (29.3)	40 (29..9)
No	123 (70.0)	94 (70.1)
Blood sampling period		
during pregnancy	132 (75.9)	107 (79.8)
after delivery	42 (24.1)	27 (20.1)
Child characteristics		
Sex		
Male	84 (48.3)	67 (50.0)
Female	90 (51.7)	67 (50.0)
Gestational age (days) [a]	276.4±8.3	276.4±8.5
Birth weight (g) [a]	3112.5±357.9	3098.6±323.3
Birth length (cm) [a]	48.2±1.7	48.2±1.6
Breast-feeding (month)		
<3	72 (41.4)	51 (38.0)
≥3	102 (58.6)	83 (62.0)
BSID II mental index score (MDI) [a]	90.5±5.7	84.2±12.0
BSID II psychomotor index score (PDI) [a]	90.2±10.3	86.4±10.9
Index of Child Care Environment [b]	22.2±3.9	28.2±3.5

[a]mean±SD. [b]perfect score is 30 and 38 points in 6 months and 18 months, respectively.

Table 2.

Concentration of PFOS and PFOA (ng/mL) in maternal blood (n=174) whose infants had Bayley test score

	Mean (SD)	Minimum	Percentile			Maximum
			25th	50th	75th	
PFOS	6.2 (2.7)	1.6	4.4	5.7	7.4	16.2
PFOA [a]	1.3 (0.8)	not detected	0.8	1.2	1.7	4.3

Concentration of PFOS and PFOA (ng/mL) in maternal blood (n=134) whose infants had Bayley test score

	Mean (SD)	Minimum	Percentile			Maximum
			25th	50th	75th	
PFOS	6.2 (2.6)	1.70	4.4	5.8	7.4	16.2
PFOA [a]	1.3 (0.8)	not detected	0.8	1.2	1.8	4.3

[a] For subjects with a level below the detection limit, a value equal to half the detection limit were used.

Table 3. BSID II MDI and PDI development scores at 6 months of age for infants in relation to the levels of PFCs in maternal blood (n=174).

	MDI			PDI		
	beta	(95% CI)	p-value	beta	(95% CI)	p-value
PFOS						
crude	0.037	(-3.26 to 5.43)	0.624	-0.016	(-8.62 to 6.96)	0.833
Adjusteda	0.036	(-4.02 to 6.08)	0.688	-0.004	(-0.391 to 0.371)	0.959
PFOA						
crude	0.003	(-2.89 to 3.03)	0.961	-0.030	(-6.38 to 4.21)	0.687
Adjusted a	-0.033	(-4.16 to 2.88)	0.721	0.011	(-5.31 to 6.08)	0.893

adjusted for gestational age, parity, maternal age, smoking and alcohol intake during pregnancy, caffeine during pregnancy, maternal education level, blood sampling period, breast feeding, score, Index of Child care Environment, and total dioxin levels (TEQ, WHO 2005).

Table 4. BSID II MDI and PDI development scores at 6 months of age for infants in relation to the levels of PFCs in maternal blood by sex stratification.

Boys (n=84)	MDI			PDI		
	beta	(95% CI)	p-value	beta	(95% CI)	p-value
PFOS						
crude	-0.080	(-8.58 to 3.96)	0.466	-0.041	(-12.21 to 8.32)	0.707
adjusted[a]	-0.064	(-9.84 to 6.26)	0.659	0.178	(-3.62 to 19.01)	0.179
PFOA						
crude	0.087	(-2.38 to 5.52)	0.431	0.009	(-6.20 to 6.74)	0.934
adjusted[a]	0.139	(-2.09 to 7.72)	0.368	0.063	(-5.87 to 9.33)	0.650
Girls (n=90)	MDI			PDI		
	beta	(95% CI)	p-value	beta	(95% CI)	p-value
PFOS						
crude	0.136	(-2.11 to 10.03)	0.198	0.002	(-11.63 to 11.90)	0.981
adjusted[a]	0.077	(-5.08 to 9.55)	0.545	0.029	(-11.27 to 14.48)	0.804
PFOA						
crude	-0.094	(-6.56 to 2.48)	0.373	-0.055	(-10.97 to 6.43)	0.605
adjusted[a]	-0.317	(-12.5 to -0.95)	**0.023**	0.096	(-6.59 to 14.46)	0.458

Adjusted for gestational age, parity, maternal age, smoking and alcohol intake during pregnancy, caffeine during pregnancy, maternal education level, blood sampling period, breast feeding, Index of Child Care Environment, and total dioxin levels (TEQ, WHO 2005).

Table 5. BSID II MDI and PDI development scores at 18 months of age for infants in relation to the levels of PFCs in maternal blood (n=134).

	MDI			PDI		
	beta	(95% CI)	p-value	beta	(95% CI)	p-value
PFOS						
crude	0.082	(-5.77 to 16.5)	0.342	-0.033	(-12.08 to 8.15)	0.701
adjusted [a]	-0.002	(-17.8 to 17.4)	0.985	-0.030	(-18.0 to 14.2)	0.815
PFOA						
crude	-0.090	(-10.9 to 3.3)	0.298	-0.065	(-8.9 to 4.0)	0.455
adjusted [a]	-0.155	(-16.5 to 3.8)	0.219	-0.135	(-14.2 to 4.3)	0.295

[a] adjusted for gestational age, parity, maternal age, smoking during pregnancy, alcohol during pregnancy, caffeine during pregnancy, maternal education, blood sampling period, breast feeding, caffeine during pregnancy, maternal education, blood sampling period, breast feeding, Index of Child Care Environment, and total dioxin levels (TEQ, WHO 2005).

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