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The Effect of Prenatal Methylmercury Exposure on the GSH Level and Lipid Peroxidation in the Fetal Brain and Placenta of Mice

CHIHO WATANABE, YUICHI KASANUMA, YASUSHI DEJIMA[1] and HIROSHI SATOH

Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai 980-8575, [1]*Department of Human Ecology, School of Health Sciences, Faculty of Health Sciences, Kyorin University, Mitaka 181-8611*

WATANABE, C., KASANUMA, Y., DEJIMA, Y. and SATOH, H. *The Effect of Prenatal Methylmercury Exposure on the GSH Level and Lipid Peroxidation in the Fetal Brain and Placenta of Mice.* Tohoku J. Exp. Med., 1999, **187** (2), 121-126 —— Effect of prenatal exposure to methylmercury (MeHg) on the glutathione (GSH) levels and lipid peroxidation in the fetal brain was examined. Pregnant ICR mice were injected with 3 mgHg/kg of MeHg on gestational day 12, 13 and 14 (G12-14). On the G14 or G17, the fetal brains were removed and their GSH levels and thiobarbituric acid-reactive substances (TBARS) levels were determined. On the G17, GSH level of MeHg-treated fetal brain was significantly higher than that of the control brain; the TBARS level showed the similar trend but the difference was not significant. These results indicated that the prenatal MeHg treatment disturbed the normal GSH level in the fetal brain and warranted further investigation on the significance of this GSH perturbation. ———— methylmercury; mice; glutathione; lipid peroxidation; fetal brain

Methylmercury (MeHg) is a potent neurotoxin that has caused several episodes of human poisoning. Since it was discovered that the fetus is much more sensitive to the neurotoxic effects of this metal than adults (Clarkson 1993), the potential neurobehavioral outcome of *in utero* exposure to MeHg has been examined in several fish-eating populations (Grandjean et al. 1995, 1997; Myers et al. 1997; Davidson et al. 1998).

Although the neurotoxic mechanism of MeHg has been the focus of much research, a firm conclusion regarding the primary toxic insult has yet to be drawn.

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Present address for Chiho Watanabe, Department of Human Ecology, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan.

For reprint request, contact C. Watanabe, Ph.D. at the address above.

It has been proposed that MeHg exerts its neurotoxicity via the production of radical oxygen species (ROS). We examined the effect of MeHg on several selenoenzymes in the fetal brain (Watanabe et al. 1998), since it is known that MeHg and selenium interact toxicologically, and found that MeHg severely depressed glutathione peroxidase activity in the fetal brain (Watanabe et al. 1998). Since the major proposed function of this enzyme is the sequestration of peroxides, including hydrogen peroxide and lipid peroxides, inhibition of its activity may enhance oxidative stress in the fetal brain.

Therefore, this study examined whether *in utero* exposure to MeHg enhances oxidative stress in the fetal mouse brain. As an index of oxidative stress, the level of thiobarbituric acid-reactive substances (TBARS) was measured in the placenta and fetal brain. In addition, the glutathione (GSH) content of the fetal brain was examined, because the GSH concentration is known to increase after a challenge by chemicals that promote the generation of radicals. In adult mice, MeHg administration resulted in an elevated GSH level (Li et al. 1996). Monoamine concentrations in the offspring were measured to assess whether the MeHg treatment exerted the neurotoxic outcome.

Materials and Methods

Pregnant ICR mice at gestational day 11 (G11; G0 was defined as the day when the vaginal plug was confirmed) were purchased from a commercial breeder (Nippon SLC Co., Ltd., Hamamatsu). On G12, 13, and 14, the mice were given either MeHg (3 mgHg/kg/day) or saline s.c. The dose was so determined, based on the previous studies of our own (Yin et al. 1997) and others (Su and Okita 1976; Nishikido et al. 1987; O'Kusky et al. 1988; Cagiano et al. 1990), that it should induce neurobehavioral disturbances in the newborn, but should not produce gross maternal toxicity.

The pregnant mice were dissected on either G14 or G17, under ether anesthesia. In the former case, dissection took place 3–6 hours after the final injection of MeHg. After sampling blood from the jugular vein, the placenta and fetal brain were removed and frozen immediately. Separated plasma and tissues were kept at −80°C until assay. In a separate experiment, dams with or without MeHg treatment were allowed to deliver their offspring and the offspring were sacrificed on the 23rd postnatal day under ether anesthesia, and the brain was dissected. To measure the monoamine levels, the dissected brain was processed immediately as described below.

Lipid peroxidation

TBARS were used as an index of lipid peroxidation in the placenta and fetal brain. TBARS were measured using the method of Uchiyama (Uchiyama and Mihara 1978) with a slight modification. Briefly, a 5% tissue homogenate was incubated with or without 1 mM ascorbate for 60 minutes at 37°C. After

centrifugation, the supernatant was reacted with thiobarbituric acid in acidic conditions. The resulting pink-colored substances were quantified spectrophotometrically using tetraethoxypropane as the standard.

Glutathione

The reduced form of GSH was measured fluorometrically (Cohn and Lyle 1966). Briefly, the tissue was homogenized in trichloroacetic acid containing EDTA. After centrifugation, the supernatant was mixed with o-phthal aldehyde and vortexed. The fluorescence was measured exactly 30 minutes after mixing.

Brain monoamine concentrations

Concentrations of monoamines and metabolites in the brains of offspring mice were measured on the 23rd postnatal day by HPLC/ECD (Wagner et al. 1982). The brain tissues were removed and sonicated immediately in an acidic solution containing EDTA, Na-metabisulfite, and deoxyepinephrine as an internal standard. The resulting suspension was centrifuged and the supernatant was filtered (pore size = 0.45 micrometer) and injected into HPLC/ECD as described elsewhere (Watanabe et al. 1997).

Statistics

The differences between the means were tested using Tukey's multiple comparison, after a one-way analysis of variance (ANOVA) using a statistical package (JMP version 3; SAS Institute Inc., Cary, NC, USA). The level of significance in each of these analyses was $p < 0.05$ unless otherwise indicated.

Results

TBARS increased in the fetal brain during development. On G14, the TBARS levels were 187 ± 22 ($n = 6$) and 166 ± 19 nmol/g ($n = 8$) (wet tissue) in the control and the MeHg-treated groups, respectively. On G17, TBARS were 445 ± 18 ($n = 8$), 497 ± 20 nmol/g ($n = 7$) in the control and MeHg-treated groups, respectively. The value with MeHg treatment was higher, although the difference was not significant ($p = 0.077$).

The GSH concentrations in the fetal and maternal brains on G17 are shown in Table 1. The GSH level in the MeHg-treated fetal brain was higher than that in the control brain, although the difference was small. In the dam's brains, no significant difference was found between the treatment groups.

The norepinephrine concentration decreased in the brains of weaning offspring, although the difference was not statistically significant; the concentrations were 199 ± 67 and 137 ± 24 ng/g brain in the control and MeHg-treated groups, respectively ($p = 0.092$).

TABLE 1. *GSH (reduced) concentration of G17 fetal brain and dam brain after MeHg treatment*

	n	Fetal brain	Maternal brain
Control	8	1.28±0.03	1.38±0.13
MeHg	8	1.41±0.03*	1.61±0.28

Values are in n mol/g brain. Mean and S.D. are shown.
*Significantly different from the control group ($p<0.05$).

DISCUSSION

In related experiments using the same dose regimen, we determined that the Hg concentration in the G17 fetal brain was around 50 nmol/g tissue (Watanabe et al. 1998), which was enough to cause neurobehavioral deficits after birth (Yin et al. 1997). The monoamine levels suggested that the dose used was marginally neurotoxic to the offspring of treated dams.

Previous studies have assessed the effects of MeHg on the brain GSH concentration, and reported that the concentration was increased (Li et al. 1996), unchanged (Yasutake and Hirayama 1994), or even decreased (Sarafian and Verity 1991; Yee et al. 1993). It should be noted that the GSH response in two of these studies (Yee et al. 1993; Li et al. 1996) was different, even though the studies used a similar time course, the same strain of mice, and a similar dose regimen. Ours is the first study to assess the effect of *in utero* exposure to MeHg on the GSH level in the fetal brain. We observed a small, but significant, increase. The increase may result from enhanced oxidative stress in the fetal brain, as was suggested for the increase of GSH in MeHg-treated adult brains (Li et al. 1996). Since the difference was so small, however, further confirmation of this increase and elucidation of its toxicological significance are necessary. Since previous reports showed that the response depends on the interval between exposure and GSH determination, the time course of the effect needs to be studied to fully assess the impact of MeHg treatment on the GSH level.

In our study, increased TBARS were not detected in the fetal brain. Increased lipid peroxidation (Yonaha et al. 1983), or increased generation of radical oxygen species such as hydrogen peroxide and superoxide anion (Yee and Choi 1994) have been reported after MeHg exposure, although none of these studies examined prenatal exposure. Lipid peroxidation is a remote endpoint in terms of the sequence of events following a toxic insult by MeHg. Indeed, in vivo experiments detected ROS generation at doses lower than those required to enhance lipid peroxidation (Yonaha et al. 1983; Yee and Choi 1994). Therefore, we might have obtained evidence of enhanced oxidative stress if we had measured ROS generation rather than lipid peroxidation. In addition, it is possible that the increase in GSH prevented lipid peroxidation from occurring. Again, this

point should be clarified by delineating the time course of the change in GSH.

In conclusion, these results indicate that *in utero* exposure to MeHg, which subsequently resulted in neurotoxicity, altered the GSH level in the fetal brain. Although direct evidence of enhanced oxidative stress was not obtained, further examination of the role of oxidative stress in the neurotoxicity of prenatal MeHg exposure is warranted.

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