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Strain Difference in Methylmercury Transport across the Placenta

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It is well documented that methylmercury (MeHg) easily penetrates the placental barrier and affects the developing fetuses in human beings and laboratory animals (Matsumoto et al. 1965; Suzuki et al. 1967; Amin-Zaki et al. 1974; Harada 1978; Kajiwara and Inouye 1986 1992; Aschner and Clarkson 1988; Inouye and Kajiwara 1988 1990). Previously, we have reported that MeHg is transferred through the placenta as MeHg-cysteine (MeHg-Cys) conjugate by the neutral amino acid transport that carries methionine (Met) and phenylalanine (Phe) in rat fetuses (Kajiwara et al. 1996). This mechanism on the transmembrane movement of MeHg is the same as those at the blood-brain barrier of both fetal and adult rats (Hirayama 1980 1985; Aschner and Clarkson 1988; Aschner 1989).

In the present experiment, MeHg transport was examined using pregnant guinea pigs. Guinea pigs have a long gestation period, and they provide a good animal model of fetal Minamata Disease (Inouye and Kajiwara 1988). Furthermore, their large umbilical cords offer the opportunity to collect continuously the fetal blood from the same fetuses. The present experiment shows the strain differences between rats and guinea pigs in both the rate of accumulation of MeHg into fetal blood and the competitive effects of the neutral amino acids on the MeHg transport through the placenta.

MATERIALS AND METHODS

Animals. Hartley guinea pigs at 8 weeks of gestation (Kyudo, Kumamoto) were used. The experimental procedure was adopted from that in the previous report (Kajiwara et al. 1996). Briefly, dams were anesthetized with pentobarbital (27 mg/kg), then both sides of the subclavian vein were exposed by small skin incisions.

Mercury accumulation in fetal blood. In the control group, phosphate-buffered saline (PBS) was injected into the left vein at the volume of 10 ml/kg. Just after injection of PBS, MeHgCl or MeHg-Cys solution in PBS was injected into the right

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vein at a dose of 0.8 mgHg/kg (0.5 ml/kg). Five, 10, 15, 30, 60 and 120 minutes, respectively, after MeHg injection, an aliquot (1.2 ml) of the maternal blood was collected from the vein in the mesometrium. A small aliquot (0.15 ml) of fetal blood was collected from the umbilical cord of each fetus. There were usually three to four fetuses in each dam. Three dams were used in each group.

Co-injection with neutral amino acids and probenecid. 0.2 M L-methionine (Met) or 0.2 M L-phenylalanine (Phe) were dissolved in PBS. The amino acid solution was injected into the left vein at a dose of 2 mmol/kg. 25 mM Probenecid (Pro) in PBS was injected similarly at a dose of 250 μmol/kg. Then MeHg was injected into the right vein. Both maternal and fetal blood were collected in the same way as in the control group. Hg and SH-compounds were determined by the method reported previously (Kajiwara et al. 1996).

RESULTS AND DISCUSSION

The Hg level of both maternal blood cells and blood plasma were shown in Figure 1A. The concentration of MeHg in the maternal blood cells gradually decreased, but that of plasma was almost constant during the experimental period.

MeHg was easily transported from maternal blood to fetal blood, and accumulated with time (Figure 1B). In the fetal blood, most MeHg distributed in the blood cells, and there was little Hg in the blood plasma (< 0.01 μg/ml). Although the MeHg level in the maternal blood plasma was the same as in rats, the rate of MeHg transport in guinea pigs was one sixth of that in rats (Kajiwara et al. 1996).

Phe administration did not influence the Hg levels in fetal blood the same as in the control. In the Met group, Hg levels increased 2.5 times higher than in the control (Figure 2). In the rat placenta, MeHg transport was effectively suppressed to 20% and 70% of control level by the coinjection of Phe and Met, respectively (Kajiwara et al. 1996). Thus there was clear difference between both species. Administration of probenecid, an inhibitor of organic acid transporter, did not affect the accumulation of Hg in the fetal blood (Figure 2).

Maternal administration of MeHg as MeHg-Cys conjugate increased the Hg levels in the fetal blood 4 times higher than by MeHg alone (Figure 2). Thus Cys was used as the carrier in MeHg transport in the placenta, supporting the previous results in rats (Kajiwara et al. 1996).

The plasma concentration of cysteine (Cys) and glutathione (GS) are shown in Table 1. The concentrations of both reduced and total Cys were significantly higher in the dams administered Met than in the control dams. Met would not have the competitive effect in MeHg transport (Figure 2); in fact, it would cause the synthesis of Cys in maternal blood, thereby accelerating the MeHg uptake.

Table 1. Cysteine (Cys) and glutathione (GS) levels (μM) in maternal blood plasma after MeHg injection alone or MeHg co-injection with methionine (Met) in guinea pigs. The reduced (r-) and total (t-) Cys and GS were measured separately.

	r-Cys	t-Cys	r-GS	t-GS
Control	10.7 ± 3.1	59.3 ± 17.5	0.61 ± 0.17	2.1 ± 0.8
Met	21.8 ± 6.4**	151.8 ± 57.2**	0.51 ± 0.28	1.4 ± 1.1

**: significantly different from control group ($p < 0.01$, Student's t-test).

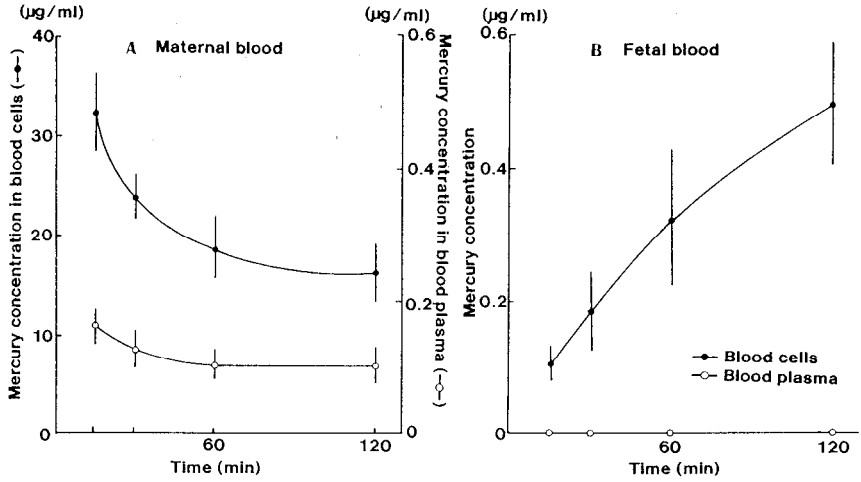

Figure 1. Changes of Hg levels in maternal blood following i.v. administration of MeHg (A). The accumulation of MeHg in the fetal blood cells was shown in B. The MeHg in fetal blood plasma was not detected (less than 0.01 μg/ml).

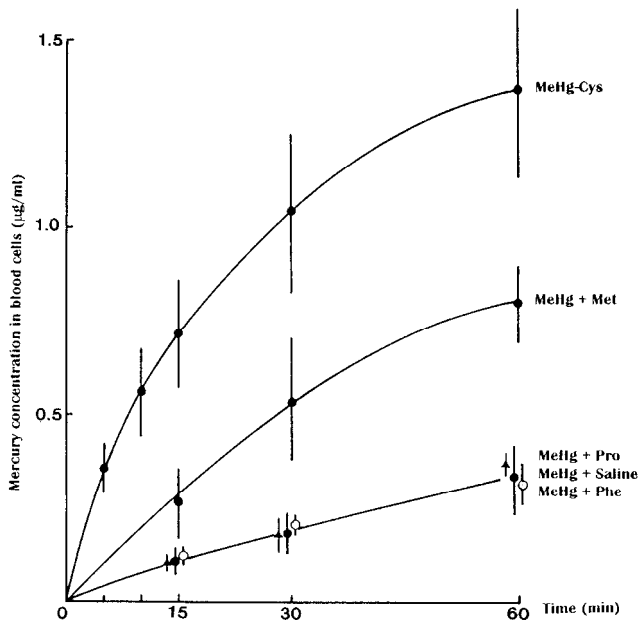

Figure 2. Effects of neutral amino acids or probenecid on the accumulation of MeHg in fetal blood cells after maternal i.v. injection of MeHg. There was no effect of the coinjection of Phe on the accumulation of MeHg in fetal blood. In the Met group, the Hg level is 2.5 times higher than MeHg alone. Four times higher accumulation of MeHg was observed following the maternal i.v. administration of MeHg-Cys than that of MeHg alone.

In the rat placenta, we earlier proposed the following mechanism of MeHg transport through the placenta (Kajiwara et al. 1996). MeHg in the maternal plasma, at least partly binds with low molecules of the thiols, such as Cys and GS. MeHg-GS or MeHg-Cys-Gly would not be transferred into fetal blood via the organic acid transport, and they would have to be degraded into MeHg-Cys. MeHg-Cys complex is exclusively transported into fetal blood plasma *via* L-type neutral amino acid carrier. Little MeHg in fetal blood plasma suggests that once MeHg is transported into the fetal plasma, it rapidly moves into the fetal blood cells.

The results obtained here in guinea pigs supported the above hypothesis.The rate of MeHg accumulation, however, in fetal blood was one sixth of that in rats (Kajiwara et al. 1996). And MeHg transport was not only suppressed by the co-injection with Phe and Met, it was increased by Met (Figure 2). Why were such differences observed in both species? One reason might be the concentration of GS. The concentration of both reduced and total GS are six and eight times lower in guinea pigs than in rats, respectively (Kajiwara et al. 1996). At the chorionic villus, MeHg-GS seems to be actively degraded into MeHg-Cys by γ-GTP and aminopeptidase, then transferred into fetal capillary, as well as in the brush border in the kidney (Yasutake et al. 1989, Hiarayama et al. 1991). Thus, GS should have an important role in the MeHg transfer through the brush border in the chorionic villus, so the lower GS concentration would cause a lower accumulation of MeHg in the fetal blood of guinea pigs. The reason why the suppressive effect of co-injection of MeHg with Met and Phe did not occur in guinea pigs is not within the scope of the present study. This report, however, suggests the importance of studing the placental transfer of MeHg using some species, including human cells *in vitro*.

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