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Inhibition of Implantation Caused by Methylmercury and Mercuric Chloride in Mouse Embryos *In Vivo*

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Methylmercury, an environmental pollutant, produces a wide spectrum of fetotoxic effects in men and laboratory animals. Experimental studies have shown that the exposure to methylmercury in the gestation period causes fetal death, gross malformation, growth retardation of the fetuses, and stillbirth (Spyker and Smithberg, 1972; Su and Okita, 1976; Inouye and Murakami, 1975; Inouye et al., 1985, 1988; Fuyuta et al., 1978; Yasuda et al., 1985). Although the effects of methylmercury on fetuses have been well documented, only a few experiments have been performed on the embryo toxicity at the early gestation periods. Because the embryos at preimplantation period are known to be highly sensitive to methylmercury in vitro and in vivo (Matsumoto and Spindle, 1982; Matsumoto et al., 1984; Kajiwara and Inouye, 1986), in the present experiment, the embryonic development after implantation was investigated following treatment with methylmercury during the preimplantation period. Since the previous report showed that methylmercury and inorganic mercury were different in their manifestation of toxicity on preimplantation embryos in vivo (Kajiwara and Inouye, 1986), the effects of methylmercury and mercuric chloride on embryos were investigated in vivo in the present study.

MATERIALS AND METHODS

Animals employed were from a closed colony of Kud:ddY mice. The animals were housed in an air-conditioned room (23+2 °C) and kept under an alternating 12-hr. light/dark schedule. A solid diet CA-1 (CLEA Japan), and tap water were made available ad libitum. Nulliparous females, 8 to 12 weeks of age, were paired with males overnight, and the following morning females with vaginal plugs were taken as being in day 0 of pregnancy.

Methylmercuric chloride (MMC) and mercuric chloride (MC) were purchased from Wako Pure Chemical Industries, Ltd. The purity of MMC and MC is over 98% and 99.5%, respectively. MMC and MC were dissolved in physiological saline for administration to animals at a dose of 0.15 ml/30 g body weight. Pregnant females were given MMC or MC intravenously through the caudal vein on day 0 (9:30 - 10:30 am) of pregnancy. The doses were 2.5, 5.0, 10.0 and 20.0 mgHg/kg body weight of MMC, and 1.0, 2.0 and 2.5 mgHg/kg of MC. Mice of the control group were given physiological saline solution. Embryos were observed on day 5 of gestation (immediately after implantation) or on day 12 of gestation (middle of gestation) as follows. For observation on day 5 of gestation, five females were used in each dose group. Pregnant mice were deeply anesthetized with pentobarbital and the blood was collected from the inferior vena cava for mercury analysis. Oviducts and uteri were removed and fixed with Bouin's solution. The specimens were dehydrated, embedded

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in paraffin, serially sectioned and stained with HE. The number of normal and abnormal embryos were microscopically checked in all serial sections. Abnormality was defined as underdeveloped embryos (not reaching egg cylinder stage) and collapsed embryos, and embryos with or without decidual membrane were also observed. For observation on day 12 of gestation, ten females were used in each group. After blood sampling, the uteri were opened and examined for the number of implants, early deaths and late deaths. Living fetuses were removed and immersed in physiological saline. They were fixed with 10% buffered neutral formalin, examined for gross malformations under a dissecting microscope, and then weighed. An aliquot of blood was centrifuged at 3,000 rpm. Analyses of total mercury contents in both maternal blood and blood plasma were performed by oxygen combustion-gold amalgamation method (Jacobs et al., 1960).

Table 1. Effects of MMC and MC during preimplantation periods on the development of mouse embryos; observation on day 5 of gestation.

Mercury and Control	Dose (mgHg/kg)	No. of treated dams	No. of observed dams	No. of embryos	No. of abnormal embryos without decidua	No. of abnormal embryos with decidua	No. of abnormal embryos Total[b]	No. of normal egg cylinder[a]	Diameter of egg cylinder[c]
Control	0.0	5	5	66 (13.2±1.1)	0	6	6 (9.1)	60 (12.0±1.6)	59.2±8.7
MMC	2.5	5	5	54 (10.8±1.6*)	0	5	5 (9.3)	49 (9.8±2.8)	62.2±9.5
	5.0	5	5	55 (11.0±1.6)	0	3	3 (5.5)	52 (10.4±1.7)	59.5±6.9
	10.0	5	5	61 (12.2±1.5*)	2	13	15 (24.6)	46 (9.2±5.4)	61.9±8.2
	20.0	5	5	53 (10.6±1.1*)	0	8	8 (15.1)	45 (9.0±1.6)	58.4±8.7
MC	1.0	5	5	45 (9.0±2.4*)	0	5	5 (11.1)	40 (8.2±2.2)	60.8±9.0
	2.0	5[d]	5	36 (7.2±2.8*)	9	8	17 (47.2)	19 (3.8±5.2)	57.8±7.9
	2.5	7	5	35 (7.0±4.1)	35	0	35 (100)	0 (0.0)	-

a; Numbers in parentheses are calculated on the basis of the litter as sample unit and given as means±S.D.
b; Numbers in parentheses are percentages of total embryos
c; Data are shown in micrometers, given as mean±S.D.
d; two of dams died
*; Significant decrease from control group by Student's t-test, $p<0.05$

Table 2. Effects of MMC and MC during preimplantation periods on the development of mouse embryos; observation on day 12 of gestation.

Mercury and Control	Dose (mgHg/kg)	No. of treated dams	No. of observed dams	No. of implants	Fetal mortality Early	Fetal mortality Late	Fetal mortality Total[b]	No. of living fetuses[a]	Fetal weight
Control	0.0	10	10	117 (11.7±4.0)	4	4	8 (6.8)	109 (10.9±4.0)	109±25
MMC	2.5	10	10	86 (8.6±5.1)	1	1	2 (2.3)	84 (8.4±5.1)	94±12*
	5.0	10	10	70 (7.0±5.9*)	2	0	2 (2.9)	68 (6.8±5.7*)	88±10*
	10.0	10	10	90 (9.0±2.4*)	3	34	37 (41.1)	53 (5.3±5.7*)	93±11*
	20.0	10	10	80 (8.0±5.1)	7	21	28 (35.0)	52 (5.2±5.5)	73±16
MC	1.0	10[d]	10	127 (12.7±1.8*)	6	4	10 (7.9)	117 (11.7±2.1*)	106±15*
	2.0	12[e]	10	68 (6.8±6.3*)	0	1	1 (2.1)	67 (6.7±6.2*)	78± 8*
	2.5	15	10	44 (4.4±6.2)	0	4	4 (18.2)	40 (4.0±5.8)	53± 8

a; Numbers in parentheses are calculated on the basis of the litter as sample unit and given as mean±S.D.
b; Numbers in parentheses are percentages of total implants
c; Data are shown in milligrams, given as mean±S.D.
d; Two of dams died
e; Five of dams died
*; Significant decrease from control group by Student's t-test, $p<0.05$

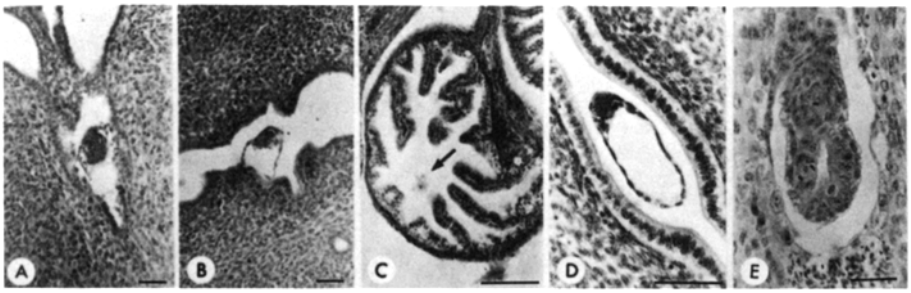

Figure 1 Photomicrographs of normal and abnormal embryos from MMC-treated (A,B), MC-treated (C,D), and control dams (E). Pregnant females were i.v. administered mercurials on day 0 of pregnancy. Embryos were histologically observed on day 5 of gestation. A, abnormal egg cylinder in decidua; B, abnormal blastocyst in decidua; C, uncleaved egg in oviduct; D, blastocyst in uterus without decidua; E, normal egg cylinder. Scales; A, B, D and E = 50 μm, C = 100 μm.

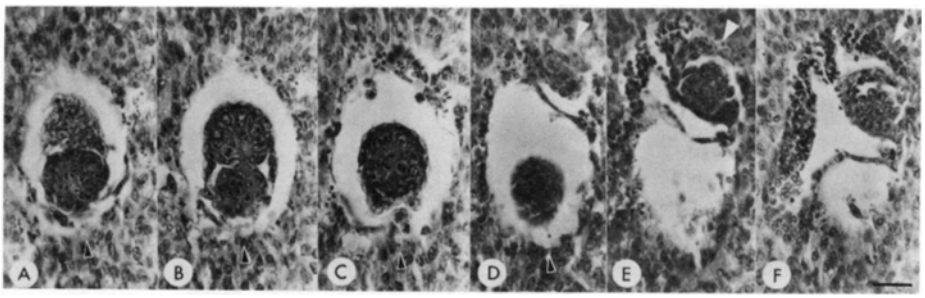

Figure 2 Abnormal implantation induced by 20 mgHg/kg methylmercury treatment. Figures A-F are photographs of serial sections, showing two embryos in one implantation site. One embryo (black arrow heads) is normal and the other embryo (white arrow heads) is abnormal. Scale = 50 μm.

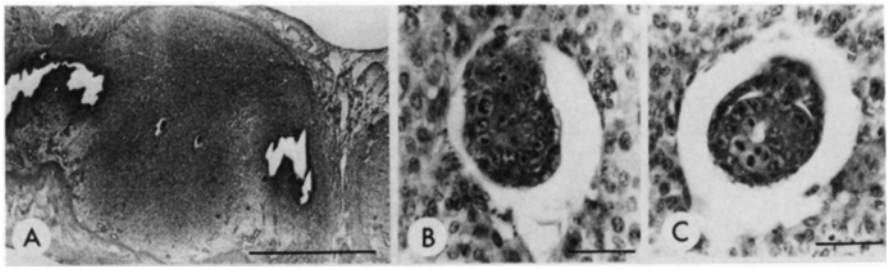

Figure 3 Abnormal implantation induced by 20 mgHg/kg methylmercury treatment. Figure A shows two egg cylinders in one decidua (Scale = 1 mm). Figures B and C show the high magnifications of each embryo (Scale = 50 μm).

RESULTS AND DISCUSSION

Observations on day 5 (Table 1, Fig. 1). Maternal treatment with either MMC or MC caused a decrease in the number of embryos, especially at 2.0 and 2.5 mgHg/kg MC. In the groups of high MMC dosage (10 and 20 mgHg/kg), abnormal embryos with decidual swellings were observed frequently; 13/61 embryos in 10 mgHg/kg group and 8/53 embryos in 20 mgHg/kg group (Table 1). Underdeveloped egg cylinder and blastocyst surrounded by decidual swellings are shown in Figs. 1a and 1b. Two cases in which two embryos implanted close to one another were found in 20 mgHg/kg MMC-treated dams (Figs. 2, 3). In one case, there were two embryos, one normal and the other abnormal in an implantation site (Fig. 2). In another case, there were two egg cylinders in one decidual swelling (Fig. 3).

In the 2.0 and 2.5 mgHg/kg MC treated groups, blastocysts without decidua (delay of implantation) in the uterine lumen were observed frequently; 9/36 embryos in the 2.0 mgHg/kg group and all embryos in the 2.5 mgHg/kg group (Table 1). These abnormal blastocysts without decidua are shown in Fig. 1d. In this case, no formation of a crypt in the uterine lumen was observed. The diameters at the embryonic region of normal egg cylinder in both MMC and MC treated groups were no different from those in the control groups (Table 1).

Observation on day 12 (Table 2). Numbers of implants, dead fetuses, living fetuses, fetal weight and malformed fetuses following maternal treatment with mercury compounds are summarized in Table 2. There were significantly fewer implants in the groups treated with 10 and 20 mgHg/kg of MMC. In these MMC-treated groups a remarkable increase in fetal mortality was noted; 37/90 fetuses in 10 mgHg/kg group and 28/80 fetuses in 20 mgHg/kg group. Fetal deaths occurred in the late gestational period (Table 2). There was significant decrease in fetal weight in all groups treated with MMC, but not in a group given 1.0 mgHg/kg MC (data not shown).

Numbers of implants were decreased in the groups of 2.0 and 2.5 mgHg/kg MC, but not in the 1.0 mgHg/kg MC group. In contrast with MMC-treated groups, fetal death after implantation was quite rare in MC-treated groups. Surviving fetuses decreased significantly in number and fetal weight was reduced by 2.0 and 2.5 mgHg/kg MC, but not at 1.0 mgHg/kg MC.

Mercury levels in maternal blood (Table 3). Total mercury concentra-tions in the maternal blood and plasma were summarized in Table 3.

Table 3. The concentration of mercury (total mercury) in the maternal blood and blood plasma after i.v. administrations of MMC and MC on day 0 of pregnancy.

Mercury	Dose (mgHg/kg)	on day 5 of pregnancy		on day 12 of pregnancy	
		Blood	Blood plasma	Blood	Blood plasma
MMC	2.5	0.94 ± 0.11	0.29 ± 0.06	0.33 ± 0.20	0.10 ± 0.02
	5.0	2.0 ± 0.5	0.61 ± 0.08	0.45 ± 0.12	0.13 ± 0.04
	10.0	4.2 ± 1.1	1.5 ± 0.4	1.6 ± 0.5	0.55 ± 0.24
	20.0	6.8 ± 0.9	2.0 ± 0.4	3.1 ± 1.0	1.1 ± 0.4
MC	1.0	0.05 ± 0.01	0.03 ± 0.0	0.09 ± 0.09	0.02 ± 0.01
	2.0	0.22 ± 0.08	0.07 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
	2.5	0.41 ± 0.12	0.16 ± 0.08	0.11 ± 0.06	0.04 ± 0.01

Data are shown in μg/ml

The results demonstrated clearly that after maternal treat with both mercurials in vivo during the preimplantation period, the mouse embryo failed to develop after implantation, resulting in the early and late fetal death or the decrease in number of implants.

Different manifestation of embryo-toxicity were observed between the two mercurials. In MC-treated groups, the examination on day 5 showed a decrease in the number of embryos in uteri and oviducts (Table 1), and most surviving embryos were blastocysts without decidual swelling (Fig. 1d). Previous study (Kajiwara and Inouye,1986) showed some preimplantation embryos were collapsed or delayed in development after treatment with MC. These collapsed embryos had been absorbed and disappeared by gestational day 5 in the present study. Subsequently, the surviving embryos which were observed as blastocysts on day 5 may resorb, resulting in the fewer implants observed on day 12. In the MMC-treated groups, but not in the MC-treated groups, an increased incidence of fetal mortality was observed on day 12 of gestation, and almost a half of fetuses were dead in the high dosage groups (10 and 20 mgHg/kg). Some fetal deaths might be caused following abnormal implantation, i.e., abnormal blastocysts or egg cylinders with decidua observed on day 5 of gestation (Table 1 and Figs. 1a,b). For instance, when embryos were treated with mercurials at the preimplantation period, MC caused failure of implantation while MMC caused abnormal implantation or abnormal development after implantation.

The exposure of preimplantation embryos to other teratogenic agents, such as mitomycin-C and N-methyl-N-nitrosourea, caused a decrease in the number of implants, and fetal mortality at term (Takeuchi, 1984; Nagao et al., 1986). In the present experiment, these two effects were also induced with mercury compounds.

Accumulation of inorganic mercury in an animal body after ingestion of methylmercury has been suggested (Norseth and Clarkson, 1970a,b; Norseth and Brendeford, 1971; Norseth, 1972; Magos and Bulter, 1976; Omata et al., 1980; Komsta-Szumska et al., 1983). From this standpoint, studies on mercury toxicity must be conducted for inorganic mercury as well as methylmercury. In the previous report (Kajiwara and Inouye, 1986), we showed the difference between these two mercurials in the manifestation of embryo toxicity during preimplantation periods. The present results indicated that toxicities of both mercurials were also different after the implantation period.

It is interesting that abnormal implantation of twin embryos in one implantation site or in one decidua, were observed in 20 mgHg/kg MMC-treated dams (Figs. 2a,b). In general, during implantation period the embryos are spaced at regular intervals each other (Block, 1966). Because two embryos in one decidua is extremely rare in normal embryogenesis (Teiler, 1972), one must carefully determine if the present abnormal implantations were caused by methylmercury. But the high incidence (two cases after treatment of five dams in 20 mgHg/kg MMC group) and the existence of many abnormal blastocysts or egg cylinders suggest that these abnormal twin embryos were caused by methylmercury treatment.

Although methylmercury is known to cause gross malformations, such as cleft palate and hydronephroses when fetuses were treated at mid- or late-gestation periods (Spyker and Smithberg, 1972; Inouye and Murakami, 1975; Fuyuta et al., 1978; Kajiwara and Inouye, 1987), the incidence of malformations was very low in the fetuses observed on day 12 in the present experiment. It is noted that 2 fetuses from dams treated with methylmercury had ectopic heart (data not shown), but this malformation is not reported to be methylmercury-induced.

It is uncertain at present whether the failure of implantation is due to embryonic disability, or to maternal dysfunction of uterus. In this aspect, embryo-transfer experiments may be required to clarify the effects of mercury during pre- and post-implantation, because of the close-relationship between embryo and uterus after implantation.

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REFERENCES

Fuyuta M, Fujimoto T, Hirata S (1978) Embryotoxic effects of methylmercuric chloride administrated to mice and rats during organogenesis. Teratology, 18:353-366.

Greene RM, Kochhar DM (1973) Spatial relations in the oral cavity of cortisone-treated mouse fetuses during time of secondary palate closure. Teratology, 8:153-162.

Inouye M, Murakami U (1975) Teratogenic effect of orally administered methylmercuric chloride in rats and mice. Congenital Anomalies 15:1-9.

Inouye M, Murao K, Kajiwara Y (1985) Behavioral and neuropathological effects of prenatal methylmercury exposure in mice. Neurobehav Toxicol Teratol 7:227-232.

Jacobs MB, Yamaguchi S, Goldwater LJ, Gilbert H (1960) Determination of mercury in blood. Amer Ind Hyg Assoc J 21:475-480.

Kajiwara Y, Inouye M (1986) Effects of methylmercury and mercuric chloride on preimplantation mouse embryos in vivo. Teratology, 33:231-237.

Kajiwara Y, Inouye M (1987) Effects of maternal treatment with methylmercury on the manifestation of cleft lip in CL/Fr mice. Congenital Anomalies 27:17-22.

Komsta-Szumska E, Czuba M, Reuhl KR, Miller DR (1983) Demethylation and excretion of methyl mercury by the guinea pig. Environ Res 32: 247-257.

Magos L, Butler WH (1976) The kinetics of methylmercury administered repeatedly to rats. Arch Toxicol 35:25-39.

Matsumoto N, Spindle A (1982) Sensitivity of early mouse embryos to methylmercury toxicity. Toxicol Appl Pharmacol 64:108-117.

Matsumoto N, Spindle A, Katayama S, Kubo H (1984) Culture and transfer of embryos as a testing system for embryo-toxicity of chemicals. Congenital Anomalies 24:353-372.

Nagao T, Ishizuka Y, Mizutani M (1986) Effects of mitomycin C treatment before implantation on development of mouse embryo. Congenital Anomalies 26:93-101.

Norseth T, Clarkson TW (1970a) Studies on the biotransformation of ^{203}Hg-labeled methyl mercury chloride in rats. Arch Environ Health 21:717-727.

Norseth T, Clarkson TW (1970b) Biotransformation of methylmercury salts in the rat studied by specific determination of inorganic mercury. Biochem Pharmacol 19:2775-2783.

Norseth T, Brendeford M (1971) Intracellular distribution of inorganic and organic mercury in rat liver after exposure to methylmercury salts. Biochem Pharmacol 20:1101-1107.

Norseth T (1972) Biotransformation of methyl mercuric salts in the rat with chronic administration of methyl mercuric cysteine. Acta Pharmacol Toxicol 31:138-148.

Omata S, Sato M, Sakimura K, Sugano H (1980) Time-dependent accumulation of inorganic mercury in subcellular fractions of kidney, liver, and brain of rats exposed to methylmercury. Arch Toxicol 44:231-241.

Spyker JM, Smithberg M (1972) Effects of methylmercury on prenatal development in mice. Teratology 5:181-190.

Su M, Okita GT (1976) Behavioral effects on the progeny of mice treated with methylmercury. Toxicol Appl Pharmacol 38:195-205.

Takeuchi IK (1984) Teratogenic effects of methylnitrosourea on pregnant mice before implantation. Experientia 40:879-881.

Teiler, K (1972) The house mouse: developmental and normal stages from fertilization to 4 weeks of age. Springer-Verlag press, pp 21-23.

Yasuda Y, Datu AR, Hirata S, Fujimoto T (1985) Characteristics of growth and palatal shelf development in ICR mice after exposure to methylmercury. Teratology 32:273-286.

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