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Developmental Effects of Methylated Arsenic Metabolites in Mice

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Arsenic occurs naturally throughout the environment, generally at low levels. Certain areas, such as parts of Taiwan, Mexico, Chile, Argentina, India, and the U.S. (Borum and Abernathy 1994; Cantor 1996) contain natural mineral deposits from which relatively high levels of inorganic arsenic may leach into well water. In addition to natural sources, application of arsenical pesticides, herbicides, and defoliants, burning of coal, and smelting of certain ores can introduce arsenic into the environment (Hood 1985), and arsenic is a contaminant at several sites on the U.S. National Priorities List of waste disposal sites (ATSDR 1993). Nearly 32 million pounds of arsenicals, principally arsenic trioxide, were imported into the U.S. in 1989, with the greatest use in the production of wood preservatives (ATSDR 1993). Human exposures tend to be oral and chronic, largely from the diet and drinking water (ATSDR 1993).

The potential for developmental toxicity of inorganic arsenic has been experimentally investigated in several species of laboratory animals, e.g., mice (Hood and Bishop 1972; Hood 1972; Domingo et al. 1995), rats (Beaudoin 1974), and hamsters (Ferm and Carpenter 1968; Hood and Harrison 1982), with dosing typically by ip or iv injection on single gestation days. With the exception of a recent report by Nemec et al. (1998) reports of oral dosing (e.g., Hood et al. 1978; Baxley et al. 1981) generally involved single high dose gavage exposures. Relatively few studies have examined the developmental toxicity potential of arsenic metabolites, and no studies have been done with mice, the most common subject of tests of inorganic arsenic effects.

Exposure of pregnant animals to a toxic dose of arsenic by injection results in considerably greater effects than would be caused by the same dose administered orally (Hood 1972; Hood et al. 1978; Baxley et al. 1981; Hood and Harrison 1982). The greater degree of maternal biotransformation to methylated metabolites, along with slower arsenic uptake by both mother and conceptus and lower peak blood levels, is probably responsible for the lower toxicity of oral exposure (Hood et al. 1987; 1988). The present study investigates the developmental toxicity potential of the major arsenic metabolites of man and the common laboratory animals, i.e., dimethylarsinic acid [DMAA, cacodylic acid,

$(CH_3)_2As(O)OH$] and methanearsonic acid [MAA, $CHsAsO(OH)_2$]. In addition to arsenic metabolism, environmental sources of these arsenicals include use of sodium dimethylarsinic acid as a pesticide and a cotton desiccant (Hood 1985) and monosodium methanearsonate as an herbicide (Naqvi et al. 1990; Keese and Camper 1994). Dimethylarsinic acid has been assessed for its developmental toxicity potential in the rat and mouse (Rogers et al. 1981), and both the sodium salt of that compound (SDMA, sodium cacodylate) and disodium methanearsonate (DSMA) have been tested in the hamster (Hood et al. 1982). However, there appear to be no published developmental toxicity assays employing acute dosing with either SDMA or DSMA to asses the influence of developmental timing of exposure in the mouse, the species most often studied for prenatal effects of inorganic arsenic exposure. We herein report that while skeletal and neural tube defects could be induced in the offspring of pregnant mice acutely exposed to the sodium salts of both methylated arsenic metabolites, the doses were extremely high, and in or near the maternally toxic range.

MATERIALS AND METHODS

Random-bred albino CD-1 mice (Charles River Breeding Laboratories, Raleigh, NC) were maintained in animal rooms controlled for temperature (22 ± 2°C), humidity (40-60%), and light (12/12 hr light/dark cycle) in an AAALAC-approved laboratory animal facility. They were housed and mated in shoe box type cages with hardwood bedding and' given Wayne Lab Blox (Allied Mills, Chicago, IL) and water *ad libitum.* Observation of a copulation plug designated gestation day 1 (GD 1), and mated females were then individually housed.

Mated females were randomly assigned to one of the following: (1) 7 groups treated with sodium dimethylarsinate dissolved in H_2O, (2) 7 groups treated with disodium methanearsonate dissolved in H_2O, (3) 7 vehicle control groups given 0.9% saline, or (4) a single untreated control group. All treatments were administered on one of GD 8 through 14, in a volume of 10 ml/kg. Dams dosed on one of days 8-10 with SDMA were given intraperitoneal (ip) injections of 1200 mg/kg, while those treated on one of days 11-14 were given 800 mg/kg. Dams dosed on one of days 8-11 with DSMA were given intraperitoneal injections of 1500 mg/kg, while those treated on one of days 12-14 were given 1200 mg/kg. The decreased doses used for treatments on the later gestation days were necessitated to prevent the maternal mortality observed when dams at the later gestation times were given the 1200 (SDMA) or 1500 (DSMA) mg/kg doses as those used to treat the mice dosed earlier in gestation. Intraperitoneal injection was used to allow more accurate quantification of the delivered dose and because mammalian metabolites of inorganic arsenic would largely be produced internally, rather than absorbed from the gut.

On GD 18, mated females were euthanized with CO_2, and their litters were examined for prenatal mortality. Live fetuses were examined for gross defects

and weighed. Approximately half of the fetuses from the dams injected on one of GD 8 ,9, or 10 were cleared and stained with alizarin red S by the method of Crary (1962) and examined for skeletal abnormalities.

For statistical analysis, the litter was used as the experimental unit. One-way analysis of variance (ANOVA, model I) was used for comparisons of treatment effects on the continuous variable, i.e., fetal weight. In cases of significance ($P \leq 0.05$) with ANOVA, the Student-Neuman-Kuels multiple range test was used to compare groups and controls. Incidence data (percent dead or resorbed, grossly malformed, or skeletally malformed) were analyzed by means of individual chi-square tests for comparison of each treated group and its vehicle control.

RESULTS AND DISCUSSION

Treatment on GD 9 with 1200 mg/kg SDMA resulted in a significant decrease in mean fetal weight (Table 1). GD 8, 9, or 10 treatment had no clear effect on prenatal mortality or visceral malformations, but day 8 or 9 SDMA exposures were associated with small nonsignificant increases in the incidences of gross malformations, primarily exencephalies, and significant increases in skeletal malformations (fused ribs). Treatment on later gestation days with 800 mg/kg SDMA did not result in gross or visceral malformations, but GD 13 or 14 treatment resulted in significant increases in the incidences of dead or resorbed fetuses. Treatment resulted in several maternal deaths, particularly at the high dose and on later gestation days, when the pregnant dams weighed more and thus received larger absolute amounts of SDMA. Maternal toxicity may thus have been a factor in the effects seen in the developing offspring.

These findings are in contrast to those of Rogers et al. (1981), who gavage dosed mice on GD 7-16 with dimethylarsinic acid (DMA). An increased incidence of cleft palate at the two higher doses employed (400 and 600 mg/kg/day), but not at the low dose (200 mg/kg/day), was the only malformation they observed. There were also decreases in the number of caudal skeletal ossification centers, in the incidence of supernumerary ribs, and in mean fetal weight at the middle and high doses, along with increased maternal and fetal mortality at the high dose. In rats, Rogers et al. (1981) observed irregular palatine rugae in fetuses from dams dosed with DMA at 30, 40, 50, or 60 mg/kg/day. They also found decreased maternal and fetal survival and weight gain, along with decreased numbers of sternal and caudal ossification centers in fetuses at the higher doses.

Hood et al. (1982) noted that 1000 mg/kg SDMA given ip to hamsters on one of GD 8-12 caused high incidences of embryonic resorption, and gross malformations were seen in high incidence after GD 9 or 10 treatment. Similar treatments with a somewhat lower dose (900 mg/kg) were also associated with high levels of prenatal mortality and gross malformations. Malformations observed were primarily cleft palate, cleft lip, exencephaly, and various limb

Table 1. Effects on mouse development of maternal intraperitoneal dosing with sodium dimethylarsinate (SDMA) on single gestation days.

Treatment			Litters	Mean implantations	Dead or resorbed	Fetal weight	Grossly malformed	Malformed skeletons
Agent	Dose (mg/kg/day)	Gestation day	(No.)	(No.)	(%)	(g ± SE)	(%)	[ratio (%)]
SDMA	1200	8	13	12.5	11.7	1.01 ± 0.02	3.5	8/61 (13.1)*
Saline[1]		8	15	12.5	6.9	1.07 ± 0.01	0	0/96 (0)
SDMA	1200	9	22	13.1	6.9	0.97 ± 0.01*	2.6	23/137 (16.8)*
Saline		9	12	12.2	4.1	1.10 ± 0.01	0	0/77 (0)
SDMA	1200	10	15	12.1	9.3	1.00 ± 0.01	0	6/80 (7.5)
Saline		10	11	12.0	5.3	1.10 ± 0.01	0	0/70 (0)
SDMA	800	11	10	12.1	3.3	1.01 ± 0.01	0	NA[2]
Saline		11	9	13.6	8.2	1.08 ± 0.01	0	NA
SDMA	800	12	10	12.9	6.2	1.08 ± 0.01	0	NA
Saline		12	10	11.8	5.9	1.12 ± 0.01	0	NA
SDMA	800	13	9	11.1	36.0**	1.02 ± 0.01	0	NA
Saline		13	10	11.8	11.0	1.03 ± 0.01	0	NA
SDMA	800	14	10	11.9	26.1**	1.18 ± 0.02	0	NA
Saline		14	10	13.0	6.9	1.12 ± 0.01	0	NA
Untreated			21	11.8	4.8	1.03 ± 0.01	0	NA

[1]Controls injected with 0.9% saline.

[2]Fetuses from dams treated after gestation day 10 were not examined for skeletal defects.

*Significantly different from control treated with saline on the same gestation day ($P \leq 0.05$).

Table 2. Effects on mouse development of maternal intraperitoneal dosing with disodium methanearsonate (DSMA) on single gestation days.

Treatment			Litters	Mean implantations	Dead or resorbed	Fetal weight	Grossly malformed	Malformed skeletons
Agent	Dose (mg/kg/day)	Gestation day	(No.)	(No.)	(%)	(g ± SE)	(%)	[ratio (%)]
DSMA	1500	8	15	12.8	13.5	0.95 ± 0.02*	3.6	13/89 (14.6)*
Saline[1]		8	15	12.5	6.9	1.07 ± 0.01	0	0/96 (0)
DSMA	1500	9	20	12.0	38.6*	0.96 ± 0.02*	3.4	19/68 (27.9)*
Saline		9	12	12.2	4.1	1.10 ± 0.01	0	0/77 (0)
DSMA	1500	10	18	11.1	44.0*	0.91 ± 0.02*	0.9	15/56 (26.8)*
Saline		10	11	12.0	5.3	1.10 ± 0.01	0.8	0/70 (0)
DSMA	1500	11	10	12.6	69.0*	0.95 ± 0.01*	0	NA[2]
Saline		11	9	13.6	8.2	1.08 ± 0.01	0	NA
DSMA	1200	12	10	13.2	34.1*	0.91 ± 0.01*	0	NA
Saline		12	10	11.8	5.9	1.12 ± 0.01	0	NA
DSMA	1200	13	10	12.8	16.4	1.04 ± 0.01	0	NA
Saline		13	10	11.8	11.0	1.03 ± 0.01	0	NA
DSMA	1200	14	14	12.1	35.9*	0.95 ± 0.01*	0	NA
Saline		14	10	13.0	6.9	1.12 ± 0.01	0	NA
Untreated			21	11.8	4.8	1.03 ± 0.01	0	NA

[1]Controls injected with 0.9% saline.

[2]Fetuses from dams treated after gestation day 10 were not examined for skeletal defects.

*Significantly different from control treated with saline on the same gestation day ($P \leq 0.05$).

defects. Some skeletal malformations (primarily fused ribs) were also seen, especially at the higher dose. Both SDMA dose levels were associated with significant maternal mortality.

According to the present study and that of Rogers et al. (1981), the effects of acute exposure of the mouse conceptus to the dimethylated arsenical differ somewhat from those following repeated oral dosing. These results also suggest that the rat conceptus differs from that of the mouse in its response to the arsenic metabolite, and both the adult rat and its developing offspring are considerably more sensitive to the lethality of DMA than are comparable life stages of mice. Comparison of these data with those of Hood et al. (1982) indicate that the hamster is intermediate between the mouse and rat in sensitivity to dimethylated arsenic.

Treatment with 1500 mg/kg DSMA on GD 9, 10, or 11 or with 1200 mg/kg on GD 12 or 14 resulted in significantly increased incidences of prenatal mortality (Table 2). DSMA treatment on every gestation day except GD 13 was associated with a significant decrement in mean fetal weight. The incidence of gross malformations (exencephalies) appeared to have been elevated by treatment on GD 8 or 9, but the differences from control values were nonsignificant. No visceral malformations were seen, but GD 8, 9, or 10 DSMA exposure significantly increased the incidence of skeletal malformations. As was the case with SDMA, DSMA injection at the higher dose and on later treatment days was frequently maternally lethal. Thus, the observed developmental toxicity may have been confounded with maternal toxicity.

According to a previous study on the hamster, maternal ip injection with a 500 mg/kg dose of DSMA appeared to decrease prenatal survival, but the decrease was statistically significant only for GD 12 treatment (Hood et al. 1982). Fetal weights were also significantly decreased by treatment on three of the five gestation days, but the incidences of both gross and skeletal malformations were low and did not differ significantly from control values. Maternal mortality ranged from 0 to 30 percent. According to these results and those of the current study, the hamster is also more sensitive than the mouse to effects of the monomethylated arsenic metabolite, at least with regard to maternal and fetotoxicity.

Stunted growth and developmental abnormalities have also been reported in *Xenopus laevis* embryos exposed to monomethylated arsenic given as monosodium methanearsonate (MSMA) (Schultz and Dumont 1984). Exposure of *Xenopus* embryos to an MSMA concentration of 46 mg/L resulted in 50% abnormal embryos, while 225 mg/L was required to induce 50% mortality; defects involving the eyes and the musculature predominated.

In the present study, both dimethylarsinic acid (SDMA) and methanearsonic acid (DSMA), the major metabolites of inorganic arsenic in humans and various other

mammals, were administered as their sodium salts to pregnant mice. Both arsenicals induced prenatal mortality and malformations in the developing offspring following ip maternal treatment on single gestation days. However, the doses eliciting adverse prenatal effects were extremely high and in the maternally toxic range. These data lend support to the assumption that prenatal toxicity seen following exposure to inorganic arsenicals is due to either direct or maternally-mediated effects of the parent compound, rather than to effects of the methylated metabolites.

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