Bull. Environ. Contam. Toxicol. (1991) 47:682–688

Bulletin of Environmental Contamination and Toxicology

Effects of Meso-2,3-Dimercaptosuccinic Acid (DMSA) on the Teratogenicity of Sodium Arsenate in Mice

M. A. Bosque, J. L. Domingo, J. M. Llobet, and J. Corbella*

Laboratory of Toxicology and Biochemistry, School of Medicine, University of Barcelona, San Lorenzo 21, 43201 Reus, Spain and *Department of Toxicology, School of Medicine, University of Barcelona, 08036 Barcelona, Spain

Arsenic is ubiquitous and is present in rock, soil, water, and living organisms. Therefore, it is impossible for humans or animals to avoid exposure (Neiger and Osweiler 1980). Toxicity of arsenic has long been of concern due to the frequent use of arsenicals as herbicides, insecticides, rodenticides, paint pigments, wood preservatives, and from the wastes derived from the production of several metals and as a by-product of the uses of fossil fuels (Baxley et al. 1981). Environmental arsenic exposure has received attention primarily because of disease resulting from ingestion of water containing this element. Acute ingestion of arsenic may result in immediate gastrointestinal symptoms, but polyneuropathy represents the subacute sequela (Goebel et al. 1990). Chronic effects, primarily from human epidemiological studies, associate arsenic with degenerative, inflammatory, and neoplastic changes of the skin, respiratory system, liver, cardiovascular system, blood, lymphatic system, nervous system, and reproductive system (Neiger and Osweiler 1989).

On the other hand, arsenate and arsenite the main forms of inorganic arsenic in the environment are embryotoxic and teratogenic in golden hamsters (Ferm and Carpenter 1968; Willhite 1981), mice (Hood and Bishop 1972; Hood et al. 1978; Lindgren et al. 1984; Morrissey and Mottet 1983), rats (Beaudoin 1974; Hood et al. 1977) and humans (IARC 1980). It has also been demonstrated that both arsenate and arsenite interfere with normal growth and development of early somite stage (postimplantation) mouse embryos when added to the culture medium (Chaineau et al. 1990).

Although the effects of arsenic on mammalian development are now well established, very few data on the protective activity of different chelators against embryotoxicity and teratogenicity of arsenic are available (Hood and Pike 1972; Hood and Vedel 1984). Chelating agents may interact with teratogen metals to augment or ameliorate their actions. Hood and Pike (1972) demonstrated that a single dose of 2,3-dimercaptopropanol (BAL) was capable of affording a degree of protection to arsenate exposed fetal mice. Subcutaneous treatment with 50 mg/kg of BAL 4 hr after arsenate

Send reprint requests to Dr. Jose L. Domingo at the above address.

reduced the frequency or severity of malformations compared with the effects of arsenate alone. However, BAL has several drawbacks: it is not water soluble, injection is painful, and toxic reactions are common (Aposhian 1983). In recent years, meso-2,3-dimercaptosuccinic acid (DMSA), a water soluble compound analogous to BAL, is receiving growing attention in the USA and Western Europe. Results of a number of different investigations in rodents have led to the conclusion that DMSA is much less toxic than BAL (Aposhian 1983; Aposhian and Aposhian 1990). Moreover, DMSA has been reported to be effective in inducing arsenic excretion (Aposhian 1983; Graziano 1986; Aposhian and Aposhian 1990).

In the present study, the protective effects of DMSA in alleviating the embryotoxic and teratogenic effects of sodium arsenate were evaluated in mice.

MATERIALS AND METHODS

Dibasic sodium arsenate ($Na_2HAsO_4.7H_2O$) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Adult Swiss mice (Letica, Barcelona, Spain) weighing 27-30 g were used. Female mice were mated with males (2:1) overnight and examined the following morning for copulatory plugs. The day on which a vaginal plug was found was designated as day 0 of gestation. Animals received food (Panlab rodent chow, Barcelona, Spain) and water **ad libitum.** Pregnant mice were divided into five groups which consisted of one positive control group, one negative control group, and three groups which received different concentrations of DMSA. Rooms were maintained at a temperature of 22 + 2ºC, a relative humidity of approximately 40-60% and a 12-hr light/dark cycle.

Each pregnant mouse, in both the positive and in the DMSA-treated groups were given a single ip dose of 45 mg/kg of dibasic sodium arsenate on day 8 of gestation. This day was expected to result in a maximum level of embryotoxic and teratogenic effects (Hood and Bishop 1972; Hood et al. 1977; Morrisey and Mottet 1983). Subsequently, the animals in the positive control group were given 0.9% saline solutions by sc injection, and those in the experimental groups received DMSA dissolved in 5% sodium bicarbonate solutions. A series of three dose levels (37.5, 75, and 150 mg/kg) of DMSA at four successive time intervals (0, 24, 48, and 72 hr) after sodium arsenate treatment were administered by ip injection. The doses are not teratogenic by themselves (Domingo et al. 1988). The animals in the negative control group were pretreated with 0.9% saline, and then posttreated with sodium bicarbonate solutions.

The dams were sacrificed on day 18 of pregnancy. Approximately one-half of the surviving fetuses were fixed in Bouin's solution, and sectioned by hand with a razor blade in order to observe their visceral anomalies. The other half of the fetuses were stained with Alizarin Red S for their skeletal examination.

Fetal body weights were analyzed by ANOVA followed by Duncan's multiple comparison procedure. Frequency data, such as prenatal mortality and incidence of malformed fetuses were analyzed using Fisher's test for uncorrelated proportions. Values were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Data concerning the effects of DMSA on arsenate-induced embryotoxicity in mice are presented in Table 1. Intraperitoneal administration of 45 mg/kg of sodium arsenate on day 8 of pregnancy significantly increased the number of early resorptions per litter, whereas the number of live fetuses per litter and the average fetal body weight were significantly decreased compared to the negative control group.

Treatment with DMSA at 37.5 mg/kg/day for four days significantly reduced the number of early resorptions per litter, although the number of live fetuses was significantly lower than that corresponding to the arsenate-untreated group. However, when DMSA was administered at 75 or 150 mg/kg/day there were no significant differences between these groups and the negative control group in the number of live, dead and resorbed fetuses, or in the fetal body weight.

The effects of DMSA on arsenate-induced fetal abnormalities in mice are summarized in Table 2. Exophthalmos and exencephaly were the major gross anomalies observed in the arsenate-treated groups, whereas fusion of ribs, bipartite sternebrae, and decreased ossification of supraoccipital bone, carpus and tarsus were the most frequently observed skeletal defects. No significant differences were seen in the number of internal malformations or variations between the positive and the negative control groups. Although treatment with DMSA at 37.5, 75 or 150 mg/kg reduced all types of defects, only at the highest dose there were no significant differences in the number of total external abnormalities as compared to the negative control group. Also, no significant differences in the number of total skeletal abnormalities between the arsenate-untreated group and the DMSA-treated groups were observed at doses of 75 and 150 mg/kg.

The objective of this study was to evaluate the ability of DMSA to prevent the developmental toxicity of arsenate. A single 45 mg/kg intraperitoneal injection of sodium arsenate to pregnant mice on day 8 of pregnancy produced essentially similar results as those reported previously. Embryolethality was evidenced by increased prenatal mortality, whereas decreased fetal body weights as well as exophthalmos, exencephaly, fusion of ribs, and decreased ossification of supraoccipital bone, tarsus and carpus were the most frequent signs of fetotoxicity (including teratogenicity).

The results above reported indicate that DMSA treatment at 75 or 150 mg/kg/day for four days had a significant protective effect against arsenate-induced embryolethality. Also, administration of

Table 1. Effects of DMSA on arsenate-induced embryofetotoxicity in mice (means ± SD)

	Groups				
Sodium arsenate (mg/kg)	0 (0.9% saline)	45	45	45	45
DMSA (mg/kg/day)	0 (5% $NaHCO_3$)	0 (5% $NaHCO_3$)	37.5	75	150
No. of litters	10	11(4)*	10(2)*	9	10
No. total implants/ litter	12.7±2.4	11.3±4.1	9.4±4.7	12.2±2.3	12.9±2.0
No. live fetuses/ litter	12.1±2.0[2]	6.7±5.4[b]	7.0±5.6[a]	11.3±3.1[1]	11.9±2.1[2]
No. early resorptions/ litter	0.3±0.7[3]	4.3±3.6[c]	1.7±3.5	0.6±0.9[2]	0.6±0.9[2]
No. late resorptions/ litter	0.3±0.5	0.3±0.6	0.5±0.5	0.3±0.7	0.2±0.4
No. dead fetuses/ litter	0.0±0.0	0.0±0.0	0.1±0.2	0.0±0.0	0.2±0.2
Fetal body weight (g)	1.17±0.05[3]	1.01±0.08[c]	1.12±0.14	1.12±0.07[2]	1.14±0.04[3]

*In parentheses, number of litters wholly resorbed. [a,b,c]Significantly different from arsenate-untreated group (negative control): $P<0.05$, $P<0.01$, $P<0.001$, respectively. [1,2,3]Significantly different from arsenate-treated group (positive control): $P<0.05$, $P<0.01$, $P<0.001$, respectively.

Table 2. Effects of DMSA on arsenate-induced external and skeletal abnormalities*

	Groups				
Sodium arsenate (mg/kg)	0 (0.9% saline)	45	45	45	45
DMSA (mg/kg/day)	0 (5% $NaHCO_3$)	0 (5% $NaHCO_3$)	37.5	75	150
External abnormalities					
No. of fetuses observed/ No. of litters	121/10	74/7	70/8	102/9	119/10
Facial hemorrhages	1(1)	0	2(2)	1(1)	1(1)
Hemorrhages in limbs	0	0	0	0	1(1)
Dorsal hemorrhages	0	2(2)	0	1(1)	1(1)
Abdominal hemorrhages	0	2(2)	0	0	0
Exencephaly	0^{2}	$4(3)^{b}$	$2(2)^{a}$	0^{2}	0^{2}
Exophthalmos	0^{3}	$16(6)^{c}$	$4(2)^{b,2}$	$5(3)^{a,2}$	0^{3}
Total external abnormalities	$1(1)^{3}$	$20(7)^{c}$	$8(5)^{c,1}$	$7(4)^{a,3}$	$3(3)^{3}$
Skeletal abnormalities					
No. of fetuses observed/ No. of litters	68/10	44/7	40/8	52/9	60/10
Supraoccipital bone, absence	0^{1}	$4(3)^{a}$	2(2)	0^{1}	0^{1}
decreased ossification	$2(2)^{3}$	$16(6)^{c}$	$3(2)^{2}$	0^{3}	0^{3}
Carpus, decreased ossification	0^{3}	$21(7)^{c}$	$6(4)^{c,2}$	$3(2)^{3}$	$1(1)^{3}$
Tarsus, decreased ossification	$4(2)^{3}$	$27(7)^{c}$	$7(4)^{3}$	$3(2)^{3}$	$3(2)^{3}$
Bipartite sternebrae	0^{1}	$9(4)^{a}$	2(2)	$1(1)^{1}$	0^{1}
Fusion of ribs	0^{3}	$18(6)^{c}$	$3(2)^{a,3}$	$5(1)^{a,3}$	$1(1)^{3}$
Total skeletal abnormalities	$4(2)^{3}$	$27(7)^{c}$	$10(4)^{b,3}$	$8(3)^{3}$	$3(2)^{3}$

*In parentheses, number of affected litters.
^{a,b,c}Significantly different from arsenate-untreated group (negative control): $P<0.05$, $P<0.01$, $P<0.001$, respectively. ^{1,2,3}Significantly different from arsenate-treated group (positive control): $P<0.05$, $P<0.01$, $P<0.001$, respectively.

DMSA at 37.5, 75 or 150 mg/kg/day significantly reduced the incidence of external and skeletal malformations and variations associated with arsenate treatment.

In a previous investigation, Hood and Pike (1972) reported that subcutaneous treatment with 50 mg/kg of BAL diminished the incidence of arsenate-induced gross malformations and growth retardation when given 4 hr after arsenate. However, the rate of resorbed fetuses as well as the percentage of skeletal malformations were not significantly reduced. 50 mg/kg of BAL and 75 mg DMSA/kg are equimolar amounts. In our study, 75 mg DMSA/kg significantly reduced the prenatal mortality and the incidence of gross and skeletal abnormalities caused by arsenate. It indicates that DMSA is more effective than BAL in the prevention of arsenate-induced embryotoxicity and teratogenicity in mice.

In recent years, it has been demonstrated that DMSA is as effective as BAL (dose for dose) in inducing arsenic excretion and more effective than BAL in reducing the arsenic content of tissues (Graziano 1986; Aposhian and Aposhian 1990; Kreppel et al. 1990). With regard to the detoxication mechanism of DMSA, it has been shown that arsenic administered as arsenite or arsenate relatively easily passes the placenta and can be found in various fetal organs in rodents as well as in monkeys (Gerber et al. 1982; Lindgren et al. 1984; Hood et al. 1987). Thus, it is possible that DMSA (like BAL) acts to increase the rate of arsenic excretion reducing the embryonic exposure to such a low level that arsenic is incapable of exerting embryofetotoxic effects on the fetuses. Due to the absence of a placental barrier for arsenic (Gerber et al. 1982; Hood et al. 1987), it seems that arsenic compounds may explain part of the "unaccountable" early abortions due to the general toxicity of metals (Chaineau et al. 1990). In contrast to BAL, DMSA offers encouragement with regard to its therapeutic potential for pregnant women exposed to pentavalent arsenic.

Acknowledgments. This study was supported by the CICYT (Spain), grant SAL90-0074.

REFERENCES

Aposhian HV (1983) DMSA and DMPS-water soluble antidotes for heavy metal poisoning. Annu Rev Pharmacol Toxicol 23: 193-215

Aposhian HV, Aposhian MM (1990) **meso**-2,3-Dimercaptosuccinic acid: chemical, pharmacological and toxicological properties of an orally effective metal chelating agent. Annu Rev Pharmacol Toxicol 30: 279-306

Baxley MN, Hood RD, Vedel GC, Harrison WP, Szczech GM (1981) Prenatal toxicity of orally administered sodium arsenite in mice. Bull Environ Contam Toxicol 26: 749-756

Beaudoin AR (1974) Teratogenicity of sodium arsenate in rats. Teratology 10: 153-158

Chaineau E, Binet S, Pol D, Chatellier G, Meininger V (1990) Embryotoxic effects of sodium arsenite and sodium arsenate on mouse embryos in culture. Teratology 41: 105-112

Domingo JL, Paternain JL, Llobet JM, Corbella J (1988) Developmental toxicity of subcutaneously administered **meso**-dimercaptosuccinic acid in mice. Fundam Appl Toxicol 11: 715-722

Ferm VH, Carpenter SJ (1968) Malformations induced by sodium arsenate. J Reprod Fertil 17: 199-201

Gerber GB, Maes J, Eykens B (1982) Transfer of antimony and arsenic to the developing organism. Arch Toxicol 49: 159-168

Goebel HH, Schmidt PF, Bohl J, Tettenborn B, Krämer G, Gutmann L (1990) Polyneuropathy due to arsenic intoxication: biopsy studies. J Neuropathol Exp Neurol 49: 137-149

Graziano JH (1986) Role of 2,3-dimercaptosuccinic acid in the treatment of heavy metal poisoning. Med Toxicol 1: 155-162

Hood RD, Bishop SL (1972) Teratogenic effects of sodium arsenate in mice. Arch Environ Health 24: 62-65

Hood RD, Pike CT (1972) BAL alleviation of arsenate-induced teratogenesis in mice. Teratology 6: 235-238

Hood RD, Thacker GT, Patterson BL (1977) Effects in the mouse and rat of prenatal exposure to arsenic. Environ Health Perspect 19: 219-222

Hood RD, Thacker GT, Patterson BL, Szczech GM (1978) Prenatal effects of oral versus intraperitoneal sodium arsenate in mice. J Environ Pathol Toxicol 1: 857-864

Hood RD, Vedel GC (1984) Evaluation of the effect of BAL (2,3-dimercaptopropanol) on arsenite-induced teratogenesis in mice. Toxicol Appl Pharmacol 73: 1-7

Hood RD, Vedel GC, Zaworotko MJ, Tatum FM, Meeks RG (1987) Distribution, metabolism and fetal uptake of pentavalent arsenic in pregnant mice following oral or intraperitoneal administration. Teratology 35: 19-25

IARC (1980) Carcinogenesis of arsenic and arsenic compounds. IARC Monogr Evalu Carcinog Risks Hum 23: 37-141

Kreppel H, Reichl FX, Szinicz L, Fichtl B, Forth W (1990) Efficacy of various dithiol compounds in acute As_2O_3 poisoning in mice. Arch Toxicol 64: 387-392

Lindgren A, Danielsson BRG, Dencker L, Vahter M (1984) Embryotoxicity of arsenite and arsenate: distribution in pregnant mice and monkeys and effects on embryonic cell in vitro. Acta Pharmacol Toxicol 54: 311-320

Morrissey RE, Mottet NK (1983) Arsenic-induced exencephaly in the mouse and associated lesions during neurulation. Teratology 28: 399-411

Neiger RD, Osweiler GD (1989) Effect of subacute low level dietary sodium arsenite on dogs. Fundam Appl Toxicol 13: 439-451

Willhite CC (1981) Arsenic-induced axial skeletal (disraphic) disorders. Exp Molec Pathol 34: 145-158

Recieved January 31, 1991; accepted May 8, 1991.