TOXICOLOGY AND APPLIED PHARMACOLOGY 73, 1–7 (1984)

Evaluation of the Effect of BAL (2,3-Dimercaptopropanol) on Arsenite-Induced Teratogenesis in Mice[1]

RONALD D. HOOD AND GINGER C. VEDEL-MACRANDER

Developmental Biology Section, Department of Biology, The University of Alabama, Tuscaloosa, Alabama 35486

Received February 10, 1983; accepted October 21, 1983

Evaluation of the Effect of BAL (2,3-Dimercaptopropanol) on Arsenite-Induced Teratogenesis in Mice. HOOD, R. D., AND VEDEL-MACRANDER, G. C. (1984). *Toxicol. Appl. Pharmacol.* **73**, 1–7. The effect of the chelating agent dimercaprol (BAL) on the embryotoxic and teratogenic effects of arsenite (As^{3+}) was determined. BAL (sc, 30 mg/kg) was administered to pregnant CD-1 mice, either 8 and 4 hr prior to or 4 and 8 hr after a 12-mg/kg ip dose of arsenite; other females received a single sc injection of 60 mg/kg BAL concurrently with the arsenite. Treatments were given on Gestation Day 9 or 12 (copulation plug = Day 1). Controls received sc corn oil or ip H_2O, with or without arsenite or BAL. Arsenite treatment caused gross and skeletal malformations and prenatal deaths, while controls were unaffected. When BAL was given prior to arsenite on Day 9, incidences of prenatal mortality and skeletal malformation were significantly diminished, and on Day 12, BAL protected against fetocidal effects of arsenite when given concurrently with the arsenite. No other significant protective effects against arsenite toxicity were seen due to BAL; however, concurrent BAL treatment on Day 9 appeared to result in decreased fetal mortality and a decline in skeletal malformations. BAL given following arsenite on Day 9 afforded no significant protection against the arsenic, although an apparent decrease in gross and skeletal malformations was suggestive of such an effect. According to these results, BAL is unlikely to have a practical beneficial effect on the arsenite exposed conceptus, because it must be administered prior to the teratogen (or perhaps simultaneously with it) to be effective.

Inorganic arsenite (As^{3+}) is a teratogen in the mouse, hamster, and chick (Hood, 1972; Birge and Roberts, 1976; Peterkova and Puzanova, 1976; Baxley *et al.*, 1981; Harrison and Hood, 1981; Willhite, 1981). Arsenate (As^{5+}) has similar effects on the conceptus; however, when used at doses equally toxic to the mother, the pentavalent form is capable of producing a higher malformation rate than is the trivalent state (Ferm and Carpenter, 1968; Hood and Bishop, 1972; Hood *et al.*, 1978). It has also been established that both arsenite and arsenate reach the mammalian conceptus following maternal exposure, although arsenite is the more toxic of the two (Hood *et al.*, 1981, 1982).

Previous studies of agents that provide protection against arsenic teratogenesis (e.g., selenium, BAL) dealt with arsenate (Holmberg and Ferm, 1969; Hood and Pike, 1972). Neither, however, addressed the question of whether the teratogenesis of trivalent arsenic could be similarly affected. Pregnant animals treated with arsenite and an arsenic chelator should not respond identically to those similiarly exposed to a chelator and arsenate. Factors such as differences in presumed mechanism of action of As^{3+} and As^{5+} (sulfhydryl poisoning versus substitution for phosphate, respectively), retention (arsenite is retained longer), and metabolism (arsenite metabolism results in a greater proportion of the mono-

[1] This work was supported by Grant R01 OH00912 from the National Institute of Occupational Safety and Health.

0041-008X/84 $3.00

valent metabolite) of the two arsenic species in pregnant mice (Hood *et al.*, 1981, 1982) argue for such a concept. BAL (British Anti-Lewisite) is an arsenic chelator frequently used to reduce the toxic effects of arsenic in man and domestic animals (Levine, 1970). Because BAL might be administered to an arsenic intoxicated pregnant woman, we employed it to determine if it could alleviate arsenite-induced teratogenesis in mice.

METHODS

Mature (27 to 35 g) CD-1 albino mice (Charles River Breeding Laboratories, Wilmington, Mass.) were mated, and the day a vaginal plug was seen was considered Gestation Day 1. Mated mice were housed in shoe box cages with hardwood chip bedding and given feed (Wayne Lab Blox, Allied Mills, Chicago, Ill.) and water *ad libitum*. Mice were kept on a 12/12-hr light/dark cycle at 22 ± 2°C and 40 to 60% relative humidity.

BAL was administered at 4-hr intervals, based on the 4-hr regimen common in human chelation therapy (Grollman, 1965). Pregnant mice were subjected to one of the following on Days 9 or 12 of gestation: (1) aqueous sodium arsenite (12 mg/kg ip in a volume of 10 ml/kg body wt) or (2) BAL in corn oil (CO) 8 and 4 hr prior to, concurrently with, or 4 and 8 hr following arsenite. The BAL was given sc at two sites in the nape of the neck, at a dose of 30 mg/kg per injection. The total BAL dose was 60 mg/kg, with a volume of 3 ml/kg body wt. The amounts of BAL given were based on previous results with BAL and arsenate (Hood and Pike, 1972). Additional groups of control females were given the solvent vehicles (distilled water or CO) alone or in combinations, with or without BAL, while another group remained untreated (for details, see Tables 1–3). The gestation days for arsenite treatment were chosen based on previous data (Hood, 1972). Day 9 treatment was expected to result in a maximum level of malformed fetuses and Day 12 treatment in a high level of prenatal mortality. Use of both days allowed testing of a wider range of possible outcomes.

Mice were killed with ether on Day 18 of pregnancy and their litters examined for prenatal mortality. Live fetuses were observed for gross external defects and weighed. Two-thirds of the available fetuses were then cleared and stained by the method of Crary (1962) and examined for skeletal defects. Fetuses were not examined for visceral anomalies, since previous results indicated their frequency would be low (Baxley *et al.*, 1981). Statistical analyses on fetal weights were done by ANOVA followed by SNK multiple range tests (Winer, 1971) corrected for unequal replicate numbers (Nie *et al.*, 1975). Malformation and prenatal mortality data were analyzed by the rank sum procedure of Wilcoxon and Wilcox (1964). The litter was the treatment unit on which all analyses were based.

RESULTS

Treatment with arsenite alone resulted in a high rate of prenatal mortality, whether treatment was given on Day 9 (58%) or 12 (87%) of gestation (Tables 1 and 2). Gross and skeletal malformations, predominately exencephalies, open eyes, and fused ribs, were also observed following Day 9 treatment with arsenite alone, but there was no significant effect on fetal weight. No malformations were associated with Day 12 arsenite treatment. Treatment with arsenite and corn oil resulted in effects similar to those seen with arsenite alone, and solvent controls were similar to untreated controls (Table 3). The only exceptions were significantly lower fetal body weights seen in the Day 9 groups given arsenite and corn oil concurrently or given arsenite followed by corn oil.

When BAL was given twice on Day 9 prior to arsenite, it had a protective effect, allowing enhanced survival of the conceptus. A protective effect with regard to skeletal malformations was also associated with the Day 9 BAL pretreatment. Mortality of conceptuses exposed to BAL concurrently with arsenite on Day 9 appeared to be less than that observed in litters exposed to arsenite alone, although the difference observed was not statistically significant (Table 1). There were no statistically significant protective effects of BAL when given after Day 9 arsenite exposure, although the apparent decrease in the frequency of gross malformations and skeletal defects is suggestive of such an effect (Table 1). The data from Day 9 thus indicate that the outcome of BAL treatment was a protective effect, if an effect occurred at all, although not all results were statistically significant. Additionally, the types of malformations seen were similar in the arsenite treated groups whether or not they were also exposed to BAL.

Concurrent administration of BAL and arsenite on Day 12 of gestation resulted in protective effects with regard to fetal survival;

TABLE 1

THE EFFECT OF BAL TREATMENT ON OUTCOME OF EXPOSURE OF PREGNANT MICE TO SODIUM ARSENITE ON GESTATION DAY 9

Treatment						Grossly malformed		Malformed skeletons		
									Fetuses affected[b]	
Type	Dose (mg/kg)	Litters (N)	Implants ($\bar{X} \pm$ SE)	Dead or resorbed (%)	Mean fetal weight (g ± SE)	No. litters affected	% fetuses affected[a]	No. litters affected	ratio	(%)
As[c]	12	23 (5)[d]	12.2 ± 0.66	50	0.94 ± 0.05[e]	9	14.3[e]	6	27/81	33
					Pretreated					
Corn oil × 2[f] + As	12	21 (1)	11.7 ± 0.78	52[e]	0.88 ± 0.06[e,g]	6	6.7[e,g]	5	29/106	27[e]
BAL × 2[f] + As	12	21 (1)	11.0 ± 0.83	18	0.95 ± 0.03[e,g]	6	5.3[e,g]	3	9/174	5
					Concurrent					
As + corn oil	12	20	12.4 ± 0.68	44[e,g]	0.77 ± 0.05[e]	7	19.4[e,h]	7	32/121	26[e,g]
As + BAL	12 60	21	11.4 ± 0.64	28[e,g]	0.93 ± 0.05	5	6.4[e,h]	6	21/146	14[e,g]
					Post-treated					
As + corn oil × 2[i]	12	21 (9)	10.3 ± 1.07	51[e,h]	0.84 ± 0.03	7	14[e,j]	4	22/88	25[e,h]
As + BAL × 2[i]	12 30	29	11.4 ± 0.51	38[e,h]	0.96 ± 0.03[e]	7	6[e,j]	10	28/190	15[e,h]

[a] Malformed fetuses as a proportion of total live fetuses.

[b] Fetuses with malformed skeletons as a proportion of those examined.

[c] Given ip.

[d] Numbers in parentheses indicate deaths of additional dams.

[e] Not significantly different from litters exposed to arsenite alone.

[f] Given sc in two doses 8 and 4 hr prior to arsenite.

[g,h,j] Values for prenatal mortality, weight, or malformations sharing the same superscripts in a given column and treatment category (pre-, post-, or concurrently treated) did not differ ($p > 0.05$).

[i] Given sc in two doses 4 and 8 hr after arsenite.

TABLE 2

THE EFFECT OF BAL ADMINISTERED WITH SODIUM ARSENITE TO PREGNANT MICE ON GESTATION DAY 12[a]

Treatment					
Type	Dose (mg/kg)	Litters (N)	Implants ($\bar{X} \pm$ SE)	Dead or resorbed (%)	Mean fetal weight (g ± SE)
As[b]	12	24 (6)[c]	10.0 ± 0.77	87	0.92 ± 0.07
		Pretreated			
Corn oil × 2[d] + As	12	20 (2)	9.0 ± 0.01	73[e]	0.92 ± 0.03[e,f]
BAL × 2[d] + As	12	21 (6)	10.1 ± 0.01	71[e]	0.95 ± 0.11[e,f]
		Concurrent			
As + corn oil	12	20 (9)	8.8 ± 0.01	95[e]	0.97 ± 0.13[e,g]
As + BAL	12 60	21 (10)	12.9 ± 0.52	29	0.97 ± 0.03[e,g]
		Post-treated			
As + corn oil × 2[i]	12	25 (5)	10.3 ± 0.01	83[e]	1.03 ± 0.10[e,i]
As + BAL × 2[i]	12 30	23 (4)	9.0 ± 0.09	81[e]	1.04 ± 0.07[e,i]

[a] No significant incidences of gross or skeletal malformations were seen.
[b] Given ip.
[c] Numbers in parentheses indicate deaths of additional treated dams.
[d] Given sc in two doses 8 and 4 hr prior to arsenite.
[e] Not significantly different from litters exposed to arsenite alone.
[f,g,h] Values for prenatal mortality, weight, or malformations sharing the same superscripts in a given column and treatment category (pre-, post-, or concurrently treated) did not differ ($p > 0.05$).
[i] Given sc in two doses 4 and 8 hr after arsenite.

however, no protection was observed when BAL was administered prior to or after arsenite (Table 2).

No statistically significant adverse effects attributable to BAL were observed following any treatment on either Day 9 or 12. Although in some cases (e.g., BAL concurrent with or following H_2O, Day 9), the prenatal mortality data were suggestive of an embryotoxic effect of BAL, the decreased survival was neither significant nor was it consistent.

DISCUSSION

Exposure of pregnant mice to sodium arsenite by ip injection produced similar outcomes to those seen previously by Hood (1972). Decreased fetal survival was the most prominent outcome following all arsenite treatments, although exposure to arsenite on Day 9 also resulted in gross external and skeletal malformations. The apparent decrease in prenatal growth following corn oil treatment on Day 9 (concurrent with or following arsenite) was unexpected, however, since no such effect was seen in the similar groups given BAL in corn oil, or in any other control group.

BAL had no significant teratogenic or fetotoxic effect. This finding agrees with the results of Hood and Pike (1972), who had injected BAL sc at a dose of 50 mg/kg in SAF/ICR mice on Day 9 alone. Nevertheless, the

TABLE 3

DATA FROM PREGNANT CONTROL MICE GIVEN BAL, VEHICLE, OR NO TREATMENT[a]

Treatment		Litters	Implants	Dead or resorbed	Mean fetal weight[c]
Day[b]	Type	(N)	(N ± SE)	(%)	(g ± SE)
9	H_2O[d]	16	10.9 ± 0.60	4	1.04 ± 0.04
	H_2O[d], corn oil[e]	52	13.2 ± 0.40	8	1.01 ± 0.02
	H_2O[d], BAL[e]	49	13.1 ± 0.31	11	1.02 ± 0.02
12	H_2O[d]	16	12.8 ± 0.50	8	0.97 ± 0.03
	H_2O[d], corn oil[e]	55 (1)[f]	12.8 ± 0.04	11	1.00 ± 0.02
	H_2O[d], BAL[e]	49 (1)	12.9 ± 0.26	10	1.03 ± 0.02
Untreated		21	11.8 ± 0.76	5	1.06 ± 0.05

[a] No significant incidences of gross or skeletal malformations were seen in these controls.
[b] Gestation day of treatment.
[c] No statistically significant differences were seen.
[d] Given ip.
[e] Given sc.
[f] Numbers in parentheses indicate deaths of additional treated dams.

current results are dissimilar to those obtained by Hood and Pike (1972) with regard to protective effects of BAL against pentavalent arsenic in pregnant mice. In the earlier study, only concurrent treatment protected against skeletal defects, and all treatments (50 mg/kg BAL 4 hr before, concurrent with, or 4 hr after 40 mg/kg ip sodium arsenate) had a protective effect against gross malformations. In addition, all three treatments protected against inhibition of fetal growth caused by arsenate. Decreased fetal growth was not observed with arsenite treatment in the present study; therefore, no protective effect could be compared. Protection was not seen against arsenite-induced malformations, and only pretreatment significantly affected the incidence of skeletal defects. Since the two studies were dissimilar with respect to strain of mouse, valence state of arsenic, and dose and timing of BAL treatment, the results would not be expected to be identical. Also, the pharmacokinetics of arsenite and arsenate in the mouse conceptus following maternal injection late in gestation differ (Hood *et al.*, 1981, 1982). Initial uptake was similar following treatment with arsenic at either valence state, but arsenite was retained at greater levels from 4 to 24 hr after treatment. Nevertheless, it is of interest that in both the current study with As(III) and that of Hood and Pike (1972) with As(V), at least some of the adverse effects of inorganic arsenicals on the conceptus were diminished.

Timing of arsenic exposure during pregnancy appeared to be a factor in the outcome of BAL treatment of arsenic-exposed litters. For example, BAL given prior to arsenite decreased prenatal mortality on Gestation Day 9 but not on Day 12. In the current study, BAL was given following arsenite to determine if therapy might be of benefit after arsenite exposure. The two additional treatments (BAL given prior to or concurrent with arsenite) were used to ascertain if the influence of BAL on arsenite treated animals was dependent on the order in which exposure to the two agents occurred. The possibility of such a result was suggested by findings with BAL and arsenate (Hood and Pike, 1972) and with cadmium, selenium, and arsenate (Holmberg and Ferm, 1969).

Previous studies with rats and rabbits found that 80% of an injected dose of BAL enters the circulation within the first hour, with high

blood levels persisting for at least two additional hours (Peters *et al.*, 1947; Spray *et al.*, 1947). The persistence of BAL in the conceptus, however, could be greater than that in the adult. For BAL to protect the developing mouse against the embryolethal effect of arsenite, it apparently must be present prior to or concurrent with maternal arsenite exposure. This outcome agrees with the finding that parenterally administered arsenite given to pregnant mice attains peak levels in the maternal blood within minutes after injection and is found in significant amounts in the fetus within the first hour (Hood *et al.*, 1982). If teratogenic outcome is correlated with peak levels of arsenic in the conceptus, it should be important to have a protective agent present concurrently with or in advance of the attainment of such levels.

Skeletal defects induced by arsenite consisted of pairs of ribs joined at their proximal ends and scrambled vertebral ossification centers. In the fetuses exposed to BAL and arsenite concurrently or to arsenite followed by BAL, the severity of the arsenite-induced skeletal malformations observed was similar in the presence or absence of BAL treatment (by severity, we mean the relative numbers of anomalous ribs or vertebrae per fetus and their degree of malformation). In the arsenate study of Hood and Pike (1972), however, the severity of such malformations (though not the frequency of affected fetuses) was decreased by BAL treatment. This observation may be a further indication of basic differences in the metabolism or distribution of tri- and pentavalent arsenic in the developing organism.

The ability of BAL to protect the developing mouse fetus against at least some harmful effects of arsenite offers only modest encouragement with regard to its therapeutic potential for pregnant women exposed to trivalent arsenic. This conclusion is apparent because treatment with BAL was not effective when exposure to arsenite occurred before BAL was given, although initial exposure to the arsenic is obviously the most likely scenario in man. Nevertheless, with higher doses of BAL or with treatment given at different time intervals, the beneficial effect might be enhanced. The protective effects of 2-mercaptopropionyl glycine (Tiopronin), given in multiple high doses following exposure of pregnant mice to methylmercuric chloride, lend support to such a possibility (Fujimoto *et al.*, 1979).

Although pregnant women may be chronically exposed to arsenic because of their occupation or their environment (Nördstrom *et al.*, 1978), acute arsenic exposure is most commonly due to accidental or intentional poisoning. Such an incidence of arsenic poisoning of a pregnant woman was described by Lugo *et al.* (1969) where maternal ingestion of arsenic trioxide (As^{3+}) resulted in death of the offspring. In less severe cases, BAL or similar therapy with 2,3-dimercapto-1-propanesulfonic acid or dimercaptosuccinic acid (Tadlock and Aposhian, 1980) might offer some benefit to the unborn child. Nevertheless, it should be kept in mind that Nishimura and Takagaki (1959) reported teratogenesis in mice given a very large dose of BAL (1200 mg/kg). Thus, if high doses were administered to a pregnant woman in an attempt to protect her conceptus, the BAL might have a potential for teratogenicity in its own right. Clinicians should therefore be cautious in their use of BAL therapy in cases of known or potential pregnancy, weighing potential benefit versus harm to both mother and offspring. The usual therapeutic doses for human adults are, however, only in the range of 2.5 to 5 mg/kg. Thus, it seems unlikely that a teratogenic dose would be reached, unless the human conceptus is strikingly more sensitive to BAL than is the developing mouse.

REFERENCES

BAXLEY, M. N., HOOD, R. D., VEDEL, G. C., HARRISON, W. P., AND SZCZECH, G. M. (1981). Prenatal toxicity of orally administered sodium arsenite in mice. *Bull. Environ. Contam. Toxicol.* **26**, 749–756.

BIRGE, W. J., AND ROBERTS, O. W. (1976). Toxicity of metals to chick embryos. *Bull. Environ. Contam. Toxicol.* **16**, 319–324.

CRARY, D. D. (1962). Modified benzyl alcohol clearing of alizarin stained specimens without loss of flexibility. *Stain Tech.* **37,** 124–125.

FERM, V. H., AND CARPENTER, S. J. (1968). Malformations induced by sodium arsenate. *J. Reprod. Fertil.* **17,** 199–201.

FUJIMOTO, T., FUYUTA, M., KIYOFUGI, E., AND HIRATA, S. (1979). Prevention by tiopronin (2-mercaptopropionyl glycine) of methylmercuric chloride-induced teratogenic and fetotoxic effects in mice. *Teratology* **20,** 297–302.

GROLLMAN, A. (1965). *Pharmacology and Therapeutics.* Lea & Febiger, Philadelphia.

HARRISON, W. P., AND HOOD, R. D. (1981). Prenatal effects following exposure of hamsters to sodium arsenite by oral or intraperitoneal routes. *Teratology* **23,** 40A. (Abstract).

HOLMBERG, R. E., AND FERM, V. H. (1969). Interrelationships of selenium, cadmium, and arsenic in mammalian teratogenesis. *Arch. Environ. Health* **18,** 873–877.

HOOD, R. D. (1972). Effects of sodium arsenite on fetal development. *Bull. Environ. Contam. Toxicol.* **7,** 216–222.

HOOD, R. D., AND BISHOP, S. L. (1972). Teratogenic effects of sodium arsenate in mice. *Arch. Environ. Health* **24,** 62–65.

HOOD, R. D., AND PIKE, C. T. (1972). BAL alleviation of arsenate-induced teratogenesis in mice. *Teratology* **6,** 235–238.

HOOD, R. D., THACKER, G. T., PATTERSON, B. L., AND SZCZECH, G. M. (1978). Prenatal effects of oral versus intraperitoneal sodium arsenate in mice. *J. Environ. Pathol. Toxicol.* **1,** 857–864.

HOOD, R. D., VEDEL, G. C., ZAWOROTKO, M. J., TATUM, F. M., AND FISHER, J. G. (1981). Arsenate distribution in po or ip treated pregnant mice and their fetuses. *Teratology* **23,** 42A. (Abstract).

HOOD, R. D., VEDEL, G. C., ZAWOROTKO, M. J., AND TATUM, F. M. (1982). Distribution of arsenite and methylated metabolites in pregnant mice. *Teratology* **25,** 50A. (Abstract).

LEVINE, W. G. (1970). Heavy metal antagonists. In *The Pharmacological Basis of Therapeutics* (L. S. Goodman and A. Gilman, eds.), pp. 944–957. Macmillan Co., New York.

LUGO, G., CASSADY, G., AND PALMISANO, P. (1969). Acute maternal arsenic intoxication with neonatal death. *Amer. J. Dis. Child.* **117,** 328–330.

NIE, N. H., HALL, C. H., JENKINS, J. G., STEINBRENNER, K., AND BRENT, D. H. (1975). *SPSS. Statistical Package for the Social Sciences.* McGraw-Hill, New York.

NORDSTRÖM, S., BECKMAN, L., AND NORDENSON, I. (1978). Occupational and environmental risks in and around a smelter in northern Sweden. III. Frequencies of spontaneous abortion. *Hereditas* **88,** 51–54.

NISHIMURA, H., AND TAKAGAKI, S. (1959). Developmental anomalies in mice induced by 2,3-dimercaptopropanol (BAL). *Anat. Rec.* **135,** 261–267.

PETERKOVÁ, R., AND PUZANOVÁ, L. (1976). Effect of trivalent and pentavalent arsenic on early developmental stages of the chick embryo. *Folia Morphol.* **24,** 5–13.

PETERS, R. A., SPRAY, G. H., STOCKEN, L. A., COLLIE, C. H., GRACE, M. A., AND WHEATLEY, G. A. (1947). The use of British anti-lewisite containing radioactive sulphur for metabolism investigations. *Biochem. J.* **41,** 370–373.

SPRAY, G. H., STOCKEN, L. A., AND THOMPSON, R. H. S. (1947). Further investigations on the metabolism of 2,3-dimercaptopropanol. *Biochem. J.* **41,** 362–366.

TADLOCK, C. H., AND APOSHIAN, H. V. (1980). Protection of mice against the lethal effects of sodium arsenite by 2,3-dimercapto-1-propane-sulfonic acid and dimercaptosuccinic acid. *Biochem. Biophys. Res. Commun.* **94,** 501–507.

WILCOXON, F., AND WILCOX, R. A. (1964). *Some Rapid Approximate Statistical Procedures.* Lederle Laboratories, Pearl River, New York.

WILLHITE, C. C. (1981). Arsenic-induced axial skeletal (disraphic) disorders. *Exp. Molec. Pathol.* **34,** 145–158.

WINER, B. J. (1971). *Statistical Principles in Experimental Design,* pp. 191–195. McGraw-Hill, New York.