Bull. Environm. Contam. Toxicol. 29, 671–678 (1982)

Effects of Prenatal Arsenite Exposure in the Hamster

Ronald D. Hood and William P. Harrison

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

Arsenic is the twentieth most abundant of the Earth's crustal elements. Although the majority of environmental arsenic is present as the pentavalent form (arsenate), arsenite (As^{+3}) is found in some cases (COMMITTEE ON MEDICAL AND BIOLOGIC EFFECTS OF ENVIRONMENTAL POLLUTANTS 1977). The most concentrated source of arsenite is arsenic trioxide, As_2O_3. This compound is produced commercially (HAMMOND AND BELILES 1980) and is a by-product of certain nonferrous metal smelters (NELSON 1977). Arsenite has also been used as a pesticide (FOWLER et al. 1979) and in the electronics industry. Although less generally abundant in the human environment, the trivalent state is more toxic than the pentavalent (HAMMOND AND BELILES 1980).

Arsenate has been the subject of numerous teratological investigations (FERM AND CARPENTER 1968; HOOD AND BISHOP 1972; BEAUDOIN 1974), but relatively few have examined the effect of exposure of developing offspring to arsenite. Among these are the reports of BAXLEY et al. (1981) and MATSUMOTO et al. (1973), who tested the effects of oral arsenite treatment on pregnant mice and that of HOOD (1972), who employed the intraperitoneal treatment route. In the only report dealing with the hamster, WILHITE (1981) used only intravenous treatment given on one gestation day.

Since it has been conjectured that man is more sensitive to arsenic toxicity than is the mouse (COMMITTEE IN MEDICAL AND BIOLOGICAL EFFECTS OF ENVIRONMENTAL POLLUTANTS 1977), a more sensitive animal model may be needed for arsenic teratogenicity testing. Pilot studies identified the hamster conceptus as especially sensitive to arsenite. Thus, the current study was performed to assay the effects of prenatal arsenite exposure in pregnant hamsters.

0007-4861/82/0029-0671 $01.60

MATERIALS AND METHODS

Outbred golden hamsters of the Lak:LVG (SYR) strain were obtained from Charles River Breeding Laboratories. The animals were housed in solid bottom cages with hardwood chip bedding. They were kept at a temperature of 22 ± 2° C with 40-60% relative humidity and a 12/12 hr light/dark cycle (5:00 a.m. - 5:00 p.m.). Food (Wayne Lab Blox) and water were available ad libitum. Mature (130 g^+) receptive females were mated overnight with experienced males, housed individually, and randomly assigned to treatment groups. The day following copulation on the previous evening was considered gestation day one.

Groups of at least 10 pregnant females were given a single dose of sodium arsenite dissolved in deionized distilled water. Treatment was given by gavage with a dose of 25 mg/kg on gestation days 8, 11, or 12, or a dose of 20 mg/kg on days 9 or 10. Additional females were dosed by intraperitoneal injection with 5 mg/kg sodium arsenite on one of gestation days 8, 11, or 12 or a dose of 2.5 mg/kg on days 9 or 10. Controls were given water only in a like manner or remained untreated. The dose levels used were based on results of pilot studies where the 5 (ip) and 25 (po) mg/kg doses, if given on gestation days 9 or 10, resulted in totally resorbed litters.

On day 15, mated females were killed by overdose of ether and their litters were examined for prenatal mortality, gross, visceral, and skeletal malformations and the fetuses weighed. Maternal liver and body weights were also obtained for calculation of liver/body weight ratios. Skeletal examinations were preceeded by clearing and staining with alizarin Red S by the method of CRARY (1962) and viscera were examined by the technique of STAPLES (1974). Fetal weight data were analyzed for treatment effects by ANOVA followed by a Student-Newman-Keuls multiple range test (WINER 1971), and percentage data were compared by the rank sum method of WILCOXON AND WILCOX (1964).

RESULTS AND DISCUSSION

Treatment of pregnant hamsters with a 20 mg/kg oral dose of sodium arsenite on one of gestation days 9 or 10, or with 25 mg/kg on day 11, had no signficant effect on prenatal growth or survival (Table 1). When hamsters were treated similarly with the higher dose on days 8 or 12, however, prenatal deaths increased, and growth was inhibited in day 12 treated fetuses. No morphological

TABLE 1

EFFECTS ON HAMSTER DEVELOPMENT OF MATERNAL EXPOSURE TO SODIUM ARSENITE BY GAVAGE ON ONE OF GESTATION DAYS 8-12.

Treatment Day	Treatment Type	Dose (mg/kg)	Litters (No.)	Implantations (No.)	Fetal Weights (g ± S.E.)	Prenatal Mortality (%)	Malformations (Gross, Visceral or Skeletal)
8	As^{+3}	25	10(1)[1]	11.4	1.91 ± 0.03	26.3*	None
	H_2O		10(1)	12.2	1.98 ± 0.02	0.8	
9	As^{+3}	20	10	11.4	1.86 ± 0.02	1.8	
	H_2O		10	11.1	1.95 ± 0.03	1.8	
10	As^{+3}	20	10(1)	12.2	1.86 ± 0.02	6.6	
	H_2O		10	12.0	1.95 ± 0.03	1.7	
11	As^{+3}	25	10(2)	12.2	1.66 ± 0.03	7.4	
	H_2O		10	11.8	1.63 ± 0.03	1.7	
12	As^{+3}	25	10(3)	12.4	1.25 ± 0.03**	36.3**	
	H_2O		10	11.7	1.95 ± 0.02	0.0	
Untreated			13	12.8	1.89 ± 0.02	3.0	

*,** Significantly different from corresponding solvent (H_2O) treated control ($P < 0.05$ or $P < 0.01$, respectively).

[1] Numbers in parentheses indicate additional females that died following treatment.

defects were observed in any fetuses from orally treated mothers. Seven of 57 arsenite gavaged mothers died, compared with only 1 of 51 solvent controls and none of 13 untreated females. Such results suggest that acute exposure of the hamster conceptus to arsenite during the period of major organogenesis is not teratogenic at doses toxic to the mother and approaching the maternal lethal dose.

When pregnant hamsters were given sodium arsenite by single intraperitoneal injections with doses of 2.5 mg/kg (gestation days 9 or 10) or 5 mg/kg (days 8, 11 or 12), fetal growth was significantly inhibited only by day 11 or 12 treatment (Table 2). Prenatal mortality was significantly increased following maternal dosing on days 8 or 11 only, although the data for day 10 and 12 treatments suggest such an effect. Gross malformations were seen only in fetuses exposed to arsenite on days 8 or 9. These consisted of micromelia, syndactyly, micrognathia and encephalocoel (day 8) and micromelia, facial malformation and twisted hindlimb (day 9). Skeletal malformations (fused ribs) were seen only following treatment on days 8 or 10. No visceral malformations were found and there were no deaths among the ip injected females.

The data on malformations failed to reveal stastically significant increases in either gross or skeletal defects. The few malformed fetuses seen were all clustered in the treatment groups dosed on days 8, 9 or 10, however; those are the gestation days when treatment would be most likely to alter developmental events and lead to anatomical anomalies in the hamster. FERM (1967), in fact, has suggested that the rapid differentiation occurring on day 8 of pregnancy in this species makes that day a uniquely sensitive period for detecting teratogenic insults. Additionally, no defects were seen in any of the fetuses from orally treated or untreated dams. Thus it appears likely that the malformations observed were treatment related, although much larger numbers of litters would be required to achieve statistical significance with such low malformation incidence rates.

The results of WILHITE (1981) with intravenously injected hamsters treated on gestation day 8 only lend support to the liklihood that the ip treatment used in the present study increased the incidence of malformations. He reported observing gross defects at dose levels of 5 or 10 mg/kg, although the number of animals treated was small. WILHITE also observed arsenite-induced prenatal mortality, but presented no data on fetal weights.

TABLE 2

EFFECTS ON HAMSTER DEVELOPMENT OF MATERNAL EXPOSURE TO SODIUM ARSENITE BY INTRAPERITONEAL INJECTION ON ONE OF GESTATION DAYS 8-12.[1]

Treatment Day	Treatment Type	Dose (mg/kg)	Litters (No.)	Implantations (No.)	Fetal Weight (g ± S.E.)	Prenatal Mortality %	Grossly Malformed %	Grossly Malformed (No.)	Malformed Skeletons %	Malformed Skeletons (No.)
8	As^{+3}	5	11	10.5	1.85 ± 0.02	24.3*	3.4	(3)	4.2	(2)
	H_2O		7	13.9	1.75 ± 0.03	7.2	0.0	(0)	0.0	(0)
9	As^{+3}	2.5	11	13.0	1.82 ± 0.03	4.9	1.5	(2)	0.0	(0)
	H_2O		6	12.2	1.81 ± 0.04	4.1	0.0	(0)	0.0	(0)
10	As^{+3}	2.5	10	11.1	1.80 ± 0.03	16.2	0.0	(0)	1.8	(1)
	H_2O		6	13.0	1.84 ± 0.03	5.1	0.0	(0)	0.0	(0)
11	As^{+3}	5	10	14.1	1.65 ± 0.02**	37.6**	0.0	(0)	0.0	(0)
	H_2O		8	15.2	1.86 ± 0.02	0.8	0.0	(0)	0.0	(0)
12	As^{+3}	5	11	12.5	1.61 ± 0.02**	17.4	0.0	(0)	1.5	(1)
	H_2O		7	12.7	1.87 ± 0.03	1.1	0.0	(0)	0.0	(0)
Untreated			13	12.8	1.89 ± 0.02	3.0	0.0	(0)	0.0	(0)

[1] No visceral malformations were seen.

*,** Significantly different from corresponding solvent (H_2O) treated control ($P < 0.05$ or $P < 0.01$, respectively).

Data on maternal liver/body weight ratios indicated no change due to treatment effects, with the exception of the day 12 orally treated group. This evidence of maternal toxicity may have been related to the larger doses given to these females. Females weigh more on day 12 of pregnancy due to the extra weight of their growing uterine contents. Since much of a gavaged dose passes through the liver immediately after uptake from the gut, such treatment may have resulted in damage to that organ. There is, of course, always the possibility that the apparent effect was not actually due to a treatment effect. That is because the liklihood of a difference that was actually due to chance variation being declared significant in a statistical analysis increases as the number of means compared increases.

The current results suggest that acute oral exposure of the pregnant hamster is not likely to result in teratogenesis at doses better tolerated by the mother. These findings confirm the similar outcome seen by BAXLEY et al. (1981) in mice gavaged with sodium arsenite doses of 40 or 45 mg/kg. Such treatment resulted in maternal and fetal deaths and some inhibition of prenatal growth. BAXLEY et al. (1981) also reported a low incidence of gross malformations, only one fetus with rib defects, and at doses comparable to those used in the current study (20 mg/kg) they found no effect on pregnant mice or their offspring.

The data of BAXLEY et al. (1981) also indicate that mice are more tolerant of arsenite than are hamsters. They found maternal death rates at oral doses of 40 mg/kg only moderately higher than those associated with the 20-25 mg/kg doses in the hamster. Our previous work with mice dosed intraperitoneally (HOOD 1972) lends further support to the concept that mice are the more tolerant of the two species with regard to arsenite toxicity, as mice required doses of 10 mg/kg before they exhibited obvious increases in prenatal deaths or inhibition of fetal growth. At ip doses of 10-12 mg/kg, however, mice exhibited significant teratogenic effects, with increased incidences of both gross and skeletal malformations.

Both the current results and the previously discussed work of HOOD (1972) and BAXLEY et al. (1981) are also indicative that arsenite is significantly less toxic when administered orally than when given intraperitoneally. Even at doses comparably toxic to the mother, oral treatment is also less likely to result in developmental defects in the offspring. We have seen a similar relationship with regard to oral versus ip administered sodium arsenate as well (HOOD et al. 1978). The greater prenatal toxicity of ip arsenite is apparently

due to the more rapid as well as quantitatively greater uptake by mother and fetus we have seen following this mode of administration (HOOD et al. 1982). Results from the same study have shown that a significant amount of the arsenic reaching the fetus may have been altered by the mother to methylated metabolites following dosage by either the oral or ip route, adding yet another factor that can influence the outcome of exposure to arsenite.

According to the data presented, the hamster conceptus can be affected by both orally and intraperitoneally administered maternal treatment with inorganic trivalent arsenic; however, oral dosing is significantly less effective. In neither case is the embryo greatly more sensitive to arsenite than is the pregnant mother. Such results predict that protection of adult humans from arsenite toxicity would protect the developing offspring as well. It must be kept in mind, however, that human offspring may be more sensitive than those of rodents to arsenite and possible paternally-mediated effects remain to be investigated.

ACKNOWLEDGMENT

This work was supported by grant No. OH 00912 to R.D.H. from the National Institute for Occupational Safety and Health.

REFERENCES

BAXLEY, M. N., R. D. HOOD, G. C. VEDEL, W. P. HARRISON, and G. M. SZCZECH: Bull. Environ. Contam. Toxicol. 26, 749 (1981).

BEAUDOIN, A. R.: Teratology 10, 153 (1974).

COMMITTEE ON MEDICAL AND BIOLOGICAL EFFECTS OF ENVIRONMENTAL POLLUTANTS: Arsenic. Washington: National Academy of Sciences 1977.

CRARY, D. D.: Stain Tech. 37, 124 (1962).

FERM, V. H.: Lab. Animal Care 17, 452 (1967).

FERM, V. H., and S. J. CARPENTER: J. Reprod. Fert. 17, 199 (1968).

FOWLER, B. A., N. ISHINISHI, K. TSUCHIYA, and M. VAHTER: Arsenic. In L. Friberg, G. F. Nordberg, and V. B. Vouk, EDS. Handbook on the Toxicology of Metals. Amsterdam: Elsevier/North-Holland, p. 293 (1979).

HAMMOND, P. B., and R. P. BELILES: Metals. In J. Doull, C. D. Klaassen, and M. O. Amdur, EDS. Casarett and Doull's Toxicology. New York: Macmillan, p. 409 (1980).

HOOD, R. D.: Bull. Environ. Contam. Toxicol. 7, 216 (1972).

HOOD, R. D., AND S. L. BISHOP: Arch. Environ. Health 24, 62 (1972).

HOOD, R. D., G. C. VEDEL, M. J. ZAWOROTKO and F. M. TATUM: Teratology 25, 50A (1982).

HOOD, R. D., G. T. THACKER, and B. L. PATTERSON: J. Environ. Pathol. Toxicol. 1, 857 (1978).

MATSUMOTO, N., T. OKINO, H. KATSUNUMA and S. IIJIMA: Congen. Anom. Curr. Lit. 13, 175 (1973).

NELSON, K. W.: Environ. Health Perspect. 19, 31 (1977).

STAPLES, R. E.: Teratology 9, A37 (1974).

WILCOXON, F., and R. A. WILCOX: Some rapid approximate statistical procedures. Pearl River, NY: Lederle Laboratories 1964.

WILHITE, C. C.: Exp. Mol. Pathol. 34, 145 (1981).

WINER, B. J.: Statistical principles in experimental design. New York: McGraw-Hill 1971.

Accepted October 17, 1982