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Evaluation of Arsenic Metabolites for Prenatal Effects in the Hamster

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Organoarsenicals, primarily cacodylic acid (dimethylarsinic acid), methanearsonic acid, and their salts, have been widely used as herbicides for cotton defoliation, poisoning broadleafed lawn grasses, and the like (PEOPLES et al. 1979). Cacodylate and its sodium salt have also been employed as a military herbicide in Southeast Asia in a formulation known as Agent Blue (ANONYMOUS 1981). Only since the work of LAKSO and PEOPLES (1975) and CRECELIUS (1977) has it been known that mammals, including man, metabolize inorganic arsenic by methylation. More recently, such mammalian arsenic methylation has been confirmed in humans and in a variety of other species (CHARBONNEAU et al. 1979 and 1980; ODANAKA et al. 1980; BUCHET et al. 1981; HOOD et al. 1982).

Although the parent inorganic arsenicals are known to be capable of causing adverse effects on mammalian embryos (FERM and CARPENTER 1968; HOOD 1972; HOOD and BISHOP 1972; BEAUDOIN 1974; HOOD et al. 1978; BAXLEY et al. 1981), relatively less is known about the potential of methylated arsenicals for teratogenicity and fetotoxicity. Thus, the current study was designed as part of an ongoing investigation of the metabolism and prenatal effects of arsenicals. It was performed to determine the sensitivity of the developing hamster to mono- or dimethylarsenic for comparison with other animal models and to complement studies of the metabolism, pharmacokinetics, and prenatal effects of inorganic arsenicals.

MATERIALS AND METHODS

Golden hamsters of the outbred Lak:LVG (SYR) strain were purchased from Charles River Breeding Laboratories and kept in shoebox type cages with ground corn cob bedding. Environmental parameters included a temperature

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of 22 ± 2° C with 40-60% relative humidity. A 12/12 hr light/dark cycle was used, with lights on from 5:00 a.m. to 5:00 p.m., to facilitate mating in the afternoon. The hamsters were fed Wayne Lab Blox (Allied Mills) and water ad libitum. Mature (130-160 g) females in estrus were placed overnight with experienced males, randomly assigned to treatment groups, and individually housed. Day one of pregnancy was considered to be the day following mating during the previous night.

Ten or more pregnant females per group were injected intraperitoneally with a single dose of either 900 or 1,000 mg/kg disodium methanearsonate (DSMA) or 500 mg/kg sodium cacodylate (NaCA) in deionized distilled water. Treatments were administered on one of gestation days 8-12. Solvent controls were treated similarly with a sodium chloride solution equimolar to the DSMA solution or were left untreated. The dose levels were chosen due to the results of pilot studies and approximated the minimum lethal doses for pregnant females.

Mated females were sacrificed by overdose with chloroform on gestation day 15, their livers were removed and weighed, and their liver/body weight ratios were calculated. Their uterine contents were examined for live, dead, or resorbed fetuses, and living fetuses were examined for gross defects and weighed. One third of the fetuses from each litter were examined for visceral anomalies by the method of STAPLES (1974) and the remaining fetuses were cleared in a KOH solution, stained with alizarin red S (CRARY 1962), and examined for skeletal defects. Data on fetal weights were subjected to ANOVA and differences among means tested by use of the technique of Student-Newman-Keuls (WINER 1971). Percentage data were compared by rank sum tests (WILCOXON and WILCOX 1964).

RESULTS AND DISCUSSION

Neither sodium cacodylate nor disodium methanearsonate treatment resulted in altered maternal liver/body weight ratios. Nevertheless, the 900 and 500 mg/kg doses for NaCA and DSMA, respectively, did result in some maternal deaths. Thus the treatments given were above the maternal MLD and must be considered acutely toxic.

TABLE 1

EFFECTS IN PREGNANT HAMSTERS OF INTRAPERITONEAL TREATMENT WITH 1000 MG/KG SODIUM CACODYLATE (NaCA)

Treatment Day	Litters[1]	Litters Resorbed	Prenatal Mortality	Live Fetuses	Fetal Wt.	Grossly Malformed		Malformed Skeletons	
	#	#	%	#	$\bar{X}$ ± S.D.	#	(%)	Ratio	(%)
8	7(1)	7	100.0	0.0					
9	7	4	82.5	2.0	1.39 ± .06	10	(71.4)	3/10	(30.0)
10	4(2)	1	72.9	3.2	1.22 ± .08	5	(38.5)	6/9	(66.7)
11	4(2)	4	100.0	0.0					
12	3	3	100.0	0.0					

[1] Numbers in parentheses represent deaths of additional treated females.

Data from a pilot study employing a 1000 mg/kg dose of NaCA are presented in Table 1. Although the maternal death rate was comparable to that seen at the 900 mg/kg dose, all fetuses from mothers treated on days 8, 11 or 12 were resorbed.

Acute dosing with 900 mg/kg NaCA invariably resulted in high levels of fetal wastage (Table 2), with the greatest effects being seen due to treatment on days 8 or 9 of gestation. Fetal stunting followed NaCA treatment for all days except day 8. Gross malformations were also observed in fetuses from all NaCA treatment groups except the one for day 12, and particularly in the day 9 or 10 treated groups. Malformations consisted of cleft palate, cleft lip, micromelia, syndactyly, exencephaly, and talipes. A few edematous fetuses were also seen. Skeletal malformations, mainly fused ribs, were detected in the day 8, 9, and 10 NaCA treatment groups. The numbers of surviving fetuses were so low in both the day 8 and 9 groups, however, that there were not enough fetuses to examine to allow a meaningful statistical analysis of the data on skeletal malformations. The same was true of the day 8 gross malformation data as well.

Results of treatment with 500 mg/kg DSMA indicate a degree of fetotoxicity (Table 3). Day 12 treatment was associated with decreased survival to gestation day 15, and treatment on any of days 9, 10, or 12 was followed by fetal stunting. Only 3 grossly malformed fetuses were seen, consisting of a fetus with exencephaly (day 8), two fetuses with lateral cleft lips (day 9) and one with a short muzzle (day 12). In addition, one fetus was seen with fused ribs in the day 8 DSMA group.

The results seen for fetal growth following acute maternal exposure to the sodium salt of cacodylic acid were similar to those following chronic treatment by ROGERS et al. (1981), who dosed pregnant mice on days 7-16 by gavage with cacodylic acid (CA) at levels up to 600 mg/kg/day. ROGERS et al. (1981) also found an increase in prenatal deaths at the high dose, delayed ossification, decreased numbers of supernumerary ribs and an increase in incidence of cleft palate. The finding of cleft palate was similar to the current results in the hamster. In the ROGERS et al. (1981) study, rats

TABLE 2

EFFECTS IN PREGNANT HAMSTERS OF 900 MG/KG SODIUM CACODYLATE GIVEN INTRAPERITONEALLY

Treatment Day	Agent	Litters[1]	Litters Totally Resorbed	Prenatal Mortality	Live Fetuses	Fetal Wt.	Fetuses Grossly Malformed		Malformed Skeletons	
		#	#	%	#	$\bar{X}$ ± S.D.	#	(%)	Ratio	(%)
8	NaCA	10	5	82.8*	2.1	1.95 ± 0.08	3	(14.3)	3/15	(20)
9	NaCA	10(1)	7	94.8*	0.6	1.56 ± 0.24	6	(100.0)*	2/5	(40)
10	NaCA	11(1)	3	55.6*	5.4	1.51 ± 0.04	26	(44.1)*	2/40	(5)
11	NaCA	11(3)	5	54.6*	5.4	1.50 ± 0.04	2	(3.4)	0/40	(0)
12	NaCA	14(2)	1	29.1*	8.4	1.57 ± 0.02		(0)	0/83	(0)
Solvent	Control[2]	20	0	2.8	10.3	1.89 ± 0.05		(0)	0/146	(0)
	Untreated	13	0	3.0	12.5	1.89 ± 0.02		(0)	0/109	(0)

[1] Numbers in parentheses represent deaths of additional treated females.

[2] Controls received NaCL solution equimolar to the disodium methanearsonate on one of days 8-12. Results did not differ by days and were pooled.

* Differs from saline controls ($P < 0.05$).

TABLE 3

EFFECTS IN PREGNANT HAMSTERS OF 500 MG/KG DISODIUM METHANEARSONATE (DSMA) GIVEN INTRAPERITONEALLY

Treatment Day	Treatment Agent	Litters[1]	Litters Totally Resorbed	Prenatal Mortality	Live Fetuses	Fetal Wt.	Fetuses Grossly Malformed		Malformed Skeletons	
		#	#	%	#	$\bar{X}$ ± S.D.	#	(%)	Ratio	(%)
8	DSMA	10(1)	0	6.3	10.5	1.87 ± 0.02	1	(1.0)	1/72	(1.4)
9	DSMA	10(1)	0	7.8	10.6	1.48 ± 0.03*	2	(1.9)	0/73	(0)
10	DSMA	10(0)	0	10.9	10.6	1.54 ± 0.04*	0	(0)	0/74	(0)
11	DSMA	10(1)	0	10.2	10.6	1.87 ± 0.02	0	(0)	0/74	(0)
12	DSMA	11(3)	1	20.5*	9.5	1.51 ± 0.05*	1	(3.8)	0/73	(0)
8-12	Control[2]	20	0	2.8	10.3	1.89 ± 0.05	0	(0)	0/146	(0)
	Untreated	13	0	3.0	12.5	1.89 ± 0.02	0	(0)	0/109	(0)

[1] Numbers in parentheses represent deaths of additional treated females.

[2] Controls received NaCl solution equimolar to the DSMA on one of days 8-12. Results did not differ by days and were pooled.

* Differs from saline controls ($P < 0.05$).

were also treated with CA. Females were dosed on gestation days 7-16 with up to 60 mg/kg/day. Lower doses were used in the case of rats, as they proved to be less tolerant of the treatment than were mice. CA treated rats also displayed decreased fetal survival and weight gain, delayed ossification and altered numbers of supernumerary ribs. They failed to exhibit cleft palate but exhibited a dose-related increase in the incidence of misaligned palatine rugae.

In the only study observed in the literature where pregnant hamsters were exposed to methylated arsenicals, WILHITE (1981) used dose levels of 20, 50 and 100 mg/kg for both CA and dimethylarsinic acid given intravenously on gestation day 8 only. He reported no dose-dependent increases in prenatal mortality. Low incidences of gross or skeletal malformations were seen, particularly in the CA treated groups, and no fetal weight data were given.

Although WILHITE (1981) used only 5 litters per dose level, his observation of fetal malformations associated with iv doses of cacodylate appear to lend support to our findings of malformations following ip treatments with the sodium salt. Both studies suggest that acute exposure to CA is likely to be teratogenic in the hamster, particularly at relatively high dose levels. Nevertheless, both studies also suggest that DSMA is not a potent teratogen when given acutely to hamsters, as the incidences of developmental defects was low, even at doses toxic or lethal to the mothers.

The data of ROGERS et al. (1981) on mice indicate that this species, too, is relatively resistant to cacodylate-induced teratogenesis, while the rat is much more susceptible. This greater sensitivity of rats to a methylated arsenic metabolite may, however, be related to the fact that rats handle alkylarsenic differently from other mammalian species on which data are available, including man (POMROY et al. 1980). The rat appears to retain methylated arsenicals for long periods of time. The data of YAMAUCHI et al. (1980), for example, show a retention of high levels of arsenic metabolites at 120 days after administration of a dose of arsenic trioxide. Additionally, ODANAKA et al. (1980) reported that at 48 hours after treatment, rats retained in their blood 44% and 39% of a dose of inorganic arsenic (arsenic acid) after oral or iv administration, respectively. The retained arsenic was almost entirely methylated, primarily in the dimethyl form. These values for retained percent of dose for the rat compare with 0.1% and < 0.2% for mice and < 0.1% and 0.1% for hamsters in the same study. Thus, if exogenously administered dimethylated arsenic is retained by the rat to a similar degree as

that produced endogenously during metabolism of inorganic arsenic, a higher proportion of the maternal dose may reach the conceptus over time. Such data also support the assumption that the rat is a poor model for studies of the effects of methylated arsenicals, as has been previously suggested in the case of inorganic forms of arsenic (COMMITTEE 1977).

The results of the current study of pregnant hamsters reinforce the concept that methylation of arsenic greatly reduces its toxicity to adult mammals, with the exception of the rat. Both mono- and dimethylated arsenicals also exhibited less toxicity to the hamster conceptus than would be expected for inorganic arsenic. Inferences based on animal models other than the rat thus uniformly suggest that methylated arsenicals are not likely to be significant teratogens in man, as the developing mammal has not been shown to be uniquely sensitive to their toxic effects.

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