Effects of Sodium Arsenite on Fetal Development

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Introduction

Arsenic compounds have been introduced in large quantities into the environment for over a hundred years through such processes as smelting, the manufacture and use of paints and industrial chemicals and through agricultural uses (2,3,10). The possible biological consequences of such practices are, however, not fully known.

Disodium methylarsenate has been shown to be teratogenic in the chick (1). According to Ferm and Carpenter (5) and Holmberg and Ferm (7), sodium arsenate is a potent teratogen in the golden hamster. Hood and Bishop (9) have also presented preliminary data on teratogenicity of sodium arsenate in the mouse. Although it can be seen that the teratogenicity of arsenate in a variety of organisms is now well documented, the only report relative to arsenite is found in a preliminary study (8). The results of the completed study are reported here.

It had been thought that the tetravalent arsenate ion is reduced *in vivo* to trivalent arsenite before it has its toxic effect in mammalian systems. It now appears, however, that the redox equilibria *in vivo* favor oxidation, and that little if any transformation to arsenite may occur (6). Arsenite is thought to act mainly by reacting with sulfhydryl groups and thus poisoning sulfhydryl enzymes, such as pyruvate oxidase, or reacting with α-lipoic acid to form a cyclic thioarsenite. Arsenate, conversely, is not thought to be a sulfhydryl poison, but rather to uncouple oxidative phosphorylation and to interfere with phosphorus metabolism (11). Since the previous studies cited have demonstrated teratogenic effects of arsenate, the present work was undertaken to determine if arsenite might also be a teratogen and if its effects would be similar to or differ from those of arsenate.

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Materials and Methods

Randombred albino Swiss-Webster mice were used. Nulliparous females were mated by overnight exposure to males and mating was detected by observation of the vaginal plug. The day on which a plug was noted was considered to be day one of gestation. Treatment consisted of single intraperitoneal injections of sodium arsenite ($NaAsO_2$) dissolved in distilled H_2O on one of days 7-12 of gestation. Dose levels were either 10 or 12 mg arsenite per kg body weight. Controls were injected with an equivalent volume of distilled H_2O. All females were sacrificed by an overdose of ether or by cervical dislocation on day 18 of gestation, and the uterine horns exposed. Numbers of live, dead, or resorbed fetuses were recorded. All remaining fetuses were then removed from the extraembryonic membranes, weighed and examined for grossly observable developmental anomalies. One-third of the fetuses obtained from each litter were subjected to the procedure described by Crary (4) for clearing and staining of skeletons. The skeletons were then examined for evidence of anomalies. Differences in fetal weights, malformations and mortality between treated and control fetuses were subjected to analysis by the method of Newman-Keuls (12).

Results and Discussion

Single intraperitoneal injections of sodium arsenite resulted in a significant increase in fetal deaths for all days (7-12) and both dose levels (10 or 12 mg/kg) investigated. Most fetal deaths occurred shortly after treatment, as judged by the degree of resorption and by presence of a discharge of blood through the mother's vagina (which is indicative of fetal death). Arsenite treatment tended to decrease fetal weights (Table 1). The decrease in fetal weights was dependent on both day and dose level, with the higher dose tending to have the greater effect. Intraperitoneal injection of mice with a 45 mg/kg dose of sodium arsenate has also been found to cause greatly increased prenatal mortality and decreased fetal growth (9).

TABLE 1

Effects of Sodium Arsenite on Fetal Development in Mice

Treatment Day	Dose (mg/kg)	No. of Litters[a]	Total Implantations	Litters Totally Resorbed	% Resorbed or Dead	% Malformed[b]	Fetal Weights (gm± s.e.)
7	10	6	71	3	50.70[d]	0	1.02±0.02[d]
	12	7	81	0	34.57[d]	1.89	0.91±0.02[d]
8	10	6(1)	72	2	48.61[d]	8.12[d]	0.81±0.03[d]
	12	8(1)	90	6	92.22[d]	0	0.78±0.04[d]
9	10	9	116	0	19.83[d]	13.98[d]	0.96±0.01
	12	8	99	6	77.78[d]	27.27[d]	0.92±0.04[d]
10	10	9(1)	106	6	87.74[d]	7.69[d]	0.95±0.02[d]
	12	6(1)	71	5	84.51[d]	36.37[d]	0.57±0.03[d]
11	10	9	103	4	59.22[d]	0	0.95±0.01[d]
	12	6(4)	63	6	100.00[d]	-	-
12	10	6(1)	55	4	75.38[d]	0	1.05±0.03
	12	6(4)	65	6	100.00[d]	-	-
Control[c]		36	394	0	1.78	0	1.05±0.01

[a] Nos. in parenthesis indicate additional females treated but not surviving to gestation day 18.

[b] As % of intact fetuses (does not include skeletal defects).

[c] Controls injected with distilled H_2O on one of gestation days 7-12.

[d] Significantly different from the controls, $P < 0.05$.

TABLE 2

Sodium Arsenite-Induced Fetal Anomalies in Mice. Day vs Response

Anomaly	Gestation day administered/Dose level[a]											
	7/12		8/10		9/10		9/12		10/10		10/12	
	N	%	N	%	N	%	N	%	N	%	N	%
Exencephaly			1/37	2.70	11/93	11.83	5/22	22.73				
Microcephaly	1/53	1.89	1/37	2.70								
Micrognathia	1/53	1.89	1/37	2.70	11/93	11.83	5/22	22.73				
Agnathia			1/37	2.70								
Exophthalmos					1/93	1.08						
Anophthalmos			1/37	2.70								
Open Eye					8/93	8.60	4/22	18.18			3/11	27.27
Displaced Pinna			1/37	2.70								
Umbilical Hernia			1/37	2.70								
Amelia									1/13	7.69		
Bent Tail			1/37	2.70							1/11	9.09
Short or Missing Tail					2/93	2.15					2/11	18.18
Rib Defects			1/12	8.33	11/22	50.00	3/7	42.86	1/5	20.00	3/7	42.86
Vertebral Fusion					2/22	9.09	1/7	14.29	1/5	20.00		
Anomalous Vertebrae					2/22	9.09	1/7	14.29	1/5	20.00		

[a] N = No. abnormal/total fetuses observed.

Examination of fetuses for gross malformations indicated the period of greatest susceptibility to teratogenic effects to be from gestation days 8 through 10 (Table 2). Both before and after this time, there were no observable defects with the exception of one fetus from the group treated with the 12 mg dose on day 7. Hood and Bishop (9) reported a high percentage of fetal malformations in mice treated with arsenate on these same days; however, a small number of malformations were also observed due to treatment on days 6, 7 or 11.

The most common defects associated with arsenite treatment were exencephaly, micrognathia and open eye. Bent, shortened or missing tails were also noted in several fetuses. No other anomaly was represented by more than a single fetus. The entire range of developmental alterations produced by arsenite can also be induced in mice by arsenate, with exencephaly, micrognathia, open eye and tail defects being among the most common in both cases (9). The only major differences are the relatively high incidences of exophthalmia and umbilical hernia in arsenate-treated fetuses compared to those treated with arsenite. Also, a barely sublethal dose of arsenate (45 mg/kg) produced a higher incidence of terata per day of treatment than did a similarly toxic dose of arsenite (10-12 mg/kg) when treatment occurred on gestation days 7, 8 or 9. Treatment of pregnant hamsters with intravenous arsenate (20 mg/kg) on gestation day 8 also produced a high incidence of exencephaly, micrognathia and open eye (5). In addition, Holmberg and Ferm (7) observed encephalocele, cleft palate-lip, microanophthalmia and ear malformations in arsenate-treated hamsters.

Arsenite exposure was also associated with skeletal anomalies. Other than the diminution in size of the lower jaw in micrognathic individuals and skull anomalies associated with exencephaly, the major skeletal defects observed were fusion of the ribs and fused or missing vertebral ossification centers. These phenomena were seen only in fetuses subjected to treatment on days 8-10 in the case of rib defects and 9 and 10 for vertebral anomalies (Table 2). In arsenate-treated mice (9), similar effects were seen, with both rib and vertebral terata occurring in mice treated on days 8-10.

The similarity of types of developmental anomalies produced by arsenate and arsenite tends to support the original theory that there is interconversion of arsenate to arsenite, which is then the toxic form. Another point in favor of such a conversion is the fact that arsenite is effective at a much lower dose level than is arsenate (10 vs 45 mg/kg). Similar terata can, however, be caused by agents which probably have differing modes of action, particularly if the end result is the same (e.g., causing death or damage to actively dividing cells in the same areas of the embryo and at the same times during development).

Summary

Single intraperitoneal injections of sodium arsenite were given to albino Swiss-Webster mice on one of days 7-12 of gestation. Two dose levels were used: 10 or 12 mg/kg. Arsenite treatment resulted in high rates of fetal deaths and tended to decrease fetal weights compared with H_2O injected controls. Arsenite induced a variety of fetal malformations on gestation days 7-10; the most common were exencephaly, micrognathia, open eye, tail defects and skeletal anomalies of the ribs and vertebrae. Defects were similar to but less numerous than those caused by sodium arsenate.

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