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The Concentration and Chemical Status of Arsenic in the Early Placentas of Arsenate-Dosed Hamsters

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We determined the concentration and chemical status of arsenic in the placentas of hamsters following continuous exposure via the osmotic minipump to minimally and frankly teratogenic doses of arsenate. Close to 70% of the placental arsenic is bound to macromolecules, two-thirds of which is dialyzable. The remaining 30% of arsenic consists of low molecular weight species, predominantly inorganic arsenic. This mix is the same for minimally teratogenic and frankly teratogenic doses of arsenate. © 1987 Academic Press, Inc.

INTRODUCTION

Arsenic, in the form of arsenate, is a known teratogen in mammals (Ferm and Carpenter, 1968; Hood and Bishop, 1972). To provide a teratogenic response, a critical concentration of a teratogen should exist at sensitive sites in the materno-embryonic unit during organogenesis. In the specific case of the hamster the requisite concentration and chemical status of arsenic must be present at vulnerable sites during the eighth day of gestation (Ferm, 1965). The location and chemical nature of the teratogen-sensitive sites have not been identified for arsenate, although placenta and early embryo itself are likely candidates (Hanlon and Ferm, 1977; Morrissey and Mottet, 1983).

Regardless of the locale of the arsenic-sensitive sites, maternal blood must be the agent of delivery. We have recently described the relationship beween the maternal blood concentration and chemical status of arsenic and the production of teratogenic lesions following continuous dosing of pregnant hamsters with arsenate (Hanlon and Ferm, 1986). In this study our intention has been to determine the concentration and chemical status of arsenic in the placenta during early organogenesis following continuous exposure of the hamsters to minimally and frankly teratogenic doses of arsenate.

MATERIALS AND METHODS

1. Reagents

Analytical grade reagents were used. Aqueous solutions were prepared using high-quality deionized water. Disodium arsenate (recrystallized) was used to prepare arsenate solutions which were radiolabelled with carrier-free arsenic-74 purchased from Amersham/Searle in the form of the arsenate salt. Radiolabelled solutions contained 0.5 μCi/μmole of arsenate.

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2. Animals

Pregnant Syrian hamsters (LGV strain) were obtained from Charles River Laboratories in Wilmington, MA. The protocols for breeding, timing, and the critical stages of embryogenesis in this species have been described (Ferm, 1967).

3. Dosing and Sampling Protocols

Osmotic minipumps (Alzet, Model 2001) were filled and implanted in pregnant hamsters on Day 6 of gestation, following a method described by Ferm and Hanlon (1985). This model osmotic minipump holds up to 250 μl of fluid and is designed to deliver 1.0 μl of solution per hour at 37°C over the duration of its operation (8 days).

Experimental animals were segregated into three groups comprising 8 to 10 animals each. One group was implanted with minipumps containing 0.642 M radiolabelled sodium arsenate. A second group received minipumps containing 0.482 M radiolabelled arsenate and a third group was implanted with minipumps containing 0.321 M radiolabelled arsenate. Individuals were weighed at the time of implant and immediately before sacrifice on the morning of Day 8 of gestation. Animals were sacrificed by CO_2 inhalation 48 hr after implanting the minipumps. Maternal blood samples were obtained by cardiac puncture. Placentas were obtained by dissection. Placentas from each dam were pooled for further study. Minipumps were removed from sacrificed dams and their contents were radioassayed to determine the actual dose of arsenate delivered during the exposure period.

4. Radioassay of Arsenic-74

Total arsenic content of samples was obtained by radioassay of weighed fractions in glass tubes (85 × 15 mm) using the Beckman Gamma 5500. The amount of arsenic present in a given sample was calculated by comparing its radioactivity with that of measured amounts of arsenic solutions of known specific activity.

5. Preparation of Placentas

Placentas were homogenized in three volumes of ice-cold 10 mM Tris (Cl), pH 8.0, using a glass homogenizer equipped with a power-driven Teflon piston. Whole homogenates were used in certain experiments. In others, soluble and insoluble placental fractions were obtained by centrifuging homogenates at 24,000*g* for 20 min at 4°C.

6. Availability of Arsenic in Placental Tissues

Gel filtration experiments on placental homogenate supernatants were performed using Sephadex G-25 (fine) as the support medium. Details of this method have been previously reported (Hanlon and Ferm, 1986).

Placental homogenates were made in 2% trichloroacetic acid (TCA) and placed on ice for 10 min. Samples were then centrifuged at 2000*g* and supernatant and pellet were separated. The pellet was suspended in an equal volume of 2% TCA and recentrifuged. This process was repeated. Supernatants and washed pellets

were radioassayed. TCA supernatants were saved for quantitative analysis of low molecular weight arsenic metabolites as described below. TCA pellets were suspended in 10 mM Tris (Cl), pH 8.0, then dialyzed for 24 hr against this same buffer using the Crowe–Englander Dialyser apparatus (A. H. Thomas). Dialysate solutions were concentrated for analysis of arsenic metabolites by rotary evaporation under reduced pressure with slight heat (35°C).

7. Quantitative Analysis of Arsenic Metabolites

Identification and quantitation of radiolabelled arsenic metabolites were carried out using ion exchange chromatography. Inorganic arsenic (As_i) and methylated arsenic species in the TCA supernatant samples and in the concentrated dialysate samples were separated and quantified using a modification of the cation exchange method of Tam *et al.* (1978). In some TCA supernatant samples arsenate was separated from other arsenic species by Dowex 2 anion exchange chromatography; the details of this method have been described (Hanlon and Ferm, 1986). TCA supernatant samples were adjusted to pH 8.0 prior to chromatography.

RESULTS

1. Arsenic Concentration in Placentas of Continuously Exposed Hamsters

Arsenic concentrations in placentas and maternal blood of hamsters in the three treatment groups are shown in Table 1.

The average body weight of dams in each treatment group was near 140 g. There was no significant change in body weights during the exposure period. Differences in individual body weights for hamsters in each treatment group created a range for doses delivered over the 48-hr period of exposure (see Table 1). However, no dose-dependent differences in the arsenic concentrations of placentas or of maternal blood samples were noted within a given treatment group. All arsenic-74 in the minipumps was in the form of arsenate immediately prior to loading and at the end of the exposure period.

2. The Molecular Weight Disposition of Arsenic in Placentas of Continuously Exposed Hamsters

High-speed centrifugation of placental homogenates showed that close to 50% of total placental arsenic was soluble and the remainder was associated with the

TABLE 1

THE CONCENTRATION OF ARSENIC IN PLACENTAS AND MATERNAL BLOOD OF HAMSTERS ON THE MORNING OF DAY 8 OF GESTATION RESULTING FROM 48-HR CONTINUOUS EXPOSURE TO ARSENATE

Treatment group	Arsenate concentration in minipump (M)	Dose range of arsenate (μmoles/kg dam)	μmoles arsenic/kg	
			Placentas	Maternal blood
I	0.321	106–114	5.07 ± 0.67	4.04 ± 0.50
II	0.482	160–175	10.20 ± 1.30	6.06 ± 0.27
III	0.642	200–223	17.20 ± 0.66	8.46 ± 0.47

washed pellet. These conditions prevailed in all three levels of arsenic exposures. Gel filtration studies carried out on supernatants of placental homogenates revealed that approximately 50% of the soluble arsenic of placentas eluted near the excluded volume of a Sephadex G-25 column. This arsenic peak was heavily skewed toward the included volume. A smaller arsenic peak was present at 1.0 included volume.

Treatment of placental homogenates with 2% TCA produced a soluble arsenic fraction (As-Sol) and an insoluble arsenic fraction (the TCA pellet). The validity of the TCA precipitation method, as a measure of arsenic bound to macromolecules and As-Sol, was tested by comparing values for each arsenic fraction obtained by the TCA method with values obtained using 50% acetone and 50% ethanol precipitation methods. The three approaches gave the same results. We used the TCA method because it eliminates the need to reduce the volume of supernatant prior to resin chromatography analysis of the As-Sol fraction. TCA does not interfere with the Dowex 2 analysis for arsenate provided the pH of As-Sol samples is adjusted to 8.0 and the assay conditions described by Hanlon and Ferm (1986) are adhered to. Prolonged dialysis of the resuspended TCA pellet material yielded data for arsenic reversibly bound to macromolecules (As-RB) and arsenic irreversibly bound to macromolecules (As-P) (Fig. 1).

3. Arsenic Metabolites in Continuously Exposed Placentas

The chemical status of low molecular weight arsenic species in placentas on the morning of the eighth day of gestation is given in Table 2. "Free" arsenic represents the arsenic contribution of TCA supernatants (As-Sol). "Total" arsenic rep-

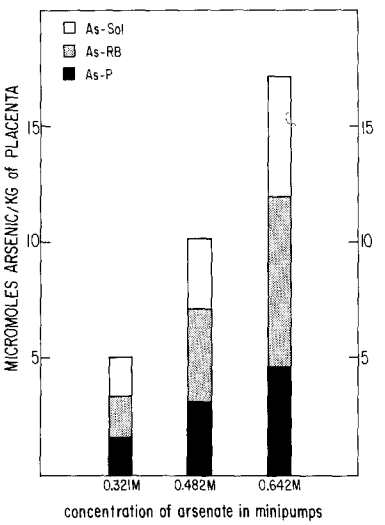

FIG. 1. The contribution of low molecular arsenic (As-Sol), arsenic reversibly bound to macromolecules (As-RB), and arsenic irreversibly bound to macromolecules (As-P) to the total arsenic concentration in placentas of arsenate-dosed hamsters. Data are for samples collected on the morning of Day 8 of gestation, 48 hr postminipump implant. Values represent averages for pooled placenta samples from 8 to 12 dams.

resents the As-Sol and the As-RB fractions combined. Inorganic arsenic (As_i) dominates the low molecular weight arsenic pool in the placentas following all three exposures. Methylated arsenic was entirely in the form of dimethylarsinate (DMA). Low counting rates of placental samples from the two lower-dose regimens allowed resolution into As_i and DMA species only. Further resolution of As_i into arsenate and arsenite was achieved for the high-dose regimen As-Sol fraction (see footnote, Table 2).

DISCUSSION

The intent of this study was to determine the chemical disposition of a known teratogen in placental tissue early in the period of organogenesis following exposure to minimally and frankly teratogenic doses of the agent. Our choice of arsenate was based on its specific teratogenic properties (Ferm and Hanlon, 1985) and on its potential as an environmental threat.

Arsenate produces teratogenic lesions in the hamster when it is administered acutely early in organogenesis (Ferm and Carpenter, 1968) or subchronically prior to and during organogenesis (Ferm and Hanlon, 1985). Our study of the disposition of placental arsenic proceeds directly from a previous investigation of the concentration and chemical status of arsenic in the maternal blood of hamsters exposed subchronically to minimal and frankly teratogenic doses of arsenate (Hanlon and Ferm, 1986).

The centrifugation data and gel filtration findings indicate that most of the placental arsenic of hamsters continuously dosed with arsenate is bound to macromolecules. A more quantitative appraisal of bound arsenic was obtained in the TCA precipitation experiments. Close to 70% of the total placental arsenic coprecipitated with proteins in all three dose regimens. We have previously shown that, under the dosing conditions used in this study, all the maternal blood plasma arsenic is unbound, or bound so weakly to macromolecules that it is immediately available for exchange (Hanlon and Ferm, 1986). This fact could explain why the total arsenic concentrations in the placentas were higher than the arsenic concentrations in maternal blood for all three treatment groups (see Table 1).

While most of the arsenic in hamster placentas is associated with macromolecules, a considerable portion of the bound arsenic is exchangeable and presum-

TABLE 2
CONCENTRATIONS OF LOW MOLECULAR WEIGHT ARSENIC METABOLITES IN PLACENTAS OF ARSENATE-DOSED HAMSTERS ON THE MORNING OF DAY 8 OF GESTATION

Concentration of arsenate in minipump (M)	Total arsenic (from Table 1)	μmoles of low molecular weight arsenic/kg			
		Inorganic arsenic		Methylated arsenic	
		Free	Total	Free	Total
0.321	5.07 ± 0.67	1.27 ± 0.30	2.94 ± 0.50	0.500 ± 0.200	0.608 ± 0.218
0.482	10.20 ± 1.30	2.23 ± 0.18	6.12 ± 0.61	0.852 ± 0.040	1.020 ± 0.300
0.642	17.20 ± 0.66	4.30 ± 0.50[a]	10.50 ± 1.50	1.030 ± 0.200	2.060 ± 0.180

[a] Dowex 2 chromatography of four pooled placental samples gave 40% arsenate and 60% arsenite.

ably available to the arsenic metabolic pool. Data shown in Fig. 1 indicate that the relative contributions of As-Sol, As-RB, and As-P remain unchanged over a greater than threefold increase in the total placental arsenic concentration. This could mean that arsenic concentrations in placentas for all three exposures are well below that which would saturate macromolecular arsenic binding sites. More likely, our data reveal a quite active arsenic metabolism in placenta. This conjecture is supported by a number of studies recently reviewed by Vahter (1983).

Our concentration and chemical status data for arsenic in the placentas of hamsters early in organogenesis should be considered in the context of the teratogenic responses elicited in hamsters subjected to the same exposure regimens (Ferm and Hanlon, 1985). Animals in treatment groups I, II, and III were exposed to amounts of arsenate which produce less than 5, 8.3, and 50.9% fetal abnormalities. The dramatic increase in teratogenic response is not predicted from the relative concentrations of total arsenic or of low molecular weight arsenic in the placentas of hamsters in the three treatment groups (see Table 2). Increases in DMA concentrations are not pertinent in any case, since administration of DMA at 10× the dose of arsenate employed in group III has no teratogenic effect in hamsters (V. H. Ferm, unpublished data). Arsenite may be the specific teratogenic form of arsenic, given its chemical reactivity toward macromolecular nucleophiles found in biological systems (Squibb and Fowler, 1983), and it is 60% of the free As_i component of placentas from group III dams. Possibly the arsenite contribution to the As_i pool is much greater in the placentas of group III animals, but this is speculation.

We do not know the origin of the arsenic metabolites in the placenta. The concentrations and chemical species mix of arsenic could result from *in situ* metabolic activity or from selective absorption from maternal blood, or both. Continuous exposure to arsenate does result in the appearance of arsenite and DMA in hamster maternal blood (Hanlon and Ferm, 1986). On the other hand, the early placenta should have the capacity to metabolize arsenate to arsenite and DMA via catalyzed reduction and methylation processes such as those that occur in the hepatic tissues of mammals (Vahter, 1983).

CONCLUSION

Our investigation has revealed the concentrations and chemical status of arsenic in placentas of hamsters receiving minimally and frankly teratogenic doses of arsenate delivered continuously by means of osmotic minipumps. Differences in total placental arsenic concentrations, inorganic arsenic, and methylated arsenic do not correlate directly with the production of teratogenic lesions. To our knowledge, this is the first report of the chemical composition of arsenic in the placental tissues of animals resulting from experimental exposure to any form of arsenic.

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