Exposure to Inorganic Arsenic Metabolites during Early Human Development

Gabriela Concha,* Gerardo Vogler,† Dora Lezcano,† Barbro Nermell,* and Marie Vahter*†

*Institute of Environmental Medicine, Karolinska Institutet, Box 210, 171 77 Stockholm, Sweden; and †Hospital San Antonio de los Cobres, Salta, Argentina

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Arsenic exposure via drinking water has been associated with cancer of the skin and various internal organs, as well as hyperkeratosis, pigmentation changes, and effects on the circulatory and nervous systems (U.S. EPA, 1988; Chen et al., 1992; Smith et al., 1992). Reproductive effects of inorganic arsenic are well documented in animal experiments. Administration of high doses of arsenite or arsenate to rodents prior to or during organogenesis results in multiple malformations, decreased prenatal growth rate, and increased mortality (Ferm et al., 1971; Hood and Bishop, 1972; Beaudoin, 1974; Hood et al., 1978; Morrissey and Mottet, 1983; Nagymajtenyi et al., 1985; Ferm and Hanlon, 1985). In particular, neural tube defects appear to be a consistent outcome (Morrissey and Mottet, 1983; Willhite and Ferm, 1984; Golub, 1994).

Despite the large number of people being exposed throughout their life, however, there are very few data on the reproductive effects of arsenic in humans. In a couple of studies, prenatal exposure to acute, very high doses of arsenic has resulted in miscarriage and early neonatal death (Lugo et al., 1969; Bolliger et al., 1992). An increased frequency of malformations, spontaneous abortions, and low birth weights has been observed among children of female employees in a copper smelter (Nordström et al., 1979a, b), but due to the multiple chemical exposure the role of arsenic is not clear. There are also a few reports indicating associations between adverse reproductive outcome and exposure to arsenic in drinking water (Zierler et al., 1988; Aschengrau et al., 1989; Börzsönyi et al., 1992); however, the causal relationship is not well ascertained. Data on placental transfer of arsenic in cases of no apparent maternal toxicity are even more scarce. In women without known exposure to arsenic, the concentration of arsenic in cord blood was similar to that in maternal blood (Kagey et al., 1977). However, the form of arsenic in blood was not determined. The main exposure form of arsenic in food is arsenobetaine, which can be present in high concentrations in various seafood (Edmonds and Francesconi, 1987) and give rise to elevated blood arsenic concentrations (Foà et al., 1984; Yamauchi et al., 1992). This form of arsenic is rapidly excreted in the urine and much less toxic than inorganic arsenic (Vahter et al., 1983).

We have recently reported on high concentrations of arsenic in blood and urine of native Andean women and children living in an area in northern Argentina with about 200 μg As/liter in the drinking water (Vahter et al., 1995a; Concha et al., 1998a), although they showed less of the metabolite methlyarsenonic acid...
in Women in Late Gestation (Average Gestational Week 39) up to 4.4 Months Postpartum*

<table>
<thead>
<tr>
<th>Time after delivery</th>
<th>N</th>
<th>B-As (µg/liter)</th>
<th>U-As(_\text{mef}) (µg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before delivery</td>
<td>11</td>
<td>10 (5.6–13)(^c)</td>
<td>325 (116–439)</td>
</tr>
<tr>
<td>2.8 weeks postpartum</td>
<td>10</td>
<td>11 (7.7–25)</td>
<td>234 (169–762)</td>
</tr>
<tr>
<td>2.5 months postpartum</td>
<td>8</td>
<td>15 (7.5–33)</td>
<td>255 (180–704)</td>
</tr>
<tr>
<td>4.4 months postpartum</td>
<td>9</td>
<td>16 (8.9–24)</td>
<td>301 (49–527)</td>
</tr>
</tbody>
</table>

* B-As, blood arsenic concentration; U-As\(_\text{mef}\), urinary arsenic metabolites.

\(^{c}\) Adjusted to a density of 1.014 g/ml (average of all urine samples).

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**RESULTS**

The concentration of arsenic in cord blood (median, 9.0 µg/liter; range, 6.0–12 µg/liter) was almost as high as that in maternal blood in late gestation (median 11 µg/liter, Table 1). As shown in Fig. 1, there was a significant association between arsenic concentrations in maternal and cord blood ($r^2 = 0.62$, $p = 0.004$). The median arsenic concentration in the placenta was 34 µg/kg wet wt (range, 17–54 µg/kg, $N = 11$).

Maternal blood arsenic concentration (B-As) increased significantly from about 11 µg/liter at delivery to about 16 µg/liter 4.4 months postpartum ($p = 0.015$; Table 1). In contrast, U-As\(_\text{mef}\) was slightly higher in late gestation compared with the postpartum period. There was a significant correlation between maternal B-As and U-As\(_\text{mef}\) 2.8 weeks and 2.5 months postpartum ($r^2 = 0.57$, $p = 0.009$, and $r^2 = 0.69$, $p = 0.005$, respectively), but not in late gestation and 4.4 months postpartum ($r^2 = 0.28$, $p = 0.088$, and $r^2 = 0.22$, $p = 0.186$).

In general, the concentrations of total arsenic in maternal milk were low, on average 3.1 µg/kg (Table 2). There was no change in the concentrations during the lactation period. There was no association between arsenic concentrations in...
maternal blood and breast milk. In the infants, U-As$_{\text{met}}$ decreased significantly ($p = 0.025$) during the first 4.4 months of life (Fig. 2). The average concentration of U-As$_{\text{met}}$ in the infants at birth (range, 51–196 µg/liter) constituted 23% of that in maternal urine, but only 9% at 4.4 months after birth (range, 13–98 µg/liter). Five infants receiving breast milk only had significantly lower U-As$_{\text{met}}$ (median, 17 µg/liter) than those fed formula in addition to breast milk (median, 103 µg/liter; $p = 0.036$).

As shown in Table 3, essentially all the arsenic in the blood plasma of women in late gestation, as well as their newborns, was present as DMA. Very few samples had detectable amounts of inorganic arsenic and/or MMA. DMA was the major form of arsenic also in maternal urine before parturition and in the first infant urine after birth (Table 4). There was a significant decrease in the percentage DMA in maternal urine from late gestation to 4.4 months postpartum ($p < 0.001$). The concentrations of arsenic in most urine samples of the infants a couple of weeks or more after birth were too low for speciation. Also, the arsenic concentrations in milk were too low for speciation.

The body weight at birth was 3000 g on average (range, 2000–3750 g). Only two infants weighed less than 2500 g at birth. There was no significant association between the arsenic concentrations in maternal blood or cord blood and body weight at birth ($r^2 = 0.27$, $p = 0.088$, and $r^2 = 0.07$, $p = 0.400$, respectively). Neither was there any gender difference in the percentage arsenic metabolites in the first urine of the infants.

**DISCUSSION**

The concentrations of arsenic in cord blood, 9.2 µg/liter on average, were about as high as in maternal blood just before delivery. This shows that arsenic is readily transferred across the placenta to the fetus. The blood arsenic concentrations were clearly elevated. For comparison, the blood arsenic concentration in people with no known exposure to arsenic is about 1 µg/liter (Concha et al., 1998a).

An important finding was that essentially all arsenic in maternal and cord plasma was in the form of DMA, the end product of inorganic arsenic metabolism. Also, the percentage DMA in maternal urine in late gestation and first infant urine was significantly higher than in the postpartum period. Previous reports on urinary arsenic metabolites in human subjects exposed to inorganic arsenic show consistently average values of 60 to 70% DMA (see, e.g., the review by Hopenhayn-Rich et al., 1993). Thus, the results indicate that arsenic methylation increases during pregnancy and that DMA is the main form of arsenic being transferred to the fetus. This is important from a toxicological point of view, as DMA is much less toxic to the embryo and fetus than inorganic arsenic. In studies on mice

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**TABLE 2**

Concentrations of Arsenic in Breast Milk of Women up to 4.4 Months Postpartum

<table>
<thead>
<tr>
<th></th>
<th>Total arsenic in milk (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N$</td>
</tr>
<tr>
<td>2.8 weeks postpartum</td>
<td>10 3.0 (2.3–4.8)*</td>
</tr>
<tr>
<td>2.5 months postpartum</td>
<td>8   2.8 (1.9–5.5)</td>
</tr>
<tr>
<td>4.4 months postpartum</td>
<td>9   3.4 (2.3–5.5)</td>
</tr>
</tbody>
</table>

*Median (range).*
and hamsters, about 10 times higher doses of DMA than of inorganic arsenic were required to induce malformations (Rogers et al., 1981; Hood et al., 1982). Our previous studies in the Andean village have indicated that the children excrete less DMA in the urine, indicating that they are more sensitive to arsenic-induced toxicity than are the adults (Concha et al., 1998a). If so, the increased arsenic methylation during pregnancy is highly protective for the developing organism.

An increased rate of methylation of arsenic may explain the observed lower B-As and higher U-As met in late gestation, compared with the postpartum period or with nonpregnant women in the same area (Vahter et al., 1995a). DMA has a higher rate of excretion and lower tissue retention than inorganic arsenic (Buchet et al., 1981a, b; Vahter et al., 1984; Marafante et al., 1987). However, the mechanism of the increased arsenic methylation is not clear. The arsenite methyltransferase has not been fully characterized, but has been isolated from tissues of several mammalian species, e.g., rabbit, rat, mouse, hamster, pigeon, and rhesus monkey, which also methylate arsenic in vivo (Aposhian et al., 1997). Enzyme activity could not be detected in the liver of the marmoset monkey and chimpanzee (Aposhian et al., 1997), which is in agreement with the lack of arsenic methylation in these species (Vahter and Marafante, 1985; Vahter et al., 1995b). There are no reports on such enzyme activity in human tissues, but, so far, all human populations studied excrete MMA and DMA in urine, giving strong support for methylation being an enzymatic process also in humans. If so, the results of the present study might indicate an induction of the arsenite methylation. Interestingly, the activity of several methyltransferases increases during pregnancy, probably because several endogenous methylation reactions, e.g., DNA methylation and protein methylation, are crucial for normal mammalian development (Paik et al., 1991; Jaenisch, 1997).

The median arsenic concentration in placenta was 34 μg/kg wet wt, i.e., about three times the blood concentration. Women living close to a smelter in Bulgaria had an average arsenic concentration in placenta of 23 μg/kg, as compared with 7 μg/kg in a nonsmelter area (Tabacova et al., 1994). The effect of arsenic accumulation on placental function is not known.

Despite the high arsenic concentrations in maternal blood and urine, the concentration in maternal milk was low, on

### Table 3

<table>
<thead>
<tr>
<th>Subject</th>
<th>%Inorg. As</th>
<th>%MMA</th>
<th>%DMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;d.l.</td>
<td>&lt;d.l.</td>
<td>&gt;95</td>
</tr>
<tr>
<td>2</td>
<td>&lt;d.l.</td>
<td>&lt;d.l.</td>
<td>&gt;95</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>1.3</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>&lt;d.l.</td>
<td>&lt;d.l.</td>
<td>&gt;95</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>&lt;d.l.</td>
<td>&lt;d.l.</td>
<td>&gt;95</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>17</td>
<td>64</td>
</tr>
<tr>
<td>8</td>
<td>&lt;d.l.</td>
<td>&lt;d.l.</td>
<td>&gt;95</td>
</tr>
<tr>
<td>9</td>
<td>&lt;d.l.</td>
<td>&lt;d.l.</td>
<td>&gt;95</td>
</tr>
<tr>
<td>10</td>
<td>&lt;d.l.</td>
<td>&lt;d.l.</td>
<td>&gt;95</td>
</tr>
<tr>
<td>11</td>
<td>&lt;d.l.</td>
<td>&lt;d.l.</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>

* <d.l., under detection limit.

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### Table 4

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>%Inorg. As</th>
<th>%MMA</th>
<th>%DMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before delivery</td>
<td>11</td>
<td>6.9 (1.9-19)*</td>
<td>4.7 (1.1-11)</td>
<td>87 (78-96)</td>
</tr>
<tr>
<td>2.8 weeks postpartum</td>
<td>10</td>
<td>13 (4.7-29)</td>
<td>9.3 (5.9-17)</td>
<td>78 (58-89)</td>
</tr>
<tr>
<td>2.5 months postpartum</td>
<td>8</td>
<td>29 (15-38)</td>
<td>9.2 (3.9-14)</td>
<td>64 (48-76)</td>
</tr>
<tr>
<td>4.4 months postpartum</td>
<td>9</td>
<td>20 (17-74)</td>
<td>7.3 (0.7-12)</td>
<td>69 (24-79)</td>
</tr>
<tr>
<td>Infants</td>
<td>10</td>
<td>9.2 (1.6-24)</td>
<td>1.4 (&lt;d.l.3-6)</td>
<td>90 (76-98)</td>
</tr>
</tbody>
</table>

* Median (range).
average 3.4 μg/kg. This is similar to a previous finding on arsenic concentrations in maternal milk (Concha et al., 1998b).

As a result of breastfeeding milk with low arsenic concentrations, U-As\textsubscript{net} concentrations in newborn infants decreased slowly during the first 4.4 months after birth. The highest U-As\textsubscript{net} concentrations were detected in three infants who were given formula in addition to breast milk. It can be calculated that breastfeeding provides about 3 μg arsenic per day, while formula prepared from the local water would provide about 200 μg arsenic/day. Thus, the low content of arsenic in maternal milk is an additional important reason for long breastfeeding periods in areas with high arsenic exposure.

ACKNOWLEDGMENT

We thank all women participating in the study.

REFERENCES


