Induction of tumors of the liver, lung, ovary and adrenal in adult mice after brief maternal gestational exposure to inorganic arsenic: promotional effects of postnatal phorbol ester exposure on hepatic and pulmonary, but not dermal cancers

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Arsenic is a recognized human carcinogen and development of rodent models remains a critically important research objective. Since gestation can be a period of high sensitivity to chemical carcinogenesis, we have performed a series of transplacental carcinogenicity studies in mice with inorganic arsenic. In this study, groups of pregnant C3H mice received drinking water containing sodium arsenite (NaAsO2) at 0, 42.5 and 85 p.p.m. arsenic ad libitum from days 8 to 18 of gestation. These doses of arsenic were well tolerated. Dams delivered normally and at weaning (4 weeks) offspring were randomly put into groups (n = 25) of males or females according to maternal dose. In an attempt to promote skin cancers initiated by transplacental arsenic, duplicate groups of control or arsenic exposed offspring were topically exposed to 12-O-tetradecanoyl phorbol-13-acetate (TPA; 2 μg/0.1 ml acetone, twice/week) from 4 to 25 weeks of age. Irrespective of TPA exposure, male offspring showed arsenic-induced dose-related increases in hepatocellular carcinoma incidence and multiplicity, as well as increases in adrenal tumor incidence and multiplicity. In female offspring, an increase in epithelial ovarian tumors occurred with arsenic exposure regardless of TPA exposure. Females also showed pre-neoplastic lesions of the reproductive tract, including hyperplasia of the uterus and oviduct, after arsenic but independent of TPA exposure. Although TPA had no effect on skin tumors, it promoted arsenic initiated liver tumors in females and lung tumors in both sexes. Thus, inorganic arsenic, as a single agent, can consistently act as a complete transplacental carcinogen in mice, inducing tumors at multiple sites, and as a tumor initiator in some tissues. Skin tumors were not initiated by arsenic in mouse fetuses possibly indicating tissue-specific mechanisms of action. This study indicates that gestation is a period of high sensitivity to arsenic carcinogenesis.

Introduction

Inorganic arsenic continues to be a major concern in the US and throughout the world largely based on its carcinogenic potential after occupational or environmental exposure (1-7). Inorganic arsenic exposure in humans is causally associated with development of malignancies in various tissues, including the skin, liver, urinary bladder, lung and prostate (1-7). Epidemiological studies have repeatedly shown clear dose-response relationships between environmental arsenic levels and human cancer incidence (2-4,6,7). The primary source of arsenic in most human populations is the drinking water, where inorganic forms predominate (2-4,7). Appropriately assigning the level of risk associated with consumption of drinking water containing elevated levels of arsenic is of critical importance, and recently, because of heightened concern about the carcinogenic potential of arsenic, the United States Environmental Protection Agency significantly lowered the safe drinking water levels for the metalloid. Clearly unsafe levels of arsenic are found in the drinking water from many areas throughout the world (2-4,7). However, recent evidence indicates that the even more moderate levels of arsenic, which can be found in drinking water in the US may carry a significant carcinogenic risk in human populations (8,9) comparable in magnitude with that posed by exposure to environmental radon or second-hand tobacco smoke (9).

Definition of carcinogenic mechanisms can be a key in assessing the potential risk posed by a given level of exposure to a carcinogen. Although, exposure to inorganic arsenic in vitro can cause a wide variety of molecular events often associated with the oncogenic process, such as genotoxicity, DNA methylation errors, diminished DNA repair, suppression of apoptosis, etc. (5,6,10), none of these potential mechanisms has been established definitively as a mechanism of arsenic-induced carcinogenesis. In the final analysis, it is very difficult to actually define carcinogenic mechanism by in vitro study alone. On the other hand, the chemical induction of tumors in rodents can be invaluable because they are unarguably pertinent to a carcinogenic process. While inorganic arsenic is unequivocally carcinogenic in humans (1-7), tumor induction in animals by inorganic arsenic has, until rather recently, been difficult to demonstrate. In this regard, the work of Germolec et al. (11,12), and subsequently Rossman et al. (13), have provided important models for arsenic-induced skin cancers, a primary target site of arsenic carcinogenesis in humans (2,6). In these experiments, co-exposure of oral inorganic arsenic together with other treatments resulted in skin cancers (11-13). Specifically, adult Tg.AC (H-ras mutated) transgenic mice develop skin tumors after co-exposure to oral inorganic arsenite and dermal application of the tumor promoting phorbol ester, 12-O-tetradecanoyl phorbol-13-acetate (TPA) (11,12), while combined ultraviolet irradiation and oral arsenite increases the incidence and multiplicity of skin cancers in adult hairless mice when compared with irradiation alone (13). In both systems, inorganic arsenic given alone did not induce dermal cancers or tumors elsewhere (11-13). These mouse skin models (11-13) represent critical advances that point to co-promotional or co-carcinogenic

Abbreviations: DMA, dimethylarsinic acid; TPA, 12-O-tetradecanoyl phorbol-13-acetate.
effects of inorganic arsenic in dermal carcinogenesis, and will probably provide mechanistic insight for inorganic arsenic-induced dermal cancers. However, inorganic arsenic has many potential targets in humans (2,6) and there is no a priori reason to believe that mechanisms in the skin would necessarily apply to all, or in fact, any other tissues.

Thus, development of additional rodent models for inorganic arsenic carcinogenesis remains a critically important research objective. Based on the knowledge that the period of gestation in rodents can be a time of high sensitivity to chemical carcinogenesis (14), which includes sensitivity to inorganic agents other than arsenic (15–18), we reported recently that maternal oral exposure to inorganic arsenic during gestation in mice resulted in a strong carcinogenic response in the offspring after they had become adults (19). Specifically, exposure of pregnant mice to well tolerated levels of sodium arsenite in the drinking water from gestation days 8 to 18 induced in the offspring dose-related increases in malignant and/or benign tumors at multiple sites, including the liver, ovaries, adrenals and lungs (19). The tumor spectrum induced by arsenic in this transplacental carcinogenesis study included malignant tumors of the liver (19), which is recognized as a target tissue for cancer associated with environmental arsenic exposure in humans (2). What is perhaps most startling about these results is that the brief duration (10 days) of maternal inorganic arsenic exposure was clearly carcinogenic to the offspring after they had reached adulthood and well after any arsenic exposure had ended (19), indicating that this is indeed a period of very high sensitivity. An analogous sensitivity to inorganic arsenic in humans during gestation would be most alarming.

Because of its potential impact, the action of inorganic arsenic as a transplacental carcinogen deserves additional study. Thus, the primary goal of the present study was to confirm the initial findings about the carcinogenic potential after gestational maternal exposure to inorganic arsenic (19). In addition, successful dermal models of inorganic arsenic carcinogenesis appear to require co-exposure to additional treatments, such as the tumor promoter, TPA (11,12). Therefore, we sought to determine if transplacental arsenic initiated carcinogenic events in the fetal skin by the use of postnatal exposure to dermal applications of TPA.

Materials and methods

Chemicals

Sodium arsenite (NaAsO₂) was obtained from Sigma Chemical Co. (St Louis, MO) and dissolved in sterile distilled water to the desired concentrations in the drinking water at the specified final concentrations as parts per million (p.p.m.) arsenic.

Animals, dosage selection and treatments

Animal care was provided in accordance with the US Public Health Policy on the Care and Use of Animals as defined in the Guide to the Care and Use of Animals (NIH Publication 86-23). Mice were housed in a standard barrier facility, at a temperature of 68–72°F and with a relative humidity of 50 ± 5% and a 12 h light/dark cycle. A basal diet (NIH-31 Open Formula, 6% Modified; Teklad Standard Diets, Madison, WI) and water (unmodified or modified as below) were provided ad libitum. The NCI-Frederick animal facility, where the biopsy portion of the present study was conducted, and its animal program are accredited by the American Association for Accreditation of Laboratory Animal Care. Mice were obtained from the Animal Production Area, NCI-Frederick, DCT Animal Program, Frederick, MD.

Dosage selection in the present work was based on a prior transplacental carcinogenesis study in which pregnant C3H/HeNCr (C3H) mice were treated with 0 (control), 42.5 or 85 p.p.m. arsenic as sodium arsenite in the drinking water from gestation days 8 to 18 and carcinogenic response was evaluated in the resulting offspring (19). In this work, neither maternal drinking water consumption nor body weight of the pregnant mice was altered by these levels of arsenic in the drinking water (19). Furthermore, transplacental exposure to arsenic at these levels did not reduce body weights in any group of offspring over the course of the experiment. The data establish the doses used in the present study as being well tolerated to both the maternal animal and the resulting offspring.

Thus, a total of 60 timed primigravid female C3H mice were randomly divided into three groups of 10 each with two separate groups at each dosage given drinking water containing sodium arsenite (NaAsO₂) at 0 (control), 42.5 or 85 p.p.m. arsenite ad libitum from days 8 to 18 of gestation. Dams were allowed to give birth, and litters were culled to no more than eight at 4 days postpartum. Offspring were weaned at 4 weeks and then randomly put into separate groups (n = 25) of males and females according to maternal exposure level. One group each of control or arsenic exposed (42.5 or 85 p.p.m.) offspring were exposed to TPA (2 μg/0.1 ml acetone, twice/week to a shaved area of dorsal skin) for 21 weeks after weaning in an attempt to promote skin cancers possibly initiated by arsenic. Duplicate groups (control and 42.5 or 85 p.p.m. arsenite) had vehicle (acetone) applied for 21 weeks after weaning to a shaved area of dorsal skin. The offspring received no additional arsenic treatment after birth. However, given a biological half-life for inorganic arsenic of ~4 days (2), some transplacental exposure of the offspring to arsenic may have occurred in this treatment protocol, although the excretion of arsenic into the breast milk is considered low (2). The dams were discarded after weaning and the offspring were observed for the next 104 weeks.

The level of TPA exposure was selected based on a prior transplacental carcinogenesis skin tumor initiation/promotion study in which prenatal cisplatin exposure together with the same postnatal TPA dosage and frequency markedly enhanced cisplatin-induced skin tumor incidence and multiplicity in mice (17).

Clinical data

Individual dam body weights were recorded between day 8 and day 18 of gestation. Water consumption of the dams was recorded in (ml/mouse) during the arsenic exposure period on days 16 and 18 of gestation. Individual neonate weights were recorded at birth and at 1 week intervals thereafter until weaning (week 4). After being placed into the appropriate gender-based treatment groups, the body weights of the offspring were recorded at 5 week intervals. In the offspring, clinical signs were checked daily and mice were killed when significant clinical signs developed or at 104 weeks of age. The area of dorsal skin, which had been used for TPA exposure was carefully inspected during the daily checks for any signs of neoplastic growth.

Pathology

A complete necropsy was performed on all moribund animals, animals found dead or on mice at the terminal death. The following tissues were taken and processed by standard techniques for histological analysis: gonads (ovaries or testes), uterus, oviduct, liver, kidneys, lung, adrenal, spleen, skin and all grossly abnormal tissues. Particular attention was paid to any skin lesions within the area used for TPA applications. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin for histological analysis. Pathological assessments were performed in a blind fashion. In addition, uterine proliferative lesions were scored in a blind fashion from each animal as absent = 0, mild = 1, moderate = 2, severe = 3, adenoma = 4 to obtain a mean severity score. The vast majority of all tumors observed in the current study occurred after 26 experimental weeks, well after the mice had reached adulthood and long after arsenic exposure had ceased.

Data analysis

Data are given as incidence (number of affected mice/total number of mice available for examination) or as mean ± SEM, as appropriate. A probability level of P ≤ 0.05 was considered to indicate a significant difference in all cases. In defining the incidence of mice bearing benign or malignant tumors in cases of multiple tumors in the same tissue, the animal is assigned to the appropriate group based on its most advanced lesion. Tissue-specific total tumor incidence is defined as those mice bearing at least one benign or malignant tumor in a given organ. Tumor multiplicity (either total or malignant tumors only) is defined as the number of tumors per mouse either of a particular organ or inclusively at any site. In pair-wise comparison of lesion incidence, a one-sided Fisher’s exact test was used. To analyze dose-related trends in incidence, a χ² test for trend was used. For multiple comparisons of mean tumor multiplicity, lesion severity or survival data, two-sided Dunnett’s test was used, while a linear trend test based on equally distributed doses was used to define dose-related trends. Incidence is based on numbers of animals available for observation, and any loss of animals to observation was due primarily to autolysis that was considered too advanced for appropriate diagnosis.
Results

Pregnant C3H mice were treated with 0 (control), 42.5 or 85 p.p.m. arsenic as sodium arsenite in the drinking water from gestation days 8 to 18, and the offspring were then exposed to TPA (2 μg/0.1 ml acetone, twice/week) or vehicle (acetone) for 21 weeks after weaning (at 4 weeks of age) to a shaved area of dorsal skin and carcinogenic response was evaluated over the next 2 years. Maternal body weight or drinking water consumption were not altered by the inclusion of arsenic in the drinking water (not shown). The transplacental exposure to arsenic did not reduce body weights in any group of male offspring over the course of the experiment (not shown). Occasional small body weight reductions (11–13%) occurred in female offspring but only after 95 weeks of age or more and only in the highest dosage groups (85 p.p.m. arsenic). There were no TPA-related effects on body weight during the course of the experiment in either male or female offspring. For instance, at 25 weeks of age (when TPA exposure ended) body weight in female offspring not given TPA (n = 25/group) was 35.5 ± 0.7 (mean ± SEM in g) in controls, 35.6 ± 0.7 in the 42.5 p.p.m. arsenic group and 36.5 ± 1.0 in the 85 p.p.m. arsenic group, while in the female offspring also exposed to TPA it was 35.6 ± 0.6 in controls, 33.2 ± 0.6 in the 42.5 p.p.m. arsenic group and 35.5 ± 0.8 in the 85 p.p.m. arsenic group. Similarly, in male offspring at 25 weeks of age body weight in groups not given TPA (n = 25/group) was 42.1 ± 0.6 (mean ± SEM in g) in controls, 40.2 ± 0.5 in the 42.5 p.p.m. arsenic group and 40.7 ± 0.7 in the 85 p.p.m. arsenic group, while in the male offspring also exposed to TPA it was 40.6 ± 1.1 in controls, 42.6 ± 0.6 in the 42.5 p.p.m. arsenic group and 40.9 ± 1.2 in the 85 p.p.m. arsenic group. These data establish the doses of arsenic and/or TPA used in the present study as being well tolerated to both the maternal animal and the resulting offspring.

Average survival was unaltered in male or female offspring exposed to the various levels of arsenic with the single exception of a reduction in survival of males given the highest dose of arsenic (85 p.p.m.) without subsequent TPA (Table I). This group of males had the highest rate of hepatic malignancies (see below), which may have accounted for a reduction in survival. There were no reductions in survival related to TPA exposure in male or female offspring.

Although TPA was used with the intent to promote skin lesions initiated by gestational arsenic exposure in the present study, the phorbol ester had no effect whatsoever on skin malignancies. Skin tumors were not induced to a significant degree by arsenic alone in either male or female mice, regardless of additional TPA treatment. Indeed, skin tumors were a rare event in the present study and only one epithelial tumor and three mesenchymal tumors occurred within or near the skin in the total of 270 male and female mice available for pathological analysis. None of these tumors occurred at the site of TPA application. Skin tumors in male mice included a squamous cell carcinoma located on the leg of an animal exposed to 42.5 p.p.m. arsenic during gestation and subsequently to TPA. Skin tumors in female mice included a sarcoma of the skin and a subcutaneous sarcoma in the control group (0 p.p.m. arsenic) without TPA exposure, and a subcutaneous sarcoma in the group exposed to 42.5 p.p.m. arsenic with subsequent TPA.

On the other hand, gestational arsenic exposure had a profound effect on development of liver cancer in male offspring (Table II). Transplacental exposure to arsenic induced a marked, dose-related increase in the incidence of hepatocellular carcinoma in male offspring, regardless of postnatal TPA exposure. There was a 3.3-fold increase over control in hepatocellular carcinoma incidence at the highest arsenite dose (85 p.p.m.) in mice not receiving dermal TPA, while a 3.5-fold increase occurred in male mice exposed to this dose of arsenic and also exposed to TPA. Categorization of tumor incidence was determined by the most advanced lesion in the individual animal and the incidence of hepatic adenoma, when considered in the context of coexistent carcinoma in the same liver, was unaltered by arsenic treatment and/or TPA exposure. However, many arsenic-treated animals bearing carcinomas also had adenomas. This is reflected in the ‘nominal’ rate of hepatic adenoma, which is the incidence of adenoma without consideration of the presence of carcinoma in the same liver. The nominal rate of adenoma showed significant dose-related increases with arsenic dose in male mice regardless of additional TPA exposure. The incidence of hepatic tumors of any type (total tumors) similarly showed a significant, dose-related increase with arsenic dose regardless of additional TPA exposure. Liver tumor multiplicity (tumors/mouse) was significantly increased at both doses of arsenic in animals not exposed to TPA and at the highest dose of arsenic in mice also exposed to TPA. Significant, arsenic dose-related trends occurred for hepatocellular carcinoma incidence, nominal hepatocellular adenoma incidence, total hepatocellular tumor incidence and tumor multiplicity data that were independent of TPA exposure. A single hepatoblastoma occurred in a male mouse given 85 p.p.m. arsenic alone.

In female offspring, liver tumors were induced in response to gestational arsenic exposure but only in conjunction with postnatal TPA treatment (Table III). Gestational arsenic exposure alone did not modify hepatocellular tumor incidence or multiplicity in female mice. However, when female mice exposed to arsenic during gestation also received postnatal TPA applications increases in total hepatocellular tumor incidence
Table II. Liver tumors in male offspring exposed during gestation to inorganic arsenic

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Hepatocellular tumorsc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>Adenoma</td>
</tr>
<tr>
<td>0 p.p.m.</td>
<td>ns (ns)</td>
</tr>
<tr>
<td>42.5 p.p.m.</td>
<td>2 (2)</td>
</tr>
<tr>
<td>85 p.p.m.</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Trend P</td>
<td>ns (0.077)</td>
</tr>
</tbody>
</table>

The designation ns indicates no significant trend occurred (P > 0.05).

Table III. Liver tumors in female offspring exposed during gestation to inorganic arsenic

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Hepatocellular tumorsc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>Adenoma</td>
</tr>
<tr>
<td>0 p.p.m.</td>
<td>ns (ns)</td>
</tr>
<tr>
<td>42.5 p.p.m.</td>
<td>2 (2)</td>
</tr>
<tr>
<td>85 p.p.m.</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Trend P</td>
<td>ns (0.077)</td>
</tr>
</tbody>
</table>

The designation ns indicates no significant trend occurred (P > 0.05).

Table IV. Epithelial ovarian tumors in female offspring exposed during gestation to inorganic arsenic

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Ovarian tumorsc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>Adenoma</td>
</tr>
<tr>
<td>0 p.p.m.</td>
<td>ns (ns)</td>
</tr>
<tr>
<td>42.5 p.p.m.</td>
<td>4d</td>
</tr>
<tr>
<td>85 p.p.m.</td>
<td>4d</td>
</tr>
<tr>
<td>Trend P</td>
<td>ns (0.054)</td>
</tr>
</tbody>
</table>

The designation ns indicates no significant trend occurred (P > 0.05).
Table V. Adrenal adenomas in male offspring exposed during gestation to inorganic arsenic

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Pulmonary tumorsc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>TPA</td>
</tr>
<tr>
<td>0 p.p.m.</td>
<td>–</td>
</tr>
<tr>
<td>42.5 p.p.m.</td>
<td>–</td>
</tr>
<tr>
<td>85 p.p.m.</td>
<td>–</td>
</tr>
<tr>
<td>Trend P =</td>
<td>0.029</td>
</tr>
<tr>
<td>0 p.p.m.</td>
<td>+</td>
</tr>
<tr>
<td>42.5 p.p.m.</td>
<td>+</td>
</tr>
<tr>
<td>85 p.p.m.</td>
<td>+</td>
</tr>
<tr>
<td>Trend P =</td>
<td>0.070</td>
</tr>
</tbody>
</table>

See Table II for details.

Table VI. Lung tumors in male offspring exposed during gestation to inorganic arsenic

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>Pulmonary tumorsc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>TPA</td>
</tr>
<tr>
<td>0 p.p.m.</td>
<td>–</td>
</tr>
<tr>
<td>42.5 p.p.m.</td>
<td>–</td>
</tr>
<tr>
<td>85 p.p.m.</td>
<td>–</td>
</tr>
<tr>
<td>0 p.p.m.</td>
<td>+</td>
</tr>
<tr>
<td>42.5 p.p.m.</td>
<td>+</td>
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<tr>
<td>85 p.p.m.</td>
<td>+</td>
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See Table II for details.

Specifically, pulmonary tumor incidence was increased in the male offspring exposed to 42.5 p.p.m. arsenic followed by TPA to a rate 5-fold over the incidence in control. No other significant differences occurred in lung tumor formation in male mice. In females the incidence of pulmonary adenomas increased at the highest dose of arsenic and there was a clear arsenic dose-related trend across all doses but only in animals receiving postnatal TPA exposure (Table VII). In addition, two pulmonary adenocarcinomas occurred in females, which were both in arsenic-exposed animals receiving TPA. In female mice exposed only to arsenic no significant changes in pulmonary tumor incidence occurred (Table VII).

Transplacental exposure to arsenic induced a marked increase in the incidence of uterine epithelial hyperplasia (cystic) and total uterine proliferative lesions (defined as epithelial hyperplasia plus epithelial tumors) at both doses of arsenic that was independent of TPA exposure (Table VIII). The average severity of uterine lesions was also increased at both doses of arsenic regardless of any additional TPA exposure. Strong, arsenic dose-related trends occurred in the incidence of uterine hyperplasia, total uterine proliferative lesions and severity of uterine lesions that were unaffected by postnatal TPA exposure. Although mild hyperplasia of the uterine epithelium was seen in control mice and was considered to be an age-related lesion, more severe hyperplasia only seldom occurred in controls. Benign smooth muscle tumors (leiomyomas) occurred occasionally but were unrelated to any treatment. Following transplacental exposure to arsenic, a marked increase in hyperplasia of the oviduct occurred at the highest dose (85 p.p.m.) in female mice (Table VIII) that was likewise independent of TPA exposure. Clear arsenic dose-related trends occurred in the incidence of oviduct hyperplasia with or without additional TPA treatments. One arsenic-treated (42.5 p.p.m.) mouse also exposed to TPA had an oviduct adenoma.

Transplacental arsenic exposure had effects on tumor multiplicity of any organ (tumors per mouse) or malignant tumor multiplicity of any organ (Figures 1 and 2). In male mice the average number of tumors per mouse and of malignant tumor per mouse were significantly increased at both doses of arsenic.
regardless of any additional TPA treatment (Figure 1). In female mice, the average number of tumors per mouse was significantly increased but only at the highest dose of gestational arsenic (85 p.p.m.) and only with additional TPA exposure (Figure 2). The increases in liver and lung tumors seen in this group (see Tables III and VII) probably contributed to this increase.

Other tumors occasionally occurred that were apparently not due to any treatment. For those spontaneous tumors not already mentioned, in female mice this included eight hemangioma (two in liver, uterus and ovary; one in leg and oviduct), five lymphoma, four mammary gland adenocarcinoma, three forestomach squamous cell carcinoma, three sarcoma (two peritoneal wall and one leg), a melanoma and a myxoma of the cervix, and a Harderian gland adenoma. In male mice this included eight hemangioma (three heart, two liver and one kidney, small intestine and spleen), two lymphoma, two lipoma (kidney, small intestine), a sarcoma of the liver, a Harderian gland adenoma, a Harderian gland carcinoma, a hemangiosarcoma of the liver, a thyroid adenoma, a preputial gland fibrosarcoma, a renal pelvic papilloma and a transition cell carcinoma of the ureter.

**Discussion**

The results of the present study clearly demonstrate that oral maternal inorganic arsenic exposure in mice has a very significant carcinogenic impact on the offspring as adults, even though they are not exposed to any additional arsenic. Indeed,
brief inorganic arsenic exposure during gestation in the present study induced a variety of tumors in the offspring, including aggressive hepatic malignancies, which is consistent with the development of liver cancer in human populations exposed to elevated levels of environmental arsenic (2). Strong dose–response relationships occurred between the level of maternal inorganic arsenic exposure and tumors in the offspring in several tissues, including malignant tumors of the liver. This is analogous to dose–response relationships seen between environmental arsenic levels and cancers in humans (2).

In all, the ‘trans-maternal’ exposure to arsenic alone in the present work lead to a tumor response in the offspring as adults. Together, these results show that exposure to arsenic during in utero development, even though the exposure is limited in duration (10 days), produces a strong carcinogenic response in the offspring long after arsenic exposure has ceased. In human exposure situations, particularly in areas where elevated levels of inorganic arsenic in the drinking water lead to endemic arsenicalism, it is probable that all stages in life are similarly exposed, which would include exposure during pregnancy. It appears arsenic freely passes the human and animal placenta (2,23,24), so trans-maternal arsenic exposure is a plausible route in humans. The consistently observed susceptibility of the mouse fetus to development of tumors as adults after in utero arsenic exposure (19; and present study) may prognosticate a similar sensitivity in humans. This is a rather alarming possibility, particularly if one considers that there could be genetically based population differences in sensitivity to arsenic carcinogenesis (25). Additional study in exposed human populations should be performed to define any such sensitivity, and intervention activities, such as supplying uncontaminated drinking water to pregnant women living in areas with high environmental arsenic, may be advisable.

In the present study inorganic arsenic exposure via the maternal system did not induce skin cancers in the offspring, regardless of TPA treatment. TPA is an effective skin tumor promoter after initiation with a variety of carcinogens (17,26). TPA has been shown to act as a co-promoter in the skin with inorganic arsenic in adult Tg.AC (H-ras mutated) transgenic mice (11,12). Transgenic K6/ODC mice are highly sensitive to organic skin carcinogens, but show a limited response to inorganic arsenic (27). Another study has shown inorganic arsenic acts to enhance dermal carcinoma formation after UV irradiation in adult mice (13). As these dural effects of inorganic arsenic have been shown when arsenic was given to adult mice (11–13), there may be factors in a more fully developed adult skin that are required for inorganic arsenic to have a significant carcinogenic impact. The present results indicate that skin cancers were not initiated by arsenic exposure in the mouse fetus. Since transplacental arsenic exposure was an effective transplacental carcinogen in several other tissues this may indicate that distinct, tissue-specific mechanisms of action exist for arsenic.

The inorganic forms of arsenic predominate in the drinking water and are the most common forms of arsenic exposure in humans (2). Inorganic arsenic can undergo a series of enzymatic methylations in humans and most rodents, eventually forming a dimethylated (dimethylarsinic acid; DMA) compound (2,6,28–30). Chronic exposure to DMA can act as a
tumor promoter or, in some instances, as a complete carcinogen in rodents (31–38). In addition, trivalent methylated arsenicals can be direct acting genotoxic species in vitro (39). However, significant questions arise as to exactly how exposure to methylated species would duplicate inorganic arsenic exposure (40). In this regard, even though DMA is generated from inorganic arsenic in humans and rodents, it is not the actual agent to which humans are exposed as methylated species rarely occur in drinking water (2). Furthermore, arsenic conversion to DMA is not universal in all cells. For instance, normal human epidermal keratinocytes, a suspected target cell population of arsenic in the skin, do not form DMA upon exposure to inorganic arsenic (41). In addition, in the human prostate epithelial cell line, RWPE-1, inorganic arsenic methylation is undetectable (M.Stybol, personal communication), although these cells can be malignant transformed by inorganic arsenic in vitro (42). So it is possible that a methylated form may not be the carcinogenic species for all target sites of arsenic carcinogenesis. Indeed, there is no compelling reason to suspect that arsenic acts through the same carcinogenic mechanism in all of its various, and rather diverse, target tissues. We currently have studies underway to assess distribution and speciation after inorganic arsenic exposure in pregnant mice, but the present data do not allow for definition of the ultimate carcinogenic species during gestational exposure. The strain of mice used in the present study methylate inorganic arsenic as adults (43) so studies using DMA as a potential transplacental carcinogen could shed further light on this issue.

In the present study TPA acted as a tumor promoter in the liver of female offspring and in the lung of male and female offspring that had been exposed to arsenic in utero. Although TPA is best known as a promoter of skin cancers in mice, it can also promote lung and liver tumors (44–47). For instance, repeated i.p. injection of TPA in mice promotes dimethyl-nitrosamine-induced lung and liver tumor formation indicating TPA can have systemic promoting effects (44). Several studies show also transplacental exposure to various organic carcinogens in mice followed by postnatal TPA treatments can promote liver and lung tumors in the offspring (45–47). Transplacental initiation of mice with 7,12-dimethylbenz[a]anthracene during gestation followed by postnatal topical application of TPA promotes liver and lung tumors in the offspring (45,46). Similarly, the offspring of mother treated with 2-acetylaminofluorene and given repeated i.p. injections of TPA after birth show a 4.4-fold increase in hepatoma formation when compared to the offspring of mothers injected with AAF alone (47). Thus, the results of the present study would be consistent with TPA promotion of lung and liver tumors initiated by in utero exposure to arsenic, and, by implication, would indicate that arsenic can act as a tumor initiator in these organs. Had TPA exposure in the present study been by a systemic route instead of through dermal application, it is possible that the promotion response in liver or lung would have been accordingly more pronounced.

The reproducible formation of tumors following oral inorganic arsenic exposure as a single agent as seen in the present study is noteworthy in several respects. Inorganic arsenic has been viewed as a paradoxical human carcinogen, with strong evidence of human carcinogenic potential but limited evidence for animal carcinogenesis. Furthermore, the case has been made that, as animal models for studying the carcinogenesis of inorganic arsenic alone have proven elusive, it is probably not a strong tumor initiator. That a relatively short period of inorganic arsenic exposure during gestation can reproducibly produce tumors in a variety of tissues in the absence of any other treatments (19; and present study), provides convincing evidence that inorganic arsenic alone can act as a complete carcinogen in animals. The present study provides evidence that TPA promoted tumors in the liver and lung of mice after in utero arsenic exposure, indicating arsenic may well have acted as a tumor initiator. In addition, concordance with tumor target (liver, lung) and type (hepatocellular carcinoma) occurred between this transplacental mouse model and human studies of inorganic arsenic carcinogenesis (2,48,49). The further development of this animal model should provide insight into the carcinogenic mechanisms of inorganic arsenic. Investigating the possibility that humans have a similar sensitivity to the in utero effects of arsenic should be a research priority.

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References

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