Arsenite Is a Cocarcinogen with Solar Ultraviolet Radiation for Mouse Skin: An Animal Model for Arsenic Carcinogenesis

Toby G. Rossman, Ahmed N. Uddin, Fredric J. Burns, and Maarten C. Bosland

Nelson Institute of Environmental Medicine and Kaplan Cancer Center, New York University School of Medicine, 57 Old Forge Road, Tuxedo, New York 10987

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Although epidemiological evidence shows an association between arsenic in drinking water and increased risk of skin, lung, and bladder cancers, arsenic compounds are not animal carcinogens. The lack of animal models has hindered mechanistic studies of arsenic carcinogenesis. Previously, this laboratory found that low concentrations of arsenite (the likely environmental carcinogen) which are not mutagenic can enhance the mutagenicity of other agents, including ultraviolet radiation (UVR). This enhancing effect appears to result from inhibition of DNA repair by arsenite. Recently we found that low concentrations of arsenite disrupted p53 function and upregulated cyclin D1. These results suggest that the failure to find an animal model for arsenic carcinogenesis is because arsenite is not a carcinogen per se, but rather acts as an enhancing agent (cocarcinogen) with a genotoxic partner. We tested this hypothesis with solar UVR as carcinogenic stimulus in hairless Skh1 mice. Mice given 10 mg/l sodium arsenite in drinking water for 26 weeks had a 2.4-fold increase in yield of tumors after 1.7 KJ/m² UVR three times weekly compared with mice given UVR alone. No tumors appeared in mice given arsenite alone. The tumors were mostly squamous cell carcinomas, and those occurring in mice given UVR plus arsenite appeared earlier and were much larger and more invasive than in mice given UVR alone. These results are consistent with the hypothesis that arsenic acts as a cocarcinogen with a second (genotoxic) agent by inhibiting DNA repair and/or enhancing positive growth signaling.

Key Words: arsenic; skin; tumor; mice; ultraviolet radiation.

Arsenic contamination of drinking water is a worldwide problem. It is especially severe in the Bengal region of India and in parts of Bangladesh, Taiwan, Chile, Argentina, Mexico, and China, but is also of concern in other countries, including the Western states of the United States and in small areas of New England and the Midwest (Guha-Mazumder et al., 1998; Tondel et al., 1999; Chiou et al., 2001; Hopenhayn-Rich et al., 1996; Garcia-Vargas et al., 1994; Lewis et al., 1999; Karagas et al., 2001; Welch et al., 1999). Epidemiologic evidence strongly implicates exposure to arsenic in the causation of cancers of the lung, skin, and bladder and possibly other organs (IARC, 1980; National Research Council, 2000). However, numerous attempts for over 30 years to find an animal model for arsenic carcinogenesis have mostly failed (reviewed in IARC, 1980; Wang and Rossman, 1996; National Research Council, 2000). This is particularly true for oral exposure, where arsenic compounds alone fail to induce tumors in animals (Kanisawa and Schroeder, 1967; Milner, 1969; Germolec et al., 1997). Thus, arsenic has remained one of the few examples of a well-established human carcinogen (on the basis of epidemiological evidence) which has failed to induce cancers in animals.

Understanding the mechanism of arsenic carcinogenesis has been hindered by this lack of an animal model. Recently, the US Environmental Protection Agency (USEPA) suggested a limit of 10 µg/l (reduced from 50 µg/l) arsenic in drinking water (Environmental Protection Agency, 2000). Because no animal model was available at the time to establish mode of action, the USEPA based the decision for risk assessment using the default assumption that carcinogens do not have a threshold and that excess risk is proportional to dose at low values (low dose linearity).

The increased cancer risk observed in epidemiological studies is attributed mainly to the presence of inorganic trivalent arsenic (arsenate) (IARC, 1980), although pentavalent inorganic arsenic is readily converted to the trivalent form in vivo (reviewed in Rossman, 1998; National Research Council, 2000). Arsenite is not significantly mutagenic at endogenous loci in bacterial or mammalian cells at concentrations giving high levels of survival (reviewed in Rossman, 1998). For this reason, arsenite is sometimes considered a tumor promoter. There is little evidence for this view, because negative results have been obtained in bioassays testing arsenite for promotional activity (Milner, 1969; Germolec et al., 1997). Arsenic compounds were also not carcinogenic to animals when tested at reasonable doses as complete carcinogens or as initiators (IARC, 1980; National Research Council, 2000).

Low concentrations of arsenite, which are not mutagenic, are
ARSENITE IS A MOUSE COCARCINOGEN

ON. Female hairless mice (Crl: SK1-hrBR) aged 3 weeks at the beginning of the experiment, were purchased from Charles River Laboratories (Portage, MI). They were housed 5 to a cage in solid-bottom plastic cages with wire mesh covers and fed autoclaved Purina lab chow 5001 and distilled water ad libitum. Room illumination was on an automated cycle of 12 h light and 12 h dark, and room temperature was maintained at 22–25°C. All experiments conducted were approved by the Institutional Animal Care and Use Committee of New York University School of Medicine.

A total of 40 mice were divided into four different groups as follows: Group 1, controls (no arsenite, no UVR), 5 mice; Group 2, arsenite only, 5 mice; Group 3, UVR only, 15 mice; Group 4, arsenite + UVR, 15 mice.

Arsenite and UVR exposure. Mice in Groups 2 and 4 received sodium arsenite (Sigma Biochemicals, St. Louis, MO) via drinking water at a concentration of 10 mg/l beginning at 21 days after birth. This corresponds to ~5770 µg/l arsenic. Water consumption was determined at weekly intervals. The weight of each mouse was determined at approximately 5-week intervals.

Three weeks after the start of the arsenite (or control water) exposure, mice were monitored at various times during the experiment. The mice were monitored at various times during the experiment for the presence of skin tumor-like lesions visible to the naked eye. The number of tumor-like lesions of diameter equal to or larger than 1 mm and persisting at least 2 weeks after appearance was recorded for each animal separately. Tumor yield was calculated at each time as the number of tumors per mouse. The mice were euthanized 182 days after the start of the UVR by carbon dioxide asphyxiation, and all tumors were biopsied for analysis. The volumes of all tumors was calculated using the standard formula length × width × height × 0.5236.

RESULTS

The concentration of arsenite (10 mg/l) used in the drinking water had no effect on the growth of the mice, compared to control mice (Fig. 1). The mice drinking the arsenite-containing water consumed a similar amount of water compared with mice drinking water without arsenite, and appeared equally active and healthy. There were no deaths in any group of mice for the duration of the experiment.

The dose of UVR was chosen to be approximately one-half the MED for these mice (Halliday et al., 2000). A slight reddening of the skin was observed after the first UVR exposure, but not in subsequent exposures. No effect of the UVR on growth, physical activity, or general health was seen.

No skin tumors were seen in control mice (Group 1) or in mice exposed to arsenite alone (Group 2), confirming the lack of carcinogenicity by oral arsenite alone observed in other studies. The first tumors in the combined arsenite + UVR group (Group 4) occurred at 8 weeks after the start of the UVR exposure (Figs. 2a and 3), whereas the first tumors in mice

 able to enhance the mutagenicity of ultraviolet radiation (UVR) (Lee et al., 1985; Li and Rossman, 1991). Evidence suggests that this comutagenic effect of arsenite is due to inhibition of repair of UVR-induced DNA damage (Hartwig et al., 1997). Comutagenic effects of arsenite for mutagens other than UVR have also been demonstrated by us and others, and there is evidence implicating inhibition of DNA repair (both base and nucleotide excision repair) as the mechanism for these effects as well (reviewed in Rossman, 1998). However, no specific repair enzyme has been found to be sensitive to the concentrations of arsenite used in these experiments (Li and Rossman, 1989a,b; Hu et al., 1998). These observations have led to the hypothesis that the effects on DNA repair by arsenite may be a result of faulty DNA damage-inducible signaling which controls DNA repair (Rossman, 1999). In a test of this hypothesis, this laboratory has recently shown that in cells treated with arsenite and ionizing radiation the p53-dependent increase in p21 expression, normally a block to cell cycle progression, was absent (Vogt and Rossman, 2001). We suggest that the absence of normal p53 functioning along with increased positive growth signaling (Germolec et al., 1997; Barchowsky et al., 1999; Trouba et al., 2000; Chen et al., 2001; Vogt and Rossman, 2001). We suggest that the absence of normal p53 functioning along with increased positive growth signaling in the presence of DNA damage may result in defective DNA repair and account for the comutagenic effects of arsenite.

Based on arsenite’s comutagenicity, we have previously hypothesized that arsenite might act as a cocarcinogen, for example in the case of skin cancer with UVR as carcinogenic stimulus (Li and Rossman, 1991). Here we show that arsenite does indeed act as a cocarcinogen by potentiating the ability of solar UVR to induce skin cancer in hairless mice.

METHODS

Animals. Female hairless mice (Crl: SK1-hrBR) aged 3 weeks at the beginning of the experiment, were purchased from Charles River Laboratories (Portage, MI). They were housed 5 to a cage in solid-bottom plastic cages with wire mesh covers and fed autoclaved Purina lab chow 5001 and distilled water ad libitum. Room illumination was on an automated cycle of 12 h light and 12 h dark, and room temperature was maintained at 22–25°C. All experiments conducted were approved by the Institutional Animal Care and Use Committee of New York University School of Medicine.

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Three weeks after the start of the arsenite (or control water) exposure, mice in Groups 3 and 4 were irradiated with a bank of four parallel Westinghouse solar FS-20 lamps three times per week at a dose of 1.7 KJ/m². According to manufacturer’s specification, 85% of the lamp output was in the UVB (290–320 nm) range, <1% in the UVC (<290 nm) range, 4% in the UV-A (320–400 nm) range, and the remainder was in the visible (>400 nm) range. The UVR dose rates were measured with a IL1400A digital radiometer/photometer equipped with a SEL240 UVB-1 detector (International Light, Inc., Newburyport, MA). The UVR dose chosen was reported to be approximately 50% of the minimal erythemic dose (MED) for these mice (Halliday et al., 2000). To avoid intensity loss, the UVR dose was checked every month, and kept constant during the experiment by altering the exposure time.

Tumors. The mice were monitored at various times during the experiment for the presence of skin tumor-like lesions visible to the naked eye. The number of tumor-like lesions of diameter equal to or larger than 1 mm and persisting at least 2 weeks after appearance was recorded for each animal separately. Tumor yield was calculated at each time as the number of tumors per mouse. The mice were euthanized 182 days after the start of the UVR by carbon dioxide asphyxiation, and all tumors were biopsied for analysis. The volumes of all tumors was calculated using the standard formula length × width × height × 0.5236.

Pathological analysis. Collected tumor-like lesions were fixed for formalin, processed, and embedded in paraffin. Five-micrometer-thick sections were stained using hematoxylin and eosin (H and E). Tumors were classified as follows: squamous cell carcinomas (SCC) had nests of atypical cells invading into the dermis or deeper subcutaneous tissue; the term keratinocytic intraepidermal neoplasia was used for small tumors with acanthotic and hyperplastic growth of the epidermis with cells of varying degrees of atypici-

ity, but no invasive growth through the basement membrane into the dermis (Cockerell, 2000); papilloma was applied to papillomatous growths of epidermal cells without cellular atypicity or invasion of epidermal tumor cells into dermis and either a sessile or exophytic appearance (Bogoslov, 1994); fibrosarcoma refers to malignant tumors of connective tissue; hyperplasia refers to nonmalignant excessive growth of tissue with varying degree of cellular atypicity without invasion. Minimally invasive and highly invasive SCC were distinguished. A tumor was considered to be minimally invasive when only one or a few invading strands of cells had broken through the basement membrane.

Statistical analysis. Differences in the total number of tumors was tested by the Wilcoxon rank–sum test and differences in their histological grades were analyzed by Fisher’s exact test. Differences in the distribution of tumor volumes were analyzed by the χ² test. The distribution of time-to-first-tumor was analyzed by the Kolmogorov–Smirnov test.
given UVR alone (Group 3) did not appear until week 12. The earlier appearance of tumors in Group 4 compared with Group 3 is statistically significant. The Kolmogorov–Smirnov Test was used to test for equality of the distribution of time-to-first-tumor in mice from Groups 3 and 4. The null hypothesis was rejected \((P = 0.0018)\). Although at the end of the experiment (26 weeks of UVR) every mouse in both the combined or UVR alone group had at least one tumor, the time until 100% tumor incidence was also much reduced in the combined group; after 19 weeks of UVR exposure, 100% mice in the combined group (Group 4) had tumor compared to 33.3% mice given UVR alone (Group 3). The percentage of tumor-bearing mice in the UVR alone group took 25 weeks to reach 100%.

Tumors developed only in the UVR-exposed regions of skin (i.e., backs) of the mice in either group. Nine tumor-like lesions in the UVR alone Group 3 and 21 in the UVR + arsenite Group 4 regressed and were not included in the count. The total number of tumors in Group 3 was 53 (the numbers for each mouse are 4, 4, 2, 4, 2, 6, 3, 5, 2, 6, 5, 2, 3, 3) and in Group 4 was 127 (the numbers for each mouse are 9, 3, 3, 6, 19, 6, 8, 5, 15, 12, 3, 9, 8, 12, 9). The hypothesis that these 2 samples can be samples from the same random distribution was tested by the Wilcoxon rank–sum test and rejected \((P = 0.0006)\). Eighteen of the 127 tumors in Group 4 (in 11 mice) had extremely rapid growth rates, often reaching a 4- to 5-mm diameter within less than a week, and eventually exceeding 10 mm in diameter. Two mice in this group developed tumors with ulcerating centers necessitating euthanasia after 18 weeks of UVR. Thus, the total number of tumors in the combined treatment group (127) is underestimated (Table 1).

The tumor yields over time are shown in Fig. 3. The tumor yield in mice exposed to arsenite + UVR was increased 5.3-fold at week 18 and 2.4-fold at week 26 after the start of UVR, compared with the yield in mice exposed to UVR alone.

![Graph showing growth of female weanling (21 days) Skh1 mice continually exposed or not to 10 mg/l sodium arsenite in drinking water. Mice were weighed at various times and the mean ± standard errors were calculated \((N = 20\) for each group).](image1)

**FIG. 1.** Growth of female weanling (21 days) Skh1 mice continually exposed or not to 10 mg/l sodium arsenite in drinking water. Mice were weighed at various times and the mean ± standard errors were calculated \((N = 20\) for each group).

![Early tumors in mice exposed to UVR + arsenite. (a) These were the first two tumors (later diagnosed as squamous cell carcinomas) that appeared after 8 weeks of UVR + arsenite. Photograph was taken after 11 weeks of UVR exposure. (b) The same tumors (top and bottom mice) became large and ulcerating by 14 weeks. The center mouse shows a small tumor later classified as keratinocytic intradermal neoplasia (arrow), that appeared at week 11 and remained almost the same size at week 14 when this photograph was taken.](image2)

**FIG. 2.** Early tumors in mice exposed to UVR + arsenite. (a) These were the first two tumors (later diagnosed as squamous cell carcinomas) that appeared after 8 weeks of UVR + arsenite. Photograph was taken after 11 weeks of UVR exposure. (b) The same tumors (top and bottom mice) became large and ulcerating by 14 weeks. The center mouse shows a small tumor later classified as keratinocytic intradermal neoplasia (arrow), that appeared at week 11 and remained almost the same size at week 14 when this photograph was taken.
The final volumes of all tumors, regardless of tumor type, are depicted in Fig. 4. It is clear that the mice in the combined treatment (arsenite + UVR) group had much larger tumors than those treated with UVR alone. Based on the $\chi^2$ test, the two distributions of final tumor volumes are significantly different ($P < 0.0001$).

All tumors observed in this experiment were examined histopathologically. The types of tumors found in Groups 3 (UVR) and 4 (UVR + arsenite) are shown in Table 1 and Fig. 5. There were no significant differences in the distribution of tumor types except for a higher malignant phenotype of tumors in the combined arsenite + UVR group. Most of the tumors were SCC with variable degrees of invasiveness and differentiation. In the combined treatment group, 64 of 127 (50.4%) of the tumors were highly invasive SCC (moderate to well differentiated) exhibiting typical keratin pearls (Fig. 5a) and invasion into deeper structures (Figs. 5a and 5b), whereas in the UVR alone group only 14 of 53 (26.4%) of the tumors were invasive SCC. A large number of small tumors (volumes 1–100 mm$^3$) from both the arsenite + UVR group and the UVR alone group showed the characteristics of SCC (Figs. 5c and 5d). In addition, a number of tumors from both groups have characteristics of SCC with invasive cancer cells not going through deeper tissues. These were classified as minimally invasive SCC (Fig. 5e) (38 of 127 in the combined group and 25 of 53 in the UVR alone group). Keratinocytic intradermal neoplasia (Fig. 5f), papilloma, fibrosarcoma, and premalignant hyperplasia were also seen (Table 1).

**DISCUSSION**

Results of this study clearly demonstrate that arsenite augments the carcinogenic effect of UVR in the skin of the hairless mouse. It was recently reported that in Tg.AC (H-ras mutated) transgenic mice, arsenite can act as a co-promoter by accelerating the onset and growth rate of benign skin tumors in combination with TPA as a promoter (Germolec et al., 1998). This report is the first demonstration that arsenite can potentiate the onset and growth of malignant skin tumors induced by a genotoxic carcinogen in a strain of mice that is wild type with respect to oncogenes and tumor suppressor genes. These data support the hypothesis that arsenite acts as a cocarcinogen for the mouse skin, when given along with carcinogenic doses of chronic UVR.

No tumors occurred on the skin of control mice or mice that received only arsenite in the drinking water. Upon autopsy, no tumors were noted in any other organs in any of the mice. Recently, it was found that trivalent monomethyl arsenic metabolites are more toxic than arsenite (Styblo et al., 2000; Petrick et al., 2000), suggesting that these metabolites may play an important role in arsenic carcinogenesis. It has also been shown that high concentrations of the herbicide and metabolite dimethylarsinic acid can act as a tumor promoter and possibly as a complete bladder carcinogen in the female rat.

### TABLE 1

**Histopathological Analysis of Tumors**

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>UVR alone</th>
<th>UVR + arsenite</th>
<th>Significance $^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell carcinoma (highly invasive)</td>
<td>14 (26.4)</td>
<td>64 (50.4)</td>
<td>$P = 0.003$</td>
</tr>
<tr>
<td>Squamous cell carcinoma (minimally invasive)</td>
<td>26 (49.1)</td>
<td>38 (29.9)</td>
<td>$P = 0.017$</td>
</tr>
<tr>
<td>Keratinocytic intraepidermal neoplasia</td>
<td>10 (18.9)</td>
<td>16 (12.6)</td>
<td>NS$^b$</td>
</tr>
<tr>
<td>Fibrosarcoma</td>
<td>1 (1.9)</td>
<td>1 (0.79)</td>
<td>NS</td>
</tr>
<tr>
<td>Papilloma</td>
<td>2 (3.8)</td>
<td>4 (3.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>0 (0)</td>
<td>4 (3.1)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>53</td>
<td>127</td>
<td></td>
</tr>
</tbody>
</table>

$^*$ Fisher’s exact test.

$^b$ Not significantly different ($P > 0.05$).
(reviewed in Kenyon and Hughes, 2001). Since mice can methylate arsenite to dimethylarsinic acid as the major urinary metabolite (Vahter, 1981), the obligate trivalent intermediate monomethylarsonous acid must also be formed. It is thus possible that some or all of these arsenic metabolites may play a role in the arsenite cocarcinogenesis seen here, but the lack of tumors at sites other than UVR-treated skin is evidence that the amounts of metabolites formed from 10 mg/l arsenite in drinking water were not sufficient to act as complete carcinogens.

The sodium arsenite concentration (10 mg/l) used in the drinking water corresponds to ~5770 µg/l arsenic, which is only 4.4 times higher than the highest concentration (1300 µg/l) found in Nevada drinking water (Warner et al., 1994) and only 1.7 times higher than the highest concentrations (3400 µg/l) found in drinking water in the West Bengal region of India (Guha Mazumder et al., 1998).

When the experiment ended at 182 days, tumors in the arsenite + UVR group outnumbered those in the UVR only group by 2.4:1. Tumors in the combined treatment group were much larger (Fig. 4) and more invasive (Table 1, Fig. 5) than in the UVR alone group, suggesting an arsenite-induced stimulation of the growth rate of the SCCs. These data are consistent with, but do not constitute proof of, our hypothesis that arsenite acts as a cocarcinogen by inhibiting DNA repair and up-regulating positive growth signaling. Inhibiting DNA repair should result in a higher rate of conversion from DNA damage (in this case by UVR) to mutations, leading to increased tumorigenicity. Up-regulating positive growth signaling should also lead to increased mutagenesis and, in addition, to increased tumor growth. Both greater tumor yield and larger tumor size are seen in mice treated with arsenite in the experiments reported here. Preliminary data show the up-regulation of cyclin D1 in the skin of mice treated with arsenite, and experiments to demonstrate inhibition of DNA repair by arsenite in vivo are planned.

It is often stated that the skin cancers seen in arsenic-exposed individuals differ from those associated with sunlight exposure in that arsenic-associated cancers develop in “unexposed” parts of the body. This statement is based on data from the southwest Taiwan arsenic endemic area (Yeh et al., 1968) where it was claimed that 48.14% of “lesions” (including keratosis as well as cancers) appeared on “unexposed surfaces,” defined as “palms, soles, extremities, and trunk.” The most frequent site of lesions in males was the back, an area that cannot be said to be unexposed in a subtropical region where the main occupations are farming, fishing, and salt production from sea water, and where men usually work wearing only shorts; children also dress in little more than shorts (Lung-Chi Chen, personal communication). If we consider as unexposed parts of the body the axilla, groin, genitals, buttock, palm, and sole (the latter two because the thick keratin layer prevents sunlight penetration), then only 5.2% of skin cancers appeared in unexposed parts of the body in men (calculated from Yeh et al., 1968). The frequency of skin cancers in this population is 2.9-fold higher in males than in females (Tseng et al., 1968). The explanation often given for this difference is that men work outside more than women, and therefore may drink more arsenic-contaminated water. However, as pointed out by Tseng et al. (1968), this raises the question of why there is a sex ratio of 1:1 for hyperpigmentation and keratosis, two important markers of arsenic exposure. Some women work at farming, but because fair skin is seen as desirable, women usually cover more of their bodies and wear hats. Body clothing worn in warm climates does not generally completely block UVR. While the average men’s shirt transmits 20% of solar UVR, the light gauzy weaves preferred by women may allow 50% penetration (World Health Organization, 1979). It should also be pointed out that in order for human skin cancers to be the result of combined arsenic + UVR exposure, it is not necessary that the cancers appear on the most UVR-exposed parts of the body. In fact, an argument can be made that in the case of severe DNA damage, inhibition of DNA repair might lead to cell death, and not to increased tumorigenesis.

In addition, UVR may not be arsenic’s only partner in inducing skin cancer. There are a number of important con-
FIG. 5. Histopathology of the tumors (H and E-stained paraffin sections). (a) One of the squamous cell carcinomas shown in Fig. 2 (volume >1000 mm³) appearing in a mouse in the combined arsenite + UVR group after 8 weeks of exposure. A large keratin pearl is shown by the arrow (30×). (b) Higher power magnification of (a) showing typical keratinizing pearls (arrows) and invasion of tumor cell nests into subcutaneous structures (arrowheads) (150×). (c) One small tumor (volume = 15 mm³) from the arsenite + UVR group showing two small keratin pearls (arrow), a typical characteristic of squamous cell carcinoma (150×). (d) Higher magnification of (c) showing small strands of atypical cells invading the dermis (arrowheads) (300×). (e) A minimally invasive SCC from a mouse in the UVR alone group. Unlike the large SCCs shown in (a) and (b), this minimally invasive SCC had only a few small strands of malignant cells invading the epidermis (arrowheads), while the remainder of the tumor did not clearly break through the basement membrane (not shown) (300×). (f) Keratinocytic intraepidermal neoplasia from a mouse in the UVR + arsenite group. These, usually small, tumors consisted of atypical epidermal cells that were entirely confined to the epidermis and did not show any evidence of dermal invasion through the basement membrane (60×).
founding variables that must be considered. Genetic susceptibility to arsenic carcinogenesis may vary in different populations (see discussion in Gebel, 2000). Other carcinogens in the environment, such as polycyclic aromatic hydrocarbons, may also be involved in skin as well as lung and bladder cancers (Boffetta et al., 1997). Humic acid byproducts in drinking water in Taiwan may contribute to its carcinogenesis (Lu et al., 1986). Skin cancer rates in Taiwan are higher for arsenic-exposed individuals who work in salt fields, are carriers of hepatitis B virus with liver dysfunction, or have poor nutritional status (Hsueh et al., 1995). Deficiencies in vitamin B12, folate, vitamin B6 (pyridoxine), niacin, ascorbate, α-tocopherol, iron, or zinc are associated with increased DNA damage (Ames, 2001). Both folic acid and selenium have been shown to protect against arsenic toxicity (Gebel, 2000; Ruan et al., 2000) and selenium protects against arsenic-induced genotoxicity in human lymphocytes (Beckman and Nordenson, 1986).

Since arsenic exposure is also associated with lung and bladder cancers in humans, it is possible that a cocarcinogenic arsenic exposure protocol may lead to animal models for these cancers as well. Arsenic appears to act synergistically in lung carcinogenesis with tobacco use in occupationally exposed workers (Pershagen et al., 1981; Hertz-Picciotto et al., 1992), and cigarette smoking in the arsenic endemic area of Taiwan was 40% (Chen et al., 1985). Cigarette smoking was independently associated with increased risk of urinary tract cancers in Taiwan (Chiou et al., 2001). Arsenic also enhances lung cancer risk from radon gas in tin miners (Xuan et al., 1993).

The development of an animal model for arsenic-related skin carcinogenesis now makes it possible to evaluate the mechanisms underlying the dose/response relationship and to determine the proper model for risk assessment. Animal models will also allow molecular analysis of arsenic’s cocarcinogenicity, determination of the role of specific environmental agents that may act as arsenite’s partners in carcinogenesis, assessment of the effects of dietary deficiencies in arsenite cocarcinogenesis, and for development of chemopreventive strategies for arsenic-exposed populations.

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Arsenic toxicity is enzyme specific and its effects on ligation are not caused by the direct inhibition of DNA repair enzymes. **Mutat. Res.** **408**, 203–218.


