Our laboratory has shown that arsenite markedly increased the cancer incidence caused by solar-simulation ultraviolet radiation (UVR) in hairless mouse skin. The relationship between arsenite concentration in drinking water and the yield of squamous cell carcinomas in UVR-exposed mouse skin was linear up to 5 mg/L. UVR is a complete carcinogen that readily induces skin cancer (Zhuang et al. 2000). Although UVR induces a wide range of DNA damage, such as protein–DNA crosslinks, oxidative base damage, single-strand breaks, and double-strand breaks (de Gruijl et al. 2001), the major DNA damage is cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs). Both of the latter lesions are repaired by nucleotide excision repair (NER) (Mitchell et al. 1985). Failure to repair CPDs and 6-4PPs leads to the signature mutations consistent with cyclobutane pyrimidine dimers and 6-4 photoproducts (6-4PPs) and apoptosis were measured using the enzyme-linked immunosorbent assay and the two-color TUNEL (terminal deoxynucleotide transferase dUTP nick end labeling) assay, respectively. The results showed that arsenite reduced the repair rate of 6-4PPs by about a factor of 2 at 5.0 µM and had no effect at 2.5 µM. UVR-induced apoptosis at 24 hr was decreased by 22.64% at 2.5 µM arsenite and by 61.90% at 5.0 µM arsenite. Arsenite decreased the UVR-induced caspase-3/7 activity in parallel with the inhibition of apoptosis. Colony survival assays of the 291.03C cells demonstrated a median lethal concentration (LC50) of arsenite of 0.9 µM and a median lethal dose (LD50) of UVR of 0.05 kJ/m2. If the present results are applicable in vivo, inhibition of UVR-induced apoptosis may contribute to arsenite’s enhancement of UVR-induced skin carcinogenesis. Key words: apoptosis, arsenite, mouse keratinocyte, photodamage repair, skin cancer, solar-simulation UVR. Environ Health Perspect 113:983–986 (2005). doi:10.1289/ehp.7846 available via http://dx.doi.org/[Online 15 April 2005]

Arsenite-Induced Alterations of DNA Photodamage Repair and Apoptosis After Solar-Simulation UVR in Mouse Keratinocytes in Vitro

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Arsenite is a toxic metal that has been shown to have various effects on health, including its role in the development of skin cancer. In this study, the authors investigated the effects of arsenite on DNA photodamage repair and apoptosis in mouse keratinocytes after solar-simulation ultraviolet radiation (UVR). They found that arsenite reduced the repair rate of 6-4 photoproducts (6-4PPs) and inhibited apoptosis. These findings suggest that arsenite may contribute to skin cancer progression, and insect to environmental health research.
methanol. We counted colonies and determined the percentage of survival as the ratio of treated to control X 100. The survival after exposure to solar-simulation UVR was determined similarly at 7 days after single doses of 0.0, 0.05, 0.10, 0.20, and 0.30 kJ/m².

Measurement of CPDs and 6-4PPs in genomic DNA by ELISA. We isolated genomic DNA using the QIAamp Blood Kit (QIAGEN Inc., Valencia, CA). DNA concentrations were calculated from the absorbance at 260 nm measured by a Beckman DU 650 spectrophotometer (Beckman Instruments, Fullerton, CA). We determined the quantities of CDPs and 6-4PPs by enzyme-linked immunosorbent assay (ELISA) as described by Mori et al. (1991). In brief, Falcon polyvinylchloride flat-bottom 96-well assay plates (Becton Dickinson Labware, Franklin Lakes, NJ) precoated with 1% proteamine sulfate (Sigma) were incubated with purified genomic DNA (15 ng for CPD detection and 150 ng for 6-4PP detection) in PBS at 37°C for 20 hr. For CPD detection, we used the TDM-2 antibody, and for 6-4PP detection we used the 64M-2 antibody (both antibodies were generously provided by T. Mori, Nara, Japan). After adding biotinylated F(ab´)2 goat anti-mouse IgG fragments and streptavidin-peroxidase (Zymed, San Francisco, CA), we measured the optical density from 492 nm using a Bio Assay Reader HTS7000. EMEM mixed with the same volume of Apo-one Homogenous Caspase-3/7 reagent served as a negative control.

Results

Toxicity of arsenite and UVR to 291.03C mouse keratinocytes. Figure 1 shows the effects of arsenite (Figure 1A) and solar-simulation UVR (Figure 1B) on clonal survival of 291.03C mouse keratinocytes. The median lethal dose (LD50) of UVR was 0.05 kJ/m². There was no measurable colony survival at UV doses > 0.30 kJ/m². The median lethal dose (LD50) of sodium arsenite was 0.9 µM. Arsenite did not show significant lethality < 0.5 µM and showed nearly 100% lethality > 5.0 µM.

In a previous study (Burns et al. 2004), hairless mice were fed sodium arsenite in drinking water at concentrations ranging from 1.25 mg/L (9.6 µM) to approximately 10 mg/L (77.0 µM), and solar spectrum UVR exposure was applied to the dorsal skin at 1.0 kJ/m² three times weekly. The arsenite concentrations and solar UVR dose used in the present in vitro study were 2.5 and 5 µM and 0.3 kJ/m². These two arsenite concentrations were estimated to be equivalent to 26 and 52% of the lowest arsenite concentration (1.25 mg/L) used in the in vivo carcinogenesis study (Burns et al. 2004).

Arsenic effects on DNA photodamage repair. The two photolesions, CPDs and 6-4PPs, were detected by ELISA. The 6-4PPs were 80% removed by 12 hr, whereas CPDs were not removed > 10% by 24 hr (Figure 2). According to the regression analysis of the data, arsenite showed no significant effect on CPDs repair. The 6-4PP repair rate after UVR was 11.95%/hr; when combined with 2.5 µM or 5.0 µM arsenite, the 6-4PP repair rates were 11.3% and 6.19%/hr, respectively. Arsenite slowed the 6-4PP repair rate by 48% at 5.0 µM, but no difference was detected at 2.5 µM.

Arsenite inhibits UVR-induced apoptosis. Figure 3 shows that at 24 hr after UVR alone (0.30 kJ/m²) the percentage of apoptotic cells was 27.6% (Figure 3D). When UVR-treated cells were incubated in 2.5 µM or 5.0 µM arsenite, the percentage of apoptotic cells decreased to 21.4% (77.36% of UVR only; Figure 3E) and 10.5% (38.1% of UVR only; Figure 3F), respectively. Untreated control cells showed few apoptotic cells (Figure 3A), whereas 5.0 µM arsenite only showed 4.9% apoptotic cells at 48 hr after treatment.
(Figure 3B) and 8.1% at 60 hr (Figure 3C) after treatment. Apoptosis was not detected at 0 hr and 14 hr after UVR and was 51.56, 39.42, and 36.47% at 36 hr after UVR, UV + 2.5 µM arsenite, and UV + 5 µM arsenite, respectively (data not shown), indicating that apoptosis is progressing with the time. As shown in Figure 3, the R3 population was 5.28% (UVR alone), 3.71% (UVR + 2.5 µM arsenite), and 1.32% (UVR + 5.0 µM arsenite), indicating that apoptosis is more extensive after treatment with UVR alone compared with UVR plus arsenite.

The caspase-3/7 activities 24 hr after UVR are shown in Figure 4. Arsenite decreased the UVR-induced caspase-3/7 activity to 88.48% at 2.5 µM and to 58.83% at 5 µM. Arsenite alone did not affect the caspase level significantly. These results are consistent with the results in Figure 3 indicating arsenite inhibited UVR-induced apoptosis (Figure 4).

**Discussion**

The photoproducts (CPDs and 6-4PPs) produced by UVR may lead to mutations and cancer development if the damage is not removed from the DNA. There are two mechanisms for a cell to remove DNA damage: repairing the DNA damage or inducing apoptosis. Arsenite indeed increased the mutagenicity of UVR in Chinese hamster V79 cells (Li and Rossman 1991). As reported here, mouse keratinocytes did not repair UVR-induced CPDs efficiently, and arsenite did not affect the DNA photodamage repair rates significantly. The apoptosis inhibiting activity of arsenite may have converted a greater amount of DNA damage to mutations without substantially affecting DNA repair. If so, these findings might help to explain why skin cancer in mice is markedly increased by prolonged exposure to the combination of UVR and dietary arsenite.

Although it has been reported that arsenite inhibits DNA repair in a variety of cell types (Hartwig et al. 1997; Li and Rossman 1989; Yager and Wiencke 1997), the present study in a mouse keratinocyte cell line (Figure 2) shows little effect of arsenite on the removal of UVR-induced photoproducts from the genomic DNA except the reduced 6-4PP repair rate at 5 µM. In normal human epidermal keratinocytes (NHEK, Cambrex BIO Science, Walkersville, MA), 6-4PPs were removed at a rate of 30%/hr, whereas CPDs were removed at a rate of 2%/hr after 0.3 kJ/m² solar-simulation UVR (data not shown). The mouse 291.03C keratocyte line exhibited a 6-4PP removal rate of 13%/hr and a CPD removal rate of < 0.4%/hr (Figure 2). The repair rates of mouse keratinocytes were not > 20% for CPDs and > 40% for 6-4PPs compared with those of human keratinocytes.

There are two subpathways of NER: transcription-coupled repair (TCR) and global genomic repair (GGR). TCR refers to the preferential repair of transcribed strands of active genes, and GGR refers to repair anywhere else in the DNA. Many rodent cells have normal TCR, which is very important for clonal survival, but are deficient in GGR of CPDs, which is more important for suppressing mutagenesis (Hanawalt 2001). Because the ELISA method used here detects GGR, these results confirm that mouse keratinocyte line 291.03C performs GGR of DNA photodamage less efficiently than do human keratinocytes.

| Apoptosis (%) | Caspase (%) |
|---------------|-------------|
| UVR           | 100         |
| UV + 2.5 µM arsenite | 77.36      |
| UV + 5 µM arsenite | 38.19      |
| 5 µM arsenite  | 17.89      |
| Control       | 2.68       |

Figure 4. The effect of sodium arsenite on caspase-3/7 activity in 291.03C cells treated with 2.5 and 5.0 µM arsenite for 24 hr and then exposed to 0.3 kJ/m² solar-simulation UVR. Twenty-four hours after UVR, caspase-3/7 activity was measured as described in “Materials and Methods.” The data show a close parallel with the apoptosis data from Figure 3. Data shown are mean ± SD (n = 3).
UVB can trigger apoptosis by damaging the DNA and activating the death receptors on the cell surface (Kulms and Schwarz 2000). Arsenite can induce Fas/FasL-dependent apoptosis at higher concentrations (≥ 5.0 µM) in primary human keratinocytes (Liao et al. 2004). The results reported here show that 5.0 µM arsenite produced a small increase (8%) in apoptosis after 60 hr of treatment, whereas a single 0.30 kJ/m² dose of solar-simulation UVR produced 27% apoptosis by 24 hr and 51% by 36 hr, indicating that apoptosis increased gradually after exposure to UVR. Paradoxically, arsenite at the 5.0 µM concentration produced a small incidence of apoptosis by itself, but it was inhibitory when combined with a strongly apoptotic dose of UVR (Figure 3). The caspase results generally confirmed the apoptosis results obtained by flow cytometry. At 36 hr after UVR + 5.0 µM arsenite, apoptosis increased to 37% (30% less than UVR alone), indicating that arsenite delayed the onset of the apoptosis but did not prevent it completely.

In a previous study of Chinese hamster V79 cells (Li and Rossman 1991), the combination of UVB (0.2 kJ/m²) and sodium arsenite (10 µM and 15 µM) increased the mutation rates by 1.65-fold and 2.06-fold respectively, whereas survival was decreased to 43 and 11.8%, respectively. The inhibition of apoptosis may help to explain the higher mutation rates in the presence of arsenite. In conclusion, arsenite lessened the rate of DNA repair and inhibited apoptosis at 24 hr after a single exposure of mouse keratinocyte line 291.03C to 0.3 kJ/m² of solar-simulation UVR. The consequences of this delay on mutation rates will be investigated in future studies.

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