Arsenic upregulates MMP-9 and inhibits wound repair in human airway epithelial cells

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Arsenic upregulates MMP-9 and inhibits wound repair in human airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 295: L293–L302, 2008. First published June 6, 2008; doi:10.1152/ajplung.00134.2007.—As part of the innate immune defense, the polarized conducting lung epithelium acts as a barrier to keep particulates carried in respiration from underlying tissue. Arsenic is a metalloid toxicant that can affect the lung via inhalation or ingestion. We have recently shown that chronic exposure of mice or humans to arsenic (10–50 ppb) in drinking water alters bronchiolar lavage or sputum proteins consistent with reduced epithelial cell migration and wound repair in the airway. In this report, we used an in vitro model to examine effects of acute exposure of arsenic (15–290 ppb) on conducting airway lung epithelium. We found that arsenic at concentrations as low as 30 ppb inhibits reformation of the epithelial monolayer following scrape wounds of monolayer cultures. In an effort to understand functional contributions to epithelial wound repair altered by arsenic, we showed that acute arsenic exposure increases activity and expression of matrix metalloproteinase (MMP)-9, an important protease in lung function. Furthermore, inhibition of MMP-9 in arsenic-treated cells improved wound repair. We propose that arsenic in the airway can alter the airway epithelial barrier by restricting proper wound repair in part through the upregulation of MMP-9 by lung epithelial cells.

arsenic; matrix metalloproteinase; cell migration; 16HBE14o- cells; airway epithelial barrier

ARSENIC IS A NATURALLY OCCURRING metalloid found in water, soil, and air. Exposure to inorganic arsenic occurs worldwide via environmental (e.g., contaminated drinking water, air, food, domestic fuel sources) and occupational exposures (e.g., smelting industries, pesticide production). In addition to its association with nonmalignant diseases, arsenic is of major worldwide health concern because of its carcinogenic potential in humans (5, 8, 22a, 43). More recently, there has been growing evidence that ingestion of arsenic also leads to noncarcinogenic lung disease (14, 33, 41, 44). Mouse models have provided evidence that single doses of ingested arsenic can lead to collection in the lung within 1 h, and chronic low-dose exposure leads to consistently elevated arsenic concentrations in the lung (21, 22, 24, 25). However, specific molecular and cellular mechanisms that lead to lung disease from low-dose arsenic exposure are not clearly elucidated.

Arsenic has been implicated in promoting alterations in growth and proliferation pathways, apoptotic pathways, DNA repair mechanisms, immunosurveillance, and stress-response pathways (1, 9, 31, 32, 40). Although chronic exposure to moderate and/or high levels of arsenic in drinking water may lead to the development of disease in humans, the effects at low-dose are inferred mostly from models of high-dose exposure. A variety of cellular signaling pathways have been implicated to be altered by arsenic exposure including reactive oxygen species production, cellular phosphorylation events, mitogen-activated protein kinase (MAPK) signaling, NF-κB activation, cellular proliferation, and apoptosis, among others (reviewed in Ref. 37). As in animal studies, direct effects in cellular studies are confounded by the wide range of arsenic used. However, an intriguing effect of arsenic exposure is the alteration of cellular migration (15, 52).

In high-throughput protein screening experiments with low-dose arsenic exposure, we found reduced expression of proteins associated with cellular migration in mouse lung tissue (26) and alteration of a specific wound repair protein marker in mouse bronchoalveolar fluid (27). Additional microarray experiments on lung tissue from mice fed low-dose arsenic revealed several changes in extracellular matrix (ECM) protein expression and a large increase in matrix metalloproteinase (MMP)-9 expression (26). In human studies for low-level arsenic exposure lung biomarkers, we found an increase in the ratio of MMP-9 to tissue inhibitor of matrix metalloproteinase (TIMP)-1 in collected sputum samples (23). MMPs are responsible for ECM degradation among other proteolyses. MMP-9 is the most prominently studied MMP in the lung and has been associated with a variety of lung diseases (4). The imbalance between MMP-9 and TIMP-1 is considered to contribute to the progression of airway remodeling in part due to changes in epithelial wound response (4, 6, 12, 29, 39, 47, 48).

In this study, we examined the effects of arsenic exposures on a human bronchiolar epithelial cell line (16HBE14o-). We found that as arsenic concentration increases, the ability for 16HBE14o- cells to repair monolayers in culture is inhibited. Furthermore, arsenic concentration is increased, MMP-9 secretion and activity also increased, and this upregulation of MMP-9 is in part responsible for altered wound response. In conclusion, arsenic directly affects signaling pathways that contribute to cell migration, and remodeling of the airway, and in this manner may cause or exacerbate lung disease.

MATERIALS AND METHODS

Materials. Minimum essential medium with Earle’s salts (MEM), Hanks’ balanced saline solution, l-glutamine, penicillin, streptomycin, amphotericin, FBS, LIVE/DEAD Viability/Cytotoxicity Kit,

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TRIzol reagent. Platinum SYBR Green qPCR SuperMix-UDG kit, Quant-iT and the OliGreen quantification kit were purchased from Invitrogen (Carlsbad, CA). Fibronectin and type I collagen were purchased from Becton-Dickinson (Franklin Lakes, NJ). Lechner and LaVeck (LHC) basal medium and BSA were purchased from Bio-Source International (Camarillo, CA). Antibodies to MMP-2, MMP-9, TIMP-1, and MMP inhibitor GM6001 were purchased from Calbiochem (La Jolla, CA). iScript cDNA synthesis kit was from Bio-Rad (Hercules, CA). Primers for real-time quantitative RT-PCR experiments were from Integrated DNA Technologies (Coralville, IA). All other chemicals were of the highest biochemical quality and purchased from Sigma-Aldrich (St. Louis, MO), VWR (West Chester, PA), or Fisher Scientific (Pittsburgh, PA).

16HBE14o- cell culture. 16HBE14o- cells are a SV40 transformed human bronchial epithelial cell line (18) and were obtained through the California Pacific Medical Center Research Institute (San Francisco, CA). 16HBE14o- cells were expanded in tissue culture flasks before culture on 15-mm glass coverslips. Flasks (2 mL) and coverslips (250 μL) were coated initially with matrix coating solution (consisting of: 88% LHC basal medium, 10% BSA (from 1 mg/mL stock), 1% bovine collagen type I (from 2.9 mg/mL stock), and 1% human fibronection (from 1 mg/mL stock solution)) and incubated for 2 h at 37°C, after which the coating solution was removed and cultureware allowed to dry for at least 1 h. 16HBE14o- cells were plated onto the matrix-coated cultureware at a concentration of 1 × 10⁴ cells/cm². Cells were cultured in 250–350 μL of control growth medium (CGM; Eagle’s MEM supplemented with 10% FBS, 2 mM L-glutamine, penicillin, streptomycin, and amphotericin) at 37°C in a 5% CO₂ atmosphere. CGM was replaced every other day until the cells reached confluence.

Scrape wound repair assays. 16HBE14o- cells were grown to confluence on matrix-treated glass coverslips in CGM as described above. CGM was removed and replaced with fresh CGM (0 ppb arsenic) or CGM supplemented with arsenic (30, 60, or 290 ppb as sodium arsenite; for comparison, 1 μM ≈ 75 ppb) arsenic for 24 h, after which growth medium was removed and replaced with new medium (with or without arsenic). A bent tip of a 21G syringe needle was sterilized and used to introduce a wound across the entire cell monolayer. Coverslips were mounted onto an Olympus IX70 microscope in epifluorescence mode with appropriate filters. Images were captured with Chemidoc XRS system and Quantity-One software. TBS-380 mini-fluorimeter (Turner BioSystems, Sunnyvale, CA). For quantitative PCR, 100 ng of total cDNA per reaction was amplified with a Platinum SYBR Green qPCR SuperMix-UDG kit according to the manufacturer’s instructions in a Rotor-Gene 3000 real-time thermal cycler (Corbett Robotics, San Francisco, CA), under the following conditions: initial hold for 2 min at 95°C and hold for 2 min at 95°C followed by 35–45 cycles consisting of denaturation 15 s at 94°C, anneal 30 s at 60°C for MMP-9 or 54°C for TIMP-1; extension for 45 s at 72°C; and melt from 72°C-99°C (1°C/s). Human gene-specific primer pairs were designed using MacVector software. Primer pairs used in this study included: MMP-9 forward: 5’-CGG TGA TTT ACG AGC CCT TT-3’; MMP-9 backward: 5’-ACC AAA CTG GAT GAC GAT GTC TG-3’; TIMP-1 forward: 5’-ACT GAT GGT GGG TGG ATG AGT AAT-3’; TIMP-1 backward: 5’-AGC AAC AAC AGG ATG CCA GAA G-3’. Individual analyses were performed in triplicate on cDNA samples obtained from at least three separate isolations for each experiment.

Immunoblot experiments were performed to assess amounts of MMP-9 and TIMP-1 protein in conditioned medium. Conditioned media from above were thawed, and equal amounts of protein from experiments with 0, 60, or 290 ppb arsenic were run on 7.5% (MMP-9) or 10% (TIMP-1) SDS-PAGE gels under nonreducing conditions. Proteins were transferred overnight to nitrocellulose and blocked with primary antibodies specific for human MMP-9 or TIMP-1, followed by washes and appropriate HRP-linked secondary antibodies. Blots were developed with the SuperSignal West Femto kit (Pierce, Rockford, IL) per the manufacturer’s instructions. Band density was determined with the Chemidoc XRS system and Quantity-One software.

Gelatin zymography experiments to assess activity of MMP-9 were performed on conditioned media from multiple scrape wounds described above. Conditioned medium was thawed, and 50 μL was mixed with 2× sample buffer (to a final buffer concentration of: 0.125 M Tris, pH 6.8, 2% SDS, 10% glycerol, and 0.05% bromophenol blue) for 15 min at room temperature. Samples were electrophoresed on 10% SDS-polyacrylamide gels containing 0.1% gelatin under nonreducing conditions. Gels were washed in a 2.5% Triton-X 100 solution two times for 15 min each to remove SDS. Gels were then incubated in developing buffer (50 mM Tris, 0.2 M NaCl, 5 mM CaCl₂, and 0.02% Brij 35, pH 7.5) for 30 min at room temperature, followed by 12 h at 37°C with shaking. Gels were incubated in Coomassie blue solution (0.25% wt/vol Coomassie blue R230 in 50% methanol, 10% acetic acid, and 40% H₂O₂) and then a destaining solution (50:10:40 methanol:acetic acid:H₂O₂) to visualize undigested gelatin. Gelatinase activity corresponded to areas of clearance of the gelatin (i.e., low Coomassie blue staining) from the native gel. Images
of each gel were captured on the Chemidoc XRS system and analyzed with Quantity-One software as previously described.

To determine effects of the MMP inhibitor GM6001 on wound closure in the presence or absence of arsenic, cells were grown to confluence in CGM as described. CGM was then removed, and cells were treated with either fresh CGM or arsenic-supplemented CGM that included GM6001 (in DMSO) or a DMSO control. After 24 h, pre-wound medium was removed and replaced with new medium supplemented with GM6001 or DMSO. Multiple scrape wound assays were performed as described above.

Statistics. All statistical analyses were evaluated with GraphPad software (San Diego, CA). Multivariate comparisons were done with a one-way ANOVA with Tukey’s multiple comparison posttest; pairwise comparisons were done with a two-tailed Student’s t-test. A value of P < 0.05 was used to establish a significant difference between samples. Dose responses to increasing arsenic concentrations were evaluated using linear regression analysis; P values are given within the text. Figures are graphed ± SE unless otherwise noted.

RESULTS

Arsenic slows wound repair of human airway epithelial cells in vitro. Because in vivo biomarker data suggested that chronic arsenic exposure altered cell migrations and wound repair in the lung (23, 26, 27), we examined whether arsenic had an acute effect on wound healing of human airway epithelial cells in culture. In these experiments, 16HBE14o- cells were grown without arsenic until confluence. At that time, cultures were treated with growth medium supplemented with arsenic at 0, 30, 60, or 290 ppb. After 24 h, a single scratch wound was introduced into the culture, and repair of the wound was monitored over 4 h at 37°C (with or without arsenic). Under these conditions and in the absence of arsenic, the denuded area was largely repaired by migrating 16HBE14o- cells within 4 h (Fig. 1A). Increasing concentrations of arsenic from 30 to 290 ppb slowed wound repair in a dose-dependent manner. To quantify wound repair, experiments were repeated in fully supplemented medium (Fig. 1B). In the absence of arsenic in the growth medium, the area of the wound significantly decreased each hour until 80.8 ± 2.5% (n = 41) of the wound area was filled in at 4 h post-wounding. Similar to results observed in Fig. 1A, increased arsenic concentration displayed a dose-dependent inhibition of wound repair (P = 0.0003 at 3 h; P = 0.0007 at 4 h). In the presence of 30 ppb arsenic, the amount of wound closure at 4 h (48.3 ± 12.5%; n = 12) was significantly less than in the control. In the presence of 60 ppb arsenic, the amount of wound closure was only 24.3 ± 14.9% (n = 27) of the original wound after 2 h, a value significantly less than in control experiments, in which 58.5 ± 2.8% of the wound was covered at this point. Compared with the amount of wound healing in the arsenic-free medium, a significantly larger wound area persisted at 3 and 4 h in cultures incubated with 60 or 290 ppb. The delayed wound response was most dramatic at the highest arsenic concentration tested. In the presence of 290 ppb arsenic, wound area expanded to 109.2 ± 5.0% (n = 15) of the original area within 1 h and showed only 16.5 ± 18.5% healing by 4 h. The observed inhibition of wound healing could be attributed to a reduction in cellular division, an increase in cellular death along the wound area, or a reduction in cellular spreading and migration. The short period of analysis and the slow duplication time of cells along the wound edge (data not shown) ruled out a prominent role for cellular division in these studies. To evaluate a role for cellular death, a live/dead assay was used to examine cells along the wound edge for up to 5 h following wounding (Fig. 2). At the time of wounding, a high number of dead cells were detected at the wound edge in the arsenic-free cultures (26.0 ± 4.2 cells/mm). This value was not significantly different from those in the 30 ppb (24.1 ± 2.2), 60 ppb (26.3 ± 3.6), or 290 ppb (24.6 ± 3.9)-treated cultures. The number of dead cells at the wound edge was reduced within 1 h of healing in the arsenic-free cultures (8.8 ± 1.3 cells/mm) as well as in the arsenic-treated cultures (11.5 ± 1.6 at 30 ppb; 12.5 ± 1.9 at 60 ppb; and 11.9 ± 1.6 at 290 ppb). Although these values were significantly different from those in the arsenic-matched cultures at the time of wounding, there were no significant differences among arsenic treatments at 1 h. At the 3- and 5-h time points, there were further reductions in the number of dead cells in all treatments, but no significant differences among the arsenic treatments at either of these...
times. In summary, the presence of arsenic for 24 h resulted in a significant reduction in the ability of human airway epithelial cells to repair scrape wounds in an in vitro model without an increase in cellular cytotoxicity; data suggested an arsenic-induced alteration in cellular migration.

MMP-9 mRNA expression, protein expression, and activity increase in human airway epithelial cells following exposure to arsenic. Because protease activity allows for degradation of extracellular matrix proteins and can contribute to cellular spreading and migration, we assayed whether arsenic exposure altered MMP-9 expression in 16HBE14o- cells. We used real-time RT-PCR to evaluate mRNA expression in confluent 16HBE14o- cells exposed to low-dose arsenic (Fig. 3). At 5 days of exposure, cells remained confluent at all arsenic concentrations tested (not shown) and displayed a dose-dependent increase in MMP-9 mRNA (Fig. 3A: \( P = 0.0002 \)). Because the activity of MMP-9 is also dependent on inhibitors produced by the cell, we also evaluated the mRNA expression of TIMP-1.
the major inhibitor of MMP-9. Unlike the MMP-9 expression, TIMP-1 mRNA expression was relatively stable at 60 ppb but displayed a sharp decrease after exposure to 290 ppb arsenic (Fig. 3B). Together, these changes in mRNA expression resulted in an effectively large increase in the MMP-9/TIMP-1 mRNA expression ratio in human airway epithelial cells in response to arsenic.

To better relate MMP-9/TIMP-1 expression patterns to cellular physiological changes observed in Fig. 1, we used the multiple scrape wounding method to assay specific protein changes in MMP-9 in response to arsenic during wounding. Similar to the single scrape model, the multiple scrape wounding assay resulted in a dose-dependent arsenic inhibition of wound repair (Fig. 4; \( P < 0.0001 \)). We evaluated MMP-9 expression with immunocytochemistry of 16HBE14o- cells at the time of wound and continued throughout the monolayer repair process over 12 h (Fig. 5). At the time of wounding (i.e., after 24-h exposure to arsenic), a high percentage of cells exposed to 290 ppb arsenic displayed MMP-9 reactivity, whereas the 0- and 60-ppb-treated cells showed minimal staining. At 6 and 12 h following the wound, a pattern of MMP-9 reactivity in response to increasing arsenic concentrations developed, with highest expression near the wound sites associated with highest arsenic exposure. Although these results are not quantifiable, they suggest an increase in intracellular MMP-9 expression in response to acute arsenic exposure.

Because MMP-9 effects on cell migration are largely extracellular, we evaluated the effects of arsenic on MMP-9 protein release into the medium with immunoblots of conditioned media from 24-h pre-wound exposure (data not shown) and 12-h post-wound exposure to arsenic (Fig. 6). MMP-9 protein expression was increased almost 4-fold in conditioned medium that contained 60 ppb and more than 15-fold in conditioned medium that contained 290 ppb arsenic when compared with arsenic-free conditioned medium. Unlike the dose-dependent increase observed in the MMP-9 results (\( P = 0.007 \)), immunoblots of conditioned media using TIMP-1 antibodies showed a similar expression of TIMP-1 protein concentration in 0, 60, or 290 ppb arsenic-supplemented conditioned media. These data are consistent with a dose-dependent, arsenic-induced increase in MMP-9/TIMP-1 protein ratio and a subsequent increase in extracellular MMP-9 activity.

Although protein evaluation of MMP-9 and TIMP-1 suggested increased MMP-9 function, MMP-9 protein is secreted from cells as a pro-enzyme before it is cleaved into an active form. To ensure that the detected MMP-9 protein changes represented an increase in the functional enzyme, we used gelatin zymography to directly evaluate MMP-9 activity in the conditioned media from above (Fig. 7). Conditioned media collected from 16HBE14o- cultures after 48-h exposure to arsenic and just before wounding showed a dose-dependent increase in MMP-9 activity (\( P < 0.0001 \); Fig. 7A). A slight increase in MMP-9 activity in conditioned media supplemented with 60 ppb arsenic and a significant increase in conditioned media supplemented with 290 ppb arsenic were also evident in the conditioned media collected after 12 h of wound repair, although collection at this time point was near the limit of detection (Fig. 7B). Unlike the activity of MMP-9, the activity of MMP-2 was stable across arsenic concentrations before and in response to wounding (data not shown).

To determine whether the observed increased MMP-9 activity in arsenic-treated 16HBE14o- cultures directly contributed to alteration of wound repair, we assayed wound healing response to multiple scrape wounded cultures in the presence of the MMP inhibitor GM6001. Individual monolayer cultures
were treated for 24 h with growth medium supplemented with 290 ppb arsenic and either 10 μM GM6001 in DMSO or DMSO as a control. Monolayers were then subjected to multiple scrape wounds and provided fresh growth medium. As in Fig. 4, individual cultures were stained with crystal violet to evaluate the amount of wounding and wound repair at the time of wound (0 h) and following 4 or 12 h of recovery at 37°C (Fig. 8). Because the addition of DMSO alone reduced MMP-9 expression and activity (not shown), only the 290-ppb arsenic concentration was tested. By 4 h of recovery, the 290-ppb-treated cultures underwent a wound expansion (∼39.2 ± 19.1% wound repair) that was partially repaired in the GM6001-treated samples (1.8 ± 5.9%). By 12 h of recovery, the DMSO controls subjected to 290 ppb arsenic had largely closed the wound expansion, but still had not progressed past the initial wound area (∼8.1 ± 9.3%). In contrast, the GM6001-treated samples had repaired a significant portion of the original wound (31.3 ± 11.9%). The inhibition of MMP-9 was sufficient to significantly improve wound repair in the arsenic treatments tested in this assay.

DISCUSSION

It has long been accepted that high exposure to arsenic through ingestion can lead to tissue damage and cancer, including that of the lung. We have recently begun to evaluate the effects of chronic, low-dose arsenic (i.e., <100 ppb) exposure on lung tissue using protein and mRNA analyses from mouse models (26, 27) and sputum analyses from human populations (23, 27). Combined findings from these studies suggested that ingestion of arsenic may alter wound response and specifically, MMP-9/TIMP-1 ratios in the lung (23, 26, 27). In this report, we used acute arsenic exposure with an in vitro human cell model in an attempt to directly examine potential cellular mechanisms of arsenic exposure in the lung epithelium. We found that arsenic (30–290 ppb) inhibited reformation of the epithelial monolayer following scrape wounds of monolayer cultures. This alteration in wound repair was associated with increases in activity and expression of MMP-9 without increases of TIMP-1 protein expression. Furthermore, inhibition of MMP-9 resulted in improved epithelial cell wound repair response when cells were exposed to 290 ppb arsenic. Our results are consistent with the hypothesis that arsenic alters the airway epithelial barrier by restricting proper wound repair in part through the increased MMP-9/TIMP-1 ratios derived from lung epithelial cells.

We used 16HBE14o- cells to model the airway epithelium in part because they are one of the few human conducting airway cell lines capable of forming functional barriers (16, 17, 45). Within this study, we focused on the contribution of cell migration to the reestablishment of airway epithelial confluency in cell cultures using wound repair assays that limit the contributions of cell proliferation and were similar to those with 16HBE14o- in previous reports (20, 30, 34, 36, 49). Independent of the paradigm that included a single scrape wound or multiple scrape wounds, we found that arsenic significantly inhibited wound repair in a dose-dependent manner, and this inhibition could not be attributed to cytotoxicity. These previous studies with 16HBE14o- cells, in addition to other bronchial epithelial cell line or primary cultured cells (10, 20, 34–36), have established prominent roles for epidermal growth factor receptors and TGF-β in airway epithelial repair that may be altered in asthmatic-derived tissue and additionally have highlighted contributions of small GTPases, growth factors, and growth factor receptors to cellular migration, but have not implicated roles for proteases or evaluated the toxic effects of arsenic that lead to altered wound healing.

MMP-9 expression has been associated with airway epithelial wound repair in primary cultured cells and in vivo (6, 13, 29). Similar to results reported in primary cultured human respiratory epithelial cells (HRECs) (6) and human bronchial epithelial cells (HBECs) (29), MMP-9 immunoreactivity of 16HBE14o- cells reported herein (Fig. 5) was shown to be highest in cells at or near the wound edge during in vitro wound repair. To show that MMP-9 was important in cell migration during respiratory wound repair, migration of HRECs could be blocked by antibodies that neutralized MMP-9, and MMP-9 expression in HBECs directly coincided with the speed of migration. In a humanized xenograft model, MMP-9 is expressed throughout airway epithelial wound healing and cell differentiation, and blocking of this enzyme resulted in a dysregulated repair (13). Our results on 16HBE14o- cells in arsenic-free medium are consistent with findings from these studies, as MMP-9 is normally upregulated after wounding and is closely associated with cells near the wound edge. In contrast with these reports, we were able to observe dysregulated wound repair when MMP-9 is overexpressed in airway epithelial cells, i.e., in the presence of arsenic. Furthermore, we were able to test the effects of neutralizing MMP-9 overexpression in an effort to restore normal MMP-9 function and repair. The upregulation of MMP-9 activity and protein ex-
pression in 16HBE14o- cells contrasts with zymograph analysis of MMP-2, which stays at a low level of activity in arsenic-free cells or those treated with arsenic (data not shown). At this time, multiple scrape wounds were applied to the culture, and conditioned medium collected at 12 h postwounding. The conditioned media were evaluated for MMP-9 and TIMP-1 protein via immunoblot. A: increased arsenic levels resulted in a 3.8-fold increase of MMP-9 protein expression in the 60-ppb samples and a 16.8-fold increase in protein expression in the 290-ppb samples. B: arsenic levels did not affect the protein levels of TIMP-1 in the conditioned medium. Together, the MMP-9/TIMP-1 ratio in the area surrounding the lung epithelial cells was increased as arsenic concentration was increased in the medium. Such an increase is consistent with an increase in MMP-9 activity. Data were graphed ± SE; *significant difference in protein expression compared with 0-ppb samples; †significant difference in protein expression compared with 60 ppb samples (P < 0.05). As, arsenic.

Fig. 6. Arsenic exposure increased MMP-9 protein expression. Monolayers of 16HBE14o- cells were grown to confluence and treated for 24 h with arsenic-free medium (0 ppb) or arsenic-supplemented media (60 or 290 ppb). At this time, multiple scrape wounds were applied to the culture, and conditioned medium collected at 12 h postwounding. The conditioned media were evaluated for MMP-9 and TIMP-1 protein via immunoblot. A: increased arsenic levels resulted in a 3.8-fold increase of MMP-9 protein expression in the 60-ppb samples and a 16.8-fold increase in protein expression in the 290-ppb samples. B: arsenic levels did not affect the protein levels of TIMP-1 in the conditioned medium. Together, the MMP-9/TIMP-1 ratio in the area surrounding the lung epithelial cells was increased as arsenic concentration was increased in the medium. Such an increase is consistent with an increase in MMP-9 activity. Data were graphed ± SE; *significant difference in protein expression compared with 0-ppb samples; †significant difference in protein expression compared with 60 ppb samples (P < 0.05). As, arsenic.

Fig. 7. Arsenic exposure increases MMP-9 activity in lung epithelial cells. Confluent monolayers of 16HBE14o- cells were grown and treated for 48 h with arsenic-free medium (0 ppb) or arsenic-supplemented media (60 or 290 ppb). A: representative gelatin zymograms of conditioned media from 0, 60, and 290 ppb and corresponding densitometry are shown. An arsenic-dependent dose-response of increased MMP-9 activity (light band at 92 kDa) was observed (P < 0.0001). B: representative gelatin zymograms of conditioned media from 16HBE14o- cells after multiple scrape wounding and 12-h recovery are shown. A slight increase in the 60-ppb sample and a significant increase in the 290-ppb sample were detected. Data are graphed ± SE; *significant difference in expression from 0-ppb samples; †significant difference in expression from 0-ppb samples (P < 0.05).
As2O3 was shown to reduce MMP-9 expression, as measured by semiquantitative RT-PCR and ELISA (53). As in the previous report, the ovarian carcinoma cells displayed a reduced migration in a dose-dependent response to As2O3. As2O3 has also been used to prevent radiation-enhanced tumor invasions in cervical cancer cells (CaSki) (46). In addition to the obvious differences in cellular migration, machinery regulation among these carcinogenic cell lines, and ones used in our studies, the specific interaction of arsenic within the cell is influenced by the form of arsenic. Differences in cellular toxicity of trivalent and pentavalent inorganic and methylated arsenicals are well documented in both rat and human cells, including normal human bronchial epithelial cells (42). Although there are few direct comparison studies between cellular effects of arsenite and As2O3, it has been shown that low doses of these two arsenicals have differing effects on radiation-induced apoptosis in a human lymphoblastoid cell line (19). Thus, it is not unreasonable to believe that the chemistry or biochemistry of these compounds can lead to different cellular physiology.

Coordination of cellular migration involves an array of cellular processes that can be affected by arsenic on a variety of levels. Which process is altered by arsenic is dependent on time and concentration of arsenic exposure, as well as on the form of arsenic that is presented to the cell and the form that can be converted by the cell. We have concentrated on exposures in the range of 0 to 290 ppb to model potential damage that can result from concentrations similar to those that are encountered in chronic environmental exposures such as in drinking water. It is important to note that in January, 2006, the Environmental Protection Agency reduced the allowable arsenic level in drinking water for municipalities in the United States from 50 ppb down to 10 ppb. Yet, even at these low doses, we and others have shown distinct changes in mouse lung protein and mRNA expression (3, 26, 27). We have shown that mice chronically exposed to 10–50 ppb arsenic in drinking water increase mRNA for MMP-9 and a battery of proteins consistent with alterations in cell migration (26), as well as for altered bronchiolar lavage proteins (27) that included a biomarker for reduced wound healing [receptor for advanced glycation end products (RAGE)]. We have additionally reported that low-dose arsenic exposure (∼20 ppb) in drinking water results in changes in specific wound repair proteins (e.g., RAGE and the MMP-9/TIMP-1 ratio) recovered from human sputum (23, 27). The work reported herein helps clarify initial cellular changes in human lung epithelial cells that, albeit on a more acute and at slightly higher concentrations than reported in the animal and human studies, may contribute to low-dose arsenic effects in the lung. Further work to examine specific pathways initiated by arsenic species and how they contribute to MMP-9 upregulation and inhibition of cell migration should allow insight into how arsenic contributes to compromise of lung function.

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ARSENIC INHIBITS AIRWAY EPITHELIAL REPAIR

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