Arsenic Exposure Accelerates Atherogenesis in Apolipoprotein E$^{-/-}$ Mice

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Epidemiologic studies have shown an association between elevated arsenic levels in drinking water and an increased risk of atherosclerosis and vascular diseases. The studies presented here were performed to evaluate the atherogenic potential of arsenic using a well-established and controlled animal model of human atherosclerosis, mice deficient in apolipoprotein E (ApoE), and *in vitro* systems including primary human vascular cells. Wild-type and ApoE-deficient mice were exposed to 20 or 100 μg/mL sodium arsenite in drinking water for 24 weeks. As assessed morphometrically, the size of grossly discernible lesions covering the intimal area of aorta were increased significantly in arsenic-treated ApoE-deficient mice compared with nontreated transgenic mice. This effect was not associated with increased levels of serum cholesterol but was accompanied by an accumulation of arsenic in the vessel wall. Introduction of cocoa butter into the diet for 2 weeks resulted in higher serum cholesterol levels and only slight increases in the lesion size in control or arsenic-exposed ApoE-deficient mice. There were no lesions observed in the wild-type C57BL6 mice, resistant to atherosclerosis, whether they received arsenic or control drinking water. *In vitro* studies, including primary aorta endothelial or smooth muscle cells, were conducted to evaluate whether arsenic induces cellular mechanisms relevant to atherogenesis such as endothelial dysfunction, lipid oxidation, and smooth muscle cell proliferation. Arsenic treatment does not modulate endothelial cell–mediated lipid oxidation or smooth muscle cell proliferation but induced the expression of genes coding inflammatory mediators, including interleukin-8. Induction of endothelial inflammatory activity may play a role in arsenic-related vascular effects. *Key words:* arsenic toxicity, cardiovascular toxicity, environmental factors, vascular diseases. *Environ Health Perspect* 111:1744–1748 (2003). doi:10.1289/ehp.6332 available via *http://dx.doi.org/* [Online 19 August 2003]

Trivalent and pentavalent forms of inorganic arsenic are ubiquitous elements found in nature that result in significant human exposure. Dermal or pulmonary exposure to arsenic can also occur occupationally in the mining/smelting, agriculture, or microelectronics industries (Enterline and Marsh 1982; Wall 1980). Although historically arsenic is considered a potent human hazard because of its neoplastic outcomes, increasing epidemiologic evidence indicates a link between arsenic exposure and risk to vascular diseases related to atherosclerosis. Increased mortality from ischemic heart disease was first reported in copper smelter workers exposed to arsenic (Axelson et al. 1978; Lee and Fraumeni 1969; Welch et al. 1982). Further, exposure to arsenic in artesian well water has been associated with blackfoot disease as well as increased mortality from cardiovascular diseases (Chen et al. 1996, 1988; Wu et al. 1989). Blackfoot disease clinically and pathologically resembles severe peripheral arteriosclerosis, such as Burger's disease (Tseng 1989). Recently, a dose–response relationship between carotid atherosclerosis and long-term exposure to arsenic in drinking water has been reported in a population with blackfoot disease, with researchers using ultrasonographic evaluation of the superficial carotid artery (Wang et al. 2002). Arsenic-related ischemic heart diseases in humans have not been related to atherogenic serum lipid profiles (Hsueh et al. 1998), and the underlying pathophysiologic mechanisms are still not established.

Progress in understanding risk factors leading to atherosclerosis has resulted from the development of specific genetic mouse models that make the mouse—which normally is very resistant to atherosclerosis—highly susceptible to this disease. One of these models is deficiency of the gene coding apolipoprotein E (ApoE), the primary ligand for removal of plasma lipoproteins from the blood. ApoE deficiency leads to severe atheroma formation remarkably similar in anatomical predilection and histopathologic features to human atherosclerosis (Nakashima et al. 1994; Palinski et al. 1994). Although different risk factors may modulate atherogenesis, a series of critical events including endothelial dysfunction/activation, oxidation of lipids, and smooth-muscle cell proliferation are currently considered major pathogenic mechanisms required for atheroma formation and acceleration (Ross 1999). In this study, we determined whether arsenic exposure influences the progression of atherosclerotic plaque formation in ApoE$^{-/-}$ mice. Further, the effects of arsenic on molecular events relevant to the major pathogenic mechanisms of atherosclerosis were evaluated in human vascular cell culture systems.

Materials and Methods

Experimental animals. Female B6.129P2-ApoE mice and female C57BL6 mice were obtained from Jackson Laboratory (Bar Harbor, ME). All animals were housed in polycarbonate cages containing hardwood chip bedding at room temperature (21 ± 2°C) on a 12-hr light/dark cycle and were provided chow and water *ad libitum.* Groups of 4-week-old mice were provided 20 or 100 μg/mL sodium arsenite (Sigma Chemical Co., St Louis, MO) in their drinking water for up to 24 weeks. The mice were fed under two regimens: regimen 1, a commercial mouse chow diet (LM-485; Harlan, Indianapolis, IN); or regimen 2, regular chow followed by a cocoa butter diet for 2 weeks (15.8% fat; TD 88051; Harlan).

Preparation of mouse aortas and quantification of atherosclerosis. After blood collection, mice were sacrificed by CO_2 asphyxiation, and the aortic tree was perfused for 10 min with ice-cold phosphate-buffered saline containing 20 mM butylated hydroxytoluene (BHT) and 2 mM ethylenediaminetetraacetic acid (EDTA) (pH 7.4) by inserting a cannula into the left ventricle and allowing free efflux from an incision in the vena cava. To allow for fixation, perfusion was continued for 10 min with formal–sucrose (4% paraformaldehyde, 5% sucrose, 20 mM BHT, and 2 mM EDTA, pH 7.4). The aorta and its main branches were dissected from the aortic valve to the iliac bifurcation. After removal of the surrounding adventitial tissue, the aorta was opened longitudinally and stained with Sudan IV. The extent of atherosclerosis was determined by using the *en face* method, as described previously (Nakashima et al. 1994; Palinski et al. 1994). Quantitative analysis of the lesion size was performed by first capturing aorta images with a Sony DXC-960MD three-chip charge-coupled device color video camera (Sony, Tokyo, Japan) connected to an Olympus SZX12 dissection microscope (Olympus, Melville, NY) and then analyzing the data with Simple 32 software (Imaging Systems, Cranberry Township, PA).

Arsenic determination in tissues. The aortic tree and heart from control or arsenic-treated mice were quick-frozen in acid-free

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vials and stored at –70°C. The tissue samples were digested by addition of 6 N HCl at 80°C for 16 hr in a specially designed reaction vessel. Analyses of arsenic tissue levels were performed by Battelle Marine Sciences Lab (Seqium, WA) using an atomic absorption method (Crecelius 1998). Quality control was established through calibration and testing of the hydride generation, purging, and detection systems.

Cell cultures. Cryopreserved, primary human aortic endothelial cells (HAECs) and aortic smooth muscle cells (AoSMC) were purchased from Clonetics Corp. (San Diego, CA) and grown in 25-cm^2 tissue culture flasks in endothelial cell-growth medium (EGM-2) or smooth-muscle growth medium (SmGM-2), respectively, supplied by Clonetics Corp. The cells were grown at 37°C in a humidified, 95% air/5% CO_2 atmosphere.

Nuclear extracts and electrophoresis mobility shift assay. Nuclear proteins were prepared from aliquots of 1×10^7 cells according to the method of Schreiber et al. (1989). DNA binding reactions and electrophoresis mobility shift assays (EMSAs) were performed as described previously (Simeonova et al. 1997). Briefly, the 5′ ends of the double-stranded oligonucleotides were labeled with γ-^{32}P-ATP (New England Nuclear/Dupont, Boston, MA), using 6–10 U of T4 polynucleotide kinase (USB/Amersham, Cleveland, OH). Binding reactions (30 µL) were performed on ice for 30 min in reaction mixtures containing 10 µg nuclear proteins, 20 mM Tris-HCl (pH 7.8), 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 5 mM dithiothreitol, 50 µg/mL bovine serum albumin, 2 µg poly(dI-dC)·poly(dI-dC), 10% glycerol, and approximately 0.1 ng (2×10^5 cpm) specified probe. For detection of activating protein (AP)-1 DNA-binding activity, an oligonucleotide was obtained from Santa Cruz (Santa Cruz, CA) containing a consensus sequence of AP-1 (5′-CGC TTG ATG ACT CAG CCG GAA-3′) or nuclear factor (NF)-κB (5′-AGT TGA GGG GAC TTT CCC AGG C-3′). Protein-DNA complexes were separated on a 5% nondenaturing polyacrylamide gel. Gels were electrophoresed at 125 V in 50 mM Tris–50 mM boric acid/1 mM EDTA, dried, and autoradiographed overnight. The autoradiograms were scanned with a computerized laser densitometer (Eagle Eye II Image Analysis System, Stratagene, La Jolla, CA). These results were examined using One Dscan gel analysis software (Scanalytics, Fairfax, VA). For characterization of DNA binding activity, nuclear protein extracts were preincubated for 1 hr before adding labeled probe, with either excess unlabeled oligomers or 2 µg/mL antibodies to different subunits of the AP-1 or NF-κB family (Santa Cruz Biotechnology, Santa Cruz, CA).

Gene expression studies. RNA from cell cultures was extracted using the Rneasy total RNA kit (Qiagen, Santa Clarita, CA). RNA purity and concentration were assessed by determining A_{260}/A_{280} absorption. cDNA was synthesized from 1 µg RNA. Conventional reverse transcriptase (RT)-PCR amplification was performed as previously described (Simeonova et al. 1997), using PCR primers for glyceraldehyde 3-phosphate dehydrogenase (G3PDH) and interleukin 8 (IL-8) (Clontech Laboratories Inc., Palo Alto, CA). The PCR products were visualized by ultraviolet illumination after electrophoresis through 2.0% agarose (Ultra-Pure, Sigma) at 60 V for 80 min and staining in Tris borate/EDTA buffer (89 mmol/L Tris, 89 mmol/L boric acid, 2.5 mmol/L EDTA, pH 8.2) containing 0.5 mg/mL ethidium bromide. Real-time PCR for IL-8 and 29S/rRNA were performed using predeveloped primers and probes (TaqMan assay reagents; PE Applied Biosystems, Foster City, CA) on an ABI Prism 7700 Sequence detector (PE Applied Biosystems). This method involved obtaining C_T values for the transcript of interest, normalizing to the housekeeping gene 29S/rRNA and comparing the relative increases between control and experimental samples.

Low-density lipoprotein oxidation. We incubated 200 µg/mL sterile human plasma low-density lipoprotein (LDL; Calbiochem, La Jolla, CA) with HAECs for 3 or 24 hr in serum-free medium. Simultaneously with LDL exposure, the cells were treated with 20 µM $CuSO_4$, 250 µM H_2O_2, or 2–50 µM sodium arsenite. The oxidation state of LDL was assayed by determining thiobarbituric acid–reactive substances using a commercially available kit (LPO-586; R&D, Minneapolis, MN).

Cell mitogenesis. Human AoSMCs were seeded in 96-well, flat-bottomed culture plates at a concentration of 1×10^3 cells/well. The next day (~40% confluent) the culture media were replaced with basal media (without growth factors), and the cells were exposed to concentrations of sodium arsenite for 48–72 hr. During the last 4 hr of incubation, ^{3}H-thymidine (6.7 Ci/mmol; New England Nuclear/DuPont, Boston, MA) was added at a concentration of 0.5 µCi/well. The cells were detached from the plates by addition of 0.1% trypsin and collected onto glass-fiber filters using an automated cell harvester (Skatron, Sterling, VA). Cellular incorporation of ^{3}H-thymidine was determined by liquid scintillation counting.

Statistical analysis. All experiments were replicated, and representative findings are shown. Statistical significance was determined by one-way analysis of variance. When the *F*-value was significant, the means were compared using Fisher's post hoc analysis.

Results

To evaluate the effects of arsenic exposure on atherogenicity, female ApoE$^{-/-}$ or C57BL/6 wild-type mice were exposed to sodium arsenite in drinking water for 24 weeks and fed either regular chow (regimen 1) or a regular chow for 22 weeks followed by a cocoa butter (fat) diet for 2 weeks (regimen 2). Controls received water not containing arsenic. Arsenic exposure enhanced atherosclerotic lesion formation in ApoE$^{-/-}$ mice under both diet regimens, compared with nonarsenic control groups. Wild-type C57BL/6 animals exposed to 100 µg/mL arsenic for 24 weeks and fed diet regimen 2 did not develop atherosclerotic lesions (Figure 1E and F). Sudanophilic lipid–rich lesions in ApoE$^{-/-}$ mice were typically found in the vicinity of the aortic valve and the small curvature of the arch at the aortic root. Because among the individual segments of the same aorta, the smallest coefficient of variation occurred in the segment that contained the most extensive lesions (Palinski et al. 1994), the thoracic part of the aorta was used for quantitative assessment. Quantitative computer-assisted image analysis revealed a dose-dependent increase in the area of aortic lesions (Figure 2). For example, in mice provided diet regimen 2, the 20 or 100 µg/mL concentrations of sodium arsenite induced an average 1.6- fold and 2.3-fold increase ($p < 0.02$) in the lesion size, respectively, compared with controls under the same regimen. Introduction of the fat diet for 2 weeks (regimen 2) resulted in slight but not significant increases of the atherosclerotic lesion area in both control and experimental mice exposed to arsenic.

Body weight and serum cholesterol levels were not significantly different between arsenic and control animals administered a regular diet (Table 1). Substitution of the regular diet with a high-fat diet for 2 weeks (regimen 2) resulted in a marked increase in cholesterol levels in control and arsenic-treated mice but no significant differences in body weight. Exposure to 20 µg/mL sodium arsenite had no additional effect on cholesterol levels in mice receiving regimen 2, whereas exposure to 100 µg/mL sodium arsenite induced a slight but significant increase ($p < 0.02$) in the serum cholesterol levels compared with the control mice.

To evaluate whether atherosclerosis progression is associated with accumulation of arsenic in the cardiovascular tissue, atomic absorption analysis was conducted. ApoE$^{-/-}$ mice that received 20 or 100 µg/mL sodium arsenic for 24 weeks showed a dose-dependent increase in total arsenic tissue accumulation, representing a 5-fold and 8.5-fold increase, respectively. (Figure 3A). Arsenic tissue accumulation depended on the exposure duration because at 12 weeks significant accumulation

was observed only with the high concentrations of arsenic (100 μg/mL). ApoE$^{-/-}$ and C57BL/6 wild-type mice demonstrated similar arsenic tissue levels at least during the 12 weeks of arsenic exposure (Figure 3B).

To evaluate whether arsenic induces events in endothelial cells relevant to atherogenic potential, we used HAEC cultures. Because oxidative modifications of lipoproteins in artherial tissue are a widely accepted hypothesis of atherogenesis (Steinberg et al. 1989), and because arsenic can alter the cellular redox state [reviewed by Pi et al. (2002)], the effect of arsenic on LDL oxidation was examined. Incubation of endothelial cells with sodium arsenite (2–50 μM), in contrast to treatment with $CuSO_4$ or H_2O_2, did not modify LDL oxidation, as measured by melonaldehyde (MDA) formation (Figure 4A). Furthermore, $CuSO_4$, but not arsenic, increased H_2O_2-induced MDA formation indicating that under these experimental conditions arsenic does not effect the oxidation state of LDL. Neointimal monocyte infiltration is an important early step in atheroma formation, mediated in part by inflammatory mediators such as adhesion molecules and chemokines, including IL-8 [reviewed by Ross (1999)]. To evaluate whether arsenic modulates the expression of inflammatory mediators, RNA was prepared from arsenic-treated endothelial cells and examined by reverse transcriptase PCR (RT-PCR) for IL-8 gene expression (Figure 4B). We obsersved a dose-dependent increase in IL-8 mRNA transcripts at 2 and 5 hr after arsenic treatment. These results were confirmed and statistically analyzed by real-time RT-PCR (Figure 4C). IL-8 expression was significantly induced by arsenic without significant changes in the housekeeping gene (29S). EMSAs were conducted to determine whether the increased expression of IL-8 was associated with DNA binding activity of the major transcription factors responsible for their regulation, AP-1 and NF-κB. Endothelial cells exposed to 5 or 50 μM sodium arsenite showed a dose-dependent increase in AP-1 and NF-κB DNA binding activity (Figure 4C). Tumor necrosis factor (TNF)-α was used as a positive control.

In connection with the role of smooth muscle cell proliferation in atherosclerosis [reviewed by Ross (1999)], and cell-growth potential of arsenic demonstrated previously for human keratinocytes and uroepithelial cells (Germolec et al. 1998; Simeonova et al. 2000), the effect of arsenic on AoSMC proliferation was evaluated. Exposure to sodium arsenite for 72 hr did not induce an increase in thymidine uptake, whereas platelet-derived growth factor (PDGF), used as a positive control, induced a significant increase in the mitogenic activity of AoSMCs under the same conditions. The thymidine uptake (cpm) of cells untreated, treated with arsenic at 0.1, 0.5, and 2 μM, or treated with PDGF at 10 ng/mL, was 1,593 ± 115, 1,576 ± 76, 1,627 ± 156, 1,051 ± 129, and 4,182 ± 171, respectively.

Discussion

ApoE$^{-/-}$ mice have been used extensively to study the development of atherosclerotic lesions and to identify potential risk factors. The wide acceptance of this model is largely based on its ability to produce lesions that show similarities in distribution and histopathologic characteristics to those found in humans (Breslow 1996). Although the quantification of atherosclerosis has been the source of considerable debate, the *en face* method in mice has been well characterized and is generally accepted (Nakashima et al. 1994; Palinski et al. 1994). This model has

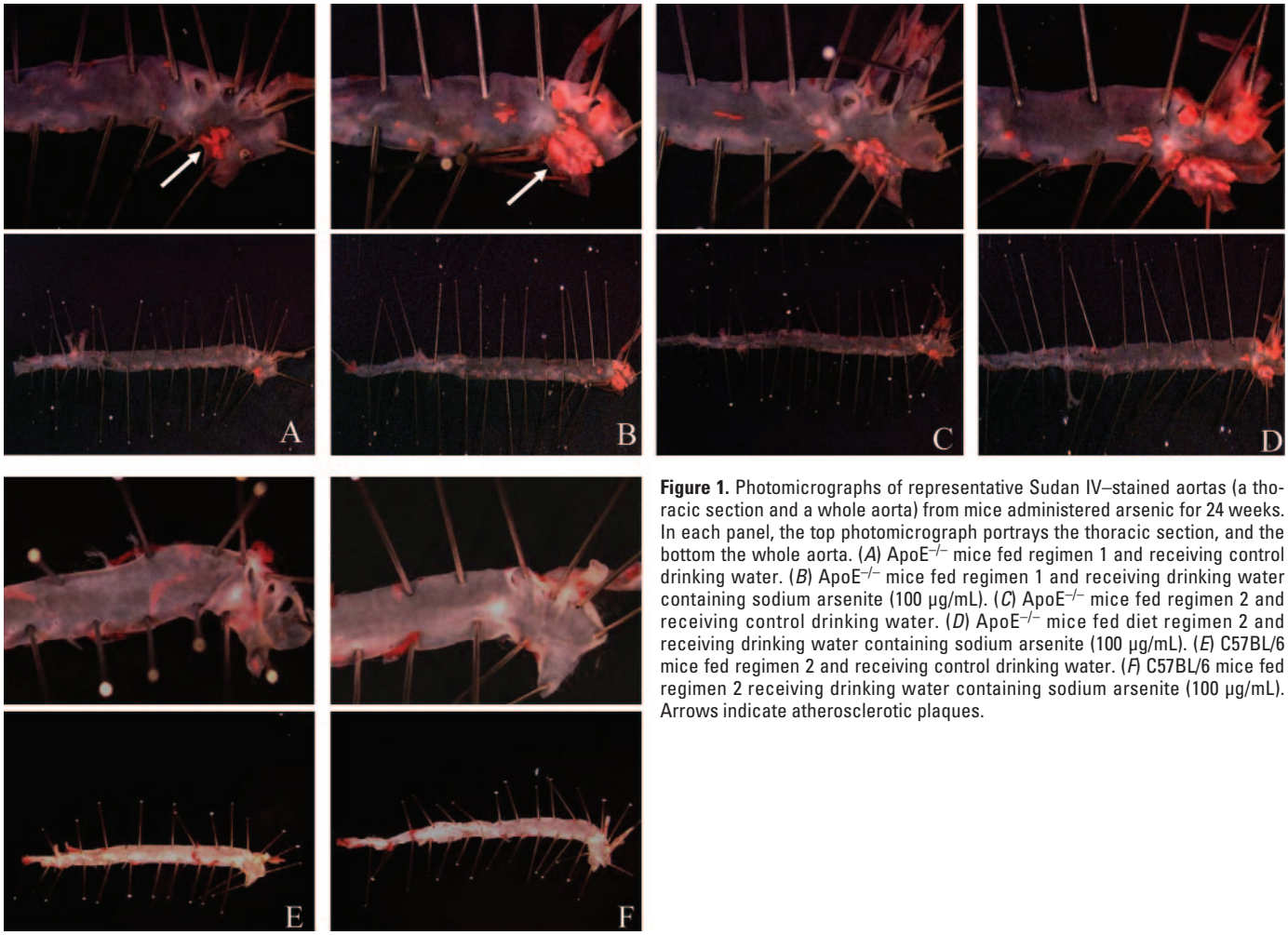

Figure 1. Photomicrographs of representative Sudan IV–stained aortas (a thoracic section and a whole aorta) from mice administered arsenic for 24 weeks. In each panel, the top photomicrograph portrays the thoracic section, and the bottom the whole aorta. (*A*) ApoE$^{-/-}$ mice fed regimen 1 and receiving control drinking water. (*B*) ApoE$^{-/-}$ mice fed regimen 1 and receiving drinking water containing sodium arsenite (100 μg/mL). (*C*) ApoE$^{-/-}$ mice fed regimen 2 and receiving control drinking water. (*D*) ApoE$^{-/-}$ mice fed diet regimen 2 and receiving drinking water containing sodium arsenite (100 μg/mL). (*E*) C57BL/6 mice fed regimen 2 and receiving control drinking water. (*F*) C57BL/6 mice fed regimen 2 receiving drinking water containing sodium arsenite (100 μg/mL). Arrows indicate atherosclerotic plaques.

been recently used to evaluate successfully the atherogenic risk of sidestream cigarette smoke and angiotensin II (Daugherty et al. 2000; Gairola et al. 2001; Weiss et al. 2001). Arsenic exposure, through drinking water, was found here to increase atheroma formation in ApoE$^{-/-}$ mice in parallel with increasing levels of arsenic in the vessel wall. Just as arsenic-related ischemic heart diseases in humans are not associated with serum lipid profiles (Hsueh et al. 1998), exposure of ApoE$^{-/-}$ mice to arsenic accelerated the atheroma formation without altering cholesterol levels, except when the higher dose of arsenic was combined with an atherogenic diet. Introduction of an atherogenic diet for 2 weeks induced severe hypercholesterolemia without a significant effect on the lesion size. If the atherogenic diet was continued for longer than 4 weeks, significant increases in cholesterol blood levels and lesion size were observed. However, the effect of arsenic on atherosclerotic lesion size, under these conditions, was diminished probably because it was masked by the diet effects (data not shown). The atherogenic effect of arsenic was not observed in wild-type mice, although they accumulate similar levels of arsenic in the cardiovascular tissue. Consistent with this paradigm, angiotensin II has a strong atherogenic potential in ApoE$^{-/-}$ mice but cannot trigger atheroma development in normal mice (Weiss et al. 2001). The wild-type mice are resistant to atherosclerosis, but atheroma formation can be triggered by a long-term (> 24 weeks) high-fat diet. Under these conditions, arsenic exposure could accelerate the atherogenesis in C57BL/6 mice. Similarly, it has been suggested that arsenic accumulates in skin or bladder tissue, major targets of arsenic carcinogenicity, but acts as a tumor promoter rather than a complete carcinogen (Germolec et al. 1998; Rossman et al. 2001).

The mechanisms whereby arsenic accelerates atherosclerosis are probably multifactorial. *In vitro* studies with endothelial cell cultures suggest that arsenic can cause direct endothelial dysfunction through oxidant formation. For example, Barchowsky et al. (1996, 1999) demonstrated that exposure of endothelial cells to arsenite induces NF-κB activation and DNA synthesis through reactive oxygen species, whereas Tsai et al. (2001) showed that arsenite decreases Fas ligand expression through oxidant formation. Other investigators have also demonstrated that arsenic disrupts cell proliferation in endothelial cell cultures (Chen et al. 1990). Bunderson et al. (2002) recently reported that arsenic increases peroxynitrite formation in bovine aortic endothelial cells as well as the inflammatory mediator cyclooxygenase-2. We observed that arsenic induces expression of genes coding for inflammatory mediators including IL-8 in HAECs. Atherogenesis requires local chemokine production for regulating migration and activation of leukocytes. Historically, IL-8 is a potent neutrophil chemoattractant (Terkeltaub et al. 1998) but participates in atherosclerosis as an endothelial growth factor (Koch et al. 1992) and a chemoattractant of smooth muscle cells (Yue et al. 1993), T cells (Lloyd et al. 1996), and monocytes (Gerszten et al. 1999). The expression of inflammatory mediators by arsenic likely occurs through activation of transcription factors, including NF-κB and AP-1. In HAECs, as well as

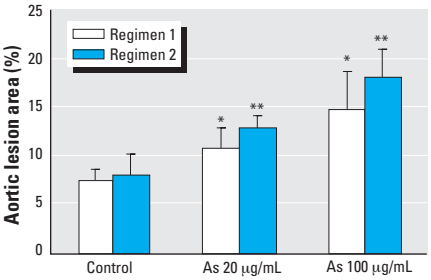

Figure 2. Percentage of aortic lesion areas in ApoE$^{-/-}$ mice fed regimen 1 or regimen 2 and receiving control drinking water or water containing sodium arsenite (As) at concentrations of 20 and 100 μg/mL. Each value represents the mean ± SE of 10 animals.
*$p < 0.02$; **$p < 0.01$.

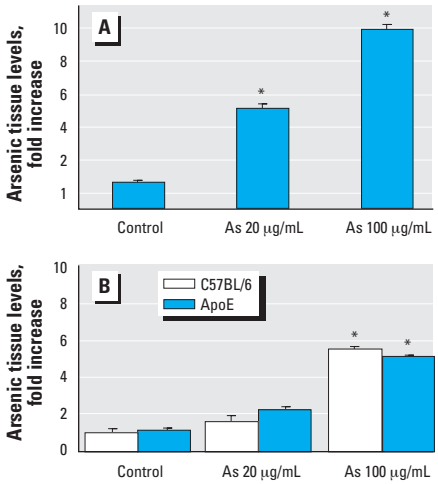

Figure 3. Arsenic levels in cardiovascular tissue. The data are presented as *n*-fold change from the control levels (ApoE 0.024 ± 0.002 and C57BL/6 0.03 ± 1.2 μg/g tissue). Each value represents the mean ± SE of four animals. (*A*) ApoE$^{-/-}$ mice fed regimen 1 receiving control drinking water or water containing 20 and 100 μg/mL sodium arsenite (As) for 24 weeks. (*B*) ApoE and C57BL/6 mice fed regimen 1 receiving control drinking water or water containing 20 and 100 μg/mL sodium arsenite (As) for 12 weeks.
*$p < 0.001$.

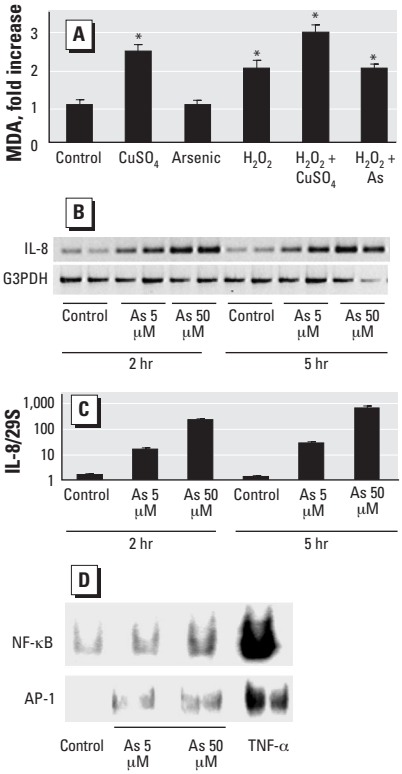

Figure 4. Evaluation of arsenic-induced cellular effects related to atherogenesis. (*A*) Effect of sodium arsenite on LDL oxidation in HAECs. Cells were cultured in serum-free medium containing 200 μg/mL LDL for 3 hr. $CuSO_4$ (20 μM), H_2O_2 (250 μM), or sodium arsenite (10 μM) was simultaneously added. The oxidation state of LDL was assayed by the determination of MDA formation and the results presented as an *n*-fold increase from the control (LDL). Each value represents the mean ± SE of four cultures. (*B*) RT-PCR analysis of IL-8 expression in HAECs treated for 2 or 5 hr with sodium arsenite. Cultures were performed in quadruplicate, and representative samples are shown. (*C*) Real-time PCR analysis of IL-8 gene expression normalized to the 29S/rRNA expression from corresponding samples. Each value represents the mean ± SE of four cultures. (*D*) NF-κB and AP-1 DNA binding activity in HAECs treated with sodium arsenite. Nuclear extracts from control or arsenic-treated cells (micromoles) were evaluated by EMSA using a ^{32}P-labeled double-stranded oligonucleotide probe. As a positive control, cells were treated for 1 hr with human recombinant TNF-α (100 ng/mL) and evaluated by EMSA.
*$p < 0.001$.

Table 1. Body weight and total plasma cholesterol under regimens 1 and 2.

			Arsenic	
	Regimen	Control	20 μg/mL	100 μg/mL
Body weight (g)	1	24.3 ± 2.0	26 ± 3.2	23.4 ± 2.4
	2	23.0 ± 2.3	23 ± 2.8	22.0 ± 2.2
Cholesterol (mg/dL)	1	634 ± 47	569 ± 45.4	533 ± 9.0
	2	3,225 ± 47	3,129 ± 84.3	4,071 ± 438 *

Each value represents the mean ± SE of 10 animals.
*$p < 0.01$.

keratinocytes and urinary bladder cells (Barchowsky et al. 1996; Burleson et al. 1996; Simeonova et al. 2000), arsenic is a potent activator of AP-1 and NF-κB DNA binding activity. Furthermore, AP-1 and NF-κB can regulate IL-8 expression, because the IL-8 gene contains multiple binding sites for these transcription factors (Ahmad et al. 1998), and both are subject to redox-dependent regulation (Flohe et al. 1997).

The affinity of inorganic arsenic for vicinal sulfhydryl groups is thought to be responsible for arsenic accumulation in tissues rich in sulfhydryl-containing molecules (Lindgren et al. 1982; Yamauchi and Yamamura 1983). We demonstrated that arsenic accumulates in the cardiovascular tissue, and this could be a prerequisite for endothelial dysfunction. Consistently, tissue accumulation through sulfhydryl interactions has been suggested to be involved in arsenic-mediated skin and bladder toxicity [reviewed by Simeonova and Luster (2000, 2002)]. Alternatively, it is possible that arsenic induces endothelial dysfunction by indirect mechanisms. In this respect, arsenic has been shown to modulate the coagulation status by enhancing the aggregation activities of platelets (Lee et al. 2002). Another factor responsible for atherogenicity is oxidative modifications of LDL (Steinberg et al. 1989). Free transition metal ions, such as copper, have been shown, although only *in vitro*, to stimulate the lipoprotein oxidation by endothelial cells (Witztum and Steinberg 1991). Although arsenic is not a transition metal, it has been demonstrated to have oxidative potential, including induction of reactive oxygen species in porcine aortic endothelial cells (Barchowsky et al. 1999). However, we were unable to demonstrate that arsenic, in contrast to copper ion or hydrogen peroxide, modulates the oxidation of LDL in aortic endothelial cells.

In conclusion, exposure to arsenic in drinking water accelerates the atherosclerosis formation in ApoE$^{-/-}$ mice, and this effect is accompanied with arsenic accumulation in the vessel wall. Although endothelial cell activation is a potential mechanism in arsenic-related vascular effects, the precise mechanisms, especially the role of oxidative stress, need to be further evaluated.

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