Arsenic-Induced Dysfunction in Relaxation of Blood Vessels

Moo-Yeol Lee,[1] Byung-In Jung,[1] Seung-Min Chung,[1] Ok-Nam Bae,[1] Joo-Young Lee,[1] Jung-Duck Park,[2] Ji-Sun Yang,[3] Hyomin Lee,[3] and Jin-Ho Chung[1]

[1]College of Pharmacy, Seoul National University, Seoul, Korea; [2]College of Medicine, Chung-Ang University, Seoul, Korea; [3]National Institute of Toxicological Research, Seoul, Korea

Several epidemiological studies have suggested that exposure to arsenic is strongly correlated with the development of cardiovascular diseases such as hypertension. To determine whether arsenic affects vasomotor tone in blood vessels, we investigated the effect of arsenic on vasorelaxation using isolated rat aortic rings in an organ-bath system. Treatment with arsenite inhibited acetylcholine-induced relaxation of the aortic rings in a concentration-dependent manner, whereas several other arsenic species did not have any effect. Consistent with these findings, the levels of guanosine 3′,5′-cyclic monophosphate (cGMP) in the aortic rings were significantly reduced by arsenite treatment. In cultured human aortic endothelial cells, treatment with arsenite resulted in a concentration-dependent inhibition of endothelial nitric oxide synthase (eNOS). In addition, higher concentrations of arsenite decreased the relaxation induced by sodium nitroprusside (an NO donor) and 8-Br-cGMP (a cGMP analog) in aortic rings without endothelium. These *in vitro* results indicate that arsenite is capable of suppressing relaxation in blood vessels by inhibiting eNOS activity in endothelial cells and by impairing the relaxation machinery in smooth muscle cells. *In vivo* studies revealed that the reduction of blood pressure by acetylcholine infusion was significantly suppressed after arsenite was administered intravenously to rats. These data suggest that an impairment of vasomotor tone due to arsenite exposure may be a contributing factor in the development of cardiovascular disease. *Key words:* **arsenic, arsenite, blood vessels, cardiovascular disease, endothelial nitric oxide synthase, nitric oxide, vasorelaxation.** *Environ Health Perspect* **111:513–517 (2003). doi:10.1289/ehp.5916 available via *http://dx.doi.org/* [Online 16 December 2002]**

Arsenic is a ubiquitous element found in several forms in foods and environmental media, such as soil, air, and water. The primary route of human exposure is through ingestion of arsenic-contaminated foods and drinking water. Although foods contain substantial levels of arsenic, it is primarily in organic form and of relatively low toxicity compared to the inorganic forms (NRC 1999). In contrast, the predominant form in drinking water is inorganic arsenic, which is both highly toxic and readily bioavailable. Chronic ingestion of arsenic-contaminated drinking water is therefore considered the major pathway behind the risk to human health (Bagla and Kaiser 1996).

In humans, chronic arsenic exposure has been associated with diverse health effects including cancer, hyperkeratosis, diabetes, and cardiovascular disease (Bates et al. 1992; Col et al. 1999; Engel et al. 1994; Tseng et al. 2000). Cardiovascular effects associated with high levels of arsenic in drinking water include atherosclerosis, hypertension, cerebrovascular diseases, ischemic heart disease, and peripheral vascular disorders such as blackfoot disease (resulting from gangrene caused by obstruction of peripheral blood vessels) (Chen et al. 1988; Chiou et al. 1997; Rahman et al. 1999; Tseng 1977; Wang et al. 2002). In a previous study we demonstrated that arsenic increased the susceptibility of platelets to aggregate, resulting in enhanced risk of arterial thrombosis, which could be a causal factor in the development of cardiovascular disease (Lee et al. 2002). However, the diversity of cardiovascular diseases arising from chronic arsenic exposure also raises the possibility that arsenic may alter the normal functioning of blood vessels, which are involved in various cardiovascular disorders.

Blood vessels maintain a balanced vasomotor tone mediated through biochemical signaling between endothelial cells and smooth muscle cells (Moncada et al. 1991). Endothelial cells can produce nitric oxide (NO) by endothelial nitric oxide synthase (eNOS), which causes vascular relaxation through guanosine 3′,5′-cyclic monophosphate (cGMP) synthesis by the activation of guanylate cyclase within the smooth muscle cells (Ignarro 1989). Impairment of these cellular functions disrupts vascular homeostasis, leading to excessive vasoconstriction, which could ultimately contribute to various vascular diseases (Bell et al. 1998). In fact, altered vasomotor tone can lead to acute vasospasm, microcirculatory ischemia, and increased systemic blood pressure (Alexander 1995; Luscher et al. 1993; Sellke et al. 1997).

Previous studies have implied that arsenic can alter normal vasomotor function. Carmignani et al. (1985) reported that chronic administration of arsenite to rats and rabbits caused significant increase in peripheral vascular resistance, which suggests that arsenite may induce impaired vascular function. Pi et al. (2000) showed that the concentration of nitrite/nitrate in the blood, which is indicative of endogenous NO levels, was significantly lower in an arsenic-exposed population than in the normal population. These studies imply that arsenic might disrupt normal vascular function. Therefore, in the present study we investigated the effects of arsenite on relaxation of blood vessels by using isolated aortic rings in an organ-bath system in an effort to provide new insight into arsenic-induced vascular dysfunction.

Materials and Methods

Materials. The following chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA): sodium arsenite (As^{3+}), sodium arsenate (As^{5+}), dimethylarsinic acid (DMA), acetylcholine, phenylephrine, sodium nitroprusside (SNP), 8-Br-cGMP, and Dowex AG50W-X8 (100–200 mesh). Monomethylarsonic acid (MMA) was obtained from Chem Service (West Chester, PA, USA), and a cGMP radioimmunoassay kit was obtained from Amersham (Buckinghamshire, UK). Human aortic endothelial cells (HAEC) and the endothelial cell growth media (EGM) kit were purchased from Clonetics Corporation (Walkersville, MD, USA). Minimum essential media (MEM) was supplied by Life Technologies (Rockville, MD, USA), and all other reagents used were of the highest purity available.

Animals. We used male Sprague-Dawley rats (Dae Han BioLink, Chungbuk, Korea) weighing 300–400 g in all experiments. Before the experiments, the animals were acclimated for 1 week in a laboratory animal facility maintained at constant temperature and humidity with a 12-hr light/dark cycle. Food and water were provided *ad libitum*.

Preparation of blood vessels in organ bath. Rats were killed by decapitation and then exsanguinated. We carefully isolated the thoracic aorta and cut it into ring segments. Aortic rings without endothelium were prepared by gently rubbing the intimal surface of the aortic rings with a wooden stick. The aortic rings were treated with As^{3+} or the vehicle (saline) in

Address correspondence to J-H. Chung, College of Pharmacy, Seoul National University, Shinrim-dong San 56–1, Kwanak-Gu, Seoul 151–742, Korea. Telephone: 82 2 889 7856. Fax: 82 2 885 4157. E-mail: jhc302@plaza.snu.ac.kr

We thank J.Y. Han and S.M. Lee for technical assistance and K.T. Kang and S.J. Lee for valuable comments, help, and discussions.

This work was supported by Eco-Technopia 21 project of the Ministry of Environment and by the National Toxicology Program of National Institute of Toxicological Research in Seoul, Korea.

Received 29 July 2002; accepted 16 December 2002.

MEM with 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in a 95% air/5% CO_2 incubator for 14 hr. The rings were then mounted in four-channel organ baths filled with Krebs-Ringer solution (pH 7.4): 115.5 mM NaCl, 4.6 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25.0 mM $NaHCO_3$, and 11.1 mM glucose. The organ baths were continuously gassed with 95% O_2/5% CO_2 and maintained at 37°C. The rings were stretched gradually to an optimal resting tension of 2 g and equilibrated for 30 min. We measured the change in tension isometrically with Grass FT03 force transducers (Grass Instrument Co., Quincy, MA, USA) and recorded the change using the AcqKnowledge III computer program (BIOPAC Systems Inc., Goleta, CA, USA).

To investigate the effect of As^{3+} on endothelium-dependent relaxation, acetylcholine was used as an agonist. The aortic rings were precontracted submaximally by adding phenylephrine, and acetylcholine was then cumulatively added to the organ bath to obtain concentration-relaxation curves. To examine the effects of As^{3+} on the NO donor-induced relaxation and on direct cGMP-dependent relaxation, sodium nitroprusside was used as the NO donor and 8-bromoguanosine 3′,5′-cyclic monophosphate (8-Br-cGMP) was used as the cGMP analog. Relaxation was expressed as the percent decrease in contractile tone elicited by phenylephrine.

Determination of eNOS activity. We determined eNOS activity by measuring the conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline. These amino acids were separated by anion exchange chromatography using a modification of the method of Bredt and Snyder (1990). We maintained HAEC (six to eight passages) in the EGM kit at 37°C in a 95% air/5% CO_2 incubator. Before the experiments, 4×10^4 cells were seeded into 12-well plates and grown for 48 hr. After the HAEC were treated with various concentrations of As^{3+} for 14 hr, they were incubated for 20 min in HEPES buffer (pH 7.4): 140 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 5.0 mM glucose, 25 mM HEPES, and 3 µCi/mL [^{3}H]-L-arginine. The cells were washed twice with ice-cold Ca^{2+}-free buffer containing 5 mM EDTA, and then 0.3 M $HClO_4$ was added. The tissue extract was then neutralized with 3 M K_2CO_3 and centrifuged at 12,000 × *g* for 2 min; the supernatants were applied to columns containing Dowex AG50W-X8 (Na^+ form), and the eluted [^{3}H]-L-citrulline was measured by scintillation counting. We determined protein content by the method of Lowry et al. (1951).

Measurement of superoxide anion generation. We determined generation of superoxide anions by lucigenin-induced chemiluminescence. Aortic rings were placed in a Krebs-Ringer solution continuously gassed with 95% O_2/5% CO_2 and allowed to equilibrate for 30 min at 37°C. A mixture of 2 mL Krebs-Ringer solution with 0.25 mM lucigenin was prepared in a scintillation tube and mixed with various concentrations of As^{3+}. Aortic rings were then added to each tube, and chemiluminescence was measured for 60 min using a luminometer (Berthold, Germany).

Measurement of cGMP levels. We treated rat aortic rings with As^{3+} or saline for 14 hr as described previously (Lee et al. 2001). For the experiments in which we examined cGMP levels stimulated by an agonist, 10^{-7} M acetylcholine was added to the organ bath for 1 min. The reactions were immediately stopped with liquid nitrogen, and the tissue was homogenized in 1 mL ice-cold 6% trichloroacetic acid. The homogenate was centrifuged at 13,600 × *g* for 15 min at 4°C. The supernatant was extracted with water-saturated ether. We assayed extracts for cGMP levels by radioimmunoassay (RIA) using a [^{125}I]-cGMP RIA kit (Amersham) according to the procedure described by the manufacturer. We determined protein content from the pellet according to the method of Lowry et al. (1951).

Measurement of blood pressure change induced by acetylcholine. Rats were anesthetized with phenobarbital (50 mg/kg, intraperitoneal). A catheter of polyethylene PE-50 tubing (Clay Adams, Sparks, MD, USA) filled with heparinized saline (100 U/mL) was placed in the carotid artery for the measurement of blood pressure, and a catheter of polyethylene PE-10 fused to PE-50 tubing was placed in the jugular vein for the administration of drugs. Catheters were tunneled subcutaneously and exteriorized at the back of the neck. Wounds were sutured and cleaned with alcohol. We began experiments after a 1-day recovery period. On the day of the experiment, the arterial catheter was connected to a pressure transducer (BIOPAC Systems Inc.), and blood pressure was measured using the AcqKnowledge III computer program. Blood pressure was allowed to stabilize for a minimum of 30 min before treatment began. To determine the effects of As^{3+} on blood pressure reduction induced by acetylcholine, we administered As^{3+} solution (1 mg/kg) by an intravenous bolus injection into the jugular vein. In the controls, equivalent amounts of saline were injected. After 2 hr, the rats were infused with 10 µg/kg/min acetylcholine for 2 min via the jugular vein, and the change in blood pressure in response to acetylcholine was monitored simultaneously. Infusions were performed with a Harvard syringe pump (Southnatick, MA, USA) at a rate of 0.1 mL/min.

Statistical analysis. We calculated the means and standard errors of means for all treatment groups. The data were subjected to one-way analysis of variance followed by Duncan's multiple range test to determine which means were significantly different from the control. In all cases, a p value of < 0.05 was used to determine significance.

Results

To determine whether arsenic affects relaxation of blood vessels, we treated intact aortic rings with various concentrations of As^{3+} for 14 hr. Phenylephrine was applied to precontract the rings, followed by cumulative addition of acetylcholine to obtain concentration–relaxation curves (Figure 1A). As^{3+} suppressed the relaxation induced by acetylcholine in a concentration-dependent manner. We also investigated the effects of the pentavalent inorganic species (As^{5+}) and two major metabolites, MMA and DMA, on the inhibition of acetylcholine-induced vasorelaxation (Figure 1B). However, As^{3+}, MMA, and

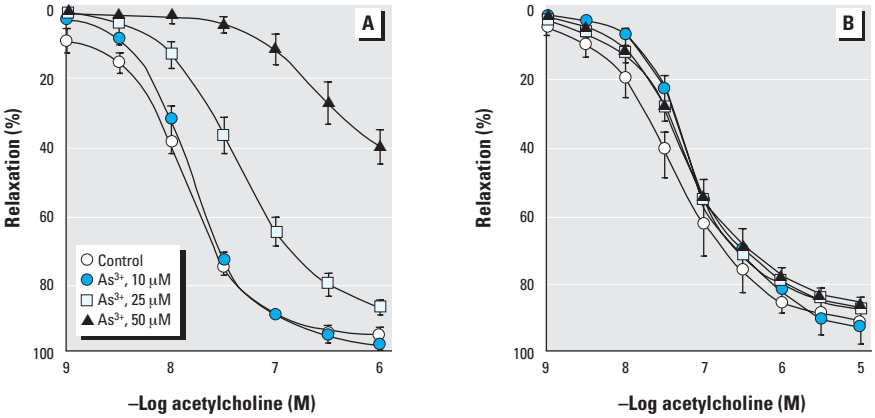

Figure 1. Inhibitory effect of As^{3+} on relaxation of aortic rings induced by acetylcholine. After intact aortic rings were treated with (*A*) various concentrations of As^{3+} and (*B*) other species of arsenic (100 µM each) for 14 hr, 1 µM phenylephrine was added to precontract the aortic rings and then increasing concentrations of acetylcholine were added in a cumulative manner. Concentration–response curves to acetylcholine were determined with relaxation expressed as the percent decrease in contractile tone elicited by phenylephrine. The values of curves are means ± SEM of four to five independent experiments.

DMA failed to inhibit the acetylcholine-induced relaxation.

Acetylcholine stimulates eNOS to produce NO in endothelium, which results in the relaxation of smooth muscle (Wanstall et al. 2001). To examine whether As^{3+} inhibits NO synthesis by endothelial cells, we investigated the effect of As^{3+} on eNOS activity in cultured HAEC. As^{3+} reduced eNOS activity in a concentration-dependent manner (Figure 2A), suggesting that As^{3+} can suppress vascular NO production in endothelial cells. Others have reported that As^{3+} can generate superoxide anions in cultured endothelial cells and smooth muscle cells (Lynn et al. 2000; Smith et al. 2001). Superoxide anions interact with NO to form peroxynitrite, resulting in suppression of vascular relaxation by blocking the NO pathway (Pryor and Squadrito 1995). To determine if superoxide anions generated by As^{3+} may play a role in the suppression of vascular relaxation, we treated aortic rings with intact endothelium with As^{3+}, and superoxide production was evaluated by measuring lucigenin-induced chemiluminescence. Increased chemiluminescence was not observed at any concentration of As^{3+} tested (Figure 2B).

NO released from endothelium elicits vascular relaxation by increasing the levels of cGMP in smooth muscle (Robertson et al. 1993). To determine the effects of As^{3+} on the NO-dependent pathway, we measured the accumulation of cGMP in aortic rings. The basal cGMP levels in the aortic rings were significantly decreased by As^{3+} treatment in a concentration-dependent manner (Figure 3A). After stimulation with 10^{-7} M acetylcholine, As^{3+} again significantly reduced cGMP levels, even though the cGMP levels were 30 times greater due to the acetylcholine stimulation (Figure 3B). However, unlike the results for acetylcholine-induced relaxation (Figure 1A), no difference in cGMP level was found between the 25- and 50-μM As^{3+} treatments, suggesting that arsenic may interfere with some relaxation mechanism in addition to inhibiting endothelium-dependent NO production.

Therefore, using aortic rings without endothelium, we examined the effect of As^{3+} on relaxation induced by SNP, a direct NO-releasing agent. Treatment with As^{3+} inhibited vasorelaxation induced by SNP to a lesser extent (Figure 4A) than the vasorelaxation induced by acetylcholine. As^{3+} at a concentration of 25 μM did not suppress SNP-induced relaxation, but 50 μM As^{3+} showed significant inhibition. Consistent with this finding, when we investigated the effect of As^{3+} on relaxation induced by the cGMP analog, 8-Br-cGMP in aortic rings without endothelium, only 50 μM As^{3+} resulted in significant reduction of cGMP analog-induced relaxation (Figure 4B). These results suggested that 50 μM As^{3+} could interfere with the cGMP-dependent relaxation machinery of smooth muscle in blood vessels.

To verify the effects of As^{3+} on blood vessels *in vivo*, we monitored the change in blood pressure after intravenous infusion of acetylcholine into conscious rats (Figure 5). An intravenous bolus of As^{3+} had no effect on basal blood pressure (Figure 5A). When rats were infused with 10 μg/kg/min acetylcholine 2 hr after As^{3+} treatment (Figure 5C), the reduction of blood pressure induced by acetylcholine was significantly suppressed compared to the control group (10.8 ± 3.5 vs. 31.3 ± 4.7 mmHg) (Figure 5B). These results suggest that As^{3+} caused suppression of vasorelaxation induced by acetylcholine *in vivo*. This confirms the previous *in vitro* results shown in Figure 1A.

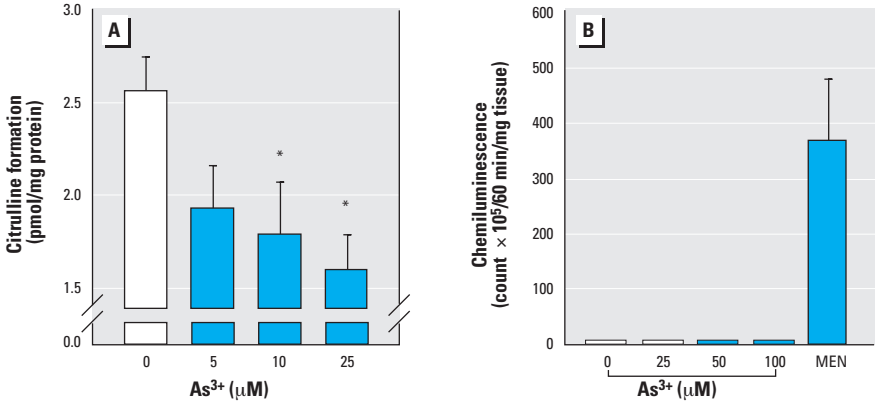

Figure 2. Effects of As^{3+} on eNOS activity in human aortic endothelial cells and superoxide production in aortic rings. (*A*) After As^{3+} was added to HAEC for 14 hr, the activity of eNOS was determined by the formation of L-citrulline from L-arginine, as described in "Materials and Methods." (*B*) Several concentrations of As^{3+} were added to aortic rings and superoxide anions was measured by lucigenin-induced chemiluminescence. Menadione (MEN; 10 μM) was used as positive control. Values are means ± SEM of (*A*) five to six and (*B*) three independent experiments.
*Significant difference from control ($p < 0.05$).

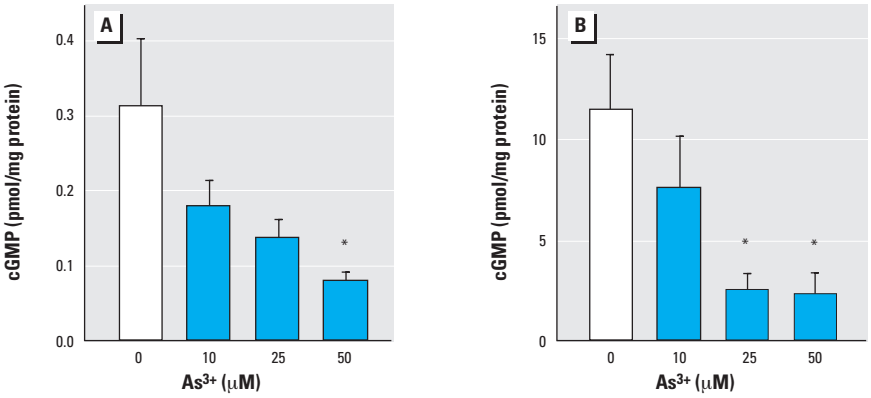

Figure 3. Effects of As^{3+} on cGMP levels of aortic rings. After aortic rings were treated with As^{3+} for 14 hr, (*A*) cGMP levels in basal state and (*B*) cGMP levels accumulated by 10^{-7} M acetylcholine were determined by RIA method as described in "Materials and Methods." Values are means ± SEM of five to seven independent experiments.
*Significant differences from corresponding control ($p < 0.05$).

Discussion

This is the first study to evaluate the effects of As^{3+} on vasomotor function *in vitro* and *in vivo*. We have shown that As^{3+} inhibits the vascular relaxation induced by acetylcholine in a concentration-dependent manner and that possible mechanisms are the inhibition of NO production in endothelial cells and the suppression of cGMP-dependent relaxation mechanisms in smooth muscles. These *in vitro* results were consistent with *in vivo* results (Figure 5) in which As^{3+} suppressed the reduction of blood pressure by acetylcholine in conscious rats. Our findings correlate well with a previous study reporting that serum concentrations of NO metabolites were decreased in a population exposed to arsenic in drinking water (Pi et al. 2000). In addition, this As^{3+}-induced suppression *in vitro* was still observed after the removal of residual As^{3+} by changing the incubation medium, suggesting that the As^{3+}-induced effect was irreversible and that damaged blood vessels might not recover even after As^{3+} has disappeared from the bloodstream.

Several epidemiologic studies have reported that arsenic-contaminated drinking water causes various types of cardiovascular disease such as blackfoot disease, atherosclerosis, cerebrovascular disorders, and hypertension (Chen et al. 1988; Chiou et al. 1997; Rahman et al. 1999; Tseng 1977; Wang et al. 2002). Recently, the effect of arsenic on platelets has been suggested as a key mechanism in the development of these cardiovascular diseases (Lee et al. 2002). Blood vessels, however, are another tissue important in the development of cardiovascular diseases. Dysfunction in blood vessels disrupts the balance in vasomotor tone between relaxation and contraction, with vasoconstriction predominating and ultimately leading to possible increased risk for development of vascular diseases such as hypertension and atherosclerosis (Lefer et al. 1991; Luscher et al. 1993; Rubanyi 1993; Vanhoutte 1997). Because our data showed that As^{3+} could suppress endothelium-dependent vasorelaxation with subsequent changes in vasomotor tone in blood vessels, we propose arsenic-induced dysfunction in blood vessels as an alternative mechanism for arsenic-associated cardiovascular disease observed in human populations.

Treatment with As^{3+} did not result in concentration-dependent inhibition of vasorelaxation induced by SNP (Figure 4A) compared to the results obtained by acetylcholine (Figure 1A). As^{3+} at a concentration of 25 μM did not inhibit SNP-induced relaxation but did inhibit acetylcholine-induced relaxation, suggesting that 25 μM As^{3+} may interfere with NO production in endothelial tissue. This conclusion is supported by our finding that As^{3+} can significantly inhibit eNOS activity in cultured human aortic endothelial cells (Figure 2A). In addition to interfering with NO generation in aortic endothelium, higher concentrations of As^{3+} may also disrupt the relaxation mechanisms in smooth muscles. Several lines of evidence support this view. First, only 50 μM concentrations of As^{3+} showed an inhibitory effect on SNP-induced relaxation in aortic rings without endothelium (Figure 4A). Second, unlike acetylcholine-induced vasorelaxation (Figure 1A), levels of acetylcholine-stimulated cGMP in aortic rings showed no difference between treatments with 25 or 50 μM As^{3+} (Figure 3B). Third, treatment with 50 μM As^{3+} resulted in significant reduction of cGMP analog-induced vasorelaxation in aortic rings without endothelium (Figure 4B). These results suggest that impairment of NO generation in endothelium and impairment of cGMP-dependent relaxation mechanisms in smooth muscles depended on the concentrations of As^{3+} that blood vessels were exposed to.

As^{3+} is reported to stimulate the formation of reactive oxygen species (ROS) in vascular endothelial and smooth muscle cells, mainly via NADH/NADPH oxidase (Lynn et al. 2000; Smith et al. 2001); thus, the generation of superoxide could elicit the reduced relaxation due to elimination of NO. Therefore, we considered the possibility that superoxide production is involved in suppression of vasorelaxation by As^{3+}. However, we detected no significant increase in ROS generation in intact aortic rings when measuring superoxide anions with the chemiluminescent probe lucigenin (Figure 2B). These contradictory results might be explained by the differences in experimental systems (we used aortic rings in an organ-bath system and the previous studies used a cell culture system), but the exact reason for this discrepancy is currently unknown. In any case, our results suggest that superoxide generation does not play an important role in the suppression of vasorelaxation by As^{3+}.

Our study revealed that higher concentrations of As^{3+} inhibited cGMP-dependent relaxation of smooth muscle in aortic rings isolated from rats. Arsenic is well known to induce heat-shock proteins (Del Razo et al. 2001), a large family of proteins whose expression is usually induced by cellular stress. Knoepp et al. (2000) reported that smooth muscle relaxation induced by SNP and forskolin, a adenylate cyclase activator, was inhibited by cellular stresses such as heat shock and As^{3+} via inhibition of the phosphorylation of heat shock protein 20, which is a regulatory component of the actin-associated cytoskeleton. However, those effects were observed in bovine carotid artery, whereas our aortic rings were isolated from rats. Furthermore, 0.5 mM As^{3+} was used in those experiments, which was 10 times higher than the concentrations used in our experiment. In fact, treatment with 0.5 mM As^{3+} resulted in complete impairment of phenylephrine-induced precontraction in rat artery (data not shown), and thus the relaxation experiment by acetylcholine was not feasible. Therefore, it appears inappropriate to apply the explanation of Knoepp's group to our observation that 50 μM As^{3+} could suppress the relaxation

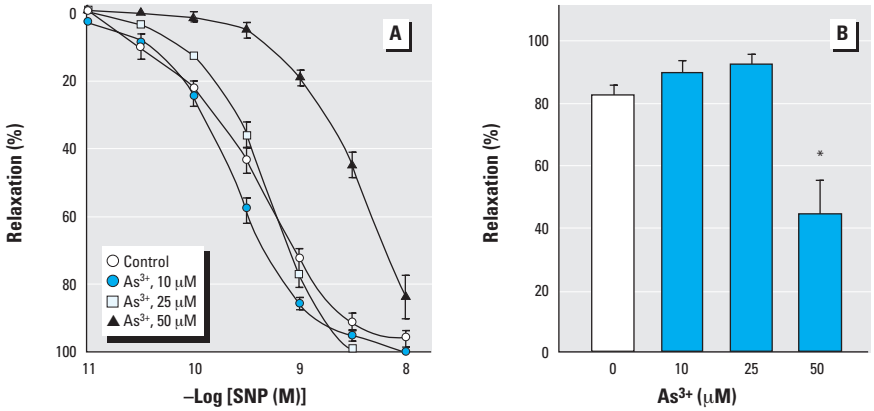

Figure 4. Effects of As^{3+} on relaxation induced by sodium nitroprusside and 8-Br-GMP in aortic rings without endothelium. After aortic rings without endothelium were treated with As^{3+} for 14 hr, 1 μM phenylephrine was added to precontract the aortic rings and then relaxation was elicited by (*A*) cumulative addition of SNP, NO donor and (*B*) 30 μM 8-Br-cGMP, a cGMP analog. Relaxation is expressed as percentage of decrease in contractile tone elicited by phenylephrine. Values are means ± SEM of four independent experiments.
*Significant difference from control ($p < 0.05$).

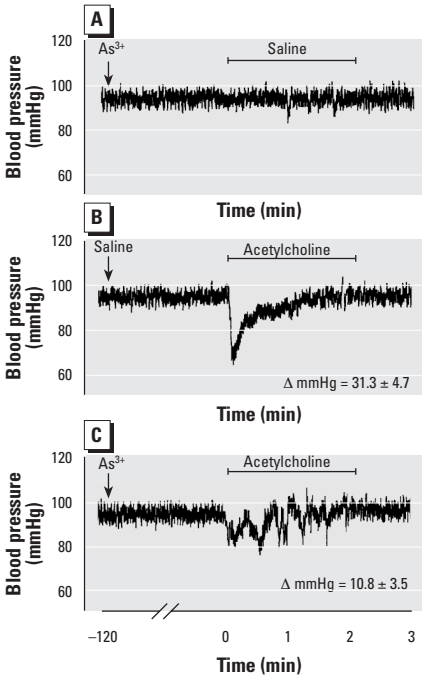

Figure 5. Effect of intravenously administered As^{3+} on decrease in blood pressure by acetylcholine infusion in rats. (*A*) Blood pressure changes by As^{3+} bolus and saline infusion were monitored. Decrease in blood pressure by acetylcholine infusion (10 μg/kg/min) was measured via carotid artery 2 hr after an intravenous bolus of (*B*) saline or (*C*) As^{3+} [1 mg/kg]. Infusions were performed by Harvard syringe pump at a rate of 0.1 mL/min for 2 min (indicated by line). Data are representative tracings of five independent experiments.

machinery in smooth muscles. The exact mechanism remains to be identified.

Previous study reported that the normal concentration of arsenic in human plasma is 2.4 ± 1.9 μg/L and that this level may be increased up to 38 μg/L with chronic arsenic exposure (Heydorn 1970). Our *in vivo* arsenic experiment showed significant suppression of acetylcholine-induced vasorelaxation after only a 2-hr exposure (Figure 5), at which time arsenic reached its plasma level of less than 100 μg/L (data not shown). This experimental arsenic exposure was therefore not more than a factor of 3 greater than arsenic levels to which human blood vessels are exposed after chronic intake of arsenic-contaminated drinking water. Considering the facts that humans are more sensitive to arsenic toxicity than are several other species (Chan and Huff 1997), the concentration of arsenic used in the current investigation could be well within the range to induce cardiovascular disease when humans drink arsenic-contaminated water for weeks or even years.

From this study, we determined that As^{3+} caused altered vascular tone by decreasing vasorelaxation. The inhibitory effects may be due to suppression of NO production mediated by eNOS inhibition in endothelial cells and interference of cGMP-dependent relaxation machinery in smooth muscles (Figure 6). In our *in vivo* study, As^{3+} treatment of rats blocked the acetylcholine-induced hypotensive effect. These results confirm our *in vitro* observations and suggest that reduced vasorelaxation may be a contributing factor in development of cardiovascular diseases in populations exposed to arsenic.

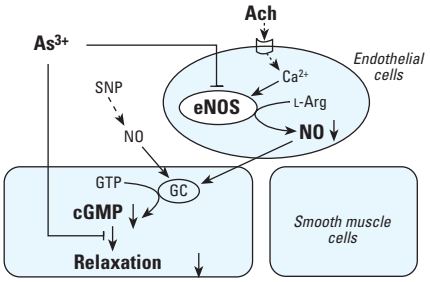

Figure 6. Proposed mechanism for inhibitory effect of As^{3+} on vasorelaxation. Abbreviations: Ach, acetylcholine; GC, guanylate cyclase; GTP, guanosine triphosphate. As^{3+} causes altered vasomotor tone by decreasing vasorelaxation induced by Ach. This could be due to suppression of NO production mediated by eNOS inhibition in endothelial cells and interference of cGMP-dependent relaxation machinery in smooth muscles.

References

Alexander RW. 1995. Hypertension and the pathogenesis of atherosclerosis: oxidative stress and the mediation of arterial inflammatory response: a new perspective. Hypertension 25:155–161.

Bagla P, Kaiser J. 1996. India's spreading health crisis draws global arsenic experts. Science 274:174–175.

Bates MN, Smith AH, Hopenhayn-Rich C. 1992. Arsenic ingestion and internal cancers: a review. Am J Epidemiol 135:462–476.

Bell DM, Johns TE, Lopez LM. 1998. Endothelial dysfunction: implications for therapy of cardiovascular diseases. Ann Pharmacother 32:459–470.

Bredt DS, Snyder SH. 1990. Isolation of nitric oxide synthetase, a calmodulin-requiring enzyme. Proc Natl Acad Sci U S A 87:682–685.

Carmignani M, Boscolo P, Castellino N. 1985. Metabolic fate and cardiovascular effects of arsenic in rats and rabbits chronically exposed to trivalent and pentavalent arsenic. Arch Toxicol Suppl 8:452–455.

Chan PC, Huff J. 1997. Arsenic carcinogenesis in animals and in humans: mechanistic, experimental, and epidemiological evidence. J Environ Sci Health, Part C: Environ Carcinog Ecotoxicol Rev 15:83–122.

Chen CJ, Wu MM, Lee SS, Wang JD, Cheng SH, Wu HY. 1988. Atherogenicity and carcinogenicity of high-arsenic artesian well water. Multiple risk factors and related malignant neoplasms of blackfoot disease. Arteriosclerosis 8:452–460.

Chiou HY, Huang WI, Su CL, Chang SF, Hsu YH, Chen CJ. 1997. Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 28:1717–1723.

Col M, Col C, Soran A, Sayli BS, Ozturk S. 1999. Arsenic-related Bowen's disease, palmar keratosis, and skin cancer. Environ Health Perspect 107:687–689.

Del Razo LM, Quintanilla-Vega B, Brambila-Colombres E, Calderon-Aranda ES, Manno M, Albores A. 2001. Stress proteins induced by arsenic. Toxicol Appl Pharmacol 177:132–148.

Engel RR, Hopenhayn-Rich C, Receveur O, Smith AH. 1994. Vascular effects of chronic arsenic exposure: a review. Epidemiol Rev 16:184–209.

Heydorn K. 1970. Environmental variation of arsenic levels in human blood determined by neutron activation analysis. Clin Chim Acta 28:349–357.

Ignarro LJ. 1989. Heme-dependent activation of soluble guanylate cyclase by nitric oxide: regulation of enzyme activity by porphyrins and metalloporphyrins. Semin Hematol 26:63–76.

Knoepp L, Beall A, Woodrum D, Mondy JS, Shaver E, Dickinson M, et al. 2000. Cellular stress inhibits vascular smooth muscle relaxation. J Vasc Surg 31:343–353.

Lee JY, Bae ON, Chung SM, Lee MY, Chung JH. 2001. Menadione induces endothelial dysfunction mediated by oxidative stress and arylation. Chem Biol Interact 137:169–183.

Lee MY, Bae ON, Chung SM, Kang KT, Lee JY, Chung JH. 2002. Enhancement of platelet aggregation and thrombus formation by arsenic in drinking water: a contributing factor to cardiovascular disease. Toxicol Appl Pharmacol 179:83–88.

Lefer AM, Tsao PS, Lefer DJ, Ma XL. 1991. Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. FASEB J 5:2029–2034.

Lowry OH, Rosenberg NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275.

Luscher TF, Tanner FC, Tschundi MR, Noll G. 1993. Endothelial dysfunction in coronary artery disease. Annu Rev Med 44:395–418.

Lynn S, Gurr JR, Lai HT, Jan KY. 2000. NADH oxidase activation is involved in arsenite-induced oxidative DNA damage in human vascular smooth muscle cells. Circ Res 86:514–519.

Moncada S, Palmer RM, Higgs EA. 1991. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacol Rev 43:109–142.

NRC (National Research Council). 1999. Chemistry and analysis of arsenic species in water, food, urine, blood, hair, and nails. In: Arsenic in Drinking Water (National Research Council, ed). Washington, DC:National Academy Press, 27–82.

Pi J, Kumagai Y, Sun G, Yamauchi H, Yoshida T, Iso H, et al. 2000. Decreased serum concentrations of nitric oxide metabolites among Chinese in an endemic area of chronic arsenic poisoning in inner Mongolia. Free Radic Biol Med 28:1137–1142.

Pryor WA, Squadrito GL. 1995. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. Am J Physiol 268:L699–L722.

Rahman M, Tondel M, Ahmad SA, Chowdhury IA, Faruquee MH, Axelson O. 1999. Hypertension and arsenic exposure in Bangladesh. Hypertension 33:74–78.

Robertson BE, Schubert R, Hescheler J, Nelson MT. 1993. cGMP-dependent protein kinase activates Ca-activated K channels in cerebral artery smooth muscle cells. Am J Physiol 265:C299-C303.

Rubanyi GM. 1993. The role of endothelium in cardiovascular homeostasis and diseases. J Cardiovasc Pharmacol 22(suppl 4):S1-S14.

Sellke FW, Boyle EM, Verrier ED. 1997. The pathophysiology of vasomotor dysfunction. Ann Thorac Surg 64:S9-S15.

Smith KR, Klei LR, Barchowsky A. 2001. Arsenite stimulates plasma membrane NADPH oxidase in vascular endothelial cells. Am J Physiol Lung Cell Mol Physiol 280:L442–L449.

Tseng CH, Tai TY, Chong CK, Tseng CP, Lai MS, Lin BJ, et al. 2000. Long-term arsenic exposure and incidence of non-insulin-dependent diabetes mellitus: a cohort study in arseniasis-hyperendemic villages in Taiwan. Environ Health Perspect 108:847–851

Tseng WP. 1977. Effects and dose-response relationships of skin cancer and blackfoot disease with arsenic. Environ Health Perspect 19:109–119.

Vanhoutte PM. 1997. Endothelial dysfunction and atherosclerosis. Eur Heart J 18(suppl E):E19-E29.

Wang CH, Jeng JS, Yip PK, Chen CL, Hsu LI, Hsueh YM, et al. 2002. Biological gradient between long-term arsenic exposure and carotid atherosclerosis. Circulation 105:1804–1809.

Wanstall JC, Jeffery TK, Gambino A, Lovren F, Triggle CR. 2001. Vascular smooth muscle relaxation mediated by nitric oxide donors: a comparison with acetylcholine, nitric oxide and nitroxyl ion. Br J Pharmacol 134:463–472.