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Research Article

Differential influences of various arsenic compounds on glutathione redox status and antioxidative enzymes in porcine endothelial cells

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Abstract. The cellular response and detoxification mechanisms in porcine endothelial cells (PAECs) to arsenic trioxide (As_2O_3), sodium arsenite ($NaAsO_2$) and sodium arsenate (Na_2HAsO_4) were investigated. $NaAsO_2$ at 20 μM for 72 h increased Cu/Zn superoxide dismutase activity resulting in elevated intracellular hydrogen peroxide levels, but As_2O_3 and Na_2HAsO_4 did not. Trivalent arsenic compounds increased intracellular oxidized glutathione (GSSG) and total glutathione (GSH) and cellular glutathione peroxidase (cGPX) and glutathione S-transferase (GST) activity, but not glutathione reductase activity. The increased cGPX activity resulted in an elevated cellular GSSG content. Na_2HAsO_4 increased the cellular GSSG level at 72 h compared to controls. These results imply that the increased GSH content responding to the oxidative stress by trivalent arsenic compounds may be mainly related to the regulation of GSH turnover. The increased GST activity implies that the elevated intracellular GSH level responding to the oxidative stress may be used to conjugate arsenic in PAECs and facilitate arsenic efflux.

Key words. Arsenic; antioxidative enzyme; glutathione redox status; hydrogen peroxide; porcine endothelial cell.

Arsenic is ubiquitous in our environment and the accumulation of this element in ground water and plants poses a health risk to both humans and animals [1]. Various forms of arsenic circulate in the environment and in living organisms and arsenic-induced toxicity is associated with inorganic arsenic, especially trivalent arsenic compounds [2]. The toxic effects of arsenic on animals and humans are important issues for investigation because it is a potential carcinogen [3]. Exposure to arsenic compounds in animals and humans has been reported to cause various health effects, such as Blackfoot disease, ischemic heart disease, vascular diseases, hypertension, diabetes, hepatic damages and cancers [4–9]. Mild oxidative stress may induce cellular regulations of antioxidative enzymes and glutathione (GSH) [10]. The elevation of cellular GSH levels exerts a protective effect in counteracting oxidative stress [11]. Research data indicate that arsenic-induced oxidative stress, such as hydrogen peroxide formation and lipid peroxidation, is associated with a change in intracellular GSH level [12]. Hydrogen peroxide production is associated with arsenic toxicity [13] and can injure vascular endothelium [14].

Several enzymes are involved in the detoxification of arsenic compounds. Superoxide dismutases (SODs) are

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ubiquitous metalloproteins that play a major protective role in living organisms by catalyzing the dismutation of superoxide into hydrogen peroxide and molecular oxygen. There are different types of SODs characterized by the redox-active metals at the catalytic site. In mammalian systems, copper/zinc SOD (Cu/Zn-SOD) is located primarily in the cytosol and nucleus whereas manganese SOD (Mn-SOD) exists mainly in mitochondria [15].

GSH and the GSH-related enzymes, glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione S-transferase (GST), are important antioxidants that play crucial roles in the detoxification of arsenic. Glutathione (L-r-glutamyl-L-cysteinylglycine) is a biologically active thiol tripeptide that plays an important role detoxifying exogenous compounds and oxidative insults [16]. GPX is a family of four selenoproteins that incorporates selenium as selenocysteine (Se-Cys) into the amino acid sequence. These enzymes exhibit different substrate specificities and collaborate to provide the main enzymatic defense system against oxidative stress in mammalian cells. Cellular GPX (cGPX) prevents the formation of reactive oxygen species (ROS) by catalyzing the decomposition of hydrogen peroxide with the concomitant oxidation of GSH to GSSG [17]. GSH reductase is the other important enzyme responsible for the availability of GSH in the GSH redox cycle by catalyzing the reduction of GSSG to GSH utilizing NADPH [18]. GST comprises a family of isozymes with various substrate specificities and amino acid sequences which are involved in metabolic detoxification by catalyzing the reaction between GSH and electrophilic substrates, preventing toxic damage in several tissues [19, 20].

Most arsenic studies have focused on the antileukemic effect of arsenic trioxide and the toxic effect of sodium arsenite in malignant cells [12, 13, 21, 22]. However, the effects of different arsenic compounds on primary culture cells and transformed cells may be different and need to be investigated. Arsenic is a known endothelial toxin and has a direct effect on endothelial cells resulting in vascular disorders and endothelial cell dysfunction [5, 23]. Therefore, vascular endothelial cells were used to investigate arsenic toxicity.

The mechanism for detoxifying arsenic-induced oxidative damage in endothelial cells remains unclear. The GSH redox cycle has been proposed to be a more important detoxification pathway for hydrogen peroxide in cells or tissues than catalase [24]. In the present study, the effects of various arsenic compounds on the glutathione redox cycle and the antioxidative enzymes in primary culture of porcine aortic endothelial cells (PAECs) were investigated. This information could help to elucidate the differential mechanisms induced by various arsenic compounds in the PAECs.

Materials and methods

Materials

Arsenic trioxide (As_2O_3), sodium arsenite ($NaAsO_2$), sodium arsenate (Na_2HAsO_4), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), xylenol orange (3, 3′-bis[N, N-di(carboxymethyl)-aminomethyl]-o-cresolsulfone-phthalein, sodium salt), nitroblue tetrazolium (NBT), GPX, GR, GSH, GSSG, 1-chloro-2,4-dinitrobenzene (CDNB), 5, 5′-dithiobis-2-nitrobenzoic acid (DTNB) and reduced β-NADPH were purchased from Sigma (St Louis, Mo.). The media and reagents for cell culture were obtained from Hyclone (Logan, Utah) and GIBCO Life Technologies (Grand Island, N. Y.). The ECL detection system and the protein markers were purchased from Amersham/Pharmacia (Arlington, Ill.). 2-Vinylpyridine was obtained from Aldrich (St Louis, Mo.). Molecular Probes (Eugene, Ore.) was the source for Dil-AcLDL (1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate). The anti-Cu/Zn-SOD and anti-GST pi polyclonal antibodies were purchased from Calbiochem-Novabiochem Corporation (San Diego, Calif.), and the anti-GPX polyclonal antibody was obtained from United States Biological (Swampscott, Mass.). Horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Bio-Rad (Richmond, Calif.).

Isolation and identification of primary PAECs

Endothelial cells were isolated according to the procedure of Sharma and Davis [25] with modification. Fresh segments of thoracic aorta from 7-month-old pigs were collected. A thin layer was gently scraped from each arterial lumen by sterile surgical blades and transferred into a sterile flask to incubate with 1 mg/ml collagenase (type II) at 37 °C for 10 min to release the endothelial cells. The mixture was centrifuged and the supernatant discarded. The pellets were washed twice in sterile basal medium with additional antibiotics and plated in sterile tissue culture dishes with culture medium (basal medium supplemented with 10% FBS) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The primary culture of PAECs was passed and maintained routinely in culture medium at 37 °C and the culture medium was changed every 2–3 days until the cells reached confluence. PAECs were identified by a Dil-AcLDL uptake assay and subsequently examined by fluorescent microscopy [26]. Microscopic examination was used to observe cell morphology and monitor cell growth. The 10th through 12th passages of PAEC primary cultures were used for investigating arsenic toxicity.

Cytotoxicity assay

PAECs were plated onto 96-well plates at a density of 1×10^4 cells/well. After overnight culture, the medium was replaced with the fresh medium containing various

concentrations of As_2O_3, $NaAsO_2$ or Na_2HAsO_4 and the plates were incubated at 37 °C for up to 72 h. At the end of the experiments, MTT (0.5 mg/ml medium) was added to each well and the plates were then incubated at 37 °C for 4 h. After adding an equal volume of solubilization solution (10% SDS in 0.01 M HCl) to each well, the plates were read on a microplate reader at 590 nm.

Cell treatments

Stock solutions of 20 mM As_2O_3 (in 0.1 M NaOH), $NaAsO_2$ and Na_2HAsO_4 were prepared. Five different concentrations (0, 1, 5, 10 and 20 μM) of As_2O_3, $NaAsO_2$ and Na_2HAsO_4 were added to cells and the culture plates were incubated at 37 °C for 24, 48 and 72 h. Following three washes with ice-cold phosphate-buffered saline (PBS), an appropriate amount of homogenate buffer [20 mM Tris (pH 7.5), 0.25 M sucrose, 1 mM EGTA, 5 mM EDTA, 1 mM PMSF, 50 mM beta-mercaptoethanol and 25 μg/ml leupeptin; pH 7.4] was added to each plate and the cells were scraped into centrifuge tubes using a rubber policeman. After sonication for 10 s on ice, the mixtures were centrifuged at 17,000 g for 10 min at 4 °C. Protein concentration of cell cytosols was determined by the Bradford assay [27] using bovine serum albumin as a standard. The cell cytosols were then used for all analyses.

Cellular arsenic contents

The cell cytosols were injected onto the SIMMA-6000 (Perkin Elmer, Norwalk, Conn.) graphite atomic absorption spectrophotometer with an autosampler [28] and the calibration was carried out by the standard addition method.

Intracellular hydrogen peroxide level

The intracellular H_2O_2 content was determined by a modification of the method described by Zhou et al. [29]. The measurement was based on the oxidation of ferrous to ferric ions by H_2O_2 under acidic conditions that in turn form a stable colored complex with xylenol orange that can be measured spectrophotometrically at 560 nm at room temperature.

Intracellular antioxidative enzyme activities

For measurement of Cu/Zn-SOD activity, the cell cytosols were extracted with ethanol/chloroform to inactivate Mn-SOD and Fe-SOD, and then Cu/Zn-SOD activity was determined spectrophotometrically based on the inhibition of the reduction of NBT by SOD [30]. One SOD activity unit (U-525) was defined as the activity that doubled the rate of the autoxidation background ($V_S/V_C = 2$) at 37 °C.

GPX activity was determined by an enzyme-coupled method with GR, utilizing hydrogen peroxide as substrate [31] with a DU Series 60 Spectrophotometer (Beckman Instruments, Fullerton, Calif.) at 30 °C. The rate of decrease in the NADPH concentration was followed at 340 nm over the 3-min period at 30-s intervals.

GR activity was determined by monitoring the oxidation of NADPH consumed in the reduction of GSSG by the change at 340 nm [32]. The rate of decrease in the absorbance at 340 nm was monitored over the 3-min period at 30-s intervals.

GST activity was determined spectrophotometrically at 340 nm by monitoring the formation of the conjugated GSH and CDNB at 25 °C [19]. The rate of increase in the absorbance at 340 nm was monitored over the 3-min period at 30-s intervals.

Total GSH and GSSG contents

Total GSH contents in cell cytosols were determined with the modification of the enzymatic recycling assay [33] by using GR, NADPH and DTNB to form a spectrophotometrically detectable product at 412 nm [34]. For the measurement of GSSG content, 2-vinylpyridine was added to scavenge GSH from cell cytosols prior to the assay [35]. The GSSG content was then determined based on the reaction with DTNB following the reduction of GSSG to GSH by GR. Total GSH and GSSG was estimated by monitoring the rate of formation of chromophoric product at 412 nm over the 3-min period at 30-s intervals.

Western blotting analysis

Cell cytosols (40–50 μg protein/lane) were electrophoretically separated on 7.5–15% gradient SDS-polyacrylamide gels as described by Laemmli [36]. Proteins were transferred onto the nitrocellulose membrane (0.2 μm) in transfer buffer (25 mM Tris, 192 mM glycine and 20% methanol) at 4 °C according to the method of Towbin et al. [37]. After transfer, membranes were incubated with blocking solution [5% non-fat dry milk in TTBS (0.05% Tween 20 in TBS; pH 7.5)] at 25 °C for 1 h, then hybridized with various polyclonal antibodies for 2 h at room temperature. Following three washes with TTBS, membranes were incubated with the HRP-conjugated secondary antibodies. Membranes were washed three times with TTBS and rinsed once with TBS. The specific binding of antibodies onto the membranes was detected by the ECL detection system. The relative amounts of specific antibodies present in various samples were estimated by densitometric scanning of the X-ray film, and analyzed by the Stratagene Eagle Eye Image System (Merck, La Jolla, Calif.).

Statistical analysis

All results were normalized to cellular protein content and the data were examined for equal variance and normal distribution prior to statistical analysis. Mean values were compared by analysis of variance (ANOVA) with Fisher's least significant difference (LSD) method for comparing groups [38]. A significance level of 5% was adopted for all comparisons.

Results

Isolation and identification of PAECs

Endothelial cells were isolated from porcine thoracic aorta and the primary culture of PAECs was used as experimental material in this study. Results from the uptake of Dil-AcLDL showed that PAECs were brilliantly fluorescent, while the fluorescent intensities of other cell types (rat L8 myoblasts and porcine smooth muscle cells) were near background level (data not shown).

Effect of arsenic compounds on PAEC cytotoxicity

As_2O_3- and $NaAsO_2$-induced cytotoxicities in PAECs were observed ($p < 0.05$) at a concentration as low as 0.625 μM at 72 h (fig. 1 A, B). Na_2HAsO_4 did not induce ($p > 0.05$) cytotoxicity in PAECs (fig. 1 C).

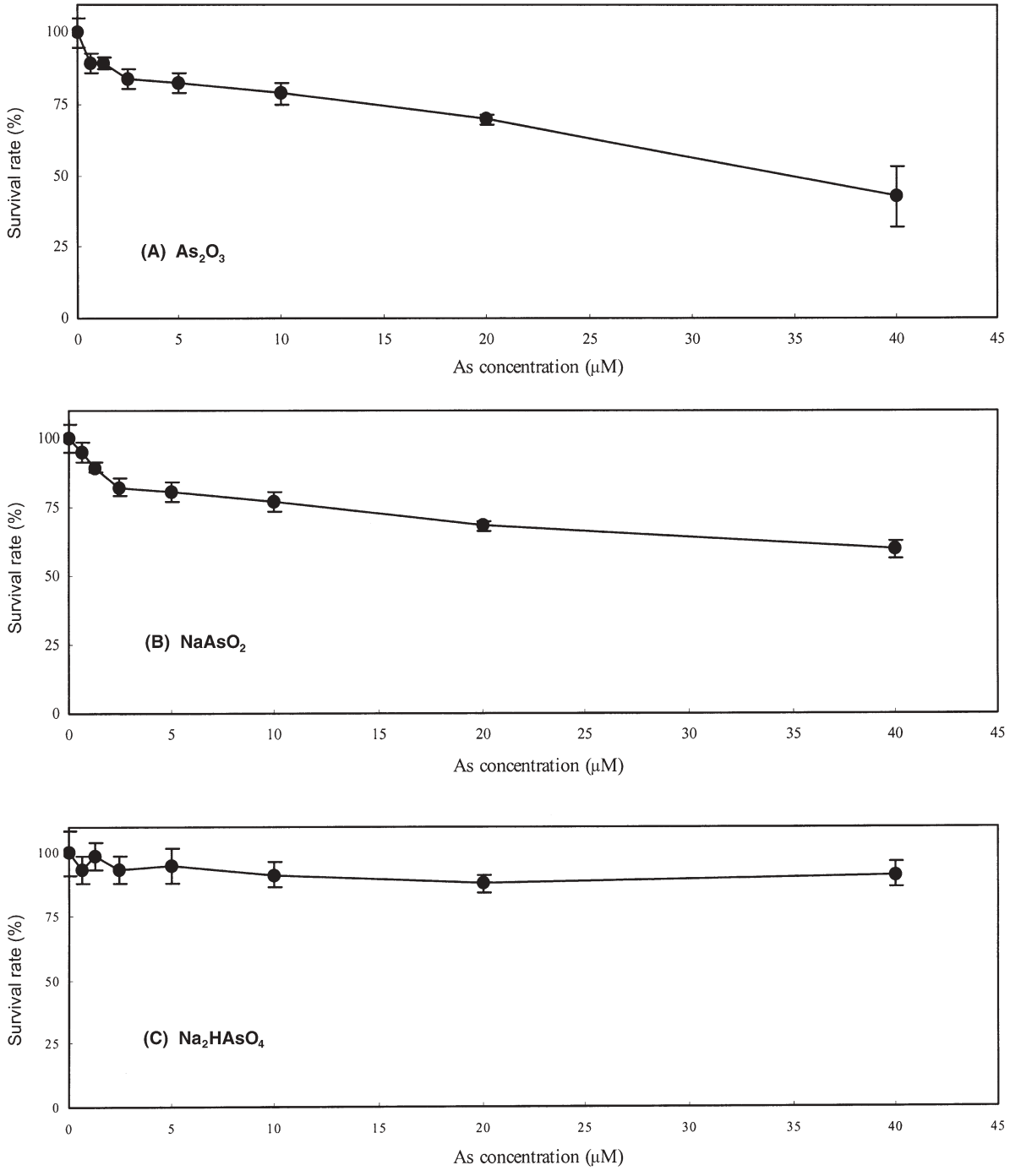

Figure 1. The cytotoxic effect of As_2O_3 (*A*), $NaAsO_2$ (*B*) and Na_2HAsO_4 (*C*) on PAECs at 72 h exposure. Each data point represents the mean ± SE of three replicates.

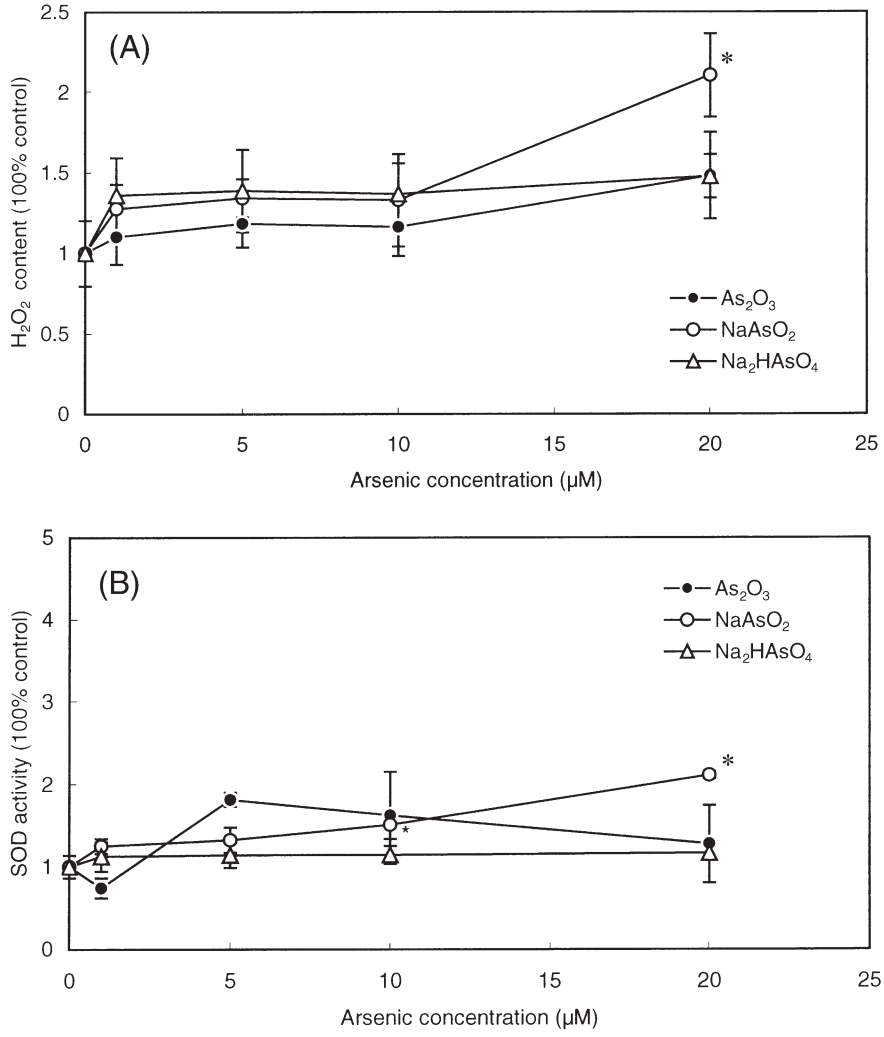

Figure 2. The effect of arsenic compounds (As_2O_3, $NaAsO_2$ and Na_2HAsO_4) on cellular H_2O_2 contents (*A*) and Cu/Zn-SOD activities (*B*) in PAECs at 72 h exposure. Each data point represents the mean ± SE of three replicates. An asterisk indicates a significant difference compared to the control in the $NaAsO_2$-treated group ($p < 0.05$).

Effect of arsenic compounds on H_2O_2 content and SOD activity

Intracellular H_2O_2 content (fig. 2A) and SOD activity (fig. 2B) were increased by 20 μM $NaAsO_2$ treatment for 72 h only ($p < 0.05$), but not by As_2O_3 or Na_2HAsO_4 treatment ($p > 0.05$). No significant differences ($p > 0.05$) were found among arsenic compounds at any concentrations for 24 or 48 h (data not shown).

Effect of arsenic compounds on GSH and GSSG contents

The intracellular GSH level was initially elevated ($p < 0.05$) by As_2O_3 (5 μM) and $NaAsO_2$ (10 μM) treatments at 24 h, then continuously increased ($p < 0.05$) at arsenic concentrations as low as 1 μM at 72 h (fig. 3A). The stimulatory effect of Na_2HAsO_4 on the GSH level was only observed at 72 h ($p < 0.05$). There was no difference ($p > 0.05$) in GSH content among arsenic compounds at 48 h (data not shown). The intracellular GSSG level was initially elevated ($p < 0.05$) by As_2O_3 and $NaAsO_2$ treatments at concentrations as low as 1 μM at 24 h, then increased ($p < 0.05$) continuously at 48 h and 72 h (fig. 3B). The effect of Na_2HAsO_4 on the increased GSSG level was observed only at higher arsenic levels (10 and 20 μM) at 72 h ($p < 0.05$).

Effect of arsenic compounds on the activity of antioxidative enzymes

The GPX activity in PAECs (fig. 4A) was increased by As_2O_3 (1 μM) or $NaAsO_2$ (5 μM) treatment only at 72 h ($p < 0.05$), but was not affected by Na_2HAsO_4 treatment ($p > 0.05$). The GR activity in PAECs (fig. 4B) was not affected by arsenic treatment at 72 h ($p > 0.05$). The GST activity in PAECs (fig. 4C) was increased by As_2O_3

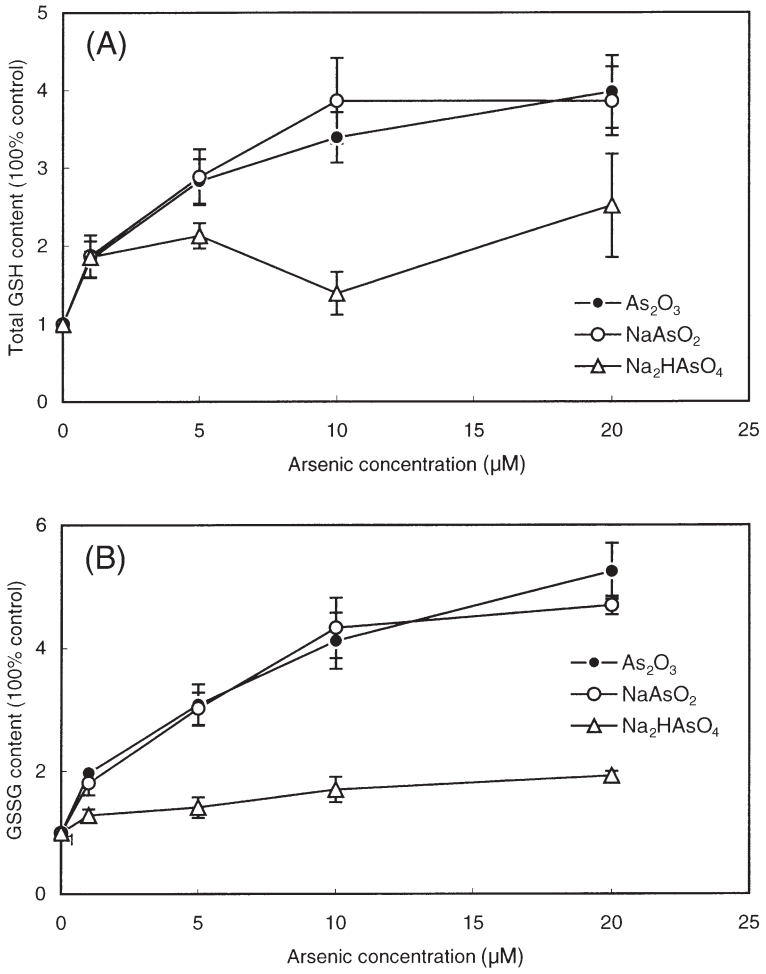

Figure 3. The effect of arsenic compounds (As_2O_3, $NaAsO_2$ and Na_2HAsO_4) on total GSH levels (*A*) and intracellular GSSG levels (*B*) in PAECs at 72-h exposures. Each data point represents the mean ± SE of three replicates.

(20 μM) and $NaAsO_2$ (10 and 20 μM) treatments at 72 h ($p < 0.05$), but no difference was observed in Na_2HAsO_4 treatment groups ($p > 0.05$). There was no significant effect ($p > 0.05$) on antioxidative enzyme activity among arsenic treatments at 24 or 48 h (data not shown).

Western blotting analysis on antioxidative enzymes

The results of Western blotting analysis are shown in figure 5 and table 1. The protein level for Cu/Zn-SOD (table 1) was not affected by 20 μM arsenic compounds at any treatment period ($p > 0.05$). The trivalent arsenic compounds at 20 μM did not affect the protein level for GST-pi (table 1), while 20 μM Na_2HAsO_4 treatment for 48 h increased the protein level for GST-pi ($p < 0.05$). Arsenic treatment at 20 μM for 48 h (table 1) significantly increased the protein level for GPX ($p < 0.05$).

Effect of arsenic compounds on cellular arsenic contents

The cellular arsenic content (fig. 6) was increased by 5 μM As_2O_3 at 48 and 72 h ($p < 0.05$), but decreased by 10 and 20 μM As_2O_3 at 48 and 72 h ($p < 0.05$) compared to levels at 24 h. There was no significant difference ($p > 0.05$) in cellular arsenic contents following $NaAsO_2$ or Na_2HAsO_4 treatment at all durations.

Discussion

Cytotoxicity assays indicated that sodium arsenite and arsenic trioxide demonstrated similar cytotoxicity on PAECs at concentrations lower than 40 μM and PAECs treated with 20 μM As_2O_3 and $NaAsO_2$ showed similar survival rates. Sodium arsenate did not induce cytotoxicity on PAECs. This may be due in part to the low cellular uptake of sodium arsenate which competes with phosphates for cellular transport [39, 40]. As_2O_3 and $NaAsO_2$ are trivalent arsenic compounds; however, the oxidative stress induced by these two arsenic compounds is different. $NaAsO_2$ induced an increase in SOD activity resulting in elevated H_2O_2 production in PAECs, whereas the

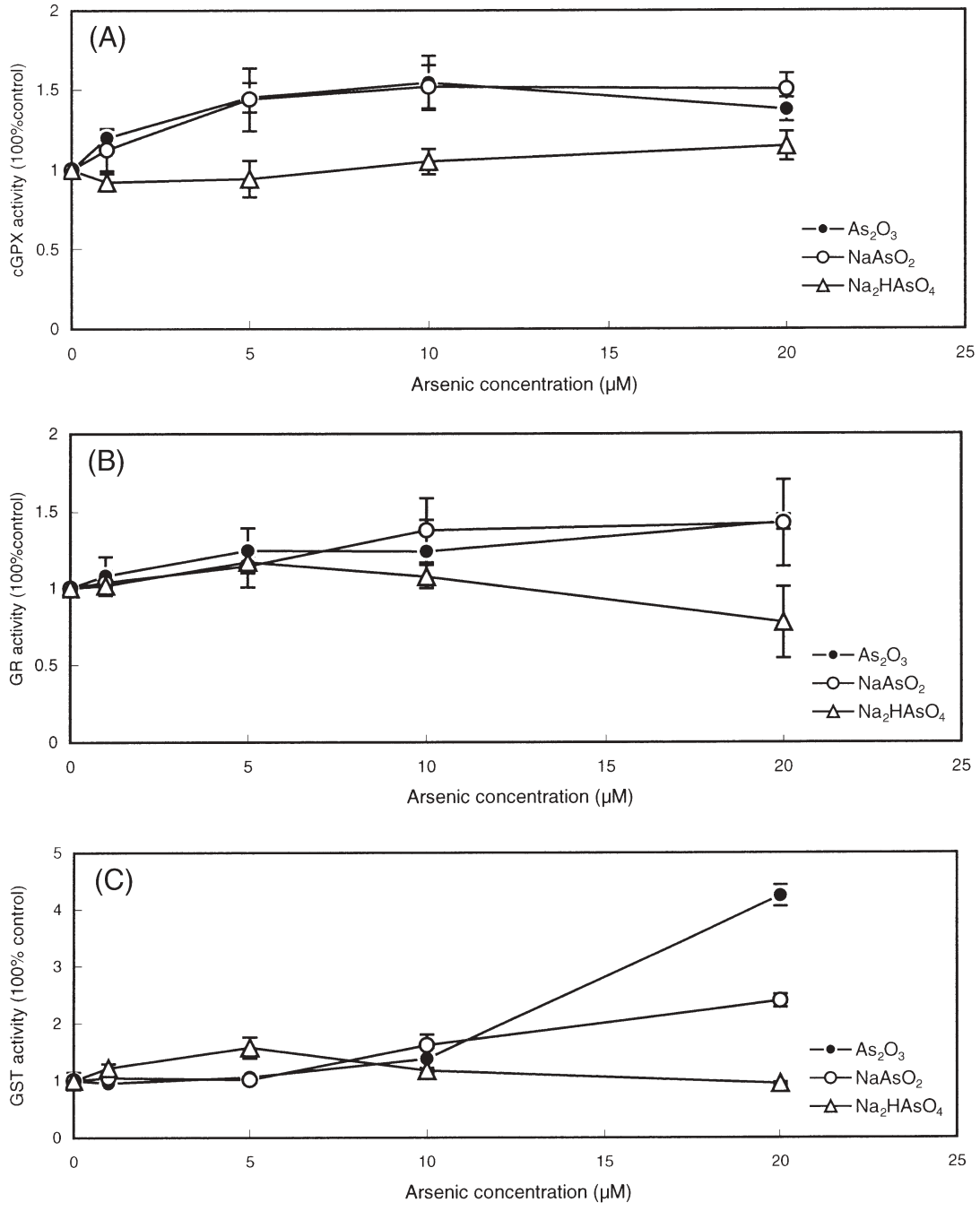

Figure 4. The effect of arsenic compounds (As_2O_3, $NaAsO_2$ and Na_2HAsO_4) on cGPX (*A*), GR (*B*), and GST (*C*) activities in PAECs after 72 h exposure. Each data point represents the mean ± SE of three replicates.

oxidative stress induced by As_2O_3 and Na_2HAsO_4 appeared to be H_2O_2 and SOD independent. The increased SOD activity by $NaAsO_2$ treatment may result from post-translational regulation since the protein level of SOD was not altered by arsenic treatment.

The intracellular GSSG level is an indicator of cellular oxidative stress. Both trivalent arsenic compounds, As_2O_3 and $NaAsO_2$, at a concentration as low as 1 μM for 24 h induced cellular oxidative stress. This resulted in an elevated intracellular GSSG content caused by increased cGPX activity. The oxidative stress increased continuously up to 72 h. Although pentavalent arsenic compounds are regarded as less toxic than the trivalent arsenic compounds [2], a high-concentration (10 and 20 μM) Na_2HAsO_4 treatment for 72 h induced cellular oxidative stress, which resulted in an increased GSSG level. The increased cGPX activity associated with the increased GSSG level by arsenic compounds may result from in-

Table 1. Scan units from Western blotting analysis (fig. 5) for SOD, GST-pi and GPX.

	Control	Arsenic trioxide (As_2O_3)	Sodium arsenite ($NaAsO_2$)	Sodium arsenate (Na_2HAsO_4)
SOD				
24 h	115 ± 2	110 ± 1	107 ± 2	114 ± 3
48 h	118 ± 4	151 ± 19	145 ± 3	132 ± 9
72 h	139 ± 8	172 ± 18	194 ± 29	191 ± 20
GST-pi				
24 h	151 ± 12	154 ± 6	136 ± 4	120 ± 3
48 h	112 ± 0	119 ± 6	138 ± 11	175 ± 10*
72 h	180 ± 10	168 ± 3	192 ± 4	154 ± 9
GPX				
24 h	134 ± 10	137 ± 0	135 ± 2	148 ± 10
48 h	148 ± 9	181 ± 5*	194 ± 3*	178 ± 9*
72 h	210 ± 12	231 ± 14	207 ± 8	182 ± 7

Each data point represents means ± SE of three replicates. An asterisk indicates a significant difference ($p < 0.05$) compared to the control among treatments for the same duration.

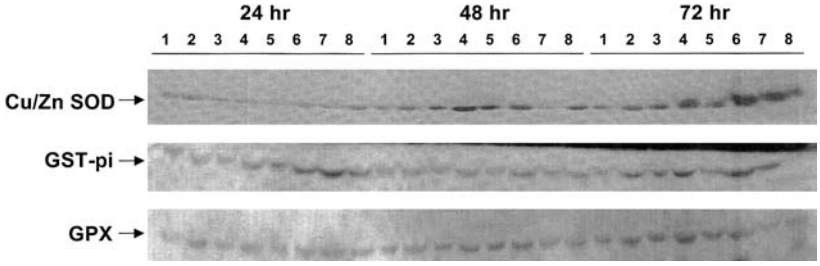

Figure 5. Western blot analysis for Cu/Zn-SOD, GST-pi and GPX in PAECs treated with different arsenic compounds for 24, 48 and 72 h exposures. Lanes 1, 2, control group without arsenic treatment; lanes 3, 4, 20 µM As_2O_3-treated group; lanes 5, 6, 20 µM $NaAsO_2$-treated group; lanes 7, 8, 20 µM Na_2HAsO_4-treated group.

creased GPX gene translation, resulting in an elevated GPX protein level.

One of the major mechanisms postulated for arsenic detoxification by living organisms involves GSH-dependent methylation and protein binding [41]. GSH plays a critical role in both the enzymatic and nonenzymatic reduction of pentavalent trivalent to arsenicals and in the complexation of arsenicals to form arsenothiols during the methylation process [42]. Recent evidence indicates that methylated metabolites generated during biotransformation of arsenic compounds are toxic both in vivo and in vitro [43, 44], and these metabolites inhibit reduction of GSSG, resulting in an altered redox status of cells [45]. Thus, whether biomethylation of arsenic compounds is beneficial to living organisms as the major detoxification pathway for arsenic is not clear. Complexing inorganic trivalent arsenic with GSH produces arsenotriglutathione that can inhibit purified yeast GR in vitro [32]. The inhibitory effect on GR could impair the GSH redox cycle [45]. Arsenic exposure did not affect GR activity in this study, which may result from the inhibitory effect of arsenotriglutathione.

GSH is an important antioxidant in living organisms and elevated GSH levels appear to be the defense response against arsenic-induced oxidative stress [46, 47]. The trivalent arsenic compounds, As_2O_3 and $NaAsO_2$, first increased the intracellular GSH level at 24 h and then continuously up to 72 h. Na_2HAsO_4 increased the GSH level only after 72 h treatment. Arsenic treatment did not affect GR activity; thus, the increased total GSH content in response to the oxidative stress by arsenic compounds may be related to the regulation of GSH turnover.

Some metals or toxins are taken up or removed from cells by GSH-mediated pathways [48]. GSH and arsenic form a complex and are exported outside the cells to reduce metal toxicity [49]. The trivalent arsenic compounds, As_2O_3 and $NaAsO_2$, but not Na_2HAsO_4, increased GST activity in PAECs at 72 h. GST was involved in the conjugation of GSH with arsenic, and thus increased GST activity by trivalent arsenic compounds suggests that an elevated intracellular GSH level in response to the oxidative stress may be used to conjugate arsenic in PAECs and facilitate the efflux of arsenic.

The intracellular arsenic levels after exposure to different arsenic compounds were not the same. There was no change in cellular arsenic contents by $NaAsO_2$ or Na_2HAsO_4 treatment over 72 h regardless of arsenic concentration, whereas, the cellular arsenic contents follow-

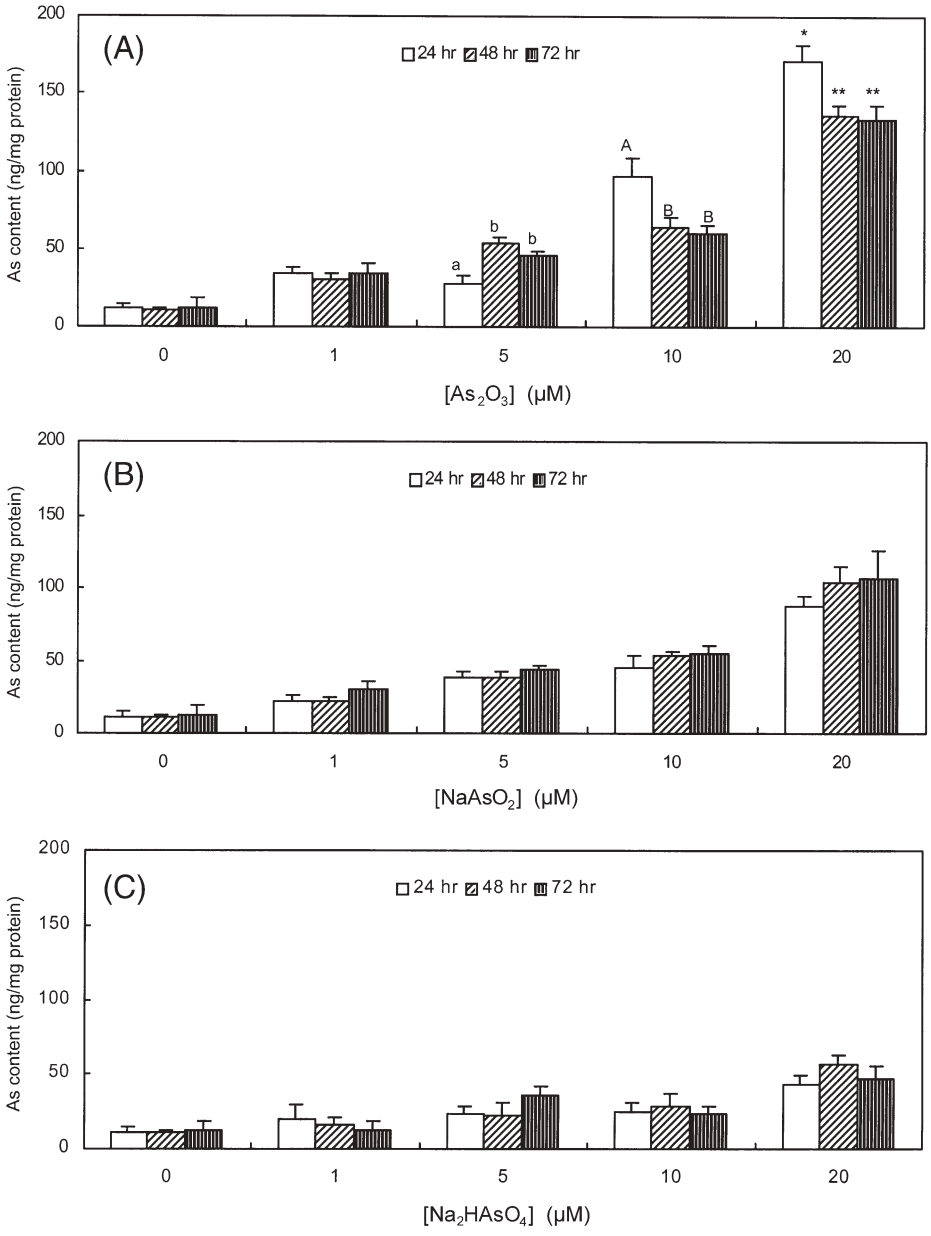

Figure 6. The intracellular arsenic contents in PAECs exposed to different concentrations of As_2O_3 (*A*), $NaAsO_2$ (*B*) and Na_2HAsO_4 (*C*). Each data point represents the mean ± SE of three replicates. Different letters and symbols indicate significant differences within each treatment concentration for a given time period ($p < 0.05$).

ing As_2O_3 treatment were different over time: they were increased by As_2O_3 treatment at low concentration (5 μM) for 48 and 72 h, but were decreased at high concentration (10 or 20 μM) for 48 and 72 h compared to that for 24 h. These results suggest that As_2O_3 treatment at a high concentration may induce the active efflux system to facilitate the exclusion of intracellular arsenic out of the cellular compartment. Moreover, the cellular arsenic contents for Na_2HAsO_4 treatment were approximately one-half that for $NaAsO_2$ treatment at the same concentration. This may result from the low cellular uptake of Na_2HAsO_4 due to its competition with phosphates for cellular transport [39] and/or the induction of an active efflux system by Na_2HAsO_4 treatment, which contributes to the less toxic effect of Na_2HAsO_4 exposure.

The in vitro effect of arsenic compounds on various purified antioxidative enzymes and cell lysates has been reported and the results in this study incubating arsenic compounds with partially purified enzymes in vitro did not indicate the direct interaction between arsenic compounds used in this study (20 μM) and the GSH-related enzymes, GPX, GR and GST (data not shown), consistent with findings from other researchers [40, 45]. Therefore, the cellular regulation of GSH-related antioxidative en-

zymes by arsenic compounds in this study did not result from the direct chemical interaction between arsenic and enzymes. This implies that the GSH-related antioxidative enzymes may not be the direct targets of arsenic intoxication. There were indications that apoptosis induced by arsenic was related to the cellular GSH redox system, and this induced apoptosis in leukemic cells is dependent on the activity of enzymes regulating cellular hydrogen peroxidase levels [12, 13]. Based on the results in this study, different arsenic compounds appeared to regulate these antioxidative enzymes at different stages during gene expression.

However, living organisms are complicated and the change in GSH level observed during cellular oxidative stress may represent either a single or combination of effects through the various mechanistic paths. In light of these finding, further investigation of other mechanisms related to arsenic detoxification is needed, including but not limited to changes in GSH turnover and the GSH efflux system by arsenic compounds. After understanding the mechanisms involved in the arsenic-induced oxidative stress, arsenic-induced toxicity may be modulated.

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