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Lifelong inorganic arsenic compounds consumption affected blood pressure in rats

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Abstract

Chronic arsenic exposure is a known risk factor for cardiovascular disease and has a strong correlation with hypertension. Oxidative stress may be one of the major contributors to arsenic-induced hypertension. To investigate the antioxidative and CYP systems through which inorganic arsenic compounds may contribute to blood pressure elevation in rats, we administered 50 ppm arsenic (as arsenite and arsenate) in drinking water to Wistar rats for 200 successive days. Systolic blood pressure was determined every 20 days, and blood samples and tissues were collected at each time point for biological analysis. Compared to the control group, weight gain in the arsenic-exposed animals was slightly but significantly lower, whereas the relative weights of the various tissues was higher. Blood pressure was elevated until day 80 in both arsenic groups followed by a time-dependent change in the antioxidative enzyme system. The hypertensive effect remained until day 200 for arsenite when the change by arsenate was minimized. Patterns of antioxidative enzyme change differed between arsenite and arsenate. However, the most common marker of hypertension, the angiotensin-converting enzyme, showed no significant change in either arsenic group. CYP4A was highly expressed in both arsenic groups, particularly in the arsenite group. These results indicate that low but chronic arsenic exposure might cause elevated blood pressure and antioxidative interference. Furthermore, CYP4A might be more important than ACE in contributing to arsenic-induced hypertension.

Keywords: Arsenite; Arsenate; Hypertension; Angiotensin-converting enzyme; CYP4A; 20-HETE

1. Background

Arsenic is a notorious human toxicant that is derived from the natural environment. Many epidemiological studies have revealed that people are more likely to have vascular diseases when living for many years in areas where aquifers or well water are contaminated with inorganic arsenic (as arsenite and arsenate) (Lilienfeld, 1988; Engel et al., 1994; Chiou et al., 1997). High prevalence of carotid atherosclerosis, ischemic heart disease, and cerebrovascular disease have been reported for chronic exposure to arsenic in water (Chen et al., 1996; Chiou et al., 1997; Wang et al., 2002). As hypertension is one of the major risk factors for vascular diseases, several studies further indicate that subsequent arsenic exposure significantly increases hypertension prevalence

Abbreviations: 20-HETE, 20-hydroxyeicosatetraenoic acid; ACE, angiotensin-converting enzyme; BCIP/NBT, 5-bromo-4-chloro-3-indolyl phosphate alkaline phosphatase substrate; CYP, cytochrome P450; ELISA, enzyme-linked immunosorbent assay; FAP, furylacrylloylphenylalanine; FAPGG, *N*-[3-(2-furyl)acrolyl]-L-phenylalanine; GP, glutathione peroxidase; GSSG, oxidized glutathione; GPx, glutathione peroxidase; HRP, horseradish peroxidase; NO, nitric oxide; MDA, malondialdehyde; PBST-20, phosphate-buffered Tween 20; PMSF, phenylmethylsulfonyl fluoride; RAA, renin–angiotensin–aldosterone; SBP, systolic blood pressure; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; XO, xanthine oxidase.

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(Rahman et al., 1999; Tseng, 2002; Simeonova and Luster, 2004; Chen et al., 2007). Studies have also reported that residents living in arsenic-polluted areas have statistically higher blood pressure (Chen et al., 1995; Rahman et al., 1999).

Because chronic arsenic toxicity interferes with more than 200 enzymes in humans (Ratnaike, 2003) and considering the intricate etiology of hypertension, mechanisms of arsenic effect on the precursory process of vascular dysfunction–hypertension remain a puzzle. Many biological factors contribute to hypertension such as the renin–angiotensinogen system and some cytochrome P450-derived (CYP) arachidonic acid metabolites (i.e., CYP4A catalyzed production of 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acid) have garnered attention for regulating blood pressure and the pathology of chronic cardiovascular disease (Honeck et al., 2000; Fleming, 2001). For example, one of the possible mechanisms for inorganic arsenic causing vascular dysfunction is that it causes oxidative stress which might lead to hypercoagulation, endothelial injury, and smooth muscle cell proliferation (Tseng, 2002). Most studies have focused on the vascular changes caused by arsenite (Jiang et al., 2002); however, few studies have mentioned the effects of chronic arsenic exposure on hypertension via tissue biomarker changes, such as the rennin and oxidative defensive system. Using a lifelong non-lethal dosage of arsenic in rats, we assessed the antioxidative and CYP systems as potential pathways by which inorganic arsenic compounds may contribute to blood pressure elevation.

2. Materials and methods

2.1. Experimental animals

Twenty-four 6-week-old male Wistar rats were housed and acclimated in stainless steel cages at room temperature (23 ± 2 °C) and at a 12-h light/dark cycle. Laboratory rodent chow (Labdiet® #5001 rodent chow, US) and water were provided ad libitum. After 1 week, separate groups of rats were provided with 50 ppm sodium arsenite or sodium arsenate (Sigma Chemical, St. Louis, MO), or pure water as the control ($n = 8$) in their drinking water for six months. The amount of water consumed was recorded every day; the body weight gain was observed every week for each animal. Tail-cuff detection of systolic blood pressure (SBP) was measured every 20 days until day 120 and tail venous blood was collected every 40 days until the end of the experiment. After six months, all animals were sacrificed, the kidney and liver were sectioned, and abdominal blood was collected for further analysis.

2.2. Systolic blood pressure determination

Animals were kept in a quiet, humidity- and temperature-stable room with stainless cages. The non-invasive blood pressure analyzer system (Model 179, IITC, US) was used to detect the tail SBP of the rats under overnight fasting every 40 days at 4:00 PM. The diagnostic values were recorded as means of three consecutive time determinations for each time interval.

2.3. Angiotensin-converting enzyme (ACE) activity and protein production

A commercial enzyme-linked immunosorbent assay (ELISA) kit (cat. no. ACE100, Chemicon, Temecula, CA) was used for ACE protein detection. Plasma samples and tissue homogenates were loaded into a 96-well plate which was coated with anti-ACE, and a horseradish peroxidase (HRP)-conjugated second antibody was added to produce color representing the amount of ACE protein at a wavelength of 450 nm (Labsystems Multiskan RC., Haverhill, MA). ACE activity was analyzed by converting, *N*-[3-(2-furyl)acrolyl]-L-phenylalanine (FAPGG), a substrate of ACE, to furylacrylloylphenylalanine (FAP) and glycylglycine. Because FAPGG has a maximal absorbance at 340 nm (by an ELISA reader), decreased absorbance of FAPGG represents the ACE activity (cat. no. 305-10, Trinity Biotech, Wicklow, Ireland) (Labsystems Multiskan RC.).

2.4. Activities of antioxidant enzyme systems

Glutathione peroxidase (GP), through the catalysis of glutathione oxidation, produces oxidized glutathione (GSSG) in whole blood and liver. In the presence of NADPH, GSSG reduces to glutathione and makes $NADP^+$ from NADPH, which is detectable at a wavelength of 340 nm (RANSEL, Randox Laboratories, UK) by spectrophotometry (He_λ IOS_α, Thermo Electron Corporation, US). The commercial analytical kits (RANSOD, Randox Laboratories) use xanthine and xanthine oxidase (XO) to produce superoxide radicals which are either consumed by superoxide dismutase (SOD) in specimens or react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The dye can be detected ay 505 nm by spectrophotometry (He_λ IOS_α, Thermo Electron Corporation). SOD activity was measured by the degree of inhibition of the reaction. After the catalysis of XO, xanthine turns into the superoxide anion (O^{2-}) and uric acid. In the presence of HRP, the remainder produces a red color that can be determined with the Amplex red reagent. We used a fluorescence ELISA reader to detect (excitation/emission) the red color expressed as units/g protein (model 1420, Victor2TM Multilabel Counter, Perkin–Elmer, Woodbridge, ON, Canada). For the determination of catalase (CAT), methanol was used to form hydrogen peroxide in samples which can be hydrolyzed by CAT. The remaining unhydrolyzed hydrogen peroxide reacts with 4-amino-3-hydrainzino-5-mercapto-1,2,4-triazole to form purplish formaldehyde which is detected at 540 nm and 25 °C by spectrophotometry (He_λ IOS_α, Thermo Electron Corporation). When free radicals attack polyunsaturated fatty acids, lipid peroxidation is evoked by a chain reaction. Its secondary product, malondialdehyde (MDA), reacts with thiobarbiturates (TBARs) and a red reactive substance forms that can be detected with fluorescence ELISA (with excitation at 531 nm and emission at 590 nm; model 1420, Victor2TM Multilabel Counter, Perkin–Elmer).

2.5. CYP4A protein expression

Tissue microsomes were obtained according to the protocol of Ishizuka et al. (2003). The tissues (kidney and liver) were sectioned and weighed for CYP4A protein analysis. Each of the sectioned tissues was homogenized with 3 mL sucrose–potassium buffer that contained 250 mM sucrose, 10 mM potassium phosphate buffer (pH 7.7), 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF). After centrifugation at 3000*g* and 4 °C for 5 min, the supernatant was drawn and re-centrifuged at 9000*g* and 4 °C for 15 min, and the final precipitation was obtained after centrifugation at 10,000*g* and 4 °C for 60 min, and redissolved with glycerol potassium buffer containing 100 mM potassium buffer (pH 7.5), 30% glycerol, 1 mM dithiothreitol, and 0.1 mM PMSF. Samples were frozen in liquid nitrogen and stored at −80 °C. The protein concentration was measured by a commercial assay (Bio-Rad DC Protein assay, Hercules, CA) using bovine serum albumin as a standard.

Microsome proteins from tissues were separated by 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (10 × 8 cm) at 100 V for 3.5 h. The protein on the gel was transferred to a polyvinylidene difluoride membrane for 16 h. Afterwards, the membrane was incubated in rabbit polyclonal antiserum, to which rat CYP4A cross-reacts with 4A1, 4A2, and 4A3 (1:3000, BioTrend, Germany), for 2 h. After blocking with

bovine serum albumin and washing with phosphate-buffered Tween 20 (PBST-20), the membrane was immersed in an alkaline phosphatase-coupled secondary antibody (1:5000, Jackson ImmunoResearch, West Grove, PA) for 2 h. The final color was developed by incubation in 5-bromo-4-chloro-3-indolyl phosphate, BCIP/NBT alkaline phosphatase substrate (Sigma Chemical Co., St. Louis, MO). Protein expression was confirmed by triplicate analysis in each group. The relative intensity of CYP 4A was calculated by comparing it to the intensity of actin (Chemicon, Temecula, CA). Image-Pro plus version 4.5 was used for image processing.

2.6. Nitric oxide generation and peroxynitrite production

The final products of NO measured in vivo were nitrate and nitrite. Before measurement, 400 μL of a plasma sample was ultrafiltered through a 10- or 30-kDa molecular weight cut-off filter (Microcon® YM-30, Millipore, US). In addition, hepatic and renal tissues were homogenized in phosphate buffer (pH 7.4), and the supernatants were collected after a series of ultracentrifugation (10,000*g* for 20 min and 100,000*g* for 30 min). Total nitrate and nitrite levels were measured using a commercial colorimetric assay kit (cat. no. 780001, Cayman Chemical, Ann Arbor, MI) for the following processes: nitrate reductase was used to convert nitrate to nitrite with additional Griess reagent for 3 h; nitrite was then converted to a deep purple azo compound with absorbance at 550 nm. Peroxynitrite reacts with the phenolic ring of tyrosine to form nitrotyrosine; a commercial peroxynitrite ELISA kit (Nitrotyrosine-EIA Assay, BIOXYTECH®, Foster City, CA) was used for nitrotyrosine measurement. A solid-phase monoclonal antibody was precoated in a 96-well microplate to capture the antigen, detected with biotin-labeled goat polyclonal antinitrotyrosine. Tetramethylbenzidine substrate was added, and a yellow color was produced (at a wavelength of 450 nm) after streptavidin peroxidase conjugation to the biotin-labeled second antibody which can be detected by an ELISA reader (Labsystems Multiskan RC., Haverhill, MA). Lyophilized nitrotyrosine was reconstituted as the standard to give a standard curve for calculating nitrotyrosine amounts in specimens.

2.7. Determination of serum triglycerides and cholesterol

A commercial kit (cat. No. TR210, Randox Laboratories) was used to determine serum triglycerides. Lipases and glycerol kinase were used for enzymatic hydrolysis, and glycerol-3-phosphate is produced in such reactions. When triglycerides react with glycerol-3-phosphate oxidase, hydrogen peroxide forms and shows a red color in the presence of 4-aminophenazone, detectable at 500 nm (He_{λ} IOS_{α}, Thermo Electron Corporation). The enzymatic endpoint method was used for total cholesterol analysis (cat. no. CH200, Randox Laboratories). After enzymatic hydrolysis and oxidation, hydroxyl peroxide is formed and reacts with 4-aminoantipyrine by which total cholesterol is determined in the presence of the indicator, quinoimine, and detected by spectrophotometry (He_{λ} IOS_{α}, Thermo Electron Corporation) at 500 nm. Also, 0.55 mM phosphotungstic acid and 25 mM magnesium chloride were used for the HDL-cholesterol precipitant (cat. no. CH203, Randox Laboratories) whereas 50,000 IU/L heparin and 0.064 mM sodium citrate were used for the LDL-cholesterol precipitant (cat. no. CH1350, Randox Laboratories). The cholesterol precipitant was determined according the method described above.

2.8. Statistical analysis

We calculated means and standard deviations for the three treatment groups. One-way ANOVA was used to analyze differences between different types of arsenic in water and the control. A p-value of <0.05 represents a statistically significant difference. Linear regression was performed to evaluate the correlation between time and SBP in three groups. Three regression equations were also compared to evaluate the change by arsenics.

3. Results

3.1. Arsenic exposure and growth

In order to investigate the extent of arsenic exposure, we recorded the daily water intake of animals during the experiment. The average daily water intake in the arsenite, arsenate, and control groups were 39.0 ± 2.7, 35.8 ± 3.2, and 36.9 ± 3.0 mL, respectively. There were no significant differences in water intake volume between animals in the three treatment groups. The amounts of inorganic arsenic compounds in both the arsenite and arsenate groups were 1.95 and 1.79 mg on average, respectively. Interestingly, arsenite consumption affected animal growth slightly; however, the arsenate group showed no difference in growth over the 200 days of exposure (Table 1). Organ weights differed significantly in both groups, especially in the arsenite group for which the liver, kidneys, heart, and lungs were significantly heavier than those of the controls; in contrast, the arsenate group presented a significant weight difference only in the liver.

3.2. Blood pressure and ACE

Changes in SBP among groups are shown in Fig. 1a; blood pressure gradually increased with time. Animals that drank arsenic-containing water began to display significantly higher blood pressures on day 80, which continued to increase until day 120. We lack blood pressure data after day 120 due to limitations of the non-invasive blood pressure detection system. We were unable to get the larger tails into the detection sensor ring after day 120. Systolic blood pressure elevation was significant related to time in all three groups (data not shown). With exclusion of aged change, we compared and analyzed the linear regression equations of three groups, the results told that arsenic had a significant different slope (arsenite *vs.* control, $p < 0.0001$; arsenate *vs.* control, $p = 0.0256$) and intercepts (arsenite *vs.* control, $p < 0.0001$; arsenate *vs.* control, $p = 0.0058$, adjusted means) from controls (Fig. 1b). Furthermore, we also found arsenite mainly influenced after 60 days treatments to the end of the experiment (slope: arsenite *vs.* control, $p < 0.0001$; arsenate *vs.* control, $p = 0.159$; intercepts: arsenite *vs.* control, $p < 0.0001$; arsenate *vs.* control, $p = 0.0012$, adjusted means); whereas arsenate affected before 60 days (slope: arsenite *vs.* control, $p = 0.3351$; arsenate *vs.* control, $p = 0.0204$; intercepts: arsenite *vs.* control, $p = 0.0693$; arsenate *vs.* control, $p = 0.0051$, adjusted means). Effects of arsenic on ACE activity remained consistent in most of the collected organs except the liver.

3.3. Antioxidative status

Data of plasma antioxidative enzyme activities were presented as percentage changes within the experiment (Fig. 2). Changes of SOD activity were significant higher in arsenite group than controls in 0–120 days and declined

Table 1
Mean body weight change, tissue weight, systolic blood pressure, and ACE protein activity for the experimental groups

	Control	Arsenate	Arsenite
Body weight gain (g)	169.9 (13.5)	196.1 (16.1)	115.7 (17.5)*
Organ weight			
Liver			
Wet weight (g)	8.7 (0.5)	12.7 (0.8)*	10.2 (1.1)
Relative weight (%)	2.1 (0.10)	2.8 (0.10)*	2.9 (0.20)*
Kidneys			
Wet weight (g)	1.2 (0.1)	1.4 (0.1)*	1.2 (0.1)
Relative weight (%)	0.29 (0.01)	0.31 (0.01)	0.35 (0.00)*
Heart			
Wet weight (g)	1.2 (0.0)	1.3 (0.1)*	1.1 (0.1)
Relative weight (%)	0.29 (0.01)	0.28 (0.00)	0.31 (0.01)*
Lungs			
Wet weight (g)	1.9 (0.1)	1.8 (0.1)	2.1 (0.1)
Relative weight (%)	0.46 (0.02)	0.41 (0.01)	0.57 (0.01)*
Serum ACE protein (ng/mL)	189.6 (14.1)	165.9 (11.3)	182.2 (9.6)
ACE activity			
Lung (U/g protein)	5.19 (0.65)	4.37 (0.79)	5.65 (1.66)
Liver (mU/g protein)	18.1 (3.4)	11.1 (2.9)*	10.7 (2.7)*
Kidney (mU/g protein)	751.3 (104.5)	773.5 (132.8)	672.0 (82.5)

Values are presented as the mean ± SE.
Relative weight (%) = (organ wet weight (g)/100 g body weight) × 100.
* $p < 0.05$ comparing to control group.

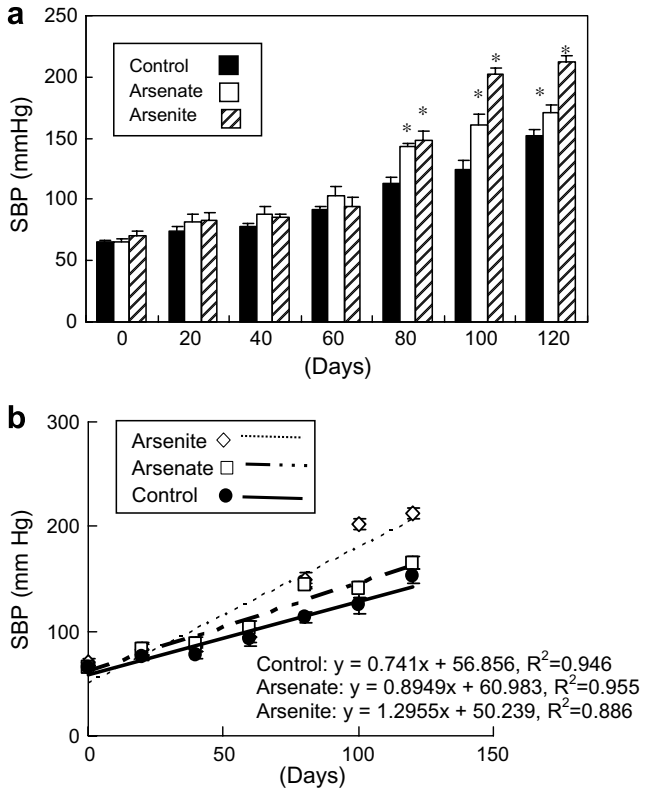

Fig. 1. Systolic blood pressure (SBP) changes with chronic arsenic exposure. (a) SBP for the length of the study for the three groups; (b) linear regression of SBP during 120 days in the three groups. (*Significant difference between groups at the same time interval ($p < 0.05$).)

at day 120 in the arsenite group (Fig. 2a). Changes of GPx activity were significant lower in arsenate group than arsenite and controls during the treatment. (Fig. 2b). Changes of catalase activity in all groups were declined during the experiment, however, there was a significant lower trend showed in arsenate group (40 days, $p < 0.05$) then in arsenite group (120–200 days, $p < 0.05$) while compared with controls (Fig. 2c). Each enzyme's activity fluctuated during the 200-day experimental period, even in the control group. Table 3 revealed plasma antioxidative enzyme activity during the study. SOD activity was lower on days 40 and 80 in the arsenite group, then continued to decline after day 80 with an increase in blood pressure. GPx activity was higher on day 40, and this trend continued until day 200 in the arsenite group. Interestingly, CAT activity increased on day 120 with arsenate consumption.

3.4. Plasma and hepatic lipids

Both arsenite and arsenate consumption affected lipid profiles; however, the mechanism seemed to act in different ways via alterations in the antioxidative status. Plasma lipids were subsequently elevated on days 160 and 200 in both arsenic groups (Fig. 3a and b). In addition, lipids did not accumulate in the liver of the arsenic groups. The hepatic cholesterol and triglyceride levels were lower in the arsenic groups than in the controls (Table 2). Meanwhile, the increased MDA production showed on day 120 in arsenate group and elevated on days 160 and 200 in arsenic treated groups statistically (Fig. 3c).

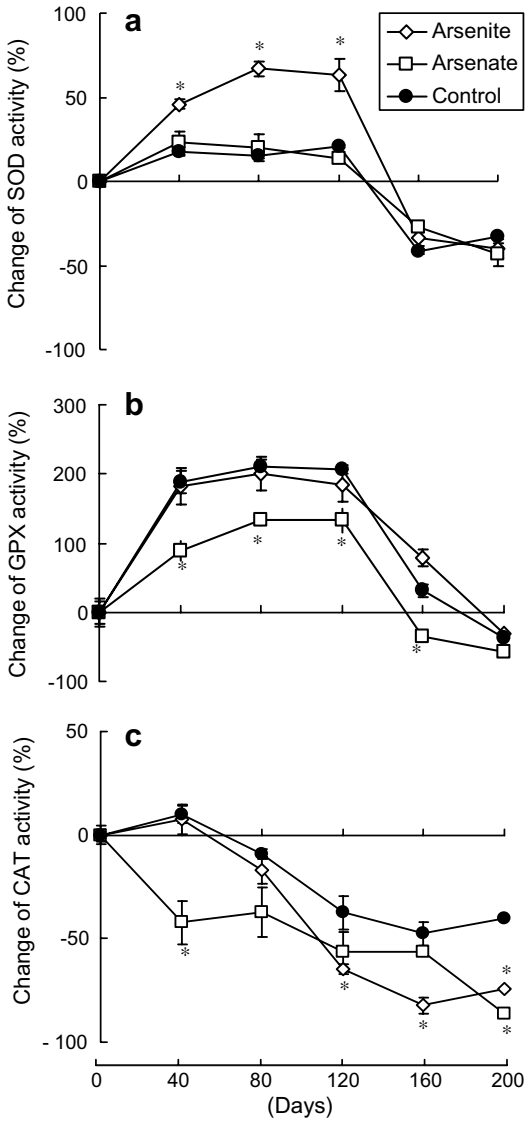

Fig. 2. Percentage changes of plasma antioxidative enzyme systems against day 0 in each group. (a) Superoxide dismutase; (b) glutathione peroxidase; (c) catalase. Numbers on *Y*-axis represented different duration within the study. (*Significant difference from controls at the same time point ($p < 0.05$).)

3.5. CYP4A expression, nitric oxide formation, and nitrotyrosine levels

In addition to ACE, CYP4A is another possible candidate for a hypertensive marker (Huang et al., 2005). Fig. 4 shows significant increase in expression of the CYP4A protein with arsenite exposure in hepatic and renal tissues. This is the first report that lifelong arsenic exposure is highly and positively related to CYP4A protein expressions in liver and kidney. The results showed significantly lower levels of NO in the livers of the arsenite group (Table 2). In terms of NO metabolism, arsenite possessed high modulation for NO production. In the arsenite group, circulating and hepatic levels of NO were dramatically lower, whereas renal NO levels were increased. Nitrotyrosine represents the amount of peroxynitrite which is formed when free radicals react with NO. There were no consistent results of nitrotyrosine contents with NO production.

4. Discussion

The etiology of primary hypertension is believed to be a multifactorial process. Chronic arsenic exposure is considered to be an oxidative stress, and is thought to be a risk factor for hypertension (Wang et al., 2002; Lee et al., 2003). However, no animal studies have explained how chronic arsenic exposure affects the blood pressure system or have verified whether a primarily hypertensive animal model by arsenic can be established. Herein, we report evidence that, after adjusting for age, lifelong arsenite/arsenate consumption causes higher SBP. It is noteworthy that arsenic caused larger amplitude of change due to the significantly larger change slope (Fig. 2b). The increasing trend persisted in the arsenite group suggesting that arsenite may produce an irreversible change in blood pressure. Thus, this tendency implies that age, arsenic exposure, and other factors may contribute to the phenomenon of a fluctuating increase in the SBP. Interestingly, while comparing the regression equations of treatment groups that arsenate impact the blood pressure system before 60 days and arsenite influenced the system later. These phenomena might imply the influence on SBP by arsenic was varied from species. The time-matched relationship between SBP and changes of antioxidative enzyme activity should be considered.

Inorganic arsenic compounds may contribute to abnormal blood pressure which may ultimately lead to cardiovascular disease (Lee et al., 2003). The increased production of free radicals and impaired individual antioxidative enzymes have been well documented (Gurr et al., 2003). In this study, we observed nearly continuous changes in antioxidative enzymes. Although changes in the oxidative systems had no significant correlations with blood pressure, time-dependent variations did develop. Some data suggest that superoxide anion production is increased in blood vessels of animals exposed to arsenic compounds (Gurr et al., 2003; Pi et al., 2003). SOD plays a role in the conversion of superoxide to hydrogen peroxide. Both GPX and CAT react with hydrogen peroxide, however, GPX is more sensitive than CAT. GPX reacts under lower oxidative stress, while CAT acts under higher stress (Redon et al., 2003). This might explain why GPX more readily reacted to the stress caused by arsenic. In addition, glutathione is a major substrate for arsenate reduction in hepatic tissues (Aposhian et al., 2004). To meet the challenge of the extra demand for arsenate conversion, more and urgent GPX activity was expressed, whereas arsenite does not have the same requirement. Consequently, activity changes in GPX with arsenite exposure could simply be a response to oxidative stress. In addition, plasma CAT activity was elevated in an effort to balance the increasing depletion of GPX. Furthermore, the results

Table 2
Antioxidative enzymes, nitric oxide and peroxynitrite generation activities in the experimental groups

	Control	Arsenate	Arsenite
Liver			
SOD[a] (U/mg protein)	19.1 (1.1)	24.5 (4.1)	8.6 (2.6)*
GPx[b] (U/100 mg protein)	60.6 (9.8)	163.1 (41.2)*	95.4 (24.0)*
CAT[c] (nmol/min/mg protein)	16.8 (0.6)	24.1 (1.1)*	26.1 (2.6)*
XO[d] (U/g protein)	2.5 (0.1)	3.0 (0.4)*	2.0 (0.5)
MDA[e] (nmol/mg protein)	12.3 (0.6)	21.3 (2.3)*	19.7 (2.5)*
Cholesterol (μmol/g)	6.75 (0.38)	5.49 (0.26)	4.75 (0.45)*
Triglyceride (μmol/mg protein)	0.17 (0.01)	0.12(0.03)	0.07(0.01)*
Nitric oxide (μM)	9.0 (0.4)	7.5 (0.7)*	6.8 (0.6)*
Nitrotyrosine (μM)	2.19 (0.04)	2.45 (0.08)*	1.89 (0.19)*
Plasma			
Nitric oxide (μM)	5.9 (0.3)	5.8 (0.2)	4.4 (0.1)*
Nitrotyrosine (μM)	1.52 (0.02)	1.62 (0.04)	1.55 (0.08)
Kidney			
Nitric oxide (μM)	11.8 (0.4)	13.1 (1.5)*	13.0 (0.6)*
Nitrotyrosine (μM)	2.05 (0.12)	2.11 (0.07)	1.76 (0.01)*

Values are presented as the mean ± SE.
* $p < 0.05$ comparing to control group.
[a] SOD, superoxide dismutase.
[b] GPx, glutathione peroxidase.
[c] CAT, catalase.
[d] XO, xanthine oxidase.
[e] MDA, malondialdehyde.

Table 3
Plasma antioxidative enzyme activity during the experiment

	Days	0	40	80	120	160	200
SOD[a] (U/mL)	Control	211.0 ± 19.2	284.2 ± 5.2	343.4 ± 15.6	239.6 ± 10.1	135.8 ± 4.8	153.6 ± 12.0
	Arsenate	217.2 ± 12.2	268.6 ± 9.8	286.7 ± 15.0*	263.4 ± 23.3	158.9 ± 6.3	150.9 ± 10.4
	Arsenite	225.3 ± 12.4	256.4 ± 5.4*	257.2 ± 10.8*	287.8 ± 13.6	133.6 ± 13.9	153.2 ± 7.0
GPX[b] ($\times 10^3$ U/L)	Control	18.1 ± 1.9	46.6 ± 2.7	58.2 ± 2.8	52.3 ± 3.6	46.7 ± 8.4	28.80 ± 2.2
	Arsenate	23.5 ± 1.5	40.7 ± 2.4*	48.2 ± 3.2*	51.3 ± 7.8	44.0 ± 1.8	14.8 ± 2.3*
	Arsenite	21.6 ± 2.6	44.8 ± 3.3	52.5 ± 2.5	46.7 ± 2.4	37.6 ± 2.8*	26.2 ± 6.4*
CAT[c] (nmol/min/mL)	Control	127.6 ± 22.7	95.4 ± 26.6	100.0 ± 2.2	80.1 ± 10.9	85.0 ± 11.8	87.6 ± 18.1
	Arsenate	160.0 ± 18.8	120.2 ± 13.7	130.0 ± 14.8	161.4 ± 27.8*	120.0 ± 22.7*	171.5 ± 32.5*
	Arsenite	143.8 ± 15.9	141.9 ± 15.4	120.0 ± 5.1	90.7 ± 13.1	100.0 ± 2.24	160.6 ± 27.4*

Values are presented as the mean ± SE.
* $p < 0.05$ comparing to control group.
[a] SOD, superoxide dismutase.
[b] GPx, glutathione peroxidase.
[c] CAT, catalase.

revealed high specificity of trivalent and pentavalent arsenic in anitioxidative enzymes. Arsenite was more sensitive to SOD whereas arsenate was sensitive to GPX. As we did not evaluate whether the liver functioned normally in the study, further research is needed to clarify this point.

Whether arsenic exposure increases NO production still remains controversial. Pineda-Zavaleta reported that the effects on NO formation and NOS activity were altered under high-level arsenic exposure (Pineda-Zavaleta et al., 2004). Some studies suggested that arsenite might increase NO, as well as peroxynitrite (the product of nitric oxide and the superoxide anion) generation (Bunderson et al., 2004; Chien et al., 2004) in hamster ovary cells, neonatal rat brain cells, human fetal brain, and bovine aorta endothelial cells, although there were no effects on rat aortic smooth muscles and hepatocytes (Gurr et al., 2003). In our study, however, the arsenite group showed significantly lower hepatic levels of NO, but not nitrotyrosine. These findings imply that other mechanisms might be involved with arsenic-stimulated NO metabolism in different types of cells.

The renin–angiotensin–aldosterone system (RAA system) is one of the crucial systems for blood pressure regulation (Sharifi et al., 2004). Renin and ACE are the two important substances within the system. Increased production of angiotensin enhances renal reabsorption and blood vessel contraction causing high blood pressure; thus, ACE

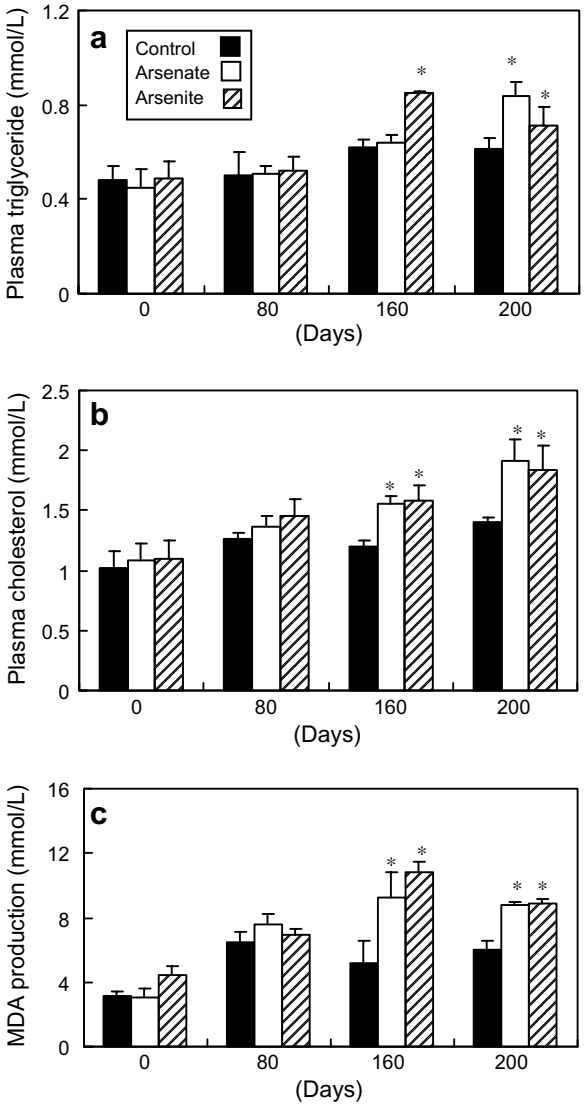

Fig. 3. Effects of arsenic exposure on serum lipids: (a) triglyceride; (b) cholesterol; (c) lipid peroxidation. (*Significant difference from controls at the same time point ($p < 0.05$).)

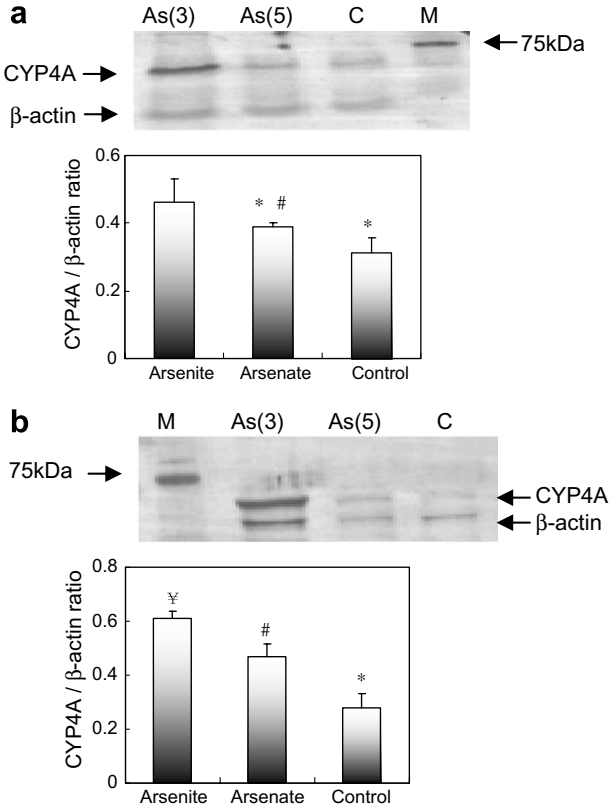

Fig. 4. CYP4A protein expression with different durations of arsenic exposure: (a) liver and (b) kidneys. The figures are based on calculation of the means and standard deviations of the results of three different membranes: As(3), arsenite; As(5), arsenate; C, control; M, marker. (*,#,¥Significant difference between groups ($p < 0.05$).)

can serve as a biomarker of hypertension (Miyazaki et al., 1988). Interestingly, there were no significant differences in renal ACE activities between the arsenic and control groups or even in the lungs, in this study. In hepatic tissues, ACE activity conspicuously declined in both arsenic groups. As described by a previous study, angiotensin II participates in hepatic inflammation and fibrosis (Kanno et al., 2005), and further evidence indicated that an ACE inhibitor causes cholestatic hepatitis (Maimon et al., 2006). It is possible that the increase in the antioxidative enzyme activities and decline in ACE activity indicates induction of an inflammatory response in hepatic tissue. We also speculate that the decline in hepatic ACE represents an adaptation of increased arsenic excretion under continuous arsenic consumption. For most of the tissues studied, we might exclude ACE as a pathogenic factor contributing to the higher blood pressure with arsenic exposure.

Due to the modulatory property of 20-HETE, renal blood flow autoregulation and CYP4A family activity play key roles in increasing the systemic blood pressure (McGiff and Quilley, 1999; Parmentier et al., 2001). Recently, CYP4A (arachidonic acid ω/ω-1 hydroxylase) overexpression has been suggested to contribute to the pathophysiology of hypertension in the SHR/WKY rat model (genetic spontaneous hypertension) (Landmesser et al., 2003) and alcohol-induced model of hypertension (Landmesser et al., 2003; Cowpland et al., 2006). CYP4A gene disruption in mice causing both androgen-sensitive and spontaneous hypertension has been reported (McGiff and Quilley, 1999; Landmesser et al., 2003). We are the first to report significantly elevated CYP4A protein expression in both the liver and kidneys with chronic arsenite exposure. Arsenate also significantly elevated CYP4A expression in the kidneys. At the same time, arsenite exposure produced a decrease in circulating NO. Pi et al. (2000) reported that there was a marked negative correlation ($r = -0.52$, $p < 0.05$) between plasma arsenic concentration and circulating NO; they found lower plasma NO concentrations in residents living where arsenic pollution was high. In a study of pregnant rats, NO modulated renal CYP4A expression and 20-HETE formation which provided an explanation of why increased glomerular filtration and

renal blood flow occurred in a normal pregnancy (Wang et al., 2003). eNOS-blockade mice showed enhanced coronary artery constriction to pressure which was attributable to increased activity of ω/ω-1 hydroxylase and CYP4A protein expression (Huang et al., 2005). As a previous study described, arsenic exposure increases superoxide production, which might indirectly disrupt eNOS formation, then decrease the release of NO, and subsequently enhance CYP4A expression (Landmesser et al., 2003; Wassmann et al., 2004). However, beyond CYP4A expression and NO metabolism, the mechanisms of arsenic-induced hypertension remain unclear; further studies are needed to clarify the hypothesis.

In this study, 50 ppm arsenite, approximately equivalent to an intake of 1.95 mg/day, in water for 200 days obviously decreased the body weight gain and elevated SBP. The same body weight-lowering effects were also seen when 100 ppm (2.24 mg/daily) of arsenite was administered for 60 consecutive days in an animal study (Shila et al., 2005). Moreover, a dose-dependent decrease of over 0–26.6 mg/kg body weight (BW) was also shown in a 30-day exposure study (Pal and Chatterjee, 2004). Contrarily, an intraperitoneal injection of 5.55 mg/kg BW arsenite for 30 continuous days produced no significant change in body weight (Schulz et al., 2002). Results of these studies suggest that the effect of arsenite on growth is affected by the exposure dosage and duration. Thus, as long as the exposure dosage of human within the contaminated area were extremely lower than it in this study (Chen et al., 1996; Tseng, 2002), the effects of yearly accumulation still could not be neglected. Possible reasons why arsenic causes body weight loss have been widely discussed: one is that arsenite may increase glucose excretion in the urine, or decrease glycogen and pyruvate synthesis (Kawaguchi, 1981; Reichl et al., 1990). Compared with arsenate, the amount by which arsenite interferes with glucose utilization is extremely low (Walton et al., 2004). For these reasons, arsenite may contribute to the blockage of cellular glucose uptake and animals exposed to such conditions for a long time might experience a change in weight.

There are some limitations need to be noticed that arsenic metabolism was slightly different from species thus the research could not provide evidence that human would experienced the extremely same mechanism like rats in arsenic-induced hypertension. When arsenic uptake in mammals, a consecutive methylation is activated to produce dimethylarsinous acid (DMA^{III}), the main arsenic metabolite in hepatic methylation which was regarded as a detoxification process in mammals (Styblo et al., 2000). Some reports showed that rat possessed higher capability to uptake and efflux DMA by red blood cells and higher methylation rate of hepatocyte than human (Shiobara et al., 2001; Styblo et al., 2000). Nevertheless, higher methylation rate did not exactly protect tissue cell from acute arsenic toxicity (Styblo et al., 2000), whether inorganic arsenicals or their methylation products have stronger toxicity on peripheral blood circulation still needs further evidences to be elucidated. Although we could not provide the SBP data after 120–200 days of treatment, the lifespan change of SBP in Wistar rats revealed that the main increase of SBP was during 18 weeks. After 18 weeks old, the SBP remained a stable trend with small turbulence instead of keeping increased (Bunag and Teravainen, 1991; Preuss et al., 1998; Laurant et al., 2000). Compared to our study, SBP was detected till 120 days (animals were 22 weeks old) thus we might presume there were no further and significant changes in the day after.

In conclusion, we successfully induced hypertension in rats by exposure to 50 ppm arsenic for 200 days and demonstrated the time-sequential changes in antioxidative enzyme systems. These data provide a picture of how chronic arsenic exposure affects the antioxidative system leading to hypertension. Moreover, arsenicals were verified to induce CYP4A expression which is involved in 20-HETE metabolism, and which may be more important than ACE in contributing to arsenic-induced hypertension.

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