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N-acetyl-L-cysteine prevents arsenite-induced cytotoxicity mainly through chelation in U937 monocytes and macrophages

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Contents

ABSTRACT

Arsenic is one of the elements in the earth's crust which is released to the environment (air, water and land) as a result of natural as well as anthropogenic activities. Arsenic-contaminated water is a big threat to public health in many Asian countries since chronic exposure to inorganic arsenic induces various biological effects including cancers in various sites and immunosuppressive status. N-acetyl-L-cysteine (NAC), a well-known antioxidant, is used as a commercially-available supplement, and it has a potential against arsenic toxicity. The present thesis describes the preventive mechanism of NAC on arsenite-induced apoptosis in U937 cells, which lack functional p53. The cytotoxicity was examined among different time sequences of NAC treatment in U937 monocytes and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-treated U937 macrophages. TPA-treated U937 macrophages were more resistant to arsenite-induced apoptosis than U937 monocytes, which might be due to the induction of Bcl-2 expression. Pretreatment with 20 mM NAC before arsenite exposure suppressed apoptosis up to 75% in U937 monocytes and 100% in macrophages, respectively. However, 6-hour NAC pretreatment and subsequent washing out of NAC from the culture medium before arsenite treatment did not inhibit the arsenite-induced apoptosis. Furthermore, post-treatment by NAC 1-hour after arsenite exposure almost completely inhibited the cytotoxic effects of arsenite in both U937 monocytes and macrophages. These results indicated that preventive effects of NAC on arsenite-induced apoptosis in U937 monocytes and U937 macrophages are mainly through chelating arsenite in culture medium.

1. INTRODUCTION:

Arsenic is recognized as a big threat to public health in many Asian countries (1-4). In Pakistan, for example, there are still many regions where the arsenic concentrations of drinking water exceed the WHO standard of 10 ppb (μg/L), and arsenic contaminations of the large water reservoirs were found to be as high as 600-700 μg/L (2-5). Most of arsenic compounds in drinking water are inorganic arsenite or arsenate (6).

1.1. Arsenic exposure:

In nature, arsenic occurs primarily in its sulfide form in complex minerals containing silver, lead, copper, nickel, antimony, cobalt, gold and iron. Arsenic is emitted to the atmosphere from both natural and anthropogenic sources. Volcanic activity is the most important natural contributor while anthropogenic sources include the emissions from the mining and smelting of base metals, low grade fuel combustion and the use of arsenic-based pesticides. High levels of arsenite in the drinking water come from arsenic-rich geological formations as well as from industrial discharges. The primary route of arsenic exposure for general population is via ingestion of contaminated food or water. Inhalation of arsenic from ambient air is generally a minor exposure route for the general population but inhalation of arsenic-containing particulates during occupational exposure is the main source of causing arsenite toxicity. This occupational exposure may be because of industrial activities where workers are or were exposed to arsenic such as coal-fired power plants, battery assembly, preparation of or work with pressure-treated wood, glass-manufacturing, and the electronics industry (7).

Chronic exposure to arsenite induces various biological effects on tissues and cells

including cancers in various sites (8), immunosuppressive status (9), and apoptosis (10). Acute ingestion of large dose of arsenic can cause muscle cramps, multi-organ failure and death while acute inhalation may result in irritation of respiratory tract. Weakness, anorexia, hepatotoxicity, irritation of the eyes, throat and respiratory tract, peripheral nervous system damage and skin disorders are common among the people who are exposed to arsenic occupationally. Chronic exposure to arsenite may leads to cardiovascular disorders, carcinogenesis and reproductive effects (11).

1.2. Arsenic toxicity and carcinogenicity:

Fatmi Z *et al.* (12) reported the burden of skin lesions of arsenicosis at higher exposure through groundwater of district Khairpur, Pakistan. An association was found between drinking arsenic-contaminated water and the risk of liver cancer (13). In particular, the impaired immune function of macrophages contributed to immune-suppression in arsenic-exposed individuals (14). Luna *et al.* (15) reported an association between arsenic exposure and the increase of nitric oxide and superoxide anion in both peripheral blood mononuclear cells and monocytes, suggesting that arsenic induced an oxidative stress in circulating blood cells. Oxidative stress plays an important role in the molecular mechanism of arsenic-induced toxicity and carcinogenesis. Exposure to arsenic will generate NO^- and O^-_2 that is subsequently converted to other more damaging reactive species such as OH radical and $ONOO^-$. The reaction and interaction of these reactive species with target molecules lead to oxidative stress, lipid peroxidation, DNA damage, and activation of signaling cascades associated with tumor promotion and/or progression (11). Another study also indicated a production of reactive oxygen species (ROS), which mediates p53 activation and apoptosis, during the

metabolism of inorganic arsenic (16). Arsenic tends to bind to the thiol-group of proteins, targeting regulatory or structural proteins. Among these proteins, the proto-oncogene c-Jun is well investigated. By binding to thiol-groups, arsenic can block Jun N-terminal kinase (JNK) phosphatase activity, resulting in an over-activation of JNK, which activates proto-oncogene c-Jun, inducing c-Jun/c-Fos mediated gene up-regulations. These up-regulated genes, including cell cycle regulation and apoptotic signaling, are strongly linked to arsenic carcinogenesis (17). There is sufficient evidence in humans for the carcinogenicity of mixed exposure to inorganic arsenic compounds, including arsenite, and arsenate. According to the recent evaluation of the International Agency for Research on Cancer, inorganic arsenic compounds cause cancer of the lung, urinary bladder, and skin. Furthermore, a positive association has been observed between exposure to inorganic arsenic compounds and cancer of the kidney, liver, and prostate (7).

1.3. Bcl-2; An anti-apoptotic factor:

Mitochondria play an important role in the regulation of cell death. They contain many pro-apoptotic proteins such as apoptosis inducing factor, Smac/DIABLO, and cytochrome c. These factors are released from the mitochondria following the formation of a pore in the mitochondrial membrane called the permeability transition pores. Mitochondrial membrane permeabilization is a critical event in the process leading to apoptosis. The permeability transition pores are thought to form through the action of the pro-apoptotic members of the Bcl-2 family of proteins, which in turn are activated by apoptotic signals such as cell stress, free radical damage or growth factor deprivation. The Bcl-2 proteins are a family of proteins involved in the response to apoptosis. Some

of these proteins (such as Bcl-2 and Bcl-xL) are anti-apoptotic, while others (such as Bad, Bax or Bid) are pro-apoptotic. When overexpressed, anti-apoptotic Bcl-2 and Bcl-xL proteins combat the pro-apoptotic function of Bax and Bak. The cell-killing activity of Bax and Bak takes place on the mitochondrial membrane, which is permeabilized in response to cell death signals. Bcl-2 and Bcl-xL can prevent cell death by blocking activation of Bax and Bak and thus inhibit cytochrome c translocation, thereby blocking caspase activation and the apoptotic process (18, 19).

1.4. U937 cell line:

The U937 cell line is an established model for monocyte/macrophage differentiation, and the absence of functional p53 in U937 monocytes is well known (20). The treatment of U937 monocytes with phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induces differentiation along the macrophage pathway (21, 22), and and TPA‐induced differentiation has been suspected to modify pharmacological effects, for example, offering resistance to apoptosis induced by a series of anticancer drugs (23,24).Some studies examined the difference in the cytotoxicity of arsenite between TPA-treated and non-treated human myeloid cells, but those results are not consistent. Sordet *et al.* (25) reported that TPA-differentiated cells showed an increased susceptibility to apoptosis induced by arsenite but others found that TPA-treated U937 macrophages were resistant to the arsenite-induced apoptosis (26, 27). This resistance might be because of activation of some antiapoptotic factor like Bcl-2 because activation of Bcl-2 by TPA treated U937 is already known (28).

1.5. Arsenite Metabolism:

According to classical view arsenate (iAs^{V}) and arsenite (iAs^{III}) in the presence of arsenic methyltransferase (Cyt19), S-adenosyl-L-methionine (SAM) and glutathione (GSH) are converted to monomethylarsonic acid (MMA^{V}), which is reduced to monomethylarsonous acid (MMA^{III}). MMA^{III} is subjected to methylation once again to form dimethylarsinic acid (DMA^{V}) under same conditions and then final reduction of DMA^{V} to dimethylarsinous acid (DMA^{III}). But new metabolic pathway of arsenite showed that first arsenic triglutathione is generated nonenzymatically from iAs^{III} and then methylated to monomethylarsonic diglutathione (MADG) and dimethylarsinic glutathione (DMAG) by SAM and Cyt19. These methylated As-GSH complexes (MADG and DMAG) are hydrolyzed and oxidized to MMA^{V} and DMA^{V}, respectively (29).

1.6. N-acetyl-L-cysteine (NAC); An efficient antioxidant:

N-acetyl-L-cysteine (NAC), a widely-used antioxidant, acts as a precursor of L-cysteine and reduced GSH (30). It is the amino acid L-Cysteine plus an acetyl ($-CO-CH_3$) group attached to the amino (NH_2) group. Amino acids which contain a sulphur group have antioxidant properties. The acetyl group makes cysteine more water-soluble, and functions to speed absorption and distribution on orally ingested cysteine. The acetyl group also reduces the reactivity of the thiol, making NAC less toxic and less susceptible to oxidation than cysteine. NAC is safe, even in large doses, and is a better source of cysteine than cysteine itself. NAC readily enters cells and is hydrolyzed to cysteine. Oral supplementation with NAC provides an alternate means of boosting intracellular glutathione via elevated intracellular cysteine. NAC is rapidly absorbed

after oral administration and reaches a maximum plasma level in 2-3 hours, with a half-life of about 6 hours. NAC readily enters cells and is hydrolyzed to cysteine. NAC treatment improved the mortality of arsenic-treated mice with a decrease of arsenic-induced oxidative stress in the tissues (31), and Reddy *et al.* (32) also indicated the beneficial role of NAC to counteract arsenic-induced oxidative stress. NAC treatment attenuates arsenic-induced oxidative stress in the liver and thus prevented apoptosis of the hepatocytes (33). Alternatively, NAC is also reported to act as a chelator (34).

2. OBJECTIVE:

The aim of the thesis is to clarify the preventive mechanism of NAC on arsenite-induced apoptosis in U937 monocytes and TPA-treated U937 macrophages.

3. MATERIALS AND METHODS:

3.1. Cells and chemicals:

The human U937 monocytic leukemia cell line (Dainippon Sumitomo Pharma, Osaka, Japan) was maintained in suspension culture of RPMI‐1640 medium (Sigma‐Aldrich, St. Louis, MO, USA) supplemented with 10% heat‐inactivated fetal bovine serum (FBS; SAFC Biosciences, Lenexa, KS, USA) at 37˚C in a humidified 5% CO2 atmosphere. U937 monocytes (initial cell concentration: 2x105 cells/ml) were differentiated to macrophages (attached to the bottom of plates) by the addition of TPA (Wako Pure Chemical Industries, Osaka, Japan) into the regular medium at a final concentration of 10 nM for 48 h. TPA stock solution (10 mM) was dissolved in dimethyl‐ sulfoxide (DMSO) and stored at ‐80˚C. Further dilutions of TPA were prepared in regular medium immediately prior to use. The final DMSO concentration did not exceed 0.1% (v/v). Sodium arsenite (Sigma‐Aldrich, Tokyo, Japan) was dissolved in distilled water to form a 100 mM stock solution, and was diluted with regular medium immediately prior to use. NAC (1 M; Wako Pure Chemical Industries) was dissolved in regular RPMI‐1640 medium without FBS, and the pH of the medium was adjusted to 7.4 with NaOH prior to use.

3.2. Arsenite and/or NAC treatments, and cytotoxicity determination:

An assessment of the cytotoxicity of arsenite was performed with an initial cell concentration of 2x105 cells/ml (100 ml/well) in 96‐well plates. The U937 monocytes and macrophages were treated with arsenite (1‐100 μM) for 24 h. Cell proliferation was determined by the trypan blue exclusion assay for U937 monocytes (35) using 0.2% trypan blue solution (Life Technologies, Tokyo, Japan) in phosphate‐buffered saline

(PBS) and the WST-8 assay for U937 macrophages using CCK-8 (Dojindo Molecular Technologies Inc., Kumamoto, Japan) according to the manufacturer's instructions. The cytotoxicity of NAC in U937 monocytes and macrophages was also determined, by treating cells with a range of concentrations of NAC (0, 1, 2, 5, 10 and 20 mM) for 25 h. The preventive effects of NAC on arsenite induced cytotoxicity were then examined. The U937 cells were treated with each concentration of NAC (0, 1, 2, 5, 10 or 20 mM) for 1 h, and then incubated in the presence of 50 μM arsenite for 24 h. To determine whether arsenite-induced cytotoxicity can be prevented by the pretreatment of cells with NAC and subse- quent wash-out, U937 monocytes were incubated with 20 mM NAC in a 12-well plate for 6 h, followed by washing twice with regular medium and centrifugation (500 x g for 3 min), prior to incubation with 50 μM arsenite in a 96-well plate for 24 h. Macrophages were incubated with 20 mM NAC in a 96-well plate for 6 h, followed by washing twice with regular medium, prior to incubation with 50 μM arsenite for 24 h. To determine the time-course dependence for the preven- tive effects of NAC on arsenite-induced cytotoxicity, NAC (final concentration: 20 mM) was added to U937 monocytes and macrophages at 0, 1, 3, 6 or 12 h following exposure to 50 μM arsenite.

3.3. Apoptosis detection:

To evaluate the apoptosis of U937 cells in the presence of arsenite, U937 monocytes with initial concentration of 2×10^5 cells/ml (100 μl/well) were plated in 8-well Lab-Tek Permanox chamber slides (Nalge Nunc, Rochester, NY, USA) with TPA for 48 hours and then treated with arsenite for further 24 h. Apoptosis was evaluated by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) using an *In Situ*

Apoptosis Detection Kit according to the manufacturer's instructions (Takara Bio, Shiga, Japan). Ultraviolet (UV) treatment is known to easily induce apoptosis in U937 cells (36). Therefore, U937 monocytes were exposed from the bottom to a 302 nm UV transilluminator M-15 (UVP, Upland, CA, USA) for 5 min at room temperature and used as a positive control for apoptosis detection of U937 cells**.**

3.4. Immunocytochemistry:

U937 monocytes and macrophages were transferred to the glass slides by a Cytospin 4 Cytocentrifuge (Thermo Fisher Scientific, Waltham, MA, USA) at 452.79 g for 5 min. Slides were dried at room temperature and cells were fixed by dipping slides in 4% paraformaldehyde for 30 min at room temperature. After rinsing with PBS (pH 7.4), non‐specific antibody binding was reduced by incubating the cells with horse serum in PBS for 30 min. The cells were then incubated for 1 h at 4˚C with a rabbit polyclonal antibody for Bcl‐2 (N‐19, 1:50 dilutions; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Following a thorough PBS wash, the slides were incubated with biotinylated rabbit antibody IgG (Vector Laboratories, Inc., Burlingame, CA, USA) for 30 min at room temperature, and then with a 1:100 dilution of the avidinbiotin‐peroxidase complex (Vectastain Elite ABC kit; Vector Laboratories, Inc.) for an additional 30 min at room temperature. The peroxidase signal was visualized by treatment with DAB Substrate‐Chromogen system (Dako, Carpinteria, CA, USA) for 10 min. The cells were then stained lightly with hematoxylin. Cytoplasmic staining was considered to indicate Bcl‐2 expression. The cells were viewed with a Axioskop 40 microscope (Carl Zeiss, Göttingen, Germany) and images were captured using a Pixera Viewfinder 3.0.1 (Pixera Corporation, Los Gatos, CA, USA). Magnification size for all

images was 200X, and hematoxylene and DAB were used as stains.

3.5. Statistical Analysis:

All values are expressed as the mean ± standard error. Statistical analysis was conducted using the Mann‐Whitney U test. All P‐values presented are two‐sided and $P<0.05$ was considered to indicate a statistically significant difference.

4. RESULTS:

4.1. Cytotoxicity of arsenite and NAC in U937 monocytes and macrophages:

The LD_{50} values of arsenite in monocytes and macrophages were 10 µM and 20 µM, respectively. Arsenite at 50 µM led to 95% and 85% cell death in monocytes and macrophages, respectively (Fig. 1A). Levels of apoptotic cell death were confirmed by TUNEL assay. Following treatment of macro- phages with 20 µM arsenite, positive signs of apoptosis were clearly observed in the nuclei of the cells (Fig. 2).

The toxicity of NAC was also determined in U937 monocytes and macrophages (Fig.1B). The cell viabilities of the two types of U937 cell were not altered following NAC treatment of ≤20 mM, then significantly declined following NAC incubation at 50 µM, for 25 h (P=0.016 and P=0.026 for monocytes and macrophages, respectively; Fig. 1B). Therefore, the concentration of 20 mM NAC was selected for use in further experiments.

4.2. Effect of NAC on arsenite-induced cytotoxicity in U937 monocytes and macrophages:

Preventive effects of NAC on the arsenite-induced apoptosis were examined. The cells were incubated with NAC-containing medium 1 h prior to arsenite administration. The 50-µM arsenite treatment alone caused 95% cell death in U937 monocytes without NAC, while only 20% cell death was observed in the presence of 20 mM NAC (Fig. 2A). The treatment with 20 mM NAC completely prevented arsenite-induced cell death in U937 macrophages (Fig. 2B). The effect of NAC on arsenite-induced apoptosis in macrophages was greater than that in monocytes (P<0.001). In U937 monocytes and macrophages pretreated with 10 mM NAC, 27% and 86% of cells were viable in

comparison with the viability of the untreated cells, respectively (Fig. 3A and B).

4.3. Effects of NAC pretreatment and wash-out on arsenite-induced cytotoxicity in U937 monocytes and macrophages:

To examine the effects of NAC pretreatment and subsequent wash-out on arsenite-induced cytotoxicity, cells were incubated with 20 mM NAC for 6 h, and then washed twice with medium prior to administration of 50 mM arsenite. The apoptosis-preventive effect of NAC was not observed in U937 monocytes or macrophages when NAC was washed out prior to arsenite administration (Fig. 4A and B). This difference in cytotoxicity between washed out and non-washed out cells was significant in both U937 monocytes and macrophages ($P<0.001$ for both types of cell).

4.4. Effects of NAC treatment after arsenite administration in U937 monocytes and macrophages:

To examine the effects of NAC treatment after arsenite administration on its cytotoxicity, NAC was added to the culture medium 0, 1, 3, 6, and 12 h after arsenite administration. In both U937 monocytes and macrophages, the arsenite-induced apoptosis was almost completely blocked by 20 mM NAC treatment within 1 h of arsenite administration (Fig. 5A and 5B).

4.5. Immunocytochemistry for Bcl-2:

To examine the mechanism of resistance to arsenite treatment in U937 macrophages, Bcl-2 expression was examined by immunocytochemical assay. Bcl-2 expression was activated in TPA-treated U937 macrophages, but no activation of Bcl-2 expression in

U937 monocytes was observed (Fig. 6).

Fig. 1

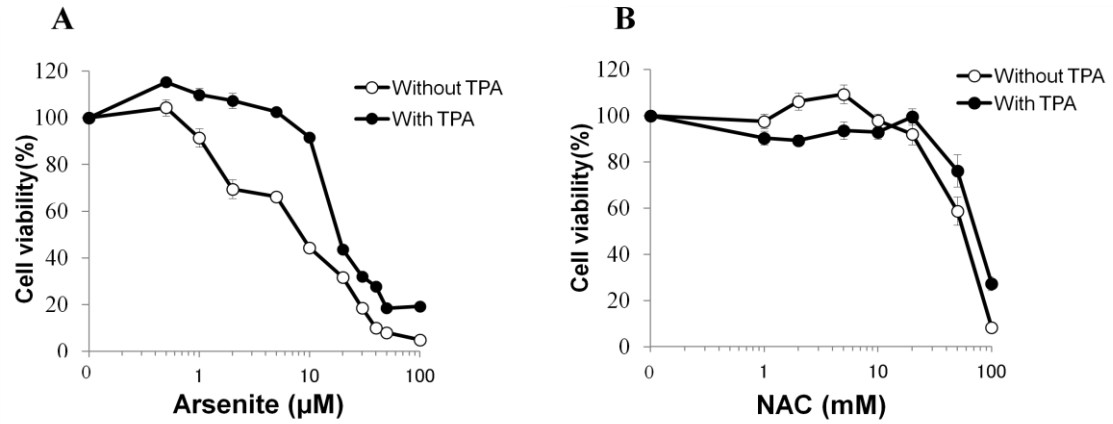


Fig. 1. Cytotoxicity of arsenite and NAC in U937 monocytes and macrophages. (a).U937 monocyte (○) and TPA-treated U937 macrophages (●) were treated with arsenite for 24 h. (b).U937 monocyte (○) and macrophages (●) were treated with NAC for 25 h. Values represent the mean ± standard error of five experiments.

Fig. 2

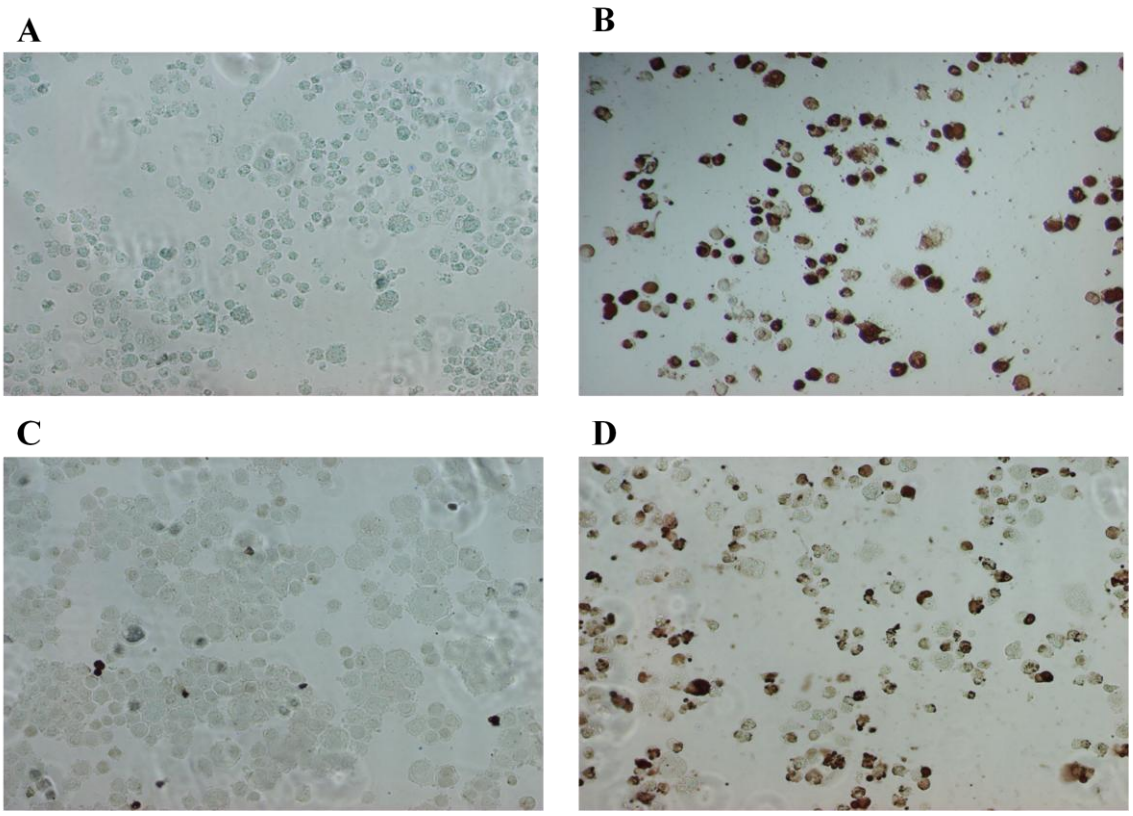


Fig.2 Apoptosis detection in U937 macrophages

Apoptosis was examined in U937 macrophages with and without arsenite. A: negative control, B: positive control, C: 0 μM arsenite, D: 20 μM arsenite. UV-treated cells were used as positive control.

Fig. 3

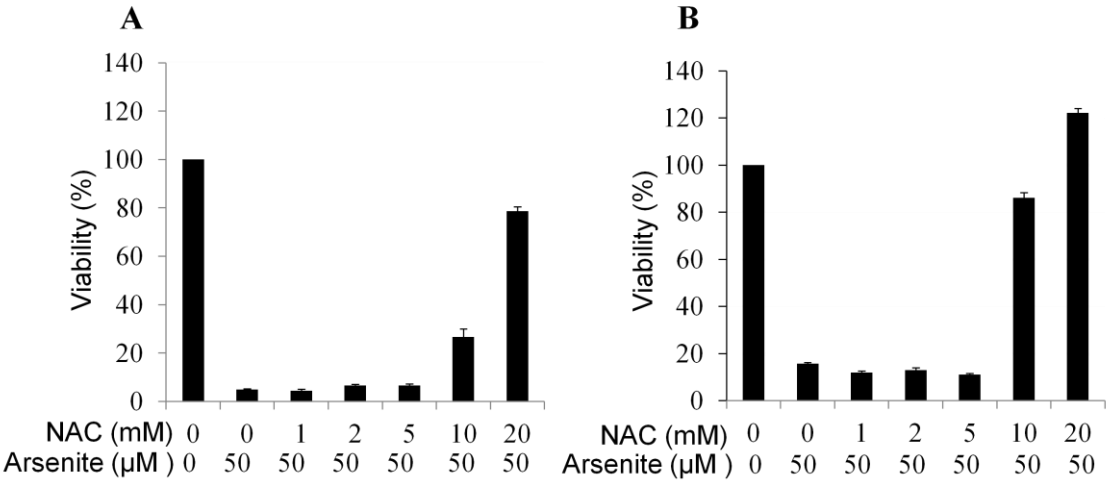


Fig. 3. Effect of pretreatment of NAC on arsenite-induced cytotoxicity in U937 monocytes and macrophages.U937 monocyte (A) and TPA-treated U937 macrophages (B) were treated with NAC for 1 h, followed by exposure with 50 mM arsenite for 24 h. Values represent the mean ± standard error of five experiments.

Fig. 4

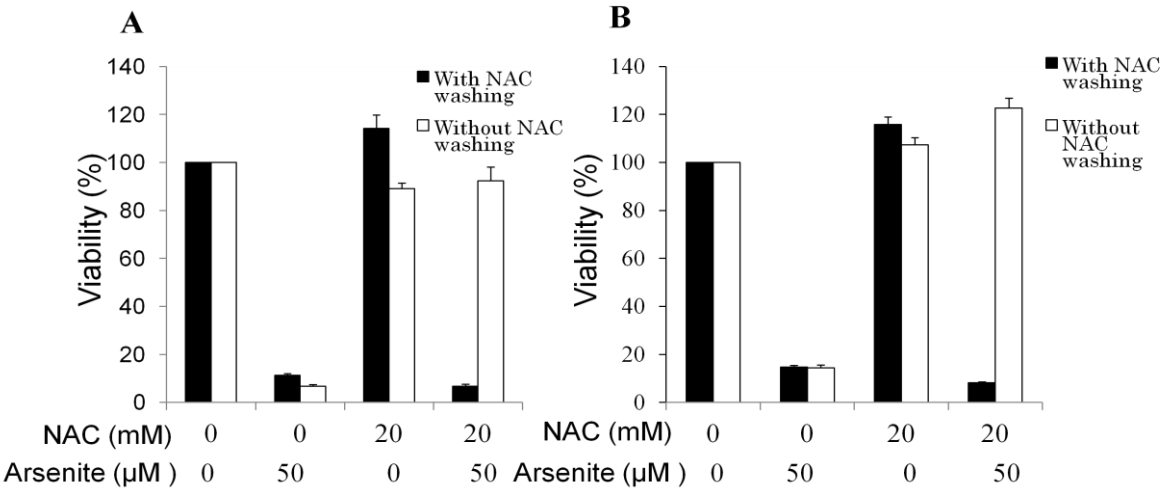


Fig. 4. Effect of NAC wash-out before arsenite-treatment in U937 monocytes and macrophages.

U937 monocytes (A) and TPA-treated U937 macrophages (B) were incubated with 20 mM NAC for 6 h, followed by washing twice with regular medium. Next, cells were incubated in the presence of 50 μM arsenite for 24 h. Values represent the mean ± standard error of five experiments.

Fig. 5

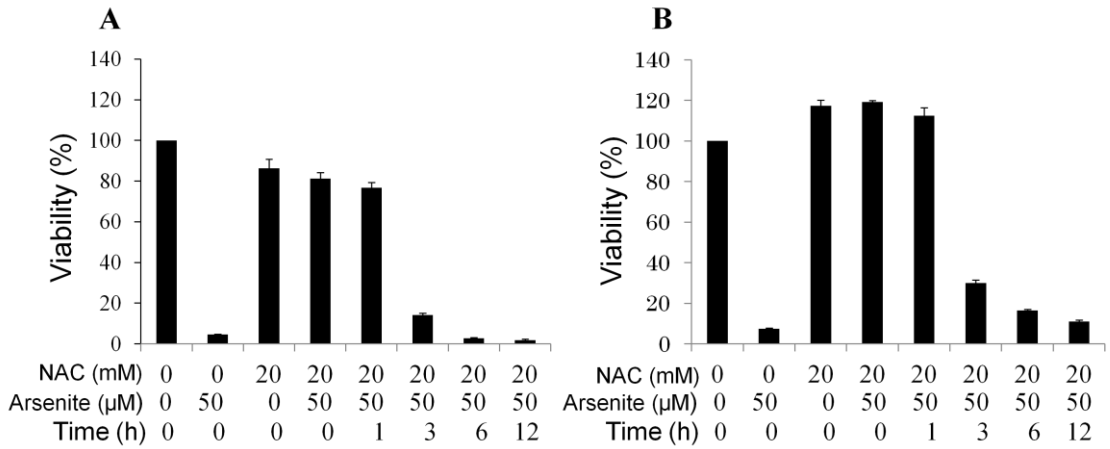


Fig. 5. Time course dependence for post-treatment of NAC in U937 monocytes and macrophages

NAC (20 mM) was added to cultured U937 monocyte (A) and TPA-treated U937 macrophages (B) at 0, 1, 3, 6 or 12 h after exposure of cells to 50 μM arsenite. Values represent the mean ± standard error of five experiments.

Fig. 6

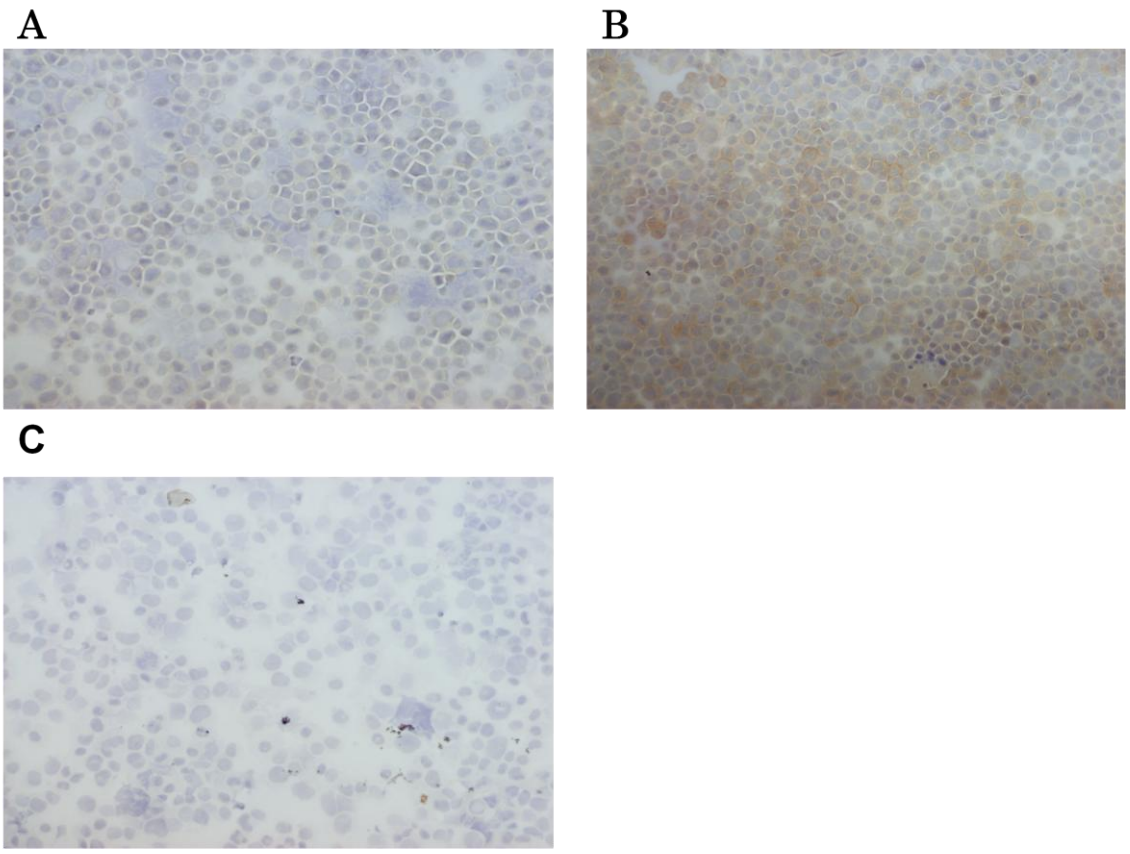


Fig. 6. Bcl-2 expression in U937 monocytes and macrophages

Bcl-2 expression was examined by immunocytochemistry in U937 monocytes (A) and TPA-treated U937 macrophages (B). (C): Negative control is U937 macrophage without Bcl-2 antibody.

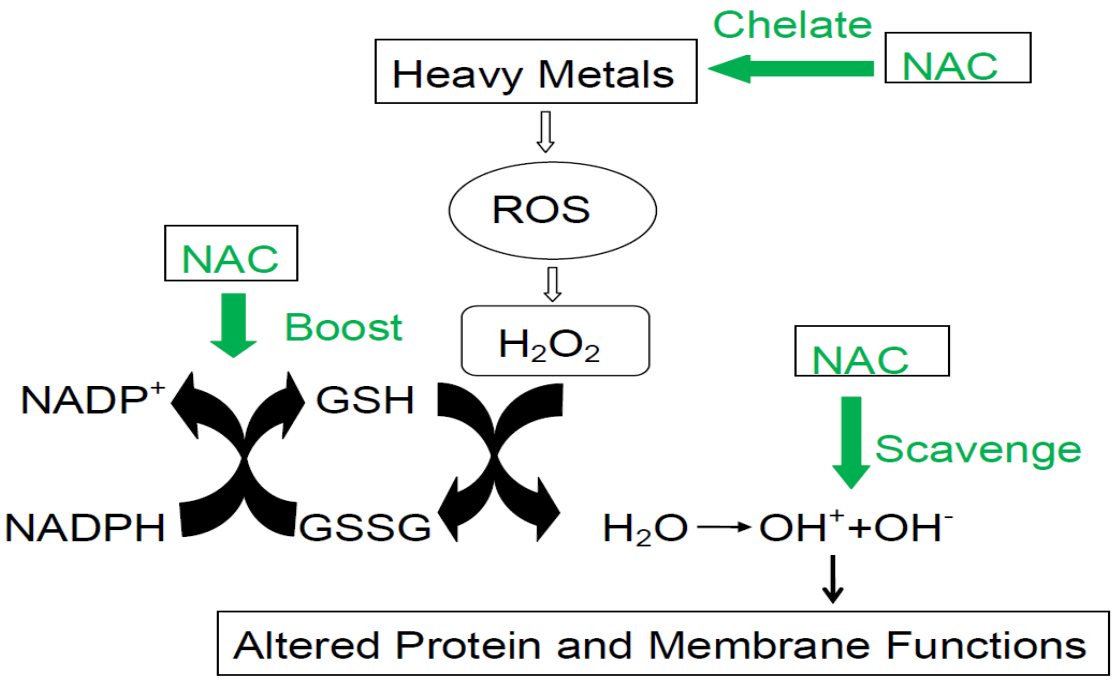
5. DISCUSSION:

In the current study, arsenite-induced apoptosis was suppressed in the presence of 20 mM NAC in U937 monocytes and macro- phages. This preventive effect was not observed when NAC was washed out from the medium prior to arsenite treatment, indicating that NAC in the medium can protect arsenite-induced apoptosis. According to a previous study, NAC can act as a chelator of metals, including chromium, lead, and boron (34). Findings of the present study indicated that the preventive mechanism of NAC in the cytotoxicity of arsenite on U937 monocytes and macrophages mainly involves chelation of arsenite in culture medium, although other studies reported non-chelation antioxidative roles of NAC in other cell lines (37). Previous studies have indicated that the preventive effects of NAC on arsenic-induced cytotoxicity may be predominantly due to antioxidative stress occurring by increasing cellular GSH levels (30). In a study in rat heart microvessel endothelial (RHMVE) cells, the effect of NAC on arsenic-induced cytotoxicity was due to the antioxidative role of non-protein thiols and not due to chelation of arsenic in the culture medium (38). The effects of extracellular NAC on the cellular levels of GSH appear to depend on cell types. For example, A549 human epithelial cells do not take up a significant amount of the NAC or GSH in the medium, while mouse glial cells are able to take up extracellular GSH (39). Furthermore, the addition of NAC to the culture medium did not increase, if any, the intracellular GSH concentration in RHMVE cells (40).

Post-treatment of NAC within 1 h of arsenite administra- tion completely inhibited the apoptosis in U937 monocytes and macrophages in the present study. It is possible that a small portion of arsenite in the medium is taken up by the U937 cells in 1 h, but it may not reach the threshold for toxicity. In U937 cells, histone deacetylase inhibitor

MS275-induced ROS production has been demonstrated to be blocked by 20 mM NAC treatment for 1 h (41). Thus, it may be possible for NAC to be used to reduce the acute toxicity of arsenite. Martin et al (42) reported a clinical application of NAC for a case in which a patient ingested a potentially lethal dose of sodium arsenate (900 mg) in a suicide attempt. In addition to the demercaprol, NAC (4 g) was administered intravenously every 4 h in total of 18 doses, and the clinical condition of the patient improved markedly within 24 h.

NAC has many functions including scavenging of free radicals, boosting GSH and Heavy metal chelation. NAC paths of action are shown in Scheme 1. The present study clearly indicates that the preventive effect of NAC on the cytotoxicity of aresenite in U937 cells is mainly through chelating arsenic in the culture medium.



Scheme 1. NAC pathway.

The results of the present study clearly indicate that the preventive effect of NAC on the cytotoxicity of arsenite in U937 cells mainly acts through chelating arsenic in the culture medium. The Environmental Protection Agency (EPA) denotes an arsenic level of <10 ppb in drinking water as safe (43). Since the cytotoxicity of 50 μM (3,750 ppm=75 g/mol x 50 μM) arsenite was almost completely inhibited by NAC as low as 20 mM, 20 μM NAC may inhibit the cytotoxic effect of arsenite at the concentration of 3.75 ppm, which is 375 times higher than the EPA standard, and even higher than the arsenic concentration of water in the areas with serious arsenic contamination of water. Supplementary intake of NAC may be valuable to prevent arsenic-induced cytotoxic effects among the inhabitants of the arsenic-contaminated regions. In experiments of the current study, the LD50 values of arsenite in U937 monocytes and macrophages were approximately 10 μM and 20 μM, respectively, indicating that U937 macrophages were more resistant to arsenite-induced apoptosis. This tendency was also observed in the presence of NAC. A number of studies have examined the difference in the cytotoxicity of arsenite between TPA-treated and non-treated human myeloid cells, but the results have been controversial. Sordet et al (25) reported that TPA-differentiated cells exhibited increased susceptibility to apoptosis induced by arsenite, which may be associated with a reduction of intracellular GSH. However, others demonstrated that TPA-treated U937 macrophages were more resistant to the arsenite-induced apoptosis than untreated cells (26, 27). Although TPA has the ability to decrease intracellular GSH in U937 monocytes, this ability was not observed following TPA-induced differentiation (27). Similarly, the resistance to apoptosis by other cytotoxic agents among differentiated myeloid cells was also reported in other studies (23, 24, 44). These findings indicate that there may be factors other than intracellular

GSH involved in the resistance to arsenic-induced apoptosis of TPA-treated U937 macro- phages, under certain circumstances.

Bcl-2, one of the key proteins in the regulation of apoptosis, is an integral membrane protein located mainly on the outer membrane of mitochondria. Overexpression of Bcl-2 prevents cells from undergoing apoptosis in response to a variety of stimuli directly on mitochondria by inhibition of cytochrome c translocation and subsequently blocking caspase activation (18, 19). In the present study, activation of Bcl-2 expression was observed in TPA-treated U937 macrophages but not in U937 monocytes. These findings are in accordance with those of a previous study indicating the up-regulation of anti-apoptotic proteins, including Bcl-2, in TPA-treated U937 macrophages (28). Other studies did not demonstrate the upregulation of Bcl-2 protein by TPA treatment in U937 cells (24, 45). Since p53 expression in U937 cells was not induced by TPA treatment in a previous study (46), the effect of TPA treatment on the pathway downstream of p53 may be involved in the sensitivity to stimuli.

6. CONCLUDING REMARKS:

The present thesis has shown the following:

1. The preventive effects of NAC on arsenite-induced apoptosis in U937 monocytes and U937 macrophages are mainly through chelating arsenite in culture medium.

2. TPA-treated U937 cells (macrophages) were more resistant to cell death as compared to monocytes. Bcl-2 activation in macrophages might be the reason of this sensitivity to arsenite exposure between U937 moncytes and macrophages.

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