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Arsenite-induced reactive oxygen species and the repression of α-tocopherol in the MGC-803 cells

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Abstract

We have investigated the action of oxidative stress in arsenite-induced apoptosis of human gastric cancer MGC-803 cells. Cells exhibited obvious characteristic of apoptosis following the treatment with 1.0 μM arsenite for 24 h. During the process, low concentration of arsenite significantly increased superoxide formation and lipid peroxidation, which was dose-dependent and was related to cell apoptosis induced by arsenite. The oxidant-dependent increase in intracellular [Ca^{2+}] level and p53 gene expression were also observed at the same time. A phospholipase C inhibitor, 1-[6-([(17β)-3-methoxyestra-1,3,5,(10)-trien-17-yl]-amino)hexyl]-2,5-dione (U73122), could block the rapid transient increase in intracellular Ca^{2+} levels, as well as the subsequent fragmentation of nuclear DNA. Addition of α-tocopherol before arsenite treatment abolished the transient increase in superoxide formation, lipid peroxidation, intracellular [Ca^{2+}] levels and p53 gene expression, and furthermore could significantly inhibited the arsenite-induced apoptosis of MGC-803 cells. These results indicate that arsenite-induced oxidative stress, which stimulate cellular signaling systems, are involved in apoptosis of MGC-803 cells.

Keywords: MGC-803 cell; Arsenite; Reactive oxygen species; α-tocopherol; Apoptosis; Ca^{2+}, intracellular

1. Introduction

Arsenite, which is widely distributed natural toxicant, has been used to treat several human diseases, such as anemia, dyspepsia and some tumors (Huang et al., 1995). In China, arsenite was identified in vitro and in vivo to be an effective drug in the treatment of acute promyelocytic leukemia (Chen et al., 1996). Recently, we have demonstrated that low concentration of arsenite inhibited growth and induced apoptosis of the solid tumors MGC-803 cells (Zhang et al., 1999, 2002). These data have renewed the interest for arsenite-containing compounds as anticancer agents and put the traditional remedy squarely in the mainstream of modern chemotherapy research. Several recent studies indicate that oxidants may be involved in arsenite toxicity. Arsenite has been shown to enhance ferritin H mRNA levels (Guzzo et al., 1994), to stimulate production of heme oxygenase (Applegate et al., 1991), an indication of oxidative stress, to increase the fluorescence intensity of dichlorofluoresin diacetate in human fibroblasts and Chinese hamster ovary (CHO-K1) cells (Wang et al., 1996; Lee and Ho, 1995). H_2O_2-resistant Chinese hamster ovary cells are cross-resistant to arsenite (Cantoni et al., 1994). Glutathione peroxidase and catalase have been shown to modulate arsenite-induced micronuclei (Wang et al., 1996, 1997). Moreover, antioxidants have been shown to reduce the sister chromatic exchange (Nordenson and Beckman, 1991), micronuclei (Wang and Huang, 1994), apoptosis (Wang et al., 1996) and cytotoxicity (Lee and Ho, 1995) in arsenite-treated cells. These findings suggest that arsenite-induced apoptosis may be triggered by reactive oxygen species (Wang et al., 1996; Watson et al., 1996; Gurr et al., 1999) and that oxidant generation during arsenite metabolism may play a role in arsenite-induced genotoxicity and apoptosis. The precise mechanism of apoptotic death, however, is still unclear. This prompted us to study the role of oxidative stress and subsequent effects in human gastric cancer MGC-803 cells induced by arsenite.

The ability of reactive oxygen species to provoke apoptosis has been associated with lipid peroxidation,

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DNA damage and alterations to protein and nuclear structures (Halliwell and Guttweidge, 1986; Korsmeyer et al., 1995). Superoxide anion (O_2^-), one of the major reactive oxygen species, is relatively harmless because it reacts with biomolecules at low rates and specific enzymes exist to remove it. Nevertheless, O_2^- can lead to the formation of the highly reactive hydroxyl radical (·OH) (Halliwell and Guttweidge, 1986). Therefore, the excess production of O_2^- may play a role in the progression of apoptosis. In this study, we measured the generation of O_2^- in MGC-803 cells, as well as lipid peroxidation, charges in [Ca^{2+}] and p53 gene expression. In addition, we found that α-tocopherol inhibits superoxide formation, lipid peroxidation, [Ca^{2+}], DNA strand breakage and p53 gene expression, all possible signal transduction pathways involved in the apoptosis of MGC-803 cells induced by arsenite.

2. Materials and methods

2.1. Cell culture and chemicals

MGC-803 cells (obtained from Beijing Medical University) were seeded in 35-mm tissue culture dishes (Costar, New York, NY, USA) and were cultured by RPMI 1640 medium (Gibco-BRL, Grand Island, NY, USA), supplemented with 10% heat-inactivated fetal bovine serum, penicillin and streptomycin and in a humidified atmosphere of 95% air/5% CO_2 at 37 °C. After seeding for 24 h, arsenite (Sigma, St. Louis, MO, USA; lotA1010) at final concentrations of 1 μM was added to the culture medium. All fluorescent probes were purchased from Molecular Probes (Eugene, OR, USA); α-tocopherol, lucigenin, etc. were purchased from Sigma. Bovine copper–zinc superoxide dismutase were obtained from Institute of Biochemistry (Academia Sinica, China).

2.2. Fluorescence microscope observation

Hoechst 33342 (Ho342) and Fluo-3/AM (Fluo-3 acetoxymethyl esters, Molecular Probes), at final concentrations of 5 and 10 μg/ml, respectively, were directly added to the culture medium of cells treated with or without arsenite and left for 30 min at 37 °C. The cells were then gently washed three times with phosphate-buffered saline (PBS, NaCl 13.7 mM, KCl 0.27 mM, Na_2HPO_4 0.43 mM, KH_2PO_4 0.14 mM, pH 7.3), and 1 ml fresh culture medium was added to every culture dish. The image fluorescence of a single cell was monitored using a fluorescence microscope (NIKON Inverted Microscope DIAPHOT 300) equipped with aquacosmos software (HAMAMATSU, Japan). Fluo-3/AM images were taken using excitation at 540-nm wavelength and recording fluorescence emission at 580-nm wavelength. After acquiring Fluo-3/AM images, Ho342 images were taken using a 340-nm excitation wavelength and a 450-nm barrier filter without changing the cell's original position in order to make control analyses of the two fluorescence images in the same cell.

2.3. Flow cytometric (FCM) analysis of apoptosis

The cells treated with different concentrations of arsenite for various times and cells of control groups were collected, centrifuged, washed in PBS and then resuspended in 1 ml PBS. Ho342 (final concentration of 5 μg/ml) was added after 30 min at room temperature (22 °C). Propidium indide (final concentration of 5 μg/ml) was then added for 30 min. Samples (10^5 cells/ml) were directly analyzed by FCM (EPICS, ELITE; Coulter, USA) with a UV laser excitation (60 mW) at 340 nm wavelength. Blue (Ho342, 480-nm wavelength) and red fluorescences (propidium indide, 600-nm wavelength) and the fluorescent ratio of 480/600 nm were measured. All data were collected, stored and analyzed by the instrument software.

2.4. Superoxide anion generation assay

In this work, chemiluminescence with lucigenin was used to demonstrate that arsenite-induced the increase of O_2^- production (Li et al., 1999). Cells treated in the presence or absence of arsenite ($4-5 \times 10^6$) were washed with PBS twice, then collected and suspended in 1 ml PBS. O_2^- was measured by monitoring the chemiluminescence after addition of 50 μM lucigenin. The kinetic curve of chemiluminescence produced in this system was immediately recorded with a computerized, high-sensitivity single-photon counter (SPC, type BPCL, manufactured at the Institute of Biophysics, Academia Sinica, China). The voltage in the photomultiplier was kept at 4000 V.

2.5. Determination of lipid peroxidation

The formation of lipid peroxidation products was assayed through the production of thiobarbituric acid chromophore in 2-ml samples of cells (2×10^6 cells) with 0.8% of 2-thiobarbituric acid (95 °C for 15 min) (Cao et al., 1996). The absorbence was read on a Hitachi U-3200 spectrophotometer using a measurement wavelength of 535 nm. The amount of thiobarbituric acid chromophore was calculated from $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ (malondialdehyde) at 535 nm. Results are expressed as nanomoles malondialdehyde equivalents per 5×10^6 cells.

2.6. Determination of single-strand DNA breakage

The single-strand DNA and double-strand DNA in MGC-803 cells were determined by hydroxylapatite batch (DNA grade, Bio-Gel HTP Bio-Rad) as previously described (Cao et al., 1992, 1994).

2.7. Determination of intracellular free calcium levels

The level of intracellular free Ca^{2+} was measured using Fura-2 as a fluorescence probe essentially by the method of Dubyak et al. (1988). Cells were collected, washed and resuspended at 10^6 cells/ml in PBS solution containing 5 mM glucose and 1 mg/ml bovine serum albumin. Cell suspensions were preincubated for 10 min at 37 °C prior to addition of 2 mM Fura-2/AM ester (Fura-2-acetoxymethyl ester). The suspension was incubated an additional 40 min at 37 °C followed by centrifugation and removal of the supernatant. The cells were then resuspended in fresh medium and incubated for an additional 10 min at 37 °C. The suspension was recentrifuged, the cell pellet resuspended at 10^6 cells/ml in ice-cold basal salt solution, and the suspension thus obtained stored on ice for up to 4 h until measurements. The fluorescence of Fura-2 excitation wavelength at 340 and 380 nm, F_{340} and F_{380}, was simultaneously recorded at the emission of 510 nm. Free cytosolic $[Ca^{2+}]$ was calculated according to the following equation:

$$[Ca^{2+}]_i = K_d[(R - R_{min})/(R_{max} - R)](F_{380.min}/F_{380.max})$$

where K_d is the dissociation constant for Ca^{2+} of Fura-2 (224 nm); $R = F_{340}/F_{380}$; $R_{max} = F_{340.\ max}/F_{380.\ max}$; $R_{min} = F_{340.\ min}/F_{380.\ min}$, $F_{340.\ max}$ and $F_{380.\ max}$ are the fluorescence excited at 340 and 380 nm, respectively, at zero $[Ca^{2+}]$. Fura-2 fluorescence was measured on a dual excitation fluorescence spectrophotometer (Hitachi F-4500) which allows simultaneous excitation of fluorescence at wavelength of 340 and 380 nm. Each experiment was repeated three times.

2.8. Western blotting

Unless otherwise indicated, cells were directly lysed in Laemmli sample buffer and boiled for 5 min. About 30 μg of proteins were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) and transferred to nitrocellulose by semidry blotting. Membranes were blocked with 10% skimmed milk in Tris buffered saline (TBS)–0.1% Tween for 1 h and incubated overnight with the antihuman P53 mouse monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA). These initial incubations were followed by incubations with antirabbit IgG (Santa Cruz). Antibody complexes were detected by chemiluminescence using the ECL kit (Amersham Life Science, Amersham, UK). An area- and densito-meter analysis of the gel bands was performed by ZEISS and VIDS all-autoanalysis system.

3. Results

3.1. Arsenite-induced apoptosis of MGC-803 cells

Fig. 1 shows typical changes in the morphology of MGC-803 cells following exposure to 1 μM arsenite for 24 h, including chromatin condensation and the formation of apoptotic bodies (Fig. 1F and H), which is consistent with

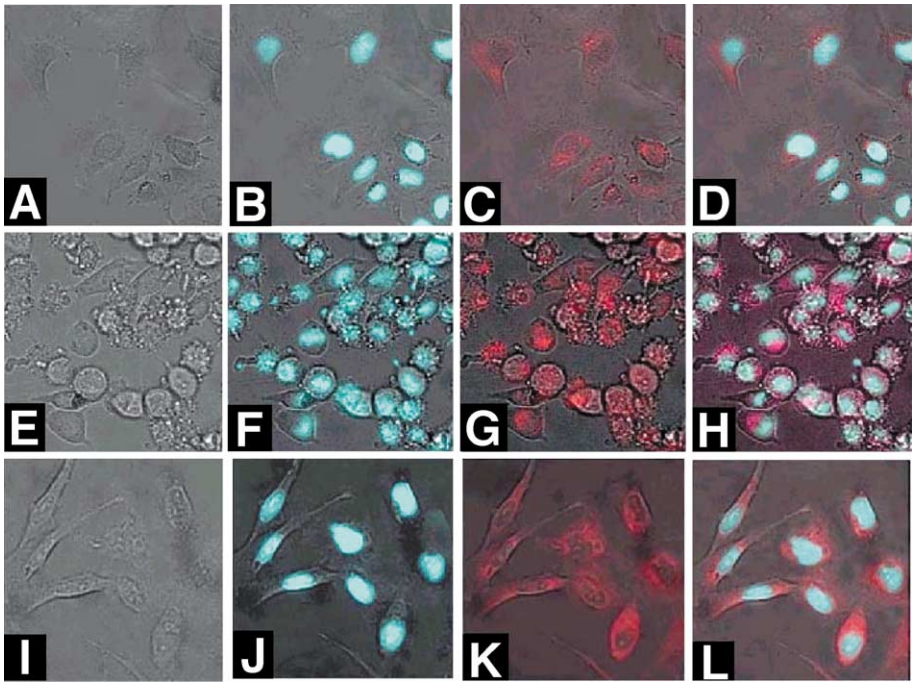

Fig. 1. Cell apoptosis and cytoplasmic $[Ca^{2+}]$ increase induced by arsenite. (A–D) Control, without any treatment. (E–H) Cells were treated with arsenite (1.0 μM final concentration, 24 h). (I–L) Cells were treated with α-tocopherol + arsenite. α-tocopherol (10 μM final concentration) was added to the culture medium 24 h before the arsenite treatment. (B, F, J) The effects of arsenite on chromatin. (C, G, K) The effects of arsenite on cytoplasmic $[Ca^{2+}]$. (D, H, L) Merge images showing the effects of arsenite on chromatin and cytoplasmic $[Ca^{2+}]$.

previous results (Zhang et al., 1999, 2000). Addition of α-tocopherol (10 μM final concentration) to the culture medium 24 h before the arsenite treatment apparently inhibit the apoptosis of MGC-803 cells induced by arsenite (Fig. 1I–L).

3.2. Arsenite stimulate the increase of superoxide production

When 50 μM lucigenin was added, cells not exposed to arsenite treatment resulted in a chemiluminescence response (Fig. 2A-b), which appears to be due to the production of endogenous O_2^-. Addition of arsenite after lucigenin could immediately produced a new lucigenin-derived chemiluminescence response, even at 0.01 μM arsenite, which gave rise to a chemiluminescence response that could be observed within 0.1 min, peaking 1 min after stimulation and rapidly declining in 3 min after stimulation (Fig. 2A-d, peak 2). Exogenous superoxide dismutase, a scavenger of O_2^-, immediately abolished the chemiluminescence response (Fig. 2A-a). Addition of α-tocopherol (10 μM final concentration) before the arsenite treatment inhibited the lucigenin-derived chemiluminescence (Fig. 2A-c) arsenite-induced

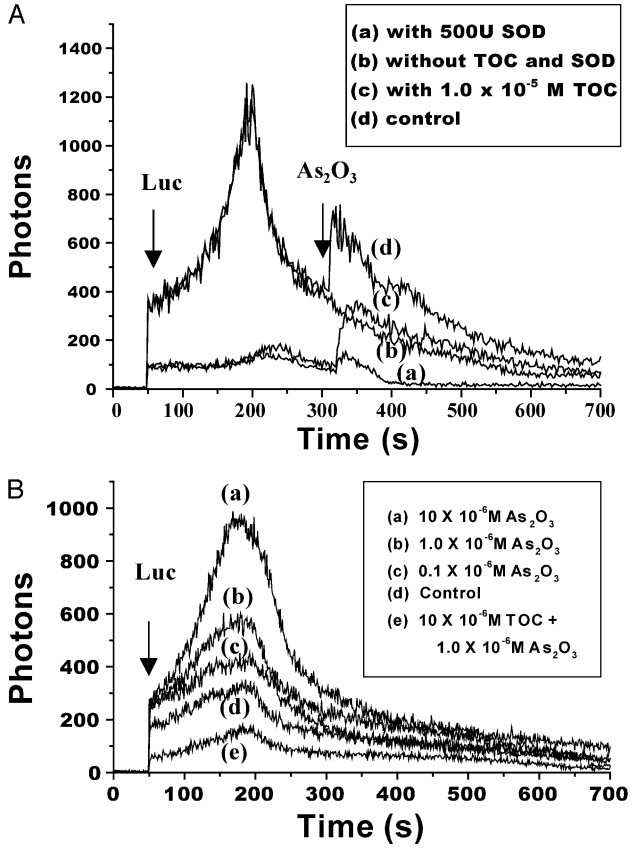

Fig. 2. Arsenite-induced increase of O_2^- production. (A) Cells treated with (a and c) or without (b and d) either α-tocopherol or superoxide dismutase (SOD); (b), not exposed to arsenite; (B) cells treated with different concentration of arsenite for 24 h. Cell number: $3-4 \times 10^6$. Control, cells not exposed to α-tocopherol and arsenite.

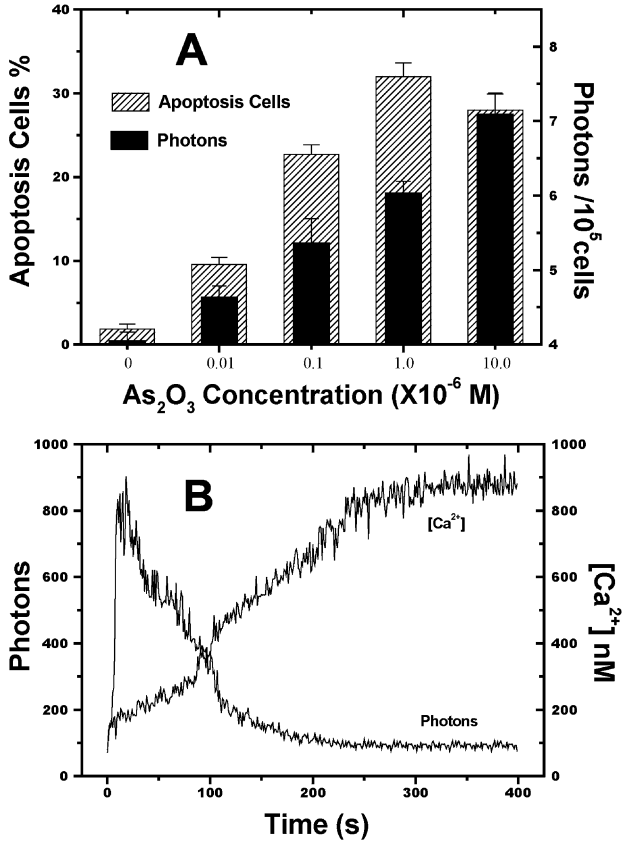

Fig. 3. The relationship between O_2^- production and cell apoptosis in MGC-803 cells (A) or rapid release of Ca^{2+} (B). There is not the chelator of extracellular Ca^{2+}, ethyleneglycol bis-2-aminoethy-ether (EGTA) in the reaction system. Photons represent sum of photon emission of 5×10^6 cells at 0–400 s. The *p* value for the difference between each group treated with arsenite and not treated controls was <0.01. No difference was obtained between a group with 1.0 μM and a group with 10.0 μM arsenite.

chemiluminescence response was concentration-dependent (Fig. 2B). No chemiluminescence was observed in cells alone (Fig. 2B). These results indicate that chemiluminescence response is related with the increase of O_2^- production induced by arsenite.

Fig. 3 shows that the arsenite-induced increase of O_2^- production was dose-dependent and increased with increase of arsenite concentration. In addition, the rapid increase of O_2^- induced by arsenite correlated with the increase of apoptotic cell (Fig. 3A). We saw marked difference in each group treated with arsenite vs. the untreated controls. The *p* value was <0.01 for apoptotic cell or O_2^- production. No difference was observed between the group exposed to 1.0 and 10.0 μM arsenite. At the same time, a rapid (<30 min) release of Ca^{2+} from both Ca^{2+} release and influx induced by arsenite was also observed (Fig. 3B).

3.3. Arsenite induced DNA-strand break by reactive oxygen species

Compared with control cells, 1 μM arsenite, with a 24-h treatment, apparently increased DNA single-strand break by

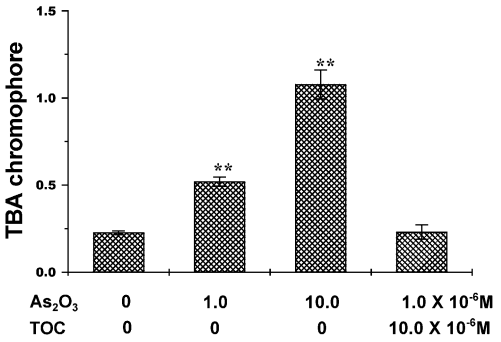

Fig. 4. Lipid peroxidation induced by arsenite and its inhibition by α-tocopherol measured as formation of TBA chromophore. **p value <0.01 compared with 0 h group.

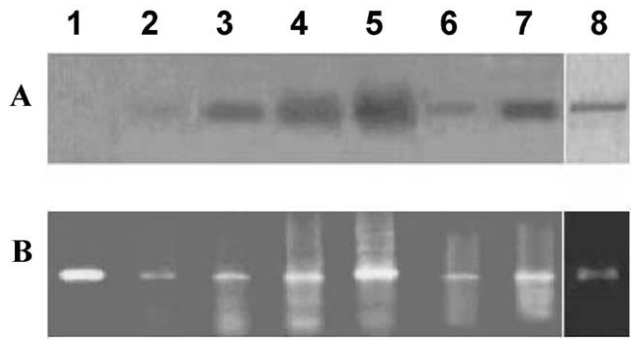

Fig. 5. Effects of arsenite on p53 protein level, p53 mRNA expression and inhibtion of this effect by α-tocopherol. (A) p53 protein level measured by western blot. (B) p53 mRNA expression by RT-PCR assay. (1) Control. (2–5) MGC-803 cells exposed to 0.00, 0.01, 0.10, 1 μM arsenite. (6) α-tocopherol (TOC, 10 μM) was added before arsenite treatment. (7) α-tocopherol (TOC) was added after arsenite treatment. (8) U73122 was added before arsenite treatment.

13%, the percent of single-strand break increased further by 15% at the concentration of 10 μM arsenite. Addition of the antioxidant α-tocopherol before arsenite treatment could effectively decrease the percent of single-strand break (to 5.2%). No direct interaction between DNA and arsenite was observed by measuring UV absorbance and by fluorescence methods (data not shown). This suggested that the reactive oxygen species contribute to the effect of arsenite on MGC-803 cells.

3.4. Arsenite increased lipid peroxidation production

As shown in Fig. 4, the amount of thiobarbituric acid chromophore in whole MGC-803 cells increased on exposure to 1 μM arsenite by approximately 1.3 times with respect to the control after 24 h of exposure and by approximately 1.7 times after exposure to 10 μM arsenite for 24 h. The thiobarbituric acid chromophore formation induced by 1 μM arsenite for 24 h was significantly suppressed by α-tocopherol.

Table 1
Arsenite-induced increase in cytoplasmic-free Ca^{2+} concentration in MGC-803 cells

Conditions	$[Ca^{2+}]$ (nM)	% baseline	p value
A			
Baseline	315 ± 94(10)	100	different from
+ Arsenite	786 ± 208(5)	291 ± 135(5)	
B			
+ Arsenite		100	–
+ Arsenite + EGTA		71 ± 11(5)	< 0.05
+ Arsenite + U73122		29 ± 10(5)	< 0.01
+ Arsenite + U73343		123 ± 29(5)	< 0.1
+ Arsenite + TOC		38 ± 9(3)	< 0.01
+ Arsenite + TOC + U73122		25 ± 12(4)	< 0.01
+ Arsenite + TOC + U73343		41 ± 8 (4)	< 0.01

* The p value for the difference between arsenite + U73122 and arsenite + U73122 was <0.01. The p value for the difference between arsenite + TOC + U73122 and arsenite + TOC + U73343 was <0.1.

** TOC: α-tocopherol. EGTA, ethyleneglycol bis 2-aminoethy-ether. U73122, 1-[6-([(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]-amino)hexyl]-2,5-dione. U73343, 1-[6-([(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl]amino)hexyl]-2,5-pyrrolidinedone.

3.5. The effect of arsenite on intracellular $[Ca^{2+}]$

Table 1 shows that arsenite induced increase of cytosolic free $[Ca^{2+}]$ in MGC-803 cells. Addition of either α-tocopherol (TOC) or α-tocopherol (TOC) + the phospholipase C inhibitor, 1-[6-([(17β)-3-methoxyestra-1,3,5,(10)-trien-17-yl]-amino)hexyl]-2,5-dione (U73122) inhibited such effect of arsenite. Similar inhibition was observed with the chelator of excellular Ca^{2+}, ethyleneglycol bis-2-aminoethy-ether (EGTA). Addition of the inactive analogue, 1-[6-([(17β)-3-methoxyestra-1,3,5,(10)-trien-17yl]amino)hexyl]-2,5-pyrrolidinedone (U73343), resulted in no charge on arsenic-induced $[Ca^{2+}]$ increase nor on its inhibition by α-tocopherol These results indicate that the $[Ca^{2+}]$ increase induced by arsenite originate from both influx of Ca^{2+} and release of the cation from the endoplasmic reticulum.

3.6. Effects of arsenite on p53 gene expression

As shown in Fig. 5, arsenite induced a concentration-dependent increase of the level of p53 protein (Fig. 5A) and p53 gene expression (Fig. 5B) in MGC-803 cells. Addition of α-tocopherol or 1-[6-([(17β)-3-methoxyestra-1,3,5,(10)-trien-17-yl]-amino)hexyl]-2,5-dione (U73122) before arsenite treatment abolished the transient increase of p53 gene expression.

4. Discussion

Oxidative stress is involved in the pathogenesis of various degenerative diseases. Apoptosis induced by a variety of stimuli is associated with increased oxidation levels in the cells (Buttke and Sandstrom, 1994). Recent studies indicate that arsenite may generate reactive oxygen

species, which are implicated as important factors in signaling mechanisms leading to its toxicity (Liu et al., 2001). Our previous experiments have demonstrated that 0.01–1 μM arsenite can inhibit growth of MGC-803 cells by inducing their apoptosis and MGC-803 cells are more sensitive to low concentration of arsenite (Zhang et al., 1999). Now, its precise mechanism of action in MGC-803 cells is still unknown. In this study, we further study the action of reactive oxygen species in the arsenite compounds on the growth of human gastric cancer MGC-803 cells.

The morphological changes, such as condensation of the cytoplasm, nuclear fragmentation, nuclear condensation and nuclear margination, are thought to be the most reliable markers of apoptosis. These were further confirmed by changes of Ho342 and PI satiability (Fig. 1), which are the characteristics in apoptotic cell death. The extent of apoptosis increased with arsenite dose as previously described by our laboratory (Zhang et al., 1999, 2000).

In this work, O_2^- was measured by the lucigenin-mediated chemiluminescence method (Li et al., 1999; Kevin and Irwin, 1993). Cells not exposed to arsenite treatment resulted in a lucigenin-derived chemiluminescence response. It has been reported that NAD(P)H oxidase activity accounts for most of the baseline lucigenin-enhanced luminescence (Suh et al., 1999; Griendling et al., 2000; Souza et al., 2000; Burchfield et al., 1999; Fukai et al., 1996; Patterson et al., 1999). Addition of arsenite could immediately enhance the lucigenin-derived chemiluminescence, which could be observed in 0.1 min after stimulation and show the dose-dependent increase. Addition of superoxide dismutase could decrease the production of O_2^-, which could determine that O_2^- exists extracellularly as superoxide dismutase cannot penetrate cells. These data suggested that the increase of O_2^- is related with arsenite stimulation and O_2^- production may come from the plasma membrane. Recent research has showed that the formation of O_2^- probably resulted from activation of NADH oxidase in human vascular smooth muscle cells induced by arsenite (Shugene et al., 2000). The similar results also showed that arsenite stimulate the NAD(P)H oxidase to generate O_2^- at or near that plasma membrane in vascular endothelial cells (Barchowsky et al., 1999; Mariano et al., 2000). Furthermore, in a variety of nonphagocytic cells, (NADPH-dependent) O_2^-/H_2O_2 generation is observed in response to divergent extracellular stimuli, such as interleukin-1, tumor necrosis factor (TNF-α) and Fas (Yoshihiro et al., 1998). These observations strongly suggest that like the NADPH oxidase system in phagocytic cells, an NADPH oxidase-like system may function as a reactive oxygen species-generating system in nonphagocytic cells. These results may explain how the production of O_2^- stimulated by arsenite in MGC-803 cells.

We assayed the contents of six solid malignant cell lines including MGC-803 cells exposed to 1 μM arsenite for 24 h. It was found that the percent of apoptosis in this cancer cell was related with the content of arsenite in the plasma membrane, not in the cell (Zhang, 1999). This result further indicated that the plasma membrane may be one of the target site of arsenite and lead to its toxicity. In this study, we observed not only membrane damage, but also oligonucleosomal fragments of cellular DNA as has been reported previously (Zhang et al., 1999), which showed the activation of nucleases and we also evidenced that no direct interaction between DNA and arsenite by UV absorbability and fluorescence methods. This data indicates that between membrane and nucleus, there must be some signal transduction pathways to activate a common apoptotic program. In this work, the formation of inositol-1,4,5-trisphosphate (IP_3) and rapid release of Ca^{2+} from intracellular pools observed could be inhibited by a inhibitor of phospholipase C, U73122, but the U73343 was without significant effect, which supported the statement that apoptosis was associated with rapid phospholipase C action and a resulting release of Ca^{2+} (Cao et al., 1999). In addition, the activation of NADPH oxidases could also increase the sensitivity of intracellular Ca^{2+} stores to IP_3 in human endothelial cells (Hu et al., 2000) and lead to the increase of intracellular Ca^{2+} which is related to the activity of nucleases. Meanwhile, the chelator extracellular Ca^{2+} EGTA could also inhibit the increase of intracellular Ca^{2+}. These suggested that the increased cytosolic [Ca^{2+}] may come from the ER and extracellular, arsenite-induced the increase of cytosolic free Ca^{2+} was related with signaling of lipid pathway in the initiation of apoptosis in MGC-803 cells.

As above mention, O_2^- production may come from the plasma membrane, suggesting that the target damage of membrane and the production of the free radicals of lipids is probably the important factor that cause the DNA damage (Cao et al., 1996) and initiates cell apoptosis. In addition, O_2^- can be converted to apoptosis-inducing hydroxyl radicals(·OH) through interaction with hypochlorous acid which is on the plasma membrane according to the formula:

$$HOCL + O_2^- \rightarrow \cdot OH + CL^- + O_2$$

The increase of DNA-strand breakage in arsenite-treated MGC-803 cells, which may result from the free radicals of lipids or ·OH and the expression of the p53 gene which is related with DSB were observed. It has been reported that arsenite induces DNA-strand breakages (Fount and Kun-Yan, 2000) and results in p53 accumulation in HFW cells through an ATM-dependent pathway (Johnson et al., 1996; Yih and Lee, 2000).

α-Tocopherol, one of the essential components of cellular defense mechanisms against endogenous and exogenous oxidants, could scavenge lipid peroxyl radicals which are the chain-carrying species and propagate lipid peroxidation. In this study, α-tocopherol was performed 24 h before arsenite treatment in order to allow diffusion into the cells. α-Tocopherol could abolished the transient increase in the production of endogenous O_2^-, arsenite-induced the production of O_2^-, lipid peroxidation, intracellular Ca^{2+} lev-

els, DNA-strand breakages, p53 gene expression and the subsequent fragmentation of nuclear DNA, as well as inhibiting arsenite-induced apoptosis of MGC-803 cells. From these data, we could conclude two subsequent effects following the production of O_2^- stimulated by arsenite and leading to apoptosis of MGC-803 cells: the first is the increase of cytosolic free Ca^{2+} may be downstream signal of lipid peroxidation and the second is the expression of p53 gene is downstream signal of oxidative stress and may also result from DNA-strand breakages (Yih and Lee, 2000; Vousden, 2000). But this does not preclude the possibility of other pathways mediated by factors other than reactive oxygen species.

In conclusion, low concentration of arsenite is able to inhibit cell growth by triggering the onset of MGC-803 cell apoptosis, and significantly increase superoxide formation and lipid peroxidation, O_2^- production and subsequent effects showed time sequence. α-tocopherol is able to inhibit this apoptosis significantly. These results indicate that arsenite-induced oxidative stress, which stimulate intracellular signaling systems, are involved in the initiation of apoptosis in MGC-803 cells.

Acknowledgements

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