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Inhibition of bovine herpesvirus-4 replication in endothelial cells by arsenite

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Abstract

The effect of arsenite pretreatment on bovine herpesvirus-4 (BHV-4) replication in bovine arterial endothelial (BAE) cells was studied. BHV-4 infectivity, including IE-2 expression, DNA replication and viral yield, were significantly reduced at nontoxic concentrations of arsenite in which cellular DNA synthesis or cell viability of BAE cells were not affected under resting and confluent conditions. This effect was accompanied by the induction of heat shock protein 70 (HSP70) and an interrupted cell cycle (causing cell cultures to accumulate at the S and G2/M phases). Actinomycin D inhibited the induction of HSP70 and reduced arsenite antiviral activity. In conclusion, cellular stress response induced by arsenite in BAE cells inhibited replication of BHV-4, and probably resulted from the induction of HSP70 and interference of cell cycle progression.

Keywords: Bovine herpesvirus-4; Endothelial cells; Arsenite; HSP70

1. Introduction

Bovine herpesvirus-4 (BHV-4) is a ubiquitous virus in cattle. BHV-4 was originally designated as bovine cytomegalovirus (CMV), primarily because many of its biological properties and growth characteristics in tissue culture closely resembled those of human CMV (Storz et al., 1984; Staczek, 1990). However, BHV-4 was shown to be more closely related to herpesvirus saimiri and Epstein–Barr virus than to CMV, thus leading to its reclassification as a member of the subfamily gammaherpesvirinae (Bublot et al., 1992; Lomonte et al., 1996). BHV-4 has a broad target cell range that causes disseminated infection and replication in numerous organs. Similar to other herpesviruses, BHV-4 frequently establishes a lifelong latent infection in the host after natural infection (Castrucci et al., 1991). Reactivation from latency can occur in response to as yet unknown stimuli.

Evidence from seroepidemiological studies, virus isolation studies of arterial tissues, and studies of viral infection in cell culture is consistent with a relationship between herpesvirus and atherosclerosis (Petrie et al., 1987; Yamashiroya et al., 1988; Melnick et al., 1995). Previously, we have demonstrated that bovine arterial endothelial (BAE) cells are susceptible to infection with BHV-4. The virus propagates rapidly in cultured BAE cells, compared to conventional Madin Darby bovine kidney cells (Lin et al., 1997). To facilitate the study of the pathogenic role of herpesvirus in atherosclerosis, we established a rabbit model of BHV-4 infection to induce and accelerate the atherosclerotic process (Lin et al., 2000). The data from that study supports the use of BHV-4 as a good model to study the herpesvirus replication in endothelial cells.

Arsenite, the trivalent version of elemental arsenic, has been documented to be a potent carcinogen. This concern is not trivial, given arsenite's significant worldwide exposure through the natural contamination of food and drinking water (Bagla and Kaiser, 1996). At the cellular and molecular levels, arsenite may alter protein structure and enzyme function via reaction with free thiol groups. One of the most prominent features of arsenite is its induction of the stress

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response that leads to increased synthesis of heat shock proteins (HSPs) (Wijk et al., 1993; Kim, 2001). Several reports have indicated that the induction of HSP expression by hyperthermia or other agents causes changes in the viral replication cycle during acute or persistent infection (Amici et al., 1994; Conti et al., 1999). The possibility that elevated levels of HSP70 may interfere with viral replication has been supported by the results of a variety of studies of antiviral activity of prostaglandins and other heat shock response inducers (Amici et al., 1994; Superti et al., 1998).

An arsenite- or thermal stress-induced stress response might result in the activation of latent viruses. For example, an increase in viral RNA production was observed following thermal stress or administration of sodium arsenite in a persistent-infection model of canine distemper virus (Oglesbee et al., 1993). Arsenite and thermal stress initiated transcription of a CMV major immediate early transcription unit in stably transfected Rat-9G cells (Geelen et al., 1987), and thermal stress activated transcription of the human immunodeficiency virus (HIV) type 1 long terminal repeat (LTR) (Geelen et al., 1988). However, reports have described arsenite interference with the replication of sendai virus (Angelidis et al., 1988), simian virus 40 (Amici et al., 1994), rotavirus (Superti et al., 1998), and human papilloma virus 16 (Zheng et al., 1999). Therefore, results to date have been inconsistent.

The ways in which the functioning of host cells becomes altered by stress during the viral infectious cycle are less clear. Arsenite can decrease the fibrinolytic activity of endothelial cells (Jiang et al., 2002). BAE cells are very susceptible to BHV-4 infection (Lin et al., 1997), and the virus enhances the atherosclerotic process in our rabbit model (Lin et al., 2000).

To clarify and probe the mechanics of the involvement of arsenite on viral replication, we have presently studied BHV-4 replication in endothelial cells.

2. Materials and methods

2.1. Reagents

Culture media, balanced salt solution, culture supplements, and trypsin were obtained from Gibco/BRL (Gaithersburg, MD, USA). Fetal calf serum (FCS) was purchased from Hyclone Laboratories (Logan, UT, USA), sodium arsenite was from Merck (Darmstadt, Germany), and both roscovitine and actinomycin D were purchased from Sigma Chemical Co. (St. Louis, MO, USA). These and other reagents specified subsequently were of the highest purity available.

2.2. Virus

The BHV-4 strain used was a Taiwanese strain isolated from persistently infected bovine arterial cell cultures (Lin et al., 1999).

2.3. Cell culture

Bovine arterial endothelial (BAE) cells were isolated from bovine carotid arteries as described previously (Lin et al., 1997; Lin et al., 1999). Briefly, cells were scraped from the intimal surface of the carotid arteries using a surgical blade. The cells were maintained as monolayers and subcultured in defined minimal essential medium (DMEM) containing penicillin (100 U/ml), streptomycin (100 μg/ml), fungizone (2 μg/ml), and 10% heat-inactivated fetal bovine serum. At confluence, the endothelial cells appeared as typical 'cobble-stone' like monolayers and incorporated 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-iodocarbocyanine perchlorate-labeled acetylated low density lipoprotein (Voyta et al., 1984). The cells were subcultured at a 1:3 ratio when cell culture achieved 80–90% confluence in 2–3 days. Ten to thirty cell passages were used.

2.4. Assay of proliferation and viability

To assay the effects of arsenite on the BAE cells under resting and proliferating conditions, either 5×10^4 or 2×10^5 cells were dispensed into each well of 24-well microplates and incubated overnight. The cells were pretreated with arsenite (2.5–50 μg/ml) for 2 h or 25 μg/ml for various time periods, washed with Hank's balanced salt solution, and subsequently cultured in the culture medium. Cell proliferation and viability was determined by the MTT method (Mosmann, 1983), with absorbance measured at 550 nm. Each experiment was performed in quadruplicate and repeated a minimum of three times.

2.5. Cell cycle analysis by flow cytometry

BAE cells were grown to 80% confluence in 6-well microplates, pretreated with 2.5, 25 or 50 μg/ml arsenite for 2 h, and washed with HBSS prior to culture in DMEM containing 2% FCS. Every 24 h, cells were harvested, washed twice with PBS, fixed in cold 75% alcohol, and stored at 4 °C until used.

Cells were washed with PBS, treated with 1% RNase A (Sigma), and stained with 100 μg/ml of propidium iodide. The distribution of the cell cycle phases with different DNA contents was determined using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA). In each sample, 10 000 cell analyses were acquired. Analysis of cell cycle distribution was performed using Modfit software (Becton Dickinson).

2.6. Viral infection

BAE cells were grown to confluence in 6-well microplates (5×10^5 cells/well), pretreated for 2 h with 2.5–25 μg/ml arsenite, and infected with BHV-4. In the actinomycin D treatment assay, BAE cells were pretreated for 60 min with 2 μg/ml actinomycin D prior to culture in the presence of

25 μg/ml arsenite for 2 h. After washing with HBSS, the cells were infected with BHV-4 at a multiplicity of infection of one. After incubation at 37 °C for 2 h, the cultures were washed with HBSS, and two ml of culture medium supplemented with 2% FCS was added. At predetermined times post infection (p.i.), the cultures were harvested and stored at −70 °C until virus titration was measured.

2.7. Virus production assay

Cell culture quantification of viruses was done utilizing the 50% tissue cell culture infectious dose ($TCID_{50}$) method (Hsiung, 1994) using monolayers in 24-well microtiter plates. Serial 10-fold viral dilutions were prepared in DMEM containing 2% FCS. Aliquots (0.2 ml) of the diluted preparation were inoculated in triplicate cell monolayers in 24-well-microtiter plates. The cells were incubated at 37 °C in a 5% CO_2 atmosphere. Plates were examined daily for 3–7 days for the appearance of cytopathic effect (CPE). The titers for each day were calculated in terms of $TCID_{50}$. Growth medium was replenished by adding maintenance medium at 4-day intervals. Cell cultures remained in satisfactory condition for at least 10 days, within which time all positive wells had developed CPE.

2.8. Viral DNA detection

Viral DNA levels were determined by DNA hybridization. BAE cells were grown to confluence in 6-well microplates (5×10^5 cells/well), pretreated with 2.5–25 μg/ml arsenite for two h, and infected with BHV-4. The cells were then harvested at 0, 12, 24, 36 and 48 h p.i.

Total DNA was isolated and 10 μg DNA (determined semi-quantitatively using spectrophotometry) was transferred to a nylon membrane, immobilized by ultraviolet (UV) crosslinking, and subjected to slot-blot hybridization using a purified ^{32}P-labeled 1.8-kb immediately-early-gene (IE-2) cDNA fragment (Van Santen, 1993). The fragment of IE-2 cDNA used for the detection of viral DNA was obtained by RT-PCR amplification. Briefly, total RNA of BHV-4 infected BAE cells was isolated and amplified with a specific pair of primers:

IE2-SE: 5′-CAAGCTTCAAACACACAGACCAG-3′
IE2-AE: 5′-CTCGAGAGGCTATTGTCAACATC-3′

The 1.8-kb fragment of PCR product was extracted from the gel by use of a DNA extraction kit (Protech Technology, Taipei, Taiwan, ROC) and labeled by [α-^{32}P]dCTP using the RadPrime DNA Labeling Kit (Gibco/BRL). The signal hybridized to each slot was quantitated and corrected for loading as measured by GAPDH hybridization.

2.9. Northern blot analysis

Total cellular RNA was isolated from cells using the RNAeasy kit (Qiagen, Valencia, CA, USA). Thirty micrograms of total RNA was separated by electrophoresis on 1% agarose–glyoxal gel, transferred to a nylon membrane, and immobilized by UV-crosslinking. The filters were prehybridized for 2 h and subsequently hybridized for 16 h with a 1.25-kb glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA or IE-2 cDNA of BHV-4 labeled by [α-^{32}P]dCTP by random priming. The filters were washed with a final stringency of 0.1 × SSC (0.15 M NaCl + 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate (SDS) at 60 °C. The cDNA-mRNA hybrids were visualized by autography. The intensity of the hybridized band was determined by Fuji Bio-imaging Analyzer BAS1000 (Fuji Photo Film Co., Tokyo, Japan).

2.10. Western analysis of p21 and HSP70

To examine the kinetics of p21 and HSP70 synthesis, BAE cells were grown to confluence in 6-well microplates (5×10^5 cells/well), pretreated with 2.5–25 μg/ml of arsenite for 2 h, washed with HBSS, and then cultured in DMEM. At the predetermined times, cells were harvested in lysis buffer (150 mM NaCl, 20 mM Tris–HCl, 1.0% Triton X-100, 0.5 mM EDTA, 50 mM NaF, 10% glycerol, 20 μg/ml of phenylmethylsulfonyl fluoride, and 1 mM sodium vanadate), and incubated on ice for 10 min with occasional vortexing. Cells were then frozen and stored at −70 °C until the entire time course was completed.

At the completion of the sampling time course, all the frozen cells were thawed, and debris was removed by centrifugation (15 000 × *g*, 10 min, 4 °C). Protein concentrations were determined using the Micro BCA protein assay reagent kit (Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's protocol. Equal amounts of protein were separated by SDS/PAGE, blotted onto nitrocellulose. HSP70 and p21 were detected by Western blot analysis using the primary mouse monoclonal antibody anti-HSP70 (CALBIOCHEM, Darmstadt, Germany) and p21 (Cell Signaling Technology, Beverly, MA, USA). After washing, the blots were probed with secondary antibody (horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin G (Calbiochem)) for 2 h at room temperature. Blots were washed three times in PBST and then developed by enhanced chemiluminescence according to the manufacturer's protocol (Pierce Biotechnology).

3. Results

3.1. Inhibition of BHV-4 lytic cycle in BAE cells by arsenite

Arsenite pretreated BAE cell lysate was examined to determine the titer of BHV-4 in terms of $TCID_{50}$. As shown in Fig. 1A, arsenite decreased the infectivity and replication titers of BHV-4 by 24 h p.i. in a dose-dependent

manner. A decrease in the concentration of arsenite resulted in a parallel decrease in the degree of viral titer inhibition. Complete inhibition of BHV-4 multiplication was observed in BAE cells pretreated with 25 μg/ml arsenite.

The inhibition of BHV-4 growth in arsenite pretreated BAE cells was seemingly not due to a change in the virus' binding capacity to endothelial cells, since there was no difference in the uptake of BHV-4 DNA between normal and arsenite-treated BAE cells as measured by quantitative PCR analysis (data not shown). Biosynthesis of BHV-4 DNA in BAE cells was significantly inhibited by arsenite pretreatment in a concentration-dependent manner (Fig. 1B) as shown by the results of slot-blot analysis of viral DNA. These results concurred with those of the virus titration studies summarized above.

To further examine the possibility that the inhibition of viral DNA replication by arsenite was mediated by reduction of immediate-early stage expression, the effect of arsenite on viral IE-2 gene expression was investigated by Northern blot analysis. As shown in Fig. 1C, pretreatment of BAE cells with arsenite inhibited the expression of IE-2 mRNA in a dose dependent manner at an early phase (6 and 12 h p.i.) of virus infection.

3.2. Effect of arsenite on the proliferation and viability of BAE cells

To determine whether the antiviral effects of arsenite could be a consequence of the compound's cytotoxic effect on BAE cells, the proliferation and viability of arsenite-pretreated cells was measured using the MTT assay. Under confluent and resting condition, the viability of BAE cells was not significantly decreased by a 2 h pretreatment of the cells with 2.5–25 μg/ml arsenite (Fig. 2A). Even an arsenite dose of 50 μg/ml failed to decrease the BAE population viability by 20%. However, a 3 h exposure of BAE cells to 25 μg/ml arsenite produced a 30% decline in viability by 48 h (data not shown). As Fig. 2B shows, under proliferating condition, a pattern indicative of a dose-dependent inhibition of the

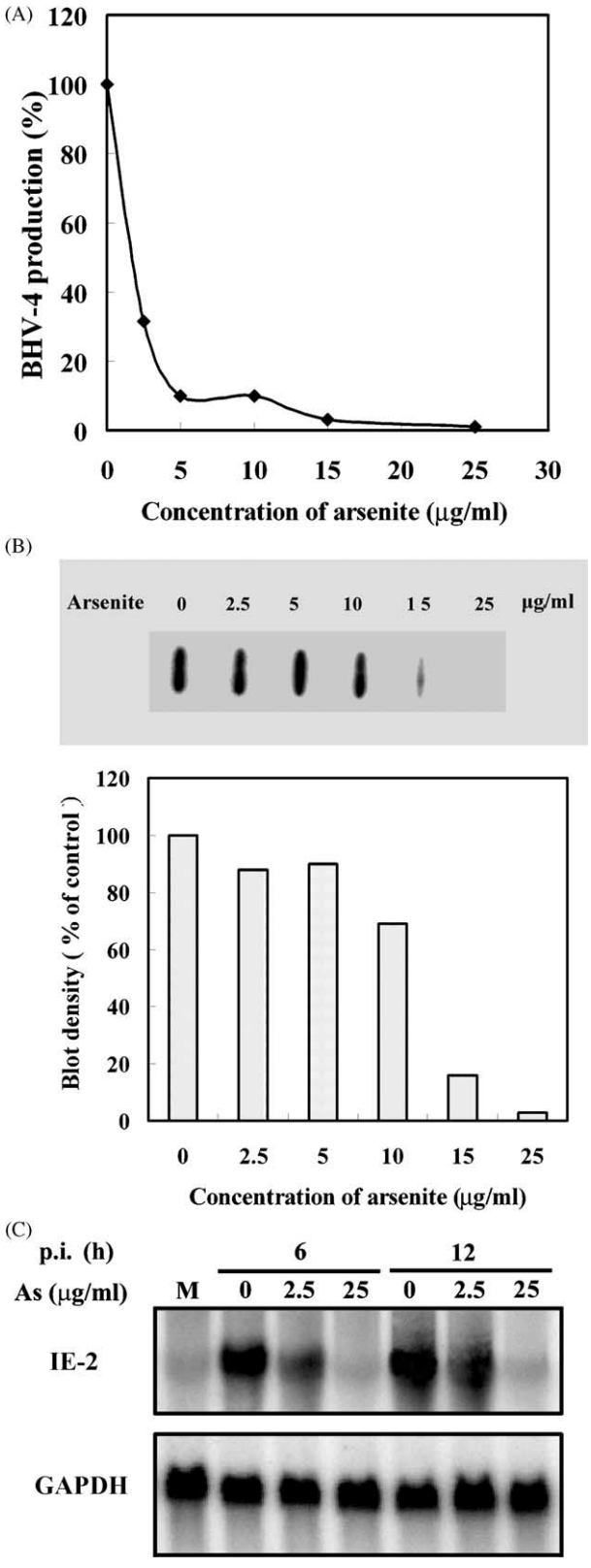

Fig. 1. Effect of arsenite on BHV-4 replication. BAE cells were pretreated for 2 h with various concentrations of arsenite and then infected with BHV-4. (A) The amount of BHV-4 in the virus-inoculated cell cultures ($TCID_{50}$) was determined by titration at 24 h p.i. The graphs show the relative titer of each sample, where 100% is the viral production of cell lysate harvested from control (no arsenite) BAE cells infected with BHV-4. The titer of the infected control was 5×10^3 TCID50/ml. Each experiment was done in triplicate, and the entire assay was performed twice. (B) The cells were harvested at 24 h p.i., analyzed and quantified for total viral DNA by slot-blotting hybridization. The signal hybridized to each slot was quantitated and corrected for loading as measured by GAPDH hybridization. (C) The cells were harvested at the indicated times p.i. and analyzed for viral mRNA expression by Northern blot analysis using a ^{32}P-labeled viral IE-2 fragment.

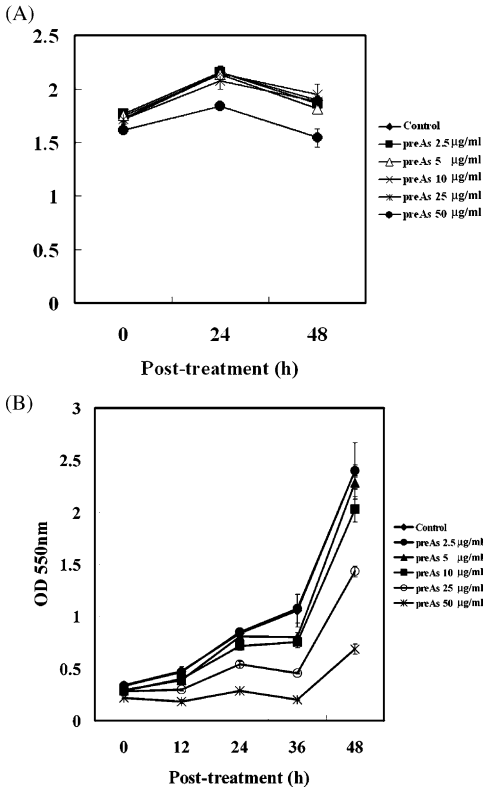

Fig. 2. Effect of arsenite on cell viability and proliferation. BAE cells were pretreated with various concentration of arsenite (2.5–50 μg/ml) for 2 h. (A) The pretreatment was performed under the resting condition, in which cells were plated at a density of 3×10^5 cells/well in 24 well-plates, or (B) under the proliferating condition, in which cells were plated at a density of 5×10^4 cells/well. The viability of the cells was measured by the MTT test.

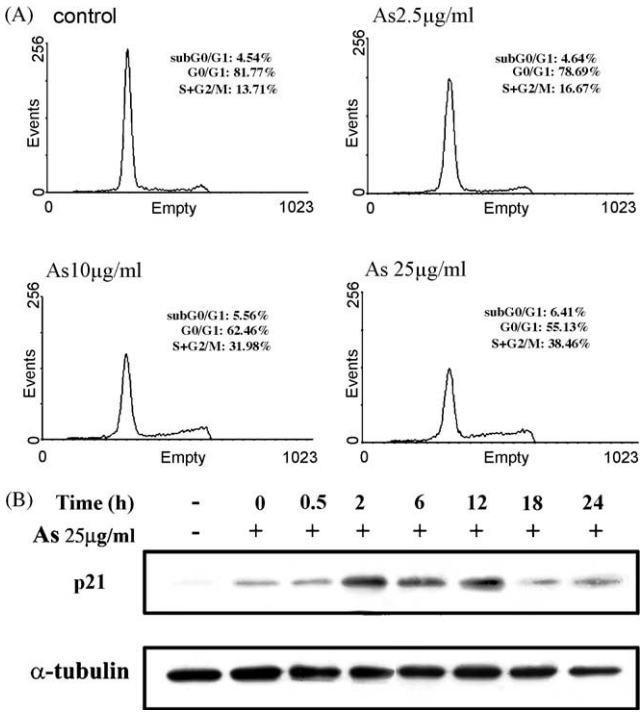

Fig. 3. Effect of arsenite on cell cycle. BAE cells were pretreated with arsenite (2.5, 10 or 25 μg/ml) and cultured in DMEM. (A) Cells were harvested at 24 h. The relative DNA contents of the cells were determined by staining with propidium iodide and flow cytometric analysis. (B) BAE cells were pretreated with arsenite (25 μg/ml) for 2 h and cell lysate was harvested at the indicated times after treatment. p21 was detected by Western blot analysis using a specific anti-p21 antibody.

rate of proliferation of BAE cells by arsenite was evident.

3.3. Effect of arsenite on cell cycle arrest by the induction of p21

To illustrate whether arsenite inhibits BHV-4 replication indirectly by interfering with the cell cycle of host cells to decrease the proliferation rate, we examined the relative DNA content of BAE cells and the percentage of the total population of BAE cells at each stage of the cycle by flow cytometry (Fig. 3A).

In the culture of arsenite-pretreated BAE cells, a dose-dependent increase in the proportion of cells at the S and G2/M phases of the cell cycle (13.71% for the control versus 16.67, 31.98, and 38.46% for cell populations treated with 2.5, 10, and 25 μg/ml arsenite, respectively) with a progressive decrease in the proportion of cells at the G0/G1 phase was observed at 24 h. To further investigate the mechanism for arsenite-induced S and G2/M arrest, we examined expression of p21, a inhibitor of cyclin-dependent kinases (cdk). As shown in Fig. 3B, arsenite induced a marked increase in the level of p21 at 2, 6, and 12 h post-treatment.

3.4. Effect of arsenite pretreatment on HSP70 expression

Pretreatment of BAE cells with 25 μg/ml arsenite for 2 h resulted in markedly elevated levels of HSP70 expression during the post-treatment times (0–24 h) as measured by Western blot analysis (Fig. 4).

3.5. Effect of arsenite and actinomycin D on BHV-4 replication

To determine whether the induction of cellular mRNA synthesis was involved in the antiviral action of arsenite, cellular mRNA synthesis was inhibited by exposure to actinomycin D to investigate the effect of HSP70 in BHV-4 infectivity. As shown in Fig. 5A, 2 μg/ml actinomycin D abolished the inhibiting effect of arsenite pretreatment on BHV-4 replication, consistent with the suggestion that the inhibition of viral replication by arsenite pretreatment is dependent on efficient cellular transcription and protein biosynthesis including HSP70. Actinomycin D-mediated inhibition of arsenite-induced HSP70 synthesis was apparent (Fig. 5B).

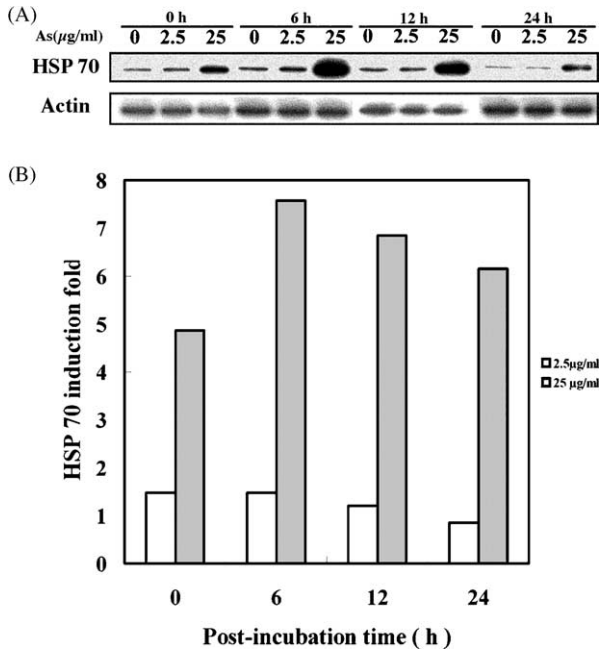

Fig. 4. Kinetics of HSP70 synthesis in BAE cells pretreated with arsenite. BAE cells were pretreated with arsenite (2.5 or 25 μg/ml) for 2 h, and the cell lysate was harvested at the indicated times. HSP70 was detected by Western blot analysis using a specific anti-HSP70 antibody, quantified by chemiluminescence, and corrected for α-actin expression on stripped blots. BAE cells were pretreated with arsenite concentrations of 2.5 μg/ml (white bars) and 25 μg/ml (black bars) as indicated. Data are presented as enhanced folds as compared to control cell values.

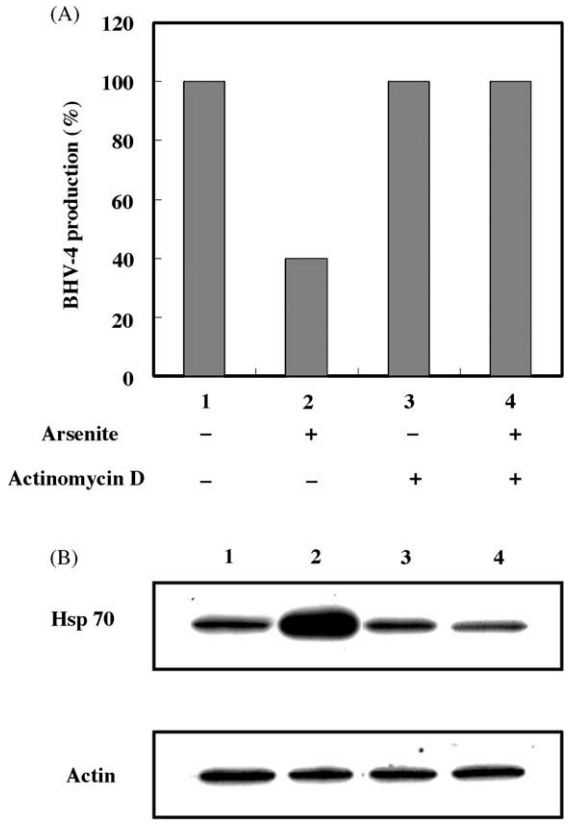

Fig. 5. Effect of arsenite and actinomycin D on BHV-4 production in BAE cells. BAE cells were pretreated with arsenite (25 μg/ml), actinomycin D (2 μg/ml), or with both arsenite and actinomycin D prior to infection with BHV-4 as described in Fig. 2. Actinomycin D was kept in the culture medium during the experiment. The cells were harvested at 12 h p.i. (A) Virus infectivity was determined by TCID50 assay. The titer of infected controls was $5 \times 10^{1.5}$ TCID50/ml. (B) HSP in each cell lysate was measured by Western blotting. BHV-4 infection was performed on BAE cells only (lane 1), cells pretreated for 2 h with arsenite (lane 2), cells pretreated for 3 h with actinomycin D (lane 3), and cells pretreated with arsenite plus actinomycin D (lane 4).

4. Discussion

Arsenite is a pleiotropic agent capable of activating a wide range of cellular responses. Long-term exposure to low levels of environmentally derived arsenite is associated with vascular diseases (Chen et al., 1996). The present study demonstrates that levels of arsenite that do not significantly damage BAE cells effectively inhibit the replication of BHV-4 in these cells. Our observations strongly indicate that this inhibitory effect results from HSP70 induction and cell cycle interference, which causes an accumulation of cells at the S and G2/M phases.

Other studies have shown that low levels of arsenite can activate NF-kB, thereby inducing oxidative stress and stimulating proliferation of endothelial cells (Barchowsky et al., 1996). An incremental increase of reactive oxygen in vascular endothelial cells exposed to high levels of arsenite causes DNA damage (Liu and Jan, 2000). Conversely, nontoxic doses of arsenite may have subtle effects on cell signaling and cell functions. For example, arsenite might induce immediate G2/M arrest that is mediated by the induction of p21, an inhibitor of cdk species (Yih and Lee, 2000). Arsenite also induces transient growth delay of NIH3T3 cells via a marked activation of p38 MAPK and p21, resulting in inhibition of cdk-2 and cdk-4 kinase activity (Kim et al., 2002).

In contrast to these deleterious effects of arsenite, arsenic trioxide (As_2O_3) has clinical benefits. It inhibits proliferation of virus-transformed cells by inducing cell cycle arrest and enhancing apoptosis (Bazarbachi et al., 1999; Zheng et al., 1999), and induces remission of acute promyelocytic leukemia and adult T-cell leukemia/lymphoma (Niu et al., 1999).

The relationship between HSP production and viral infection is becoming more widely documented. Increased HSP expression has been described in cells infected with herpes simplex, CMV and adenovirus, consistent with a common role of HSP in viral replication (Glotzer et al., 2000). By inhibiting the translation of viral proteins, HSP70 overexpression in cellular responses to different situations (such as incubation with prostaglandins or arsenite, or heat shock) might interfere the replication of sendai virus and simian virus 40 (Angelidis et al., 1988; Amici et al., 1994). While arsenite might induce cellular stress responses that in turn interfere with virus replication, some other reports

indicate that arsenite enhances human T-cell lymphotropic virus type 1 expression (Andrews et al., 1997), and increases the virus-induced cytopathic effect of measles virus (Vasconcelos et al., 1998). Investigation of the cellular responses to arsenite in conjunction with the viral gene expression might shed some light to the mechanism of arsenite in modulating the virus infectivity in host cells.

Similar to observations by other investigators, we noted a slight increase of HSP70 biosynthesis in BAE cells infected with BHV-4 for 24 h (data not shown), consistent with a suggested role of such low levels of HSP70 in viral replication (Glotzer et al., 2000). On the other hand, high expression levels of HSP70 synthesis induced by different agents exerts an antiviral function that is mechanistically complex. Viral RNA transcription can be blocked (Yamamoto et al., 1987; Bader and Ankel, 1990), and viral protein translation and maturation can be retarded by binding to the neosynthesized viral proteins (Santoro et al., 1982; Santoro et al., 1989).

In the case of BHV-4 infection in BAE cells, intracellular accumulation of HSP70 induced by arsenite inhibits BHV-4 replication (Fig. 1A and B) by blocking the transcription of the viral IE-2 gene (Fig. 1C). The observations that HSP70 synthesis increases after arsenite treatment and persists for up to 24 h supports the hypothesis that high levels of HSP70 synthesis plays a role in arsenite's antiviral effect. This involvement of HSP70 in the control of BHV-4 replication is further supported by our finding that actinomycin D, which blocks cellular protein expression including HSP70, reverses the inhibiting effect of arsenite on BHV-4 replication (Fig. 5). Similar results have been described with the antiviral activity of cyclopentenone prostaglandins (Amici et al., 1994; Superti et al., 1998). Therefore, induction of HSP70 expression by different agents could be involved in the mechanism of antiviral activity.

To exclude the possibility that arsenite-mediated antiviral activity was directly due to a toxic effect on the cells, the effect of arsenite on the proliferation and viability of BAE cells under resting or proliferating conditions was measured using the well-established MTT test (Fig. 2). Our results clearly demonstrate no toxic effect of arsenite on BAE cells under resting and confluent condition (Fig. 2A), although cell proliferation was inhibited in a dose-dependent manner (Fig. 2B).

Replication of herpesvirus is influenced by the physiological state of host cells. For example, an inhibition of cdk-2 activity suppresses CMV replication (Bresnahan et al., 1997). Inversely, viral infection of host cells induces expression of high levels of cell cycle regulators, leading to cell cycle arrest in the G2/M phase (Jault et al., 1995). Viral replication may be a consequence of the dependence of DNA synthesis on the S phase of the host cell cycle (Vanderplasschen et al., 1995). Presently, cell cycle arrest and concomitant inhibition of BHV-4 replication in arsenite-treated BAE cells occurred at the G2/M phase. Such inhibition has been similarly documented using roscovitine (Meijer et al., 1997; Mgbonyebi et al., 1998). Since roscovitine treatment of BHV-4-infected BAE cells induced cell cycle arrest at G2/M phase, resulting in the inhibition of BHV-4 infectivity (unpublished data), the antiviral activity of arsenite might be partially mediated by cell cycle arrest. This suggestion is consistent with the known dependence of BHV-4 DNA synthesis on the S phase of the host cell cycle (Vanderplasschen et al., 1995).

The synthesis of HSP70 was increased in BAE cells upon arsenite treatment. This elevated synthesis persisted for up to 24 h following arsenite exposure. These observations are entirely consistent with the hypothesis that high levels of HSP70 synthesis are involved in arsenite-mediated antiviral activity. Furthermore, we propose that arsenite induces the expression of HSP70 (Fig. 4) and p21 (Fig. 3B). The resulting inhibition of cdk-2 activity (Kim et al., 2002) would lead to cell cycle arrest at the G2/M phase (Luft et al., 2001), providing another mechanism for the suppression of viral production by arsenite.

Such a common mode of action for arsenite might be a consequence of a common signaling pathway leading to G2/M cell cycle arrest. The interference of the cell cycle progression and inhibition of the cdk activity might contribute to the inhibition of BHV-4 replication process by arsenite, especially concerning the transcription of IE-2.

The present study provides a model for studying the basic cellular response that could modulate BHV-4 replication, and provides an opportunity to investigate the effect of viral gene expression in endothelial cells. Although long-term chronic exposure of arsenite has been associated with vascular disease (Jiang et al., 2002), and exposure to high doses of arsenite can induce acute cytotoxicity including apoptosis and DNA damage (Huang et al., 1995; Watson et al., 1996), our results indicate that cellular stress response can be induced by short-term exposure to moderate doses of arsenite. This latter induction may suppress viral replication in endothelial cells.

Finally, we observed that under well-controlled conditions arsenite may be capable of inhibiting viral replication through the modulation of the cellular stress responses. If so, arsenite holds the potential of being able to control viral replication in an acute viral infection such as that caused by herpesvirus.

Acknowledgements

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