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Research Paper

Astragaloside IV inhibits adenovirus replication and apoptosis in A549 cells *in vitro*

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Abstract

Objectives Astragaloside IV, purified from the Chinese medical herb *Astragalus membranaceus* (Fisch) Bge and *Astragalus caspicus* Bieb, is an important natural product with multiple pharmacological actions. This study investigated the anti-ADVs effect of astragaloside IV on HAdV-3 (human adenovirus type 3) in A549 cell.

Methods CPE, MTT, quantitative real-time PCR (qPCR), flow cytometry (FCM) and Western blot were apply to detect the cytotoxicity, the inhibition and the mechanisms of astragaloside IV on HAdV-3.

Key findings TC_0 of astragaloside IV was 116.8 μM, the virus inhibition rate from 15.98% to 65.68% positively was correlated with the concentration of astragaloside IV from 1.25 μM to 80 μM, IC50 (the medium inhibitory concentration) was 23.85 μM, LC50 (lethal dose 50% concentration) was 865.26 μM and the TI (therapeutic index) was 36.28. qPCR result showed astragaloside IV inhibited the replication of HAdV-3. Flow FCM analysis demonstrated that the anti-HAdV-3 effect was associated with apoptosis. Astragaloside IV was further detected to reduce the protein expressions of Bax and Caspase-3 and increasing the protein expressions of Bcl-2 using western blotting, which improved the anti-apoptosis mechanism of astragaloside IV on HAdV-3.

Conclusions Our findings suggested that astragaloside IV possessed anti-HAdV-3 capabilities and the underlying mechanisms might involve inhibiting HAdV-3 replication and HAdV-3-induced apoptosis.

Keywords anti-ADVs; apoptosis; astragaloside IV; cytopathic effect; human adenovirus type 3

Introduction

Adenoviruses (ADVs), a group of viruses prevailing throughout the world, infect the membranes (tissue linings) of the respiratory tract, the eyes, the intestines and the urinary tract. ADV infection in humans can lead to many diseases, such as acute upper and lower respiratory infection, fulminating conjunctivitis, acute hemorrhagic cystitis, rheumatoid arthritis, immunodeficiency disease, cerebritis, cerebral meningitis and infant gastroenteritis.[1,2] The infectivity of ADVs is very high, especially in densely populated areas and among immune-deficient patients, such as the elderly and those with bone-marrow transplants, leukemia and AIDS.[3] In infants between 6 months and 3 years of age, ADVs can not only cause viral pneumonia, but can also give rise to long-term lung damage.[4] The six species of human adenoviruses (HAdV, genus astadenovirus, family Adenoviridae), with their 51 types, are associated with a variety of diseases affecting all organ systems.[5] As a causative agent of acute respiratory disease among HAdVs, human adenovirus type 3 (HAdV3) is an important etiological agent that can cause human respiratory and alimentary infection and has been proven to be one of main etiological agents of diseases that lead to respiratory infection in children.[6] In the absence of vaccination, a safe and effective antiviral would be welcomed by the susceptible populations. There remains a lack of specific drugs for the treatment of viral diseases, and so the search for antiviral medicines that are effective, safe and have few (if any) side effects is imperative. Traditional Chinese medicine has shown intriguing potential in this area since it is associated with, for example, abundant resources, multitargeted mechanisms of action, few side effects and no drug resistance.[7]

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Astragaloside IV, a cycloartane-type triterpene glycoside extracted from *Astragalus membranaceus* (Fisch) Bge and *Astragalus caspicus* Bieb, has been shown to have multiple pharmacological effects, including antivirus, anti-infarction, antioxidant and anti-inflammation.[8–12] As a widely used active ingredient, astragaloside IV is reported to have significant activities on different diseases, such as metabolic syndrome, diabetic peripheral neuropathy, chronic experimental asthma, heart failure, ischemic brain injury and diabetic vascular remodeling.[8,13–17] Astragaloside IV also possesses inhibitory actions on hepatitis B and coxsackievirus B3 virus.[11,18–20] In traditional Chinese folk medicine, *Astragalus membranaceus* is used in the treatment of inflammation of the respiratory tract induced by the influenza virus, ADVs and so on, in either simple recipes or complex prescriptions. However, there have been no reports of the anti-HAdV effects of *Astragalus membranaceus*. Since astragaloside IV is believed the main active constituent of *Astragalus membranaceus*, its anti-HAdV effect in A549 cells was investigated based on the following tests: cytopathic effect (CPE), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), quantitative real-time PCR (qPCR), flow cytometry (FCM) and Western blot.

Materials and Methods

Cell lines

The A549 cell line used in this study was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and preserved by the Department of Hygienic Microbiology of Harbin Medical University in China.

Cell culture

Cell culture experiments were performed with A549 cells, which were propagated under standardized conditions (37°C, 5% carbon dioxide). The cells were cultured in RPMI-1640 (Invitrogen Corp., CA, USA) and harvested by trypsin/EDTA. The culture was supplemented with 10% fetal bovine serum (FBS) and 100 μg/ml penicillin/streptomycin. For antiviral assays, the medium was supplemented with 2% FBS and the antibiotics.

Antiviral agents

Astragaloside IV (purity >98%), $C_{41}H_{68}O_{14}$, molecular weight 784.97, CAS # 84687-43-4, was purchased from the National Institute for the Control of Pharmaceutical and Biological Products. The dry substance was freshly prepared in DMSO before use. Stock preparations of the reagents were stored at –20°C.

Virus stock solutions

HAdV3 was obtained from ATCC (Manassas, VA, USA). HAdV3 were propagated on A549 cells in 75 cm^2 cell-culture flasks. They were harvested when the CPE reached more than 95%, by freezing (–80°C, 10 min) and thawing (room temperature) the cell-culture flasks three times. The supernatant was cleared by centrifugation (3000*g* for 5 min) and stored at –80°C for further use.

Determination of human adenovirus type 3 toxicity

Virus titres were determined as 50% cell culture infective dose (CCID50) on A549 cell monolayers in 96-well microtitre plates. A ten-times serial dilution of the virus-infected fluid was inoculated into a monolayer of cells and the CPE was observed. The CCID50 was calculated with the Reed–Muench method.

Determination of astragaloside IV toxicity

Different concentrations of astragaloside IV (from 0 to 2560 μM) were added to 96-well cell-culture plates containing a monolayer of A549 cells. The conventional MTT method (Sigma Chemical Co. (St. Louis, MO, USA) was used to determine astragaloside IV toxicity at 72 h exposure.[21] Optical density values were read using a microplate reader at two wavelengths (540 nm and 690 nm). The viability was expressed as percentage of non-treated control.

Cytopathic effect and MTT-based antiviral activity assay

A549 cells were seeded in 96-well plates at 1×10^4 cells/well and incubated for 24 h. Subsequently 100 μl/well 100 CCID50 HAdV3 solution was inoculated into 96-well plates, incubated for 2 h at 37°C and the virus fluid removed. Different concentrations of astragaloside IV in an atoxic range were added to the 96-well plates (200 μl/well). For each astragaloside IV concentration, six duplicate wells, a virus control group and a cell control group were tested simultaneously. The negative control wells had no cells, with culture medium only. When the virus control group CPE reached more than 75%, the cell survival rate was detected by the MTT method. Experiments were repeated three times.

Real-time quantitative polymerase chain reaction

The total intracellular HAdV3 DNA was isolated with a Column Mate Tissue/Cell gDNA Isolation Mini Kit (Watson Biotechnologies, Inc., Shanghai, China) from A549 cells. qPCR was performed on a 96-well StepOnePlus™ system (A&B Applied Biosystems, Foster City, CA, USA). HAdV3 hexon was amplified using qPCR with the following primers: the forward primer (5′-CTGGGAAATGGTCGTTAT-3′) and reverse primer (5′-ATTGATGCTGGTGAAACT-3′). This reaction produced a 195-bp PCR product. The appropriate cycle threshold was firstly determined using the automatic baseline determination feature. The dissociation analysis (melt-curve) on the reactions was then performed to identify the characteristic peak associated with primer-dimers in order to separate from the single prominent peak representing the successful PCR amplification of HAdV3 hexon DNA. Detection Kit (Ambion, Austin, TX, USA) in conjunction with real-time PCR with SYBR Green I was used to detect the amplification levels of HAdV3 hexon DNA and the expression of GAPDH was used as an internal control. All samples were run in triplicate for HAdV3 hexon or human GAPDH, respectively.

Flow cytometry analysis of apoptosis

The percentage of apoptotic A549 cells was assessed from the Annexin-V-FITC protein binding using an Annexin-V-

FLUOS Staining Kit (Roche, Mannheim, Germany). 10^6 cells were washed with PBS and centrifuged at 200*g* for 5 min. The cell pellets were resuspended in 100 µl of Annexin-V-fluorescein and propidium iodide solution and incubated for 15 min at room temperature. A quantity of 0.5 ml Hepes buffer per 10^6 cell was used in the detection and quantification of apoptotic cells by using a FACScan laser flow cytometry system (Mountain View, CA, USA), using 488 nm excitation and a 515 nm bandpass filter for fluorescein detection and a filter >600 nm for propidium iodide (PI) detection. Cell populations which were Annexin V^-/PI^- were considered to be alive, those which were Annexin V^+/PI^- were considered an early apoptotic population, and those which were Annexin V^+/PI^+ were late-stage apoptotic or necrotic.

Western blot

A549 cells (1×10^6) cultured in 100 mm^2 culture dishes were treated with various concentrations of astragaloside IV (0, 1.25, 5, 20, 80 µM) for 48 h. The protein samples were extracted from A549 cells. The protein content was determined with a BCA Protein Assay Kit (Beyotime Institute of Biotechnology, Haimen, China) using bovine serum albumin as the standard. Samples of 80 µg of protein from different experimental groups were mixed with 5 × loading buffer solution, denatured for 5 min at 100°C and separated by 12% SDS–PAGE and transferred to nitrocellulose membranes by electroblotting (300 mA for 2 h). The membranes were blocked in PBS containing 5% (w/v) skimmed milk at 37°C for 1 h. The membranes were then incubated overnight at 4°C with the primary antibody, including a rabbit polyclonal affinity purified anti-Bcl-2 antibody, a rabbit polyclonal affinity purified anti-Bax antibody and a rabbit polyclonal anti-caspase-3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membrane was washed three times (each 10 min) with PBS containing 0.5% Tween 20 (PBS-T) and followed by incubation with the secondary antibody for an additional 1 h at room temperature. The membrane was then washed three times (10 min for each wash) with PBS-T and scanned by an Odyssey Infrared Imaging System (LI-CORBiosciences, Lincoln, NE, USA) at a wavelength of 800 nm. Western blot bands were quantified using Odyssey v1.2 software by measuring the band intensity (area multiplying OD) for each group and normalizing to actin as an internal control (anti-actin antibody from Santa Cruz Biotechnology, Santa Cruz, CA, USA). The final results were expressed as fold changes by normalizing the data to the control values.

Statistical analysis

The data were processed with SAS (version 9.13). The drug lethal dose 50% concentration (LC50) and the medium inhibitory concentration (IC50) were calculated using the linear-regression method. The following calculations were used:

$$\text{A549 cell survival rate}(\%) = (\text{medicine group absorbance value} / \text{cell control group absorbance value}) \times 100\%$$

$$\text{Inhibition ratio}(\%) = 100\% - \text{survival rate}$$

$$\text{Virus inhibition ratio} = (\text{medicine treatment group absorbance value} - \text{virus control group absorbance value}) / (\text{cell control group absorbance value} - \text{virus control group absorbance value}) \times 100\%$$

$$\text{Therapeutic index}(\text{TI}) = (\text{LC50})/\text{IC50}$$

Data are expressed as mean ± SD. Statistical analyses were performed using one-way analysis of variance, and differences between means were tasted using Duncan's multiple-range tests. A significant difference was determined by a *P* value of less than 0.05 and significance was identified in each figure. If the *P* value was greater than 0.05, the data were not considered statistically significantly different.

Results

Human adenovirus type 3 toxicity assay

Compared with the normal cell group, A549 cells infected with HAdV3 became round and then clumped together. When 50% of the cells exhibited a CPE they were regarded as CPE^+ cells. If the cells did not appear to exhibit any CPE after 72 h, they were regarded as CPE^- cells. The CCID50 of ADV3 in A549 cells is $10^{4.5}$. The challenging dose of virus used in the experiments was 100 CCID50/100 µl.

Toxic effect of astragaloside IV on A549 cells

The atoxic range of astragaloside IV was assessed by measuring the TC_0 (The maximum non-toxic concentration) and LC50 using the MTT method. The 72 h toxic effect of astragaloside IV on A549 cells reduces with decreasing concentration, with a concomitant gradual increase in the cell survival rate. The TC_0 of astragaloside IV was 116.8 (µM) (Figure 1).

The anti-HAdV3 Effect of astragaloside IV assessed by CPE and MTT

The MTT method has been proven to be a sensitive and accurate method for screening anti-HAdV3 agents. A549 cells are highly susceptible to HAdV3-induced CPE, as characterized by cell swelling to round-like grape. This makes A549

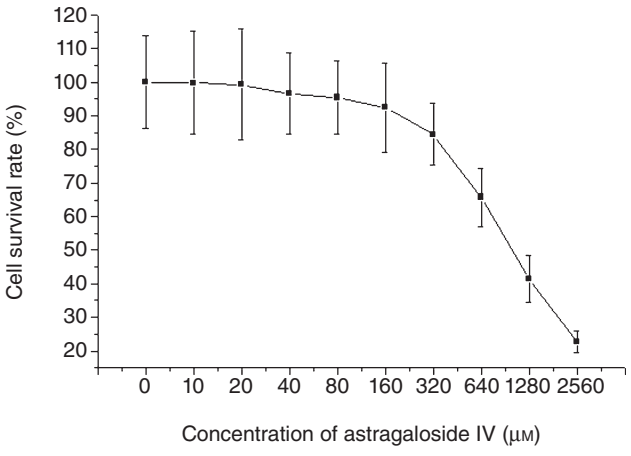

Figure 1 Toxicity of astragaloside IV to A549 cells in MTT assay.

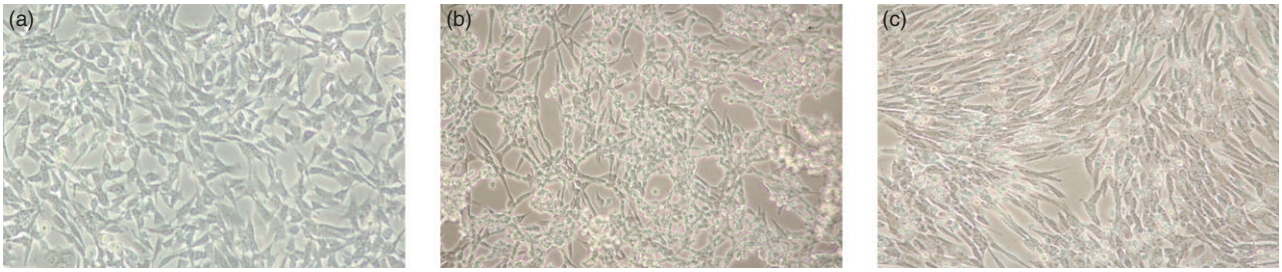

Figure 2 HAdV3-induced formation of CPE in A549 cells (×200). Representative photographs show: (a) non-infected cells; (b) cells infected with HAdV3 at an 100 CCID50 of 100 μl; (c) HAdV3-infected cells treated with astragaloside IV (20 μM) 2 h after infection.

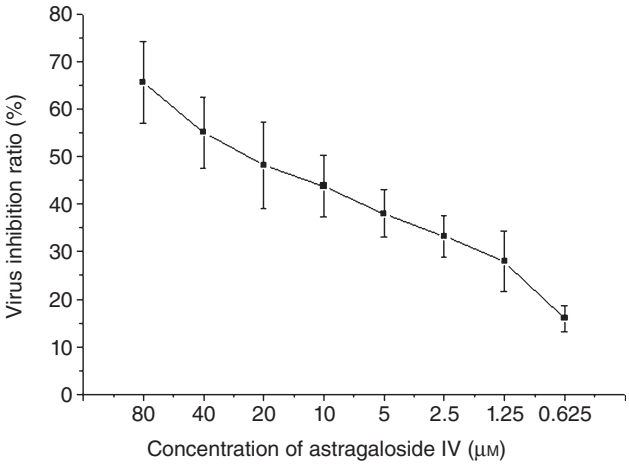

Figure 3 Inhibitory activity of astragaloside IV on CPE in A549 cells infected with HAdV3 at an 100 CCID50 of 100 μl.

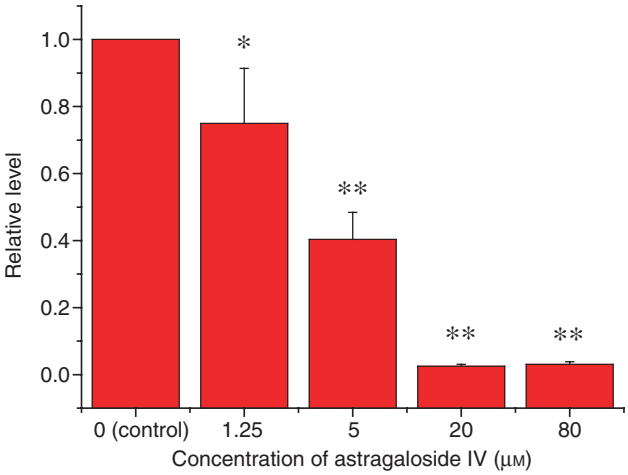

Figure 4 Effect of astragaloside IV on HAdV3 replication in A549 cells 48 h post-infection. Total DNA was extracted and viral hexon copy number was determined by quantitative real-time PCR analysis (with virus HAdV3 = 1.0). $*P < 0.05$ and $**P < 0.01$, compared with virus control group. Each column represents single concentration of astragaloside IV solution.

cells useful for rapid screening of antiviral substances. The influence of astragaloside IV on HAdV3-induced CPE was investigated in A549 cells, at different concentrations ranging from 0 to 80 μM, and the effect is shown in Figure 2. The virus inhibition rate correlated positively with the concentration of astragaloside IV (1.25–80 μM), and its range was from 15.98 to 65.68% in a concentration-dependent manner (Figure 3). IC50 was 23.85 μM, LC50 865.26 μM and the TI 36.28.

Astragaloside IV inhibits HAdV3 replication in A549 cells

The effect of astragaloside IV on HAdV3 replication was investigated in A549 cells. To assess the effects of astragaloside IV on HAdV3 replication, a virus replication reduction assay was performed, using qPCR with 0, 1.25, 5, 20 and 80 μM concentrations, at 48 h after infection. Meanwhile, the expression levels of HAdV3 represented as the radio of HAdV3/GAPDH were significantly decreased after treatment with 1.25, 5, 20 and 80 μM astragaloside IV compared with the control group ($P < 0.05$, $n = 6$). Therefore, Astragaloside IV significantly reduces HAdV3 replication in a dose-dependent manner (Figure 4).

Astragaloside IV inhibits HAdV3-induced apoptosis

To determine whether the astragaloside IV-induced decrease in viable cells occurred via apoptosis, A549 cells were analysed for apoptotic changes by FCM analysis. After 48 h infection with mock HAdV3 at 100 CCID50, the percentage of cells that were apoptotic in response to HAdV3 increased by 32.7 ± 3.64 in A549 cells (Figure 5). Astragaloside IV treatment resulted in a significant dose-dependent decrease in the number of apoptotic cells.

The effect of astragaloside IV on HAdV3 revealed by Western blotting

The potential mechanism through which astragaloside IV affects the HAdV3 was investigated by assessing the changes in protein expression in A549 cells infected with HAdV3. When the experiment was finished, the percentages of expression of Bcl-2, Bax and caspase-3 proteins were determined. The expression of Bcl-2 proteins was positively correlated with the concentration of astragaloside IV. The expression of Bax and caspase-3 proteins was negatively correlated with the concentration of astragaloside IV (Figure 6).

Discussion

Drug treatment is a main therapy for ADV infections nowadays, but there remains a lack of specific drugs for the aden-

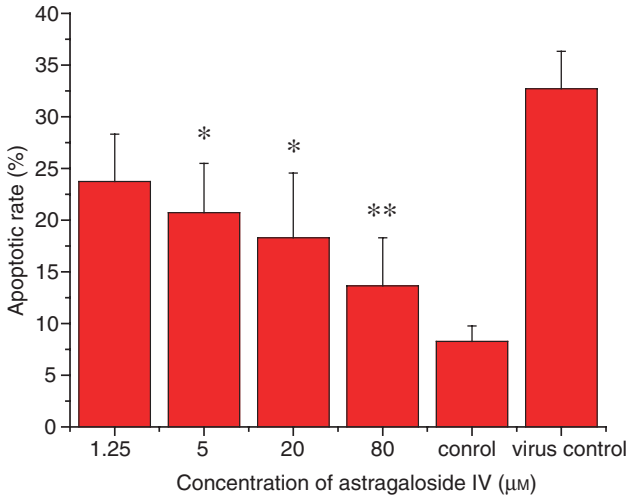

Figure 5 Investigation of apoptosis induction in HAdV3-infected A549 cells. Astragaloside IV treatment was performed 2 h after infection. Single asterisk (*) and double asterisks (**) indicate $P < 0.05$ and $P < 0.01$, compared with virus control group. The data are representative of three separate experiments. Each column represents single concentration of astragaloside IV solution.

oviral diseases without strong side effects.[7] For this reason, our study was performed to illustrate the anti-HAdV3 effect of astragaloside IV, a natural constituent from a Chinese herbal drug that has been used for thousands of years. In the present study, the inhibition rate of the virus, a replication reduction assay, the extent of apoptosis and expression of Bcl-2, Bax and caspase-3 proteins were used to determine the anti-HAdV3 effect and potential mechanism of action of astragaloside IV.

The toxicity study of astragaloside IV on A549 cells in this study indicates that astragaloside IV possesses a low cytotoxicity even at concentration as high as 160 μM. In the anti-virus test, the results from the CPE method demonstrate that in the astragaloside IV-treated HAdV3-infected groups, most of the A549 cells maintained a normal cellular shape with a normal epithelium, except for a small proportion of the cells which presented pathological changes such as shrinking, clustering, overlapping or exfoliation. The results from the MTT method showed that astragaloside IV in 1.25–80 μM concentration inhibits the growth of HAdV3, with a virus inhibition rate of 15.98–65.68%, indicating that the inhibiting activity is concentration-dependent.

In real-time PCR, it is possible to measure the amount of PCR product at any time point. The quantification of the PCR product at specific cycle numbers allows complete understanding of the PCR process with truly quantitative estimations.[22] qPCR has been applied for many different biological applications including identification of genomic DNA copy number, single nucleotide polymorphism, viral load and many others. It has become the standard test for these purposes because it is fast, sensitive and quantitative.[23]

The ratios of HAdV3/GAPDH were found to be significantly decreased in quantified hexon copy number of cells infected with HAdV3. Astragaloside IV reduced the relative hexon copy number in HAdV3 infected cells and the effect was concentration-dependent, indicating that the inhibition of astragaloside IV on HAdV3 may be owing to the antireplication effect in A549 cells.

Apoptosis, a type of gene-regulated process of cell death, is an important mechanism of organism growth and development, cell differentiation and physiological and pathological death. Some studies have indicated that ADV has a cytotoxic effect on human tumor cells, inducing apoptosis and repressed ectopic xenograft tumor growth.[13,24] In our results, the human adenovirus type 3-induced apoptosis was found in the A549 cell line, the same effect having been reported in normal airway epithelial cells.[25] The aim of this article is to describe the antiviral effect of astragaloside IV, with the use of a control group to eliminate the possible influence of the interaction of virus and cells. Astragaloside IV has been reported to reduce cell apoptosis induced by tumor necrosis factor.[13] The FCM analysis results showed that the degree of apoptosis in astragaloside IV-treated groups was clearly lower than that in the virus control groups, suggesting the anti-HAdV3 effect of astragaloside IV is possibly correlated with the apoptotic mechanism.

Some apoptosis-related studies were also conducted for further evidence for the mechanisms. A family of proteins known as caspases is typically activated in the early stages of apoptosis. Some caspases, e.g. caspase-3 and caspase-6, can activate other caspases in a cascade that eventually leads to the activation of the effector caspases.[26] Caspase-3 is also involved in the process of PARP, repairing DNA damage, releasing an enzyme named CAD which catalyses fragmentation of DNA into nucleosomal units, and mediating the anti-apoptotic effects of NO through nitrosylation and inactivation.[27,28] The Western blot result shows that the expression of the caspase-3 protein was significantly down-regulated by astragaloside IV, and with dose dependence. The decrease in expression of caspase-3 in astragaloside IV-treated groups further demonstrates that the anti-HAdV3 effect of astragaloside IV may inhibit the caspase-mediated apoptosis pathway. 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.[29,30] In our Western blotting results, the expression of bax was down-regulated by astragaloside IV, while the bcl-2 expression was simultaneously up-regulated, with dose-dependence. The differential expression of these important proteins proves that the protective effect of astragaloside IV on HAdV3-infected A549 cells may be due to the regulation of the key proteins or enzymes associated with apoptosis. Furthermore, other possible mechanisms should also be considered. Recently astragaloside IV has been reported to up-regulate matrix metalloproteinase-2 mRNA and protein expression in A549 cells.[17] In addition, the process may be due to astragaloside IV's ability to suppress growth factors and oxyradicals, and to stabilize calcium homeostasis and mitochondrial function.[31]

Conclusion

In conclusion, astragaloside IV inhibits HAdV3 replication and HAdV3-induced apoptosis, probably by inhibition of the expression of bax and caspase-3 proteins, and an increase

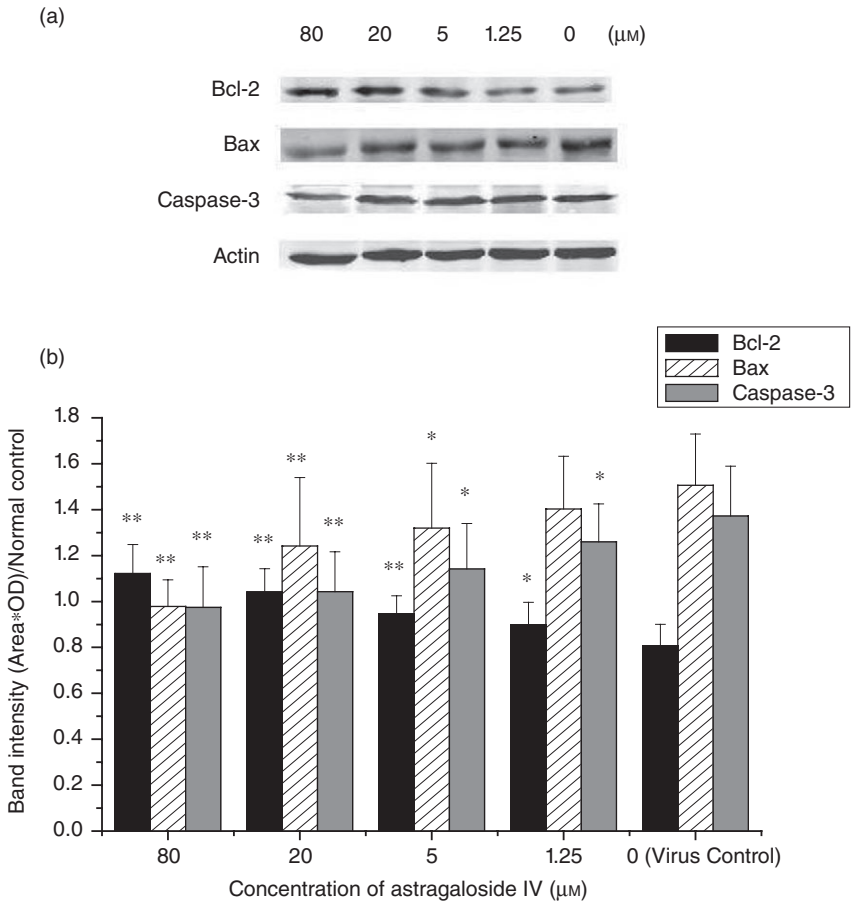

Figure 6 Expression of Bcl-2, Bax and cleaved Caspase 3 proteins as determined by Western blotting. (a) Western blotting result, lane 1, astragaloside IV (80 μM); lane 2, astragaloside IV (20 μM); lane 3, astragaloside IV (5 μM); lane 4, astragaloside IV (1.25 μM); lane 5, HAdV3 virus control (0 μM). (b) Band intensity values. Results are presented as an arithmetic mean of 3 separate experiments ± SE. Single asterisk (*) and double asterisk (**) indicate $P < 0.05$ and $P < 0.01$, respectively, Actin compared with HAdV3 group.

in the expression of bcl-2 protein. Further exploration into the mechanisms underlying the actions of this treatment can provide a theoretical foundation for the clinical application of astragaloside IV in anti-HAdV3 therapy, and opens up new thinking regarding the development and exploitation of novel, effective antiviral medicines.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Walls T *et al*. Adenovirus infection after pediatric bone marrow transplantation: is treatment always necessary? *Clin Infect Dis* 2005; 40: 1244–1249.
2. Morfin F *et al*. In vitro susceptibility of adenovirus to antiviral drugs is species-dependent. *Antivir Ther* 2005; 10: 225–229.
3. Kojaoghlanian T *et al*. The impact of adenovirus infection on the immunocompromised host. *Rev Med Virol* 2003; 13: 155–171.
4. Fernandez JA *et al*. Plasma interferon-gamma, interleukin-10 and soluble markers of immune activation in infants with primary adenovirus (ADV) and respiratory syncytial virus (RSV) infection. *Eur Cytokine Netw* 2005; 16: 35–40.
5. Swenson PD *et al*. Adenoviruses. In: Murray PR, ed. *Manual of clinical microbiology*, 8th edn, Vol. 2. Washington, DC: American Society for Microbiology Press, 2003: 1404–1417.
6. Munoz FM *et al*. Disseminated adenovirus disease in immunocompromised and immunocompetent children. *Clin Infect Dis* 1998; 27: 1194–1200.
7. Liu L *et al*. The antiadenovirus activities of cinnamaldehyde in vitro. *Lab Med* 2009; 40: 669–674.

8. Luo Y *et al*. Astragaloside IV protects against ischemic brain injury in a murine model of transient focal ischemia. *Neurosci Lett* 2004; 363: 218–223.
9. Zhang WJ *et al*. Antiinflammatory activity of astragaloside IV is mediated by inhibition of NF-kappaB activation and adhesion molecule expression. *Thromb Haemost* 2003; 90: 904–914.
10. Li ZP, Cao Q. Effects of astragaloside IV on myocardial calcium transport and cardiac function in ischemic rats. *Acta Pharmacol Sin* 2002; 23: 898–904.
11. Wang S *et al*. Anti-hepatitis B virus activities of astragaloside IV isolated from radix Astragali. *Biol Pharm Bull* 2009; 32: 132–135.
12. Fathiazad F *et al*. Cycloartane-type glycosides from the roots of *Astragalus caspicus* Bieb. *Nat Prod Res* 2010; 24: 1069–1078.
13. Zhang P *et al*. Tumor-specific, hypoxia-regulated, WW domain-containing oxidoreductase-expressing adenovirus inhibits human non-small cell lung cancer growth in vivo. *Hum Gene Ther* 2010; 21: 27–39.
14. Yuan W *et al*. Astragaloside IV inhibits proliferation and promotes apoptosis in rat vascular smooth muscle cells under high glucose concentration in vitro. *Planta Med* 2008; 74: 1259–1264.
15. Du Q *et al*. Inhibitory effects of astragaloside IV on ovalbumin-induced chronic experimental asthma. *Can J Physiol Pharmacol* 2008; 86: 449–457.
16. Zhao Z *et al*. Effects of astragaloside IV on heart failure in rats. *Chin Med* 2009; 4: 6.
17. Su C *et al*. Astragaloside IV increases MMP-2 mRNA and protein expression in human lung cancer A549 cells. *Mol Med Rep* 2009; 2: 107–113.
18. Kinjo J *et al*. Anti-herpes virus activity of fabaceous triterpenoidal saponins. *Biol Pharm Bull* 2000; 23: 887–889.
19. Zhang Y *et al*. Astragaloside IV exerts antiviral effects against coxsackievirus B3 by upregulating interferon-gamma. *J Cardiovasc Pharmacol* 2006; 47: 190–195.
20. Zhang ZC *et al*. Effect of astragaloside on cardiomyocyte apoptosis in murine coxsackievirus B3 myocarditis. *J Asian Nat Prod Res* 2007; 9: 145–151.
21. Kodama E *et al*. Application of a gastric cancer cell line (MKN-28) for anti-adenovirus screening using the MTT method. *Antiviral Res* 1996; 31: 159–164.
22. Dorak MT. *Real-Time PCR*. New York: Taylor & Francis Group, 2006: 220.
23. Stock R *et al*. A rapid quantitative PCR-based assay for testing antiviral agents against human adenoviruses demonstrates type specific differences in ribavirin activity. *Antiviral Res* 2006; 72: 34–41.
24. Figova K *et al*. Reovirus – possible therapy of cancer. *Neoplasma* 2006; 53: 457–462.
25. Singhera GK *et al*. Apoptosis of viral-infected airway epithelial cells limit viral production and is altered by corticosteroid exposure. *Respir Res* 2006; 7: 78.
26. Budihardjo I *et al*. Biochemical pathways of caspase activation during apoptosis. *Annu Rev Cell Dev Biol* 1999; 15: 269–290.
27. Brauns SC *et al*. Caspase-3 activation and induction of PARP cleavage by cyclic dipeptide cyclo(Phe-Pro) in HT-29 cells. *Anticancer Res* 2005; 25: 4197–4202.
28. Dash PR *et al*. Nitric oxide protects human extravillous trophoblast cells from apoptosis by a cyclic GMP-dependent mechanism and independently of caspase 3 nitrosylation. *Exp Cell Res* 2003; 287: 314–324.
29. Adams JM, Cory S. Life-or-death decisions by the Bcl-2 protein family. *Trends Biochem Sci* 2001; 26: 61–66.
30. Mahajan NP *et al*. Bcl-2 and Bax interactions in mitochondria probed with green fluorescent protein and fluorescence resonance energy transfer. *Nat Biotechnol* 1998; 16: 547–552.
31. Xu XL *et al*. Astragaloside IV improved intracellular calcium handling in hypoxia-reoxygenated cardiomyocytes via the sarcoplasmic reticulum Ca-ATPase. *Pharmacology* 2008; 81: 325–332.