

Alantolactone exhibited anti-herpes simplex virus 1 (HSV-1) action *in vitro*

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Summary The aim of this study was to determine the inhibitory action of alantolactone, a gradient of traditional Chinese medicine *Inulae Radix* (Tu-Mu-Xiang), on herpes simplex virus 1 (HSV-1). African green monkey kidney cells (Vero cells) were infected with HSV-1 and the protective effects of alantolactone on Vero cells were examined. At concentrations of 10^{-6} , 10^{-7} , and 10^{-8} g/mL, alantolactone did not have a marked harmful effect on the viability of Vero cells according to an MTT assay. Based on the cytopathic effect (CPE) and MTT assays, alantolactone at these concentrations exhibited antiviral action and protected cells from being damaged by HSV-1. Results indicated that alantolactone had potent anti-HSV-1 action and provided evidence for use of *Inulae Radix* in the treatment of HSV-1 infection.

Keywords: Herpes simplex virus (HSV), *Inulae Radix*, alantolactone, cytopathic effect (CPE)

1. Introduction

Viral infections are serious conditions and are usually difficult to treat at the current point in time (1). Herpes simplex virus (HSV) can primarily be divided into two serotypes, *i.e.* HSV-1 and HSV-2, and infection with HSV can lead to herpes simplex encephalitis, genital herpes, or cervical cancer, and is a risk factor for fetal congenital malformation (2,3). HSV-1 infection is a common and widespread disease that is characterized by lifelong persistence and periodic reactivation (4). Ribavirin is the one of the main drugs that is currently used to treat HSV-1 infection (5). However, mounting evidence has shown that the virus is becoming resistant to this drug due to long-term use (6,7). Consequently, the pressing challenge is to develop novel drugs to treat HSV-1 infection.

Traditional Chinese medicine (TCM) has attracted

considerable attention in the search for antivirals because TCM has obvious advantages in the treatment of viral infections and TCM has a broad range of applications (8). *Inulae Radix* (Tu-Mu-Xiang) is a typical TCM that is usually used to treat bacterial or viral infection in China. Thus, the current study examined the anti-HSV-1 action of alantolactone, a main component found in *Inulae Radix*, *in vitro*.

2. Materials and Methods

2.1. Agents

Alantolactone was obtained from Shanghai Yuanye Biological Technology Co. Ltd. (Shanghai, China). For an *in vitro* assay, alantolactone was first dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) and further diluted in RPMI-1640 media (Hyclone) before use. Injectable ribavirin was purchased from Sanjing Pharmaceutical Co., Ltd. (Harbin, Heilongjiang, China). HSV-1 was obtained from the School of Basic Medicine, Nanjing University of Traditional Chinese Medicine (Nanjing, Jiangsu, China).

2.2. Cell line and cell culture

African green monkey kidney cells (Vero cells) were

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Table 1. Anti-HSV-1 action of alantolactone evaluated with a CPE-based assay

Experiments No.	Virus-free cells	Infected cells	Ribavirin	Alantolactone concentration (g/mL)				
				10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	10 ⁻⁹	10 ⁻¹⁰
1	-	++++	-	+	-	-	++	+++
2	-	++++	-	+	+	-	++	+++
3	-	++++	-	-	-	++	+++	++++

-, none of the cells exhibited a cytopathic effect; +, 0-25% of cells exhibited a cytopathic effect; ++, 25-50% of cells exhibited a cytopathic effect; +++, 50-75% of cells exhibited a cytopathic effect; +++++, 75-100% of cells exhibited a cytopathic effect.

obtained from the School of Basic Medicine, Nanjing University of Traditional Chinese Medicine. Briefly, cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) at 37°C in a humid atmosphere (5% CO₂-95% air). Cells were harvested by brief incubation in 0.02% (w/v) ethylenediaminetetraacetic acid (EDTA) in PBS.

2.3. Assay of the cytopathic effect (CPE) of the virus

A CPE-based assay was used to determine the structural changes in the host cells that are caused by viral invasion. Common examples of CPE include rounding of infected cells, fusion with adjacent cells to form syncytia, and the appearance of nuclear or cytoplasmic inclusion bodies (9). '-' was recorded when none of the cells exhibited cytopathic effect; '+' was recorded when 0-25% of cells exhibited a cytopathic effect; '++' was recorded when 25-50% of cells exhibited a cytopathic effect; '++++' was recorded when 50-75% of cells exhibited a cytopathic effect; and '+++++' was recorded when 75-100% of cells exhibited a cytopathic effect.

2.4. MTT assay

Cells (1×10^4 per well) seeded in 96-well plates were exposed to increasing concentrations of alantolactone for specified times. After incubation for 72 h, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed by adding 20 μ L of MTT (5 mg/mL, Sigma-Aldrich) for 4 h (10). Light absorbance of the solution was measured at 490 nm on a microplate reader (Perkin-Elmer, USA).

3. Results and Discussion

3.1. Toxicity of the virus

Vero cells seeded in 96-well plates were exposed to decreasing concentrations of HSV-1 (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , or 10^{-9} of stock virus solution) for 1 h. The virus solution was then removed. The cells were subsequently cultured in RPMI-1640 media for 72 h. A CPE-based assay was used to determine the damage caused by HSV-1 in Vero cells. Results indicated that Vero cells were all infected when the dilution of the virus was above 10^{-3} , and the tissue culture 50%

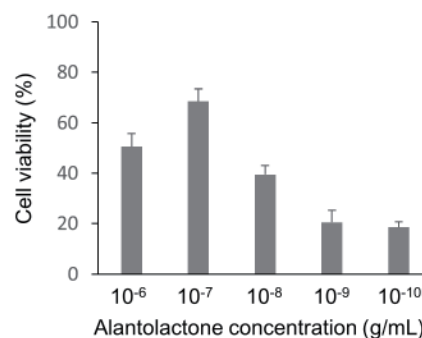


Figure 1. Alantolactone increased the viability of Vero cells infected with HSV-1.

infective dose (TCID₅₀) was calculated to be $10^{-6.33}$ according to the Reed-Muench method.

3.2. Effects of alantolactone on the viability of Vero cells

Vero cells seeded in 96-well plates were exposed to increasing concentrations of alantolactone for 72 h. The cells were then subjected to an MTT assay. Results revealed no obvious cytotoxic effects when the concentration of alantolactone was lower than 10^{-6} g/mL. In order to avoid the harmful effects of alantolactone on Vero cells, the concentration of alantolactone was set below 10^{-6} g/mL in the following antiviral assay.

3.3. Antiviral action of alantolactone on HSV-1

The effects of alantolactone on HSV-1 were determined using a CPE-based assay and an MTT assay. Results of the CPE-based assay indicated that alantolactone at a concentration of 10^{-6} , 10^{-7} , or 10^{-8} g/mL markedly inhibited viral infection (Table 1). However, alantolactone at a concentration of 10^{-7} g/mL exhibited more potent antiviral action. The reason why alantolactone had less potent antiviral activity at 10^{-6} g/mL than at 10^{-7} g/mL might be because a higher concentration of alantolactone has more of a cytotoxic effect on Vero cells, thus reducing the resistance of Vero cells to viral infection. Similar results were obtained using the MTT assay (Figure 1). Cell viability increased markedly when the alantolactone concentration was 10^{-6} , 10^{-7} , or 10^{-8} g/

mL. Alantolactone at a concentration of 10^{-7} g/mL provided the most protection from HSV-1 infection. The IC_{50} of alantolactone was determined to be $10^{-7.4}$ g/mL.

The present study examined the antiviral action of alantolactone on HSV-1. Given the possible cytotoxic effects of alantolactone on Vero cells, the effects of alantolactone on the cell viability of Vero cells were first determined using an MTT assay. Results indicated that alantolactone at concentrations below 10^{-6} g/mL did not have a marked effect on the cell viability on Vero cells. Alantolactone at a concentration of 10^{-6} , 10^{-7} , or 10^{-8} g/mL inhibited viral infection and the viability of Vero cells increased markedly at these concentrations. These results suggest that alantolactone had antiviral action against HSV-1 *in vitro* and they provide evidence for use of Inulae Radix to treat HSV-1 infection. Studies to examine the antiviral action of alantolactone *in vivo* and the mechanisms underlying the action of alantolactone are warranted in the future.

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