Phytomedicine 18 (2011) 110–118

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Activity investigation of pinostrobin towards herpes simplex virus-1 as determined by atomic force microscopy

Nan Wu[a,b,1], Yu Kong[a,b,1], Yuangang Zu[a,b], Yujie Fu[a,b,*], Zhiguo Liu[a,b], Ronghua Meng[a,b], Xia Liu[a,b], Thomas Efferth[c]

[a] Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, PR China
[b] Engineering Research Center of Forest Bio-preparation, Ministry of Education, Northeast Forestry University, Harbin 150040, PR China
[c] Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, University of Mainz, 55099 Mainz, Germany

ARTICLE INFO

Keywords:
Pinostrobin
Cajanus cajan
HSV-1
MTT-assay
AFM
In vivo

ABSTRACT

In the present study, the antiviral activity of pinostrobin towards herpes simplex virus-1 (HSV-1) was investigated by MTT assay and atomic force microscopy. Pinostrobin can inhibit HSV-1 replication with 50% effective concentration (EC_{50}) of 22.71 ± 1.72 μg/ml. MTT assay showed HSV-1 was significantly inhibited when pretreated with pinostrobin, with the inhibition of 85.69 ± 2.59%. Significant changes in morphology and size of HSV-1 were observed by atomic force microscopy (AFM) in response to pinostrobin treatment. AFM topography and phase images showed that with increasing time, the envelope was shedded and damaged, finally leading to virus inactivation. With increasing concentration, pinostrobin caused a gradual leakage, also contributing to breakage of the envelope and virus inactivation. Treatment effect of oral pinostrobin *in vivo* showed that pinostrobin (50 mg/kg/dose) possesses definite therapeutical effect in the development of lesion score. In general, the results showed that AFM represents a powerful technique for the investigation of morphology and size of HSV-1 treated by antiviral agents. AFM is applicable to study chemically induced morphological changes at the nanometer level.
© 2010 Elsevier GmbH. All rights reserved.

1. Introduction

Herpes simplex virus-1 (HSV-1) consists of a lipid envelope with embedded proteins and an icosahedral capsid of protein containing a double-stranded DNA genome. Between the membrane and the capsid are tegument proteins of various sizes. The intact virion is about 150–200 nm in diameter (Stannard et al., 1986). HSV-1 could cause a variety of diseases in humans, sometimes it may even lead to life-threatening conditions especially in immuno-compromised patients (Chuanasa et al., 2008; Su et al., 2008). According to epidemiological surveys, the HSV-1 infection rate has continuously increased in most countries. In current years, nucleoside analogues such as acyclovir (ACV) and other nucleoside derivatives (*e.g.* penciclovir, valaciclovir, famciclovir, and ganciclovir and others) have been approved for treatment of HSV-1 infections worldwide (Galasso et al., 1997; Leung and Sacks, 2000; Brady and Bernstein, 2004). However, the appearance of ACV-resistant viruses became a severe problem. The failure of treatment is also due to the relapse of latent viruses. Consequently, there is an urgent need for more effective and less toxic drugs.

Flavonoids are phenolic plant constituents, which are abundantly present in high concentrations in whole grains and other legumes. Flavonoids show a number of favorable pharmacological properties such as anti-oxidant, anti-inflammatory, anti-carcinogenic, and anti-malarial activities (Tewtrakul et al., 2009; Chang et al., 2008; Khaomek et al., 2008). For example, myricetin inhibits the growth of multidrug-resistant *Burkholderia cepacia*, vancomycin-resistant enterococci and other medically important microorganisms (Xu and Lee, 2001). Several flavonoids exhibit good antiviral activities. Quercetin is an efficient HIV1-protease and reverse transcriptase inhibitor (Xu et al., 2000; Spedding et al., 1989). Hesperidin, prevents cells against invasion of rotaviruses (Bae et al., 2000). As a matter of fact, screening antiviral agents from natural products has become a hot spot of research in the world in recent years.

Pinostrobin (Fig. 1) is a natural flavanone with notable antispamodic, anti-*Helicobacter pylori* as well as anti-tumor activity (Walle et al., 2001; Bhamarapravati et al., 2006; Le Bail et al., 2000). However, the anti-herpes properties of pinostrobin have not been elucidated yet.

With the advent of AFM technology, a novel dimension in microscopy has been reached allowing much more detailed investigations (Kiselyova et al., 2003). AFM is based on the raster scanning

* Corresponding author at: Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, PR China.
Tel.: +86 451 82190535; fax: +86 451 82102082.
E-mail addresses: yujie_fu2002@yahoo.com, bao_doubao@163.com (Y. Fu).
[1] Both the authors contributed equally to this work.

0944-7113/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2010.07.001

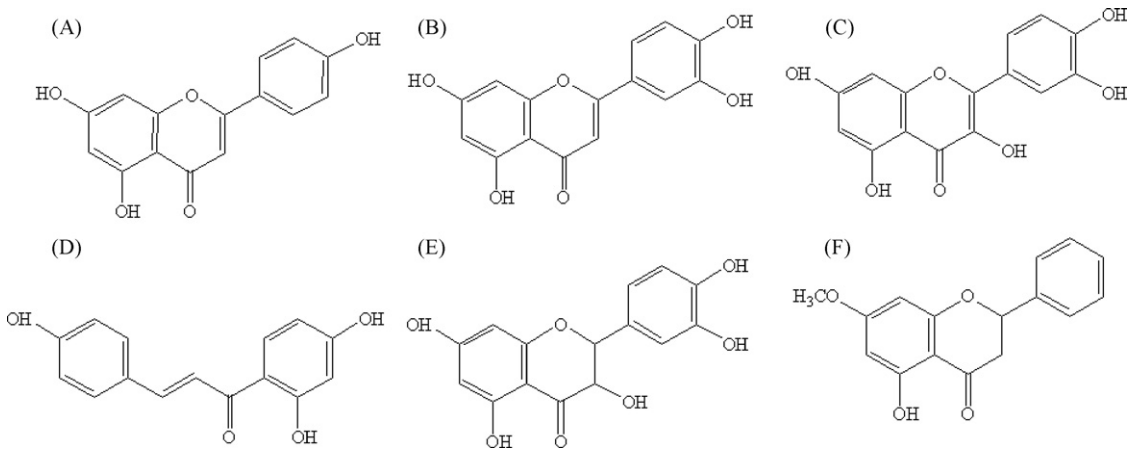

Fig. 1. Structure of apigenin (A), luteolin (B), quercetin (C), isoliquiritigenin (D), taxifolin (E) and pinostrobin (F).

of a sharp probe that interacts locally with the surface of a specimen. The most remarkable feature of AFM is high-resolution imaging of soft, living objects which allows the study of single viral particles, *e.g.* growth of viral crystals, packing of viral DNA, etc. (Wang et al., 2007; Dubrovin et al., 2007). To our knowledge, AFM has not been used to analyze the mode of action of anti-HSV-1 activity of pinostrobin.

In this study, we first evaluated the anti-HSV-1 activity of pinostrobin, by MTT assay. To investigate the possible mechanism of anti-HSV-1 activity of pinostrobin, AFM technique was applied to detect morphology changes and size alterations of HSV-1 treated with pinostrobin in a time- and concentration-dependent manner. Furthermore, HSV-1 infection mice model was set up to investigate antiviral activity of pinostrobin *in vivo*.

2. Materials and methods

2.1. Compounds

5 flavonoids including apigenin, luteolin (flavone), quercetin (flavonol) isoliquiritigenin (chalcone); taxifolin (flavanonol) and acyclovir were purchased from Sigma (St. Louis, MO) (Fig. 1, Table 1).

2.2. Viruses and cells

HSV-1 (F strain) was purchased from Wuhan Institute of Virology, Chinese Academy of Sciences. Vero E6 cells (ATCC CCL81) were grown and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% and 2% fetal bovine serum (FBS), respectively. In order to prepare virus stocks, Vero cells were infected with HSV-1 (F strain) at a multiplicity of infection (MOI) equal to 0.1 for 1 h at 37 °C. Next, residual viruses were washed out with phosphate-buffered saline (PBS) and cells were cultured for an additional 72 h. Partially purified cell-free virus was then prepared as follows: the infected cells were lysed by three cycles of freezing and thawing, centrifuged at 2000 × *g* at 4 °C for 20 min to remove cellular debris. The clarified supernatant was then centrifuged at 14,000 × *g* for 2.5 h at 4 °C using a Beckman 35 rotor. The resultant pellet was drained thoroughly and resuspended in 350 μl of sterile TE buffer (0.01 mol/l Tris, 0.001 mol/l ethylenediaminetetraacetic acid, pH 7.6) at 4 °C overnight. The titer of virus suspension were evaluated by MTT assay and stored at −70 °C for further studies.

2.3. Purification of pinostrobin from Cajanus cajan

Pinostrobin was purified from the leaves of *Cajanus cajan* (L.) Millsp. (pigeonpea) and the method was previously reported (Wu et al., 2009). In summary, leaves of pigeonpea were first extracted in ethanol. Then, silica gel column chromatography eluting was used to isolate the active fraction. After that, the active fraction was further rechromatographied on middle-pressure silica gel columns and then purified by crystallization and recrystallization to obtain pinostrobin. The purified compound was analyzed as 5-hydroxy-7-methoxy flavanone (pinostrobin) by spectroscopy. The chemical was detected by HPLC and found to be greater than 95% pure.

2.4. Cytotoxicity assay

Monolayers of 10^4 Vero cells in 96-multiwell plates were treated with various concentrations of pinostrobin for 72 h after which 20 μl of a 5 mg/ml solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma) was dissolved in DMEM without serum and was added to the cell culture. MTT was removed after 4 h, 200 μl of DMSO was added and the optical density (OD) was read using an ELISA reader (Thermo Molecular Devices Co., Union City, USA) with a 570 nm test wavelength and a 690 nm reference wavelength (Souza et al., 2008; Denizot and Lang, 1986). The maximal non-toxic concentration (TD_0) and 50% cytotoxic concentration (CC_{50}) were calculated by linear regression analysis of the dose–response curves generated from the data.

2.5. Antiviral activity

Inhibition of virus replication was also measured by the MTT assay. Monolayers of Vero cells in 96 well plates were exposed to virus for 1 h at 37 °C. The infected cells then were added with serial dilution of pinostrobin and acyclovir. Each assay was performed in eight replicates. After incubation for 72 h at 37 °C. The cultures were measured by MTT method as described above. The concentration of test compound was determined, which inhibited virus numbers by 50% (EC_{50}).

2.6. Mode of antiviral activity

Mode of antiviral activity of pinostrobin was measured by the MTT assay (Schnitzler et al., 2008). Briefly, Cells and viruses were incubated with pinostrobin at different stages during the viral infection cycle in order to determine the mode of antiviral action. Cells were pretreated with pinostrobin before viral infec-

Download English Version:

https://daneshyari.com/en/article/5816978

Download Persian Version:

https://daneshyari.com/article/5816978

Daneshyari.com