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International Journal of Antimicrobial Agents 32 (2008) 355–359

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

Short communication

Aloe-emodin is an interferon-inducing agent with antiviral activity against Japanese encephalitis virus and enterovirus 71

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Received 7 March 2008; accepted 24 April 2008

Abstract

In this study, aloe-emodin was identified as a potential interferon (IFN)-inducer by screening compounds from Chinese herbal medicine. Aloe-emodin showed low cytotoxicity to human HL-CZ promonocyte cells and TE-671 medulloblastoma cells and significantly activated interferon-stimulated response element (ISRE) and gamma-activated sequence (GAS)-driven cis-reporting systems. Moreover, aloe-emodin upregulated expression of IFN-stimulated genes such as dsRNA-activated protein kinase and 2′,5′-oligoisoadenylate synthase. Aloe-emodin resulted in significant activation of nitric oxide production. The antiviral activity of aloe-emodin against Japanese encephalitis virus (JEV) and enterovirus 71 (EV71) was evaluated using dose- and time-dependent plaque reduction assays in HL-CZ cells and TE-671 cells. The 50% inhibitory concentration (IC_{50}) of aloe-emodin ranged from 0.50 μg/mL to 1.51 μg/mL for JEV and from 0.14 μg/mL to 0.52 μg/mL for EV71. Aloe-emodin showed clearly potent virus inhibitory abilities and achieved high therapeutic indices, in particular for HL-CZ cells. Therefore, the study demonstrated dose- and time-dependent actions of aloe-emodin on the inhibition of JEV and EV71 replication via IFN signalling responses.

Keywords: Aloe-emodin; Interferon signalling; Japanese encephalitis virus; Enterovirus 71

1. Introduction

Aloe-emodin (1,8-dihydroxy-3-hydroxymethyl-anthraquinone) is one of the natural anthraquinone derivatives from the root and rhizome of *Rheum palmatum*. Aloe-emodin has been reported to inhibit the replication of enveloped viruses including herpes simplex virus, influenza virus [1] and human cytomegalovirus [2]. In addition, other anthraquinone derivatives, including emodin, chrysophanic acid and hypericin, have demonstrated antiviral activities against hepatitis B virus [3] and poliovirus [4].

Type I and type II interferons (IFNs) are produced by leukocytes and fibroblasts in the non-specific host response to viral infection. IFNα and IFNβ show a therapeutic effect on viral infections, including coxsackievirus type A16 [5] and West Nile virus (WNV) [6]. IFNγ is involved in the activation of endogenous nitric oxide (NO) to inhibit viral replication in the macrophage and the central nervous system (CNS) [7]. Importantly, polyriboinosinic:polyribocytidylic acid (poly(I:C)), a potent IFN-inducer, has been demonstrated to improve the survival rate and to decrease tissue viral titres following enterovirus 71 (EV71) and WNV challenge in mice [6,8]. Therefore, screening for IFN-inducers from the compounds of Chinese herbal medicine is an alternative approach to identify potent antiviral agents.

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doi:10.1016/j.ijantimicag.2008.04.018

Japanese encephalitis virus (JEV) and EV71 are neurotropic viruses that spread particularly in the Asia–Pacific region [9,10]. JEV and EV71 encephalitis are inflammatory diseases of the CNS, like poliomyelitis-like acute flaccid paralysis, aseptic meningitis and encephalitis. JEV and EV71 multiply in primary sites such as monocytes and myeloid and lymphoid cells and then travel from the periphery to the CNS [9,10]. The antiviral approach against JEV and EV71 could be simultaneously set up in the same cell lines.

In this study, we aimed to identify antiviral herb compounds against JEV and EV71 in cell models such as the human TE-671 medulloblastoma cell line and HL-CZ promonocyte cell line. Aloe-emodin was identified from screening of IFN-inducers from Chinese herbal compounds showing IFN signalling induction in mammalian cells. Aloe-emodin revealed a dose-dependent effect on IFN expression and NO production as well as in vitro antiviral activities against JEV and EV71.

2. Materials and methods

2.1. Viruses and cells

JEV strain T1P1 [11] and an EV71 isolate from a throat culture were used in this study. BHK-21 cells were maintained in Modified Eagle's Medium (MEM) with 10% foetal bovine serum (FBS). TE-671 medulloblastoma cells were grown in MEM with 2 mM L-glutamine, 1 mM sodium pyruvate and 10% FBS. HL-CZ cells were incubated with RPMI 1640 medium containing 10% FBS.

2.2. Cytotoxicity test

Aloe-emodin, arecoline, catechin, shikonin, trans-cinnamic acid, hesperetin, scopoletin, daidzein and resveratrol were purchased from Sigma Chemical Co. (St Louis, MO). Cells were treated with various concentrations of each herb component for 48 h, followed by an MTT assay. Survival rates of cells were calculated as the ratio of the optical density at 570–630 nm ($OD_{570–630}$) of treated cells to the $OD_{570–630}$ of untreated cells. Quadruplicate wells were analysed for each concentration. The cytotoxic concentration showing 50% toxic effect (CC_{50}) was determined using a computer program (provided by John Spouge, National Center for Biotechnology Information, National Institutes of Health).

2.3. Enzyme-linked immunosorbent assay (ELISA) for detection of IFNα expression

Following 48 h incubation of treated cells, IFNα was measured using an ELISA with anti-IFNα monoclonal antibody in quadruplicate wells. The ELISA product was developed using a chromogen solution containing ABTS (2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate)) and hydrogen peroxide and then the absorbance at 405 nm (A_{405}) was measured.

2.4. Transient transfections of cis-reporter plasmids for signalling pathway assay

Cells were transiently co-transfected with an internal control reporter pRluc-C1 plus the indicated cis-reporting plasmid pISRE-Luc, pGAS-Luc or pNFκB-Luc (Stratagene, La Jolla, CA). Cells were treated with each herbal compound at 1 μg/mL and incubated for 4 h and then firefly and Renilla luciferase enzymes were measured using the dual Luciferase Reporter Assay System (Promega, Madison, WI) and a TROPIX TR-717 luminometer (Applied Biosystems, Foster City, CA). The relative firefly luciferase activity was normalised by Renilla luciferase and compared with untreated cells.

2.5. Quantification of IFN-stimulating gene expression using real-time reverse transcription polymerase chain reaction (RT-PCR)

Quantification of mRNA was performed with TaqMan® real-time RT-PCR (Applied Biosystems) using 2.5 μL of reverse-transcribed cDNA. Oligonucleotide primers and TaqMan® probes for human protein kinase R (PKR) were as follows: forward primer, 5′-CAACC AGCGG TTGAC TTTTT-3′; reverse primer, 5′-ATCCA GGAAG GCAAA CTGAA-3′; and probe #50 (Universal ProbeLibrary probe). The forward primer for 2′,5′-oligoadenylate synthetase (OAS) was 5′-GATGT GGTTA GGTTT ATAGCTG-3′ and the reverse primer was 5′-TTGGG GGTTA GGTTT CTGCCTTT-3′. In addition, the forward primer for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was 5′-AGCCA CATCG CTCAG ACAC-3′ and the reverse primer was 5′-GCCCCA ATACG ACCAA ATCC-3′. The TaqMan® probe for GAPDH was probe #37. The measured amounts of PKR and OAS mRNA were normalised to the amount of GAPDH mRNA.

2.6. Measurement of NO production

Cells were loaded with DAF-2 DA for 3 h at 37 °C. After rinsing three times with serum-free MEM, cells were treated with each herbal compound. Fluorescence (excitation wavelength 480 nm, emission wavelength 530 nm) was measured using a BD FACSAria analyser (BD Biosciences, San José, CA).

2.7. Dose- and time-dependent plaque reduction assays

The dose-dependent plaque reduction assay was performed using 10-fold dilutions of aloe-emodin in triplicate. Untreated and treated cells were infected at a multiplicity of infection of 1 with JEV or EV71. After 48 h of incubation at 37 °C, the cultured supernatant was harvested for determination of viral plaques using BHK-21 cell monolayers. The 50% inhibitory concentration (IC_{50}) was the concentration required for 50% inhibition of the indicated virus plaques.