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Aurantiamide acetate from baphicacanthus cusia root exhibits anti-inflammatory and anti-viral effects via inhibition of the NF-κB signaling pathway in Influenza A virus-infected cells

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ABSTRACT

Ethnopharmacological relevance: *Baphicacanthus cusia* root also names “Nan Ban Lan Gen” has been traditionally used to prevent and treat influenza A virus infections. Here, we identified a peptide derivative, aurantiamide acetate (compound E17), as an active compound in extracts of *B. cusia* root. Although studies have shown that aurantiamide acetate possesses antioxidant and anti-inflammatory properties, the effects and mechanism by which it functions as an anti-viral or as an anti-inflammatory during influenza virus infection are poorly defined. Here we investigated the anti-viral activity and possible mechanism of compound E17 against influenza virus infection.

Materials and methods: The anti-viral activity of compound E17 against Influenza A virus (IAV) was determined using the cytopathic effect (CPE) inhibition assay. Viruses were titrated on Madin-Darby canine kidney (MDCK) cells by plaque assays. Ribonucleoprotein (RNP) luciferase reporter assay was further conducted to investigate the effect of compound E17 on the activity of the viral polymerase complex. HEK293T cells with a stably transfected NF-κB luciferase reporter plasmid were employed to examine the activity of compound E17 on NF-κB activation. Activation of the host signaling pathway induced by IAV infection in the absence or presence of compound E17 was assessed by western blotting. The effect of compound E17 on IAV-induced expression of pro-inflammatory cytokines was measured by real-time quantitative PCR and Luminex assays.

Results: Compound E17 exerted an inhibitory effect on IAV replication in MDCK cells but had no effect on avian IAV and influenza B virus. Treatment with compound E17 resulted in a reduction of RNP activity and virus titers. Compound E17 treatment inhibited the transcriptional activity of NF-κB in a NF-κB luciferase reporter stable HEK293 cell after stimulation with TNF-α. Furthermore, compound E17 blocked the activation of the NF-κB signaling pathway and decreased mRNA expression levels of pro-inflammatory genes in infected cells. Compound E17 also suppressed the production of IL-6, TNF-α, IL-8, IP-10 and RANTES from IAV-infected lung epithelial (A549) cells.

Conclusions: These results indicate that compound E17 isolated from *B. cusia* root has potent anti-viral and anti-inflammatory effects on IAV-infected cells via inhibition of the NF-κB pathway. Therefore, compound E17 could be a potential therapeutic agent for the treatment of influenza.

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1. Introduction

Influenza A virus (IAV) causes a contagious respiratory disease in humans that is responsible for the annual epidemics with a high rate of morbidity and mortality worldwide. Zoonotic infections with avian influenza viruses such as subtype H7N9 or H5N1 are also serious public health concerns (Chi et al., 2013; Korteweg and Gu, 2008). Despite the availability of antiviral drugs, namely the M2 ion channel inhibitors amantadine and rimantadine, and the neuraminidase inhibitor oseltamivir, mutations or reassortment in IAV genes can result in reduced drug effectiveness or resistance (Hayden and de Jong, 2011). Therefore, new drug candidates that target the virus replication cycle are urgently needed.

Using agents that act on cellular pathways to enhance their antiviral effects may be a good antiviral strategy. Numerous host signaling pathways are induced during an infection to exert an antiviral effect, such as the nuclear factor (NF)-κB pathway, the MAPK pathway, the phosphatidylinositol-3kinase (PI3K)/AKT pathways and the TLR/RIG-I signaling cascades (Hale and Randall, 2007; Le Goffic et al., 2007; Ludwig et al., 1999; Pleschka et al., 2001). The IAV nonstructural protein 1 (NS1) activates the PI3K pathway to suppress apoptosis induction via inhibition of AKT to ensure efficient viral replication (Ehrhardt et al., 2007). During the early stages of infection, IAV-induced activation of ERK results in V-ATPase-dependent acidification of the endosomal interior for fusion (Marjuki et al., 2011). Meanwhile, influenza virus-mediated late activation of the ERK cascade is required for nuclear export of the viral ribonucleoprotein (RNP) complexes (Marjuki et al., 2006; Pleschka et al., 2001). Blockade of the ERK signaling cascade by a specific inhibitor prevented the export of vRNPs, resulting in a significant reduction in virus titer (Pleschka et al., 2001). The NF-κB pathway has been shown to upregulate pro-apoptotic mediators such as TRAIL and Fas/FasL, which mediate the apoptosis of infected cells and enhance viral RNP nuclear export (Wurzer et al., 2004). Furthermore, the p65 subunit of NF-κB is responsible for the synthesis of vRNA (Kumar et al., 2008). Therefore, cellular pathways are a promising target for novel antiviral drug development.

The immune response is another aspect of the host antiviral response that acts via cellular pathways, particularly via cytokines. Whilst cytokines are important for an effective antiviral response, cytokine dysregulation can contribute to clinical symptoms and pulmonary immunopathology (Kaiser et al., 2001; Oslund and Baumgarth, 2011). Most patients with confirmed H7N9 avian influenza virus infections developed severe pneumonia and acute respiratory distress syndrome (ARDS) similar to patients with fatal highly pathogenic avian influenza (HPAI) virus infection (Chi et al., 2013; de Jong et al., 2006). Therefore, selective inhibition of host signaling pathway may provide a novel therapeutic approach that targets both viral replication and aberrant proinflammatory responses to influenza virus infection.

B. cusia root. also name “Nan Ban Lan Gen” is an herbal medicine traditionally used as a treatment for the common cold (Tanaka et al., 2004). *B. cusia root*. contains numerous compounds including alkaloids, flavonoids, steroids and pentacyclic triterpenoids (SUN et al., 2008). Recently, we isolated Aurantiamide acetate (N-(N-benzoyl-L-phenylalanyl)-O-acetyl-L-phenylalanol) from *B. cusia root*. Aurantiamide acetate was first isolated from Piper aurantiacum (Banerji and Das, 1975). It had been reported to inhibit cysteine proteinases and exhibits anti-HBV activity (Isshiki et al., 2001; Qiu et al., 2011), anti-inflammatory activity (Suhas and Channe Gowda, 2012), anti-nociceptive activity (Liu et al., 2015) and anti-neuroinflammatory effects (Yoon et al., 2014). Here, we sought to investigate whether aurantiamide acetate isolated from *B. cusia root*. has anti-viral and anti-inflammatory activity to lessen the severity of IAV infection.

2. Materials and methods

2.1. Preparation of extracts

2.1.1. Generals

Thin layer chromatography (TLC): Kieselgel 60 F254 plates (0.2 mm thick, Merck KGaA Corporation); visualized by UV light (254 and 366 nm) and by spraying with 10% H2SO4 reagent. Column chromatography (CC): silica gel 60 (200–300 mesh, Merck KGaA Corporation) and Reverse Phase-18 (RP-18) (45 μm, Merck KGaA Corporation). Medium Pressure Preparative Liquid Chromatography: BUCHI MPLC System using a RP-18 column (SilicBond C18, 36*460 mm ID, 40–63 μm particle size (Silicycle)). Preparative and semi-preparative HPLC: Lab Alliance system with a YMC-Pack ODS-A column (10 μm, 250*10 mm) and a Vision HT C18 polar column (5 μm, 22*250 mm, Grace, USA). Optical rotations: Rudolph Research Analytical Autopol I automatic polarimeter (Na 589 nm); in MeOH. 1H- and 13C-NMR spectra: A Bruker Ascend 600 NMR spectrometer (600 and 150 MHz, resp.); in CD3OD and Pyridine-d5; at ambient temp; coupling constants J in Hz, and chemical shifts in δ [ppm]. HR-ESI-MS: Agilent 6230 accurate mass time-of-flight mass spectrometer (USA) equipped with an electrospray ion source (ESI), coupled to an UHPLC systerm performed on an Agilent 1290 system using an Eclipse XDB-C18 column (3.0*150 mm, Agilent); in m/z.

2.1.2. Plant material

The root of Baphicacanthus cusia (Nees) Bremek was collected from Honghe, Yunnan, China in October 2012. The plant was authenticated by Dr. Zhifeng Zhang (Macau University of Science and Technology).

2.1.3. Extraction and isolation

Air-dried roots of Baphicacanthus cusia (Nees) Bremek (3 kg) were cut into small pieces and refluxed with 80% aqueous EtOH (30 L, 24 L, 18 L). The extract was suspended in water, and partitioned with ethyl acetate (1 L each) and n-BuOH (1 L each) successively to yield the ethyl acetate layer (44 g), n-BuOH layer (48 g) and H2O layer (62 g). The ethyl acetate layer was subjected to silica gel CC (35*5 cm) using a gradient mixture of ethyl acetate-petroleum ether (9:1 to 5:5) as eluent to afford Fr. 1- 13. Fr. 7 (2.0 g) was separated by RP-18 CC with MeOH-H2O (0:100 to 100:0) and MeOH-CH3COCH3 (100:0 to 50:50) to give 12 sub fractions 7-1~7–12. Sub Fr. 7–8 (63.0 mg) afforded compounds E-17 (4.1 mg) by semi-preparation HPLC (MeCN-H2O).

Aurantiamide acetate (=N-(N-benzoyl-L-phenylalanyl)-O-acetyl-L-phenylalanol E17) 1H-NMR (600 MHz, CDCl3) δ: 4.35 (1H, m, H-2), 2.80 (2H, heptet, H-3), 7.20(2H, m, H-5, H-9), 7.07(2H, d, *J* =8.1 Hz, H-6, H-8), 7.16 (1H, m, H-7), 3.93 (1H, dd, J =11.3, 4.9 Hz, H-10b), 3.82 (1H, dd, J =11.3, 4.2 Hz, H-10a), 2.03 (3H, s, H-12), 4.76 (1H, q, J =5.82 Hz, H-13), 7.72 (2H, d, J =7.77 Hz, H-16, H-20), 7.44 (2H, t, J =7.81 Hz, H-17, H-19), 7.53 (1H, t, J =7.46 Hz, H-18), 3.21 (1H, dd, J =13.7, 5.91 Hz, H-21b), 3.06 (1H, dd, J =13.7, 8.5 Hz, H-21a), 7.31 (2H, m, H-23, H-27), 7.28 (2H, m, H-24, H-26), 7.25 (1H, m, H-25). 13C-NMR (150 MHz, CDCl3) δ: 170.3 (C-1), 49.5 (C-2), 37.4(C-3), 136.6(C4), 128.7 (C-5), 128.8 (C-6), 126.8 (C-7), 128.8 (C-8), 128.7 (C-9), 64.6 (C-10), 170.8 (C-11), 20.4 (C-12), 55.0 (C-13), 167.1 (C-14), 133.6 (C-15), 127.0 (C-16), 128.6 (C-17), 131.9 (C-18), 128.6 (C-19), 127.0 (C-20), 38.4(C-21), 136.7 (C-22), 128.1(C-23), 128.3(C-24), 127.2 (C-25), 128.3 (C-26), 128.1 (C-27).

2.2. Viruses, cell lines and viral infections

The cell lines used in this study were purchased from ATCC. Human lung carcinoma A549 cells and Madin–Darby canine kidney (MDCK) cells were cultured in Dulbecco's modified Eagles medium DMEM/F12(1:1) medium (Hyclone) and DMEM medium (Hyclone) respectively, both supplemented with 10% fetal bovine serum at 37 °C. A549 cells were infected with virus at the indicated multiplicities of infection