Microbial Pathogenesis 110 (2017) 240–244

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

In vitro antiviral efficacy of caffeic acid against canine distemper virus

Zong-Mei Wu [1], Zhen-Jiang Yu [1], Zhen-Qiang Cui, Lu-Yuan Peng, Hao-Ran Li, Chun-Lei Zhang, Hai-Qing Shen, Peng-Fei Yi*, Ben-Dong Fu

Department of Clinical Veterinary Medicine, College of Veterinary Medicine, Jilin University, No. 5333 Xi'an Road, Changchun, Jilin 130062, China

ARTICLE INFO

Article history:
Received 10 May 2017
Received in revised form
29 June 2017
Accepted 3 July 2017
Available online 4 July 2017

Keywords:
Canine distemper virus
Caffeic acid
Antiviral

ABSTRACT

Canine distemper (CD) is a highly contagious disease caused by the canine distemper virus (CDV), and mortality can be as high as 100%. However, there is no specific treatment for CD. In this study, the antiviral activity of the caffeic acid against CDV was evaluated in vitro. The results showed that the IC_{50} of the caffeic acid against CDV at 1 and 2 h post infection (PI) is 23.3 and 32.3 μg/mL, respectively. Consistently, at 1 and 2 h PI, the caffeic acid exhibited a reduced (23.3–57.0% and 37.2–38.1%) viral inhibitory effect in vero cells. Furthermore, the caffeic acid plus Ribavirin (RBV) has greater antiviral activity against CDV than the caffeic acid or RBV individually. In addition, the caffeic acid reduced the total viral RNA synthesis by 59–86% at 24-72 h. Therefore, our data provided the experimental evidence that the caffeic acid effectively inhibited CDV infection in vero cells, which may potentially be used to treat clinical disease associated with CDV infection.

1. Introduction

Canine distemper virus (CDV) is a single-stranded RNA belonging to the genus *Morbillivirus* family *Paramyxoviridae*. The incidence of canine distemper (CD) in canine population seems to have increased in the past decades worldwide and even though the disease is controlled by vaccination. However, many vaccinated dogs became distemper [1–3]. CDV causes systemic infections similar to but distinct from human measles in carnivores such as canines, felids, ferrets, raccoons and seals, with lethality rates, depending on the host, of up to 100% [4,5]. However, there is no specific antiviral agent for the treatment of CD. The nucleoside analogue 1-(b-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (ribavirin, RBV) is the only commercially available molecule with a well-known antiviral activity towards several members of the *Paramyxoviridae* family [6–8]. Therefore, it is necessary to explore new agent to treat CD. The antiviral activity of the caffeic acid (3, 4-dihydroxycinnamic acid) has been reported for some viruses, including influenza A virus [9]. Caffeic acid is an important phenolic compound commonly found in plants, foods, and propolis samples, particularly in the form of caffeic acid phenethyl ester [10]. It is better known for its pharmacological properties, including antimicrobial, antioxidant, anti-inflammatory, and anticancer [11,12].

We hypothesized that the caffeic acid could have inhibitory effect on CDV. Therefore, in this study, our aim was to investigate the anti-CDV activity of the caffeic acid in vitro.

2. Materials and methods

2.1. Cells and viruses

Vero cells were used for the in vitro growth of CDV. Vero cells were cultured in DMEM with 5% fetal bovine serum (FBS) (Gibco) and antibiotics (100 units/ml penicillin, and 100 μg/ml streptomycin) (Gibco, USA) at 37 °C and 5% CO_2 atmosphere incubator.

The CDV-11 strain (purchased from Qilu Animal Health Products Co., Ltd.) was propagated in vero cells using a 5% FBS medium and subsequently titrated. The viral titer was expressed as the 50% infectious dose of the tissue culture ($TCID_{50}$/mL). Assessment of CDV growth in vero cells was performed after incubation at 37 °C and 5% CO_2 for 4d.

2.2. Reagents

Caffeic acid (Purity>98%) and RBV (Purity>98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and their chemical structure

* Corresponding author.
E-mail address: yipengfei1982@126.com (P.-F. Yi).
[1] Zong-Mei Wu and Zhen-Jiang Yu contribute equally to this paper.

http://dx.doi.org/10.1016/j.micpath.2017.07.006
0882-4010/

were shown in Fig. 1. They were dissolved in solution of dimethyl sulfoxide (DMSO) (Sigma, USA). The real-time RT-quantitative PCR (qRT-PCR) and One Step PrimeScript™ RT-PCR Kit were bought from Takara, Japan. All other chemicals were of reagent grade.

2.3. Cytotoxicity assay

The cytotoxicity of the tested compounds were performed on cells using a colorimetric assay based on the MTT(3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl) (Amresco) [13]. Vero cells were cultured into 96-well plate (10^6 cells/well) of incubation at 37 °C and 5% CO_2. The cells were treated with the tested compounds diluted in DMEM at different concentrations (from 12.5 to 200 μg/ml), after 72 h washed twice with phosphate-buffered saline (PBS), and incubated with 20 μl of MTT (5 mg/mL) for 4 h, the salt formed was solubilized by adding DMSO (150 mL/well) and shaking for 10min. The optical density (OD) was determined using an EnSpire Multimode plate Reader (PE, USA) with a 490-nm excitation filter. The 50% cytotoxic concentration (CC_{50}) values were calculated at least three independent experiments.

2.4. Antiviral assay

The intracellular activity of caffeic acid against CDV was evaluated with a cytopathic effect (CPE) reduction assay on confluent vero cells, using the method of Reed and Muench. Vero cells seeded in 96-well plates were infected with CDV at 37 °C in 5% CO_2, and the tested compounds (12.5–200 μg/ml) were added at different time post infection (-1, 0, 1 and 2 h), according to the study [14]. The 50% inhibitory concentration (IC_{50}) was defined as the compound concentration required to reduce viral CPE by 50% of the virus control. The IC_{50} values of caffeic acid and RBV were calculated as the mean ± SD at least three independent experiments. The selectivity index (SI) was obtained by calculating the ratio of the CC_{50}/IC_{50}.

2.5. Time-of-addition study

To investigate the antiviral effect of caffeic acid at different stage, 96-well microplates were seeded with vero cells monolayers (10^6 cells/well), incubated with the tested compounds at different time post infection (-1, 0, 1 and 2 h), vero cells were infected with dilutions of CDV (10-10^5 $TCID_{50}$/mL). The study was according to the method described in a previous study [14]. At the presence of 100% CPE in the virus controls (approximately 72 h PI), the antiviral effect was calculated using the method of Reed and Muench. The inhibition of CDV growth by the tested compounds was expressed as the $TCID_{50}$ value at each time point. Assays were done in triplicate.

2.6. Real-time RT-quantitative PCR analysis

The reduction of viral growth in the presence of the tested compounds was evaluated using RNA quantification. Vero cells were seeded in 6-well plates were infected with 500 $TCID_{50}$ after an incubation time of 2 h at 37 °C in 5% CO_2, and vero cells were washed twice with PBS, then the tested compounds were added. After further incubation of 24, 48 of 72 h, the cells were harvested. One step Real Time PCR technique was used to quantify the copies of viral nucleic acid in the cells. The nucleotide sequences of the forward and reverse primers were 5′-TGGTCGGAGAATTTA-GAATGAACA-3′ and 5′-CACAAATCATTTCAGCAATTCTAGG-3′, respectively, and the TaqMan probe was CCAA-GATGAGTGCCACCATGAACCGCC. The reaction included a first denaturation step at 42 °C for 5 min, 95 °C for 10s, followed by 40 cycles at 95 °C for 5s, and 60 °C for 34s. Each sample and each dilution of the internal control were repeated in duplicate during the same reaction, and the relative level of RNA expression was calculated as the mean of the two measurements.

2.7. Statistical analyses

All values are expressed as means ± SD. Differences between mean values of normally distributed data were analyzed using one-way ANOVA (Dunnett's *t*-test). Statistical significance was accepted at $P < 0.05$ or $P < 0.01$.

3. Results

3.1. The cytotoxicity of caffeic acid, RBV and caffeic acid plus RBV

As shown in Fig. 2A and B, the cytotoxicity was not observed in the cells following the caffeic acid and RBV treatment for 72 h from 12.5 to 200 μg/ml compared with non-treated (control) cell. The CC_{50} values of the caffeic acid and RBV were >200 μg/ml (Table 1). There is also no cytotoxicity of the caffeic acid (32 μg/ml) plus RBV (8-16 μg/ml) and DMSO (4%) (Fig. 2C).

3.2. Caffeic acid inhibits CDV infection in vero cells

When post-infection for 1 and 2 h, the caffeic acid showed antiviral effect. When pre-infection for -1 and 0 h, no antiviral activity against CDV was observed (see Table 1).

The caffeic acid reduced viral yield at times 1 h (IC_{50} 23.3 μg/mL and SI > 8.6), and 2 h (IC_{50} 32.3 μg/mL and SI > 6.2) PI, and the efficiency of antiviral activity of RBV was demonstrated at times 1 h (IC_{50} 12.2 μg/mL and SI > 16.4), and 2 h (IC_{50} 16.1 μg/mL and SI > 12.4) PI.

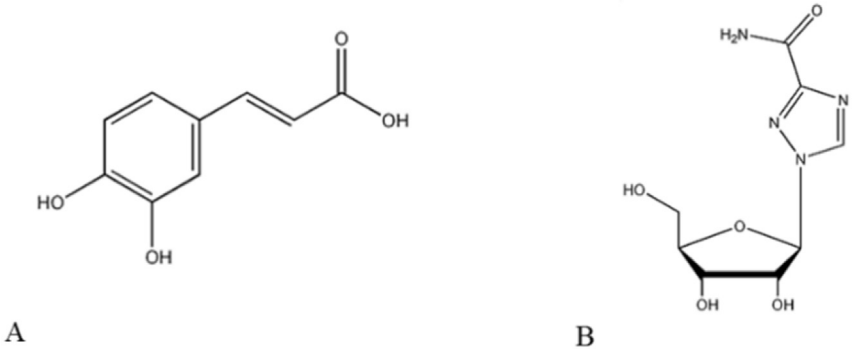

Fig. 1. Chemical structures of caffeic acid (A) and RBV (B).