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Antiviral Effect of Emodin from *Rheum palmatum* against Coxsackievirus B_5 and Human Respiratory Syncytial Virus *In Vitro*[*]

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Summary: Viral infections are the major causes of morbidity and mortality in elderly people and young children throughout the world. The most common pathogens include coxsackie virus (CV) and respiratory syncytial virus (RSV). However, no antiviral agents with low toxicity and drug resistance are currently available in clinic therapy. The present study aimed to examine the antiviral activities of emodin (an ingredient of *Rheum palmatum*) against CVB_5 and RSV infections, in an attempt to discover new antiviral agents for virus infection. The monomer emodin was extracted and isolated from *Rheum palmatum*. The antiviral activities of emodin on HEp-2 cells were evaluated, including virus replication inhibition, virucidal and anti-absorption effects, by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and plaque reduction assay (PRA). The kinetics of virus inhibition by emodin in a period of 14 h was further determined by plaque assay and quantitative real time PCR (qPCR). Cytokine (IFN-γ, TNF-α) mRNA expressions after emodin treatment (7.5, 15, 30 μmol/L) were also assessed by qPCR post-infection. The results showed that emodin had potent inhibitory activities against CVB_5 and RSV, with the 50% effective concentration (EC_{50}) ranging from 13.06 to 14.27 μmol/L and selectivity index (SI) being 5.38–6.41 μmol/L. However, emodin couldn't directly inactivate the viruses or block their absorption to cells. It acted as a biological synthesis inhibitor against CVB_4 and RSV in a concentration- and time-dependent manner, especially during the first 0–4 h post-infection. Moreover, emodin could decrease the mRNA expression of IFN-α but enhance TNF-γ expression significantly compared to the viral controls *in vitro*. Our results provide a molecular basis for development of emodin as a novel and safe antiviral agent for human enterovirus and respiratory virus infection in the clinical therapy.

Key words: emodin; antiviral effect; coxsackievirus B_5; respiratory syncytial virus

Coxsackievirus group B (CVB) infection is characterized by asymptomatic infection, undifferentiated febrile illness, or mild upper respiratory symptoms[1]. Occasionally, CVB causes inflammatory diseases of the pancreas, heart, and central nervous system[2, 3]. Coxsackievirus B_5 (CVB_5) belongs to picornaviridae family and is one of the six serotypes of CVB. It is among the top five commonly identified enterovirus (EV) types[4] and exhibits the highest annual prevalence in some countries[5]. It is associated with encephalitis and myocarditis in immunocompromised children and central nervous system (CNS) disease in older adults[6]. The typical clinical outbreak of CVB_5 results in acute meningitis, and recently CVB_5 was reported to be responsible for an outbreak of neurological hand, foot, and mouth disease in China[7].

Human respiratory syncytial virus (RSV), a member of the Paramyxoviridae family, is a common and widespread respiratory tract infectious agent, resulting in approximately 64 million infections and 160 000 deaths each year[8]. It is the most important cause of lower respiratory tract (LRT) infections such as bronchiolitis and pneumonia in young children and neonates, and it gives rise to acute LRT associated death in young children in developing countries[9]. Clinically, severe RSV infection is primarily seen in young children with naïve immune systems and/or genetic predisposition[10], patients with suppressed T-cell immunity[11], and the elderly[12].

Control over these RNA virus including CVB_5 and RSV infections and related diseases remains a public health concern. Until now, there has been no EV-specific vaccine or licensed RSV vaccine available for clinical use[13, 14]. The efficacy of therapeutic reagents for CVB_5 and RSV infection is woefully unsatisfactory. Ribavirin is licensed for treatment of virus infection but has limited efficacy and causes side effects such as hemolytic anemia. The ineffectiveness of ribavirin and other antivirals is due to the virus-induced inflammatory response generated during infection, which persists after virus replication has ended[15, 16].

Traditional Chinese Medicine (TCM) is well known in clinical research for its abundant resources, low toxicity and high efficiency. Therefore, screening for antiviral agents from the compounds of Chinese herbal medicine is beneficial. Emodin, 1, 3, 8-trihydroxy-6-methyl-anthraquinone, is an anthraquinone derivative from the roots of *Rheum palmatum*. It has been reported that emodin possesses a number of biological activities such as oxidase

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inhibition, anti-inflammatory, anti-viral, vasorelaxative, immunosuppressive, hepatoprotective and anti-tumor effects[17].

To evaluate the antiviral activity and action mechanism of emodin, such as the cellular events in viral life cycle, we investigated the emodin's effects against two human pathogenic RNA viruses (CVB_5 and RSV) in tissue culture cells in this study.

1 MATERIALS AND METHODS

1.1 Cells and Virus Stocks

HEp-2 (human laryngeal carcinoma) cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Gibco, USA), penicillin (100 U/mL), streptomycin (100 μg/mL) and 0.1% L-glutamine. The medium used for the cytotoxic and antiviral assays contained 2% of the serum. CVB_5 strain was maintained in our laboratory and propagated on HEp-2 cells. RSV was kindly provided by Professor Paton at the Department of Children's Health, Glasgow University (England). CVB_5 and RSV proliferated on HEp-2 cells and were harvested when the typical cytopathic effect (CPE) could be observed in more than 75% of the cells.

1.2 Plant Preparation and Extraction Procedure

Rheum palmatum was cultivated and collected from the high mountainous area of Qinghai province, China. The material was then extracted and purified by Department of Plant Chemistry, Hubei College of Traditional Chinese Medicine, China. Its extraction scheme is shown in fig. 1. Emodin, one of the monomers in chloroform extract from *Rheum palmatum* was extracted and isolated (fig. 1). The content of emodin in 50 g *Rheum palmatum* was 293.70±6.12 mg, or (5.87±0.15)% in terms of dried starting materials. Its purity was found to be (45.60±0.23)% in three independent experiments. Ribavirin was purchased from Tianjin Pharmaceutical Co., Ltd. (China).

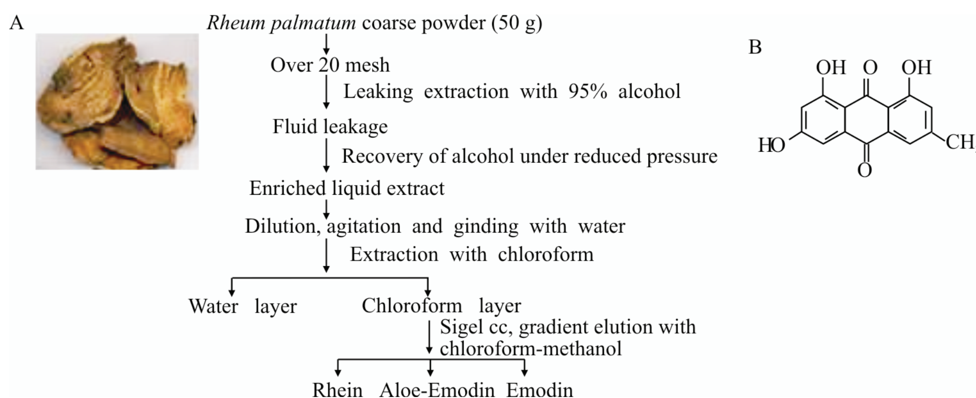

Fig. 1 Isolation and identification of emodin

A: extraction procedure of emodin from *Rheum palmatum*; B: chemical structure of emodin

1.3 Virus Titration

Virus titration was performed on 96-well microtitre plates with the 10-fold dilution of each sample by the limit dilution method. The virus titer was estimated from cytopathogenicity of cells induced by viral infection and expressed as 50% tissue culture infectious dose per milliliter ($TCID_{50}$/mL) by Reed-Muench method, which was 10^{-5} $TCID_{50}$/0.1 mL for CVB_5 and 10^{-4} $TCID_{50}$/0.1 mL for RSV[18].

1.4 Evaluation of Cytotoxicity

The quantitative colorimetric MTT [(3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] assay was performed as described previously[19]. Briefly, 10^5/mL cells were seeded in 96-well plates and cultured at 37°C in an atmosphere of 5% CO_2 incubator for 24 h until subconfluence. After removal of the growth medium, two-fold serial dilutions of emodin (5.95–185.02 μmol/L) and ribavirin (127.96–4094.83 μmol/L) were added and incubated for 72 h. Then the culture medium was removed and the 50 μL thiazolyl blue tetrazolium bromide solution (Sigma-Aldrich, USA) was added and incubated for 2–4 h until the purple precipitate was fully visible. The formazan was dissolved in dimethyl sulfoxide (DMSO), and then quantitated in a conventional microplate reader at 490 nm according to the manufacturer's protocol. The toxic dose for 50% cell death (CC_{50}) was calculated from dose-response curves on the basis of absorbance (A). Cell viability (%)=$A_{\text{treated cells}}/A_{\text{untreated cells}}\times100\%$. All data presented were results of experiments performed in triplicates.

1.5 Virus Replication Inhibition Assay

Monolayers of subconfluent HEp-2 cells were challenged with 100 $TCID_{50}$ CVB_5 (under 37°C) and RSV (under 35°C) for 2 h. After 2 h incubation for virus adsorption, the cells were washed twice with PBS and further incubated with test medium containing two-fold serial dilutions of emodin (1.875–30 μmol/L) or ribavirin (62.5–1000 μmol/L). The antiviral activity of each dilution was measured by MTT assay and plaque reduction assay (2-day incubation with CVB_5; 5-day incubation with RSV). The effective dose for 50% reduction on viral replication (ED_{50}) was determined by the percentage of inhibition, which was calculated as follows: MTT virus inhibition rate=$(A_{\text{tested}}-A_{\text{virus}})/(A_{\text{cell}}-A_{\text{virus}})\times100\%$; PRA virus inhibition rate=[(Number of plaque)$_{\text{virus}}$–(Number of plaque)$_{\text{tested}}$/(Number of plaque)$_{\text{virus}}$]×100%. The selectivity index (SI) was evaluated with the following formula: SI=TD_{50}/ED_{50}.

1.6 Virucidal Assay

Viral suspensions containing 100 $TCID_{50}$/mL of CVB_5 and RSV were either incubated with an equal volume of medium containing two-fold serial dilutions of emodin or with drug-free vehicle at 4°C for 2–6 h, respectively. Then the mixture containing virus were added in the monolayers of subconfluent HEp-2 cells at 37°C for CVB_5 or 35°C for RSV in 5% CO_2 atmosphere. After 2-h adsorption, the

mixed suspensions were removed. The cell monolayers were rinsed carefully with phosphate buffered solution (PBS) and maintained in test medium at 37°C or 35°C for 2 or 5 days. The virucidal effect was determined by an MTT assay following the procedures described above.

1.7 Drug Pretreatment before Virus Infection

Confluent HEp-2 monolayer cells were preincubated with test media in the presence or absence of two-fold serial dilutions of emodin at 37°C or 35°C in 5% CO_2 atmosphere for 2–6 h. The cell sheets were then washed with PBS twice and challenged with 100 $TCID_{50}$/mL of CVB_5 or RSV for 2 h. After incubation for virus adsorption, the cells were rinsed twice with PBS and overlaid with 2% DMEM for 2 or 5 days until typical CPE was visible. The inhibition of virus was evaluated by MTT assay as described above.

1.8 Plaque Reduction Assay

The antiviral activity of emodin was also evaluated by plaque reduction assay (PRA). Briefly, confluent HEp-2 cells grown in a 24-well culture plates were infected with CVB_5 or RSV for 2 h. After 2 h adsorption, the cells were washed twice with pre-warmed PBS and overlaid with 1.2% agarose (42–45°C) containing complete DMEM with different dilutions of emodin at 37°C or 35°C. After incubation for 2–5 days, the cells were then fixed with 10% formaldehyde for 30 min and stained with 1% crystal violet solution. The number of plaques was counted by visual examination and the percentage of plaque inhibition rate was calculated as follows: PRA virus inhibition rate = [(Number of plaque)$_{virus}$– (Number of plaquee)$_{tested}$/(Number of plaque)$_{virus}$]×100%[20].

1.9 Time of Addition Assay

Time of addition assay was performed as described elsewhere[21]. Briefly, confluent HEp-2 monolayer cells in 24-well culture plates were first infected with 100 $TCID_{50}$/mL CVB_5 or RSV at 4°C for 1 h. Then the unbound virus was rinsed from the monolayers with PBS for three times. 30 μmol/L emodin or DMEM without serum were added into wells concurrently with virus infection (0 h) or at intervals of 2, 4, 6, 10, 14 and 18 h after infection. Infected cells were scraped after 24 h and viruses were released from cells by freeze thawing three times. Cell debris was removed by centrifugation and virus titers in supernatant were determined by plaque-forming assay. The percentage of inhibition was calculated according to the following formula: Plaque formation rate (%)=Number of plaques formed$_{treated\ group}$/Number of plaques formed$_{virus\ control}$×100%.

1.10 Quantification of Viral RNA Level by Real-time PCR

Total RNAs were isolated using TRIzol Reagent (Invitrogen, USA) and reverse transcripted into cDNA, according to the manufacturer's instructions. Random primer (Sangon, China) was used for reversed transcription of the total RNA by M-MLV reverse transcriptase (Promega, China). cDNA was prepared at 25°C for 10 min, 42°C for 60 min, 94°C for 3 min and 4°C for 4 min. Then the common PCR amplification was performed with cDNA products as templates (denaturation, 94°C 5 min; annealing, 94°C 45 s; 55°C 45 s, 72°C 30 s for 30 cycles, extension 72°C 5 min). Primer pairs according to the reference[22–24] are listed in table 2. PCR products were resolved in 1.2% agarose gel, purified according to the instructions of DNA gel extraction kit and sequenced to confirm the purpose gene. Then virus (CVB_5 and RSV) gene and housekeeping gene (GAPDH) were serially diluted to obtain a standard series from 10^9 to 10 copies per mL with each step differing by 10 fold. The reaction was performed on a Bio-Rad CFX96 instrument using SYBR Green Real-time PCR Master Mix Reagent (Toyobo, Japan). Each real-time PCR reaction was performed by initial denaturation at 95°C for 3 min, then a three-step cycle procedure (denaturation, 95°C 10 s; annealing, 55°C 10 s; extension, 72°C 30 s) for 40 cycles, followed by 95°C 10 s, 65°C 5 s. The corresponding standards series were obtained after the samples were assayed for CVB_5 and RSV and GAPDH. Thus the copy numbers of samples with emodin at different time could be determined by reading off the standards series with the Ct values of the samples.

1.11 Cytokine Induction and Measurement

Cytokine (IFN-γ, TNF-α) mRNA levels in the cultured cells were measured. Confluent HEp-2 monolayer cells in a 24-well plate were infected with CVB_5 or RSV and incubated at 37°C or 35°C for 2 h. After washing with PBS, 7.5, 15, 30 μmol/L emodin or 500 μmol/L ribavirin was added in 5% CO_2 atmosphere for 48 h. Virus added samples with the same volume of DMEM were used as the virus-infected controls. The cytokine levels in the supernatants were measured with relative quantification of mRNA. Briefly, the total RNA of cells was extracted with TRIzol reagent and reverse transcripted into cDNA as described above. Primers for IFN-γ, TNF-α and the housekeeping gene GAPDH are shown in table 1. After an initial denaturation step at 94°C for 3 min, a three-step cycle procedure was carried out (30 s at 95°C, 40 cycles of 95°C for 15 s and 60°C for 45 s). The relative expression levels of the target genes were normalized to the housekeeping gene GAPDH and were calculated using the following formula[25]: Relative expression level=$2^{-\Delta\Delta CT}=2^{-[(CT\ sample-CT\ house\ keeping\ gene)-(CT\ virus-CT\ house\ keeping\ gene)]}$. Each sample was performed in at least duplicate.

Table 1 Sequences of primers for real-time RT-PCR

Primers	Sequence (5'–3')
CVB_5	(Forward) 5'- TTACGGCGAAAGCTTGAGAT-3'
	(Reverse) 5'- GTGGACGTCTGCCAACTGTA-3'[22]
RSV	(Forward) 5' -CAATGAACTAGGATATCAAGAC-3'
	(Reverse) 5'-GTCTTGATATCCTAGTTCATTG-3'
IFN-γ	(Forward) 5'-CTCAAGTGGCATAGATGTGGAAG-3'[23]
	(Reverse) 5'-GCTGGACCTGTGGGTTGTTGA-3'
TNF-α	(Forward) 5'-CACGTGACGAACCAGAAGATCTT-3'[24]
	(Reverse) 5'-GAGGGTGGTGTTCCAAGCA-3'
GAPDH	(Forward) 5'-TCATTGACCTCAACTACATGGTTT-3'
	(Reverse) 5'-GAAGATGGTGATGGGATTTC-3'

1.12 Statistical Analysis

Each set of experiments was repeated at least three times with consistent results. The data of $TCID_{50}$, CC_{50} and EC_{50} were calculated and analyzed by SPSS 13.0 software (SPSS Inc., USA). The data of different-dose treatments and the data of cytokine levels were compared and analyzed by one-way analysis of variance (one-way ANOVA) with LSD method. A P value of <0.05 was considered statistically significant.

2 RESULTS

2.1 Cellular Toxicity of Emodin

The cytotoxicites of emodin from *Rheum palmatum* for HEp-2 cells were evaluated by MTT assay. As shown in fig. 2, the cell viability was reduced with the increase of drug concentration. The estimated CC_{50} values were 76.783 and 2642.953 μmol/L for emodin and ribavirin in HEp-2 cells, respectively. Emodin at 30 μmol/L did not cause the visible changes in cell morphology or cell density, which was set as the highest non-toxic concentration in antiviral assays. Cells treated with emodin at 50 μmol/L or higher grew tardily and showed alterations in cell morphology.

2.2 Emodin Inhibited Viral Replication in a Dose-dependent Manner

The antiviral potential of emodin on CVB_5 and RSV was assessed by MTT assay and PRA using HEp-2 cells. The virus-induced CPE, normally apparent at 2nd day (CVB_5) or 5th day (RSV) post-infection, was dramatically delayed by emodin in a concentration-dependent manner. As shown in fig. 3, a noted increased antiviral effect was observed with the increase of drug concentrations. Emodin at 30 μmol/L could suppress more than 80% of CVB_5 and RSV replication in HEp-2 cells. A good concentration-dependent linear relationship (r=0.955, P<0.05 for RSV and r=0.952, P<0.05 for CVB_5) was also observed between 0–30 μmol/L emodin and the virus inhibitory rate.

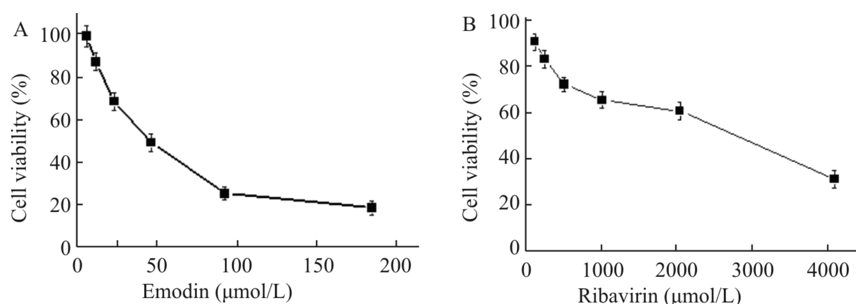

Fig. 2 Effect of emodin (A) and ribavirin (B) on cell viability of HEp-2 cells

Emodin: 1 μmol/L=270.24 ng/mL; Ribavirin: 1 μmol/L=244.21 ng/mL. Values are presented as x±s of each four detections.

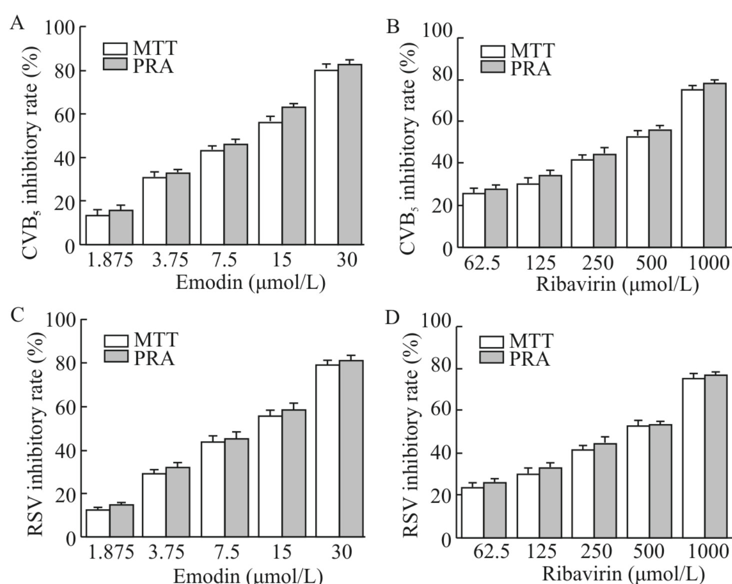

Fig. 3 Antiviral effects of emodin (A, C) and ribavirin (B, D) against CVB_5 and RSV in HEp-2 cells by MTT assay and plaque formation assay

Values are presented as x±s of each four detections.

The results of drug toxicity showed that emodin acted as a potent inhibitor against CVB_5 and RSV with an average EC_{50} value in the range of 13.06–14.27 μmol/L and SI of 5.38– 6.41 μmol/L when added post-infection (table 2). Both the EC_{50} and SI of emodin showed no significant difference from those of the positive control ribavirin (P>0.05), indicating that the anti-CVB_5 and anti-RSV activities of emodin were similar to those of ribavirin.

Table 2 The CC_{50}, EC_{50} and SI of the drugs

	Compound	EC_{50} (μmol/L)		SI	
		MTT	PRA	MTT	PRA
CVB_5	Emodin	13.77±0.52	12.11±0.95	5.58±0.41	6.34±0.25
	Ribavirin	474.35±1.64	412.01±1.23	5.57±0.60	6.41±0.28
RSV	Emodin	14.27±1.42	13.06±1.55	5.38±0.40	5.87±0.58
	Ribavirin	481.07±1.53	447.91±1.75	5.49±0.50	5.90±0.36

2.3 Emodin could not Prevent Viral Adsorption or Directly Inactivate Virus

To test if emodin had an antiviral adsorption and directly inactivating effect on CVB_5 and RSV virions, different doses of emodin were pre-incubated with cells or incubated with virus for 2–6 h before titration. The residual infectivity was further determined by plaque assay. However, no obvious antiviral adsorption or virucidal effect could be observed, and the inhibitory rate was only 6.20%–8.66% at each dosage (data not shown). There was no significant difference between emodin-treated groups and the virus control group in pre-incubation and direct inactivation assay (P>0.05).

2.4 Emodin Inhibited Virus Infection at the Initial Stage of Viral Replication

As described above, emodin treatment caused a dose-dependent reduction of CVB_5 and RSV in HEp-2 cells. To determine whether the inhibition of virus replication by emodin was time-dependent, the compound was added at the indicated time (0, 2, 4, 6, 10, 14, 18 h post-infection). The growth of CVB_5 and RSV on HEp-2 cells was measured over time by plaque assay and qPCR. Plaques formed (fig. 4A and 4C) in 0–4 h (17.5%–41.6%) were much less than those in 6, 10, 14, 18 h (88.6%–93.6%) and virus control (P<0.01). The plaques formed in emodin treated group in 6–18 h were not significantly different from those in virus control group (P>0.05). The results suggested emodin inhibited virus-specific events within the first 4 h of CVB_5 and RSV infection.

Infectious viral particles in the supernatant were also quantitated by quantitative real time PCR at 28 h post-infection. We found that emodin significantly inhibited CVB_5 and RSV replication when added 0–4 h after virus inoculation (fig. 4B). CVB_5 and RSV copies at 0, 2, 4 h expressed 0.71, 0.58, 0.47 and 0.67, 0.53, 0.44-fold lower than the virus control group (P< 0.01), a result that corroborates the PRA results of emodin against virus infection. In addition, the results of plaque-forming assay and qPCR suggested that the anti-CVB_5 and anti-RSV activity of emodin was indistinguishable (P>0.05).

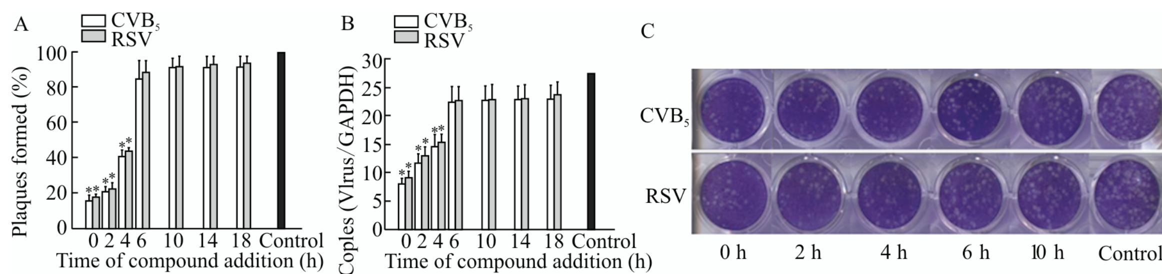

Fig. 4 Emodin inhibited virus infection at the initial stage of viral replication

A: plaque-forming assay. After 2 h at 4°C to allow virus binding but not virus entry, the infected cells were treated with 30 μmol/L emodin immediately or at 0, 2, 4, 6, 10, 14, 18 h post-adsorption. The virus titer in supernatants was determined by plaque-forming assay. Plaques formed (%)=number of plaques formed in treated group/number of plaques formed in virus control×100%. B: real-time PCR. Total viral RNAs were extracted, reverse transcribed and assayed by qPCR. Values are presented as x±s of each four detections. Control indicates viral controls. $^*P$<0.01 emodin *vs.* virus infected control. C: Plaque-forming images showing the inhibitory effect of emodin against CVB_5 and RSV under the light microscope at 0, 2, 4, 6, 10 h

2.5 Emodin Regulated the mRNA Expression of IFN-α and TNF-γ

As described above, cytokine (IFN-γ, TNF-α) levels in the cultured cells were detected by real-time PCR and the results are shown in fig. 5. The mRNA expression of IFN-γ was significantly enhanced but the expression of TNF-α mRNA was decreased in emodin-treated groups as compared with virus infected control group [P<0.05, emodin (7.5, 15 μmol/L)-treated group *vs*. virus infected control group; P<0.01, emodin (30 μmol/L)-treated group *vs*. virus infected control group]. The increase of IFN-γ mRNA and the decrease of TNF-α were not significantly different between CVB_5 and RSV treated groups (P>0.05).

3 DISCUSSION

Emodin is an anthraquinone compound consisting of three cyclic rings, which is the active constituent derived from the roots of *Rheum palmatum*. Emodin possesses antibacterial, diuretic, vasorelaxant, anti-inflammatory, anti-proliferative and anti-carcinogenic properties[26, 27]. Several studies have demonstrated the effect of emodin on the yield of herpes simplex virus[28], hepatitis B virus[29], severe acute respiratory syndrome (SARS) coronavirus[30], etc. Emodin is suspected to exhibit its antiviral activity by inhibiting casein kinase 2, which is essential for the phosphorylation of proteins in virus life cycle[31], or by disrupting the lipid bilayer to inactivate the enveloped virus[32].

The antiviral activities of emodin against viruses were investigated in this study by examining virus replication inhibition, virucidal and anti-absorption effects on HEp-2 cells by MTT and PRA method. Emodin was found to present potent antiviral activity when added after infection with EC_{50} of 12.11 μmol/L, SI of 6.34 for CVB_5 and EC_{50} of 13.06 μmol/L, SI of 5.87 for RSV, respectively.

Emodin (0–30 μmol/L) caused a dose-dependent reduction of CVB_5 and RSV in HEp-2 cells. The antiviral activities of ribavirin against CVB_5 and RSV were almost equivalent. However, emodin could not prevent HEp-2 cells from virus infection in anti-absorption assay, nor could it show virucidal effects against CVB_5 and RSV. The results implied that emodin may inhibit virus biological synthesis rather than directly inactivating the viruses or blocking their absorption to the susceptible cells *in vitro*.

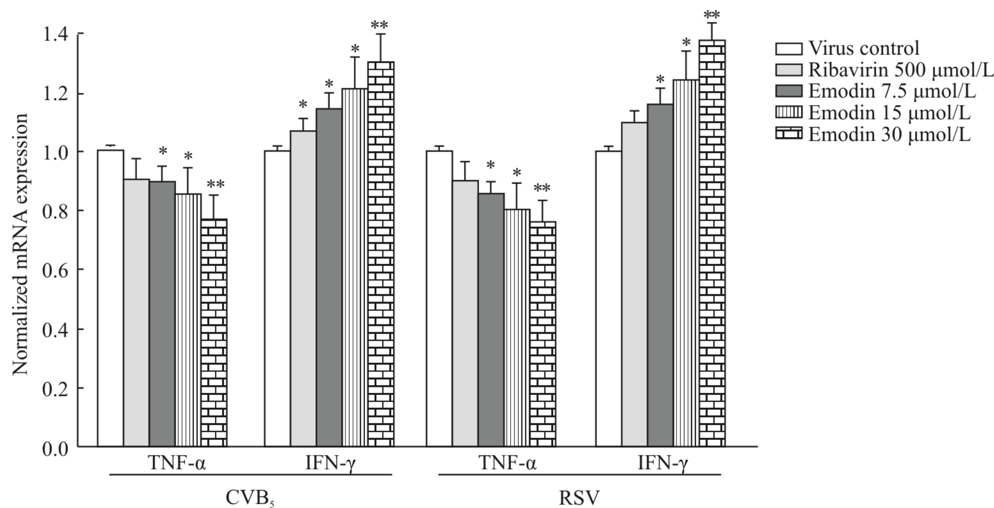

Fig. 5 Effect of emodin from *Rheum palmatum* on the expression of cytokine TNF-α and IFN-γ in the CVB_5- or RSV-infected culture supernatants

Values are presented as $\bar{x}\pm s$ of each four detections. $^{*}P<0.05$, $^{**}P<0.01$ emodin *vs.* virus infected control

To gain more insight into the mechanism by which emodin interrupts virus replication, time of addition assays were performed to determine the time point of maximum inhibition by emodin. Only 15.5%–41.6% plaques were formed when emodin was added immediately or at 2, 4 h after virus adsorption but rapidly increased to 90% when added 10 h post-infection. In qPCR study, a dramatic reduction in infectious virus copies was observed at 0–4 h post-infection, which coincided with the results of plaque formed. Our data demonstrated that emodin may interrupt the early stages of the viral replication cycle.

Cytokines, secreted by human body's cells, play a critical role in connecting the innate immunity and specific immunity in body and have been demonstrated to augment or weaken host resistance against virus infection. For example, specific expression of IFN-γ could control hepatitis B virus replication *in vitro*[33] and was both necessary and sufficient to clear measles virus in infected brain tissue[34]. Furthermore, IFN-γ could restrict cell-to-cell spread of CVB_3 in the heart to limit myocardial organ injury[35, 36] and was observed to protect pancreatic cells from invading CVB_4 by activation of resident macrophages[37]. TNF, a polypeptide cytokine produced by activated macrophages, monocytes and T cells, has been proved pivotal in triggering the inflammatory infection. For instance, TNF-α was over-expressed through activation of cardiomyocyte-specific promoter regions[38], and also enhanced the expression of a variety of adhesion molecules required for inflammatory cell infiltration of infection sites in CVB_3 -induced myocarditis[39].

Our investigation of the effect of emodin on the mRNA expression of cytokines revealed that emodin significantly increased mRNA expression of IFN-γ, but decreased TNF-α mRNA expression, demonstrating that emodin may strengthen host resistance against virus infection by enhancing the expression of IFN-γ, and prevent virus induced inflammatory responses by inhibiting TNF-α mRNA expression.

In summary, our results provide a molecular basis for development of emodin as a novel and safe antiviral agent for human enterovirus and respiratory virus infection in the clinical therapy. Emodin could inhibit the replication of CVB_5 and RSV post-infection in a concentration- and time-dependent manner and possess antiviral activities against virus by regulating cytokine (IFN-γ and TNF-α) expression. As the activation of inflammatory cytokines and virus replication share the same intercellular pathway, our finding might provide some clues for understanding the mechanism by which emodin acts on these cascades. Emodin may be an antiviral candidate with a broad spectrum of antiviral activities by targeting or interrupting viruses or virus synthesis-related proteins in the early stages of the viral replication cycle. Its modulatory function on virus-induced inflammatory cytokines, such as IFN-γ and TNF-α might contribute to the immunoregulation effect of the drug. Further studies of these pathways using microarray analysis or Western blotting analysis will be needed. Being the main component extracted from *Rheum palmatum*, emodin holds the promises to become a potential therapeutic agent for virus infection.

Conflicts of Interest Statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

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