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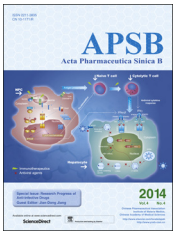

ORIGINAL ARTICLE

Curcumin inhibits the replication of enterovirus 71 *in vitro*

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Abstract Human enterovirus 71 (EV71) is the main causative pathogen of hand, foot, and mouth disease (HFMD) in children. The epidemic of HFMD has been a public health problem in Asia-Pacific region for decades, and no vaccine and effective antiviral medicine are available. Curcumin has been used as a traditional medicine for centuries to treat a diversity of disorders including viral infections. In this study, we demonstrated that curcumin showed potent antiviral effect again EV71. In Vero cells infected with EV71, the addition of curcumin significantly suppressed the synthesis of viral RNA, the expression of viral protein, and the overall production of viral progeny. Similar with the previous reports, curcumin reduced the production of ROS induced by viral infection. However, the antioxidant property of curcumin did not contribute to its antiviral activity, since *N*-acetyl-L-cysteine, the potent antioxidant failed to suppress viral replication. This study also showed that extracellular signal-regulated kinase (ERK) was

Abbreviations: CVB, coxsackieviurs B; DCFH-DA, dichloro-dihydro-fluorescein diacetate; ERK, extracellular signal-regulated kinase; EV71, enterovirus 71; GBF1, Golgi brefeldin A resistant guanine nucleotide exchange factor 1; GEF, guanine nucleotide exchange factor; HBV, hepatitis B virus; HCV, hepatitis C virus; HFMD, hand, foot, and mouth disease; HIV, human immunodeficiency virus; HPV, human papillomavirus; NAC, *N*-acetyl-L-cysteine; PARP-1, poly(ADP-ribose) polymerase; PGC-1α, peroxisome proliferator-activated receptor-gamma co-activator 1 alpha; p.i., post-infection; PI4KB, phosphatidylinositol 4-kinase class III catalytic subunit β; PI4P, phosphatidylinositol 4-phosphate; ROS, reactive oxygen species; siRNA, small interfering RNA; SLLVY-AMC, succinyl-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin; UPS, ubiquitin–proteasome system

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activated by either viral infection or curcumin treatment, but the activated ERK did not interfere with the antiviral effect of curcumin, indicating ERK is not involved in the antiviral mechanism of curcumin. Unlike the previous reports that curcumin inhibited protein degradation through ubiquitin–proteasome system (UPS), we found that curcumin had no impact on UPS in control cells. However, curcumin did reduce the activity of proteasomes which was increased by viral infection. In addition, the accumulation of the short-lived proteins, p53 and p21, was increased by the treatment of curcumin in EV71-infected cells. We further probed the antiviral mechanism of curcumin by examining the expression of GBF1 and PI4KB, both of which are required for the formation of viral replication complex. We found that curcumin significantly reduced the level of both proteins. Moreover, the decreased expression of either GBF1 or PI4KB by the application of siRNAs was sufficient to suppress viral replication. We also demonstrated that curcumin showed anti-apoptotic activity at the early stage of viral infection. The results of this study provide solid evidence that curcumin has potent anti-EV71 activity. Whether or not the down-regulated GBF1 and PI4KB by curcumin contribute to its antiviral effect needs further studies.

1. Introduction

Enterovirus 71 (EV71) is a single stranded RNA virus which belongs to genus *Enterovirus*, family Picornaviridae[1]. EV71 infection is often asymptomatic or causes mild manifestations such as fever, sore throat and general malaise[2]. However, it may cause severe and sometimes fatal complications such as encephalitis and pulmonary edema. More importantly, outbreak of EV71 infection has become a public health problem especially in the Asia-Pacific region[2–4]. However, since there is no specific anti-EV71 vaccine or medicine available, treatment for the patients with EV71 infection is simply of supportive[5]. Therefore, to identify the effective anti-EV71 drugs is urgent.

Curcumin is a natural compound extracted from turmeric (*Curcuma longa*). It has been used widely as a spice and coloring agent in food[6]. In addition, curcumin is a constituent of many herbal medicines used for diverse medical conditions[7–9]. In recent years, extensive studies have shown that curcumin has a variety of pharmacological properties, including anti-inflammation, antitumor and antioxidant[10–12]. Accumulating evidence has also demonstrated that curcumin has antiviral activity[6,13–17]. Viruses inhibited by the treatment of curcumin include hepatitis C virus (HCV), hepatitis B virus (HBV), coxsackieviurs B3 (CVB3) and human papillomavirus (HPV)[16,18–20]. It has been reported that curcumin suppresses CVB3 replication *via* the dysregulation of the ubiquitin-proteasome system (UPS)[20]. Other studies have shown that the inhibitory effect of curcumin on HCV replication is associated with the suppression of AKT and the inhibition on viral entry[13,18], while curcumin inhibits HBV replication *via* down-regulation of peroxisome proliferator-activated receptor-gamma co-activator 1 alpha (PGC-1α), a major metabolic co-activator of HBV[16]. Overall, these findings suggested that curcumin may directly block the replication machinery of viruses or regulate cellular metabolic or signaling pathways which are exploited by viruses.

Although curcumin shows inhibitory effect on a variety of viruses, whether it exerts antiviral effect on EV71 is currently unknown. In the present study, we evaluated potential antiviral activity of curcumin against EV71. To understand the involved mechanisms of the effect of curcumin on viral replication, the influence of curcumin on reactive oxygen species (ROS), apoptosis, extracellular signal-regulated kinase (ERK) pathway and UPS were observed. The expression of phosphatidylinositol 4-kinase class III catalytic subunit β (PI4KB) and Golgi brefeldin A resistant guanine nucleotide exchange factor 1 (GBF1) were also studied.

2. Materials and methods

2.1. Chemical reagents and antibodies

Curcumin, which was dissolved in DMSO before use, and *N*-acetyl-L-cysteine (NAC) were purchased from Sigma-Aldrich (St. Louis, USA). MG132 and the Reactive Oxygen Species Assay Kit were obtained from Beyotime (China). The fluorimetric substrate succinyl-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin (SLLVY-AMC) was obtained from MERCK. GBF1 small interfering RNA (siRNA), siRNA of PI4KB, and the scramble control siRNA were synthesized by Takara (Dalian, China).

The monoclonal anti-enterovirus VP1 antibody was purchased from DakoCytomation (clone5-D8/1, Denmark). Anti-PI4KB and anti-GBF1 antibodies were from Becton Dickson. Anti-ERK1/2 and anti-phospho-ERK1/2 antibodies were obtained from Cell Signaling (Danvers, USA). Anti-cleaved caspase 3, antibody against the poly (ADP-ribose) polymerase (PARP-1), and anti-p53 antibody were purchased from Santa Cruz (Dallas, USA). Anti-β-actin and the horseradish peroxidase-conjugated secondary antibodies were obtained from Zhongshan Golden Bridge (Beijing, China).

2.2. Cell culture

Vero cells and RD cells (a human rhabdomyosarcoma cell line) were maintained in the Department of Microbiology, Harbin Medical University. Cells were cultured in complete Dulbecco's modified Eagle medium (DMEM, Invitrogen, China) supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin and streptomycin) at 37 °C with 5% CO_2. Vero cells were used for the studies on EV71 infection and the effect study. RD cells were used for siRNA transfection.

2.3. Viral infection

EV71 BrCr strain was kindly offered by Professor Mingli Wang, Department of Microbiology, Anhui Medical University, China.