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Citreoviridin inhibits cell proliferation and enhances apoptosis of human umbilical vein endothelial cells

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ABSTRACT

In some areas of China, citreoviridin (CIT) is considered one of the risk factors for development of cardiovascular disease (CVD). Apoptosis of endothelial cell may induce vascular endothelium injury and atherosclerosis, which result in CVD probably. In this study, we investigated the effect of CIT on apoptosis and proliferation of human umbilical vein endothelial cells (HUVECs). The MTT assay was used to determinate HUVECs proliferation. Distribution of the cell cycle was analyzed by flow cytometry. The Annexin-V/PI staining was used to investigate cell apoptosis. Western blotting analysis was used to indicate changes in the expression level of apoptosis-related proteins. The results indicated that CIT inhibited HUVECs proliferation and the cells were arrested at G0/G1 phase, which is associated with decreased levels of cyclinD1 and increased expression of p53 and p21. The apoptosis rate of HUVECs was improved by CIT. The expression of Bcl-2 were down-regulated after CIT treatment, whereas the levels of Bax was significantly up-regulated. Furthermore, CIT-induced apoptosis was accompanied by the activation of caspase-3, -9. These findings demonstrate that CIT inhibits cell proliferation via DNA synthesis reduction and induces caspase-dependent apoptosis in HUVECs. CIT plays a pivotal role in the process of endothelial cell apoptosis, may thereby play an important role in the improvement of CVD in areas of China that have a high prevalence of CIT contamination.

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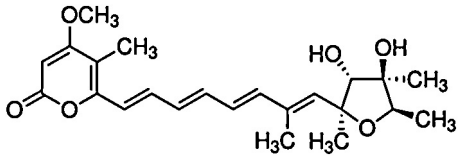

Fig. 1 – Structural formula of citreoviridin.

1. Introduction

Atherosclerosis is the lesion primarily underlies cardiovascular disease (CVD), one of the most common causes of illness and death worldwide, which is the principal causes of death in China (Hou et al., 2013; Wang et al., 2011). Generally, the endothelial surface of the vessel lumen is a relatively non-adhesive and non-thrombogenic conduit for the transportation of cellular and macromolecular constituents of blood. Apoptosis and inflammation in endothelial cells are key factors involved during atherosclerosis (Rus et al., 1996; Traub and Berk, 1998). Apoptotic endothelial cell death may distroy the endothelial monolayer and thereby contribute to vascular injury.

Citreoviridin (CIT) is one of mycotoxins derived from Penicillium strains, and the structural formula of CIT is showed in Fig. 1. CIT is thought to be the toxicological reason of acute cardiac beriberi that has prevailed in Japan and Keshan disease in China (Taatjes et al., 2008; Taylor et al., 2008; Hou et al., 2006; Nishie et al., 1988). A novel regular phenomenon was discovered that the contamination of CIT in foods was obvious in some areas in China where the prevalence of cardiovascular disease was serious. Previous research has demonstrated that the contamination levels of CIT (in grains) in target areas in China were between 4.9 μg/kg and 33.2 μg/kg (Li et al., 2009). Although studies have shown that CIT induce apoptosis in HUVECs (Hou et al., 2011), but the underlying mechanisms of its action are not completely understood.

The objective of the current study is to investigate the direct effects of CIT in inducing apoptosis to vascular endothelial cells in vitro and to explore its underlying mechanisms. Our results showed that CIT inhibited cell proliferation and induce cell apoptosis. And the stimulated effect of CIT on apoptosis of HUVECs via caspase-dependent apoptotic pathway was found. As apoptosis of endothelial cells is also a key factor during atherosclerosis we suggest that CIT can regulate apoptosis in endothelial cells during the process of atherosclerosis. And our findings may have important implications and underscore the role of CIT as a novel risk factor for atherosclerosis and CVDs.

2. Materials and methods

2.1. Reagents

Human umbilical vein endothelial cells (HUVECs) were provided by ATCC (Manassas, VA, USA). DMEM/F12 growth medium and fetal bovine serum (FBS) were purchased from GIBCO BRL (Grand Island, NY, USA). Citreoviridin (CIT) was provided by Fermentek Company (Jerusalem, Israel) and dissolved with 100% DMSO (concentration of the stock solution was 100 mg/l). Carbobenzoxy-valyl - alanyl-aspartyl-[O-methyl]-fluoromethylketone (Z-VAD-FMK) were from Promega corporation (Madison, WI, USA). 3-[4,5-Dimethylthiazol-2-y-l]-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Genview (Houston, TX, USA). Propidium iodine (PI) was purchased from Sigma Chemical Co (St Louis, MO, USA). Annexin V-FITC apoptosis detection kit, caspase-3 activity assay kit and caspase-9 activity assay kit were purchased from KeyGEN Biotech (Nanjing, P. R. China). The ECL Western Blotting Detection Reagents was purchased from Thermo Scientific Pierce (Rockford, IL, USA). Mouse anti-β-Actin, Mouse anti-Bax and rabbit anti-Bcl-2 were provided by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse anti-p21, mouse anti-p53, rabbit anti-cyclinB1 and rabbit anti-cyclinD1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse anti-caspase-3, mouse anti-caspase-8, mouse anti-caspase-9 and rabbit anti-PARP were purchased from Cell Signaling Technology (Danvers, MA, USA). The anti-NF-κB p65 primary antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). DAPI and Alexa Fluor 568 labeled anti-rabbit IgG were provided by Invitrogen (Carlsbad, CA, USA).

2.2. Cell cultures and treatments

HUVECs were cultured in DMEM medium supplemented with 10% FBS, penicillin-G (100 U/ml), streptomycin (100 μg/ml). The growth medium was changed every other day until the cells reached confluence. Cells of passage 4 and 6 were seeded in monolayers at 37 °C in a humidified atmosphere (5% CO_2 and 95% air).

According to previous experiments and the reported contamination levels of CIT in grains obtained in Northeast China (Li et al., 2009), we cultured HUVECs in relevant medium, and divided the cells into 3 groups: low level CIT treatment group (addition of 0.1 mg/l CIT in the medium), high level CIT treatment group (addition of 0.2 mg/l CIT in the medium), HUVECs in untreated group (addition of the same volume of DMSO only). In order to analyze the recovery of CIT-induced apoptosis, we pre-treated the cells with the pan-caspase inhibitor, Z-VAD-FMK (50 μM) for 1 h, then induced the cells by CIT (0, 0.1 and 0.2 mg/l) for 24 h. The procedures of drug preparation and treatment were carried out in the dark. HUVECs were further cultured at 37 °C for 24 h, 48 h or 72 h. Five replicates for each experiment were performed.

2.3. MTT assay

The growth inhibitory effect of CIT on HUVECs was assessed by MTT. Cells were seeded at a density of 1×10^3 cells/well in 96-well plates. And cells were treated with CIT at 0.1 mg/l or 0.2 mg/l for 72 h. MTT assays were performed as instructed by the manufacturer every 24 h. The absorbance was read at 490 nm using a microplate reader (Sunrise RC, Tecan, Switzerland). Relative cell viability was expressed as the percentage of the control.