Antiviral Research 141 (2017) 133–139

Contents lists available at ScienceDirect

Antiviral Research

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

Oxymatrine provides protection against Coxsackievirus B3-induced myocarditis in BALB/c mice

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ARTICLE INFO

Article history:
Received 20 September 2016
Received in revised form
21 December 2016
Accepted 18 January 2017
Available online 20 January 2017

Keywords:
Oxymatrine
Coxsackievirus B3
Antiviral activity
Viral myocarditis

ABSTRACT

Oxymatrine is the primary pharmacological component of *Sophora flavescens Ait*. In the present study, we investigated the protective effect of oxymatrine against Coxsackievirus B3-induced myocarditis in mice. Coxsackievirus B3-infected HeLa cells were treated with oxymatrine and the viral titer, as well as the degree of cellular proliferation were determined. Additionally, BALB/c mice were infected with Coxsackievirus B3 and received differing concentrations of oxymatrine. On days 5 and 12 following treatment, mice were sacrificed, and serum lactate dehydrogenase, creatine kinase-MB isozyme, and tumor necrosis factor-α levels were quantified. The heart index and degree of myocardial tissue inflammation were also assessed. On day 5, the Coxsackievirus B3 $TCID_{50}$ values of the heart tissue, and the expression of *NTR*, *IFN-*γ, and *TNF-*α genes in the myocardial tissue were measured. Our results showed that oxymatrine exhibits potent antiviral effects on Coxsackievirus B3 as 50% inhibition was achieved at a concentration as low as 0.238 mg/mL. Oxymatrine markedly reduced the viral titer and inhibited cardiac myocyte pathology exhibited in viral myocarditis. Furthermore, oxymatrine treatment reduced the expression of Coxsackievirus B3 *NTR* and mouse *TNF-*α genes compared to the controls. Therefore, our findings indicate that oxymatrine is a promising potent antiviral agent against Coxsackievirus B3-induced myocarditis.

1. Introduction

Myocarditis is defined as inflammation of the myocardium that usually follows microbial infections in the heart (Cooper, 2009). It represents one of the most challenging clinical problems in cardiology, associated with a broad spectrum of pathological triggers and a wide range of clinical presentations that vary from mild dyspnea to acute heart failure and sudden death (Cooper, 2009; Sagar et al., 2012). Myocarditis can account for as high as 10% of patients with acute heart failure from unknown causes and 12% of sudden deaths in individuals less than 40 years old (Blauwet and Cooper, 2010). Therefore, myocarditis a significant cardiovascular health problem (Blauwet and Cooper, 2010). Coxsackievirus B3 (CVB3) is an enterovirus in the genus *Picornaviridae* and is the most frequent etiological agent to induce myocarditis. CVB3 proliferates rapidly within human cardiomyocytes, entering the cell via a transmembrane coxsackievirus and adenovirus receptor that is differentially expressed on various cell types (Elamm et al., 2012; Knowlton, 2008; Pallansch, 2007). This receptor is highly expressed on cardiomyocytes and is essential for mammalian cardiogenesis (Elamm et al., 2012; Knowlton, 2008; Pallansch, 2007).

Virally-induced myocarditis is a triphasic disease involving an initial viral infection, followed by an autoimmune response, and results in the remodeling of the cardiac structure and function. Padalko et al., (2004) have been reported the viral myocarditis mice models, so it is possible to investigate the pathogenesis, prevention, and treatment of viral myocarditis *in vivo*. To date, there are no clinical available vaccines and few drugs are therapeutic in treating the disease. Most often, severe cases of viral myocarditis have a poor prognosis without a heart transplantation (Bean, 1992). To treat viral myocarditis, ribavirin has been found to exhibit potent antiviral properties essential for the clinical treatment of several virally-induced diseases (Sharma et al., 2014). However, its use remains controversial due to its questionable efficacy, side effects, and high cost. The therapeutic methods for viral myocarditis are primarily supportive, involving bed rest, vitamin C, and coenzyme

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http://dx.doi.org/10.1016/j.antiviral.2017.01.013
0166-3542/

Q10 (Elamm et al., 2012). Thus, there is an urgent need for an effective antiviral agent or cure for virally-induced myocarditis.

Sophora flavescens Ait., commonly known as Kushen in Chinese medicine grows widely in northeastern and northern China. Radix Sophorae Flavescentis (Kushen), a popular Chinese herb, has been widely and successfully used for treating angina pectoris, myocardial infarction (MI), and stroke. Oxymatrine (OMT, Fig. 1) is a preparation of alkaloid aqueous solution extracted from Kushen, and was assigned as the marker species for Kushen in the 2005 edition of Chinese Pharmacopoeia. The bioactivities of OMT, such as anti-inflammatory, antitumor, immunomodulatory, and anti-proliferative activities, have been reported (Liu et al., 2014; Xiao et al., 2014; Zhu et al., 2014). OMT has the anti-viral activities, and the role inhibiting virus, such as respiratory syncytial virus, hepatitis B virus, influenza A virus and CVB3 was reported (Ma et al., 2002, 2013; Pan et al., 2015). It was confirmed through experiments *in vitro*. In our previous study, we demonstrated that OMT is protective against aldosterone-induced cardiomyocyte injury via the inhibition of calpain and apoptosis-inducing factor signaling (Xiao et al., 2014). In addition, OMT has also shown to exhibit a protective effect towards myocardial fibrosis (Shen et al., 2011). In the present study, OMT was investigated for its anti-CVB3 activity and its potential as a treatment of viral myocarditis using both *in vitro* and *in vivo* techniques.

2. Materials and methods

2.1. Experimental animals

Specific-pathogen-free female BALB/c mice (6-weeks-old) were obtained from the Laboratory Animal Center of Guizhou Medical University (Guizhou, China). The experiments were carried out in accordance with the guidelines issued by the Ethical Committee of Guizhou Medical University (No. 1301016). This housing facility is a barrier housing facility, and it has in keeping with national standard *Laboratory Animal-Requirements of Environment and Housing Facilities (GB 14925-2010)*. The care of laboratory animal and the animal experimental operation have conforming to *Administration Rule of Laboratory Animal*.

2.2. Drug preparation

OMT (99.0% purity) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). 20 mg of OMT was dissolved in sterile distilled water. Different concentrations of an OMT solution in RPMI-1640 were used for cellular assays, and in sterile PBS for animal experiments. Ribavirin (Guizhou, PR China) was used as a positive control for OMT.

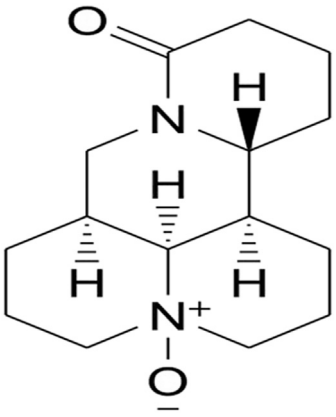

Fig. 1. The chemical structure of OMT.

2.3. Virus, cells, and media

CVB3 (Nancy strain) was purchased from the Wuhan Institute of Virology, Chinese Academy of Sciences. The virus was prepared in HeLa cells (Kunming Committee Type Culture Collection cell bank, Chinese Academy of Sciences), cultured in RPMI-1640 (Hyclone, Logan, UT, USA), and the $TCID_{50}$ was determined. HeLa cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum (FBS; Hyclone).

2.4. 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay

The cytotoxicity and antiviral activity of OMT were evaluated by MTT (Solarbio, Beijing, China) as previously described (Nishimura et al., 2002). Briefly, 10^5 cells/mL were seeded into 96-well culture plates (Costar, Cambridge, MA, USA). After 2–3 d of incubation at 37 °C with 5% CO_2, the cytotoxicity and antiviral tests were performed. The media was aspirated, cells were rinsed with PBS, and the MTT reagent (5 mg/mL) was added. The plate was incubated for an additional 4 h, and 100 μL dimethyl sulfoxide (Solarbio, Beijing, China) was added to each well. Optical densities were determined using a plate reader (EL x 800 light absorption enzyme standard instrument, BioTek, USA) at a wavelength of 492 nm.

2.5. Tissue culture infective dose ($TCID_{50}$) assays

CVB3 in the supernatants of the *in vitro* infected HeLa cells, and the homogenates of the myocardial tissue from mice were detected and titrated using the endpoint method. This involved infecting replicate HeLa cells in 96-well plates with 10-fold serial dilutions of virus-containing supernatants. The homogenates consisted of 50 mg myocardial tissue from infected mice in 500 μL of PBS. The presence of the typical cytopathic effects of CVB3 was monitored in all replicate cells for 4 d (96 h). From these data, the 50% tissue culture infective dose ($TCID_{50}$) was calculated as previously described (Condit, 2006). The rates were expressed as $TCID_{50}$ units/0.1 mL.

2.6. Cytotoxicity assays of OMT or ribavirin in HeLa cells and mice

HeLa cells were grown in the RPMI1640 medium as described above, seeded into 96-well tissue culture plate. Various concentrations of OMT (final concentrations were 0.038, 0.0076, 0.0038, 0.0019, 0.00076, and 0.00038 mol/L) or ribavirin (final concentrations were 0.04, 0.02, 0.01, 0.005, 0.0025, 0.00125, 0.000625, and 0.0003125 mol/L) were added to the medium. The cells were incubated at 37 °C with 5% CO_2 for 72 h. Cell proliferation and viability were determined using the MTT test described above. The cytotoxic concentration of OMT or ribavirin towards cells was calculated by the following formula:

$$\text{percent of surviving cells} = [OD_T/OD_C] \times 100\%$$

where OD_T and OD_C denote the absorbencies of the tested compounds and solvent control, respectively. The concentration of 50% cellular cytotoxicity (CC_{50}) of the tested compounds was calculated according to the method of Weislow et al. (1989).

Twelve mice were divided into two groups of six mice per group: 1) normal controls; and 2) OMT, 50 mg/kg/d, once daily via intraperitoneal (i.p.) injection. After three months, all mice were