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Original article

ReDuNing, a patented Chinese medicine, reduces the susceptibility to H1N1 influenza of mice loaded with restraint stress

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Abstract

Introduction: ReDuNing (RDN), a patented traditional antipyretic-detoxicate Chinese medicine, has been widely applied as an anti-inflammatory and anti-infectious drug in Chinese clinics. However, relative experimental data on anti-influenza virus and its subsequent viral pneumonia by RDN are limited. In this study, restraint stress mice model, which could mimic the physiological conditions of the population susceptible to influenza infection, was applied to investigate the protective effect of RDN against influenza.

Methods: Mice were infected with H1N1 influenza virus 3 days after restraint, and RDN was administered to mice the day after stress by intraperitoneal injection for 7 days. Body weight and mortality were recorded every day. Histopathologic changes, lung index, nucleoprotein (NP) gene expressions and inflammatory markers in the lungs were also measured.

Results: RDN administration significantly decreased lung index, ameliorated lung histopathologic damage and decreased lung NP gene expression in influenza-infected mice loaded with restraint stress. It also decreased phosphorylated inhibitor of nuclear factor kappa B (p-IκB), nuclear factor-kappa B (NF-κB) protein level and interleukin-1β (IL-1β) mRNA expression, increased interferon inducible transmembrane 3 (IFITM3) and mitochondrial antiviral signaling (MAVS) gene expression.

Conclusions: This study provided preliminary support to suggest that RDN can reduce the susceptibility to, and the severity of influenza in restraint-stressed mice. The study also supported the potential of implementing RDN as an antiviral and its impact as potential anti-influenza treatments which rebalances homeostasis in the *body* caused by stress.

Keywords: Influenza virus; Restraint stress; Pneumonia; ReDuNing

Introduction

Influenza is an acute respiratory disease caused by influenza virus which can lead to disease outbreaks in various avian and mammalian species, including humans [1,2]. In response to such outbreaks, many schools in US, Japan and other countries are closed in order to prevent the spread of influenza virus infection [3]. The clinical manifestations of pandemic H1N1 infection can range from mild, self-limiting respiratory infection to severe illness with a significant morbidity and mortality [4]. In China, several traditional Chinese medicines (TCM) are used to prevent or treat influenza infection in clinical practice [5,6]. They synergically interact with the virus and the body in order to keep

Abbreviations: RDN, ReDuNing; TCM, traditional Chinese medicines; NP, nucleoprotein; NF-κB, nuclear factor-kappa B; IL-1β, interleukin-1β; IFITM3, interferon inducible transmembrane 3; MAVS, mitochondrial antiviral signaling.

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it in a steady state [7] with few side effects, which is totally different from commonly used anti-influenza drugs. Thereby, more attention should be paid to exploring the potential of different therapies on the influenza virus and mechanisms of action.

ReDuNing (RDN), a patented TCM, has been widely used as an antipyretic and anti-inflammatory drug. In clinic, it can be used to treat common cold, cough, acute upper respiratory infection (AURI) and acute bronchitis. It also shows potential effects in the therapy of influenza and pneumonia in clinic [8]. Previous studies have disclosed its components as chlorogenic acid, secologanic acid, caffeic acid, geniposide, isochlorogenic acid, etc. Chlorogenic and neochlorogenic acid have a variety of biological functions, such as antimicrobial activity and anti-inflammatory effects [9]. Isochlorogenic acid A can restrain hepatitis B virus infection [10]. However, there is little experimental data on the anti-influenza effect of RDN due to the lack of a suitable animal model. Positive results were difficult to achieve through simple in vitro antiviral experiments. Influenza viruses can infect a lot of people, especially the newborns, the elderly, the sick, and individuals presenting fatigue or stress, who have weakened immunity [5]. Influenza in these patients might aggravate other symptoms and complications, such as chest pain, lymphadenopathy, and even pneumonia. Viral pneumonia can develop rapidly and may result in respiratory failure and death [1]. It has been reported that over 90% of deaths occur in these susceptible populations [11]. Our previous studies found that stress increases host's susceptibility to bacterial pathogens or virus, diminish the strength of immune defense, and aggravate the severity of infectious disease [12,13]. Restraint is a commonly used stressor and we have successfully used restraint stress model to evaluate anti-influenza effects of natural products [13]. In this study, we employed restraint stress mice model to investigate anti-influenza effects of RDN and its related mechanisms.

Methods

Drugs and reagents

RDN (Batch No. 100906) was provided by Jiangsu Kanion Pharmaceutical Co., Ltd (Jiangsu, China). Its chemical constitutes were determined using HPLC analysis. The injection was analyzed by Dianon HP-20 Toyopearl HW40 and preparative HPLC chromatographic columns, getting peaks of primary components in the HPLC fingerprint. The compounds' structures were identified by mass spectrometer (Bruker, USA), NMR spectrometer (Bruker, USA) and spectrophotometer (Waters, USA). As shown in Fig. 1, the main peaks in HPLC fingerprint were demonstrated as neochlorogenic acid (peak 1), chlorogenic acid (peak 2), cryptochlorogenic acid (peak 3), secologanic acid (peak 4), caffeic acid (peak 5), geniposide (peak 6), secologanin (peak 7), isochlorogenic acid B (peak 8), isochlorogenic acid C (peak 9), and isochlorogenic acid A (peak 10). Ribavirin injection was purchased from Guangzhou Baiyunshan Tianxin Pharmaceutical Co., Ltd. (Guangdong, China).

Virus

The influenza A/FM/1/47 (H1N1) virus was donated by College of Veterinary Medicine of South China Agricultural University (Guangzhou, China). The virus strain was propagated in specific pathogen-free fertilized eggs and adapted for lethality in mice after three passages in the animal. Virus containing allantoic fluid was harvested and stored in aliquots at −80 °C. The

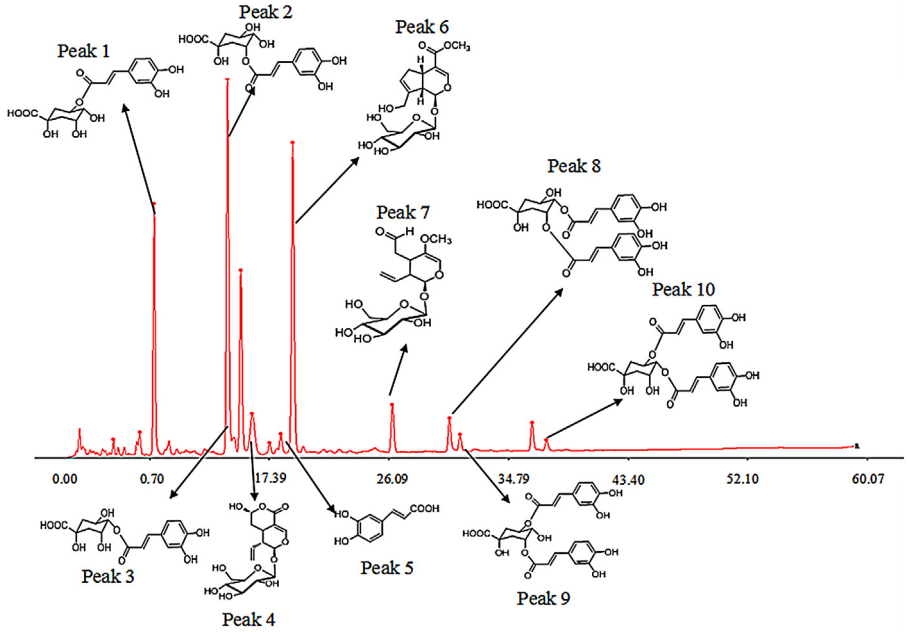

Fig. 1. Chemical profile of RDN analyzed by HPLC. Several batches of RDN were analyzed by HPLC with UV detection at 225 nm, and similar profiles were obtained. The compounds were eluted (eluent A, 0.1% phosphoric acid in water; eluent B, 0.1% phosphoric acid in methanol) at a flow rate of 0.8 mL/min using a gradient program (linear gradient from 20% B to 60% B in 50 min, and 60% for 10 min).