International Immunopharmacology 55 (2018) 237–244

Contents lists available at ScienceDirect

International Immunopharmacology

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

Review

Inhibition of myocardial hypertrophy by magnesium isoglycyrrhizinate through the TLR4/NF-κB signaling pathway in mice

Donglai Ma[a,b,d], Jianping Zhang[c], Yuanyuan Zhang[a,d], Xuan Zhang[c,d], Xue Han[a], Tao Song[a,d,e], Ying Zhang[c,*], Li Chu[a,d,e,**]

[a] School of Pharmacy, Hebei University of Chinese Medicine, Shijiazhuang 050200, Hebei, China
[b] Collaborative Innovation Center of Integrative Reproductive Disorders, Hebei University of Chinese Medicine, Shijiazhuang 050200, Hebei, China
[c] School of Basic Medicine, Hebei University of Chinese Medicine, Shijiazhuang 050200, Hebei, China
[d] Hebei key laboratory of integrative Medicine on Liver-Kidney Patterns, Shijiazhuang 050200, Hebei, China
[e] School of Basic Medicine, Hebei Medical University, Shijiazhuang 050017, Hebei, China

ARTICLE INFO

Keywords:
Magnesium isoglycyrrhizinate
Cardiac fibrosis
Isoproterenol
Cardioprotection
TLR4/NF-κB

ABSTRACT

Magnesium isoglycyrrhizinate (MgIG) is a magnesium salt of the 18-α glycyrrhizic acid stereoisomer that has exhibited hepato-protective effects and has anti-inflammatory, antioxidant, and antiviral activities. Here, we have investigated the effects and potential mechanisms of action of MgIG, with respect to myocardial fibrosis induced by isoproterenol (ISO) in mice. Mice were administered MgIG for 14 days, with concurrent ISO dosing, and were sacrificed two weeks later. Lactate dehydrogenase (LDH) and creatine kinase (CK) concentrations were measured in the blood. Pathological changes in the myocardium were observed via light microscopy. In addition, the expression of the Bax and Bcl-2 genes, and the basic fibroblast growth factor (bFGF) protein were measured via an immunohistochemical method. The RNA expression of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), c-fos, and c-jun mRNA were quantified by reverse transcription-polymerase chain reaction (RT-PCR) in the myocardial tissue. The protein expression of toll-like receptor (TLR) 4, and nuclear factor kappa B (NF-κB) (p65) were measured using Western blot assays. Compared with the control group, the ISO group showed significant increases in bFGF, Bax, Bcl-2, TLR4, and NF-κB (p65) expressions, as well as increased serum levels of LDH and CK. MgIG had a protective effect on ISO-induced myocardial fibrosis, which might be ascribed, at least in part, to the inhibition of the TLR4/NF-κB (p65) signaling pathway.

1. Introduction

Cardiac fibrosis and hypertrophy are risk factors that threaten human health. They are also independent risk factors for increased incidences of cardiovascular disease and cardiovascular disease-related mortality [1,2]. The occurrence of cardiac fibrosis and hypertrophy are closely related to chronic inflammation in vivo [3,4], as inflammatory factors directly damage the heart and blood vessels. In addition, inflammatory factors mediate and provide a final common pathway for a variety of neuroendocrine factors [5,6]. For example, inflammatory factors are involved in cardiac damage caused by the sympathetic nervous system and oxygen-free radicals.

The continuous stimulation of adrenergic receptors can promote cardiomyopathy and increase the expression levels of pro-inflammatory factors in the myocardium [7,8]. These inflammatory factors are mainly involved in the development of cardiomyopathy, and they also activate the inflammatory signaling pathway downstream of nuclear factor kappa B (NF-κB) [9]. Moreover, β-adrenergic receptor stimulation increases the expression of pro-inflammatory factors in cardiac hypertrophy and upregulates NF-κB expression [9].

The toll-like receptor (TLR) is a pathogen pattern recognition receptor that is closely related to congenital immunity [10]. The existing TLR family contains at least 12 members with different ligands. TLR4 is the main TLR that mediates the endotoxin/lipopolysaccharide response [11]. TLR4 commonly exists in the cardiovascular system and is implicated in the occurrence, development, and prognosis of cardiovascular diseases. It is a mediator of cardiac hypertrophy inflammatory signals. TLR4 activation increases NF-κB expression, and also induces the expression of a series of inflammatory factors [10]. Therefore, the TLR4/NF-κB signal transduction pathway might be an important pathway in the development of cardiac hypertrophy.

Magnesium isoglycyrrhizinate (MgIG) is a single stereoisomer

* Corresponding author.
** Correspondence to: L. Chu, School of Pharmacy, Hebei University of Chinese Medicine, Shijiazhuang 050200, Hebei, China.
E-mail addresses: yingzhang2810@163.com (Y. Zhang), chuli0614@126.com (L. Chu).

https://doi.org/10.1016/j.intimp.2017.12.019
Received 10 April 2017; Received in revised form 12 December 2017; Accepted 14 December 2017

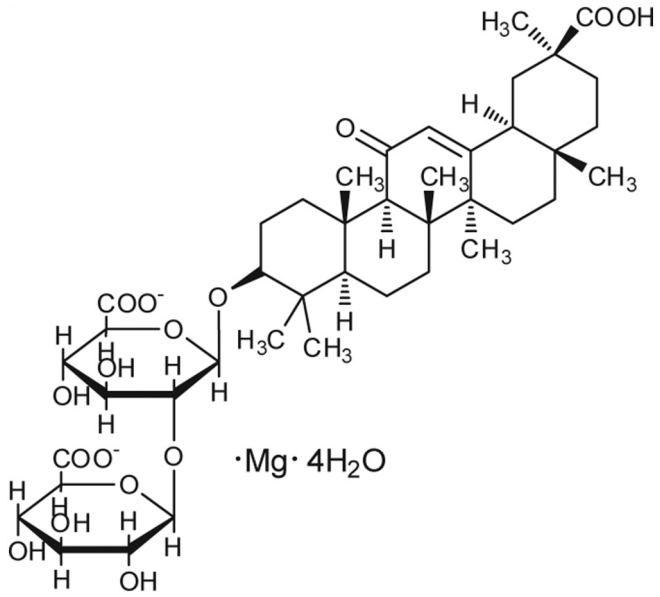

Fig. 1. Chemical structure of MgIG.

magnesium salt (Fig. 1). Studies have confirmed that glycyrrhizic acid has two 18α and 18β stereoisomers. It controls the adrenocortical hormones and possesses anti-inflammatory and immunoregulatory effects [12]. MgIG is an 18α glycyrrhizic acid with greater lipotropy than the β-isomer, and it more easily combines with the target cell receptors of receptor proteins and steroid hormones to oppose inflammation [13], protect the liver cell membranes [14], and improve liver function [12].

MgIG is refined from glycyrrhizic acid that is extracted from the roots of the licorice plant. Studies have shown the potential protective effects of licorice against ischemia–reperfusion injuries in the heart. The results of these studies clearly demonstrated that licorice mediates protective effects against myocardial infarction by ameliorating oxidative stress [15]. The active components of *Radix Glycyrrhizae Preparata* (Zhi Gancao, a licorice decoction) can markedly lower the incidence of cardiac-triggered activity, protect the myocardium from injury, and decrease the incidence of arrhythmias induced by ischemia-reperfusion, as seen in rat models [16]. A serum containing *Radix Glycyrrhizae Preparata* decreases the L-type Ca^{2+} currents (ICa-L) in a concentration-dependent manner in isolated rabbit ventricular myocytes, which may be the mechanism of action underlying the treatment of cardiac diseases [17]. In addition, previous studies have demonstrated that MgIG exerts beneficial effects on various types of organ damage and diseases [18]. However, the cardiovascular protective effects of MgIG, especially the underlying mechanisms, have not received as much attention with regard to research efforts.

Here, we have speculated that MgIG could inhibit isoproterenol (ISO)-induced cardiac fibrosis. We investigated the anti-fibrotic effects of MgIG in a series of experiments. First, we created an ISO-induced mouse model of cardiac fibrosis by exposing the subjects to long-term ISO injections. We then confirmed the results with immunohistochemical analysis, reverse transcription-polymerase chain reaction (RT-PCR), and Western blotting, and several other methods. Next, we used a series of cardiac fibrosis detection methods to detect MgIG and ISO-induced cardiac fibrosis, and determined their role in the relationship. We investigated the potential mechanisms and pathways of the anti-fibrotic effects of MgIG on ISO-induced cardiac fibrosis by analyzing the factors related to fibrosis, such as pro-inflammatory cytokines and apoptotic factors.

2. Materials and methods

2.1. Animals

Fifty Kunming mice (weight: 22.0 ± 2.0 g, age: 4–5 weeks) were purchased from the Center of Laboratory Animal of the Hebei Medical University (Shijiazhuang, China). The mice were maintained at 25 ± 2 °C and 50 ± 10% relative humidity on a 12 h light/dark cycle. The mice were given a normal pellet diet and drinking water. All animal procedures were performed in accordance with the Guidelines of Animal Experiments of Hebei University of Chinese Medical.

MgIG injections were approved by the State Food and Drug Administration (approval No. Z32020161) and the MgIG was obtained from the Chia Tai Tianqing Pharmaceutical Group Co., Ltd. (Jiangsu, China; batch no. 12050617). Isoproterenol (hydrochloride) was purchased from Amylet Scientific Inc. (Michigan, USA). Propranolol hydrochloride (99% pure) was obtained from Afar Sally Chemical Co., Ltd. (Tianjin, China).

2.2. Treatments

The Kunming mice were randomly divided into five groups ($n = 10$) including a control group (CONT, normal saline, subcutaneous and intraperitoneal 10 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$), an ISO group (subcutaneous injection of ISO, 10 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$), a propranolol group (Pro, intraperitoneal injection 40 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$), a low-dose MgIG group (L-MgIG, intraperitoneal injection 25 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$), and a high-dose MgIG group (H-MgIG, intraperitoneal injection 50 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$).

The cardiac fibrosis model was established according to slightly modified literature guidelines. All mice were subcutaneously injected with a single dose of ISO (5 $mg{\cdot}kg^{-1}$) twice a day (at 8-hour intervals) for 14 days, except for the mice in the CONT group. The CONT mice were subcutaneously injected with normal saline. The L-MgIG and H-MgIG groups received intraperitoneal administration of MgIG at 25 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ and 50 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$, respectively. The CONT group and ISO group received isovolumic normal saline intraperitoneal injection (i.p.). The Pro group received i.p. propranolol hydrochloride (40 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$). The entire experimental period lasted for 2 weeks. Blood was collected retro-orbitally and allowed to clot for 1 h at room temperature. The serum was separated by centrifugation and preserved at − 20 °C.

At the end of the two-week experimental period, the mice chests were opened to remove the hearts, which were washed with cold 0.9% sodium chloride. The auricles and peripheral connective tissues were removed, and any excess moisture was blotted with filter paper. The heart samples were subsequently placed in a 4% paraformaldehyde solution or snap frozen in liquid nitrogen.

2.3. H&E, Masson's trichrome and WGA staining

The mice heart tissue samples were fixed in 4% paraformaldehyde and embedded in paraffin. Sections (4 μm) were cut, and the samples were stained with hematoxylin-eosin (H&E), Masson's trichrome, and wheat germ agglutinin (WGA) for histological evaluation. The diagnosis of hypertrophic cardiomyopathy was made according to the current guidelines.

2.4. Immunohistochemistry

Sections were processed for immunohistochemistry after de-paraffinization. All immunohistochemistry tests were performed in duplicate, and the results used the SP-9002 Histostain™ - Plus kit (Sigma, St. Louis, IL) according to the manufacturer's instructions. To expose the target proteins, we performed an antigen retrieval using sodium citrate (pH 6.0), and 10 min of microwave treatment. Following the antigen retrieval, the tissues were blocked in 3% H_2O_2-deionized water for