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Original Article

Matrine ameliorates isoproterenol-induced acute myocardial ischemia through regulation of growth factors and RhoA/ROCK1 pathway

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Abstract: Growth factors and inflammatory Ras homolog gene family member A (RhoA)/coiled-coil containing protein kinase 1 (ROCK1) pathway have been shown to regulate isoproterenol (ISO)-induced myocardial ischemia. However, the association between matrine and myocardial ischemia remains to be elucidated. The objective of this study was to explore whether matrine ameliorates ISO-induced myocardial ischemia through modulating growth factors and RhoA/ROCK1 cell signaling. Male Sprague-Dawley rats were pretreated with matrine (50, 100 and 200 mg/kg/d) intragastrically for two days. Acute myocardial ischemia was induced in rats by subcutaneous injection of ISO (85 mg/kg/d) for two days. Histopathological changes, cardiac troponin (cTn-I) levels, mRNA and/or protein levels of growth factors, collagen 1a1 and 3a1 and RhoA/ROCK1 were measured. Short-term oral administration of matrine provided protection against ISO-induced acute myocardial ischemia. Matrine reduced ISO-induced cTn-I (from 50.43±1.79 to 35.99±3.23, $P < 0.01$) and collagen 1a1 (from 0.30±0.09 to 0.13±0.10, $P < 0.05$), and reversed ISO-induced suppression of transforming growth factor-beta1 (TGF-β1) (from 24.07±6.17 to 51.59±6.50, $P < 0.05$), insulin-like growth factor-1 (IGF-1) (from 97.73±24.19 to 146.83±37.20, $P < 0.01$). Meanwhile, it prevented ISO-induced increases in RhoA (from 0.311±0.042 to 0.124±0.031, $P < 0.01$)/ROCK1 (from 0.305±0.019 to 0.171±0.010, $P < 0.05$). Taken together, the cardioprotective effect of matrine on ISO-induced myocardial ischemia is associated with its ability of increasing levels of the IGF-1, TGF-β1, suppressing cTn-I release and inflammatory mediators RhoA/ROCK1. Therefore, matrine may be a potential medication for the treatment of myocardial ischemia.

Keywords: Matrine, acute myocardial ischemia, RhoA/ROCK1, IGF-1, TGF-β1, ISO

Introduction

Acute myocardial ischemia is an adaptive enlargement of the myocardium in response to altered stress or injury [1]. It is a major indicator for risk development of heart failure and sudden cardiac death. It causes rapid development of myocardial necrosis between coronary artery and imbalance of myocardial blood supply. Prevention of myocardial ischemia hinders the developments of myocardial necrosis and cardiac failure [2]. Matrine ($C_{15}H_{24}N_2O$), extracted and separated from the traditional Chinese herb Sophoraalopecuroides L, possesses diverse pharmacological activities. In particular, it is a potent cardioprotective agent against coronary artery ligation-induced arrhythmias, pressure overload-induced cardiac hypertrophy and fibrosis, and angiotensin II-induced hyperplasia of cardiac fibroblasts [3-5]. Our previous study has demonstrated that matrine exerts cardioprotection against isoproterenol (ISO)-induced myocardial injury through suppression of oxidative stress [6, 7]. However, the molecular mechanisms underlying its cardioprotective effect has not been clarified to date. The present study sought to explore roles of transforming growth factor-beta1 (TGF-β1) and insulin-like growth factor-1 (IGF-1) in matrine-induced cardioprotection against myocardial ischemia.

TGF-β1 is a key mediator of cardiac fibroblast activation and differentiation into all kinds of myofibroblasts. Moreover, it is a regulatory factor that controls cell growth and differentiation through the change of abundance and composition of the extracellular matrix (ECM) [8]. Vivar R and co-workers reported that addition of TGF-β1 to rabbit cardiac fibroblasts in culture resulted in stimulation of mRNAs and production of type I and III collagens [9]. These findings suggest that TGF-β1 may play an important role in the regulation of collagen synthesis in the myocardium. However, it is currently unclear whether increase in collagen mRNA expression occurs in the myocardium of ischemia. Thus, one of the aims is to determine whether collagen mRNA expression increases in the myocardial ischemia rat model, which has been shown to be responsive to the presence of TGF-β1 and which is assumed to play a critical role in cardiac fibroblast hyperplasia and myocardial ischemia. The molecular mechanism by which increases in cardiac workload lead to an increase in ECM protein production is also unknown to date. Given the observation that TGF-β1 is a major regulatory factor for ECM production, it is possible that TGF-β1 might play an important role in the interstitial space remodeling that is observed in myocardial ischemia. Moreover, Insulin-like growth factor I (IGF-I) is a polypeptide that belongs to the family of insulin. It can promote the cardiomyocyte growth, enhance the cardiac function and cause myocardial hypertrophy, cardiac failure, cardiomyocyte apoptosis and other pathological processes. IGF-1 is thought to be involved in cardiac tissue maintenance and function.

Previous reports show that Ras homolog gene family member (Rho) signaling is involved in TGF-β1-mediated upregulation of tenascin-C, although different downstream signaling pathways are regulated by TGF-β1 [10]. Rho/coiled-coil containing protein kinase1 (ROCK1) and/or other Rho downstream signaling pathways engage in differentially regulating phenotypic changes during the trans-differentiation of keratocytes into fibroblasts and myofibroblasts [10].

The mechanism by which matrine prevents myocardial ischemia is not been completely clear, and so far there have not been any reports as to whether matrine prevents cardiomyocyte hypertrophy through regulating cytokines via RhoA/ROCK1 pathway. Therefore, investigation into the mechanisms of matrine prevention of the myocardial ischemia is valuable. ISO, a non-selective β-adrenoceptor agonist, is known to induce at high doses acute irreversible myocardial injury in rats that is pathophysiologically and morphologically analogous to myocardial infarction in humans [11-13]. Our previous experiments had showed that protective effects of matrine against ISO-induced rat chronic cardiac hypertrophy and failure was related to RhoA/ROCK1 signaling pathway which are mediated by their upstream cytokines such as TGF-β1 and IGF-1 [14, 15]. The present study was designed to determine whether the changes of TGF-β1, IGF-1 and cardiac troponine (cTn-I) levels and their mRNA expressions may affect ISO-induced acute rat myocardial ischemia, and to evaluate whether Rho/ROCK1 signaling pathway may play an important role in ISO-induced acute rat myocardial ischemia.

Materials and methods

Materials

Matrine (white powder, purity > 99.8%) was purchased from Ningxia Bauhinia Pharmaceutical Co., Ltd (Yinchuan, Ningxia, China). Isoproterenol hydrochloride (ISO) was bought from Tokyo Industrial Co., Ltd. Rat IGF-1, rat TGF-β1 and rat cardiac troponin I (cTn-I) enzyme-linked immunosorbent assay kit were obtained from Cusabio biotech Co., Ltd. Total protein extraction kit and bicinchoninic acid protein assay kit were purchased from KenGen Biotechnology Co., Ltd (Nanjing, Jiangsu, China). Primary antibodies to Ras homolog gene family member A (RhoA) and coiled-coil containing protein kinase 1 (ROCK1) were bought from British Abcam. b-actin antibody and horseradish peroxidase-conjugated goat anti-rabbit IgG were purchased from Beijing Chinese Fir Golden Bridge Biotechnology Companies (Beijing, China). Reagents used were of commercial analytical grade.

Experimental animals

Adult male Sprague-Dawley rats weighing 280-320 g (300±20 g) were obtained from the Laboratory Animal Center at Ningxia Medical University (Yinchuan, China). The rats were housed in a temperature-controlled room with 12 h light-dark cycle. The rats were housed for

7 days to allow adapting to the new environment. All animal experiments were approved by the Ethics Committee of Science and Research at the General Hospital of Ningxia Medical University (Yinchuan, China), and the experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Induction of myocardial ischemia

ISO (85 mg/kg), dissolved in normal saline (NS), was subcutaneously injected to rats once daily for 2 consecutive days in order to induce experimental myocardial ischemia [16].

Experimental design

Our previous study exhibited that oral administration of matrine to rats at three different doses (50, 100 and 200 mg/kg/d, respectively) for 7 consecutive days markedly reduced the ISO-induced myocardial hypertrophy and failure [17, 18]. This cadioprotective effect was also observed in rats after oral administration of matrine at doses of 100 or 200 mg/kg/d for only 2 days [6]. Therefore, 50, 100 and 200 mg/kg/d of matrine were used for 2 days as the optimum dosages for the study. Seventy-two rats were randomly assigned to control group, ISO treatment group, matrine-alone group, low-dose, medium-dose, and high-dose matrine pretreatment groups (50, 100 and 200 mg/kg/d, respectively). There were 12 rats in each group. In control group, NS 10 ml/kg/d was subcutaneously injected to rats 1 h after intragastrical administration of NS 10 ml/kg/d; in ISO treatment group, ISO 85 mg/kg/d was subcutaneouslly injected to rats 1 h after intragastrical administration of NS 10 ml/kg/d; in matrine-alone group, NS 10 ml/kg/d was subcutaneouslly injected to rats 1 h after intragastrical administration of matrine 200 mg/kg/d; in matrine pretreatment groups, ISO 85 mg/kg/d was subcutaneouslly injected to rats 1 h after intragastrical administration of matrine 50, 100 and 200 mg/kg/d, respectively; the aforementioned medications were administered once daily to 12 rats of each group for 2 successive days.

Histopathological study

The cardiac apexes obtained from all experimental rats were cut off and fixed in 4% buffered paraformaldehyde solution. The cardiac tissues were embedded in paraffin, sectioned at 4 μm thickness and stained with hematoxylin and eosin (HE). The histological slices were observed under an optical microscope (Shenzhen, China) at a minimum of 4 microscopic fields and then images were captured. Interstitial edema, myocardial fibrosis, inflammatory cell infiltration, and cardiomyocyte hypertrophy for each image were examined and classified in light of severity of changes using scores on a scale of no abnormal findings (-), mild (+), moderate (++), and severe (+++) [7]. Histological outcomes were scored on coded slides by a pathologist in a blinded manner.

Real-time reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNA was prepared from the rat left ventricle by using Trizol Reagent (Roche Molecular Biochemicals, Mannheim, Germany). The total RNA was subjected to DNase (Roche Molecular Biochemicals, Mannheim, Germany) digestion. RT reactions were carried out using random primers with 200 units of Transcriptor First Strand cDNA Synthesis (Roche Molecular Biochemicals, Mannheim, Germany). RT-qPCR was performed with lightCycler 480 SYBR Green I Master Gene Expression Assays (Roche Molecular Biochemicals, Mannheim, Germany) using a lightCycler 480 SYBR Green I Master (Roche Molecular Biochemicals, Mannheim, Germany). The primers used were as follows: Col1a1, forward 5'-TCAAGATGGTGGCCGTTACT-3' and reverse 5'-CATCTTGAGGTCACGGCATG-3', Col3a1, forward 5'-TGGGATGCAACTACCTTGGT-3' and reverse 5'-AGGTGTAGAAGGCTGTGGAC-3', TGF-β1, forward 5'-CCTGCAAGACCATCGACATG-3' and reverse 5'-TGTTGTACAAAGCGAGCACC-3', IGF1, forward 5'-TCTCCTAGTCCCTGCCTCTT-3' and reverse 5'-TCTGTGAAGGAAGCGGCTTA-3' and GAPDH, forward 5'-GAGACAGCCGCATCTTCTTG-3' and reverse 5'-TGACTGTGCCGTTGAACTTG-3'.

The primers were synthesized by Sangon BiotechCo., Ltd (Shanghai, China). One microliter of each RT product was used for the real-time PCR. All experiments were performed in triplicate. The ΔCt values of all other samples were normalized with those obtained by the amplification of GAPDH mRNA. The $2^{\Delta\Delta Ct}$ method was used to analyze the mRNA expression levels.

Table 1. Effects of matrine on histopathology in cardiac apexes in ISO-induced myocardial ischemia rats

Group	N	Cardiomyocyte hypertrophy				Infiltration of inflammatory cells				Interstitial edema				Myocardial fibrosis			
		-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
Control	12	12	0	0	0	10	2	0	0	12	0	0	0	12	0	0	0
ISO (85 mg/kg)	10	3	1	3	3	0	2	4	4	1	6	0	3	1	0	3	6
Matrine (200 mg/kg) alone	12	12	0	0	0	8	3	1	0	12	0	0	0	12	0	0	0
Matrine (50 mg/kg) + ISO	8	0	3	5	0	0	5	2	1	0	3	1	4	0	4	4	0
Matrine (100 mg/kg) + ISO	12	0	5	5	2	0	7	2	3	10	2	0	0	0	5	5	2
Matrine (200 mg/kg) + ISO	8	0	2	4	2	0	6	1	1	1	4	2	1	0	2	4	2

The figures represent the number of rats affected. Images estimated the damage in the cardiac apexes. The histopathological changes were arbitrarily scored as follows: -, no abnormalities; +, mild; ++, moderate; +++, severe.

Enzyme-linked immunosorbent assay (ELISA)

Levels of serum IGF-1, TGF-β1 and cTn-I were measured using ELISA kits coupled with Bio-RAD 680 automatic microplate reader (Thermo Labsystems, Shanghai, China). The experimental procedures were conducted in accordance with the manufacturer's instructions.

Western blot analysis

Equal amounts (50 μg) of protein samples were loaded to SDS-PAGE gel and separated by electrophoresis. The proteins were then electrophoretically transferred to nitrocellulose membranes using an electrophoretic transfer system (Bio-Rad, CA, USA). Membranes were subdivided, and each protein of interest and β-actin were analyzed from a single transfer. The membranes were blocked with 5% (w/v) nonfat dry milk in phosphate buffer saline containing 0.1% (v/v) Tween 20 for 2 h at room temperature, then incubated with the primary antibody of RhoA (1:1400), ROCK1 (1:300) or β-actin (1:1000) overnight at 4°C, followed by Biotin-SP-conjugated AffiniPure Goat Anti-Rabbit IgG secondary antibodies, respectively. Bands on blots were observed using Super Signal West Pico Chemiluminescence Kit (Thermo Scientific, USA), and eventually exposed to radiographic films. These films were scanned, and the ratio of each protein to its corresponding β-actin band density was detected using the Quantity-One software (Bio-Rad Laboratories, CA, USA).

Statistical analysis

All the following statistical analyses were conducted with the SPSS 11.5 (Chicago, IL, USA). Quantitative values are shown as mean ± SD. Differences amongst groups were analyzed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $P < 0.05$.

Results

Mortality

All animals survived to the predetermined endpoint in the control group, matrine-alone group, and matrine medium-dose group. However, 2, 4 and 4 animals died in the ISO treatment group, 50 mg/kg and 200 mg/kg matrine groups, respectively. The mortality of rats was associated principally with ISO toxicity.

Matrine ameliorates ISO-induced myocardial ischemia

Table 1 shows the effect of matrine on the degree of histopathological changes in cardiac apexes of ISO-induced myocardial ischemia rats. Histopathological findings indicated that ISO could induce severe myocardial fibrosis, cardiomyocyte hypertrophy, infiltration of inflammatory cells and interstitial edema (**Figure 1B**); conversely, there were no changes in the myocardium in the control group rats (**Figure 1A**) and the matrine-alone group (**Figure 1C**). Matrine pretreatment considerably improved ISO-induced myocardial fibrosis, cardiomyocyte hypertrophy, infiltration of inflammatory cells and interstitial edema in comparison with the ISO treatment alone (**Figure 1D-F**, respectively).

Matrine prevents ISO-induced increases of cTn-I

The levels of cTn-I was measured using ELISA kits (**Table 2**). The results showed that cTn-I

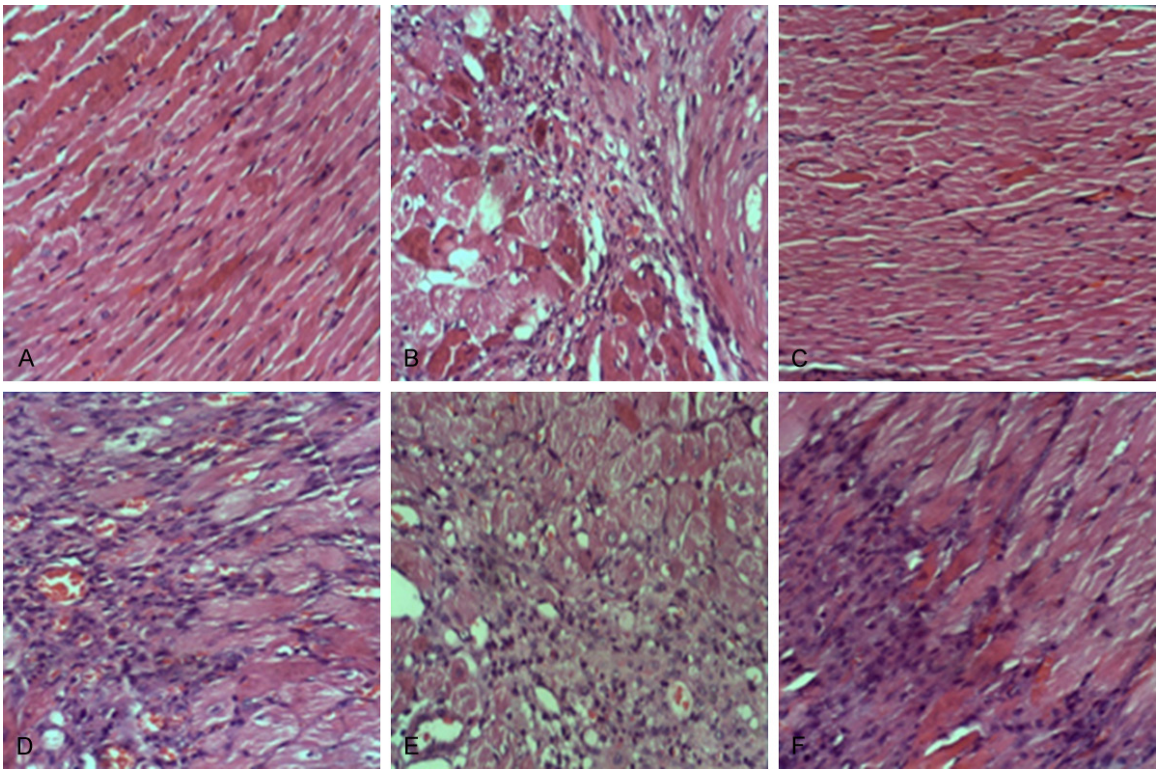

Figure 1. Representative images of rat cardiac apexes by HE staining (×400). A. Control rats. B. 85 mg/kg ISO-given rats. C. 200 mg/kg matrine alone-given rats. D. ISO- and 50 mg/kg matrine-given rats. E. ISO- and 100 mg/kg matrine-given rats. F. ISO- and 200 mg/kg matrine-given rats.

Table 2. Effects of matrine on cTn-I, IGF-1, TGF-β1 levels in ISO-induced acute myocardial ischemia rats

Group	cTn-I (pg/ml)	IGF-1 (ng/ml)	TGF-β1 (ng/ml)
Control (n=12)	33.06±2.86	142.20±21.01	51.18±18.47
ISO (85 mg/kg) (n=10)	50.43±1.79**	97.73±24.19**	24.07±6.17*
Matrine (200 mg/kg) alone (n=12)	37.87±2.63##	145.30±20.51#	49.43±27.10#
Matrine (50 mg/kg) + ISO (n=8)	37.27±5.05##	177.89±32.45##	47.31±16.67#
Matrine (100 mg/kg) + ISO (n=12)	36.96±4.05##	154.57±21.40#	50.70±23.28#
Matrine (200 mg/kg) + ISO (n=8)	35.99±3.23##	146.83±37.20##	51.59±6.50#

Note: *P < 0.05, **P < 0.01, compared with control group; #P < 0.05, ##P < 0.01, compared with ISO group.

was significantly increased after ISO treatment for 2 days, which are consistent with the finding that ISO induces myocardial ischemia. Matrine pretreatment, at all 3 doses, successfully prevented the ISO induced alterations of cTn-I.

Effects of matrine on serum IGF-1 and TGF-β1

To confirm whether ISO- and matrine-induced gene modulation correlates to the protein translation, we measured the protein levels of IGF-1 and TGF-β1 using ELISA. Corresponding to the suppression of transcription by ISO, the serum protein levels of IGF-1 and TGF-β1 were also significantly decreased in ISO alone-treated animals (**Table 2**). Consistent with the gene expression pattern, matrine significantly increased the protein levels of IGF-1 and TGF-β1 in ISO-treated animals.

ISO suppresses and matrine restores mRNA expressions of growth factors and collagens

The mRNA expressions of 2 growth factors, IGF-1 and TGF-β1, and 2 collagens, col1a1 and col3a1, were determined in left ventricular tissues using real time RT-PCR. ISO markedly suppressed the gene expressions of the 2 growth

Table 3. Effects of matrine on the mRNA expressions of IGF-1, TGF-β1, col1a1, col3a1 in the ISO-induced acute myocardial ischemia rats

Group	IGF-1	TGF-β1	col1a1	col3a1
Control (n=12)	5.42±1.30	5.75±0.66	0.11±0.07	11.87±3.78
ISO (85 mg/kg) (n=10)	2.05±1.82*	2.83±1.08*	0.30±0.09*	0.36±0.34
Matrine (200 mg·kg^{-1}) alone (n=12)	4.49±1.43	4.16±1.98	0.17±0.10	3.27±5.29
Matrine (50 mg·kg^{-1}) + ISO (n=8)	5.76±2.77$^{\#}$	7.06±2.51$^{\#\#}$	0.15±0.10$^{\#}$	2.32±4.79
Matrine (100 mg·kg^{-1}) + ISO (n=12)	7.99±3.17*,$^{\#\#}$	8.58±2.97$^{\#\#}$	0.10±0.06$^{\#\#}$	13.57±18.03
Matrine (200 mg·kg^{-1}) + ISO (n=8)	6.66±1.81$^{\#\#}$	8.21±2.49$^{\#\#}$	0.13±0.10$^{\#}$	1.64±2.11

*P < 0.05, compared with control group; $^{\#}$P < 0.05, $^{\#\#}$P < 0.01 compared with ISO group.

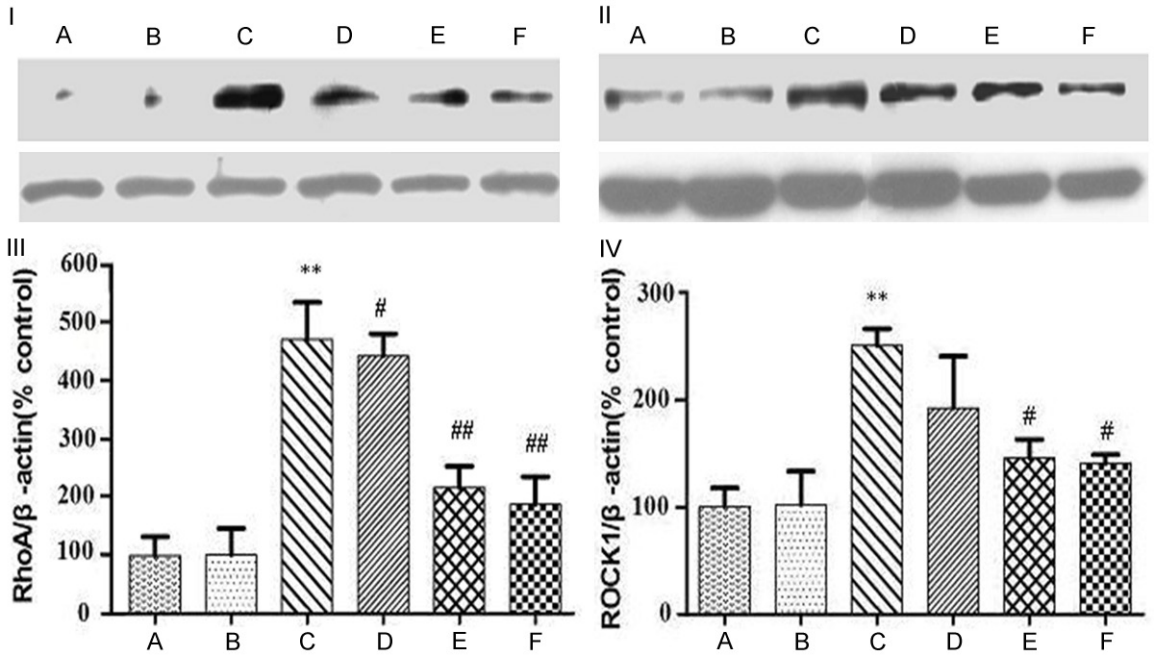

Figure 2. Effects of matrine on RhoA and ROCK1 expressions in hearts from ISO-induced myocardial ischemia rats. I and II show Western blot densitometry analysis of RhoA, ROCK1 and β-actin. A. Control group; B. Matrine-alone group; C. ISO treatment group; D. Low-dose matrine group; E. Medium-dose matrine group; F. High-dose matrine group. III and IV indicate the ratios of RhoA and ROCK1, respectively, to β-actin densitometric values, and then divided by those of control group. Values are presented as mean ± SD, n=8-12. *P < 0.05, **P < 0.01 compared with control group; $^{\#}$P < 0.05, $^{\#\#}$P < 0.01 compared with ISO group.

factors. In contrary, pretreatment with medium- and/or high-dose matrine prevented the suppression effects of ISO on these genes (**Table 3**). ISO enhanced the mRNA expression of col1a1 and matrine suppressed the overexpression of col1a1. ISO suppressed col3a1 while only matrine medium dose, not low or high doses, prevented the ISO-induced suppression. Furthermore, its best effects were achieved at a medium dose.

Influence of matrine on RhoA and ROCK1

RhoA and ROCK1 signaling pathway regulates inflammatory responses, which mediate the myocardial remodeling. The levels of RhoA and ROCK1 proteins were measured using Western blotting. Both proteins were significantly increased after ISO challenge. Administration of matrine at medium and high dosages significantly reduced the levels of RhoA and ROCK1 in ISO treated animals (**Figure 2**).

Discussion

The major findings of this study are as follows. First, short-term oral administration of matrine inhibited ISO-induced rat myocardial ischemia in the rat; second, matrine improved ISO-induced abnormalities of the serum cTn-I lev-

els; third, matrine prevented the ISO-induced suppression of transcription and translation of IGF-1 and TGF-β1; finally, matrine suppressed inflammatory RhoA-ROCK1 signaling pathway.

Subcutaneous injection of overdoses of ISO elicits myocardial ischemia and inhibition of left ventricular function, which approximates the pathological changes observed in human myocardial infarction [19]. The present research results demonstrated that ISO-induced severe injury to the myocardium in rats, accompanied with severe myocardial fibrosis, cardiomyocyte hypertrophy, infiltration of inflammatory cells and interstitial edema, which are in accordance with previous reports [17, 18, 20]. Meaningfully, matrine (50, 100 and 200 mg/kg/d) pretreatment attenuated ISO-induced histopathological damage to the heart, suggesting that matrine extends a prominent protective effect on myocardial ischemia.

Collagen I and III, predominantly synthesized by fibroblasts, were the most abundant forms of collagen in the interstitium of cardiac tissues [21]. It was reported that collagen I and III were increased in ISO-induced cardiac fibroblast proliferation and collagen synthesis [22]. Our histopathological studies proved that matrine could prevent pathological changes in cardiac tissues. Moreover, matrine (50, 100 and 200 mg/kg/d) prevented myocardial ischemia by reducing the expression of collagen I mRNA, whereas it did not affected that of collagen III among the pretreatment groups. These findings suggested that the preventive effect of matrine on change in heart injury and gene expression may not be caused by mechanical factors alone.

It is well known that cTn-I is a sensitive parameter for monitoring the development of myocardial injury [23]. In our present study, serum cTn-I levels in the ISO group rose remarkably compared to the control group. Pretreatment of matrine (50, 100 and 200 mg/kg/d) successfully prevented the serum cTn-I increase induced by the ISO, suggesting that matrine plays a protective role in ISO-induced myocardial ischemia in the rat.

The mechanism of ISO-induced myocardial ischemia has not been completely clarified. IGF-1 is an endocrine and autocrine or paracrine growth factor and a primary mediator of growth hormone [24]. In several reports, the insulin/IGF-mediated signaling appears to be able to activate RhoA and thus negatively regulates Rho/ROCK1 signaling in cancer and in metabolic settings [25]. It is not clear whether the upstream insulin/IGF-mediated survival signaling is related to the RhoA/ROCK1 in the development of rat myocardial ischemia. IGF-1 regulates cell growth, differentiation and survival of myocardial cells, and is among the most potent anti-apoptotic growth factors presented in eukaryotic cells [26]. The present study results showed that serum IGF-1 levels in the ISO-injected rats were markedly decreased compared with the control group. Matrine (50, 100 and 200 mg/kg/d) significantly increased the serum IGF-1 levels in ISO-induced rats, which is contrary to the results of our previous chronic pathological myocardial hypertrophy study [15]. To further confirm the involvement of IGF-1 in myocardial protection, its mRNA expression was detected by real time RT-RCR. The results showed that matrine (50, 100 and 200 mg/kg/d) pretreatment significantly upregulated the IGF-1 mRNA expression of the left ventricular tissue in ISO-induced rats, suggesting that the preventive effects of matrine on ISO-induced myocardial ischemia are mediated through the RhoA-ROCK1-IGF-1 pathway.

The conversion of cardiac fibroblasts to myofibroblasts is controlled by a variety of growth factors, cytokines, and mechanical stimuli [27]. TGF-β1 is a key mediator of cardiac fibroblast activation and differentiation into hypersecretory myofibroblasts [28]. In the presence of persisting injurious pathways, TGF-β1 can initiate the transformation of fibroblasts and fibrocytes into myofibroblasts, which are resistant to apoptosis [29]. Previous studies have found that the fibrosis organ had abnormal expressions of Rho and ROCK1, and inhibitors of ROCK1 could improve organ fibrosis [30]. It was suggested that TGF-β1 is one of the major cytokines in chondrocytes and also an upstream activator of ROCK1 [31]. Our current results showed that matrine (100 and 200 mg/kg/d) significantly increased serum TGF-β1 levels and its mRNA expressions, and decreased cardiac RhoA and ROCK1, indicating that the increase of TGF-β1 levels led to the decrease of RhoA and ROCK1 levels that is consistent with our previous chronic pathological myocardial hypertrophy investigation [14].

Conclusions

Our previous chronic pathological myocardial hypertrophy and failure investigation showed that seven-day intragastric administration of matrine (50, 100 and 200 mg/kg/d) provided a significant cardioprotection against ISO (5 mg/kg/d)-induced myocardial hypertrophy in the rat. This protective effect may be mediated through p-Akt/Akt, p-eNOS/eNOS, RhoA/ROCK1 and p-mTOR/mTOR signaling pathways in chronic pathological myocardial hypertrophy rats [14, 15, 32]. The present study provided experimental evidence that two-day oral administration of matrine to rats attenuated ISO-induced acute myocardial ischemia. Its molecular mechanism involved the regulation of RhoA/ROCK1 signaling pathway. ISO-induced rat myocardial ischemia is related to the increases of RhoA/ROCK1 levels. The protective effect of matrine on ISO-induced rat myocardial ischemia is associated with upregulation of transcription and translation of IGF-1 and TGF-β1, and downregulation of RhoA /ROCK1 levels. Therefore, matrine might be a potential medication for the treatment of acute myocardial ischemia.

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Disclosure of conflict of interest

None.

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References

[1] Yang M, Chen J, Zhao J and Meng M. Etanercept attenuates myocardial ischemia/reperfusion injury by decreasing inflammation and oxidative stress. PLoS One 2014; 9: 1-9.

[2] Tin LL, Beevers DG and Lip GY. Hypertension, left ventricular hypertrophy, and sudden death. Curr Cardiol Rep 2002; 4: 449-457.

[3] Xu CQ, Dong DL, Du ZM, Chen QW, Gong DM and Yang BF. Comparison of the anti-arrhythmic effects of matrine and berbamine with amiodarone and RP58866. Acta Pharm Sin 2004; 39: 691-695.

[4] Zhang YJ, Xiang MX, San J, Cheng G and Wang SS. Effect of matrine and carvedilol on collagen and MMPs activity of hypertrophy myocardium induced by pressure overload. J Zhejiang Univ Sci B 2006; 7: 245-250.

[5] Li Y, Wang B, Zhou C and Bi Y. Matrine induces apoptosis in angiotensin II-stimulated hyperplasia of cardiac fibroblasts: effects on Bcl-2/Bax expression and caspase-3 activation. Basic Clin Pharmacol Toxicol 2007; 101: 1-8.

[6] Krenek P, Kmecova J, Kucerova D, Bajuszova Z, Musil P, Gazova A, Ochodnicky P, Klimas J and Kyselovic J. Isoproterenol-induced heart failure in the rat is associated with nitric oxide-dependent functional alterations of cardiac function. Eur J Heart Fail 2009; 11: 140-146.

[7] Li XB, Wang X, Guo YF, Deng N, Zheng P, Xu QB, Wu Y and Dai GD. Regulation of endothelial nitric oxide synthase and asymmetric dimethylarginine by matrine attenuates isoproterenol-induced acute myocardial injury in rats. J Pharm Pharmacol 2012; 64: 1107-1125.

[8] Morais C, Small DM, Vesey DA, Martin J, Johnson DW and Gobe GC. Fibronectin and transforming growth factor beta contribute to erythropoietin resistance and maladaptive cardiac hypertrophy. Biochem Biophys Res Commun 2014; 444: 332-337.

[9] Vivar R, Humeres C, Ayala P, Olmedo I, Catalan M, Garcia L, Lavandero S and Diaz-Araya G. TGF-beta1 prevents simulated ischemia/reperfusion-induced cardiac fibroblast apoptosis by activation of both canonical and non-canonical signaling pathways. Biochim Biophys Acta 2013; 1832: 754-762.

[10] Chen J, Guerriero E, Sado Y and SundarRaj N. Rho-mediated regulation of TGF-beta1- and FGF-2-induced activation of corneal stromal keratocytes. Invest Ophthalmol Vis Sci 2009; 50: 3662-3670.

[11] Anandan R, Mathew S, Sankar TV and Viswanathan Nair PG. Protective effect of n-3 polyunsaturated fatty acids concentrate on isoproterenol-induced myocardial infarction in rats. Prostaglandins Leukot Essent Fatty Acids 2007; 76: 153-158.

[12] Li X, Zhou R, Zheng P, Yan L, Wu Y, Xiao X and Dai G. Cardioprotective effect of matrine on isoproterenol-induced cardiotoxicity in rats. J Pharm Pharmacol 2010; 62: 514-520.

[13] Bhuiyan MS, Shioda N, Shibuya M, Iwabuchi Y and Fukunaga K. Activation of endothelial nitric oxide synthase by a vanadium compound ameliorates pressure overload-induced cardiac injury in ovariectomized rats. Hypertension 2009; 53: 57-63.

[14] Yang WC, Fu Y, Wei SJ, Zhang WP and Dang HW. Inhibitory effects of matrine against isoproterenol-induced chronic heart failure via regulation of p-NOS/eNOS and RhoA/ROCK1 in rats. Chinese Journal of Hospital Pharmacy 2014; 34: 595-599.

[15] Fu Y, Yang WC, Wei SJ, Zhang WP and Dang HW. Inhibitory effect of matrine in rats on isoproterenol-induced cardiac hypertrophy via regulation of p-Akt/Akt protein. Chinese Journal of Hospital Pharmacy 2014; 34: 505-509.

[16] Rajadurai M and Prince PS. Preventive effect of naringin on isoproterenol-induced cardiotoxicity in Wistar rats: an in vivo and in vitro study. Toxicology 2007; 232: 216-225.

[17] Nandave M, Ojha SK, Kumari S, Nag TC, Mehra R, Narang R and Arya DS. Cardioprotective effect of root extract of Picrorhizakurroa against isoproterenol-induced cardiotoxicity in rats. Indian J Exp Biol 2013; 51: 694-701.

[18] Pook E, Grimm S, Bonin A, Winkler T and Hillen W. Affinities of mAbs to Tet repressor complexed with operator or tetracycline suggest conformational changes associated with induction. Eur J Biochem 1998; 258: 915-922.

[19] Morimoto H, Takahashi M, Izawa A, Ise H, Hongo M, Kolattukudy PE and Ikeda U. Cardiac over expression of monocyte chemoattractant protein-1 in transgenic mice prevents cardiac dysfunction and remodeling after myocardial infarction. Circ Res 2006; 99: 891-899.

[20] Nichtova Z, Novotova M, Kralova E and Stankovicova T. Morphological and functional characteristics of models of experimental myocardial injury induced by isoproterenol. Gen Physiol Biophys 2012; 31: 141-151.

[21] Zhao M, Zheng S, Yang J, Wu Y, Ren Y, Kong X, Li W and Xuan J. Suppression of TGF-beta1/Smad signaling pathway by sesamin contributes to the attenuation of myocardial fibrosis in spontaneously hypertensive rats. PLoS One 2015; 10: e0121312.

[22] Woodiwiss AJ, Tsotetsi OJ, Sprott S, Lancaster EJ, Mela T, Chung ES, Meyer TE and Norton GR. Reduction in myocardial collagen cross-linking parallels left ventricular dilatation in rat models of systolic chamber dysfunction. Circulation 2001; 103: 155-160.

[23] Korraa A, Ezzat MH, Bastawy M, Aly H, El-Mazary AA and Abd El-Aziz L. Cardiac troponin I levels and its relation to echocardiographic findings in infants of diabetic mothers. Ital J Pediatr 2012; 38: 1-8.

[24] Hull KL and Harvey S. GH as a co-gonadotropin: the relevance of correlative changes in GH secretion and reproductive state. J Endocrinol 2002; 172: 1-19.

[25] Ma T, Zhao Y, Kwak YD, Yang Z, Thompson R, Luo Z, Xu H and Liao FF. Statin's excitoprotection is mediated by sAPP and the subsequent attenuation of calpain-induced truncation events, likely via rho-ROCK signaling. J Neurosci 2009; 29: 11226-11236.

[26] Tapia GG, Razonable RR, Eckel-Passow JE, Lahr BD, Afessa B, Keegan MT, Catania J and Baddour LM. A scoring model of factors associated with Candida glabrata candidemia among critically ill patients. Mycoses 2012; 55: 228-236.

[27] Gellings Lowe N, Swaney JS, Moreno KM and Sabbadini RA. Sphingosine-1-phosphate and sphingosine kinase are critical for transforming growth factor-beta-stimulated collagen production by cardiac fibroblasts. Cardiovasc Res 2009; 82: 303-312.

[28] Lijnen PJ, Petrov VV and Fagard RH. Induction of cardiac fibrosis by transforming growth factor-beta(1). Mol Genet Metab 2000; 71: 418-435.

[29] Malli F, Koutsokera A, Paraskeva E, Zakynthinos E, Papagianni M, Makris D, Tsilioni I, Molyvdas PA, Gourgoulianis KI and Daniil Z. Endothelial progenitor cells in the pathogenesis of idiopathic pulmonary fibrosis: an evolving concept. PLoS One 2013; 8: e53658.

[30] Ji H, Tang H, Lin H, Mao J, Gao L, Liu J and Wu T. Rho/Rock cross-talks with transforming growth factor-beta/Smad pathway participates in lung fibroblast-myofibroblast differentiation. Biomed Rep 2014; 2: 787-792.

[31] Pei X, Mo Y, Ning B, Yuan Z, Peng L and Ma R. The role of TGFβ1 stimulating ROCK I signal pathway to reorganize actin in a rat experimental model of developmental dysplasia of the hip. Mol Cell Biochem 2014; 391: 1-9.

[32] Guo ZL, Fu Y, Wei SJ, Zhang WP and Dang HW. Protective effect of matrine on isoptroterenol-induced cardiac hypertrophy via the regulation of mTOR/p70S6K signaling pathway in rats. Chinese Journal of Hospital Pharmacy 2015; 35: 794-797.