Regulation of endothelial nitric oxide synthase and asymmetric dimethylarginine by matrine attenuates isoproterenol-induced acute myocardial injury in rats

Xiaobing Li*, Xiao Wanga,b, Yafang Guoa, Ning Denga,b, Ping Zhenga,b, Qingbin Xub,d, Yang Wuac and Guidong Daidbc

aDepartment of Pharmacology, School of Pharmacy, Ningxia Medical University, bNingxia Engineering & Technology Research Center for Modernization of Hui Medicine, cNingxia Research Institute of Medicine & Pharmacy and dAffiliated Hospital of Ningxia Medical University, Yinchuan, Ningxia, China

Keywords
asymmetric dimethylarginine; endothelial nitric oxide synthase; isoproterenol; matrine; myocardial ischaemia

Correspondence
Qingbin Xu or Guidong Dai, Ningxia Engineering & Technology Research Center for Modernization of Hui Medicine, 1160 Shengli Street, Yinchuan, Ningxia 750004, China. E-mail: xu-qb@163.com (for Qingbin Xu); daiguidong@163.com (for Guidong Dai)

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Abstract

Objectives This study was designed to investigate the cardioprotective effects of matrine on regulation of endothelial nitric oxide synthase (eNOS) and asymmetric dimethylarginine (ADMA) in isoproterenol-induced acute myocardial ischaemic rats.

Methods Male Sprague–Dawley rats were pretreated with matrine (200, 100 and 50 mg/kg) orally for 10 days. Acute myocardial injury was induced in rats by subcutaneous injection of isoproterenol. Serum and haemodynamic parameters, histopathological variables and expression of protein levels were analysed.

Key findings Oral administration of matrine (200, 100 and 50 mg/kg) significantly attenuated isoproterenol-induced cardiac necrosis and left ventricular dysfunction. Matrine treatment restored impaired ventricular Akt and eNOS protein expression with concomitant increased phosphorylation of Akt (Ser473) and eNOS (Ser1177), and also restored glycogen synthase kinase 3β activity, as indicated by increased phosphorylation at Ser 9. Moreover, treatment with matrine had no effect on the isoproterenol-induced elevated protein arginine methyltransferase 1 protein expression, but could significantly normalize the reduced dimethylarginine dimethylaminohydrolase 2 expression and attenuate the increased serum level of ADMA. The expression of catechol-o-methyltransferase and monoamine oxidase did not differ among all groups (all $P > 0.05$).

Conclusions Our results suggested that matrine protects against isoproterenol-induced myocardial ischaemia via eNOS and ADMA pathway.

Introduction

Ischaemic heart disease is the leading cause of morbidity and mortality from cardiovascular disease worldwide. It results from the rapid development of myocardial necrosis caused by critical imbalance between coronary blood supply and myocardial demand. Subcutaneous injection of isoproterenol, a synthetic catecholamine and β-adrenoceptor agonist, at supramaximal doses induces acute irreversible myocardial injury in rats, which pathophysiologically and morphologically resembles myocardial infarction in humans. Oxidative stress mediated by increased generation of reactive oxygen species and/or depletion of the antioxidants in the defence system, as well as nitric oxide level regulated by impaired endothelial nitric oxide synthase (eNOS) signalling pathway, have been recognized as the possible biochemical and molecular mechanism, respectively, for the myocardial damage caused by this catecholamine.

Matrine, an active alkaloid with a molecular formula of C15H24N2O (Figure 1), derived from the traditional Chinese herb *Sophora alopecuroides* L, has been shown to possess diverse pharmacological activities blended with profound antioxidant and anti-inflammatory properties. It has been shown that matrine 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. Our previous study demonstrated that matrine has a
Matrine regulates eNOS and ADMA pathway

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Nitric oxide is a free-radical species produced by the oxidation of arginine by all three isoforms of nitric oxide synthase – neuronal, inducible and endothelial nitric oxide synthase (nNOS, iNOS and eNOS, respectively). Signalling through the eNOS pathway is the principal regulator of arterial haemodynamic function and pathological myocardial ischaemia.\(^{[12–14]}\) It has been reported that high-dose isoproterenol-induced myocardial infarction markedly reduces eNOS protein expression and/or nitric oxide level,\(^{[6]}\) whereas both upregulation and downregulation of eNOS expression have been observed in isoproterenol-induced heart failure.\(^{[15,16]}\) Knocking out the eNOS gene induces myocardial ischaemia in mice, and enhancement of eNOS expression has anti-ischaemic cardiac effects, thereby inhibiting myocardial injury.\(^{[17,18]}\) In addition, targeted overexpression of eNOS attenuates cardiac hypertrophy induced by chronic isoproterenol infusions.\(^{[19]}\)

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, competitively inhibits the production of nitric oxide, and plays an important role as a predictor of cardiovascular disease events or death in patients with established coronary artery disease.\(^{[20,21]}\) Elevated serum ADMA levels were found in various animal models of cardiovascular disease;\(^{[22,23]}\) this induces vascular endothelial impairment, aggravates post-ischaemic, and deteriorates systemic, haemodynamic and organ blood flow.\(^{[24,25]}\) These observations suggest that regulation of eNOS signaling and ADMA metabolism may be a promising target for prevention of myocardial ischaemia. Therefore, the aim of this work was to describe the cardioprotective effects of matrine on the regulation of the eNOS signaling pathway and metabolism of ADMA in isoproterenol-induced myocardial injury. Considering that catecholamine is metabolized by catechol-o-methyltransferase (COMT) and monoamine oxidase (MAO), and we also investigated ventricular COMT and MAO protein expression during isoproterenol-induced cardiotoxicity in rats.

Materials and Methods

Drugs and chemicals

Matrine (white powder, purity > 99.8%) was purchased from Ningxia Bauhinia Pharmaceutical Co. Ltd (Yinchuan, China). Isoproterenol hydrochloride was bought from Sigma Chemical Co (St. Louis, USA). Rat asymmetric dimethylarginine (ADMA) enzyme-linked immunosorbent assay kit was purchased from Cusabio Biotech Co. Ltd (Newark, USA). Total protein extraction kit and bicinchoninic acid protein assay kit were purchased from KenGen Biotechnology Co. Ltd (Nanjing, China). Primary antibodies to eNOS, phosphorylated eNOS (phospho-eNOS) (Ser1177), Akt, phosphorylated Akt (phospho-Akt) (Ser473), glycogen synthase kinase 3β (GSK3β), phosphorylated GSK3β (phospho-GSK3β) (Ser9), protein arginine methyltransferase 1 (PRMT1), β-actin and secondary antibody of horseradish peroxidase-conjugated goat anti-rabbit IgG were bought from Cell Signaling Technology (Danvers, USA). Antibodies to dimethylarginine dimethylaminohydrolase (DDAH2), monoamine oxidase (MAO), catechol-o-methyltransferase (COMT) and secondary antibody of horseradish peroxidase-conjugated donkey anti-goat IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, USA). Super Signal West Pico Chemiluminescent Substrate was purchased from Thermo scientific Corporation (Rockford, USA). Other reagents used were of commercial analytical grade.

Experimental animals

The investigation conformed to 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). All protocols and surgical procedures were approved by the Institute of Animal Care and Use Committees of Ningxia Medical University.

Male Sprague–Dawley rats, 250 ± 20 g, obtained from Ningxia Laboratory Animal Centre (Ningxia, China), were used for the experiment. All rats were housed in polypropylene cages (48 cm × 35 cm × 20 cm) lined with husk, renewed every 24 h under hygienic conditions, and placed in a controlled environment under natural light and dark cycle at 24 ± 2°C for seven days before the experiments. They were allowed free access to a commercial standard rat cube diet (Beijing Keaoxieli Feed Co. Ltd, Beijing, China) and water.

Induction of myocardial ischemia

Experimental myocardial ischaemia was induced by daily subcutaneous injection of 85 mg/kg isoproterenol.
hydrochloride in the upper back of rats for two consecutive days.[11] Isoproterenol hydrochloride solutions were prepared under sterile condition with physiological saline immediately before injection and used within 30 min of preparation.

**Experimental design**

Our previous study showed that oral administration of matrine at three different doses (200, 100 and 50 mg/kg) for a period of 10 days markedly reduced the isoproterenol-induced myocardial ischaemia; this protective effect also was seen in rats after oral administration of matrine for two days at a dosage of 200 or 100 mg/kg.[11] Since matrine at a dose of 50 mg/kg for two days did not show any significant effect in isoproterenol-induced rats,[11] we used 200, 100 and 50 mg/kg of matrine for 10 days as the optimum dosages for the study.

The rats were randomly divided into five groups of 9–10 rats as follows: control group, rats received oral administration of physiological saline 10 ml/kg for 10 days; isoproterenol treatment group, rats received oral administration of physiological saline 10 ml/kg for 10 days and on the 9th day were subcutaneously injected with isoproterenol (85 mg/kg dissolved in saline, once a day at an interval of 24 h for two consecutive days); matrine pretreatment groups: rats received matrine (200, 100 and 50 mg/kg by gastric gavage, respectively) for 10 days and then on the 9th day were subcutaneously injected with isoproterenol (85 mg/kg dissolved in saline, once a day at an interval of 24 h for two consecutive days).

**Measurement of cardiac function**

Twenty-four hours after the second dose of isoproterenol, all the rats were anaesthetized intraperitoneally with urethane (1.2 g/kg) and needle electrodes were inserted under the skin for the limb lead at position II for recording of heart rate. To evaluate the cardiac left ventricular function, a polyethylene catheter (PE50) filled with heparin saline (500 U/ml) was inserted into the right carotid artery and then advanced into the left ventricle. The catheter was connected to BL-420E Biological Data Acquisition & Analysis Class (Chengdu TME Technology Co. Ltd, Chengdu, China) by a pressure transducer (Chengdu TME Technology Co. Ltd, Chengdu, China), the haemodynamic parameters of mean blood pressure, left-ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), maximum rate of developed left ventricular pressure (LV dP/dtmax) and minimum rate of developed left ventricular pressure (LV dP/dtmin), were continually recorded 5–10 min after 10 min of stabilization; the values were averaged.

**Histopathological studies**

The cardiac apexes obtained from all experimental groups were excised and fixed in 4% buffered paraformaldehyde solution. Tissues were embedded in paraffin, sectioned at 4 µm and stained with haematoxylin and eosin (H&E). The slides were evaluated under a light microscope, and then photomicrographs were taken. At least five fields for each slide were examined and graded for severity of changes using scores on a scale of no abnormal findings (−), mild (+), moderate (+++) and severe (++++) Scoring was carried out on coded samples by an experienced pathologist in a blinded manner.

**Serum asymmetric dimethylarginine estimation**

After haemodynamic parameters were measured, as described in our previous study,[19] blood was collected from the right carotid artery and transferred into tubes. After leaving to clot for 1 h at room temperature and then centrifugation at 7000 g for 10 min (4°C), serum samples were collected and stored at −80°C for assay. According to the manufacturer’s instruction, enzyme-linked immunosorbent assay was performed for the determination of serum ADMA levels by Bio-RAD 680 automatic microplate reader.

**Western blot analysis**

Hearts were rapidly dissected and washed and the ventricle was separated and frozen at −70°C for protein assays. Total proteins were isolated from cardiac tissues, homogenized in cold lysis buffer using total protein extraction kit with protease inhibitor fluid and then subjected to sonication for 5 s on ice (repeated three times), followed by centrifugation at 4°C for 10 min at 17,000 g to remove cellular debris. The protein concentration was estimated using a bicinchoninic acid protein assay reagent kit with bovine serum albumin as a standard. Equivalent amounts (100 µg) of protein samples were loaded and separated by electrophoresis on 5% or 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) and then electrophoretically transferred to nitrocellulose membranes using an electrophoretic transfer system (Bio-Rad, Hercules, USA). Membranes were subdivided, and each protein of interest and β-actin were analysed from a single transfer. The membranes were blocked with 5% (w/v) nonfat dry milk in phosphate-buffered saline (PBS) containing 0.1% (v/v) Tween 20 (PBST) for 1 h at room temperature, then incubated with primary antibody of Akt (1:1000 dilution), phospho-Akt (Ser473) (1:500 dilution), eNOS (1:400 dilution), phospho-eNOS (Ser1177) (1:500 dilution), GSK3β (1:1000 dilution), phospho-GSK3β (Ser9) (1:1000 dilution), PRMT1(1:1000 dilution), DDAH2(1:200 dilution), MAO-A/B (1:200 dilution), COMT (1:200 dilution) or β-actin (1:400 dilution) overnight at 4°C, followed by respective horseradish peroxidase-conjugated secondary antibodies. Bands on blots were visualized using Super Signal West Pico Chemiluminescence Kit, and finally exposed to radiographic films. The films were scanned, and quantitation...
of protein band density normalized to β-actin was measured using Quantity One software (Bio-Rad Laboratories).

Statistical analysis

One-way analysis of variance with Bonferroni–Dunn post-hoc test was used to assess the effects of matrine on isoproterenol-induced myocardial injury within each group using SPSS software package 11.5 (Chicago, USA). Results were expressed as mean ± SD; *P* < 0.05 was considered as statistically significant.

Results

Mortality

There was no mortality in the matrine pretreatment groups subjected to isoproterenol subcutaneous injection. Two rats died in the isoproterenol-injected group.

Table 1  Effect of matrine on body weight and heart weight in isoproterenol-induced myocardial ischaemic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Ventricular weight (g)</th>
<th>Left ventricular weight (g)</th>
<th>Relative left ventricular weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>231.4 ± 24.25</td>
<td>247.7 ± 24.95</td>
<td>0.793 ± 0.055</td>
<td>608 ± 0.059</td>
</tr>
<tr>
<td>Isoproterenol (85 mg/kg)</td>
<td>235.5 ± 27.50</td>
<td>241.5 ± 24.86</td>
<td>0.820 ± 0.069</td>
<td>640 ± 0.072</td>
</tr>
<tr>
<td>Matrine (200 mg/kg) + isoproterenol</td>
<td>226.9 ± 11.68</td>
<td>243.6 ± 12.83</td>
<td>0.808 ± 0.050</td>
<td>629 ± 0.061</td>
</tr>
<tr>
<td>Matrine (100 mg/kg) + isoproterenol</td>
<td>230.6 ± 26.57</td>
<td>238.2 ± 28.87</td>
<td>0.777 ± 0.056</td>
<td>590 ± 0.053</td>
</tr>
<tr>
<td>Matrine (50 mg/kg) + isoproterenol</td>
<td>233.4 ± 24.58</td>
<td>242.1 ± 25.49</td>
<td>0.803 ± 0.058</td>
<td>614 ± 0.048</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD, *n* = 8–9. Relative left ventricular weight is the weight of the left ventricular/rat body weight ¥ 100.

Table 2  Effect of matrine on the degree of histopathological changes in cardiac apexes in isoproterenol-induced myocardial ischaemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Myocardial necrosis</th>
<th>Infiltration of inflammatory cells</th>
<th>Interstitial oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Isoproterenol (85 mg/kg)</td>
<td>10</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Matrine (50 mg/kg) + isoproterenol</td>
<td>10</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Matrine (100 mg/kg) + isoproterenol</td>
<td>10</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Matrine (200 mg/kg) + isoproterenol</td>
<td>10</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

The figures represent the number of rats affected. Photomicrographs were used to evaluate the damage in the cardiac apexes, and the histopathological changes were arbitrarily scored as follows: −, no abnormal findings; +, mild; ++, moderate; ++++, severe.

Figure 2  Representative images of rat cardiac apexes by hematoxylin and eosin staining (× 400). (a) Control rats. (b) Rats given isoproterenol (85 mg/kg). (c) Rats given isoproterenol and 200 mg/kg matrine. (d) Rats given isoproterenol and 100 mg/kg matrine. (e) Rats given isoproterenol and 50 mg/kg matrine.
**Effect of matrine on cardiac function**

Consistent with the previous studies, isoproterenol treatment of rats resulted in left ventricular dysfunction as indicated by significant fall in values of LVSP, LV dP/dt\textsubscript{max} and LV dP/dt\textsubscript{min}, and a rise in values of LVEDP (Table 3) as compared with the control group. Oral administration of matrine increased LVSP, LV dP/dt\textsubscript{max} and LV dP/dt\textsubscript{min} in all the doses studied (200, 100 and 50 mg/kg) (Table 3). Likewise, it also prevented the increase in LVEDP caused by isoproterenol subcutaneous injection (Table 3) and improved cardiac function of the isoproterenol-treated rat. The heart rate and mean blood pressure were not significantly changed ($P > 0.05$) in any of the experimental groups (Table 3).

**Effect of matrine on ventricular Akt, endothelial nitric oxide synthase and glycogen synthase kinase 3β expression**

Compared with control, expression of Akt, eNOS and phospho-GSK3β (Ser9) was significantly decreased, and expression of phospho-Akt (Ser473) and phospho-eNOS (Ser1177) was not significantly changed in the isoproterenol-treated rats (Figures 3–5). Matrine (200, 100 and 50 mg/kg) improved the isoproterenol-induced decrease in the expression of Akt, eNOS and phospho-GSK3β (Ser9), and increased the expression of phospho-Akt (Ser473) and phospho-eNOS (Ser1177) (Figures 3, 4 and 5b). There were no marked changes in GSK3β expression among all groups (Figure 5c).

**Effect of matrine on serum asymmetric dimethylarginine level**

Compared with the control group, isoproterenol-treated rats showed a significant rise in serum ADMA, and matrine (200, 100 and 50 mg/kg) normalized the level of ADMA (Figure 6a).

**Effects of matrine on expression of cardiac protein arginine methyltransferase 1 and dimethylarginine dimethylaminohydrolase 2**

Compared with control, the expression of PRMT1 markedly increased, whereas the expression of DDAH2 significantly decreased in isoproterenol-treated rats. Matrine (200, 100 and 50 mg/kg) markedly increased the lower level of DDAH2 expression, but did not affect the increased expression of PRMT1 (Figure 6b, 6c and 6d).

**Effects of matrine on expression of monoamine oxidase and catechol-o-methyltransferase**

MAO and COMT expression in ventricular tissue were not significantly altered in any of the experimental groups (Figure 7).

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>LV dP/dt\textsubscript{max} (mmHg/kg)</th>
<th>LV dP/dt\textsubscript{min} (mmHg/kg)</th>
<th>LVSP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>Mean blood pressure (mmHg)</th>
<th>Heart rate (beat/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5749.44 H/11006 412.15 -</td>
<td>4693.57 H/11006 4693.57 -</td>
<td>371.16 -</td>
<td>131.89 -</td>
<td>6.70 -</td>
<td>10.83 -</td>
</tr>
<tr>
<td>Isoproterenol (85 mg/kg)</td>
<td>4805.46 H/11006 389.56** -</td>
<td>3822.16 H/11006 266.19** -</td>
<td>266.19**</td>
<td>120.70**</td>
<td>6.96**</td>
<td>2.88</td>
</tr>
<tr>
<td>Matrine (200 mg/kg) + Isoproterenol</td>
<td>5441.90 H/11006 175.40## -</td>
<td>4407.05 H/11006 388.56** -</td>
<td>112.17 H/11006 5.39##</td>
<td>132.17 H/11006 3.98 -</td>
<td>5.53##</td>
<td>7.78</td>
</tr>
<tr>
<td>Matrine (100 mg/kg) + Isoproterenol</td>
<td>5277.35 H/11006 182.26 H/11006 8.50##</td>
<td>470.54 H/11006 132.17 H/11006 5.39##</td>
<td>182.26 H/11006 8.50##</td>
<td>132.17 H/11006 5.39##</td>
<td>5.53##</td>
<td>7.78</td>
</tr>
<tr>
<td>Matrine (50 mg/kg) + Isoproterenol</td>
<td>5160.93 H/11006 192.48##</td>
<td>4270.62 H/11006 288.33**</td>
<td>182.26 H/11006 8.50##</td>
<td>132.17 H/11006 5.39##</td>
<td>5.53##</td>
<td>7.78</td>
</tr>
</tbody>
</table>

LV dP/dt\textsubscript{max}, maximum rate of developed left ventricular pressure; LV dP/dt\textsubscript{min}, minimum rate of developed left ventricular pressure; LVSP, left ventricular systolic pressure. Values are presented as mean ± SD, n = 8–9. *P < 0.05, **P < 0.01 compared with control; #P < 0.05, ##P < 0.01 compared with isoproterenol.
Discussion

The major findings of this study is that matrine protected against isoproterenol-induced myocardial ischaemia via regulating Akt/eNOS signalling pathways and their inhibition targets DDAH2 and ADMA.

Subcutaneous injection of supramaximal doses of isoproterenol induces subendocardial myocardial ischaemia and inhibition of left ventricular function, which closely resembles the pathological changes seen in human myocardial infarction.[3] The present investigation demonstrated that myocardium in rats subjected to isoproterenol showed severe injury, with subendocardial necrosis, infiltration of inflammatory cells and interstitial oedema, and inhibition of left ventricular diastolic and systolic function, which was in agreement with previous reports.[3,27] Interestingly, oral administration of matrine (200, 100 and 50 mg/kg) improved haemodynamic disturbance and ameliorated the histological damage of the hearts induced by isoproterenol, indicating that matrine exerts a pronounced protective effect on myocardial injury.

The mechanism of isoproterenol-induced myocardial injury is not completely understood. The formation of free radicals, as well as the accumulation of lipid peroxides, has been recognized as the biochemical mechanism[3–5] for the myocardial ischaemia caused by this catecholamine; down-regulation of eNOS expression and nitric oxide level are the possible molecular mechanisms.[6] Our previous study demonstrated that either chronic matrine (50, 100 and 200 mg/kg, respectively) administration for ten days or acute matrine (100 and 200 mg/kg, respectively) administration for two days markedly reduced isoproterenol-induced myocardial injury; this cardioprotective effect of matrine was mediated through prevention isoproterenol-induced oxidative stress.[11] Hence, the present study was undertaken to study the effect of matrine on regulation of the eNOS signaling pathway.

Figure 3  Effect of matrine on expression of Akt and phospho-Akt (Ser473) in hearts from isoproterenol-induced myocardial ischaemic rats. (a) Western blot analysis of Akt and phospho-Akt (Ser473), β-actin is shown as loading control. I = control; II = isoproterenol (85 mg/kg); III = matrine (200 mg/kg) + isoproterenol; IV = matrine (100 mg/kg) + isoproterenol; V = matrine (50 mg/kg) + isoproterenol. Quantitative data of Akt and phospho-Akt (Ser473) signals are shown as percentages of the value of control rats (b and c). Values are presented as mean ± SD, n = 8–10. **P < 0.01 compared with control; #P < 0.01 compared with isoproterenol.
pathway during isoproterenol-induced cardiotoxicity in rats.

There is evidence that the decreased eNOS activity is involved in isoproterenol-induced myocardial injury, and that the augmented eNOS signalling by treatment with alpha-mangostin accounts for cardioprotection in rat heart.\[6\] In line with the previous findings, our study showed that isoproterenol-induced myocardial ischaemic rats demonstrated a marked decrease of eNOS expression in the ventricle. Administration of matrine appeared to restore eNOS expression, which suggested that matrine could attenuate isoproterenol-induced myocardial infarction via the eNOS signalling pathway.

It is well known that eNOS and GSK3β belong to the serine/threonine protein kinases, and the upstream element, Akt phosphorylation of eNOS at Thr495, weakens the action of eNOS, whereas phosphorylation of Ser1177 is required for enhancing the eNOS activity.\[28,29\] Decreased expression of Akt and its phosphorylation at Ser473 were found in myocardial ischaemic rats.\[30\] In addition, GSK3β functions as a negative regulator of cardiac hypertrophy, and it has been reported that the phosphorylation of GSK3β could protect cardiac muscle from reperfusion injury.\[31–34\] Our present study showed that Akt expression was decreased in the heart of isoproterenol-induced myocardial ischaemia. Administration of matrine (200, 100 and 50 mg/kg) significantly increased the expression of Akt, phospho-Akt (Ser 473) and phospho-eNOS (Ser1177) in the heart. Although there was no significant change in GSK3β expression among all experimental groups, the phosphorylation of GSK3β at Ser9 was decreased in the ventricle of isoproterenol-induced myocardial ischaemic rats. Oral administration of matrine (200, 100 and 50 mg/kg) increased the phospho-GSK3β expression, thereby enhancing GSK3β activity. Our findings suggest that matrine exerts its cardioprotective effects via regulation of Akt, eNOS and GSK3β expression.

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Figure 4  Effect of matrine on expression of endothelial nitric oxide synthase (eNOS) and phospho-eNOS (Ser1177) in hearts from isoproterenol-induced myocardial ischaemic rats. (a) Western blot analysis of eNOS and phospho-eNOS (Ser1177), β-actin is shown as loading control. I = control; II = isoproterenol (85 mg/kg); III = matrine (200 mg/kg) + isoproterenol; IV = matrine (100 mg/kg) + isoproterenol; V = matrine (50 mg/kg) + isoproterenol. Quantitative data of eNOS and phospho-eNOS (Ser1177) signals are shown as percentages of the value of control rats. Values are presented as mean ± SD, n = 8–10. *P < 0.05, **P < 0.01 compared with control; #P < 0.05, ##P < 0.01 compared with isoproterenol.
ADMA is an endogenous competitive inhibitor of all isoforms of nitric oxide synthase. An elevated serum concentration of ADMA has been reported in patients with cardiovascular risk factors and heart failure, as well as in animal models of myocardial infarction, heart failure and pulmonary hypertension. ADMA originates from the degradation of post-translational methylated protein arginine residues by protein arginine methyltransferase type 1, and PRMT1 is the predominant isoform of protein arginine methyltransferase in mammalian cells. In addition, circulating ADMA is degraded in the body by DDAH1 and DDAH2. DDAH1 is typically found in tissues expressing nNOS, whereas DDAH2 predominates in tissues expressing eNOS. Elevated serum ADMA concentration and increased DDAH2 expression were found in the heart post-myocardial infarction. Our results showed that isoproterenol-induced cardiac ischaemic rats displayed elevation of ADMA level, increased expression of PRMT1, and decreased expression of DDAH2. Administration of matrine (200, 100 and 50 mg/kg) exerted no effects on elevated PRMT1 expression, but significant normalized the level of ADMA, and reduced expression of DDAH2. These results indicate that matrine decreases serum levels of ADMA via increased content of DDAH2, which may contribute to its beneficial effect on nitric oxide synthesis.

As a catecholamine, isoproterenol is metabolized and inactivated by COMT, whereas MAO inhibition may significantly antagonize isoproterenol-induced cardiomegaly. Therefore, COMT and MAO may influence the degree of ischaemia in myocardial cells induced by isoproterenol. The present study showed that matrine did not affect the amounts in which they were expressed, suggesting that the protection of cardiac muscles from ischaemia by matrine is not related to the enhanced metabolism of isoproterenol.

Our previous study showed that administration of matrine (200, 100 and 50 mg/kg, respectively) for 10 days provides...
significant cardioprotection against isoproterenol-induced cardiotoxicity through its antioxidant property. The present study demonstrated that these dosages of matrine protect against isoproterenol-induced myocardial ischaemia via the eNOS and ADMA pathways. So these particular dosages would be fixed as the optimum dosage for further studies.

In this study, we also recognize several potential limitations. In particular, we did not observe effects of matrine administration alone on rat cardiac tissues, which would show whether matrine can modulate the eNOS signalling pathway and ADMA metabolism directly, leading to cardioprotection, or whether its effect on the these pathways is specific to isoproterenol-induced injury. In addition, the isoproterenol-induced cardiac infarct size was not estimated in the present study, which would helpful to accurately evaluate myocardial ischaemia. Furthermore, the results presented here relate to in-vivo matrine treatment. Further studies should be carried out to discover the effects of acute in-vitro eNOS augmentation with matrine.

**Conclusions**

This study provided experimental evidence that administration of matrine attenuated isoproterenol-induced myocardial ischaemia in rats. The molecular mechanism responsible for this beneficial effect may involve regulation of eNOS signalling pathway as demonstrated by normalization of Akt,
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Figure 7  Effects of matrine on expression of monoamine oxidase (MAO) and catechol-o-methyltransferase (COMT) in hearts from isoproterenol-induced myocardial ischaemic rats. (a) Western blot analysis of MAO and COMT, β-actin is shown as loading control. I = control; II = isoproterenol (85 mg/kg); III = matrine (200 mg/kg) + isoproterenol; IV = matrine (100 mg/kg) + isoproterenol; V = matrine (50 mg/kg) + isoproterenol. Quantitative data of MAO and COMT signals are shown as percentages of the value of control rats (b and c). Values are presented as mean ± SD, n = 8–10.

eNOS and GSK3β protein expression, and also ADMA metabolism as demonstrated by normalization of DDAH2 expression and reduction of ADMA accumulation. Therefore, this study shows that matrine might be a potential therapeutic agent for pharmacological management of myocardial ischaemic diseases.

Declarations
Conflict of interest
The Author(s) declare(s) that they have no conflicts of interest to disclose.

References

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