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Research Paper

Cardioprotective effect of matrine on isoproterenol-induced cardiotoxicity in rats

Xiaobing Li[a], Ru Zhou[a], Ping Zheng[a], Lin Yan[a], Yang Wu[a,b], Xu Xiao[a] and Guidong Dai[a,b]

[a]Department of Pharmacology, Ningxia Medical University and [b]Ningxia Research Institute of Medicine & Pharmacy, Yinchuan, Ningxia P. R. of China

Abstract

Objectives This study was designed to explore the effect and mechanism of matrine, an active component of Chinese traditional medicine, on isoproterenol-induced acute cardiotoxicity in rats.

Methods Acute myocardial injury was induced in rats by daily subcutaneous injection of isoproterenol (85 mg/kg) for two days. Haemodynamic and biochemical parameters were measured and histopathological examination was performed.

Key findings Chronic oral administration of matrine (50, 100 or 200 mg/kg per day for 10 days) significantly reduced the release of lactic dehydrogenase, glutamic oxaloacetic transaminase and creatine kinase after isoproterenol-induced myocardial ischaemic injury, improved the left ventricular (LV) dysfunction, including increased LV systolic pressure (LVSP), maximum rate of developed LV pressure (LV dP/dt_{max}) and minimum rate of developed LV pressure (LV dP/dt_{min}), increased the activity of superoxide dismutase, catalase and glutathione peroxidase, and also decreased the content of the lipid peroxidation product malondialdehyde in plasma and myocardial tissues in rats. Acute oral administration of matrine at a dose of 100 or 200 mg/kg for two days also had a cardioprotective effect on this rat model. The protective role of matrine on isoproterenol-induced myocardial damage was further confirmed by histopathological examination. There were no significant changes in heart rate and blood pressure in all experimental groups.

Conclusions Our results suggest that matrine has a significant cardioprotection against isoproterenol-induced cardiotoxicity through its antioxidant property.

Keywords antioxidants; cardioprotection; cardiotoxicity; isoproterenol; matrine

Introduction

Matrine, an active alkaloid with a molecular formula of $C_{15}H_{24}N_2O$ (Figure 1), is purified from the traditional Chinese herb *Sophora alopecuroides* L., and has been intensively studied for its pleiotropic pharmacological effects, including anticancer, anti-inflammatory and anti-arrhythmic activity.[1–4] The traditional Chinese medicine pharmacopoeia prescribes matrine for improving cardiac function and prognosis in heart failure, although the mechanisms involved are not yet clear. Experimental studies have shown that matrine prevents cardiac hypertrophy and fibrosis induced by pressure overload *in vivo*,[5] and inhibits hyperplasia of cardiac fibroblasts induced by angiotensin II and norepinephrine.[6,7] Both in-vivo and in-vitro studies have provided evidence of the positive inotropic actions of matrine on atrial and ventricular papillary muscles.[8,9] Matrine also significantly increased the dose of aconitine needed for induction of ventricular premature and ventricular tachycardia, decreased the number of arrhythmias induced by coronary artery ligation, and increased the ventricular fibrillation threshold induced by electric stimulation in experimental animal models.[10]

It is well established that subcutaneous injection of a high concentration of isoproterenol, a synthetic adrenoceptor agonist, can deplete the energy reserve of the myocardium and induce severe oxidative stress and result in necrotic lesions in the myocardium.[11,12] Oxidative stress mediated by increased generation of reactive oxygen species or depletion of the antioxidants in the defence system plays an important role in the pathogeneses of this

Correspondence: Dr G Dai, Department of Pharmacology, Ningxia Medical University, 1160 Shengli Street, Yinchuan, Ningxia 750004, P. R. of China. E-mail: daiguidong@163.com

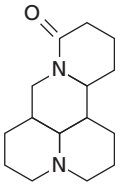

Figure 1 Chemical structure of matrine

non-invasive myocardial necrotic rat model, and the decreased myocardial compliance and inhibition of diastolic and systolic function in rats are comparable with those taking place in human myocardial infarction.[11–14]

In our preliminary studies, we found that administration of matrine for 30 days could markedly attenuate myocardial injury in hypercholesterol-fed rats, which suggests that matrine may protect the injured myocardium via its ability to decrease the total cholesterol and triglyceride content of serum, enhance the activity of antioxidative enzymes, and maintain the stability of myocardial cellular membranes.[15,16] Therefore, the purpose of this study was to elucidate whether administration of matrine could attenuate the isoproterenol-induced cardiotoxicity via improving impaired endogenous antioxidant defence mechanisms, and the time-course effect of matrine was also considered.

Materials and Methods

Drugs and chemicals

Matrine (white powder, purity > 99.8%) was purchased from Ningxia Bauhinia Pharmaceutical Co. Ltd (Ningxia, China). Isoproterenol hydrochloride was bought from Sigma Chemical Co. (St Louis, USA). Diagnostic agent kits of superoxide dismutase, malondialdehyde, creatine kinase, catalase, glutamic oxaloacetic transaminase, lactate dehydrogenase and glutathione peroxidase were obtained from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). Other reagents used in the study were of commercial analytical grade.

Animals

All the experiments were carried out with male Sprague–Dawley rats, 250 ± 20 g, supplied by Ningxia Laboratory Animal Centre (Ningxia, China). 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 a natural light and dark cycle at 23 ± 2°C for seven days before the experiment. They were allowed free access to a commercial standard rat cube diet and water. The animal centre of Ningxia Medical University approved the study.

Induction of myocardial injury

Isoproterenol was dissolved in physiological saline solution and injected subcutaneously to rats (85 mg/kg) daily for two consecutive days to induce experimental myocardial infarction.[17,18]

Experimental design

The dosages of matrine (50, 100 and 200 mg/kg, orally) were selected on the basis of the cardioprotective dose of matrine according to our previous study.[15,16] The rats were randomly divided into eight groups of nine or ten 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 physical saline 10 ml/kg for 10 days and on day nine were subcutaneously injected with isoproterenol (85 mg/kg dissolved in saline, once a day at an interval of 24 h for two consecutive days); Chronic matrine groups, rats received matrine (50, 100 or 200 mg/kg by gastric gavage) for 10 days and then on day nine were subcutaneously injected with isoproterenol (85 mg/kg dissolved in saline, once a day at an interval of 24 h for two consecutive days); acute matrine groups, rats received oral administration of physiological saline for eight days and received matrine (50, 100 or 200 mg/kg by gastric gavage) on days nine and ten, and half an hour after gastric gavage of matrine, rats 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-fours 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 (PE 50) 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 (Sichuan, China) by a pressure transducer. The haemodynamic parameters of mean blood pressure (mBP), left ventricular systolic pressure (LVSP), maximum rate of developed left ventricular pressure (LV dP/dt_{max}) and minimum rate of developed left ventricular pressure (LV dP/dt_{min}) were continually recorded for 5–10 min after 10 min of stabilization; the values were averaged.

Biochemical estimations

After haemodynamic parameters were measured, blood was collected from the right carotid artery and transferred into tubes. After having clotted for 1 h at room temperature and centrifugation at 3500 rev/min for 10 min at 4°C, serum samples were collected and stored at −80°C for further biochemical assay. The rats were sacrificed by cervical decapitation, each heart was removed rapidly, the cardiac apex was excised immediately for histopathological studies, and the remaining tissues of the ventricles were thoroughly washed with pre-chilled physiological saline and stored at −80°C. A known weight of cardiac ventricles was ground by liquid nitrogen under refrigeration, then homogenized with pre-chilled physiological saline at a ratio 1 : 9 (w/v). The homogenate was centrifuged at 3000*g* for 10 min at 4°C and the supernatant was used for biochemical assay. The content of tissue protein was determined by Coomassie brilliant blue G250. Levels of creatine kinase, catalase, glutamic

oxaloacetic transaminase, lactate dehydrogenase, superoxide dismutase, malondialdehyde and glutathione peroxidase in plasma and heart tissue were measured according to the manufacturer's instructions, and their absorbance was measured by 752 spectrophotometer (Shanghai, China).

Histopathological studies

The cardiac apex obtained from all experimental groups was excised and fixed in 4% buffered paraformaldehyde solution. Tissues were embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin (H&E). The slides were evaluated under a light microscope, and then photomicrographs were taken. A minimum of six 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 (+++).[19] Scoring was done on coded samples by an experienced pathologist in a blinded manner.

Statistical analysis

Statistical analyses of observed values were performed using one-way analysis of variance using SPSS software package 11.5. Results were expressed as mean ± SD and $P < 0.05$ was considered as statistically significant.

Results

Effect of matrine on body weight and serum diagnostic marker enzymes

The mean body weights of rats in all experimental groups showed no significant change (Table 1). Compared with control groups, the activity of lactate dehydrogenase, glutamic oxaloacetic transaminase and creatine kinase was significantly increased in the isoproterenol-treated group of rats. Chronic and acute administration of matrine (50, 100, 200 mg/kg daily, for 10 days, and 100 and 200 mg/kg daily, for two days, respectively) attenuated all the isoproterenol induced alterations of these serum diagnostic marker enzymes (Table 1).

Ventricular function assessment

Isoproterenol treatment of rats resulted in left ventricular dysfunction as indicated by a significant fall in values of LV dP/dt_{max}, LV dP/dt_{min} and LVSP as compared with the control group. Chronic matrine administration (50, 100 and 200 mg/kg, for 10 days) improved depressed cardiac function of isoproterenol-treated rats, and acute matrine administration (100 and 200 mg/kg, for two days) also showed a cardioprotective effect on ventricular function (Table 2). Acute administration of matrine at dose of 50 mg/kg to isoproterenol-treated rats did not have any significant effect on left ventricular function (Table 2). Heart rate and mean blood pressure were not significantly altered ($P > 0.05$) in any of the experimental groups (Table 2).

Effect of matrine on lipid peroxidation and antioxidant enzymes

Compared with the control group, isoproterenol-treated rats demonstrated a significant rise in the content of malondialdehyde, as well as a significant decline in the activity of superoxide dismutase, catalase and glutathione peroxidase in the tissues of cardiac ventricles and serum. Chronic and acute administration of matrine (50, 100 and 200 mg/kg, for 10 days and two days, respectively) had the potential effect of attenuating the isoproterenol-induced increase in malondialdehyde, a lipid peroxidation marker enzyme, and the isoproterenol-induced decrease in antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, was attenuated (Tables 3, 4). Acute administration of matrine for two days at dosage of 50 mg/kg did not produce any significant changes in antioxidant enzymes when compared with the isoproterenol group (Tables 3, 4).

Histopathological examination of cardiac tissue

On histopathological examination, the heart tissue in the isoproterenol-treated group showed severe subendocardial necrosis, infiltration of inflammatory cells and interstitial oedema (Table 5, Figure 2b) as compared with that in normal

Table 1 Effect of matrine on body weight and serum marker enzymes in myocardial ischaemic rats

Group	Body weight (g)		Lactate dehydrogenase	Glutamic oxaloacetic transaminase	Creatine kinase
	Initial	Final	(U/l)	(U/l)	(U/ml)
Control	231.4 ± 24.25	247.7 ± 24.95	5119.59 ± 690.00	15.01 ± 2.50	0.93 ± 0.20
Isoproterenol (85 mg/kg)	235.5 ± 27.50	241.5 ± 24.86	7360.83 ± 907.40**	32.72 ± 9.53**	1.58 ± 0.23**
Chronic matrine (50 mg/kg) + isoproterenol	233.4 ± 24.58	242.1 ± 25.49	5863.92 ± 843.51$^{*\#\#}$	19.75 ± 8.01$^{\#\#}$	1.06 ± 0.36$^{\#\#}$
Chronic matrine (100 mg/kg) + isoproterenol	230.6 ± 26.57	238.2 ± 28.87	5736.08 ± 1036.96$^{\#\#}$	21.25 ± 5.00$^{*\#\#}$	0.98 ± 0.18$^{\#\#}$
Chronic matrine (200 mg/kg) + isoproterenol	226.9 ± 11.68	243.6 ± 12.83	5530.93 ± 622.05$^{\#\#}$	19.96 ± 5.08$^{\#\#}$	0.94 ± 0.18$^{\#\#}$
Acute matrine (50 mg/kg) + isoproterenol	246.2 ± 15.59	258.0 ± 23.68	7028.35 ± 712.39**	31.05 ± 8.19**	1.16 ± 0.47$^{\#\#}$
Acute matrine (100 mg/kg) + isoproterenol	235.9 ± 17.12	231.5 ± 24.79	6481.96 ± 634.23$^{**\#}$	25.35 ± 6.29$^{**\#\#}$	1.12 ± 0.39$^{\#\#}$
Acute matrine (200 mg/kg) + isoproterenol	242.3 ± 22.74	244.1 ± 23.48	6085.05 ± 709.61$^{*\#\#}$	24.85 ± 5.90$^{**\#}$	1.03 ± 0.26$^{\#\#}$

Values are presented as mean ± SD, n = 7–10. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with isoproterenol.

Table 2 Effect of matrine on left ventricular function in isoproterenol-induced myocardial ischemic rats

Group	LV dP/dt_{max} (mmHg/s)	LV dP/dt_{min} (mmHg/s)	LVSP (mmHg)	Mean blood pressure (mmHg)	Heart rate (bpm)
Control	5749.44 ± 412.1	4693.57 ± 522.16	131.89 ± 6.70	91.38 ± 3.75	401.63 ± 19.54
Isoproterenol (85 mg/kg)	4805.46 ± 389.56**	3822.16 ± 266.19**	120.70 ± 6.96**	98.07 ± 6.53	416.22 ± 27.25
Chronic matrine (50 mg/kg) + isoproterenol	5160.93 ± 242.48$^{**\#}$	4270.62 ± 288.33$^{*\#}$	129.27 ± 3.72$^{\#}$	92.58 ± 5.69	401.22 ± 11.84
Chronic matrine (100 mg/kg) + isoproterenol	5277.35 ± 470.54$^{**\#\#}$	4311.69 ± 522.13$^{*\#}$	128.26 ± 8.50$^{\#}$	94.83 ± 7.61	401.78 ± 32.42
Chronic matrine (200 mg/kg) + isoproterenol	5441.90 ± 175.40$^{\#\#}$	4407.05 ± 347.25$^{\#\#}$	132.17 ± 5.53$^{\#\#}$	92.22 ± 6.13	408.44 ± 41.23
Acute matrine (50 mg/kg) + isoproterenol	5017.58 ± 399.01**	4083.57 ± 505.45**	126.78 ± 11.82	93.25 ± 14.62	427.00 ± 18.52
Acute matrine (100 mg/kg) + isoproterenol	5213.73 ± 154.20$^{**\#}$	4239.97 ± 264.38$^{*\#}$	128.74 ± 6.43$^{\#}$	94.07 ± 7.64	394.63 ± 17.16
Acute matrine (200 mg/kg) + isoproterenol	5297.70 ± 340.38$^{**\#\#}$	4278.38 ± 316.21$^{*\#}$	130.01 ± 6.52$^{\#\#}$	91.05 ± 8.51	406.22 ± 28.83

LVSP, left ventricular systolic pressure; LV dP/dt_{max}, maximum rate of developed LV pressure; LV dP/dt_{min}, minimum rate of developed LV 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.

Table 3 Effect of matrine on antioxidant parameters in plasma of rats with acute myocardial damage

Group	Superoxide dismutase (U/ml)	Malondialdehyde (nmol/ml)	Catalase (U/ml)	Glutathione peroxidase (U/ml)
Control	183.34 ± 10.75	2.08 ± 0.35	4.92 ± 0.64	9176.98 ± 703.77
Isoproterenol (85 mg/kg)	134.86 ± 10.08**	4.67 ± 0.56**	2.31 ± 0.41**	6893.53 ± 511.17**
Chronic matrine (50 mg/kg) + isoproterenol	156.35 ± 10.00$^{**\#\#}$	3.58 ± 0.87$^{**\#\#}$	3.82 ± 1.33$^{*\#\#}$	8283.45 ± 915.55$^{*\#\#}$
Chronic matrine (100 mg/kg) + isoproterenol	159.95 ± 13.57$^{**\#\#}$	3.15 ± 0.71$^{**\#\#}$	3.96 ± 1.06$^{*\#\#}$	8671.94 ± 880.51$^{\#\#}$
Chronic matrine (200 mg/kg) + isoproterenol	168.83 ± 18.28$^{**\#\#}$	3.17 ± 0.46$^{**\#\#}$	4.12 ± 0.95$^{\#\#}$	8853.24 ± 842.54$^{\#\#}$
Acute matrine (50 mg/kg) + isoproterenol	145.01 ± 17.38**	4.26 ± 0.75**	3.71 ± 0.95$^{**\#\#}$	7230.22 ± 996.42**
Acute matrine (100 mg/kg) + isoproterenol	150.70 ± 9.09$^{**\#\#}$	4.03 ± 0.64$^{**\#}$	3.97 ± 0.93$^{*\#\#}$	7697.84 ± 647.84$^{**\#}$
Acute matrine (200 mg/kg) + isoproterenol	155.21 ± 7.75$^{**\#\#}$	3.45 ± 0.44$^{**\#\#}$	4.11 ± 0.80$^{\#\#}$	8340.53 ± 616.06$^{*\#\#}$

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.

Table 4 Effect of matrine on antioxidant parameters in heart tissue of rats with acute myocardial damage

Group	Superoxide dismutase (U/mg protein)	Malondialdehyde (nmol/mg protein)	Catalase (U/mg protein)	Glutathione peroxidase (U/mg protein)
Control	528.35 ± 91.37	2.01 ± 0.88	5.81 ± 0.84	316.69 ± 28.71
Isoproterenol (85 mg/kg)	293.93 ± 32.13**	5.89 ± 0.68**	3.79 ± 0.65**	189.37 ± 26.10**
Chronic matrine (50 mg/kg) + isoproterenol	379.03 ± 48.77$^{**\#\#}$	4.41 ± 0.60$^{**\#\#}$	5.05 ± 0.37$^{*\#\#}$	274.77 ± 27.98$^{**\#\#}$
Chronic matrine (100 mg/kg) + isoproterenol	496.27 ± 82.82$^{\#\#}$	4.15 ± 1.00$^{**\#\#}$	4.93 ± 0.57$^{**\#\#}$	276.63 ± 28.23$^{**\#\#}$
Chronic matrine (200 mg/kg) + isoproterenol	483.69 ± 38.89$^{\#\#}$	4.22 ± 0.48$^{**\#\#}$	5.00 ± 0.73$^{*\#\#}$	298.36 ± 10.46$^{\#\#}$
Acute matrine (50 mg/kg) + isoproterenol	336.50 ± 69.72**	4.53 ± 1.14$^{**\#\#}$	4.41 ± 0.82**	201.84 ± 36.59**
Acute matrine (100 mg/kg) + isoproterenol	350.08 ± 37.55$^{**\#}$	4.60 ± 0.63$^{**\#\#}$	4.71 ± 0.37$^{**\#\#}$	270.04 ± 30.61$^{**\#\#}$
Acute matrine (200 mg/kg) + isoproterenol	359.85 ± 31.16$^{**\#}$	4.62 ± 1.04$^{**\#\#}$	5.21 ± 0.82$^{\#\#}$	258.10 ± 19.25$^{**\#\#}$

Values are presented as mean ± SD, n = 7–10. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with isoproterenol.

the control group (Table 5, Figure 2a). Chronic and acute administration of matrine (50, 100 and 200 mg/kg, for 10 days and 50, 100 and 200 mg/kg, for two days, respectively) demonstrated the relative degree of improvement in isoproterenol-induced subendocardial necrosis, infiltration of inflammatory cells and interstitial oedema (Table 5, Figure 2c–h).

Discussion

Our study showed that chronic and acute administration of matrine attenuated the abnormalities of serum diagnostic marker enzymes and antioxidant enzymes, the left ventricular dysfunction and the damage to the cardiac architecture in myocardial ischaemic rats induced by isoproterenol.

Cytosolic enzymes (lactate dehydrogenase, glutamic oxaloacetic transaminase and creatine kinase) are used as diagnostic enzyme markers of myocardial tissue damage. They are released from the damaged tissue to the blood stream when myocardial ischaemia occurs. The significantly elevated levels of these serum enzymes indicate the severity of isoproterenol-induced necrotic damage to the myocardial membrane.[20] Our results showed that significant elevation in the levels of lactate dehydrogenase, glutamic oxaloacetic transaminase and creatine kinase occurred in the plasma of

Table 5 General characteristics of respondents

Group	*n*	Myocardial necrosis				Infiltration of inflammatory cells				Interstitial oedema			
		−	+	++	+++	−	+	++	+++	−	+	++	+++
Control	10	10	0	0	0	10	0	0	0	10	0	0	0
Isoproterenol (85 mg/kg)	10	0	0	2	8	0	0	2	8	0	0	2	8
Chronic matrine (50 mg/kg) + isoproterenol	10	0	8	2	0	0	8	2	0	0	8	2	0
Chronic matrine (100 mg/kg) + isoproterenol	10	0	9	1	0	0	9	1	0	2	7	1	0
Chronic matrine (200 mg/kg) + isoproterenol	10	0	9	1	0	0	9	1	0	2	7	1	0
Acute matrine (50 mg/kg) + isoproterenol	9	0	2	3	4	0	2	3	4	0	2	3	4
Acute matrine (100 mg/kg) + isoproterenol	9	0	4	3	2	0	4	3	2	0	4	3	2
Acute matrine (200 mg/kg) + isoproterenol	10	0	7	1	2	0	7	1	2	0	7	1	2

The figures represent the number of rats affected. The histopathological changes were arbitrarily scored as follows: −, no abnormal findings; +, mild; ++, moderate; +++, severe.

isoproterenol-injected rats, which is in line with the findings of previous reports[18,20] and is an indication of isoproterenol-induced necrotic damage of the myocardium. This study demonstrated that chronic and acute administration of matrine (50, 100 and 200 mg/kg for 10 days, 100 and 200 mg/kg for two days, respectively) significantly lowered the isoproterenol-induced elevation in the levels of the diagnostic marker enzymes lactate dehydrogenase, glutamic oxaloacetic transaminase and creatine kinase. These results suggested that matrine offered significant cardioprotection against isoproterenol-induced myocardial injury.

To evaluate the effect of matrine on isoproterenol-induced cardiac dysfunction, haemodynamic parameters were detected in anaesthetized rats. In this study, isoproterenol-induced myocardial ischaemic rats exhibited a significant fall in LVSP, LV dP/dt_{max} and LV dP/dt_{min}. The decreased LV dP/dt_{min} indicated left ventricular diastolic dysfunction, and the decreased LVSP and LV dP/dt_{max} indicated left ventricular systolic dysfunction. These results are in line with our previous findings[19] of isoproterenol-induced left ventricular dysfunction. Administration of matrine (50, 100 and 200 mg/kg for 10 days, 100 and 200 mg/kg for two days, respectively) appeared to attenuate left ventricular systolic dysfunction as shown by the improvement in LV dP/dt_{max} and LVSP, as well as left ventricular diastolic dysfunction as shown by improvement in LV dP/dt_{min}. These results strongly support the suggestion that matrine can improve isoproterenol-induced cardiac dysfunction probably due to the inotropic effect of matrine. The in-vivo study showed that matrine has positive inotropic effects on both atria and ventricles,[9] and the in-vitro study demonstrated that matrine significantly increased the contraction of guinea-pig papillary muscle through L-type calcium channels, other than interaction with α- and β-adrenergic pathways.[8] This could be the reason for the significant improvement of haemodynamic variables following oral administration with matrine.

Supramaxial doses of isoproterenol causes myocardial ischaemia due to the generation of excessive reactive oxygen species or depression of antioxidants in the defence system. Biological membranes are sensitive to lipid peroxidation by cytotoxic free radicals. Malondialdehyde is a major lipid peroxidant end product, and increased malondialdehyde content is an indication of the severity of isoproterenol-induced necrotic damage to the heart.[18,21] Previous studies have reported[15,16] that oral administration of matrine at the dosage of 50, 100 and 200 mg/kg for 30 days could decrease the elevation of malondialdehyde content in hypercholesterol rats either concomitant with isoproterenol injection or not. The results in this study indicate that chronic and acute administration of matrine (50, 100 and 200 mg/kg for 10 days, 100 and 200 mg/kg for two days, respectively) could decrease the level of malondialdehyde induced by isoproterenol. These data further confirmed the antioxidant action of matrine against lipid peroxidation.

Free radical scavenging enzymes, such as superoxide dismutase, catalase and glutathione peroxidase,[22] form the first-line cellular defence against oxidative stress, eliminating reactive oxygen radicals, such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), and preventing the formation of the more reactive hydroxyl radical (·OH). It is well known that isoproterenol-induced myocardial injury is mediated primarily via the β-adrenergic receptor. Acute β-adrenergic receptor stimulation not only leads to generation of reactive oxygen species, but also depletes total cellular antioxidant capacity, down-regulates copper–zinc superoxide dismutase enzyme activity, protein and mRNA, and reduces glutathione level, leading to the loss of membrane integrity and induction of heart contractile dysfunction and myocyte toxicity, finally producing myocardial necrosis.[12,23] In this study, we found that the decreased activity of superoxide dismutase, catalase and glutathione peroxidase in isoproterenol-injected rats was significantly ($P < 0.01$) elevated by matrine (Tables 3, 4). These findings suggest that matrine may considerably improve cellular defence against oxidative stress. The decreased level of malondialdehyde in heart tissue and plasma of matrine (50, 100 and 200 mg/kg)-treated rats might been due to the enhanced activity of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase). It is quite possible that the free radicals induced by isoproterenol are effectively neutralized or scavenged, as a result of the antioxidant property of matrine. However, in this study, we did not investigate the effect of matrine on entrapment, dismutation or neutralization of the free radicals generated by isoproterenol; therefore, further study is needed to clarify this mechanism.

Histopathological finding of myocardium from rats undergoing oral administration of matrine (50, 100 and

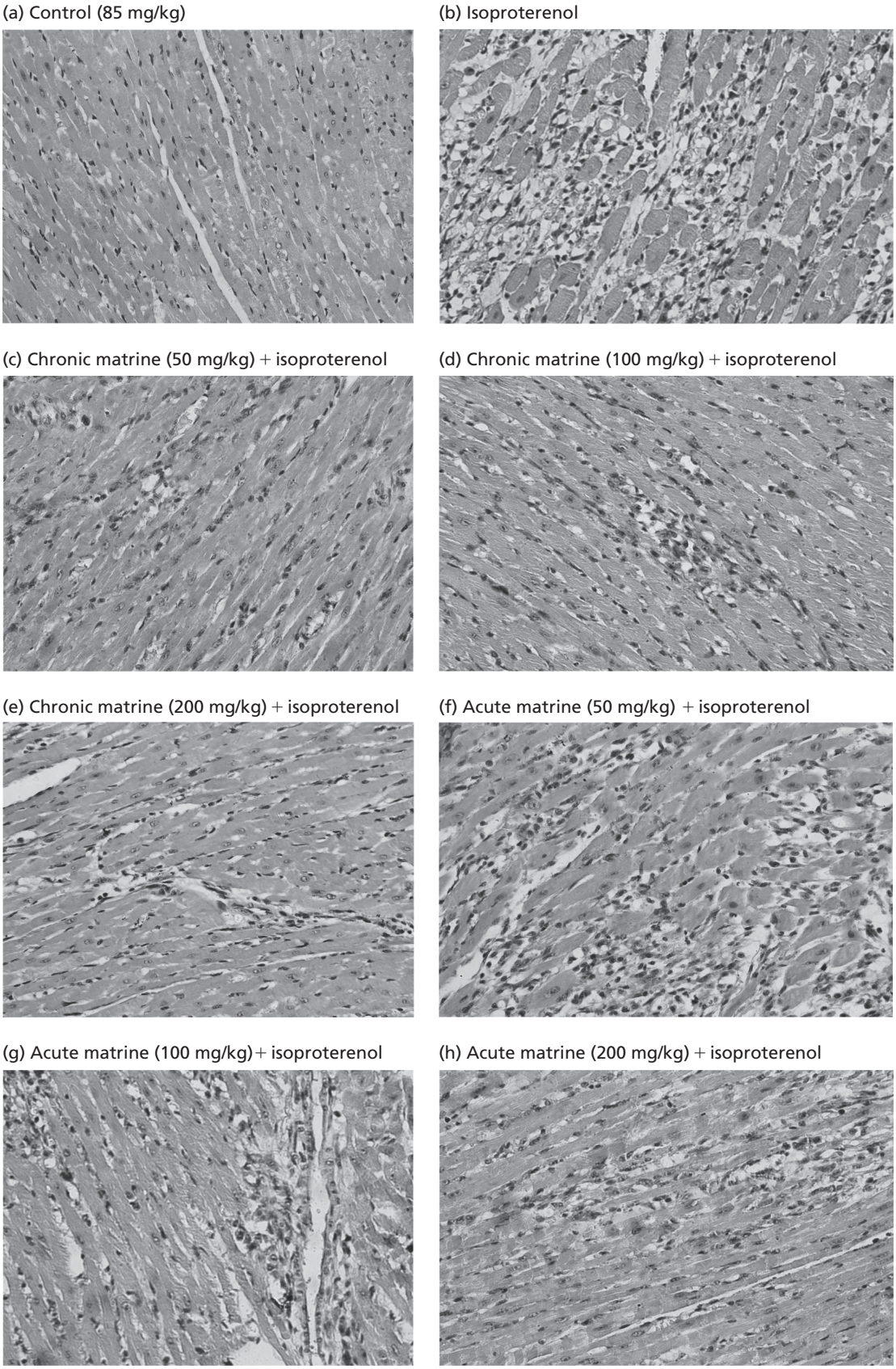

Figure 2 Histopathological changes in rat cardiac apexes. Haematoxylin and eosin ×400

200 mg/kg for 10 days, and 50, 100 and 200 mg/kg for two days, respectively) showed the preventive effect of matrine against subendocardial necrosis, infiltration of inflammatory cells and interstitial oedema seen in the myocardium of isoproterenol-treated rats (Table 5, Figure 2). These data further confirm the cardioprotective action exerted by either chronic or acute oral administration of matrine.

To understand the time-course effect of matrine on recovery from myocardial injury, study of the effect of acute and chronic matrine oral administration on isoproterenol-induced rat myocardial necrosis was incorporated into our experimental design. Our results demonstrated that chronic administration of matrine (50, 100 and 200 mg/kg, respectively) for 10 days markedly reduced the isoproterenol-induced myocardial injury; this protective effect also was seen in rats after acute matrine administration for two days at a dosage of 100 or 200 mg/kg. Our previous studies showed that oral administration of matrine for 30 days significantly attenuated myocardial ischaemia in isoproterenol-treated rats and in rats fed on a hypercholesterol diet.[15,16] Our results suggested that the protective effect of matrine on myocardial damage depends not only on the duration of administration but also on the dosage of the drug.

Conclusions

Our results suggest that matrine offers protection against isoproterenol-induced myocardial injury, characterized by decreasing cardiac marker enzymes activity, improvement of cardiac ventricular function, and attenuation of the structure of isoproterenol-induced myocardial ischaemia. The cardioprotective effect of matrine is mediated through suppression of isoproterenol-induced oxidative stress, and either acute oral administration of matrine with relatively higher dosage or chronic oral administration of matrine might be required for the cardioprotective effect to occur.

Declarations

Conflict of interest

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

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