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Effect of matrine and carvedilol on collagen and MMPs activity of hypertrophy myocardium induced by pressure overload

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Abstract: Objective: To explore the effect and mechanism of matrine (Mt.) on myocardial interstitial fibrosis induced by pressure overload. Methods: Pressure overloaded myocardial hypertrophy was produced by banding of aorta abdominalis in 67 male Sprague-Dawley rats weighing (200±15) g. The rats were assigned into one of the following groups: sham-operation control, operation control, operation group treated with matrine (15 mg/(kg·d)) and treated with carvedilol (Car.) (3.6 mg/(kg·d)) group. The rats were given drugs one day after operation. Five weeks after treatment, the left ventricular weight (LVW) was measured and the volume of myocardial cells was detected with Hematoxylin-Eosin (H-E) stain and Masson stain was used to assess the level of fibrosis of the myocardial matrix. Myocardial metalloproteinase activity was quantified with zymography, and survival rate was calculated. Results: Survival rate significantly decreased (P<0.05), LVW/BW (body weight), MMP-2 (matrix metalloproteinase-2) activity (P<0.05), size of cardiomyocytes and interstitial fibrosis obviously increased in the operation group compared with sham control group. Mt. and Car. treatment can significantly increase survival rate (P<0.05), decrease LVW/BW (P<0.05) and MMP-2 activity (P<0.05), decrease size of cardiomyocytes and interstitial fibrosis compared with operation group. But there was difference compared with sham group. Conclusion: Matrine was shown to be able to prevent cardiac remodelling of hypertrophy cardium induced by pressure overload including myocardial hypertrophy and fibrosis which may be associated with the decrease in MMP-2 activity of heart.

Key words: Matrine, Carvedilol, Myocardial, Hypertrophy fibrosis, MMP-2
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INTRODUCTION

Matrine, a monomer of traditional Chinese medicine, comes from leguminosae plants such as Kusheng, is quinoilizidine with four-loop and molecular formula of $C_{15}H_{24}N_{20}$. Matrine has been proved to have anti-arrhythmia (Xu *et al*., 2004), anti-hypoxia and decreasing heart rate effects (Zhang *et al*., 1990a; 1990b) in many animal experiments, and has the role of inducing calmness (Luo *et al*., 2001) and lowering body temperature (Tao and Wan, 1992). Traditional Chinese medicine Pharmacopoeia prescribes it for medical therapy of heart failure, for improving cardiac function and prognosis. Many documents on animal experiments proved drugs reversed heart remodelling (Cohn, 1995) through interfering with the nervous-endocrine system such as carvedilol (β-blockade) (Fung *et al*., 2003). Our purpose is to explore if matrine can affect cardiac fibrosis of hypertrophy myocardium induced by overload pressure and the causative mechanism responsible for the problem.

METHODS

Animals

Myocardial hypertrophy was established in male Sprague-Dawley rats weighting about 200 g through abdominal aorta banding partly according to the methods described by Doering *et al*.(1988). Abdominal aorta was banded by No. 7 injection pinhead with 0.7 mm diameter. The sham-operation group rats were given the same procedure except for the abdominal aorta banding.

Experimental protocol

The survival rats were arranged into four groups: sham-operation group, operation group, matrine (Mt.) and carvedilol treated (Car.) operation groups. The rats in treatment groups were given 15 mg/kg of matrine (Wang *et al*., 1996) or 3.6 mg/kg of carvedilol by gavage once per day. Mt. was present by Yanci Redbud Pharmaceutical Industries, Ltd. Ninxia, 99.6% purity material powder and carvedilol is from Roche. Rats were anesthetized with 4% hydration aldehyde chloride and the heart was arrested in diastole with 3 ml of 10% KCl injected into the carotid artery, the atria and vessels were trimmed away, LV (left ventricular) was weighed. A transversal cross-section block was cut from LV and soaked in 4% paraformaldehyde. What remained was frozen in liquid nitrogen and kept in −70 °C.

H-E and Masson stain of myocardial tissue

After 12 h in 4% paraformaldehyde, the blocks were dehydrated and embedded in paraffin, then cut into 4 μm slices which were heated overnight in 60 °C incubator, and then dewaxed and stained with H-E and Masson dye respectively. A slice chosen from each group was analyzed under 400× microscope.

MMP(s) activity determined by zymography

Extracelluar matrix metalloproteinase(s) (MMP(s)) activity was assessed according to the method described by Tyagi *et al*.(1993a; 1993b). The frozen LV tissue weighing about 100 mg was ground after it was poured into liquid nitrogen, and then put in 1 ml MMPs lyses buffer (0.3% SDS, 20 mmol/L Tris, 150 mmol/L NaCl, 0.01% Triton X-100). After centrifuging in 4 °C, the protein quantity was measured by Lowry *et al*.(1951) method. SDS-PAGE electrophoresis was used in zymography method. Glutin was added into 10% separating gel to final concentration of 1 mg/ml, and after mixing with 4× loading buffer (10% SDS, 4% sacharose, 0.25 mol/L Tris-Cl pH 6.8, 0.1% bromphenol blue), the sample was loaded into the pores of 4% condensed gel. The protein marker was loaded into the first pore (Fermentas lot 1422, molecular weight of 118000, 85000, 47000, 36000, 26000, 20000) for electrophoresis at 100 volts under 4 °C, then put the gel on ice for 60 min in 2.5% Triton X-100 for two times. The gel was incubated in incubating-solution (50 mmol/L Tris-Cl pH 8.0, 5 mmol/L $CaCl_2$, 0.02% NaN_3, 1% Triton X-100) overnight, dyed in 0.05% coomassie blue R-250 for 30 min, then washed in degraded washing solution for 3 h. The degraded density area was scanned. Sham-operation group was treated as described in reference. Actin was detected by coomassie blue at 42000.

Survival

Three days after operation, the cages were inspected every day to calculate survival rate.

Statistics

All data were expressed as mean±standard error, statistical analysis was determined using one way analysis of variance (ANOVA) followed by Student-Newman-keuls *t* test. Survival rate was analyzed by continual corrective χ^2 analysis. In all tests, differences were considered statistically significant at a value of $P<0.05$.

RESULTS

Survival analysis

In a period of 5 weeks, all rats in sham-operation group, 5 of 16 rats in Car. group, 5 of 10 rats in Mt. group, 5 of 36 rats in operation group survived, at survival rate of 31%, 50% and 14% respectively, which was analyzed by continual corrective χ^2 analysis (Table 1).

Table 1 Analysis of survival rate among groups

	Death (*n*)	Survival (*n*)	Total (*n*)	Survival rate (%)
Car.	11	5	16	31$^{\#}$
Mt.	5	5	10	50$^{\#}$
Oper.	31	5	36	14*
Sham	0	5	5	100

Sham: Sham-operation group; Oper.: Operation group; Car.: Carvedilol treated group; Mt.: Matrine treated group (Mt. vs Oper., Car. vs Oper., Sham vs Oper.); * $P<0.05$; $^{\#}$ $P<0.05$

Effect of matrine and carvedilol on cardiac remodeling

Five weeks after operation, The LVW/BW and the size of cardiomyocytes significantly increased compared with the sham operation group; the cardiac fibrosis was also obviously enhanced in the operation

group as shown in Figs.1 and 2. Matrine and carvedilol treatment significantly decreased the above indices (Table 2, Fig.3).

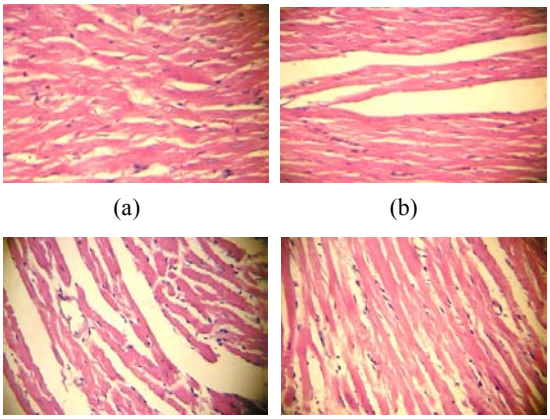

Fig.1 The size of cardiomyocytes H-E stain×400 (a) Operation group; (b) Carvedilol treated group; (c) Matrine treated group; (d) Sham group

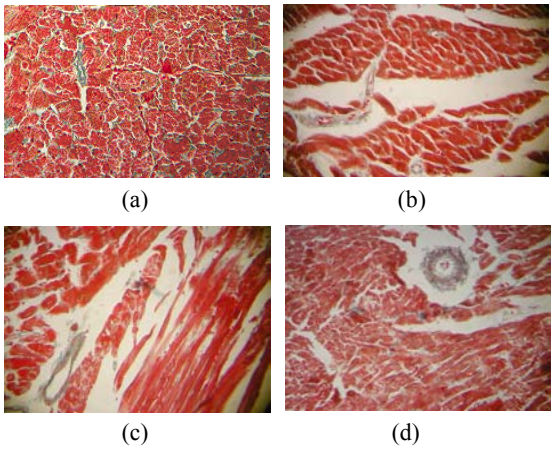

Fig.2 Cardiac fibrosis with Masson stain×400 (a) Operation group; (b) Carvedilol treated group; (c) Matrine treated group; (d) Sham group

Fig.3 Cardiac hypertrophy among matrine group (T) operation group (C) and sham group (N)

MMPs activity of myocardial tissue

Fig.4 shows that there was no change in the degrade band around 92000, which implies MMP-9, but that there was obvious change in the degrade band around 72000, which implies MMP-2. There were significant differences between Oper. group and Sham-Oper. group where MMP-2 activity became stronger ($P<0.05$). There were significant differences in two Oper.-treated groups compared with Oper. group where MMP-2 activity became weaker ($P<0.05$).

DISCUSSION

Matrine is a kind of alkaloid, coming from leguminosae plants such as Kusheng. Many animal experiments have proved its anti-arrhythmia (Li *et al*., 1996) and anti-hypoxia effects, and efficacy in rapidly slowing heart rate. Its role in inducing calmness and lowering body temperature was also proved (Zhang *et al*., 1990a; 1990b). Matrine was recently found to have the effects of anti-arrhythmias induced by aconitine, barium chloride or coronary ligation in different experimental models where the heart rate was reduced, and the P-R and QTc intervals were prolonged, and the ectopic cardiac rhythm inhibited obviously (Ai *et al*., 2000).

Matrine delay action potential duration (APD) can possibly be due to blocking of the myocardial K^+ channel (Xu *et al*., 2004). The inhibitory actions of matrine on I_{K1}, I_{Kr}, I_{Ks}, and I_{to} could promote inflow of Ca^{2+} without releasing of intracellular [Ca^{2+}], and at the same time decreasing intracellular [Ca^{2+}] while intracellular [Ca^{2+}] increases (Xie *et al*., 2004). Study on the relationship of diuresis and the pharmacokinetics of matrine, showed that the diuresis effect of matrine in rabbits is relatively weakened after its decomposition in blood (Song *et al*., 2001). Matrine was shown to become analgesia in the brain after inflow of Ca^{2+} and decrease of NO (Luo *et al*., 2001).

In vitro study showed that matrine could improve cardiovascular remodelling through inhibiting the calcium overload of vascular smooth muscular cell stimulated by Angiotensin II and partly blocking the intracellular signal transducing pathway (Li *et al*., 2000). Matrine can inhibit cardiac fibroblast proliferation induced by Angiotensin II in rats and the cellular cycle of cardiac fibroblast (Zhou *et al*., 2004)

Table 2 Effect of matrine and carvedilol on left ventricular hypertrophy (mean±*SE*, *n*=5)

Weight	Group			
	Sham-Oper.	Mt.-Oper.	Car.-Oper.	Oper.
LVW (mg)	630±5	690±4	670±1	780±6
BW (g)	310±28	308±15	249±19	26±16
LVW/*BW* (mg/g)	2.01±0.09	2.34±0.23$^{\#}$	2.54±0.19$^{\#}$	2.94±0.27*

Sham-Oper.vs Oper., Oper.vs Mt.-Oper., Oper.vs Car.-Oper.; * P<0.05; $^{\#}$ P<0.05

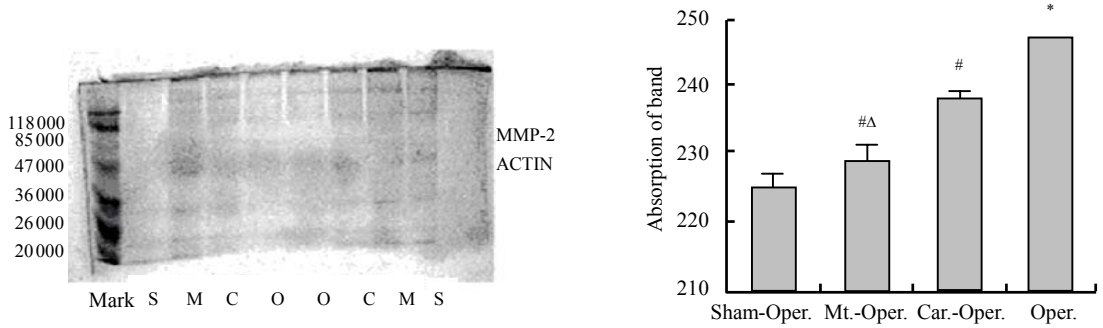

Fig.4 (a) The differences of degrade bands of 2 groups: Mark, Sham-Oper., Mt.-Oper., Car.-Oper., Oper., Oper., Car.-Oper., Mt.-Oper., Sham-Oper.; (b) The differences of degrade bands of four groups, *n*=5, mean±*SE* Sham-Oper. (224.68±1.73) vs Oper. (244.66±0.47), Oper. vs Mt.-Oper. (228.37±2.02), Oper. vs Car-Oper. (236.51±0.52), Mt.-Oper. vs Car.-Oper.; * P<0.05, compared with Sham-Oper. and Oper.; $^{\#}$ P<0.05, compared with Oper.and Oper.-treated; $^{\Delta}$ P<0.05, compared with two-treated

and nuclear antigen expression of proliferating cells induced by aldosterone (Hu *et al*., 2004). In vivo study on the role of matrine in cardiac remodelling has not been reported.

Myocardial hypertrophy is an independent risk factor. Cardiac remodelling induced by pressure overload includes myocardial hypertrophy and interstitial fibrosis. Cardiac fibroblast proliferation and extensive extracelluar collagen deposition are involved in myocardial interstitial fibrosis. Hypertrophy, apoptosis, death of cardiomyocytes, change of collagen content and level, structure turbulence of myocardial matrix, are caused by change of molecular level of many factors, then finally lead to change of pathological structure, which is also called ventricular remodeling (Leon, 2003). It is accepted that activated nervous-endocrine persistently cause myocardial remodeling (Weber, 2000).

Many worldwide reports on animal experiments proved β-blockade reverses heart remodelling through interfering with the nervous-endocrine system. Recently β-blockade has been proved effective in reducing death and complications, improving symptomatic status in chronic heart disease in worldwide large-scale, multi-center clinical trials involving carvedilol (NCT00004854. Sponsored by National Center for Research Resources). Heart failure induced by pressure overload due to aortic banding in rats is a commonly accepted animal model (Weinberg *et al*., 1994). It has been reported that light microscope observation showed that collagen stain around arterial and inter-myocardial tissue was significantly enhanced 4 weeks after operation (Wang *et al*., 2000).

Our study found that survival rate significantly increased (P<0.05), LVW/BW (P<0.05), and that size of cardiomyocytes and interstitial fibrosis obviously decreased in operation group compared with sham control group. Mt. and Car. treatment can significantly increase survival rate (P<0.05), decrease LVW/BW (P<0.05) and size of cardiomyocytes and interstitial fibrosis compared with operation group. But there was difference compared with sham group.

In order to explore the mechanism, MMP(s) were quantified by zymography (Tyagi *et al*., 1993a; 1993b). These enzymes, which degrade collagen in vivo, are mainly MMP(s), involved MMP-1, MMP-2, MMP-3, etc. Glutin was added into 10% separating gel, which is a substance that MMP-2 and MMP-9 act on. Among the MMP(s), MMP-2 and MMP-9 may

play important roles during LV remodelling. In our study, there were significant differences in Oper. group and Sham-Oper. group: where MMP-2 activity became stronger ($P<0.05$). There were significant differences in two Oper.-treated groups compared with Oper. group: where MMP-2 activity became weaker ($P<0.05$). The results suggested matrine and carvedilol prevented fibrosis of pressure overloaded myocardial hypertrophy respectively, and its mechanism may relate with MMP-2 activity of the myocardial matrix.

CONCLUSION

It was shown that matrine can prevent cardiac remodelling of hypertrophy cardium induced by pressure overload including myocardial hypertrophy and fibrosis which may be associated with the decrease in MMP-2 activity of the heart.

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