Antiarrhythmic Properties of Long-Term Treatment with Matrine in Arrhythmic Rat Induced by Coronary Ligation

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Matrine, a monomer of traditional Chinese medicine Sophora flavescent, is a potential drug for treatment of arrhythmia. The aim of the study is to elucidate the protective effects of matrine on arrhythmic rat induced by myocardial infarction (MI) and further explore underlying targets. Experiments were performed to investigate the effects of long-term oral administration of matrine on coronary ligation induced arrhythmia, measured in whole animals, via surface electrocardiogram (ECG). Whole-cell patch-clamp technique was used to record the action potential and potassium ionic currents in myocytes isolated from rat hearts. The cytoplasmic free Ca2+ concentration ([Ca2+]i) was measured using the scanning confocal microscopy. Mortality rate was 19/30 (63%) in MI group and 10/30 (33%) in matrine group (p<0.05). This represented a 1.9-fold reduction in long-term mortality rate. The prolonged action potential duration (APD) induced by MI were significantly shortened by long-term treatment of matrine. Matrine restored Kv4.2/I1, Kir2.1/I4 in rat ventricular myocytes after MI. Abnormally decreased [Ca2+]i, mediated by ischemia can be recovered by matrine. Our results suggested that long-term oral administration of matrine reduced arrhythmia and mortality. Electrophysiological experiment revealed that long-term matrine treatment played an important role in anti-arrhythmia through ionic mechanism. Knowledge of matrine from this work may provide insight into the development of new drugs for long-term myocardial infarction treatment.

Key words matrine; arrhythmia; potassium current; myocardial infarction; intracellular calcium

Matrine, an effective monomer extracted from Sophora flavescent, is one of traditional Chinese medicine. In Chinese clinical practice, it has been well documented that matrine produced a variety of biological actions, such as anthelmintic activity and anti-tumor mechanism.1—3) Recently matrine has anti-arrhythmic activity on different experimental models of arrhythmias induced by aconitine, barium chloride or coronary ligation.4—7) However, what underlying mechanisms are involved in its effectiveness is still poorly understood.

Patients experiencing a myocardial infarction (MI) are at increased risk for reinfarction, remodeling, heart failure, and sudden cardiac death in the post-infarction period. Post-infarction remodeling is an important factor for triggering ventricular arrhythmias. Patients with more extensive ventricular remodeling are at greater risk for cardiovascular fatalities. Most of these are thought to be due to ventricular tachycardia (VT) or fibrillation (VF).8,9) At present, majority of anti-arrhythmic drugs are not able to decrease mortality in these patients. On the contrary, certain drugs may actually increase rather than decrease the risk of sudden cardiac death. The observations in the cardiac arrhythmia suppression trial (CAST) led to the abandonment of some anti-arrhythmic drugs for treatment of post-MI patients. Now, most of anti-arrhythmic agents, except for amiodarone and beta-blockers, are relatively contraindicated in post-MI patients.

The question arose as to whether the long-term effects of matrine could stem from its prevention for remodeling or other mechanisms. We measured the electrophysiological changes in action potential duration (APD), inwardly rectifying potassium current (I_K1), transient outward potassium current (I_o) density, and cytoplasmic Ca2+ concentration of rat ventricular myocytes derived from 3 months after myocardial infarction. In this study, matrine is the promising drug for the treatment of long-term consequences of remodeling and reducing the risk of sudden cardiac death secondary to ventricular arrhythmias.

MATERIALS AND METHODS

Reagents Amiodarone hydrochloride (Sigma Chemical Co., U.S.A.) was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution. DMSO at 0.1% had no significant effect on ventricular myocytes. Matrine (Kangjiu Chemical Co., China) was dissolved in physiological saline to make a stock solution, which were further dissolved in phosphate buffered solution to proper concentration.

Rate Model of Myocardial Infarction One hundred and fifty adult male Wistar rats weighing 200—250 g were randomly divided into five groups: sham-operated group, myocardial infarction (MI), MI rat treated with matrine (Mat+MI 15 mg/kg) and (Mat+MI 30 mg/kg), MI rat treated with amiodarone (Amio+MI 50 mg/kg), respectively. Matrine and amiodarone were administrated 7 d before the experiments, respectively.10,11) The animals were anesthetized with sodium pentobarbital (40 mg/kg) via intraperitoneally (i.p.) then underwent ligation of the left anterior descending (LAD) coronary artery, as described previously.12) Sham-operated rats were handled in the same manner except that the coronary artery was not ligated. After surgery, rats were given antibiotics, and housed in a climate-controlled environment at an ambient temperature of 20 °C with a 12 h light/dark cycle. After operation, Mat+MI and Amio+MI group rats were treated with intragastric administration of matrine (30 mg/kg) and amiodarone (50 mg/kg) daily for continuous 3 months, respectively. Animal experiments were performed in accordance with institutional guidelines for animal use in research. The...
standard limb lead II ECG was recorded for 2 h after operation. The incidence of arrhythmias and the survival rate were registered and evaluated, and the criteria of arrhythmias was very similar to that described previously, as follows: 0 = no arrhythmia; 1 = < 10 s premature ventricular contraction (PVC) and/or VT; 2 = 11—30 s PVC and/or VT; 3 = 31—90 s PVC and/or VT; 4 = 91—180 s PVC and/or VT, <10 s reversible VF; 5 = > 180 s PVC and/or VT, >10 s reversible VF, 6 = irreversible VF. In all experiments, irreversible ventricular fibrillation was the only cause of death.

**Measurements of Hemodynamic Function** Three months after MI, rats were anesthetized for terminal study. Left ventricular end diastolic pressure (LVEDP), and time derivatives of pressure were measured during contraction (+dP/dt) and relaxation (−dP/dt) recorded on a polygraph as described previously.

**Isolation of Ventricular Myocytes** Single ventricular myocyte was isolated enzymatically as previously described in detail. Three months after surgery rats of four groups were anaesthetized and the hearts were quickly removed. Then hearts were rapidly washed in cool, oxygenated Tyrode solution in mM: 126 NaCl, 5.4 KCl, 10 N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid (HEPES), 0.33 NaH2PO4·2H2O, 1.0 MgCl2·6H2O, 1.8 CaCl2 and 10 glucose, pH was adjusted to 7.4 with NaOH, and mounted to a Langendorff perfusion apparatus and perfused retrogradely via the coronary circulation. The heart was perfused with standard Tyrode’s solution for 5 min, then switched to Ca2+-free Tyrode’s solution until it stopped beating, followed by perfusion with the same solution containing collagenase II (7.0 mg · 50 ml−1) and BSA. The peri-ischemic zone ventricular tissue was shaved and minced in the storage solution and filtered. The freshly isolated myocytes were gently centrifuged and resuspended in the KB medium (in mM: 70 glutamic acid, 15 taurine, 30 KCl, 10 KH2PO4, 10 HEPES, 0.5 MgCl2·6H2O, 1.8 CaCl2 and 10 glucose and 0.5 EGTA; pH 7.4 with KOH). All solutions were gassed with 100% oxygen and warmed to 37 ± 0.5 °C. Only single rod-shaped, Ca2+-tolerant, and quiescent cell with clear cross-striation was selected for electrophysiological recording and intracellular calcium measurement.

**Whole-Cell Patch-Clamp Recording** Currents were recorded in the whole-cell voltage-clamp mode and action potentials (APs) were recorded in the current-clamp mode, with an Axopatch-200B amplifier (Axon Instruments). Patch-clamp techniques have been described in detail elsewhere. To measure K+ currents, borosilicate glass electrodes had tip resistance of 2—4 MΩ when filled with pipette solution. For K+ currents and action potential recordings, myocytes were superfused with a standard Tyrode solution. The pipette solution for K+ current recordings contained in mM: 20 KCl, 110 potassium aspartate, 1 MgCl2, 5 Na2-ATP, 10 EGTA, and 10 HEPES, pH adjusted to 7.20 with KOH. The internal pipette solution for APs recording contained the same components as for K+ currents recording, except that EGTA was reduced to 0.05 mM. For outward K+ current recordings, NaCl was replaced by choline chloride in the external solution to eliminate the fast Ii,sc. CdCl2 (1.0 mM) was added to the external solution to inhibit Ii,sc. The capacitance and series resistance (Rs) were compensated. Recordings were low-pass filtered at 1 kHz. Junction potentials were zeroed before formation of the membrane-pipette seal in Tyrode solution. All experiments were performed at room temperature (19—21 °C).

**Western Blot** To determine the protein expression level of Kir2.1 and Kv4.2, we performed standard Western blot techniques as described previously by our laboratories. Briefly, equal amounts of protein (60 µg) were subjected to 10% SDS-PAGE and blotted to nitrocellulose. The blots were blocked in 5% nonfat milk dissolved in PBST for 3 h, then probed with Kir2.1 (Santa Cruz) and Kv4.2 (Alomone) antibodies, or GAPDH (Kangcheng Inc.) in PBST, and reacted with the second antibody (Alexa Fluor) in PBST. Immunoreactive bands were captured by the Odyssey System (LI-COR Bioscience, Lincoln, U.S.A.). Western blot bands were quantified using Odyssey v1.2 software and normalizing GAPDH as an internal control.

**[Ca2+]i Measurement** [Ca2+]i fluorescence measurement in cardiomyocytes has been described previously. The peri-ischemic zone ventricular myocytes derived from sham-operated rats were referred to as sham cells whereas those derived from MI rats were referred to as MI myocytes. Isolated single ventricular myocytes were adhered then rinsed with normal Tyrode’s solution and incubated with a working solution containing Fluo-3/AM (20 µM) and Pluronic F-127 (0.03%) at 37 °C for 45 min. The fluorescent change of the Fluo-3/AM loaded cell was detected by confocal laser scanning microscope (Fluview-FV300, Olympus, Japan) with 488 nm for excitation from an Argon ion laser and 530 nm for emission under inverted microscope with 20× objective. All [Ca2+]i measurements were made under steady-state conditions stimulating by KCl. Sixty micromolar KCl was added between 2nd and 3rd scan and the fluorescent intensities before (FI0) and after (F1) drug administration were both recorded. The change of [Ca2+]i was represented with ratio of fluorescence intensity (FI/F1). Area under the curve of [Ca2+]i values were determined using the “Area Below Curves” function in Sigma Plot 9.0. Only the cell with rod shaped and visible striations was used for experiments.

**Statistical Analyses** Data are presented as the mean ± S.E.M. and analyzed by GraphPad Prism 5.0, pCLAMP 8.0 and Sigmaplot 9.0 software. Chi-square analysis was used to compare incidence of arrhythmia and mortality among different groups. Kruskal–Wallis analysis was used to compare arrhythmia score. Statistical comparisons (performed using analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison tests or Dunnett’s method) were used as appropriate to evaluate data among groups. A two-tailed p < 0.05 was considered to be a statistically significant difference.

**RESULTS**

**Long-Term Effects of Matrine and Amiodarone on Arrhythmic Rats Induced by MI** Electrical disorders were observed after operation in 2 h. Arrhythmias, mainly of VT defined as a run of rapid ventricular deflections lasting longer than 30 s and reversible VF, was observed in the MI rats (Fig. 1A). The VT often predisposed to VF leading to sudden death. The ECG clearly showed runs of polymorphic VT and VF in MI rats. Cumulative arrhythmic durations and arrhythmia scores in MI group were significantly higher than those
in sham-operated group \((p<0.05, n=30)\). However, both of parameters in Amio+MI 50 mg/kg and Mat+MI 30 mg/kg groups were decreased compared with those in MI group (Figs. 1B, C). But, matrine 15 mg/kg had no obvious effect on arrhythmia score and arrhythmia duration in MI rats. Mortality were comparable among long-term MI, Amio+MI, Mat+MI 30 mg/kg animals (63%, 37%, 33%, \(n=30\), respectively). Mortality in MI group was increased obviously. As illustrated in Fig. 1D, both matrine and amiodarone reduced the incidence of sudden death in myocardial infarction rats (\(p<0.05, n=30\)).

**In Vivo Hemodynamic Parameters** Hemodynamic parameters measured in the four groups of rats were shown in Table 1. Compared with the sham animals, heart rate (HR) was significant decreased in MI group. Most dramatic changes were seen in left ventricular end diastolic pressure (LVEDP), \(+dP/dt\) and \(-dP/dt\) values of MI rats. As shown in Table 1, matrine 30 mg/kg and amiodarone treatment significantly lowered LVEDP in rat after MI \((p<0.05, n=8)\). The increase in LVEDP and the decrease both in \(+dP/dt\) and \(-dP/dt\) were also prevented in the Mat+MI group. Matrine improved the hemodynamic function of long-term ischemic hearts.

**Effects of Matrine and Amiodarone on Action Potential Duration Prolongation Induced by MI in Rat Ventricular Cell** Action potentials were recorded in the current-clamp mode. APD at 50% and 90% repolarization (APD\(_{50}\) and APD\(_{90}\)) were used to describe APD changes. APD\(_{50}\) (50.0±1.6 ms) and APD\(_{90}\) (65.3±1.9 ms) in long-term MI cells were all significantly prolonged \((p<0.05, n=18)\). Matrine significantly shortened APD\(_{50}\) and APD\(_{90}\) compared with MI and, and APD were nearly recovered to the normal value in Mat+MI group (data not shown), while amiodarone prolonged APD at 50% and 90% repolarization in MI rats (Fig. 2B, \(p<0.05, n=18\)).

**Long-Term Effects of Matrine and Amiodarone on Transient Outward \(I_{to}\) and Kv4.2 Protein** \(I_{to}\) was the predominant repolarizing potassium current in rat ventricular myocytes. \(I_{to}\) representative current traces from sham, MI, Amio+MI, and Mat+MI groups were shown in Fig. 3A. Peak current density \((I_{peak})\) was reduced in MI myocytes compared with that in sham myocytes. Treatment with 50 mg/kg amiodarone inhibited \(I_{peak}\), however, treatment with 30 mg/kg matrine restored \(I_{peak}\) to levels observed in sham-operated controls. \(I_{to}\) density–voltage relationship was summarized in Fig. 3B. \(I_{to}\) density at +40 mV was significantly reduced from 17.94±1.41 pA/pF \((n=19)\) in sham myocytes.

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**Table 1. Long-Term Effects of Matrine and Amiodarone on Hemodynamic Parameters during MI**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham</th>
<th>MI</th>
<th>Amio+MI</th>
<th>Mat+MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>351±9</td>
<td>328±10*</td>
<td>319±7</td>
<td>340±9</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>7.7±0.2</td>
<td>27.6±0.6*</td>
<td>19.4±0.4*</td>
<td>8.0±0.5*</td>
</tr>
<tr>
<td>(+dP/dt) (mmHg/s)</td>
<td>3965±42</td>
<td>2497±53</td>
<td>2578±34</td>
<td>3274±27*</td>
</tr>
<tr>
<td>(-dP/dt) (mmHg/s)</td>
<td>3238±63</td>
<td>2105±46*</td>
<td>2497±55</td>
<td>2035±31*</td>
</tr>
</tbody>
</table>

HR: heart rate; LVEDP: left ventricular end diastolic pressure; \(dP/dt\): time derivatives of pressure. Each value represents a mean±S.E.M. One-way ANOVA followed by Bonferroni’s post-hoc test. *\(p<0.05\) versus sham group, **\(p<0.05\) versus MI group, \(n=8\).
to 15.43±0.77 pA/pF in MI myocytes (n=18, p<0.05). \(I_\text{to}\) density was decreased to 10.54±1.41 pA/pF in rat treated with 50 mg/kg amiodarone 3 months (n=18, p<0.05), but treatment with 30 mg/kg matrine produced a marked increase in \(I_\text{to}\) density to 19.28±0.74 pA/pF (n=16, p<0.05) compared with MI myocytes. Kv4.2 level was indeed significantly reduced in MI rats compared to sham rats (\(p<0.05\)). Western blot analysis indicated that the level of Kv4.2 was decreased in peri-ischemic zone ventricular cardiomyocytes of MI rat. More potent than from sham group, \(p<0.05\) versus MI group.

**Long-Term Effects of Matrine and Amiodarone on \(I_{K1}\) and Kir2.1 Protein** Changes of \(I_{K1}\) were obtained from sham, MI, Amio+MI, and Mat+MI groups. Our data showed that the reduction of \(I_{K1}\) current density was confirmed in ventricular cardiomyocytes of MI rat. More potent \(I_{K1}\) reduction was also observed in Amio+MI group at test potential from −120 to −90 mV (n=17, \(p<0.05\)), however, in sharp contrast matrine restored the \(I_{K1}\) reduction to the level observed in sham rats (n=16, \(p<0.05\), Fig. 4). The inwardly rectifying potassium channel Kir2.1 subfamily members primarily mediate cardiac \(I_{K1}\), Kir2.1 being the major component in both cardiac chambers. Figure 4C showed Western blot analysis of membrane protein Kir2.1. Kir2.1 level was significantly reduced in MI rats compared to sham rats (n=8, \(p<0.05\)). Matrine can partly recover the expression of Kir2.1 protein. There was no significantly difference in Amio+MI and MI group.

**Effect of Matrine and Amiodarone on Decreasing [Ca^{2+}]_i Induced by MI** The [Ca^{2+}]_i was further investigated in Fluo-3/AM loaded cardiac myocytes and cells were stimulated with KCl in standard Tyrode’s solution from different four groups (Fig. 5). Intracellular calcium overload has been considered an underlying mechanism of cardiac myocytes injury during early ischemia/reperfusion. While in MI myocytes, the response of myocytes in four groups to KCl stimulation protocol. Mean data from both MI (n=16), Amio+MI (n=18), and Mat+MI (n=16) group are significantly different from sham group (n=19). \(p<0.05\) versus sham group, \(p<0.05\) versus MI group. (C) Long-term effect of matrine and amiodarone on Kir2.1 protein expression. Left: Kir2.1 protein levels from 4 group animals (n=8), data were normalized to GAPDH. Right: representative image for Kir2.1 and GAPDH. \(p<0.05\) versus sham group, \(p<0.05\) versus MI group.
electrical activity and shaped by different ion channel currents and ion transporters. The prolongation of action potential duration and ventricular arrhythmias are the characteristics of normal-zone tissues from post-infarction rat hearts. After MI, the noninfarcted myocardium undergoes significant hypertrophy and this hypertrophic response of remodeled myocardium provokes an arrhythmogenic substrates, including APD prolongation, regional differences in APD, and increased tendency to early after depolarization (EAD)-triggered activity and reentrant tachyarrhythmias. Inhibiting current by K+ channels controls the early repolarization phase 1. A reduction in the amplitude of I_{to} may contribute to action potential prolongation and arrhythmia in heart failure. It was reported that the prolongation of APD in the hypertrophic myocytes from rats after MI was due partly to the reduction of current density of both I_{to} and I_{ks}. These were similar to our results, moreover, Western blot analyses revealed a reduction of Kv4.2 protein in MI. Thus the decreased K+ channel expression was largely responsible for the prolonged APD. Cardiac I_{K1} current is a strongly inwardly rectifying K+ selective current and plays an important role in shaping the normal action potential. The cardiac I_{K1} stabilizes the resting membrane potential and is responsible for shaping the initial depolarization and final repolarization of the action potential. Studies show I_{K1} plays a role in ventricular arrhythmias, highlighted by the recently described Andersen’s syndrome and studies in the guinea pig heart model of ventricular fibrillation. The results of the present study indicated that I_{K1} current density and the level of Kir2.1 protein were downregulated by MI, which was in accordance with previous studies.

We further focused on the properties and effects of matrine and amiodarone on post-infarction rat hearts. The most striking finding of our study is that matrine obviously restored the decreased I_{K1} and I_{K1}/Kir2.1 protein in post-MI rat ventricular myocytes, suggesting that it may be one of likely mechanisms that matrine modulates electrical remodeling. However, long-term treatment of amiodarone, a class III anti-arrhythmic agent, not only reduced I_{to} but also inhibited I_{to}, I_{K1} in MI rats. Matrine corrected prolongation of APD perhaps by restoring the decreased I_{to} and I_{K1} in MI rats ventricular myocytes, while amiodarone failed to do so. This may explain the reason why long-term treatment matrine can reduce cardiovascular mortality significantly.

The prolonged APD in post-MI myocardium could be explained by the marked decrease in the density of the two outward K+ currents (I_{K1} and I_{to}) rather than by changes in the density or kinetics of the L-type Ca2+ current. These results suggested that K+ channel down-regulation is the primary determinant of APD. Furthermore, the prolonged APD provides longer time frame for Ca2+ influx and Ca2+ release from sarcoplasmic reticulum (SR), which may take part in unbalance of the Ca2+ handling. And impaired Ca2+ uptake by SR in hypertrophic cells favors the occurrence of DADs, which associated with increased incidences of arrhythmias in post-MI rats.

Cytosolic calcium overload often occurs in acute myocardial ischemia/reperfusion injury. Impaired Ca2+ uptake by SR and decay of Na+–Ca2+ exchange current induced the [Ca2+]i overload and contractile abnormalities. In our study
was reduced in long-term MI cardiac myocytes compared with sham group. It was supported by the peak systolic
\([Ca^{2+}]\), that was depressed in the MI compared with the control groups, and the \([Ca^{2+}]\) transient that was suppressed in post-MI myocytes.28–31 \([Ca^{2+}]\) transients from myocytes of heart failure were significantly smaller and decayed more slowly than those from normal hearts. \(Ca^{2+}\) uptake rates by the SR and the amount of \(Ca^{2+}\) stored in the SR were significantly reduced in myocytes of heart failure.32 The reduced \(SR Ca^{2+}\) content may be largely responsible for the smaller \([Ca^{2+}]\), transient in myocytes.31,32 High concentration of \(KCl\) causes a membrane depolarization which leads to an increased \(Ca^{2+}\) influx. When \(KCl\) depolarization, \(Ca^{2+}\) released from SR was diminished in rat myocytes after post-infarction.

Normal intracellular \(Ca^{2+}\) handling in cardiomyocytes is maintained by multiple proteins including L-type \(Ca^{2+}\) channel, \(Na^{+}/Ca^{2+}\) exchanger (NCX), ryanodine receptor 2, \(Ca^{2+}\)-ATPase.32,33 We have no enough evidence that matrine influences \(Ca^{2+}\) efflux activity, however, matrine might ex- trude excessive intracellular \(Ca^{2+}\) through NCX have and protective effect against arrhythmias. Moreover the present study shows that matrine increases contractile reserve in myocytes of post-infarction. Matrine could recover the decreased \([Ca^{2+}]\), in myocytes from 3 months MI rats, which might be one of the mechanism of their protective effects on the heart, while amiodarone failed to restore the decreased \([Ca^{2+}]\). We concluded that amiodarone partially blocked \(Ca^{2+}\) channel and \([Ca^{2+}]\), was depressed in post-infarction myocytes. Matrine elevated \([Ca^{2+}]\), in normal guinea pig myocytes, and increased the effect on \(I_{Ca-L}\) channel, moreover moderately prolonged APD.4,6,7,34 It was likely that matrine increased \([Ca^{2+}]\), by elevating the influx of \(Ca^{2+}\) through L-type \(Ca^{2+}\) channel. Our observation supported the hypothesis that matrine administration had direct beneficial effects on contractile function in myocytes from 3 months myocardial-infarction rats.

In conclusion, this anti-arrhythmic profiles of matrine differed from those of class III drugs amiodarone. These results indicated that long-term matrine treatment improved cardiac function in myocardial-infarction heart not only by restoring the reduced \(I_{Na}\) but also by recovering the decreased \(I_{K1}\) in myocardial-infarction cardiomyocytes, and by restoring the depressed amplitude of \([Ca^{2+}]\) in MI myocytes as well. The new insights that we have obtained from this study will help in the development of safer and more effective drugs.

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REFERENCES