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RESEARCH ARTICLE

Magnesium isoglycyrrhizinate inhibits L-type Ca^{2+} channels, Ca^{2+} transients, and contractility but not hERG K^+ channels

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Abstract To explore the cardiovascular protective effects of Magnesium isoglycyrrhizinate (MI), especially the underlying cellular mechanisms related to L-type calcium channels and myocardial contractility, and to examine the effects of MI on hERG K^+ current expressed in HEK293 cells. We used the whole-cell patch clamp technique, video-based edge detection and dual excitation fluorescence photomultiplier systems to explore the effect of MI on L-type Ca^{2+} currents ($I_{Ca\text{-}L}$) and cell contraction in rat cardiomyocytes. We also examined the rapidly activating delayed rectifier potassium current (I_{Kr}) expressed in HEK293 cells using a perforated patch clamp. MI inhibited $I_{Ca\text{-}L}$ in a dose-dependent manner, with a half-maximal inhibitory concentration (IC_{50}) of 0.22 mg/ml, and the maximal inhibitory effect was 61.10 ± 0.59%. MI at a concentration of 0.3 mg/ml reduced cell shortening by 24.12 ± 3.97% and the peak value of the Ca^{2+} transient by 36.54 ± 4.96%. MI had no significant influence on hERG K^+ channels expressed in HEK293 cells at all test potentials. MI exerts protective effects on the heart via the inhibition of $I_{Ca\text{-}L}$ and cell shortening in rat cardiomyocytes. However, MI had no significant influence on I_{Kr}; thus, MI may exert cardioprotective effects without causing drug-induced long QT syndrome.

Keywords Magnesium isoglycyrrhizinate · L-type Ca^{2+} currents · Myocyte shortening · Ca^{2+} transient · hERG K^+ channels · Rat cardiomyocyte

Introduction

The root of *Glycyrrhiza uralensis* Fisch (*Glycyrrhiza glabra* L. Leguminosae) is called licorice and is a native herbaceous perennial legume with a sweet flavor. It usually grows in southern Europe and parts of Asia. In addition, licorice is a traditional herbal remedy that has long been used worldwide in herbal and folk medications. Previous studies demonstrated the potential protection of licorice against ischemia–reperfusion injury (I–R) in the heart, and the results clearly demonstrated that licorice mediates protective effects against myocardial infarction through the amelioration of oxidative stress (Ojha et al. 2013). The active components of Radix Glycyrrhizae Preparata (Zhi Gancao, a licorice decoction) can markedly lower the incidence of cardiac triggered activity, protect the myocardium from injury and decrease the incidence of arrhythmia induced by I–R in rats (Li et al. 2003). A serum containing Radix Glycyrrhizae Preparata decreases the L-type Ca^{2+} currents ($I_{Ca\text{-}L}$) in isolated rabbit ventricular myocytes in a concentration-dependent manner, which may be the mechanism of action by which the preparation treats arrhythmia (Zhou et al. 2007).

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Magnesium isoglycyrrhizinate (MI) is a saponin, which is alkali-catalyzed, isomerized, salified, and refined from glycyrrhizic acid extracted from the licorice (Xing et al. 2003). The structure of MI is shown in Fig. 1. MI has been shown to possess anti-inflammatory and hepatoprotective activity (Dong et al. 2006a). In addition, previous studies demonstrated that MI exerts positive effects on multiple types of organ damages and diseases, such as lung injury, alcoholic liver disease induced by paraquat poisoning (Xiao et al. 2014), and epithelial ovarian cancers (Chen et al. 2014). However, the cardiovascular protective effects of MI, especially the underlying cellular mechanisms, remain less studied.

Increasing the levels of intracellular Ca^{2+} ($[Ca^{2+}]_i$) can aggravate myocardial contraction and promote related pathological changes, such as apoptosis (Chen et al. 2005) and hypertrophy (Frey and Olson 2003). The main feature of the cardiomyocyte response to ischemic myocardial diseases is increased contractility (Bristow et al. 1985). Moreover, drugs that suppress cardiac contractility and L-type calcium channels (LTCCs) can effectively protect against myocardial ischemic injury. Previous studies suggested that the alteration of LTCC properties, in addition to rapidly activating delayed rectifier potassium current (I_{Kr}) antagonistic activity, should be considered when evaluating the proarrhythmic potential of drugs (Kim et al. 2016). In ventricular myocytes in the heart, the suppression of the cardiac delayed rectifier K^+ current causes long QT syndrome (LQTS), which is a disease with a high risk of developing lethal ventricular tachyarrhythmias (Martin et al. 2012). However, calcium antagonists were reported to have the potential to block I_{Kr} (Chouabe et al. 1998), indicating that calcium antagonists may increase the risk of LQTS. Therefore, the assessing I_{Kr} inhibition is useful for the evaluation of drugs that are suspected of causing LQTS. We examined the effects of MI on $I_{Ca\text{-}L}$ in rat cardiomyocytes and I_{Kr} expressed in HEK293 cells using the whole-cell patch-clamp technique. We observed contractility and Ca^{2+} transients in rat cardiomyocytes using video-based edge detection and dual excitation fluorescence photomultiplier systems.

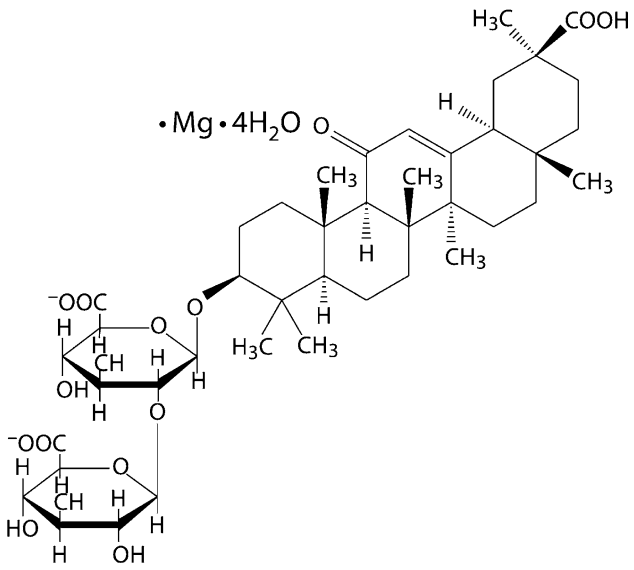

Fig. 1 Chemical structure of MI

Materials and methods

Drugs and reagents

MI was obtained from NIFDC (Batch: 100879-201302, Purity 90.8%, Beijing, China). $MgCl_2$ was obtained from Sigma-Aldrich (Batch: WXBB1001V, Purity 98%, St. Louis, MO, USA). Collagenase type II was obtained from Worthington Biochemical (Batch: 45A15451, Lakewood, NJ, USA). Verapamil (Ver) was obtained from Cayman Chemical (Batch: AS402230, Purity ≥98%, Ann Arbor, MI, USA). Unless otherwise mentioned, all the other chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents used in this study were of analytical purity.

The Ca^{2+}-free Tyrode's solution included 135 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 0.33 mM NaH_2PO_4, 10 mM glucose and 10 mM HEPES, and the pH was adjusted to 7.4 with NaOH. The enzyme solution was made with the Ca^{2+}-free Tyrode's solution and 0.6 mg/ml of collagenase type II, 0.5 mg/ml of bovine serum albumin and 30 μM $CaCl_2$. The Kreb's buffer (KB) solution included 10 mM HEPES, 40 mM KCl, 25 mM KH_2PO_4, 80 mM KOH, 20 mM taurine, 50 mM glutamic acid, 1 mM EGTA, 10 mM glucose and 3 mM $MgSO_4$ (pH adjusted to 7.2 with KOH). The intracellular pipette solution included 120 mM CsCl, 20 mM tetraethylammonium chloride, 10 mM HEPES, 5 mM Mg-ATP and 10 mM EGTA (pH adjusted to 7.2 with CsOH).

Animals

Sprague–Dawley rats weighing 200–240 g were obtained from the Experimental Animal Center (Hebei Medical University). The study was performed in accordance with the Guidelines of Animal Experiments from the Committee of Medical Ethics, National Health Department of China.

Isolation of adult rat ventricular myocytes

Individual ventricular myocytes were separated from the Sprague–Dawley rat hearts via the collagenase perfusion method, as described previously (Gao et al. 2014). Each

animal was anesthetized via an intraperitoneal injection of heparin (500 IU/kg) and ethylcarbamate (4 mg/kg). The heart was excised rapidly from the rats and cannulated onto a Langendorff apparatus for perfusion. Perfusion was performed via the aorta with an oxygenated calcium-free solution for 5 min to remove the blood. Subsequently, the heart was perfused with enzymatic solution for 18–25 min. After approximately 20 min, the heart was changed to 20 ml of Ca^{2+}-free Tyrode's solution to wash out the enzyme solution. After perfusion, the myocardial tissue was dissected into small pieces in KB solution. The cardiomyocytes were preserved in KB solution (bubbled with O_2) at a temperature of 23–25 °C for up to 1–2 h before the experiment.

Cell culture

Human embryonic kidney 293 (HEK293) cells stably transfected with the human *ether-à-go-go*-related gene (hERG) were cultured in DMEM supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 600 μg/ml of G418 and 1% penicillin/streptomycin in a humidified incubator at 37 °C at 5% CO_2. The cells were seeded on glass coverslips in a 24-multiwell plate. The assays were performed 48 h after plating.

Current recording

The experiments were performed at room temperature (22–25 °C). Whole-cell patch recordings were performed on ventricular myocytes to detect LTCCs, and the perforated patch configuration was used to record hERG K^+ currents. A level puller (Sutter Instruments, Model P-97) was used to pull the borosilicate glass electrodes. When the cells first entered the solution, whole-cell patch recordings had a resistance of 2–5 MΩ and perforated patch recordings had a resistance of 1–2 MΩ. After gigaseal and membrane rupture to establish the whole-cell configuration, the $I_{Ca\text{-}L}$ currents were recorded in voltage-clamp mode. To minimize the rundown of hERG K^+ currents, 200 μg/ml of amphotericin B was added to the pipette solution in perforated patch recordings. The Axon patch 200B amplifier and the pClamp 10.0 software (Axon Instruments, Union City, CA, USA) were used to record the currents.

Measurement of myocyte contractions

A video-based edge-detection system (IonOptix, Milton, MA, USA) was used to detect cell shortening of myocardial myocytes. Cell contractions were elicited by the frequency of field stimulation at 0.5 Hz (2 ms duration). Cell activity could be observed by an inverted microscope and a detection system placed on the glass chamber. Myocytes with clear edges were chosen to measure cell shortening before and after MI administration.

Measurement of $[Ca^{2+}]_i$ transients

The myocytes were incubated with fura-2/AM for 15 min in the dark at room temperature, as described previously (Gao et al. 2014). The fluorescence measurements were recorded using a dual-excitation fluorescence photomultiplier system (IonOptix). A 0.5 Hz field was used to stimulate myocyte contraction. Contractive myocytes were imaged through a Fluor 40 × oil objective and exposed to light, which was passed through a 340- or 380-nm filter (bandwidth measurements were ±15 nm). The emitted fluorescence was detected at 510 nm. The emitted fluorescence ratio was responded by two wavelengths (340/380) to determine an intracellular calcium concentration. Ca^{2+} transients were calibrated as described previously (Salem et al. 2010).

Data analysis

The electrophysiologic data were processed using Clampfit 10.0 software. The concentration–response curve was fitted with logistic equation: $y = A_2 + (A_1–A_2)/[1 + (x/x_0)^p]$, where y is the response; A_1 and A_2 are the maximum and minimum response, respectively, x is the drug concentration, and p is the Hill coefficient. The current activation curves were generated by plotting the normalized tail current amplitudes against the step potentials and were fitted with a Boltzmann function: $y = A/\{1 + exp[(V_h - V_m)/k]\}$, where A is the maximal current amplitude, V_h is the voltage for half-maximal activation, V_m is the test potential and k is the slope factor. The data are presented as the mean ± S.E.M. Intergroup differences were assessed using the Student's t test or a one-way ANOVA. The differences were considered significant to be at $p < 0.05$. Where ANOVA was used, a post hoc test (Bonferroni) was subsequently used if F achieved $p < 0.05$ and there was no significant variance inhomogeneity.

Results

Confirmation of $I_{Ca\text{-}L}$

$I_{Ca\text{-}L}$ was elicited via the steady-state activation protocol in rat ventricular myocytes. Verapamil (100 μM) could almost completely block the currents, indicating that these currents were Ca^{2+} currents (Fig. 2) ($p < 0.01$).

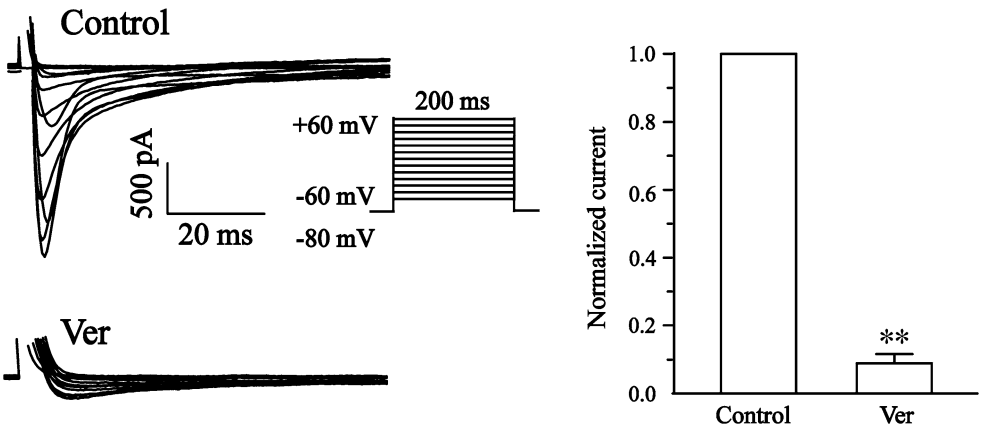

Fig. 2 Confirmation of $I_{Ca\text{-}L}$. Ver (100 μM) completely blocked $I_{Ca\text{-}L}$ in rat ventricular myocytes. Representative $I_{Ca\text{-}L}$ recordings with the steady-state activation protocol before and after application of Ver. Data are presented as mean ± S.E.M. (n = 6 cells, $^{**}p < 0.01$, compared with control)

Effects of MI on $I_{Ca\text{-}L}$

The peak of $I_{Ca\text{-}L}$ was significantly reduced after exposure to 1 mg/ml of MI ($p < 0.01$). However, the $I_{Ca\text{-}L}$ recovered after the MI was washed out with the external solution (Fig. 3a, b). These results indicate that the effect of MI on $I_{Ca\text{-}L}$ was not because of rundown, and the effect of MI on $I_{Ca\text{-}L}$ was partially reversible. Representative current recordings made with the activation protocol after the sequential administration of MI (0.01, 0.03, 0.1, 0.3 and 1 mg/ml, i.e., 0.01, 0.04, 0.12, 0.35 and 1.18 mM) are shown in Fig. 4a, b. The equation indicates that the half-maximal inhibitory concentration (IC_{50}) of MI was 0.22 mg/ml. The rates of inhibition by MI at 0.01, 0.03, 0.1, 0.3 and 1 mg/ml were 9.88 ± 0.32%, 16.45 ± 0.27%, 24.07 ± 1.49%, 45.68 ± 0.92% and 61.10 ± 0.59%, respectively (Fig. 4c).

Effects of MI on the I–V relationship of $I_{Ca\text{-}L}$

The effects of MI (0.01, 0.1, and 1 mg/ml) on the I–V relationship of the $I_{Ca\text{-}L}$ are depicted in Fig. 5. Figure 5a shows representative trace recordings that shifted upward with a significant growth in the concentration of MI (0.01, 0.1, and 1 mg/ml). Figure 5b shows the current density–voltage relationship with controls for MI (0.01, 0.1, and 1 mg/ml) and verapamil (10^{-7} M). These results demonstrated that 0.01, 0.1 and 1 mg/ml MI could markedly depress the maximum current.

Effects of MI on steady-state activation and inactivation of $I_{Ca\text{-}L}$

The voltage dependence of the steady-state activation and inactivation of $I_{Ca\text{-}L}$ in the presence or absence of MI is shown in Fig. 6. The value at $V_{1/2}$ and the slope factor (k) of the normalized activation conductance curves for the control and 0.01 mg/ml, 0.1 mg/ml, and 1 mg/ml MI were −1.46 ± 1.52 mV/9.80 ± 1.17; 0.14 ± 1.40 mV/9.56 ± 1.03; 1.04 ± 1.32 mV/9.66 ± 0.94; and −1.15 ± 1.22 mV/10.42 ± 0.92, respectively ($p > 0.05$, n = 6). The $V_{1/2}$ and the k values of the steady-state inactivation curves for the control and 0.01 mg/ml, 0.1 mg/ml, and 1 mg/ml of MI were −26.55 ± 1.00 mV/4.27 ± 0.80; −25.56 ± 0.67 mV/4.57

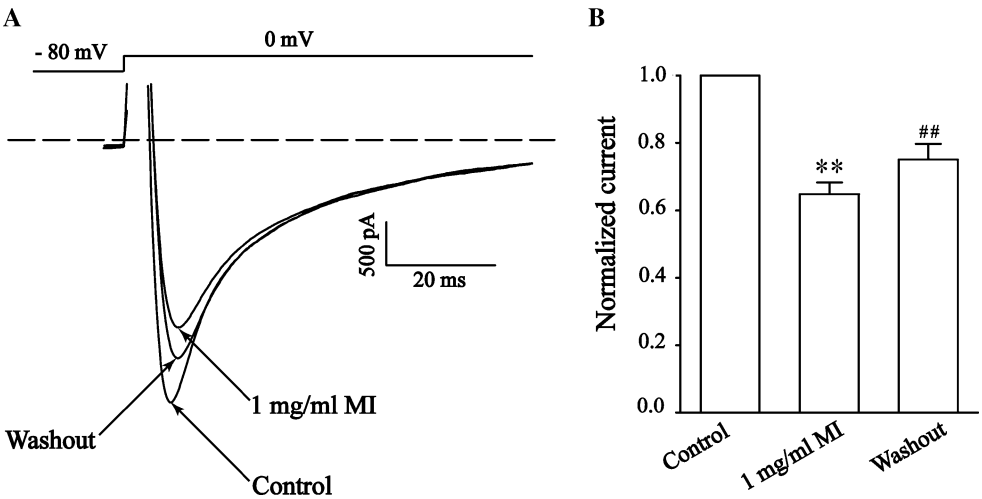

Fig. 3 Reversible effects of MI on $I_{Ca\text{-}L}$ of ventricular myocytes. Exemplary traces (**a**) and pooled data (**b**) of $I_{Ca\text{-}L}$ were recorded under control conditions, during exposure to 1 mg/ml MI and during wash out. Data are presented as mean ± S.E.M. (n = 6 cells, $^{**}p < 0.01$, compared with control, $^{\#\#}p < 0.01$, compared with 1 mg/ml MI)

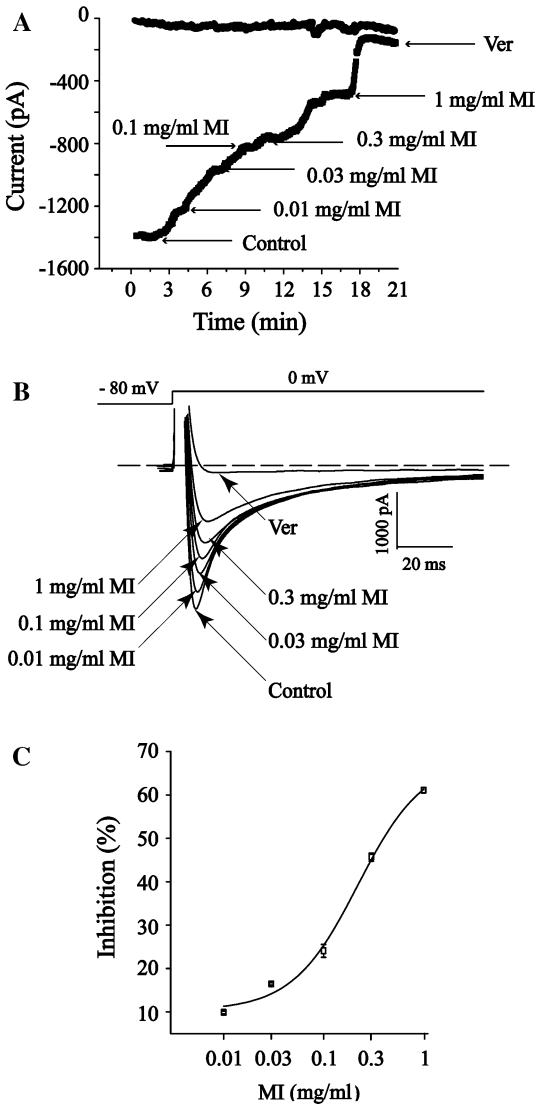

Fig. 4 Effects of MI on $I_{Ca\text{-}L}$ of ventricular myocytes. **a** Time course of $I_{Ca\text{-}L}$ recorded under the control conditions, during exposure to 0.01, 0.03, 0.1, 0.3 and 1 mg/ml MI and 100 μM Ver. **b** Original current traces in response to 0.01, 0.03, 0.1, 0.3 and 1 mg/ml MI and 100 μM Ver. **c** Concentration–response curve of MI. Data are presented as mean ± S.E.M. (n = 6–8 cells)

± 0.53; −25.51 ± 0.76 mV/4.63 ± 0.61; and −29.07 ± 0.28 mV/5.07 ± 0.27, respectively ($p > 0.05$, n = 6).

Effects of MI on cell shortening and Ca^{2+} transients

Figure 7 shows a time-based record of cell length from a representative rat ventricular myocyte before and after the administration of 0.3 mg/ml of MI. The results indicated that MI at a concentration of 0.3 mg/ml could significantly reduce cell shortening by 24.12 ± 3.97% (n = 6, $p < 0.01$) and decrease the amplitude of the Ca^{2+} transient by 36.54 ± 4.96% (n = 6, $p < 0.01$). Compared with the control group, MI at a concentration of 0.3 mg/ml had no significant effect on the time to reach 10% of the peak (Tp) and the time to 10% of the baseline (Tr) ($p > 0.05$).

Effects of $MgCl_2$ on $I_{Ca\text{-}L}$, cell shortening and Ca^{2+} transients

The peak of $I_{Ca\text{-}L}$ was slightly reduced after exposure to 0.1 and 0.3 mg/ml of $MgCl_2$ ((n = 6, $p < 0.01$). The rates of inhibition by $MgCl_2$ at 0.1, and 0.3 mg/ml were 5.12% ± 2.03% and 7.03% ± 3.22%, respectively (Fig. 8a).

Figure 8b, c shows a time-based record of cell length from a representative rat ventricular myocyte before and after the administration of 0.3 mg/ml of $MgCl_2$. Compared with the control group, $MgCl_2$ at a concentration of 0.3 mg/ml had no significant effect on cell shortening and the Ca^{2+} transient (n = 6, $p > 0.05$).

Effects of MI on I_{Kr}

The effect of MI on I_{Kr} expressed in HEK293 cells was also examined. I_{Kr} was launched using 1-s test pulses between −60 mV and +60 mV with 20 mV steps from a holding potential of −80 mV, then back to −40 mV for 3-s at 0.05 Hz. Figure 9 shows the trace of I_{Kr} recorded in one cell in the absence or presence of 1 mg/ml of MI. MI at a concentration of 1 mg/ml remained stable on the expressed I_{Kr} (1.71 ± 0.43%) at all test potentials ($p > 0.05$, n = 6).

Discussion

Previous research indicated that licorice has positive effects on heart diseases, such as I–R injury, myocardial infarction and arrhythmia (Li et al. 2003; Zhou et al. 2007; Ojha et al. 2013). Because MI is one component of licorice extract, MI may exert protective effects against myocardial ischemia in a similar way.

However, the ion channel and cellular mechanisms of MI have not been clarified systematically. In our present study, we used the patch-clamp technique to confirm that MI suppresses the $I_{Ca\text{-}L}$ of rat cardiomyocytes in a dose-dependent manner.

Ca^{2+} channels are found in all excitable cells and regulate many physiological activities, such as excitation-secretion coupling, excitation–contraction coupling and heart pace-making. An increase of the diastolic calcium concentration or cellular Ca^{2+} overload is a significant cause of many cardiovascular diseases, such as ischemic injury, hypertension, oxidant stress and glycuresis. These diseases can increase intracellular Ca^{2+} and trigger endo-calcium release in the sarcoplasmic reticulum (SR). Ca^{2+} overload

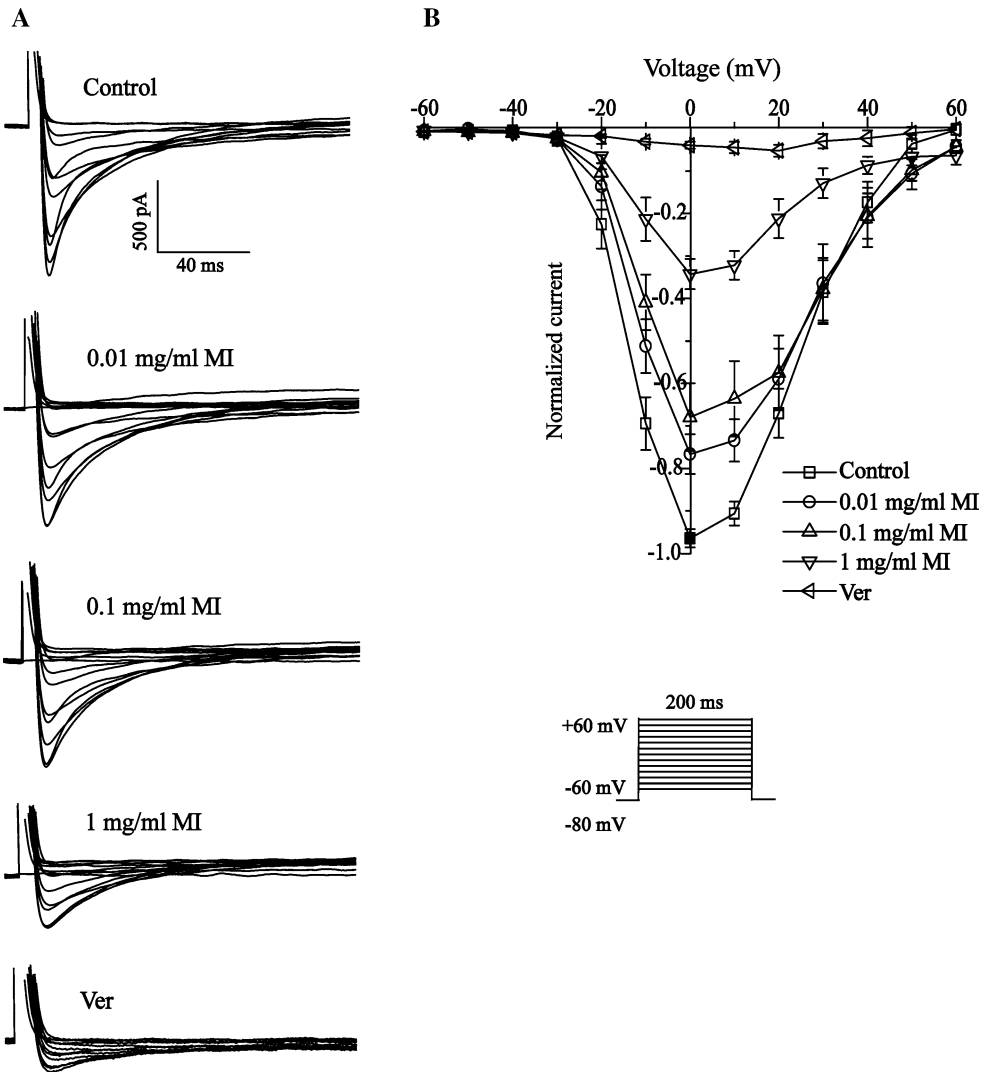

Fig. 5 Effects of MI on current–voltage (I–V) relationship of $I_{Ca\text{-}L}$. Exemplary traces (**a**) and pooled data (**b**) show effects of MI at different concentrations on the I–V relationship. Control is marked with (*square*), MI at 0.01 mg/ml with (*circle*), MI at 0.1 mg/ml with (*triangle*) and MI at 1 mg/ml with (*inverted triangle*) and Ver at 100 μM with (). Data are presented as mean ± S.E.M. (n = 6 cells). The current activation curves were generated by plotting the normalized tail current amplitudes against the step potentials and were fitted with a Boltzmann function: $y = A/\{1 + \exp[(V_h - V_m)/k]\}$, where A is the amplitude of relationship, V_h is the voltage for half-maximal activation, V_m is the test potential and k is the slope

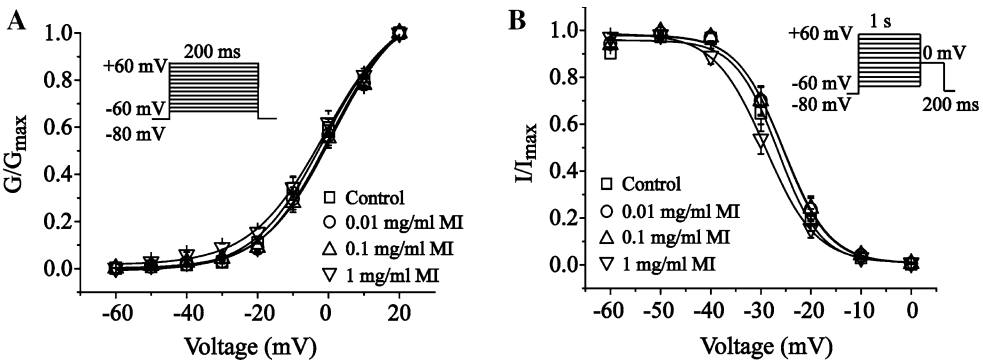

Fig. 6 Effects of MI on steady-state activation and inactivation of $I_{Ca\text{-}L}$. (A) Activation kinetics of $I_{Ca\text{-}L}$. (B) Inactivation kinetics of $I_{Ca\text{-}L}$. Control is marked with (*square*), MI at 0.01 mg/ml with (*circle*), MI at 0.1 mg/ml with (*triangle*) and MI at 1 mg/ml with (*inverted triangle*). Data are presented as mean ± S.E.M. (n = 6 cells)

is a main cause of myocardial ischemia reperfusion injury (Menown and Adgey 2001), and it is partially a result of the opening of LTCCs (Piper et al. 1998). Thus, during prolonged ischemia and reperfusion, blocking LTCCs has great significance (Woods 1991). Many studies have demonstrated that LTCCs play an important role in Ca^{2+} influx in cardiomyocytes, and modulating LTCC activity has been the focus of research interest (Bers 2002; Richard et al. 2006). In addition, LTCCs represent the main route of intracellular Ca^{2+} influx and have the properties of slow inactivation and a long opening duration (Guan et al. 2013). We found that MI could reduce the amplitude of the $I_{Ca\text{-}L}$ in a dose-dependent manner at concentrations of 0.01, 0.03, 0.1, 0.3, and 1 mg/ml, and the inhibition was partially

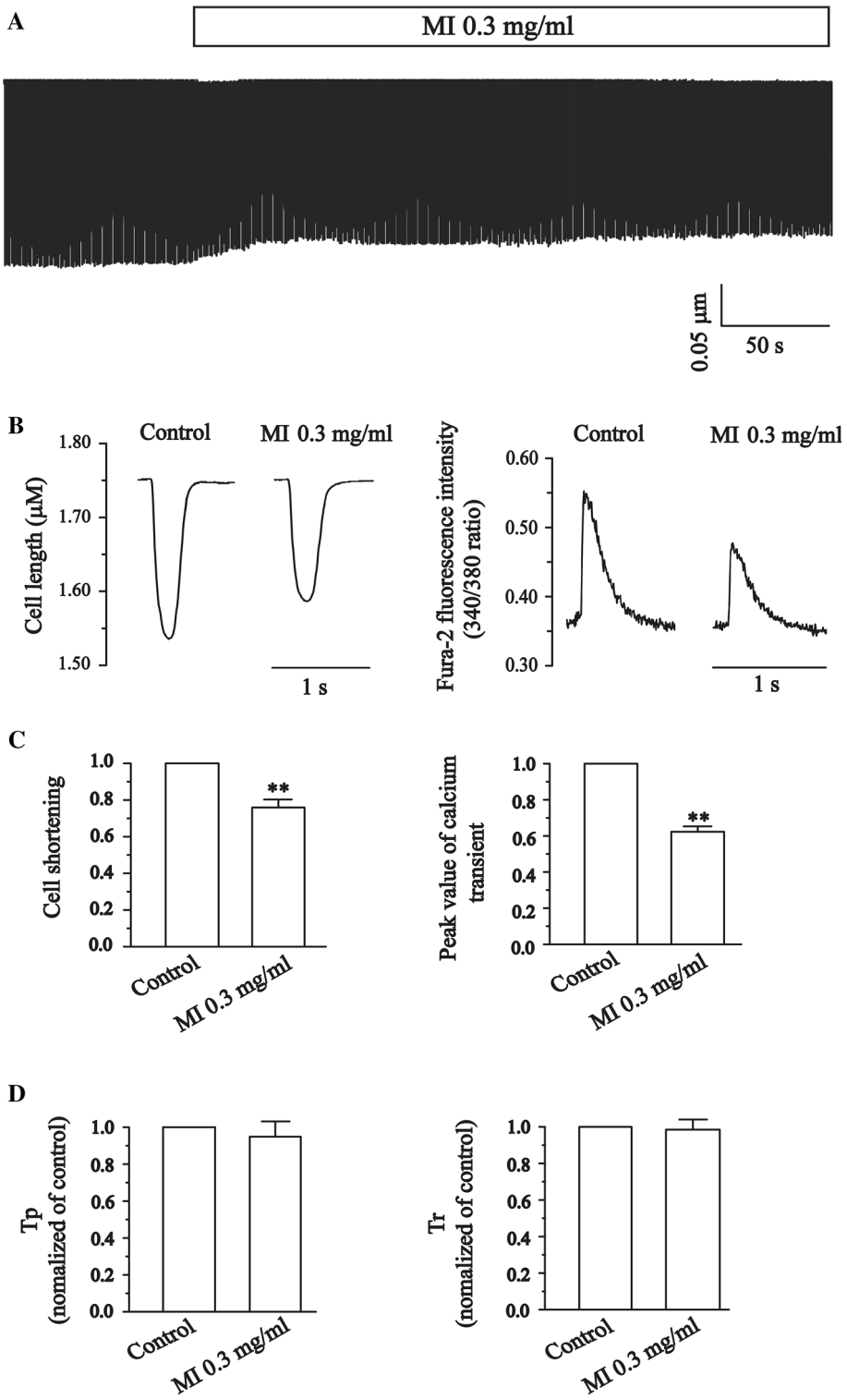

Fig. 7 Effects of 0.3 mg/ml MI on cell shortening, Ca^{2+} transient and time parameters of cell shortening. **a** Tracings demonstrating effects of 0.3 mg/ml MI in a myocyte. **b** Representative signals of cell shortening and fura-2 ratio (upper tracings). **c** Summary effects of 0.3 mg/ml MI on cell shortening and fura-2 ratio. Data are presented as mean ± S.E.M. (n = 6 cells, $^{**}p < 0.01$, compared with control). **d** Summary results of the time to 10% of the peak (Tp) and the time to 10% of the baseline (Tr) for cell shortening before and after application of 0.3 mg/ml MI. Data are presented as mean ± S.E.M. (n = 6 cells, $p > 0.05$, compared with control)

reversible (Fig. 3, 4). However, neither the current–voltage relationship nor the reversal potential of $I_{Ca\text{-}L}$ was affected (Fig. 5). MI did not alter the steady-state activation and inactivation of $I_{Ca\text{-}L}$ when the peak value of $I_{Ca\text{-}L.}$ was restrained (Fig. 6). These results demonstrate that MI suppressed the $I_{Ca\text{-}L}$ primarily by decreasing the Ca^{2+} current amplitude.

LTCCs play a major role in Ca^{2+} influx, as LTCCs can trigger Ca^{2+} release from SR Ca^{2+} stores. The reductions in the Ca^{2+} transient and the amplitude of contraction were

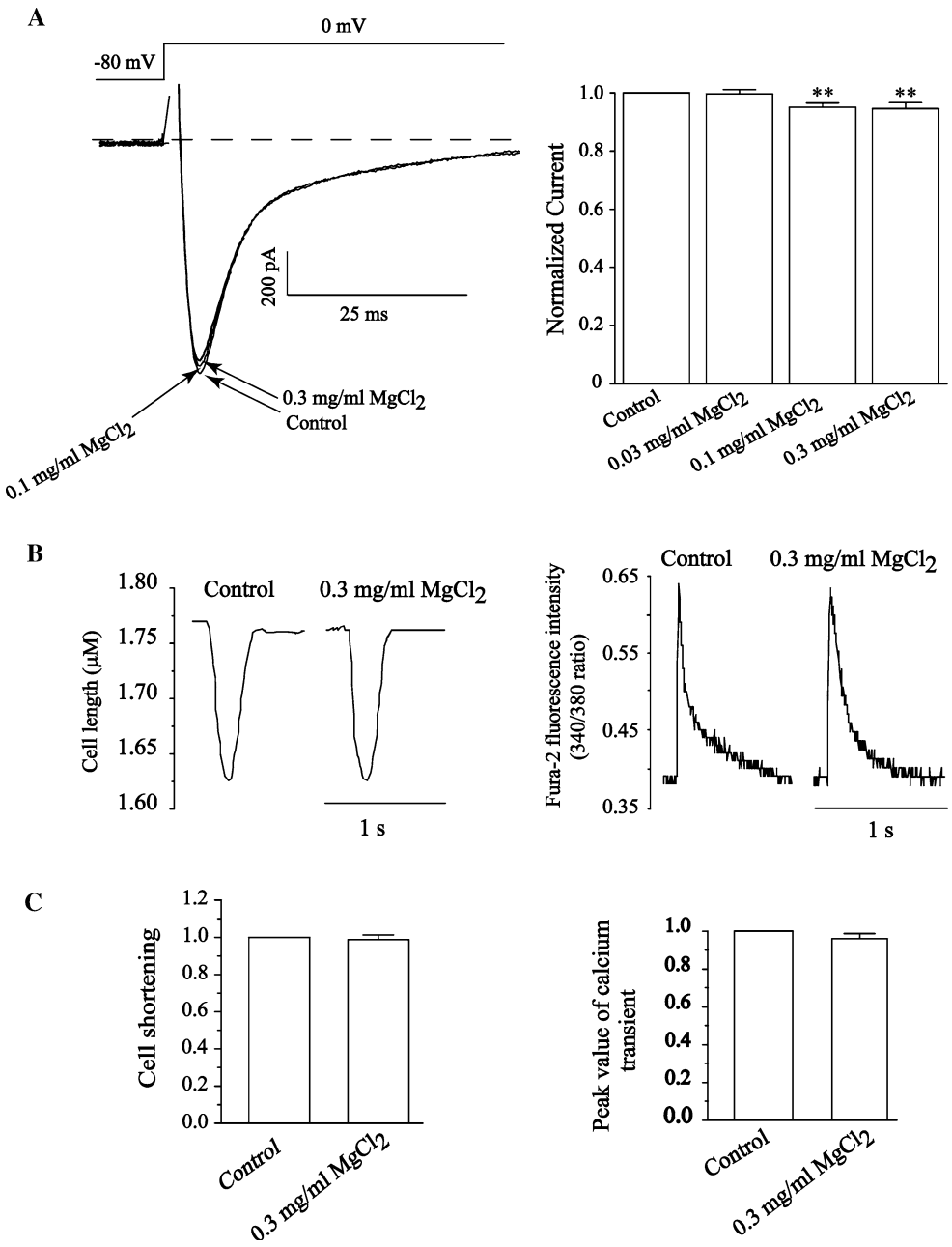

Fig. 8 Effects of $MgCl_2$ on I_{Ca-L}, cell shortening and Ca^{2+} transient of ventricular myocytes. **a** Exemplary traces and pooled data of I_{Ca-L} were recorded under control conditions, during exposure to 0.1 and 0.3 mg/ml of $MgCl_2$. Data are presented as mean ± S.E.M. (n = 6 cells, $^{**}p < 0.01$, compared with control). **b** Representative signals of cell shortening and fura-2 ratio (upper tracings). **c** Summary effects of 0.3 mg/ml MI on cell shortening and fura-2 ratio. Data are presented as mean ± S.E.M. (n = 6 cells, $p > 0.05$, compared with control)

caused by the blockade of I_{Ca-L} (Cannell and Lederer 1987; Cleemann and Morad 1991). In our study, MI had inhibitory effects on LTCCs, which resulted in cardiac negative inotropic effects. In the presence of MI, the Ca^{2+} transient and contractility was significantly reduced (Fig. 7a, b, c). In addition, we observe that MI seems to inhibit cell shortening less than I_{Ca-L}. This phenomenon is a result of the complex process of myocardial contraction, which is related to intracellular Ca^{2+} concentration and to intracellular proteins that are involved in contraction (actin and myosin) or regulation (troponin, tropomyosin, and tropomodulin) (Adamcova et al. 2006). The more detailed mechanisms of the effects of MI on myocardial contraction need to be explored in our future studies. The speed of cell contraction and cell relaxation are represented by Tp and Tr, respectively. In this study, MI at a concentration of 0.3 mg/ml had no significant effect on Tp and Tr (Fig. 7d). The reduction of the Ca^{2+} transient indicates a decrease in intracellular free Ca^{2+}. MI may inhibit LTCCs to limit Ca^{2+} entry and inhibit Ca^{2+} release from the SR. This effect is likely responsible for the negative inotropic effect of MI because cardiac muscle contraction is modulated by the amplitude of the Ca^{2+} transient (Cannell and Lederer 1987; Cleemann and Morad 1991). In clinical treatment of myocardial ischemia, reduced myocardial contractility and myocardial oxygen consumption are the most important

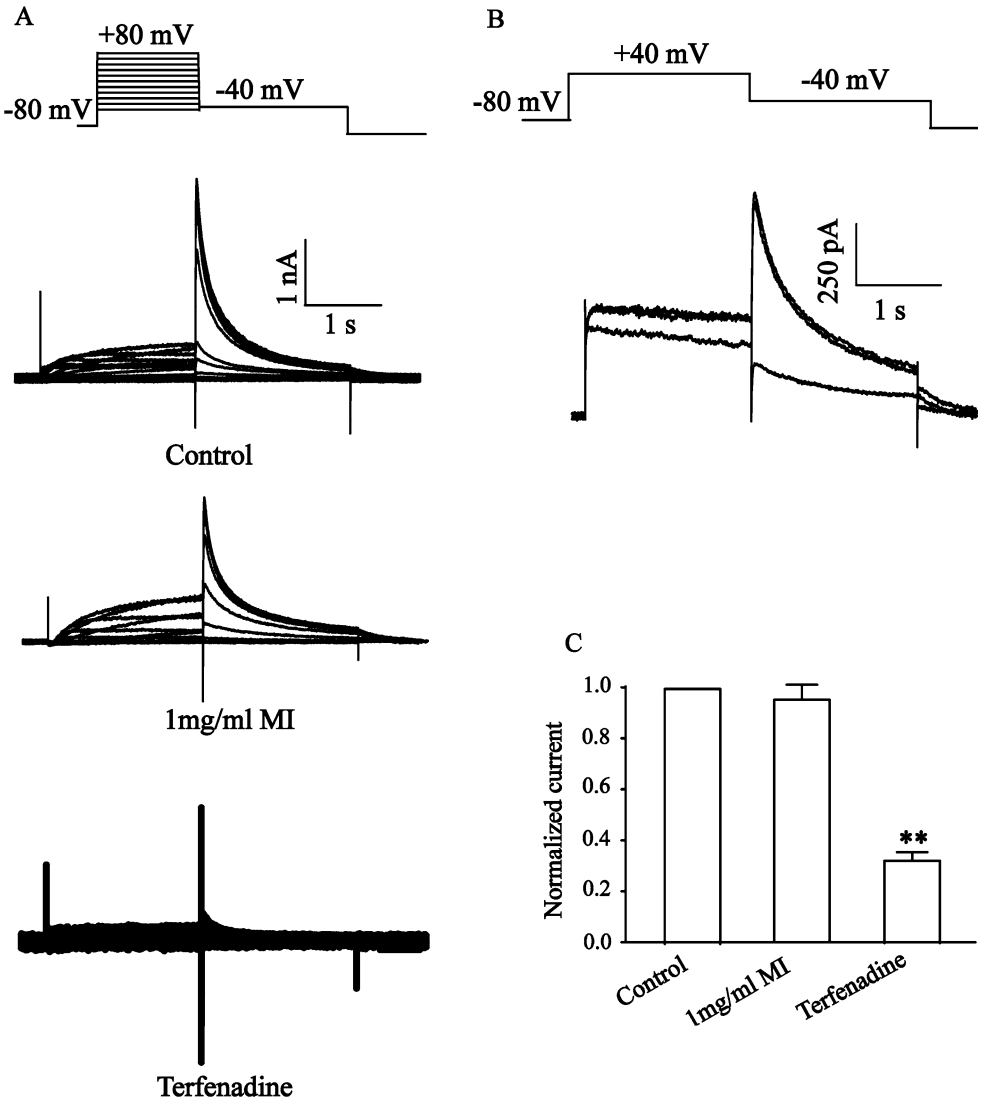

Fig. 9 Effects of MI on I_{Kr} expressed in HEK293 cells. **a** The currents traces of I_{Kr} induced by the multiple depolarization steps were shown. Cells were exposed to 1 mg/ml MI and tefenadine. **b** Exemplary currents traces of I_{Kr} induced by 0 mV depolarization step were shown. The effect of 1 mg/ml MI was shown. **c** Summary data of (b). Data are presented as mean ± S.E.M. (n = 6 cells)

cellular mechanisms (Sonnenblick et al. 1965; Crystal et al. 2013). The activity of several ATP-consuming enzymes was accelerated by elevated $[Ca^{2+}]_i$; this phenomenon may consume cellular energy stores and increase the risk of ischemic damage (Balaban 2002). The benefits of Ca^{2+} antagonists have been shown to protect against cardiac injury that is induced by Ca^{2+} overload (Balaban 2002). Clinically, many drugs used to treat patients with myocardial ischemia are related to inhibition of the LTCCs (Poole-Wilson et al. 2004; Doggrell 2005), such as nifedipine, verapamil, diltiazem et al. Our research demonstrated that MI is a Ca^{2+} antagonist and could thus be expected to exert a cardioprotective effect against ischemic damage by inhibiting $I_{Ca\text{-}L}$ and decreasing myocardial contractility.

It has been reported that higher concentration ranges of magnesium can inhibit the L-type Ca^{2+} channel in smooth muscle cells and isolated ventricles (Sharma et al. 2012; Zhao et al. 2015). The highest concentration MI used in the present experiments is 1 mg/ml (1.18 mM), which contains 0.11 mg/ml (1.18 mM) magnesium. To investigate the effects of the magnesium ion, we used $MgCl_2$ up to a high concentration of 0.3 mg/ml (3.15 mM), which is much higher than magnesium contained in high concentration MI. The experimental results show that the inhibitory effect of 0.1 and 0.3 mg/ml $MgCl_2$ on $I_{Ca\text{-}L}$ is low, at 5 and 7%, respectively (Fig. 8a), which is much weaker than that of 1 mg/ml MI (1.18 mM), which was 61%. Compared with the control group, $MgCl_2$ at concentration of 0.3 mg/ml had no significant effect on cell shortening and Ca^{2+} transients ($p > 0.05$, Fig. 8b, c). The effect of MI on $I_{Ca\text{-}L}$ and cell shortening is considered to be mainly a result of the glycyrrhizic acid ingredient rather than the effect of the magnesium ion.

Drug-induced LQTS is commonly ascribed to the blockade of I_{Kr} because of a pharmacodynamic interaction

between the drug and cardiac potassium channel proteins (ten Tusscher et al. 2004; ten Tusscher and Panfilov 2006). Drug-induced LQTS may culminate in ventricular fibrillation and polymorphic ventricular tachycardia, which cause syncope and sudden death (Holladay et al. 1997; Verkerk et al. 2005). Accordingly, compounds undergoing drug development and some commonly used calcium antagonists such as verapamil are routinely screened for their (undesired) ability to block I_{Kr} (Chouabe et al. 1998; Grandi et al. 2010). Previous studies showed that the characterizations of hERG K^+ currents stably expressed in HEK293 cells are very similar to those of native I_{Kr} in cardiomyocytes (Dong et al. 2006b). Adult rat cardiomyocytes lack native I_{Kr}. Therefore, we examined the effects of MI on I_{Kr} expressed in HEK293 cells (Fig. 9) and found that MI did not significantly affect this current, indicating that the use of MI may not cause drug-induced LQTS. MI is currently superior to other calcium antagonists that affect I_{Kr}.

This study provided evidence that MI exerts its cardiovascular protective effects by inhibiting $I_{Ca\text{-}L}$ and accordingly decreasing Ca^{2+} transients and myocardial contractility. As an LTCC blocker, MI may have a positive effect on protecting cardiomyocytes against ischemic damage and apoptosis induced by Ca^{2+} overload. Meanwhile, MI had no significant influence on I_{Kr} expressed in HEK293 cells at all test potentials; thus, MI may exert cardioprotective effects without causing drug-induced LQTS. Our study provides new ionic evidence to demonstrate that MI can decrease the $I_{Ca\text{-}L}$. Pretreatment with MI may have therapeutic benefits and may represent a promising approach to combating cardiovascular disease.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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