Cardiovascular pharmacology

Cisapride protects against cardiac hypertrophy via inhibiting the up-regulation of calcineurin and NFATc-3

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ABSTRACT

Cisapride has been shown to have electrophysiological effects on the heart. The aim of this study was to investigate whether cisapride has effects on cardiac hypertrophy. Rat and cellular models of cardiac hypertrophy were used in this study. Cell surface area (CSA), mRNA and protein expression were used to evaluate cardiac hypertrophy. Cardiac function was measured by echocardiography. Cisapride attenuated ISO-induced increase in CSA in a dose-dependent manner in cultured neonatal rat cardiomyocytes. A significant anti-hypertrophic effect was achieved by cisapride 0.01 μM (P<0.05). Cisapride repressed the increased mRNA levels of ANP, BNP, β-MHC in ISO-treated cells (P<0.05). However, mallotoxin or GR113808 did not influence anti-hypertrophic effects of cisapride. In addition, cisapride inhibited the increase of intracellular Ca 2+ ([Ca 2+ ] i) and the upregulation of protein levels of calcineurin and NFATc-3 (P<0.05) as well as prevented the downregulation of p-NFATc-3 (P<0.01) induced by ISO. Consistently, cisapride (0.5 mg/kg/day) produced inhibitory effects on cardiac hypertrophy, including the suppression of ANP, BNP, β-MHC, calcineurin, and NFATc-3; elevation of p-NFATc-3; reduction of cross-sectional area of cardiomyocytes in rat heart; and restoration of cardiac dysfunction by improving left ventricular diastolic and systolic performance. Importantly, cisapride 0.5 and 5.0 mg/kg/day did not cause prolongation of QT and QTC intervals in rats. In conclusion, cisapride possesses a prominent anti-hypertrophic property which is likely to be conferred by its ability to downregulate Ca 2+ /calcineurin/NFAT and the present data provide new insight into this drug action.

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1. Introduction

Cisapride, a gastrointestinal prokinetic agent, increases gastrointestinal motility by selectively activating serotonin (5-hydroxytryptamine, 5-HT) 4 receptor (5-HT4R) and has been used to treat gastrointestinal motility disorders (Wiseman and Faulds, 1994). However, in many countries, it has recently been withdrawn due to its potential to trigger life-threatening torsades de pointes arrhythmias (Ahmad & Wolfe, 1995) and long QT syndrome (Bran et al., 1995) by blocking human ether-a-go-go-related gene (HERG) channel, a major contributor to the repolarization of cardiomyocyte action potential. In addition to its effect on HERG channel, cisapride has been demonstrated to directly block L-type Ca 2+ current (I Ca,L ) in cardiac myocytes in a voltage-dependent manner (Chiang et al., 2003). Up to now, nearly all previous studies on cisapride have been focused on cardiac electrophysiological activities.

Cardiac hypertrophy is a physiological or compensatory response that is often associated with the cardiovascular diseases and a potent predictor of progressive heart disease, such as heart failure and sudden death (Hardt and Sadoshima, 2002). Pathological cardiac hypertrophy can be caused by hypertension, myocardial infarction, and valvular heart disease. Hypertrophy is commonly associated with the enlargement of cardiomyocytes and cardiac dysfunction, which lead to heart failure and even death. It is well known that multiple signaling pathways participate in cardiac hypertrophy, for example, the mitogen-activated protein kinase (MAPK) signaling pathway (Bueno et al., 2000), the calcineurin-nuclear factor of activated T cells (NFAT) signaling pathway (Molkentin et al., 1998), the G protein-coupled receptors signaling pathway (Wettschureck et al., 2001), the STAT3 pathway, and the PI3K/Akt/GSK-3 signaling pathway (Heinke and Molkentin, 2006). Molkentin demonstrated that Ca 2+ induces phosphorylation of calcineurin, which dephosphorylates NFAT in the nucleus and activates expression of hypertrophy-related genes to induce cardiac...
hypertrophy (Molkentin, 2004). Notably, calcineurin/NFAT signaling is also intertwined with other hypertrophic pathways, such as MAPK, glycogen synthase kinase-3β (GSK3β), p38 and JNK (De Windt et al., 2000). As mentioned above, calcineurin/NFAT signaling plays a central regulatory role in the pathological hypertrophy and heart failure. Some agents have been documented to exert anti-hypertrophic effect via mediating calcineurin/NFAT signaling (Tan et al., 2011; Ni et al., 2006).

Ca²⁺-mediated signal transduction plays a central role in the development of cardiac hypertrophy including calcineurin/NFAT signaling and cisapride can act on ICa-L, an essential element in regulation of [Ca²⁺]i by Ca²⁺-induced Ca²⁺ release. We proposed that cisapride might play a role in regulating cardiac hypertrophy. The primary aim of the present study was to examine this hypothesis.

In this study, we investigated the anti-hypertrophic action of cisapride both in vitro and in vivo experiments in rats and demonstrated that cisapride exerted its anti-hypertrophic effect via mediating Ca²⁺-dependent calcineurin/NFAT signaling pathway. However, it did not cause prolongation of QT and QTc intervals. We further revealed cisapride improved cardiac function in hypertrophic hearts. The present results provide new insight into this drug action.

2. Materials and methods

2.1. Materials

Isoproterenol (ISO) was obtained from Shanghai Hefeng Pharmaceutical Co. Ltd. (Shanghai, China). Cisapride, Mallotoxin and GR113808 were bought from Sigma-Aldrich Co. (St. Louis, MO, USA). The primers of ANP, BNP, and GR113808 were purchased from Molecular Probes Co. (Molecular Probes Inc, USA). Anti-sarcomeric actin antibody was bought from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and the phospho-NFATc-3 antibody was purchased from Abcam (Cambridge, MA, USA).

2.2. Cardiomyocytes culture and treatment

Neonatal cardiomyocytes were isolated from 1 to 3-day-old Sprague-Dawley rats. Briefly, neonatal rats were killed by decapitation and their hearts were rapidly removed. The isolated hearts were cut into 1.0 mm blocks. Then the tissues were dissociated in 0.25% trypsin at 37 °C and cell suspension was collected. After centrifugation (1500 rpm, 5 min), the isolated cells were resuspended in DMEM (Hyclone Laboratories, Utah, USA) containing 10% fetal bovine serum (Gibco, Invitrogen, CA, USA), 100 units/ml penicillin and 100 μg/ml streptomycin. Then the cells were cultured at 37 °C in humidified air with 5% CO₂ for 2 h. After fibroblast adherence, the non-adherent cardiomyocytes were seeded onto cell culture dishes. After 48 h culture, the medium was replaced with serum-free DMEM. To induce the hypertrophic response, ISO was added to culture medium at a concentration of 10 μM, which has been proven to induce cardiomyocyte hypertrophy in previous study (Tan et al., 2011). The cells were treated with ISO alone, cisapride (0.001–1.0 μM) + ISO, cisapride (0.01 μM) alone or solvent (DMSO, control) for 24 h. The medium was changed every 12 h.

2.3. Animal experiments

Healthy adult male Sprague-Dawley rats (180–200 g) used in the current study were housed in stainless steel cages with sawdust bedding. They were kept under standard animal housing conditions (temperature at 21 ± 1 °C, humidity of 55 ± 5%), 12 h dark/light cycle and allowed food and water unlimited. All experimental procedures were approved by the ethics committee of Harbin Medical University.

After acclimatization for 5 days, the rats were subcutaneously injected with ISO (2.5 mg/kg/day) or cisapride (0.5 mg/kg/day) for 10 days. Saline-injected rats served as controls.

2.4. Measurement of cell surface area

Cardiomyocytes from different treatment groups were fixed with 4% paraformaldehyde for 15 min at 37 °C. Then the cells were treated with 0.4% Triton X-100 for 90 min and blocked by goat serum for 90 min at room temperature. Next, the myocytes were stained with anti-sarcomeric actin antibody at 4 °C overnight and then stained with FITC-conjugated goat anti-mouse antibody for 1 h and visualized under a fluorescence microscope (Nikon 80i, Japan). At least 60 cells were randomly chosen from 4–5 experiments in each group for analysis of cell surface area and cross-sectional area, which were measured by Image-Pro Plus Data Analysis Program.

2.5. Quantitative real-time PCR

Total RNA was extracted using TRIzol reagent. To detect the expression level of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and β-myosin heavy chain (β-MHC) mRNAs, quantitative real-time PCR was performed using ABI 7500 fast real time PCR system (Applied Biosystems, USA). The sequences of ANP primers were forward 5’-CTCCGATAGCTGCCCTTGAAA-3’ and reverse 5’-GTACCGGAAGCTTGCCACCTA-3’; the sequences of BNP primers were forward 5’-TGCGCAAGATAGCCCCCAT-3’ and reverse 5’-GGCTCTTCTGTTAAACACCTCA-3’; the sequences of β-MHC primers were forward 5’-AACCTTCAAGTTCGGCAAGTG-3’ and reverse 5’-GACCTGGTAGAAGCTTACT-3”; the expression level of GAPDH was used as an internal control.

2.6. Measurement of resting intracellular free calcium

Cultured neonatal cardiac myocytes were stained with 5 μM Fluo-3/AM at 37 °C for 45 min. Then cells were washed with solution [containing (in mM) NaCl 145, KCl 2.8, CaCl₂ 1.0, MgCl₂ 2.0, D-glucose 10, and HEPES 10, pH 7.4] for 3 times. Fluorescent intensities of Fluo-3/AM-loaded cells were detected using laser confocal scanning analysis (Olympus FV-300, Japan) with 488 nm for excitation from an argon ion laser and 530 nm for emission. Relative alteration in intracellular free calcium ([Ca²⁺]i) was calculated by normalizing the fluorescence intensity to control group.

2.7. Western blot analysis

Total protein sample was extracted from the neonatal cardiac myocytes and left ventricular tissues of rat heart with RIPA lysis buffer (Beyotime Institute of Biotechnology, China). Protein concentration was determined by BCA Protein Assay Kit (Beyotime Institute of Biotechnology, China). The protein samples (100 μg) were fractionated by SDS-PAGE (10% polyacrylamide gels) and transferred to NC membrane (Millipore, Bedford, MA). The membranes were then blocked with milk powder at room temperature for 3 h and incubated overnight at 4 °C with the primary antibody. The following day, the membranes were washed and incubated with a secondary rabbit or mouse polyclonal antibody for 1 h at room temperature. Western blot bands were quantified using Odyssey v1.2 software.
by measuring the band intensity (Area × OD) for each group and normalizing to GAPDH.

2.8. Cardiac hypertrophy measurements

After 10 days of drug administration, all rats were sacrificed. The rat hearts were immediately dissected out and weighed. The ratio of heart weight (in milligram) to body weight (in gram; HW/BW) or the ratio of left ventricular weight (in milligram) to body weight (in gram; LVW/BW) was calculated to estimate the extent of cardiac hypertrophy.

2.9. Histological examination

The rat hearts were excised, washed with saline, fixed in 10% formalin, sectioned into 7 μm slices, and stained with hematoxylin and eosin (H&E). Cross-sectional images of cardiomyocytes were scanned at 400 × magnification to estimate the degree of cardiomyocyte hypertrophy. Cross-sectional area of cells was measured with an Image Pro-Plus 4.0 system.

2.10. Echocardiographic assessment of cardiac function

Cardiac function was evaluated in lightly anesthetized rats using a Vevo 2100 Imaging system (Visualsonics, Toronto, Canada) equipped with a 30 MHz microscan transducer. M-mode tracings were recorded in both parasternal long and short axis views. Ventricular parameters including diastolic anterior wall thicknesses, diastolic posterior wall thicknesses and left ventricular systolic internal diameters were measured. Fractional shortening (FS) and ejection fraction (EF) were calculated automatically.

2.11. Electrocardiogram recording

A standard lead II surface electrocardiogram (ECG) was recorded on the lightly anesthetized rats by a BL-420 data acquisition system (Chengdu TME technology Co., Ltd., China). The ECG was recorded for 1 h before cisapride injection and on day 10 after cisapride injection. The QT intervals and RR intervals were measured, and heart-rate corrected QT intervals (QTc) was calculated with the Bazett’s formula: QTc = QT/(RR)^1/2, which is an accepted method for correcting QT interval in rats (Hayes et al., 1994).

Fig. 1. Effects of cisapride on cell surface area in rat cardiomyocytes. (A) Representative fluorescence microscopy images of sarcomere organization (200 × magnification) in different groups. (B) Summarized data from different treatments. n = 60 cells from four experiments, **P < 0.01 vs. control (Ctl); *P < 0.05 & **P < 0.01 vs. ISO. ISO, isoproterenol (10 μM).
2.12. Statistical analysis

All experimental data are expressed as mean ± S.D. ANOVA or student’s t-test was used to compare mean values for multiple group or two group comparisons, using SPSS 13.0 software. Values of $P < 0.05$ were considered to indicate statistically significant.

3. Results

3.1. Cisapride attenuates ISO-induced cardiomyocyte hypertrophy

ISO 10 μM for 24 h was used to induce cardiomyocyte hypertrophy, an experimental model for the study of cardiac hypertrophy (Ozaki et al., 2002; Nunn et al., 2010). To determine whether cisapride inhibited isoproterenol (ISO)-induced myocardial hypertrophy, we first measured cell surface area (CSA) of cardiomyocytes. As shown in Fig. 1A, CSA has significantly increased by 157.1 ± 5.7% in ISO group, compared to the control group ($P < 0.01$). Cisapride produced inhibitory effect on the increase in CSA of hypertrophied cardiomyocytes induced by ISO in a concentration-dependent manner (Fig. 1B). A significant inhibition on the increased CSA was observed at concentration of 0.01 μM cisapride that has no electrophysiological effects in the heart at this concentration (Puisieux et al., 1996).

Atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and β-myosin heavy chain (β-MHC) are well-established markers for cardiomyocyte hypertrophy. Consistent with the CSA results, ISO induced remarkable expression of ANP, BNP, and β-MHC at the mRNA level ($P < 0.05$) (Fig. 2A–C). As expected, cisapride 0.01 μM abolished the upregulation of these markers in ISO-treated cells ($P < 0.05$) (Fig. 2A–C) and cisapride had no effects on expression of these markers in normal cells (Fig. 2A–C). These results indicate that cisapride produced anti-hypertrophic effects in neonatal rat ventricular cells.

3.2. Contribution of $Ca^{2+}$/calcineurin/NFATc3 downregulation to the anti-hypertrophic effects of cisapride

To unravel the mechanisms underlying the anti-hypertrophic property of cisapride, we first tested whether the HERG channel-blocking action of cisapride has any connection to the anti-hypertrophic effects of this agent. As shown in Fig. 3A, mallotoxin (MTX, 0.5 μM), a HERG channel activator, did not affect the changes in CSA of cardiomyocytes without treatment or treated with ISO alone or ISO + cisapride. We then went on to detect whether 5-HT receptor is involved in this anti-hypertrophic effect of cisapride. GR113808 (5-HT inhibitor, 0.5 μM) did not show the anti-hypertrophic effect of cisapride on cardiomyocytes. GR113808 did not influence the CSA of cardiomyocytes exposed to ISO or ISO + cisapride (Fig. 3B).

As $[Ca^{2+}]_i$ plays a central role in the development of cardiac hypertrophy, we then investigated whether $[Ca^{2+}]_i$ was involved in the anti-hypertrophic action of cisapride. As shown in Fig. 4, ISO 10 μM incubation significantly elevated the resting $[Ca^{2+}]_i$ in cultured neonatal cardiomyocytes ($P < 0.01$), which is attenuated by pretreatment with cisapride 0.01 μM ($P < 0.01$).

The calcium-mediated calcineurin/NFAT pathway plays a crucial role in the development of cardiac hypertrophy (Dong et al., 2010; Crabtree and Olson, 2002; Hunter & Chien, 1999). As shown in Fig. 5, ISO significantly upregulated the expressions of calcineurin and NFATc-3 ($P < 0.05$) (Fig. 5A–C) and downregulated the level of phosphorylated NFATc-3 ($P < 0.01$) at the protein level. Notably, pretreatment with cisapride inhibited ISO-induced upregulation of calcineurin and NFATc-3 ($P < 0.05$) (Fig. 5A–C) and prevented downregulation of phosphorylated calcineurin and NFATc-3 at the protein level.
NFATc-3 \((P < 0.01)\) (Fig. 5D and E). Cisapride alone had no significant effect on expression of calcineurin, NFATc-3, and phosphorylated NFATc-3. Downregulation of phosphorylated NFATc-3 reflects that dephosphorylated form of NFATc-3, an active form of NFATc-3, should be enhanced in the cardiomyocytes treated with ISO.

3.3. Anti-hypertrophic properties of cisapride in a rat model of cardiac hypertrophy

Rats were subjected to daily subcutaneous injection of ISO (2.5 mg/kg/day) for 10 days to induce cardiac hypertrophy. The ratio of heart weight (HW) or left ventricular weight (LVW) to body weight (BW) (HW/BW or LVW/BW) and cross-sectional area of cardiomyocytes, indicators of cardiac hypertrophy, were measured. As shown in Fig. 6, the ratios of HW/BW and LVW/BW have significantly increased from 3.4 \(\pm\) 0.1 to 5.4 \(\pm\) 0.1 mg/g \((P < 0.01)\) (Fig. 6A) and from 2.3 \(\pm\) 0.1 to 3.6 \(\pm\) 0.1 mg/g \((P < 0.01)\) (Fig. 6B), respectively, in ISO-treated rats compared to control. Cross-sectional area of cardiomyocytes was also significantly increased by about 1.5-fold \((P < 0.05)\) (Fig. 6C and D) in rats after ISO treatment. Administration of cisapride (0.5 mg/kg/day, subcutaneous injection for 10 days) produced an inhibitory effect on ISO-induced cardiac hypertrophy, as indicated by the decreased HW/BW (Fig. 6A) and LVW/BW ratios (Fig. 6B) as well as cardiomyocyte cross-sectional area (Fig. 6C and D). Administration of cisapride alone had no significant effects on these hypertrophic parameters in non-ISO-treated control rats (Fig. 6).

Cardiac hypertrophy markers ANP, BNP, and β-MHC mRNA levels were markedly upregulated in LV tissues from ISO-treated rats \((P < 0.05)\) (Fig. 7A–C). Cisapride completely suppressed ISO-induced upregulation of ANP, BNP, and β-MHC expression.
Cisapride had no such effects in normal rats without ISO treatment to induce hypertrophic responses (Fig. 7). Western blot analysis of LV tissues from hypertrophied hearts induced by ISO revealed that the hypertrophic factors calcineurin and NFATc-3 were remarkably increased (Fig. 8A and B) whereas phosphorylated NFATc-3 was reduced in their protein levels (Fig. 8C and D), and cisapride effectively abolished these changes (Fig. 8 A–D). These results provided a piece of evidence for the possible involvement of calcineurin/NFATc3 in the anti-hypertrophic effects of cisapride.

### 3.4. Improvement of cardiac function by cisapride in a rat model of cardiac hypertrophy

Cardiac function was evaluated by echocardiography in rats with different treatments. As shown in Table 1, LVAWd and LVPWd were increased in ISO-treated rats compared with control group (P < 0.05), indicating impaired diastolic function of the heart. Consistently, EF and FS values were significantly reduced in ISO-treated rats (P < 0.05), which is consistent with the findings from previous studies (Gan et al., 2007; Li et al., 2008; Sun et al., 2011). After administration of cisapride, the increased LVAWd and LVPWd, and the reduced EF and FS, were all significantly restored to almost normal values as in non-ISO treated control rats (P < 0.05). These data indicate that cisapride profoundly improves cardiac function by attenuating hypertrophic impairment.

### 3.5. Effects of cisapride on QT interval in rats

In order to more precisely evaluate the change of QT interval, both QT and the corrected QT (QTc) intervals were calculated on the ECG from the different groups of rats. As shown in Fig. 9, compared to control rats, cisapride (subcutaneous injection for 10 days) at 0.5 or 5.0 mg/kg/day did not cause any changes in either QT or QTc interval (Fig. 9A–D). When rats were treated with cisapride 10.0 mg/kg/day, significant prolongation of QT and QTc intervals was observed, QT interval 80.26 ± 3.66 ms for control and 93.63 ± 2.43 ms for cisapride (P < 0.01) (Fig. 9C), QTc interval...
11.07 ms for control and 253.04 ms for cisapride, respectively (P < 0.01) (Fig. 9D).

4. Discussion

In this study, we assessed the effects of cisapride on the hypertrophic response, cardiac function, and the Ca\(^{2+}\) signaling pathway in the rat model of cardiac hypertrophy and a cellular model of cardiomyocyte hypertrophy. Our results strongly suggest that cisapride possesses anti-hypertrophic properties without affecting QT and QTc intervals, reflected by the actual reduction of cell/heart size, downregulation of hypertrophic marker genes, and amelioration of ISO-induced cardiac hypertrophy and dysfunction, which is likely conferred by its ability to downregulate Ca\(^{2+}\)/calcineurin/NFAT.

An important finding in our study is that cisapride at concentration of 0.01 μM exerted significantly anti-hypertrophic effects induced by ISO in neonatal rat cardiomyocytes. Report has shown that cisapride 0.05 μM has no electrophysiological effects in cardiomyocytes and purkinje fibers (Puisieux et al., 1996). While cisapride doses higher than 0.1 μM can cause prolongation of action potential duration (APD) in cardiomyocytes (Kii and Ito, 1997), which is associated with long QT and arrhythmia. In vivo study we also observed significant anti-hypertrophic effect of cisapride at concentration of 0.5 mg/kg/day in rat. For human it would be 0.08 mg/kg/day calculated from 0.5 mg/kg/day divided by 6, a factor used to convert rat dose to human-equivalent dose. However, in human cisapride at concentration of 0.8 mg/kg/day does not cause arrhythmia by inhibiting HERG channel (Markiewicz and Vanden, 2000). Importantly, our experimental data demonstrate that cisapride at dose of 0.5 or 5.0 mg/kg/day did not cause QT or QTc interval prolongation. However, prolongation of QT and QTc intervals was observed in the rats treated with cisapride 10.0 mg/kg/day. Taken together, our study demonstrate that cisapride had profoundly anti-hypertrophic effects in vitro at concentration of 0.01 μM and in vivo at 0.5 mg/kg/day without

![Fig. 8. Effects of cisapride on expression of calcineurin and NFATc3 in a rat model of cardiac hypertrophy induced by ISO. (A, C) Representative examples of Western blot bands from different treatments. Mean data of band density of calcineurin, NFATc3 (B) and p-NFATc3 (D) in different groups. n = 4 rats; *P < 0.05 & **P < 0.01 vs. Ctl; #P < 0.05 vs. ISO.](image)

Table 1
Parameters of cardiac function in rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>ISO</th>
<th>ISO + CIS</th>
<th>CIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVAWd (mm)</td>
<td>1.95 ± 0.28</td>
<td>2.60 ± 0.07(^{\text{a}})</td>
<td>2.39 ± 0.30(^{\text{b}})</td>
<td>1.87 ± 0.13</td>
</tr>
<tr>
<td>LVIDd (mm)</td>
<td>6.05 ± 0.66</td>
<td>6.05 ± 0.46</td>
<td>5.85 ± 0.34</td>
<td>5.76 ± 0.40</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td>1.74 ± 0.29</td>
<td>2.96 ± 0.43(^{\text{a}})</td>
<td>2.26 ± 0.46(^{\text{b}})</td>
<td>1.86 ± 0.19</td>
</tr>
<tr>
<td>EF (%)</td>
<td>83.85 ± 0.96</td>
<td>77.54 ± 1.12(^{\text{a}})</td>
<td>87.14 ± 6.01(^{\text{a}})</td>
<td>85.44 ± 3.81</td>
</tr>
<tr>
<td>FS (%)</td>
<td>55.20 ± 3.94</td>
<td>46.98 ± 0.89(^{\text{a}})</td>
<td>58.46 ± 8.13(^{\text{b}})</td>
<td>55.62 ± 4.36</td>
</tr>
</tbody>
</table>

LVAWd, left ventricular anterior wall, diastolic; LVIDd, left ventricular internal dimension, diastolic; LVPWd, left ventricular posterior wall, diastolic; EF, ejection fraction; FS, fractional shortening. n = 4 rats for each group.

\(^{\text{a}}\) vs. control, P < 0.05;
\(^{\text{b}}\) vs. ISO, P < 0.05.

216.53 ± 11.07 ms for control and 253.04 ± 12.48 ms for cisapride, respectively (P < 0.01) (Fig. 9D).
effects on QT interval in rat heart, indicating that cisapride may be used as an anti-cardiac hypertrophic agent without causing long QT syndrome associated with ventricular arrhythmias and torsades de pointes.

Pathological cardiac hypertrophy can be induced by a wide array of cardiovascular stresses such as hypertension, myocardial infarction, cardiac arrhythmias, and heart failure (Lorell and Carabello, 2000). A variety of hormonal factors, including angiotensin II (AngII), phenylephrine (PE), and isoproterenol (ISO) can stimulate the hypertrophic responses in cardiomyocytes (Karliner et al., 1990; Sadoshima and Izumo, 1993). The hypertrophic responses are often accompanied by an increase in intracellular Ca\(^{2+}\) concentration that can activate multiple signaling pathways, including the calcium/calmodulin-dependent phosphatase calcineurin/NFAT pathway, the calcium/calmodulin-dependent protein kinase (CaMK), and the MAP kinases (MAPK) pathway. Among these, the calcineurin/NFAT signaling pathway appears to play a key role in the development of pathological cardiac hypertrophy. In this study, it appears that cisapride exerts its anti-hypertrophic effect through mediating Ca\(^{2+}\)-mediated calcineurin/NFATc-3 signaling pathway, as it substantially inhibited the enhancement of intracellular Ca\(^{2+}\) and the upregulation of calcineurin and NFATc-3 induced by ISO. In addition, our results show that cisapride prevented the decrease of phosphorylated NFATc-3 induced by ISO. This is indirect evidence indicating that cisapride inhibited the upregulation of dephosphorylated NFATc-3, an active form of NFATc-3, induced by ISO (MacDonnell et al., 2009). However, this study did not go further to elucidate how cisapride induces intracellular Ca\(^ {2+}\) increase and the expression of calcineurin and NFATc-3. Involvement of the Ca/calcineurin/NFATc-3 pathway in the anti-hypertrophic effect has been demonstrated. For example, scutellarin has been shown to prevent cardiac hypertrophy through inhibiting the calcineurin and CaMK II pathways (Pan et al., 2010); Tanshinone IIA was found to prevent hypertrophy of cardiomyocyte via mediating the Ca/calcineurin/NFATc3 pathway (Tan et al., 2011). It has been shown that calcium antagonists produce beneficial effects on pathological cardiac hypertrophy (Finckenberg and Mervaala, 2010). In our study, we have excluded the possible contribution of HERG channel blocking effects and 5-HT activating properties of cisapride to its anti-hypertrophic effects.

This study reveals that cisapride at low and safe concentration protects against ISO-induced cardiac hypertrophy both in vitro and in vivo models without effects on ventricular repolarization (QT interval) in the rat heart, and provides new insight into this drug action. The Ca\(^{2+}\)-mediated calcineurin/NFATc-3 signaling pathway likely confers the anti-hypertrophic property of cisapride. Whether cisapride can be used as an anti-hypertrophic agent in patients with cardiac hypertrophy need to be further explored in future studies.

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