

Department of Cardiology, The First Affiliated Hospital of Harbin Medical University, Harbin, China

Cyclosporin A induces cardiomyocyte injury through calcium-sensing receptor-mediated calcium overload

JIEBING TANG, GUIXIANG WANG, YUE LIU, YU FU, JINYU CHI, YEJING ZHU, YANRU ZHAO, XINHUA YIN

Received July 13, 2010, accepted August 18, 2010

Xinhua Yin, Department of Cardiology, The First Affiliated Hospital of Harbin Medical University, No. 23, YouZheng Street, NanGang District, Harbin 150001, China
harbin0910@yahoo.com

Pharmazie 66: 52–57 (2011)

doi: 10.1691/ph.2011.0711

The aim of this study was to investigate whether Cyclosporin-A (CsA)-induced myocardial injury is mediated by elevating the intracellular calcium concentration ($[Ca^{2+}]_i$) through the Calcium sensing receptor (CaSR). Cultured neonatal rat cardiomyocytes were treated with CsA, with or without pretreatment with the CaSR-specific antagonist NPS2390 or the CaSR-specific agonist gadolinium chloride ($GdCl_3$). At 2 h, 4 h, 6 h and 8 h after CsA treatment, the ultrastructural changes of the cardiomyocytes were observed. In addition, the lactate dehydrogenase (LDH) and creatine kinase (CK) release from the cardiomyocytes, the $[Ca^{2+}]_i$ and the level of CaSR expression were determined. With increasing time of CsA treatment, ultrastructural damage of cardiomyocytes gradually aggravated, LDH and CK release and $[Ca^{2+}]_i$ also gradually increased. CaSR mRNA and protein expression increased at 4 h after CsA treatment. Compared with CsA treatment alone, pretreatment with NPS2390 lessened the ultrastructural damage of the cardiomyocytes as well as decreased the LDH and CK release, $[Ca^{2+}]_i$ and the expression of the CaSR mRNA and protein. Conversely, pretreatment with $GdCl_3$ aggravated the ultrastructural damage of the cardiomyocytes as well as increased LDH and CK release, $[Ca^{2+}]_i$ and the expression of the CaSR mRNA and protein. These results demonstrate that CsA induced cardiomyocyte injury in a time-dependent manner. Moreover, CsA-induced cardiomyocyte injury was related to CaSR-mediated intracellular calcium overload. These findings provide new insight into the mechanisms involved in CsA-induced myocardial injury.

1. Introduction

Cyclosporin A (CsA) is an effective immunosuppressive agent that is used in preventing organ rejection and treating autoimmune disease (Borel et al. 1996). Some clinical investigations have shown that several patients who underwent orthotopic heart transplantation with CsA treatment developed severe diastolic dysfunction, and their endocardial biopsy showed myocardial fibrosis (Laczkovics et al. 1987; Siostrzonek et al. 1993). Several experimental studies have also shown that CsA can lead to myofibril disorganization and myocardial fibrosis in the rat heart (Rezzani et al. 2003; Selcoki et al. 2007). Thus, treatment with CsA is limited by its cardiotoxicity. Although the mechanism of CsA-induced cardiotoxicity remains poorly understood, some studies have shown that intracellular calcium overload could be a major cause (Florio et al. 2003). Therefore, analysis of the pathway of CsA-induced calcium overload would aid in understanding the mechanism of CsA-induced cardiotoxicity. The calcium-sensing receptor (CaSR) is a member of the G protein-coupled receptor (GPCR) superfamily. The CaSR was initially discovered in parathyroid chief cells (Brown et al. 1993), and expression was subsequently found in various tissues and organs (Brown and MacLeod 2001). Moreover, the CaSR was shown to play an important role in the regulation of systemic calcium homeostasis (Fudge and Kovacs 2004; Kifor et al. 2001). The CaSR is also involved in cell differentiation and proliferation (Quarles 1997), gene

expression (Tfelt-Hansen et al. 2003), ion channel opening (van den Hurk et al. 2005) and hormone secretion (Squires et al. 2000). Wang et al. (2003) reported that the level of the CaSR that is present in cardiac tissue and the activation of the CaSR increased $[Ca^{2+}]_i$ through the PLC-inositol, 4, 5-triphosphate (IP_3) pathway. During cardiac ischemia/reperfusion (I/R), CaSR expression increased, and the CaSR was found to be involved in calcium overload-induced cardiomyocyte apoptosis (Zhang et al. 2006). However, the relationship between the CaSR and CsA-induced myocardial injury remains unknown. Our previous study showed that CaSR mRNA level increased in rat ventricular muscle after one week of CsA administration. Therefore, we speculated that the CaSR could be directly or indirectly involved in CsA-induced myocardial injury. With this hypothesis in mind, we investigated the injury induced by CsA and the effect of CsA on CaSR expression in neonatal rat cardiomyocytes. Furthermore, we studied the role of the CaSR in CsA-induced cardiomyocyte injury using the CaSR-specific antagonist NPS2390 and the CaSR-specific agonist $GdCl_3$.

2. Investigations and results

2.1. Effect of CsA on the ultrastructure of cardiomyocytes

Transmission electron microscopy revealed a normal ultrastructure for the cardiomyocytes in the control group characterized by evenly distributed chromatin, clear mitochondrial cristae and

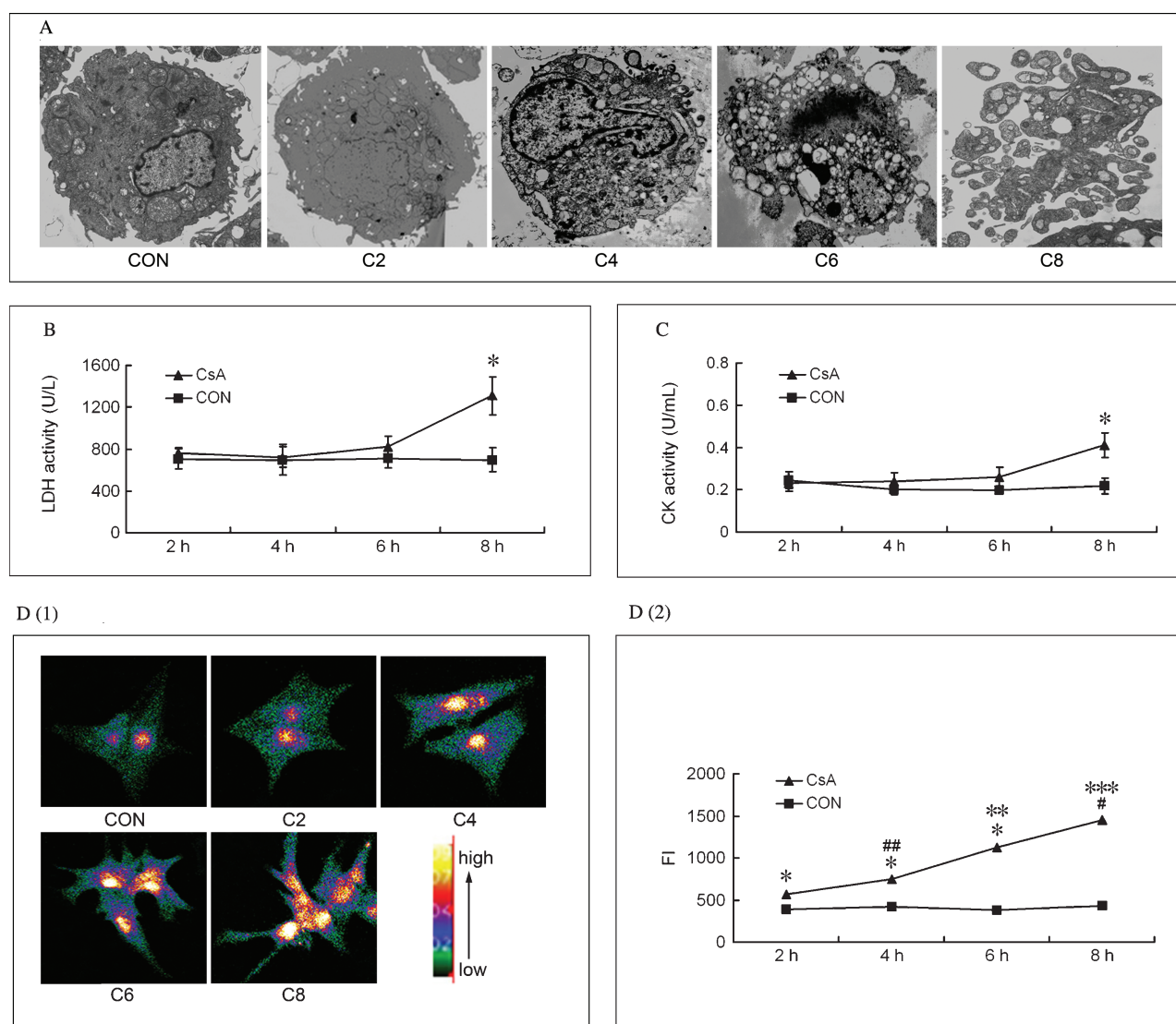


Fig. 1: Effect of CsA on neonatal rat cardiomyocytes. Neonatal rat cardiomyocytes were treated with 10 $\mu\text{g/ml}$ CsA. At 2 h, 4 h, 6 h and 8 h after CsA treatment, the ultrastructural changes of the cardiomyocytes were observed, and the LDH and CK release from the cardiomyocytes and their $[\text{Ca}^{2+}]_i$ were determined. CON: control; C2: CsA 2 h; C4: CsA 4 h; C6: CsA 6 h; C8: CsA 8 h. A. Ultrastructural changes of cardiomyocytes. The ultrastructural damage of the cardiomyocytes aggravated with treatment time, progressing from slight mitochondrial swelling to cellular apoptosis and secondary necrosis. (Original magnification: CON, C2, C6 $\times 6000$; C4, C8 $\times 8000$) B. and C. LDH and CK release from cardiomyocytes. D. $[\text{Ca}^{2+}]_i$ changes in neonatal rat cardiomyocytes. D(1). Fluorescent images of cardiomyocytes. D(2). Fluorescent intensity analysis for $[\text{Ca}^{2+}]_i$ in cardiomyocytes. * $p < 0.05$ vs. control; ** $p < 0.01$ vs. control; *** $p < 0.05$ vs. CsA 2 h; * $p < 0.05$ vs. CsA 4 h; ** $p < 0.05$ vs. CsA 6 h

plentiful muscle fibers. After 2 h of CsA treatment, slight mitochondrial swelling was observed; after 4 h, the ultrastructural changes of the cardiomyocytes included cell contraction, cytoplasmic condensation and slight nuclear pyknosis, which are characteristics of early apoptosis; after 6 h, the ultrastructural changes of the cardiomyocytes included nuclear pyknosis, mitochondrial distension, chromatin margination and the formation of apoptotic bodies, characteristics of late apoptosis; after 8 h, apoptotic bodies and secondary necrosis were seen in most cardiomyocytes (Fig. 1).

2.2. Effect of CsA on LDH and CK release from cardiomyocytes

The control group showed no differences over time in LDH and CK release from cardiomyocytes treated with DMEM for 2 h, 4 h, 6 h and 8 h. Compared with the control group, LDH and CK release did not change significantly at 2 h after CsA treatment. At 4 h and 6 h, the release was slightly but not significantly elevated ($p > 0.05$), and at 8 h, release was significantly higher than the control ($p < 0.05$) (Fig. 1).

2.3. Effect of CsA on the $[\text{Ca}^{2+}]_i$ of cardiomyocytes

Measurement of $[\text{Ca}^{2+}]_i$ using laser confocal scanning microscopy showed no differences among the $[\text{Ca}^{2+}]_i$ of cardiomyocytes treated with DMEM for 2 h, 4 h, 6 h and 8 h. The $[\text{Ca}^{2+}]_i$ of the cardiomyocytes increased to various degrees at 2 h, 4 h, 6 h and 8 h after CsA treatment compared to that in the control group ($p < 0.05$ and $p < 0.01$). Moreover, CsA increased $[\text{Ca}^{2+}]_i$ in a time-dependent manner (Fig. 1).

2.4. CaSR expression in neonatal rat cardiomyocytes

2.4.1. CaSR mRNA expression

After CsA treatment for 4 h, the level of CaSR mRNA was higher than that of the control group ($p < 0.05$). NPS pre-treatment attenuated the increase in the CaSR mRNA level ($p < 0.05$), and GdCl_3 further increased the CaSR mRNA level ($p < 0.05$) (Fig. 2).

2.4.2. CaSR protein expression

In the western blot analysis, two bands corresponding to the CaSR with relative molecular masses of 150 kD and 170–180 kD

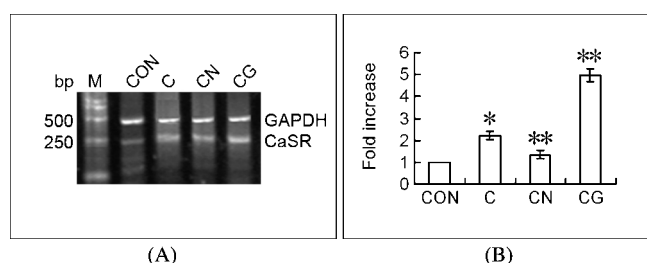


Fig. 2: CaSR mRNA expression in neonatal rat cardiomyocytes as determined by RT-PCR. M: Marker; CON: control; C: CsA; CN: CsA + NPS2390; CG: CsA + GdCl₃. A: RT-PCR result. B: Analysis of CaSR mRNA expression. The intensity of each band was quantified using densitometry, and the data were normalized to the actin signal. * $p < 0.05$ vs. control; ** $p < 0.05$ vs. CsA

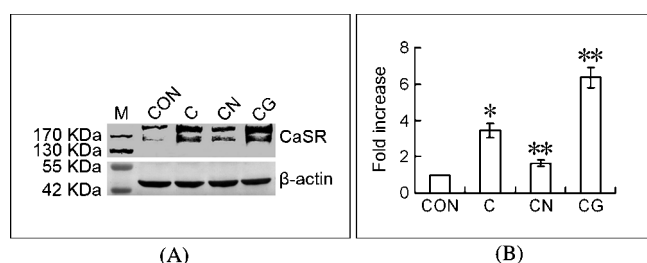


Fig. 3: CaSR protein expression in neonatal rat cardiomyocytes as determined by western blot analysis. M: Marker; CON: control; C: CsA; CN: CsA + NPS2390; CG: CsA + GdCl₃. A: Western blot result. B: Analysis of CaSR protein expression. The intensity of each band was quantified using densitometry, and the data were normalized to the actin signal. * $p < 0.05$ vs. control; ** $p < 0.05$ vs. CsA

were detected. After CsA treatment for 4 h, the CaSR protein level was higher than that of the control group ($p < 0.05$). NPS pretreatment attenuated the increased level of the CaSR protein ($p < 0.05$), and GdCl₃ further increased the CaSR protein level ($p < 0.05$) (Fig. 3).

2.5. Effect of inhibition or activation of the CaSR on CsA-induced cardiomyocyte ultrastructure damage

Compared with CsA treatment alone for 4 h, NPS pretreatment alleviated the cardiomyocyte ultrastructural damage, including mitochondrial swelling and mild nuclear swelling, and GdCl₃ pretreatment aggravated the cardiomyocyte ultrastructural damage, including the formation of apoptotic bodies and secondary necrosis (Fig. 4).

2.6. Effect of inhibition or activation of the CaSR on CsA-induced LDH and CK release

Compared with CsA treatment alone for 8 h, NPS pretreatment attenuated the increase in LDH and CK release ($p < 0.05$), and GdCl₃ pretreatment further increased LDH and CK release ($p < 0.05$) (Fig. 4).

2.7. Effect of inhibition or activation of the CaSR on CsA-induced $[Ca^{2+}]_i$ increase

Compared with CsA treatment alone for 4 h, NPS pretreatment attenuated the increase in $[Ca^{2+}]_i$ ($p < 0.05$), and GdCl₃ pretreatment further increased $[Ca^{2+}]_i$ ($p < 0.05$) (Fig. 4).

3. Discussion

CsA has been reported to have dual effects on the myocardium. Some studies have shown that CsA leads to degenerative changes

in normal rat cardiac tissues, including a great disorganization of the myofibrils and myocardial fibrosis in both the atria and ventricles (Bianchi et al. 2003; Jurado et al. 1998). Conversely, other studies have shown that CsA inhibits cardiac hypertrophy (Molkentin et al. 1998) and protects the myocardium against ischemia/reperfusion injury (Bes et al. 2005; Griffiths and Halestrap 1993) and failure (Sharov et al. 2007). Although these studies have demonstrated that CsA has opposing effects on the myocardium, CsA-induced myocardial injury cannot be neglected. In our previous studies, CsA was administered to normal rats and this treatment resulted in widespread edema, muscle fiber fracture and cardiac fibrosis in cardiac tissues. In the present study, we found that the ultrastructural damage of cardiomyocytes aggravated with increasing CsA treatment time, progressing from slight mitochondrial swelling to cellular apoptosis and secondary necrosis. Meanwhile, LDH and CK release, indicators of cardiomyocyte injury, gradually increased and were significantly higher at 8 h after CsA treatment compared with the control. This result demonstrated that CsA induced cardiomyocyte injury in a time-dependent manner.

The maintenance of intracellular calcium homeostasis plays an important role in cell growth, development and survival. Exposure to a variety of stimuli can lead to an imbalance of intracellular calcium homeostasis, including calcium overload and calcium depletion, which can lead to reversible or irreversible cell injury. Commonly, this imbalance in intracellular calcium homeostasis is manifested as calcium overload (Dong et al. 2006). Recent studies showed that intracellular calcium overload-induced oxidative stress could be important in CsA-induced myocardial injury (Florino et al. 2003). Therefore, in the present study we investigated the effect of CsA on $[Ca^{2+}]_i$. The result showed that CsA treatment increased $[Ca^{2+}]_i$ in a time-dependent manner, with $[Ca^{2+}]_i$ gradually increasing with treatment time. Meanwhile, CsA-induced cardiomyocyte injury aggravated over time. This indicated that the degree of intracellular calcium overload seemed to determine the extent of cardiomyocyte injury. Our data suggested a key role for intracellular calcium overload in CsA-induced cardiomyocyte injury. Numerous studies have shown that the CaSR is expressed throughout the cardiovascular system, such as in the myocardium (Wang et al. 2003), vascular smooth muscle (Smajilovic et al. 2006) and endothelial cells (Ziegelstein et al. 2006). Furthermore, the CaSR plays an important role in a variety of physiological and pathological conditions, such as myocardial ischemia/reperfusion (Zhang et al. 2006, 2007), cardiomyocyte apoptosis (Sun et al. 2006) and cardiac hypertrophy (Wang et al. 2008). However, there is a lack of information concerning the relationship between the CaSR and CsA-induced myocardial injury. In our study, we used the CaSR-specific antagonist NPS2390 and the CaSR-specific agonist GdCl₃ to reveal the role of the CaSR in CsA-induced myocardial injury. First, we determined the level of CaSR expression by RT-PCR and western blot analysis. During CsA-induced cardiomyocyte injury, CaSR mRNA and protein expression increased, which indicated the involvement of the CaSR in CsA-induced cardiomyocyte injury. In order to further elucidate the role of the CaSR in CsA-induced cardiomyocyte injury, neonatal rat cardiomyocytes were pretreated with NPS2390 or GdCl₃ before CsA treatment. Compared with CsA treatment alone, pretreatment with NPS2390 ameliorated the ultrastructural damage of the cardiomyocytes, decreased the release of LDH and CK and decreased the expression of the CaSR mRNA and protein. Conversely, pretreatment with GdCl₃ aggravated the ultrastructural damage of the cardiomyocytes, increased the release of LDH and CK and increased the expression of the CaSR mRNA and protein. These results suggested that the CaSR could be involved in CsA-induced cardiomyocyte injury. Recently, an

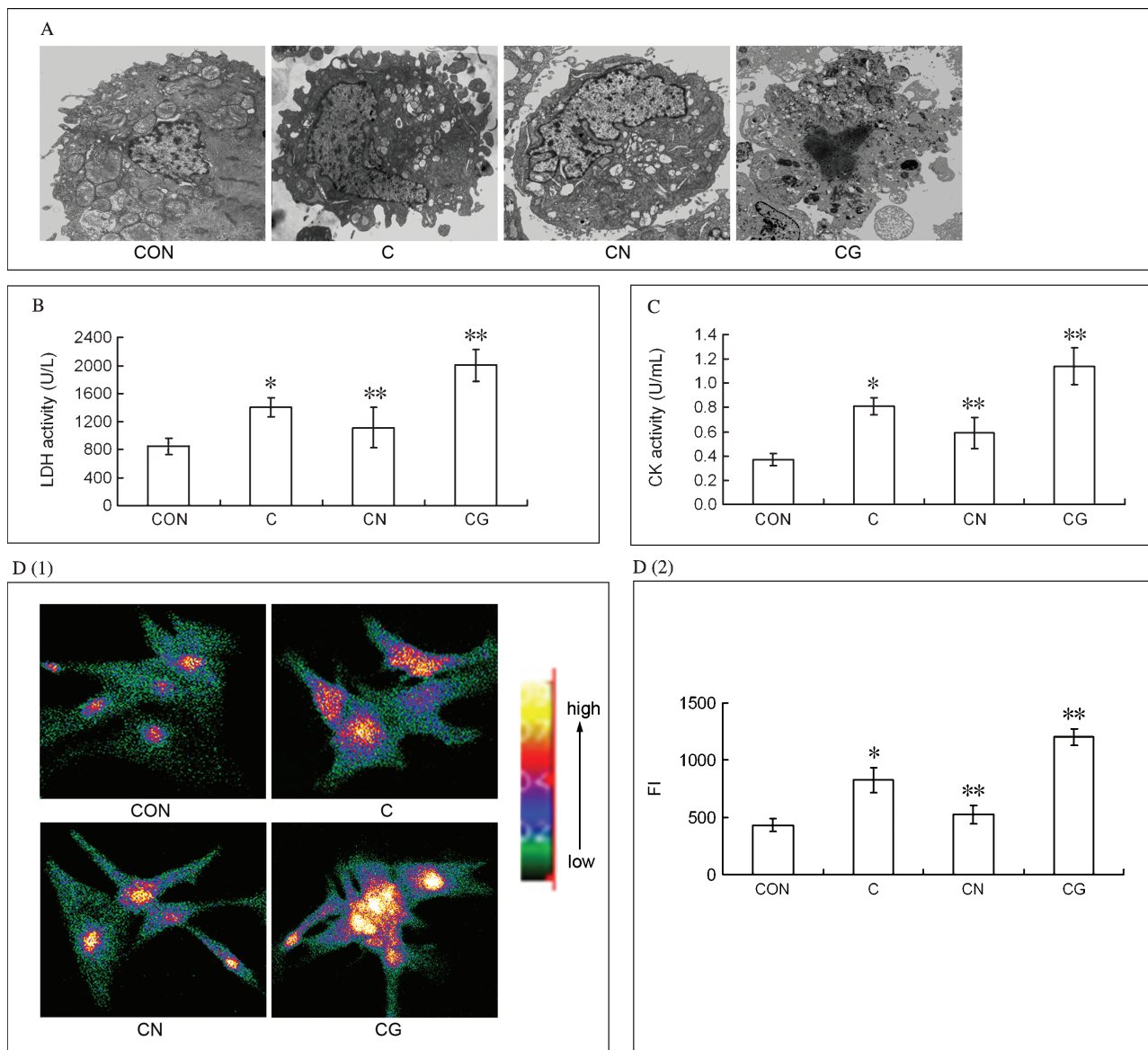


Fig. 4: Effect of the inhibition or activation of the CaSR on CsA-treated cardiomyocytes. CON: control; C: CsA; CN: CsA + NPS2390; CG: CsA + GdCl₃. A. Ultrastructural changes of cardiomyocytes. Compared with the CsA treatment alone for 4 h, pretreatment with NPS alleviated the cardiomyocyte ultrastructural damage, including mitochondrial swelling and mild nuclear swelling. Conversely, GdCl₃ pretreatment aggravated the cardiomyocyte ultrastructure damage, including the formation of apoptotic bodies and secondary necrosis. (Original magnification: CON \times 10000; C, CN, CG \times 6000) B. and C. LDH and CK release from cardiomyocytes. D. $[Ca^{2+}]_i$ changes in neonatal rat cardiomyocytes. D(1). Fluorescent images of cardiomyocytes. D(2). Fluorescent intensity analysis for $[Ca^{2+}]_i$ in cardiomyocytes. * $p < 0.05$ vs. control; ** $p < 0.05$ vs. CsA

accumulating body of evidence has shown that the CaSR was an important regulator of calcium homeostasis in cardiomyocytes (Sun et al. 2006; Wang et al. 2003; Zhang et al. 2006). Therefore, we examined the involvement of the CaSR in calcium overload during CsA-induced cardiomyocyte injury. Our findings that CsA-induced calcium overload was attenuated by NPS2390 and exacerbated by GdCl₃ suggested that the CaSR could be involved in CsA-induced calcium overload. These results demonstrated that the CaSR could mediate intracellular calcium overload, thus inducing cardiomyocyte injury. Previous studies have shown that the CsA-induced increase in the frequency of Ca²⁺ oscillation and in the level of free calcium in the cytosol is likely related to a higher probability of endoplasmic reticulum (ER) Ca²⁺-release channel openings (Bandyopadhyay et al. 2000). The binding of extracellular calcium to the CaSR could induce calcium release from the ER through the G protein-PLC-IP₃ pathway (Wang et al. 2003). One attractive hypothesis is that CsA activates the CaSR and its G protein-PLC-IP₃ pathway, followed by ER calcium release.

Calcium is an important messenger for intracellular signal transduction (Berridge et al. 2003). Cellular calcium is remarkable in its ability to bind and regulate various proteases such as phospholipase, calpain and endonuclease. An increase in $[Ca^{2+}]_i$ induces the activation of these Ca²⁺-dependent proteases, which leads to membrane phospholipid hydrolysis, cytoskeletal disruption and DNA damage, resulting in cell injury (Dong et al. 2006). Therefore, CsA-induced cardiomyocyte injury might be related to proteases activated by calcium overload. In addition, mitochondria are important organelles in the maintenance of intracellular calcium homeostasis. Upon an increase in $[Ca^{2+}]_i$, calcium uptake by the mitochondria increases and mitochondrial calcium overload ensues, resulting in cell apoptosis and cell injury (Ott et al. 2002; Schanne et al. 1979). Millane et al. (1994) found that mitochondrial calcium overload could cause CsA-induced myocardial injury. Further study is required to determine whether CaSR-mediated intracellular calcium overload results in mitochondrial calcium accumulation during CsA-induced cardiomyocyte injury.

4. Experimental

4.1. Materials

Cyclosporin A, fluo-3 acetoxymethyl ester (Fluo-3/AM), the CaSR-specific agonist GdCl₃ and the CaSR-specific antagonist NPS2390 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The lactate dehydrogenase (LDH) and creatine kinase (CK) kits were obtained from Nanjing Jiancheng Biotechnology Co., Ltd. (Nanjing, Jiangsu, China). The primary anti-CaSR antibody was obtained from Alpha Diagnostic International Inc. (San Antonio, TX, USA).

4.2. Neonatal rat cardiomyocyte culture

Neonatal rat cardiomyocytes were prepared according to a previously described method (Xu et al. 2004) with minor modifications. Briefly, hearts from 1- to 3-day-old neonatal Wistar rats (Animal Research Institute of Harbin Medical University, China) were dissected, minced and placed in a Petri dish. The tissue was trypsinized at 37 °C in D-Hanks' balanced salt solution (8.00 g/L NaCl, 0.4 g/L KCl, 0.06 g/L KH₂PO₄, 0.35 g/L NaHCO₃, 0.09 g/L Na₂HPO₄·7H₂O and 0.25% trypsin). After centrifugation (1000 rpm, 10 min), cells were collected and resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) newborn calf serum. The cells were diluted to 5 × 10⁶ cells/ml and then cultured at 37 °C in a humidified atmosphere containing 5% (v/v) CO₂.

4.3. CsA incubation

After 72 h of culture and subsequent starving in serum-free medium for 24 h, the cardiomyocytes were incubated with 10 μg/ml CsA for 2 h, 4 h, 6 h or 8 h, in 5% CO₂ at 37 °C. A control group was incubated in DMEM for the same time periods.

4.4. Preincubation with NPS2390 or GdCl₃

After 72 h of culture and subsequent starving in serum-free medium for 24 h, the cardiomyocytes were preincubated with or without NPS2390 (10 μM, 1 h) or GdCl₃ (30 μM, 2 h) and then incubated with 10 μg/ml CsA for 4 h or 8 h in 5% CO₂ at 37 °C.

4.5. Transmission electron microscopy

The ultrastructural changes of the cardiomyocytes were examined by transmission electron microscopy. Electron microscopy analysis was performed as published previously (Ho et al. 1996). After the treatment described above, the cells were fixed with 2.5% glutaraldehyde in Hank's modified salt solution and then postfixed in 1% OsO₄ in 0.1 M cacodylate buffer. The OsO₄-fixed samples were dehydrated in an ethanol series and embedded in Araldite. Ultrathin sections were stained with saturated uranyl acetate and lead citrate and then observed under a JEM-1220 electron microscope.

4.6. LDH and CK assays

The cardiomyocytes were assayed for LDH and CK release into the culture media, which are indicators of cardiomyocyte injury. After the treatment described above, the culture media samples were collected and measured spectrophotometrically using an LDH or CK kit. With this kit, LDH catalyzes lactate to yield pyruvate, which reacts with 2,4-dinitrophenylhydrazine to produce pyruvate dinitrophenylhydrazone. The change in absorbance at 440 nm was monitored with a spectrophotometer and LDH activity was expressed as U/L. CK phosphorylates creatine to yield creatine phosphate, which dissociates into inosine and inorganic phosphate. Inorganic phosphate reacts with ammonium molybdate to produce phosphomolybdic acid, which is reduced to molybdenum blue. The change in absorbance at 660 nm was monitored with a spectrophotometer and CK activity was expressed as U/ml.

4.7. Reverse transcription-polymerase chain reaction (RT-PCR) of CaSR

Total RNA was isolated from cultured cardiomyocytes using TRIzol Reagent (Invitrogen) 4 h after CsA treatment. Total RNA (2 μg) was reverse transcribed with a RT kit (Promega). The RT reaction was performed with random primers according to the manufacturer's protocol. For each RT product, aliquots of the final reaction volume were amplified in a PCR reaction with CaSR-specific primers (sense 5'-TTCGGCATCAGCTTTGTG-3', antisense 5'-TGAAGATGATTTCTGCTTCC-3') or GAPDH-specific primers (sense 5'-CTCAACTACATGGTCTACATG-3', antisense 5'-TGGCATGGACTGTGGTCATGAG-3'). Amplification was performed over 35 cycles, each consisting of 2 min at 94 °C, 40 s at 50 °C and 40 s at 72 °C. A final extension period of 2 min at 72 °C was included after the 35 cycles. The PCR products were electrophoresed on a 1.2% agarose gel and visualized with ethidium bromide. The optical density (OD) of each

band was measured using a Bio-Rad Chemi Doc™ EQ densitometer and Bio-Rad Quantity One software (Bio-Rad Laboratories, Hercules, USA).

4.8. Western blot analysis for CaSR

Western blot analysis was performed according to a previously described method (El-Hajj et al. 2007). Briefly, the cells were washed with PBS and resuspended in cold lysis buffer with PMSF. The cell lysate was incubated on ice for 30 min and centrifuged at 12,000 rpm for 15 min at 4 °C. The protein concentration of the lysate was determined with a BCA-200 protein assay kit (Beyotime). Equal amounts of protein were denatured by boiling and then electrophoresed in an 8% SDS-polyacrylamide gel. The protein was transferred to nitrocellulose. After blocking with 5% nonfat milk in TBS-T [137 mM NaCl, 20 mM Tris (pH 7.6), and 0.1% (v/v) Tween 20] for 1 h at 37 °C, the blots were incubated overnight at 4 °C with a primary antibody against CaSR (1:2000, rabbit monoclonal). The membrane was incubated with an AP-conjugated secondary antibody for 1 h at 37 °C. The volumes of the protein bands were quantified using a Bio-Rad Chemi Doc™ EQ densitometer and Bio-Rad Quantity One software (Bio-Rad Laboratories, Hercules, USA). β-actin was used as an internal control for the semiquantitative assay.

4.9. Measurement of [Ca²⁺]_i

The cardiomyocytes were loaded with 5 μmol/L of the Ca²⁺-selective fluorescent probe Fluo-3/AM for 30 min at 37 °C. Excess extracellular dye was removed by washing three times with Ca²⁺-free PBS. The fluorescence intensity for [Ca²⁺]_i was measured using a laser scanning confocal microscope (Olympus, Japan) with excitation at 488 nm and emission at 530 nm.

4.10. Statistical analysis

All results are expressed as the mean ± SEM. A one-way ANOVA was used for multiple comparisons. Differences between two groups were tested with unpaired two-tailed Student's *t* tests. A value of *p* < 0.05 was considered statistically significant.

Acknowledgments: This research was supported by the National Natural Science Foundation of China (30872387) and the Postgraduate Foundation of the National Education Ministry of China (200802260006).

References

- Bandyopadhyay A, Shin DW, Ahn JO, Kim DH (2000) Calcineurin regulates ryanodine receptor/Ca(2+)-release channels in rat heart. *Biochem J* 352 Pt 1: 61–70.
- Berridge MJ, Bootman MD, Roderick HL (2003) Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 4: 517–529.
- Bes S, Vandroux D, Tissier C, Devillard L, Brochot A, Tatou E, Duvillard L, Rochette L, Athias P (2005) Direct, pleiotropic protective effect of cyclosporin A against simulated ischemia-induced injury in isolated cardiomyocytes. *Eur J Pharmacol* 511: 109–120.
- Bianchi R, Rodella L, Rezzani R (2003) Cyclosporine A up-regulates expression of matrix metalloproteinase 2 and vascular endothelial growth factor in rat heart. *Int Immunopharmacol* 3: 427–433.
- Borel JF, Baumann G, Chapman I, Donatsch P, Fahr A, Mueller EA, Vigouret JM (1996) In vivo pharmacological effects of cyclosporin and some analogues. *Adv Pharmacol* 35: 115–246.
- Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, Hebert SC (1993) Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature* 366: 575–580.
- Brown EM, MacLeod RJ (2001) Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev* 81: 239–297.
- Dong Z, Saikumar P, Weinberg JM, Venkatachalam MA (2006) Calcium in cell injury and death. *Annu Rev Pathol* 1: 405–434.
- El-Hajj RA, McKay SB, McKay DB (2007) Pharmacological and immunological identification of native alpha7 nicotinic receptors: evidence for homomeric and heteromeric alpha7 receptors. *Life Sci* 81: 1317–1322.
- Florio S, Ciarcia R, Crispino L, Pagnini U, Ruocco A, Kumar C, D'Andrilli G, Russo F (2003) Hydrocortisone has a protective effect on CyclosporinA-induced cardiotoxicity. *J Cell Physiol* 195: 21–26.
- Fudge NJ, Kovacs CS (2004) Physiological studies in heterozygous calcium sensing receptor (CaSR) gene-ablated mice confirm that the CaSR regulates calcitonin release in vivo. *BMC Physiol* 4: 5.
- Griffiths EJ, Halestrap AP (1993) Protection by cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts. *J Mol Cell Cardiol* 25: 1461–1469.

- Ho YS, Wang YJ, Lin JK (1996) Induction of p53 and p21/WAF1/CIP1 expression by nitric oxide and their association with apoptosis in human cancer cells. *Mol Carcinog* 16: 20–31.
- Jurado F, Bellon JM, Pareja JA, Golitsin A, Millan L, Pascual G, Bujan J (1998) Effects of ischaemia-reperfusion and cyclosporin-A on cardiac muscle ultrastructure. *Histol Histopathol* 13: 761–774.
- Kifor O, MacLeod RJ, Diaz R, Bai M, Yamaguchi T, Yao T, Kifor I, Brown EM (2001) Regulation of MAP kinase by calcium-sensing receptor in bovine parathyroid and CaR-transfected HEK293 cells. *Am J Physiol Renal Physiol* 280: F291–302.
- Laczkovics A, Havel M, Teufelsbauer H, Horvath R, Schreiner W, Wolner E (1987) Cyclosporin-A induced heart failure after orthotopic heart transplantation. *Thorac Cardiovasc Surg* 35: 83–86.
- Millane T, Wilson AJ, Patel MK, Jennison SH, Holt DW, Murday AJ, Camm AJ (1994) Mitochondrial calcium deposition in association with cyclosporine therapy and myocardial magnesium depletion: a serial histologic study in heart transplant recipients. *J Heart Lung Transplant* 13: 473–480.
- Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR, Olson EN (1998) A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93: 215–228.
- Ott M, Robertson JD, Gogvadze V, Zhivotovsky B, Orrenius S (2002) Cytochrome c release from mitochondria proceeds by a two-step process. *Proc Natl Acad Sci U S A* 99: 1259–1263.
- Quarles LD (1997) Cation sensing receptors in bone: a novel paradigm for regulating bone remodeling? *J Bone Miner Res* 12: 1971–1974.
- Rezzani R, Rodella L, Dessy C, Daneau G, Bianchi R, Feron O (2003) Changes in Hsp90 expression determine the effects of cyclosporine A on the NO pathway in rat myocardium. *FEBS Lett* 552: 125–129.
- Schanne FA, Kane AB, Young EE, Farber JL (1979) Calcium dependence of toxic cell death: a final common pathway. *Science* 206: 700–702.
- Selcoki Y, Uz E, Bayrak R, Sahin S, Kaya A, Uz B, Karanfil A, Ozkara A, Akcay A (2007) The protective effect of erdosteine against cyclosporine A-induced cardiotoxicity in rats. *Toxicology* 239: 53–59.
- Sharov VG, Todor A, Khanal S, Imai M, Sabbah HN (2007) Cyclosporine A attenuates mitochondrial permeability transition and improves mitochondrial respiratory function in cardiomyocytes isolated from dogs with heart failure. *J Mol Cell Cardiol* 42: 150–158.
- Siostrzonek P, Teufelsbauer H, Kreiner G, Laczkovics A, Gossinger H, Heinz G, Stumppfen A, Mossbacher H (1993) Relief of diastolic cardiac dysfunction after cyclosporine withdrawal in a cardiac transplant recipient. *Eur Heart J* 14: 859–861.
- Smajilovic S, Hansen JL, Christoffersen TE, Lewin E, Sheikh SP, Terwilliger EF, Brown EM, Haunso S, Tfelt-Hansen J (2006) Extracellular calcium sensing in rat aortic vascular smooth muscle cells. *Biochem Biophys Res Commun* 348: 1215–1223.
- Squires PE, Harris TE, Persaud SJ, Curtis SB, Buchan AM, Jones PM (2000) The extracellular calcium-sensing receptor on human beta-cells negatively modulates insulin secretion. *Diabetes* 49: 409–417.
- Sun YH, Liu MN, Li H, Shi S, Zhao YJ, Wang R, Xu CQ (2006) Calcium-sensing receptor induces rat neonatal ventricular cardiomyocyte apoptosis. *Biochem Biophys Res Commun* 350: 942–948.
- Tfelt-Hansen J, Schwarz P, Terwilliger EF, Brown EM, Chattopadhyay N (2003) Calcium-sensing receptor induces messenger ribonucleic acid of human securin, pituitary tumor transforming gene, in rat testicular cancer. *Endocrinology* 144: 5188–5193.
- van den Hurk MJ, Jenks BG, Roubos EW, Scheenen WJ (2005) The extracellular calcium-sensing receptor increases the number of calcium steps and action currents in pituitary melanotrope cells. *Neurosci Lett* 377: 125–129.
- Wang LN, Wang C, Lin Y, Xi YH, Zhang WH, Zhao YJ, Li HZ, Tian Y, Lv YJ, Yang BF, Xu CQ (2008) Involvement of calcium-sensing receptor in cardiac hypertrophy-induced by angiotensinII through calcineurin pathway in cultured neonatal rat cardiomyocytes. *Biochem Biophys Res Commun* 369: 584–589.
- Wang R, Xu C, Zhao W, Zhang J, Cao K, Yang B, Wu L (2003) Calcium and polyamine regulated calcium-sensing receptors in cardiac tissues. *Eur J Biochem* 270: 2680–2688.
- Xu FP, Chen MS, Wang YZ, Yi Q, Lin SB, Chen AF, Luo JD (2004) Leptin induces hypertrophy via endothelin-1-reactive oxygen species pathway in cultured neonatal rat cardiomyocytes. *Circulation* 110: 1269–1275.
- Zhang WH, Fu SB, Lu FH, Wu B, Gong DM, Pan ZW, Lv YJ, Zhao YJ, Li QF, Wang R, Yang BF, Xu CQ (2006) Involvement of calcium-sensing receptor in ischemia/reperfusion-induced apoptosis in rat cardiomyocytes. *Biochem Biophys Res Commun* 347: 872–881.
- Zhang WH, Lu FH, Zhao YJ, Wang LN, Tian Y, Pan ZW, Lv YJ, Wang YL, Du LJ, Sun ZR, Yang BF, Wang R, Xu CQ (2007) Post-conditioning protects rat cardiomyocytes via PKCepsilon-mediated calcium-sensing receptors. *Biochem Biophys Res Commun* 361: 659–664.
- Ziegelstein RC, Xiong Y, He C, Hu Q (2006) Expression of a functional extracellular calcium-sensing receptor in human aortic endothelial cells. *Biochem Biophys Res Commun* 342: 153–163.