Mitochondrial calcium uniporter blocker prevents cardiac mitochondrial dysfunction induced by iron overload in thalassemic mice

Sirinart Kumfu · Siriporn Chattipakorn · Suthat Fucharoen · Nipon Chattipakorn

Abstract Iron-overload induced cardiomyopathy is a major cause of morbidity and mortality in thalassemic patients. Previous studies suggest that cardiac mitochondrial dysfunction may be involved in the pathogenesis of cardiomyopathy in thalassemia. We tested the hypothesis that iron overload causes dysfunction of cardiac mitochondria isolated from thalassemic mice. Cardiac mitochondria were isolated from the heart tissue of genetically-altered, β-thalassemic mice (HT) and adult wild-type mice (WT). Ferrous iron (Fe^{2+}) at various concentrations (0–5 µg/ml) was applied to induce iron toxicity. Pharmacological interventions, facilitated by mitochondrial permeability transition pore (mPTP) blocker, CsA, and mitochondrial Ca^{2+} uniporter (MCU) blocker, Ru360, were used to study their respective effects on cardiac mitochondrial dysfunction. Cardiac mitochondrial ROS production, mitochondrial membrane potential changes, and mitochondrial swelling were determined. Iron overload caused increased ROS production, mitochondrial depolarization, and mitochondrial swelling in a dose-dependent manner in WT and HT cardiac mitochondria. CsA decreased only ROS production in WT and HT cardiac mitochondria, whereas Ru360 completely prevented the development of cardiac mitochondrial dysfunction by decreasing ROS, mitochondrial depolarization, and swelling in both WT and HT cardiac mitochondria. Ru360, an MCU blocker, provides protective effects by preventing ROS production and mitochondrial depolarization as well as attenuating mitochondrial swelling caused by Fe^{2+} overload. These findings indicate that the MCU could be a major portal for Fe^{2+} entry into cardiac mitochondria. Therefore, blocking MCU may be an effective therapy to prevent iron-overload induced cardiac mitochondrial dysfunction in patients with thalassemia.

Keywords Cardiac mitochondria · Iron overload · Thalassemia · Mitochondrial dysfunction · MCU

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Introduction

Iron is one of many important elements essential to maintain physiological function of the cells in the

body (Hentze et al. 2004; Papanikolaou and Pantopoulos 2005). In mitochondria, iron is necessary for the synthesis of heme and iron sulfur cluster (ISC)-containing proteins involved in electron transport and oxidative phosphorylation (Levi and Rovida 2009; Richardson et al. 2010). However, excess iron levels, often found in thalassemia patients who receive regular blood transfusion (Weatherall and Clegg 2001; Olivieri 1999), can lead to increased reactive oxygen species (ROS) production via the Haber–Weiss and Fenton reactions (Bartfay and Bartfay 2000; Lekawanvijit and Chattipakorn 2009). High ROS production can damage cellular lipids, proteins, and DNA. It can also affect other cellular organelles such as lysosomes and mitochondria (Bartfay and Bartfay 2000; Lekawanvijit and Chattipakorn 2009).

In thalassemia patients, iron-overload induced cardiomyopathy is a major cause of morbidity and mortality (Lekawanvijit and Chattipakorn 2009; Zurlo et al. 1989; Wood et al. 2005). Previous studies suggest that mitochondrial dysfunction could be responsible for cardiac failure resulting from chronic iron overload (Gao et al. 2009, 2010). It has been shown that chronic iron overload can cause cumulative iron-mediated damage to mitochondrial DNA (mtDNA) and impaired synthesis and function of mitochondrial respiratory chain subunits, thus leading to the loss of respiratory capacity and cardiac dysfunction (Gao et al. 2009, 2010). Furthermore, the increase in ROS production can cause ROS concentrations to reach the threshold level which can trigger the opening of the inner membrane anion channel (IMAC) and mitochondrial permeability transition pore (mPTP) (Zorov et al. 2006; Thummasorn et al. 2011). This results in mitochondrial depolarization and mitochondrial swelling, leading to the release of cytochrome c, activation of caspase pathways, and cell death by necrosis and/or apoptosis (Zorov et al. 2006; Thummasorn et al. 2011).

Previous studies have shown that isolated rat liver mitochondria accumulate Fe^{2+} via the mitochondrial Ca^{2+} uniporter (MCU) (Flatmark and Romslo 1975). In intact cultured rat hepatocytes, it has been shown that Ru360, a highly specific inhibitor of the MCU, inhibited bafilomycin-induced quenching of mitochondrial calcein fluorescence (Uchiyama et al. 2008). This result suggested that iron uptake to mitochondrial hepatocytes was via the MCU (Uchiyama et al. 2008). However, the role of the MCU on

iron (Fe^{2+}) induced thalassemic cardiac mitochondrial dysfunction has not been investigated.

In this study, we investigated the roles of the mPTP and MCU on iron (Fe^{2+}) induced cardiac mitochondrial ROS production, mitochondrial membrane potential changes ($\Delta\Psi$), and mitochondrial swelling in isolated cardiac mitochondria from wild type (WT) and thalassemic (HT) mice. MCU and mPTP blockers were used to determine the roles of these channels in Fe^{2+}-induced cardiac mitochondrial dysfunction of thalassemic mice. We tested the hypothesis that mPTP and MCU blockers could provide protective effects by preventing ROS production and mitochondrial depolarization as well as attenuating mitochondrial swelling caused by Fe^{2+} overload.

Materials and methods

Animal models

Two types of adult C57/BL6 mice (3–6 months old): wild type (mu$\beta^{+/+}$, WT) and heterozygous β^{KO} type (mu$\beta^{th-3/+}$, HT) were used in this study (Incharoen et al. 2007; Kumfu et al. 2011; Jamsai et al. 2006, 2005). All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Medicine, Chiang Mai University, and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All animals were housed in controlled temperature and humidity rooms with 12-hour light–dark cycles.

Preparation of cardiac mitochondria

Mice were deeply anesthetized with Zoletil (20 mg/kg body weight), injected intraperitoneally (Lee et al. 2011), after which the hearts were removed, finely minced, and homogenized in ice-cold buffer containing (in mmol/l) sucrose 300, TES 5 and EGTA 0.2, pH 7.2. The homogenates were centrifuged at $800 \times g$ for 5 min and the supernatant was collected and centrifuged at $8,800 \times g$ for 5 min. The resulting mitochondrial pellet was resuspended in ice-cold buffer and centrifuged once more at $8,800 \times g$ for 5 min. Finally, the mitochondrial pellet was resuspended in a respiration buffer (containing 100 mM KCl, 50 mM sucrose,

10 mM HEPES, 5 mM KH_2PO_4, pH 7.4 at 25 °C). Protein concentration was determined according to the bicinchoninic acid (BCA) assay (Thummasorn et al. 2011).

Experimental protocols

Isolated cardiac mitochondria from the hearts of WT and HT rats were used in all experiments. The first protocol investigated the dose-dependent effects of iron treatment on cardiac mitochondrial dysfunction. Ferric ammonium citrate (FAC), at various concentrations ranging from 0, 1.25, 2.5 and 5 μg/ml, co-administered with 1-mM ascorbic acid serving to represent the ascorbate-reduced form of iron (Fe^{2+}), were applied to both types of isolated cardiac mitochondria for 5 min. In the second protocol, the mechanism of Fe^{2+} induced cardiac mitochondrial dysfunction was investigated. Pharmacological intervention, facilitated by mPTP and MCU blockers, was used. To determine the roles of the mPTP and MCU on Fe^{2+} induced cardiac mitochondrial dysfunction, the isolated cardiac mitochondria (0.1 mg/ml) were exposed to mPTP blocker [Cyclosporin A (CsA)] 5 μM, and MCU blocker (Ru360) 10 μM for 5–10 min before being loaded with Fe^{2+} at 5 μg/ml for 5 min. The concentrations of both blockers have been shown to be effective in preventing mitochondrial dysfunction caused by oxidative stress (Thummasorn et al. 2011; Yarana et al. 2012). Lastly, the assessments on ROS production, mitochondrial membrane potential changes, and mitochondrial swelling were performed according to the experimental protocols.

Identification of cardiac mitochondria with electron microscopy

Electron microscopy was used to identify isolated cardiac mitochondria (Chelli et al. 2001; Thummasorn et al. 2011). Isolated cardiac mitochondria were fixed in 2.5 % glutaraldehyde in 0.1-M cacodylate buffer, pH 7.4 at 4 °C. After rinsing in cacodylate buffer, the mitochondrial pellets were fixed in 1 % cacodylate-buffered osmium tetroxide for 2 h at room temperature then dehydrated in a graded series of ethanol. The mitochondria were then embedded in Epon–Araldite. Ultra-thin sections were cut with a diamond knife, placed on copper grids, stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope.

Determination of cardiac mitochondrial ROS production

ROS production was measured with the dye dichlorohydrofluorescein diacetate (DCFDA) (Novalija et al. 2003; Thummasorn et al. 2011). After pharmacological and iron treatment, all groups of isolated cardiac mitochondria were incubated with 2-μM DCFDA at 25 °C for 20 min. ROS levels were determined via a fluorescent microplate reader at λ_{ex} 485 nm and λ_{em} 530 nm.

Determination of cardiac mitochondrial membrane potential change

Mitochondrial membrane potential change ($\Delta\Psi$m) was measured with the dye 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1) (Di Lisa et al. 1995; Thummasorn et al. 2011). After pharmacological and iron treatment, all groups of isolated cardiac mitochondria were stained with JC-1 (310 nM) at 37 °C for 30 min. Mitochondrial membrane potential was determined as fluorescence intensity via the use of a fluorescent microplate reader. JC-1 monomer (green) fluorescence was excited at the wavelength of 485 nm and the emission detected at 530 nm. JC-1 aggregate form (red) fluorescence was excited at 485 nm and the emission detected at 590 nm. The change in mitochondrial membrane potential was calculated as the ratio of red to green fluorescence. Mitochondrial depolarization was indicated by a decrease in the red/green fluorescence intensity ratio.

Determination of cardiac mitochondrial swelling

Isolated cardiac mitochondria from all groups were incubated in respiration buffer (containing 100-mM KCl, 50-mM sucrose, 10-mM HEPES, 5-mM KH_2PO_4, pH 7.4 at 37 °C) with the addition of 10-mM pyruvate/malate. After pharmacological and iron treatment, cardiac mitochondrial swelling was detected as a decrease in the absorbance of the suspension at 540 nm using a spectrophotometer for 30 min (Ruiz-Meana et al. 2006; Thummasorn et al. 2011).

Data analysis

All data were presented as mean ± SEM. Student's t test was performed for group comparisons. A P value <0.05 was considered statistical significance.

Results

Effects of iron (Fe^{2+}) at various concentrations on cardiac mitochondria

Under iron-loaded conditions, ROS levels in both types of cardiac mitochondria were significantly increased in a dose-dependent manner (Fig. 1a, b). Consistent results were found in mitochondrial membrane potential change ($\Delta\Psi$m). When iron (Fe^{2+}) was added to the mitochondrial suspension, mitochondrial depolarization was observed in both types of cardiac mitochondria as indicated by a significant decrease in

mitochondrial membrane potential in a dose-dependent manner (Fig. 1c, d). Moreover, applying iron (Fe^{2+}) to both types of cardiac mitochondria resulted in significantly decreased absorbance (Fig. 1e, f), suggesting that iron (Fe^{2+}) can significantly increase mitochondrial swelling in a dose dependent manner.

Comparison between cardiac mitochondria of WT and HT mice was determined, and our data demonstrated that the ROS levels in HT cardiac mitochondria were significantly higher than those in WT cardiac mitochondria at iron (Fe^{2+}) concentrations of 1.25 μM (HT 10.10 $\pm$ 0.37 vs. WT 8.90 $\pm$ 1.57, $P < 0.05$), 2.5 μM (HT 14.28 $\pm$ 0.88 vs. WT 10.86 $\pm$ 2.11,

Fig. 1 Effects of iron (Fe^{2+}) at various concentrations on (**a**, **b**) cardiac mitochondrial ROS production, (**c**, **d**) mitochondrial membrane potential change and (**e**, **f**) mitochondrial swelling. WT (**a**, **c**, **e**) and HT (**b**, **d**, **f**) cardiac mitochondria ($n = 5$/group) were treated with various concentrations of iron (Fe^{2+}) at 0, 1.25, 2.5, 5 μg/ml. *$p < 0.05$ versus M, #$p < 0.05$ versus MFe1.25, †$p < 0.05$ versus MFe2.5
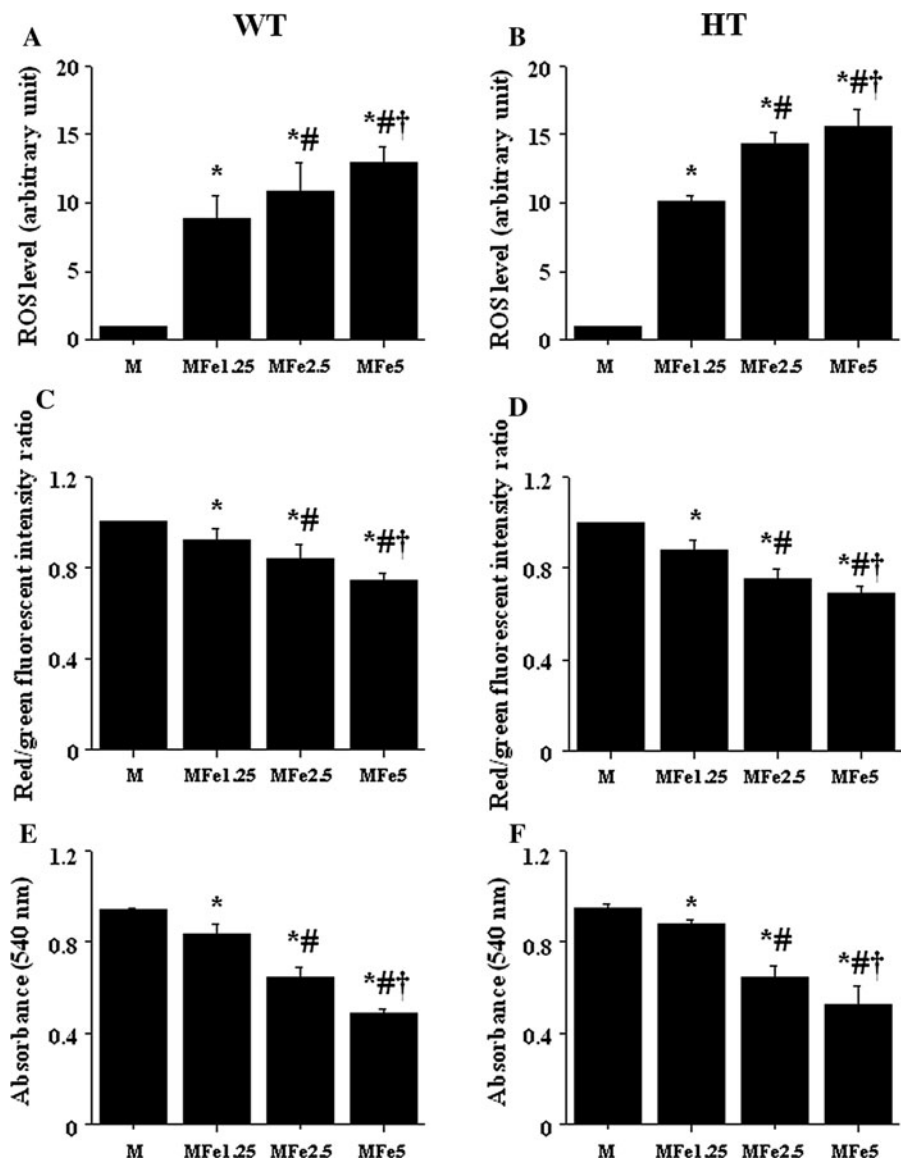
$P < 0.05$), 5 μM (HT 15.55 ± 1.28 vs. WT 12.95 ± 1.17, $P < 0.05$) (Fig. 1a, b). Furthermore, the red/green fluorescent intensity ratio in HT cardiac mitochondria was also significantly lower than that in WT cardiac mitochondria (i.e. indicating more depolarization) at iron (Fe^{2+}) concentrations of 1.25 μM (HT 0.88 ± 0.04 vs. WT 0.92 ± 0.05, $P < 0.05$), 2.5 μM (HT 0.76 ± 0.04 vs. WT 0.84 ± 0.07, $P < 0.05$), 5 μM (HT 0.69 ± 0.03 vs. WT 0.74 ± 0.03, $P < 0.05$) (Fig. 1c, d). However, the levels of mitochondrial swelling in both types of cardiac mitochondria were equivalent at the same dose of iron (Fe^{2+}) treatment (Fig. 1e, f).

Effects of mPTP blocker (CsA) and mitochondrial Ca²⁺ uniporter blocker (Ru360) on ROS levels

The pretreatment of cardiac mitochondria with CsA and Ru360 alone did not increase ROS production in either type of cardiac mitochondria (Fig. 2). When iron (Fe^{2+}) was added to the mitochondrial suspension, the ROS levels were significantly increased in comparison to the control group [mitochondria alone (M)] (Fig. 2). Treatment with CsA and Ru360 caused a significant decrease in ROS production in both types of cardiac mitochondria compared to the iron (Fe^{2+}) treated group (Fig. 2). Furthermore, treatment with Ru360 attenuated the increase in ROS levels more than CsA (Fig. 2).

Effects of pharmacological interventions on changes in mitochondrial membrane potential (ΔΨm)

The ΔΨm in CsA and Ru360 pretreated cardiac mitochondria did not differ from the control group (Fig. 3). When iron (Fe^{2+}) was applied to the
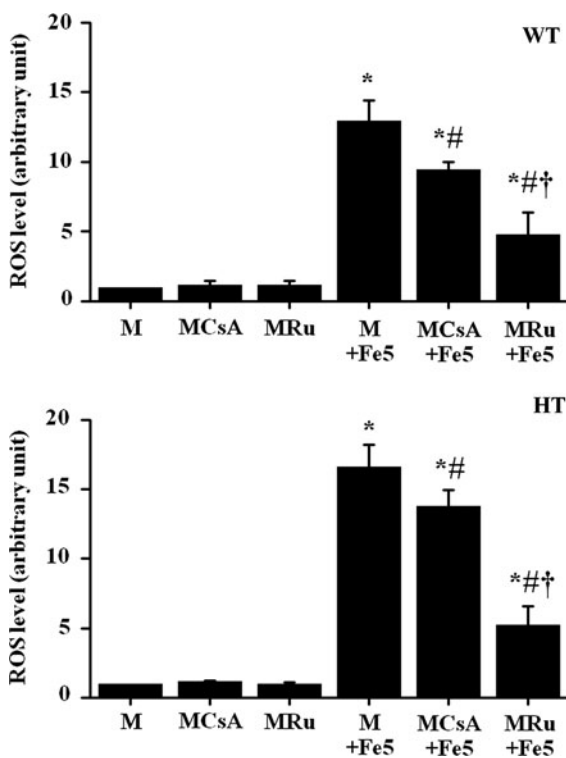

Fig. 2 Effects of pharmacological interventions on ROS level in WT and HT cardiac mitochondria ($n = 5$/group). WT and HT cardiac mitochondria ($n = 5$/group) were exposed to mPTP blocker (CsA) 5 μM and MCU blocker (Ru360) 10 μM for 5–10 min, before being loaded with iron (Fe^{2+}), at 5 μg/ml for 5 min. *$p < 0.01$ versus M, #$p < 0.05$ versus M + Fe5, †$p < 0.05$ versus MCsA + Fe5
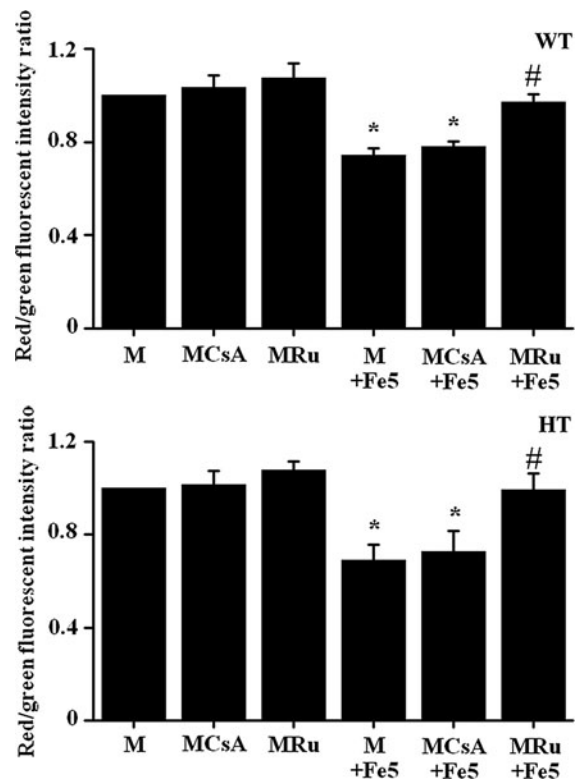

Fig. 3 Effects of pharmacological interventions on mitochondrial membrane potential change in WT and HT cardiac mitochondria ($n = 5$/group). WT and HT cardiac mitochondria ($n = 5$/group) were exposed to mPTP blocker (CsA) 5 μM and MCU blocker (Ru360) 10 μM for 5–10 min, before being loaded with iron (Fe^{2+}), at 5 μg/ml for 5 min. *$p < 0.01$ versus M, #$p < 0.01$ versus M + Fe5

mitochondrial suspension, mitochondrial depolarization was observed as indicated by a marked decrease in mitochondrial membrane potential. When Ru360 was applied to mitochondria prior to iron (Fe^{2+}) application, $\Delta\Psi m$ was significantly improved in comparison with the iron (Fe^{2+}) treated group (Fig. 3). However, treatment with CsA did not attenuate the $\Delta\Psi m$ in either type of cardiac mitochondria compared to the iron (Fe^{2+}) treated group (Fig. 3).

Effects of pharmacological interventions on cardiac mitochondrial swelling

CsA and Ru360 were chosen due to the fact that they can effectively prevent ROS production. Ru360 can also reduce $\Delta\Psi m$ after iron (Fe^{2+}) application. Pretreatment with CsA and Ru360 alone did not change the absorbance levels in either type of cardiac mitochondria (Fig. 4). When iron (Fe^{2+}) was applied
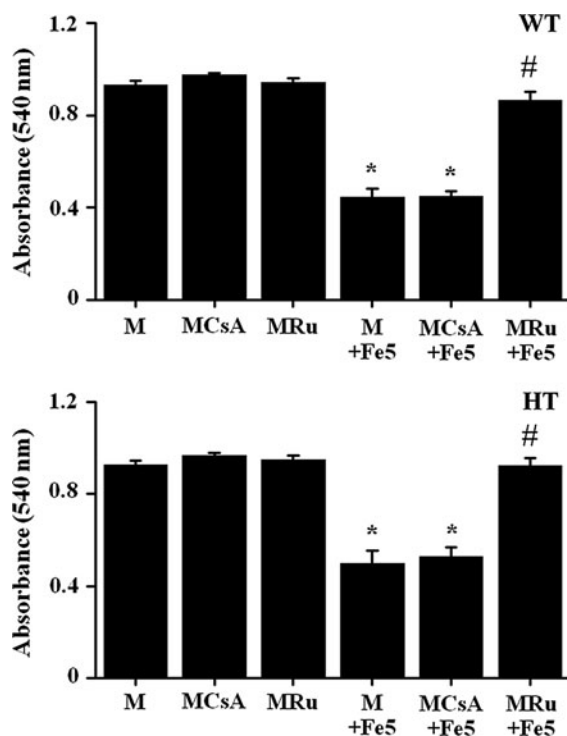

Fig. 4 Effects of mPTP blocker (CsA) and MCU blocker (Ru360) on cardiac mitochondrial swelling in WT and HT cardiac mitochondria ($n = 5$/group). WT and HT cardiac mitochondria ($n = 5$/group) were exposed to mPTP blocker (CsA) 5 μM and MCU blocker (Ru360) 10 μM for 5–10 min, before being loaded with iron (Fe^{2+}), at 5 μg/ml. $*p < 0.01$ versus M, $\#p < 0.01$ versus M + Fe5

to the mitochondrial suspension, absorbance was markedly decreased compared to the control group (Fig. 4). Pretreatment of cardiac mitochondria with Ru360 prior to iron (Fe^{2+}) application attenuated the reduction in absorbance compared to the iron (Fe^{2+}) group (Fig. 4). However, pretreatment with CsA prior to iron (Fe^{2+}) application could not prevent the absorbance reduction in the cardiac mitochondria (Fig. 4).

Identification of the cardiac mitochondria with electron microscopy

A transmission electron microscope was used to identify the cardiac mitochondria. WT cardiac mitochondria are shown in Fig. 5a and HT cardiac mitochondria are shown in Fig. 5b. Applying iron (Fe^{2+}) to HT cardiac mitochondria caused mitochondrial swelling with markedly unfolded cristae (Fig. 5c). When Ru360 was applied to cardiac mitochondria prior to iron (Fe^{2+}) application, the morphology of the cardiac mitochondria was well preserved (Fig. 5d), indicating the effectiveness of Ru360 in preventing morphological change in cardiac mitochondria from iron (Fe^{2+}) application.

Discussion

The major findings in the present study are that (i) iron overloaded conditions lead to an increase in ROS production, change in mitochondrial membrane potential ($\Delta\Psi m$), and mitochondrial swelling in a dose dependent manner in both WT and HT cardiac mitochondria, (ii) pretreatment with CsA and Ru360 decreased ROS production in both WT and HT cardiac mitochondria, and (iii) only pretreatment with Ru360 completely attenuated the $\Delta\Psi m$ and prevented mitochondrial swelling in both WT and HT cardiac mitochondria.

Mitochondrial Ca^{2+} handling plays an important role in energy production and various cellular signaling processes (Hoppe 2010; O'Rourke et al. 2005). Previous studies suggest that mitochondrial Ca^{2+} uptake is regulated by the MCU and its Ca^{2+} sensing regulatory subunit mitochondrial calcium uptake 1 (MICU1) (Perocchi et al. 2010), the rapid mode of mitochondrial Ca^{2+} uptake (RAM), and mitochondrial ryanodine receptors (mRyR) (Hoppe 2010; O'Rourke
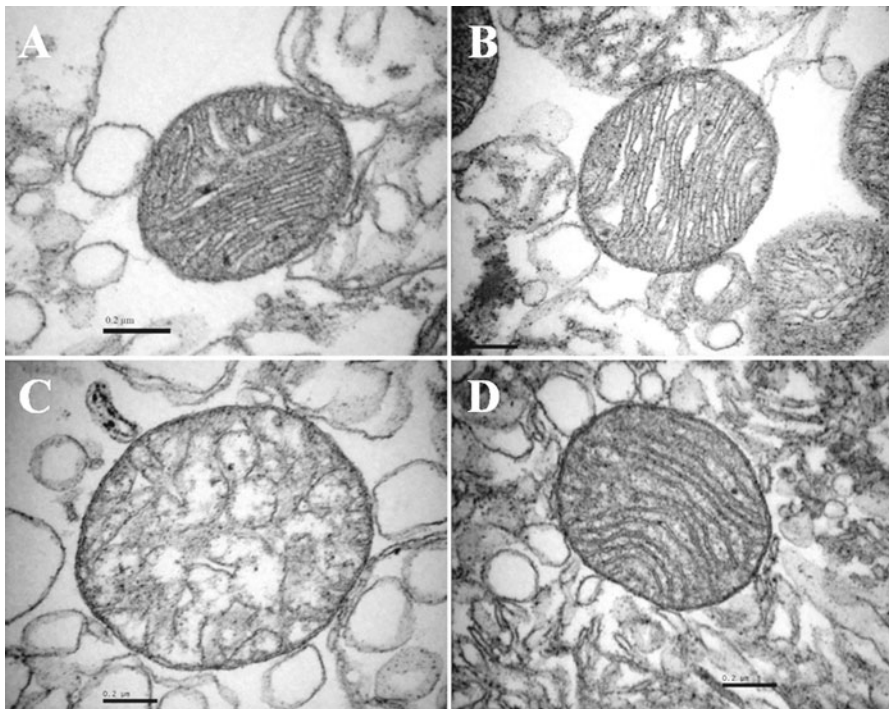
Fig. 5 Identification of the cardiac mitochondria with electron microscopy; **a** WT cardiac mitochondria, **b** HT cardiac mitochondria, **c** HT cardiac mitochondria treated with 5 µg/ml iron (Fe^{2+}) and **d** HT cardiac mitochondria pretreated with Ru360, 10 µM followed by 5 µg/ml iron (Fe^{2+})

et al. 2005). MCU are primarily used for Ca^{2+} transport (Kirichok et al. 2004). However, they may be able to transport many other divalent cations. Previous studies have shown that isolated rat liver mitochondria accumulate Fe^{2+} via the MCU (Flatmark and Romslo 1975). In intact cultured rat hepatocytes, it has been shown that Ru360, a highly specific inhibitor of the MCU, inhibited bafilomycin-induced quenching of mitochondrial calcein fluorescence (Uchiyama et al. 2008). This result suggested that iron uptake to mitochondrial hepatocytes was via the MCU (Uchiyama et al. 2008). However, the role of the MCU on iron (Fe^{2+}) induced thalassemic cardiac mitochondrial dysfunction has not been investigated. Our present study demonstrated for the first time that the protective effects of Ru360 on ROS production and mitochondrial depolarization resulted in the attenuation of Fe^{2+}-induced cardiac mitochondrial morphological change and mitochondrial swelling in thalassemic cardiac mitochondria. These findings were consistent with previous studies in isolated rat liver mitochondria (Flatmark and Romslo 1975) and intact cultured rat hepatocytes (Uchiyama et al. 2008).

These findings further emphasize the important role of the MCU as a major portal for Fe^{2+} entry into cardiac mitochondria.

The opening of the mPTP allows the mitochondrial inner membrane to become nonselectively permeable to all solutes of molecular masses up to approximately 1500 Da (Kim et al. 2003; Zorov et al. 2006). Calcium ions, oxidative stress, and numerous reactive chemicals have been shown to induce the onset of the mitochondrial permeability transition (MPT), whereas CsA and pH < 7 inhibits the opening of the mPTP (Zorov et al. 2006; Kim et al. 2003). After MPT onset, mitochondrial depolarization and swelling could be observed, leading to the release of cytochrome c, activation of caspase pathways, and cell death by necrosis and/or apoptosis (Kim et al. 2003; Zorov et al. 2006). Cyclophilin D (CypD) is the component of the mPTP that can interact and be inhibited by CsA (Kim et al. 2003; Zorov et al. 2006). In the present study, CsA, an mPTP blocker, slightly decreased ROS production but did not prevent ΔΨm and mitochondrial swelling induced by Fe^{2+} application. These findings suggest that the mPTP is not mainly involved

in Fe^{2+} entry into mitochondria or in inducing cardiac mitochondrial dysfunction in either WT or HT cardiac mitochondria. Although CypD underlies the mPTP inhibition by CsA, a CsA-independent mPTP has been demonstrated to exist in CypD-deficient mitochondria (Forte and Bernardi 2005; Bernardi and Forte 2007). Therefore, our findings did not rule out the possibility that Fe^{2+} may enter cardiac mitochondria via CsA-independent mPTP. Nevertheless, whether or not CsA-independent mPTP are involved in the Fe^{2+} entry is not known and requires further investigation. The limitation in the present study was that we examined only in isolated cardiac mitochondria. The findings from this study could be similar or different if cardiomyocytes isolated from the WT and HT hearts were used.

In summary, our present study demonstrated that under iron overload conditions, Fe^{2+} enters cardiac mitochondria, leading to cardiac mitochondrial dysfunction. Ru360, an MCU blocker, provides protective effects by preventing ROS production and mitochondrial depolarization as well as attenuating mitochondrial swelling caused by Fe^{2+} overload. These findings indicate that the MCU could be a major portal for Fe^{2+} entry into cardiac mitochondria, leading to cardiac mitochondrial dysfunction in iron overloaded conditions.

References

Bartfay WJ, Bartfay E (2000) Iron-overload cardiomyopathy: evidence for a free radical-mediated mechanism of injury and dysfunction in a murine model. Biol Res Nurs 2(1):49–59

Bernardi P, Forte M (2007) The mitochondrial permeability transition pore. Novartis Found Symp 287:157–164 (discussion 164–159)

Chelli B, Falleni A, Salvetti F, Gremigni V, Lucacchini A, Martini C (2001) Peripheral-type benzodiazepine receptor ligands: mitochondrial permeability transition induction in rat cardiac tissue. Biochem Pharmacol 61(6):695–705

Di Lisa F, Blank PS, Colonna R, Gambassi G, Silverman HS, Stern MD, Hansford RG (1995) Mitochondrial membrane potential in single living adult rat cardiac myocytes exposed to anoxia or metabolic inhibition. J Physiol 486(Pt 1): 1–13

Flatmark T, Romslo I (1975) Energy-dependent accumulation of iron by isolated rat liver mitochondria. Requirement of reducing equivalents and evidence for a unidirectional flux of Fe(II) across the inner membrane. J Biol Chem 250(16):6433–6438

Forte M, Bernardi P (2005) Genetic dissection of the permeability transition pore. J Bioenerg Biomembr 37(3):121–128

Gao X, Campian JL, Qian M, Sun XF, Eaton JW (2009) Mitochondrial DNA damage in iron overload. J Biol Chem 284(8):4767–4775

Gao X, Qian M, Campian JL, Marshall J, Zhou Z, Roberts AM, Kang YJ, Prabhu SD, Sun XF, Eaton JW (2010) Mitochondrial dysfunction may explain the cardiomyopathy of chronic iron overload. Free Radic Biol Med 49(3):401–407

Hentze MW, Muckenthaler MU, Andrews NC (2004) Balancing acts: molecular control of mammalian iron metabolism. Cell 117(3):285–297

Hoppe UC (2010) Mitochondrial calcium channels. FEBS Lett 584(10):1975–1981

Incharoen T, Thephinlap C, Srichairatanakool S, Chattipakorn S, Winichagoon P, Fucharoen S, Vadolas J, Chattipakorn N (2007) Heart rate variability in beta-thalassemic mice. Int J Cardiol 121(2):203–204

Jamsai D, Zaibak F, Khongnium W, Vadolas J, Voullaire L, Fowler KJ, Gazeas S, Fucharoen S, Williamson R, Ioannou PA (2005) A humanized mouse model for a common beta0-thalassemia mutation. Genomics 85(4):453–461

Jamsai D, Zaibak F, Vadolas J, Voullaire L, Fowler KJ, Gazeas S, Peters H, Fucharoen S, Williamson R, Ioannou PA (2006) A humanized BAC transgenic/knockout mouse model for HbE/beta-thalassemia. Genomics 88(3):309–315

Kim JS, He L, Lemasters JJ (2003) Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. Biochem Biophys Res Commun 304(3):463–470

Kirichok Y, Krapivinsky G, Clapham DE (2004) The mitochondrial calcium uniporter is a highly selective ion channel. Nature 427(6972):360–364

Kumfu S, Chattipakorn S, Srichairatanakool S, Settakorn J, Fucharoen S, Chattipakorn N (2011) T-type calcium channel as a portal of iron uptake into cardiomyocytes of beta-thalassemic mice. Eur J Haematol 86(2):156–166

Lee DW, Lee TK, Cho IS, Park HE, Jin S, Cho HJ, Kim SH, Oh S, Kim HS (2011) Creation of myocardial fibrosis by transplantation of fibroblasts primed with survival factors. Am J Physiol Heart Circ Physiol 301(3):H1004–H1014

Lekawanvijit S, Chattipakorn N (2009) Iron overload thalassemic cardiomyopathy: iron status assessment and mechanisms of mechanical and electrical disturbance due to iron toxicity. Can J Cardiol 25(4):213–218

Levi S, Rovida E (2009) The role of iron in mitochondrial function. Biochim Biophys Acta 1790(7):629–636

Novalija E, Kevin LG, Eells JT, Henry MM, Stowe DF (2003) Anesthetic preconditioning improves adenosine triphosphate synthesis and reduces reactive oxygen species formation in mitochondria after ischemia by a redox dependent mechanism. Anesthesiology 98(5):1155–1163

Olivieri NF (1999) The beta-thalassemias. N Engl J Med 341(2):99–109

O'Rourke B, Cortassa S, Aon MA (2005) Mitochondrial ion channels: gatekeepers of life and death. Physiology (Bethesda) 20:303–315

Papanikolaou G, Pantopoulos K (2005) Iron metabolism and toxicity. Toxicol Appl Pharmacol 202(2):199–211

Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE, Mootha VK (2010) MICU1 encodes a mitochondrial EF hand protein required for Ca(2+) uptake. Nature 467(7313):291–296

Richardson DR, Lane DJ, Becker EM, Huang ML, Whitnall M, Suryo Rahmanto Y, Sheftel AD, Ponka P (2010) Mitochondrial iron trafficking and the integration of iron metabolism between the mitochondrion and cytosol. Proc Natl Acad Sci USA 107(24):10775–10782

Ruiz-Meana M, Garcia-Dorado D, Miro-Casas E, Abellan A, Soler–Soler J (2006) Mitochondrial Ca^{2+} uptake during simulated ischemia does not affect permeability transition pore opening upon simulated reperfusion. Cardiovasc Res 71(4):715–724

Thummasorn S, Kumfu S, Chattipakorn S, Chattipakorn N (2011) Granulocyte-colony stimulating factor attenuates mitochondrial dysfunction induced by oxidative stress in cardiac mitochondria. Mitochondrion 11(3):457–466

Uchiyama A, Kim JS, Kon K, Jaeschke H, Ikejima K, Watanabe S, Lemasters JJ (2008) Translocation of iron from lysosomes into mitochondria is a key event during oxidative stress-induced hepatocellular injury. Hepatology 48(5):1644–1654

Weatherall DJ, Clegg JB (2001) Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ 79(8):704–712

Wood JC, Enriquez C, Ghugre N, Otto-Duessel M, Aguilar M, Nelson MD, Moats R, Coates TD (2005) Physiology and pathophysiology of iron cardiomyopathy in thalassemia. Ann N Y Acad Sci 1054:386–395

Yarana C, Sanit J, Chattipakorn N, Chattipakorn S (2012) Synaptic and nonsynaptic mitochondria demonstrate a different degree of calcium-induced mitochondrial dysfunction. Life Sci 90(19–20):808–814

Zorov DB, Juhaszova M, Sollott SJ (2006) Mitochondrial ROS-induced ROS release: an update and review. Biochim Biophys Acta 1757(5–6):509–517

Zurlo MG, De Stefano P, Borgna-Pignatti C, Di Palma A, Piga A, Melevendi C, Di Gregorio F, Burattini MG, Terzoli S (1989) Survival and causes of death in thalassaemia major. Lancet 2(8653):27–30