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Alpha lipoic acid protects heart against myocardial ischemia–reperfusion injury through a mechanism involving aldehyde dehydrogenase 2 activation

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ABSTRACT

Recent studies demonstrate that alpha lipoic acid can prevent nitroglycerin tolerance by restoring aldehyde dehydrogenase 2 (ALDH2) activity and ALDH2-mediated detoxification of aldehydes is thought as an endogenous mechanism against ischemia–reperfusion injury. This study was performed to explore whether the cardioprotective effect of alpha lipoic acid was related to activation of ALDH2 and the underlying mechanisms. In a Langendorff model of ischemia–reperfusion in rats, cardiac function, activities of creatine kinase (CK) and ALDH2, contents of 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) were measured. In a cell model of hypoxia–reoxygenation, the apoptosis, ALDH activity, reactive oxygen species level, 4-HNE and MDA contents were examined. In the isolated hearts, ischemia–reperfusion treatment led to cardiac dysfunction accompanied by an increase in 4-HNE and MDA contents. Pretreatment with lipoic acid significantly up-regulated myocardial ALDH2 activity concomitantly with an improvement of cardiac dysfunction and a decrease in 4-HNE and MDA contents, these effects were blocked by the inhibitor of ALDH2. Similarly, in the cultured cardiomyocytes, hypoxia–reoxygenation treatment induced apoptosis accompanied by an increase in the production of reactive oxygen species, 4-HNE and MDA. Administration of lipoic acid significantly up-regulated cellular ALDH2 activity concomitantly with a reduction in apoptosis, production of reactive oxygen species, 4-HNE and MDA, these effects were reversed in the presence of ALDH2 or PKCε inhibitors. Our results suggest that the cardioprotective effects of lipoic acid on ischemia–reperfusion injury are through a mechanism involving ALDH2 activation. The regulatory effect of lipoic acid on ALDH2 activity is dependent on PKCε signaling pathway.

1. Introduction

Following acute myocardial ischemia, restoring coronary blood flow (the so-called reperfusion) with the rapid use of pharmacological or mechanical interventions, such as thrombolytic treatment, angioplasty or coronary bypass surgery, is essential to salvage viable myocardium (Luo et al., 2009; Quintana et al., 2004). Paradoxically, reperfusion itself can result in myocyte death, a phenomenon referred to as "reperfusion injury". Necrosis and apoptosis are the two major distinct types of cardiomyocyte death caused by ischemia and reperfusion (Armstrong, 2004; Eefting et al., 2004; Gottlieb, 2011). The excessive death of cardiomyocytes ultimately leads to cardiac dysfunction.

The exact mechanisms underlying ischemia–reperfusion injury are not fully elucidated. Oxidative stress is considered as one of the key factors that contribute to ischemia–reperfusion injury (Braunersreuther and Jaquet, 2011). Reactive aldehydes, such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA), are produced during oxidative stress as the major end products of lipid peroxidation and are accumulated during ischemia–reperfusion (Conklin et al., 2007; Renner et al., 2005). Reactive aldehydes are highly toxic and react with proteins to form various adduct, leading to dysfunction of proteins and in turn cellular injury (Marchitti et al., 2007). The mitochondrial enzyme aldehyde dehydrogenase 2 (ALDH2) plays a major role in detoxification of reactive aldehydes in a range of organs and cell types (Endo et al., 2009). Therefore, ALDH2-mediated detoxification of reactive aldehydes may represent an endogenous mechanism against myocardial injury during ischemia–reperfusion (Budas et al., 2009).

Alpha lipoic acid, a natural dithiol compound with excellent antioxidant properties, is known as a co-factor for mitochondrial dehydrogenase (Dudek et al., 2008; Wenzel et al., 2007). There are reports that alpha lipoic acid is able to reduce myocardial injury and preserve cardiac

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function during ischemia–reperfusion (Freisleben, 2000; Schonheit et al., 1995). However, the underlying mechanisms are not fully understood. Recent studies demonstrate that alpha lipoic acid can prevent nitroglycerin tolerance by restoration of ALDH2 activity (Wenzel et al., 2007). Considering the critical role of ALDH2 in detoxification of reactive aldehydes during ischemia–reperfusion, we therefore speculate that the cardioprotective effects of alpha lipoic acid against ischemia–reperfusion injury are related to activation of ALDH2.

In the present study, using a Langendorff model of ischemia–reperfusion in rats, we evaluated the correlation between the cardioprotective effect of alpha lipoic acid and ALDH2. Using a cell model of hypoxia–reoxygenation, we further explored the role of ALDH2 in the beneficial effect of alpha lipoic acid on hypoxia–reoxygenation injury. Because protein kinase C epsilon (PKCε) is reported to participate in ALDH2 activation (Budas et al., 2010; Churchill et al., 2009), we therefore explored whether the regulatory effect of alpha lipoic acid on ALDH2 activity is also involved in PKCε signaling pathway.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 200–250 g were obtained from Laboratory Animal Center, Xiang-Ya School of Medicine, Central South University, China. The animals were fasted for 24 h before the experiments, with free access to tap water. The study was performed following the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996) and experiments were approved by the Central South University Veterinary Medicine Animal Care and Use Committee.

2.2. Isolated heart experiments

Rats were anesthetized by intraperitoneal administration of sodium pentobarbital (60 mg/kg). The heart was rapidly excised and placed in ice-cold Krebs–Henseleit buffer solution containing NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and glucose 11.0 mM (Peng et al., 2000; Zanesco et al., 1999). The heart was attached to a Langendorff apparatus via the aorta for retrograde perfusion with Krebs–Henseleit buffer solution. The perfusate was equilibrated with 95% O_2 and 5% CO_2, maintained at 37 °C and pH 7.4. Perfusion pressure was maintained at 85 cm H_2O. All hearts were treated with global ischemia for 60 min followed by reperfusion for 30 min except the control group. The isolated hearts of control group were perfused with Krebs–Henseleit buffer solution continuously for 120 min. A water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle via the mitral valve and the volume was adjusted to achieve a stable left ventricular end-diastolic pressure of 2–3 mm Hg during initial equilibration. Left ventricular pressure (systolic and diastolic) and the maximal rates of LV pressure decay and development ($\pm dp/dt_{max}$) were continuously monitored. The resulting electrical signals were digitized by a MacLab (ADInstruments, Barcelona, Spain) analogue-to-digital converter and recorded by a PowerLab system (ADInstruments Shanghai Trading Co, Ltd, Shanghai, China). The left ventricular developed pressure (LVPd = LV systolic pressure − LV diastolic pressure) was calculated. Coronary flow was measured by timed collection of coronary effluent and samples of coronary effluent were collected at 5 min of reperfusion for determination of creatine kinase activity.

All isolated hearts were initially equilibrated for 10 min and were randomly allocated to five groups (n = 6 per group): (1) The control group (perfused with Krebs–Henseleit buffer solution continuously for 120 min), (2) the ischemia–reperfusion group (subjected to 60 min of global ischemia followed by 30 min of reperfusion), (3) the daidzin + ischemia–reperfusion group (pretreated with daidzin {Sigma-Aldrich, St. Louis, USA, dissolved in DMSO, 10^{-8} M, the final concentration of DMSO was 0.1%} 20 min before ischemia), (4) the low dose (L) alpha lipoic acid (Sigma-Aldrich, St. Louis, USA, dissolved in DMSO, the final concentration of DMSO was 0.1%) or (5) the high dose (H) alpha lipoic acid + ischemia–reperfusion group (pretreated with 10^{-8} M or 5×10^{-8} M alpha lipoic acid before ischemia), (6) the high dose (H) alpha lipoic acid + daidzin + ischemia–reperfusion group (pretreated with daidzin for 10 min followed by alpha lipoic acid for 10 min before ischemia) and (7) the vehicle + ischemia–reperfusion group (pretreated with DMSO for 20 min before ischemia, the final concentration of DMSO was 0.1%) (Fig. 1A). Here, daidzin, a selective inhibitor of ALDH2, was chosen to explore whether the cardioprotection afforded by alpha lipoic acid was involved in ALDH2 and the vehicle group was set to rule out the possible effect of DMSO on cardiac function.

2.3. Cell experiments

Rat heart-derived H9c2 cells were seeded at constant density ($1\times10^4/cm^2$) and grown to 70–80% in Dulbecco's Modified Eagle Medium (DMEM, GIBCO-BRL, Shanghai, China) containing 10% fetal bovine serum and antibiotics. Cells were washed with phosphate buffered saline (PBS) and rendered quiescent in 1% serum DMEM for 24 h prior to experiments. Cells were divided into 6 groups (n = 8 per group): (1) the control group (cultured in normal condition), (2) the hypoxia–reoxygenation group (subjected to 24 h of hypoxia (gas mixture of 1% O_2, 94% N_2, 5% CO_2) followed by 12 h of reoxygenation) (Hu et al., 2007), (3) the alpha lipoic acid + hypoxia–reoxygenation group (pretreated with 10^{-5} M alpha lipoic acid before reoxygenation), (4) the alpha lipoic acid + cyanamide + hypoxia–reoxygenation group (pretreated with 10^{-5} M alpha lipoic acid and 10^{-4} M cyanamide before reoxygenation), (5) the alpha lipoic acid + daidzin + hypoxia–reoxygenation group (pretreated with 10^{-5} M alpha lipoic acid and 10^{-5} M daidzin

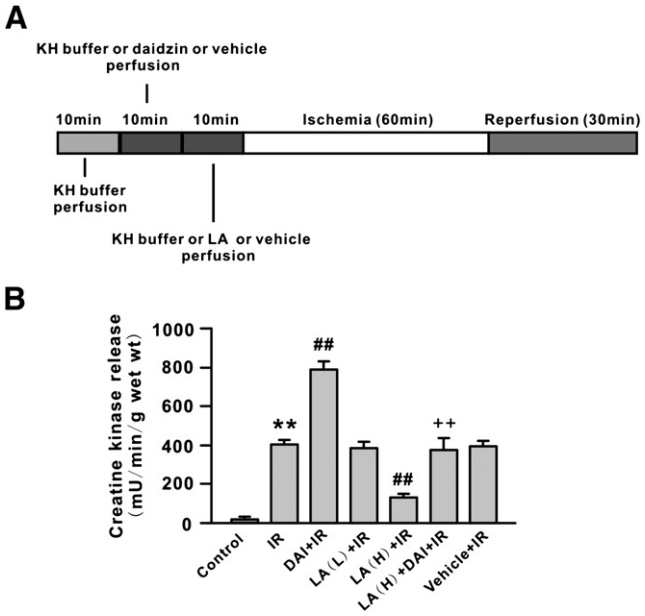

Fig. 1. Effect of alpha lipoic acid on creatine kinase release following myocardial ischemia–reperfusion. A. The protocol for isolated heart experiments. The time point and duration for different treatments are indicated. B. Creatine kinase activity in each group. All values were expressed as means ± S.E.M. (n = 6 in each group). KH buffer: Krebs–Henseleit buffer; IR: ischemia–reperfusion; DAI + IR: daidzin (10^{-8} M) + ischemia–reperfusion; LA (L) + IR: alpha lipoic acid (10^{-8} M) + ischemia–reperfusion; LA (H) + IR: alpha-lipoic acid (5×10^{-8} M) + ischemia–reperfusion; LA (H) + DAI + IR: alpha lipoic acid (5×10^{-8} M) + daidzin (10^{-8} M) + ischemia–reperfusion; Vehicle + IR: DMSO + ischemia–reperfusion. $^{**}P<0.01$ vs Control; $^{\#\#}P<0.01$ vs IR; $^{++}P<0.01$ vs LA (H) + IR.

before reoxygenation), (6) the alpha lipoic acid + εV1-2 + hypoxia–reoxygenation group (pretreated with 10^{-5} M alpha lipoic acid and 10^{-6} M εV1-2 before reoxygenation) and (7) the vehicle + hypoxia–reoxygenation group (DMSO was added to culture medium before reoxygenation, the final concentration of DMSO was 0.1%). Hypoxia treatment was conducted in a CO_2/O_2 incubator (Forma 3131, Thermo Fisher Scientific, Auburn, USA). The percentage of CO_2 (volume/volume) inside the incubator chamber is automatically regulated by CO_2 gas through a CO_2 sensor whereas the percentage of O_2 and N_2 are automatically regulated by N_2 gas through an O_2 sensor.

2.4. Measurement of creatine kinase activity

The creatine kinase activity in the coronary effluent from the heart at 5 min of reperfusion was measured spectrophotometrically by a commercial kit (Biosino Bio-Technology, Beijing, China) following the instruction offered by the supplier.

2.5. Measurement of caspase-3 activity

Assay of caspase-3 activity was performed according to the manufacturer's instruction (Beyotime, Shanghai, China). Briefly, 10 μl of cell lysates was mixed with 90 μl of reaction solution containing caspase-3 substrate (Ac-DEVD-pNA) and incubated for 60 min at 37 °C. The absorbance was read at 405 nm. The enzyme activity was expressed as U/g protein and 1 U of enzyme was defined as the amount of enzyme required to cleave 1.0 nmol Ac-DEVD-pNA per hour at 37 °C.

2.6. Measurement of MDA contents

The MDA contents were determined by thiobarbituric acid (TBA) method with slight modification (Beyotime, Shanghai, China). Briefly, 0.5 ml of tissue homogenate or cell lysates was mixed with 3 ml of 1% phosphoric acid and 1 ml of 0.67% TBA. The mixture was incubated at 95 °C for 60 min and cooled down subsequently. In order to extract the MDA, 375 μl N-butanol was added and vortexed vigorously for 10 s. After centrifuging, the upper N-butanol layer was transferred to a glass tube. The absorbance of the butanol phase was measured at 532 nm. The MDA content was expressed as nmol/mg protein.

2.7. Measurement of 4-HNE content

4-HNE is able to bind to proteins and form stable adducts, which is generally used to represent the content of 4-HNE. A commercially available ELISA kit (R&D, Minneapolis, USA) was used for the measurement of HNE-protein adducts content. Briefly, 100 μl of tissue homogenate or cell lysates was added to a 96-well protein binding plate and incubated at 37 °C for 2 h. Then the 4-HNE protein adducts were probed with an anti-HNE-His antibody, followed by an HRP conjugated secondary antibody. After adding stop solution, the absorbance of each well on a microplate was read at 450 nm immediately. The HNE-protein adducts content was determined by comparing with a standard curve that was prepared from predetermined HNE-BSA standards. The 4-HNE content was expressed as ng/g protein.

2.8. Measurement of ALDH2 activity

Assay of ALDH2 activity was performed according to the manufacturer's instruction (GenMed Scientifics Inc., Wilmington, USA). In brief, the ALDH2 enzymatic activity was measured at 25 °C in 1 ml reaction system containing 33 mM sodium pyrophosphate (pH 8.8) containing 0.8 mM NAD+, 15 μM propionaldehyde, and 0.1 ml of tissue homogenate or cell lysates. Production of NADH was determined spectrophotometrically by monitoring the change of absorbance intensity at 340 nm every 30 s for 5 min. ALDH2 reaction rates were expressed as μmol NADH/min/mg protein.

2.9. Analysis of cellular apoptosis

The method of Hoechst staining was used to evaluate cellular apoptosis (Spreafico et al., 2008). The procedure for Hoechst staining was performed following the manufacturer's instruction (Beyotime, Shanghai, China). Briefly, the H9c2 cells were fixed for 15 min in 4% paraformaldehyde, washed in PBS, air dried, and incubated at room temperature for 5 min with 1 μg/ml Hoechst 33258, a bisbenzimide cell-permeant dye that fluoresces bright blue on binding to DNA. Stained cells were washed twice with PBS and imaged under a fluorescent microscope (excitation, 350 nm; emission, 460 nm). Twenty random high-power fields from each sample were chosen and blindly quantified. The number of apoptosis cells was presented as percentage of the total cells.

2.10. Statistical analysis

SPSS software (version 10.0) was used for statistical analysis. Data were expressed as mean ± S.E.M. and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons. The significant level was chosen as $P<0.05$.

3. Results

3.1. Alpha lipoic acid treatment improves cardiac function and decreases CK release caused by ischemia–reperfusion

There was no significant difference in basal values of LVPd, $\pm dp/dt_{max}$ and coronary flow among the experimental groups. Sixty minutes ischemia and 30-min reperfusion caused a marked decrease in cardiac function (LVPd, $\pm dp/dt_{max}$ and coronary flow) and a significant increase in creatine kinase release. High-dose alpha lipoic acid (5×10^{-8} M) treatment significantly improved cardiac function and decreased creatine kinase release, these effects (except the effect on coronary flow) were reversed by daidzin, a specific inhibitor of ALDH2 (Table 1, Fig. 1). Ischemia–reperfusion-induced myocardial injury (except the decrease in coronary flow) was aggravated in the presence of daidzin alone. Compared to the ischemia–reperfusion group, vehicle treatment did not show any significant effect on cardiac function and CK release (Table 1, Fig. 1).

3.2. Alpha lipoic acid treatment reduces cardiomyocyte apoptosis caused by hypoxia–reoxygenation

Hypoxia–reoxygenation treatment significantly increased the percentage of apoptotic cells in H9c2 cells. Alpha lipoic acid (10^{-5} M) treatment significantly reduced hypoxia–reoxygenation-induced cellular apoptosis (Fig. 2A, B). In agreement with the results of the Hoechst staining, hypoxia–reoxygenation significantly up-regulated caspase-3 activity in H9c2 cells, which was significantly inhibited by alpha lipoic acid treatment (Fig. 2C). The beneficial effects of alpha lipoic acid on hypoxia–reoxygenation-induced apoptosis were reversed in the presence of daidzin, cyanamide (another inhibitor of ALDH2) or εV1-2 (a specific inhibitor of PKC-ε). Compared to the hypoxia–reoxygenation group, vehicle treatment did not show any significant effect on cellular apoptosis and caspase-3 activity (Fig. 2).

3.3. Alpha lipoic acid treatment increases ALDH2 activity

As displayed in Fig. 3, compared to the control group, ischemia–reperfusion treatment did not show significant effect on ALDH2 activity. High-dose alpha lipoic acid treatment significantly increased ALDH2 activity in myocardium, this increase was attenuated by pretreatment with daidzin. Consistent with the findings in isolated heart experiments, hypoxia–reoxygenation treatment did not show significant effect on ALDH2 activity in cultured H9c2 cells. Alpha lipoic acid

Table 1
Effect of alpha-lipoic acid on cardiac function following ischemia–reperfusion.

	n	Pre-ischemia	Reperfusion (min)		
			10	20	30
Left ventricular developed pressure (mm Hg)					
Control	6	92±3	92±4	92±4	92±4
I/R	6	86±7	16±3[a]	24±3[a]	31±5[a]
+Daidzin	6	86±9	12±2	15±6[b]	18±4[b]
+LA (L)	6	87±2	15±4	25±4	36±3
+LA (H)	6	91±8	45±13[b]	69±11[b]	71±8[b]
+LA (H)+daidzin	6	81±3	26±7[c]	43±8[c]	43±5[c]
+Vehicle (DMSO)	6	96±3	13±3	26±3	36±3
$+dp/dt_{max}$ *(mm Hg/s)*					
Control	6	3172±263	3312±303	3268±297	3257±294
I/R	6	2632±238	333±57[a]	541±85[a]	891±108[a]
+Daidzin	6	2873±371	264±47[b]	301±43[b]	527±86[b]
+LA (L)	6	2832±133	390±70	526±96	810±95
+LA (H)	6	2694±330	1246±385[b]	1934±294[b]	2090±260[b]
+LA (H)+daidzin	6	2812±148	638±208[c]	1159±186[c]	1139±110[c]
+Vehicle (DMSO)	6	2970±217	303±40	550±85	828±84
$-dp/dt_{max}$ *(mm Hg/s)*					
Control	6	1633±72	1744±100	1696±106	1702±104
I/R	6	1531±218	260±41[a]	353±63[a]	590±75[a]
+Daidzin	6	1377±195	166±18[b]	228±25[b]	349±79[b]
+LA (L)	6	1565±108	307±51	373±58	532±59
+LA (H)	6	1644±186	937±187[b]	1137±181[b]	1207±159[b]
+LA (H)+daidzin	6	1341±76	481±112[c]	771±111[c]	718±70[c]
+Vehicle (DMSO)	6	1775±90	256±38	409±49	559±33
Coronary flow (ml/min)					
Control	6	13.1±1.0	13.0±1.0	12.9±1.0	13.0±0.4
I/R	6	14.9±0.9	6.8±0.7[a]	6.8±0.7[a]	6.6±0.7[a]
+Daidzin	6	13.2±2.4	9.1±3.5	8.4±2.8	8.6±2.9
+LA (L)	6	11.0±1.3	6.7±0.7	6.3±0.98	5.8±1.0
+LA (H)	6	14.5±0.6	12.1±1.1[b]	10.7±0.9[b]	10.0±0.8[b]
+LA (H)+daidzin	6	11.6±1.6	12.4±2.8	10.3±2.0	10.0±2.1
+Vehicle (DMSO)	6	12.7±1.0	7.4±0.9	7.1±0.8	6.5±0.6

Values are means±S.E.M. IR: ischemia–reperfusion; LA (L): alpha-lipoic acid (10^{-8} M); LA (H): alpha-lipoic acid (5×10^{-8} M).
[a] $P<0.01$ vs Control.
[b] $P<0.01$ vs IR.
[c] $P<0.01$ vs LA (H)+IR.

treatment significantly increased ALDH2 activity, this increase was reversed in the presence of daidzin, cyanamide or εV1-2 (Fig. 4). Compared to the ischemia–reperfusion or hypoxia–reoxygenation group, vehicle treatment did not show any significant effect on ALDH2 activity (Figs. 3 and 4).

3.4. Alpha lipoic acid treatment reduces ischemia–reperfusion or hypoxia–reoxygenation-induced aldehydes and reactive oxygen species production

Both 4-HNE and MDA are generally accepted markers for oxidative stress. In the isolated heart experiments, ischemia–reperfusion treatment significantly increased 4-HNE and MDA contents in myocardium. High-dose alpha lipoic acid treatment markedly decreased ischemia–reperfusion-induced aldehyde production, this decrease was suppressed by pretreatment with daidzin (Fig. 5). In the cultured H9c2 cells, hypoxia–reoxygenation treatment dramatically increased reactive oxygen species level, 4-HNE and MDA contents. Alpha lipoic acid treatment significantly attenuated hypoxia–reoxygenation-induced aldehydes and reactive oxygen species production, these effects were reversed in the presence of daidzin, cyanamide or εV1-2 (Fig. 6). Compared to the ischemia–reperfusion or hypoxia–reoxygenation group, vehicle treatment did not show any significant effect on 4-HNE, MDA or reactive oxygen species production (Figs. 5 and 6).

4. Discussion

In this study, by using a Langendorff model of ischemia–reperfusion or a cell model of hypoxia–reoxygenation, we evaluated the beneficial effects of alpha lipoic acid on ischemia–reperfusion or hypoxia–reoxygenation-induced injury and explored whether these effects were involved in ALDH2 activation. The results clearly showed that ischemia–reperfusion or hypoxia–reoxygenation treatment led to cardiac dysfunction or cardiomyocyte apoptosis accompanied by the increased 4-HNE and MDA contents. Pretreatment with alpha lipoic acid significantly attenuated ischemia–reperfusion or hypoxia–reoxygenation-induced cardiac dysfunction (this including decrease in LVPd, $\pm dp/dt_{max}$ and increase in CK release) or cellular apoptosis concomitantly with an increase in ALDH2 activity and a decrease in 4-HNE as well as MDA contents, these effects were blocked by the inhibitors of ALDH2 or PKCε. Unexpectedly, in the isolated hearts administration of daidzin did not attenuate the effect of alpha lipoic acid on coronary flow. A possible explanation for this discrepancy is that in addition to impairing contractility of the heart through inhibition of ALDH2 (which led to a decrease in the coronary flow), daidzin itself might be able to dilate the coronary artery (which led to an increase in the coronary flow) because daidzin was reported to exert relaxation effects on isolated rat basilar artery rings (Deng et al., in press). To the best of our knowledge, this is the first study to demonstrate that the cardioprotective effects of alpha lipoic acid are involved in, at least in part, ALDH2 activation and PKCε signaling pathway.

There are reports that reactive aldehydes are significantly accumulated during ischemia–reperfusion due to the increased oxidative stress (Conklin et al., 2007; Renner et al., 2005). These reactive aldehydes, such as 4-HNE, are highly toxic and can form protein adducts with the amino acid residues of cysteine, histidine or lysine, which lead to myocardial tissue damage and cardiac dysfunction during ischemia–reperfusion (Uchida and Stadtman, 1992). In agreement with the reports, the results from this study showed that ischemia–reperfusion or hypoxia–reoxygenation treatment significantly increased the contents of 4-HNE protein adducts concomitantly with decreased cardiac function or increased cardiomyocyte apoptosis, suggesting a positive correlation between aldehydes and ischemia–reperfusion injury.

Current strategies in clinic for myocardial infarction therapy, such as pharmacological or surgical interventions, are aimed at disrupting the occlusion and restoring coronary flow (Budas et al., 2009). However, these treatments show little direct beneficial effects on prevention of myocardial tissue damage during ischemia–reperfusion. Thus, it is a clinical priority to seek novel drugs with direct beneficial effects on reduction of ischemia–reperfusion injury (such as cardiomyocyte necrosis and/or apoptosis). Since oxidative stress is a key factor that contributes to ischemia–reperfusion injury, anti-oxidant treatment is considered as a potential strategy to prevent myocardial ischemia–reperfusion injury (Aldakkak et al., 2011; Montecucco et al., 2010). Although general antioxidants display beneficial effects on reduction of ischemia–reperfusion injury, their clinical applications are still limited (Braunersreuther and Jaquet, 2011).

Recently, ALDH2 is emerging as a key enzyme involved in protecting the heart against ischemia–reperfusion injury (Budas et al., 2009; Chen et al., 2008). In mammalian cells, reactive aldehydes are detoxified by oxidation to carboxylates, a reaction catalyzed by ALDHs. ALDH2 is one of 19 members of the ALDH gene family and localizes within the mitochondria, a major site for reactive oxygen species and reactive aldehyde generation (Vasiliou and Nebert, 2005). An inverse correlation between ALDH2 activity and infarct size has been found in a myocardial infarction model (Chen et al., 2008). Based on the role of reactive aldehydes in ischemia–reperfusion-induced myocardial injury and the role of ALDH2 in detoxification of reactive aldehydes, ALDH2 is thought as a novel therapeutic target for treating myocardial injury induced by ischemia–reperfusion. Recently, using an approach of high-throughput screen, the lab of Mochly-Rosen identified a small-molecule activator of ALDH2, ALDH2 agonist-1 (Alda-1) (Chen et al., 2008). They found that administration of

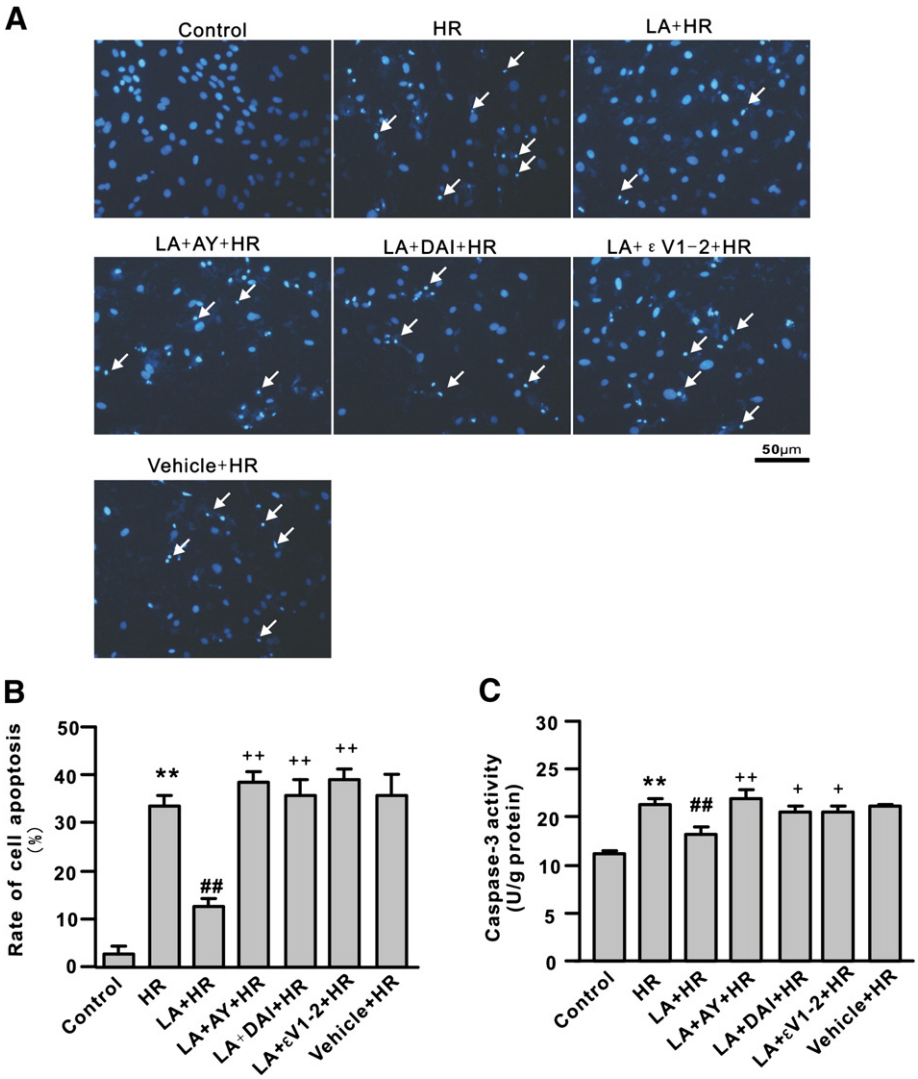

Fig. 2. Effect of alpha lipoic acid on cardiomyocyte apoptosis induced by hypoxia–reoxygenation. A. Representative images of cardiomyocyte apoptosis by Hoechst staining in each group. The apoptotic cells were indicated by the arrows. B. Percentage of apoptotic cells per total number of cardiac cells in each group. C. Caspase-3 activity in each group. All values were expressed as means ± S.E.M. (n = 8 in each group). Hypoxia–reoxygenation: hypoxia–reoxygenation; LA + hypoxia–reoxygenation: alpha lipoic acid (10^{-5} M) + hypoxia–reoxygenation; LA + AY + hypoxia–reoxygenation: alpha lipoic acid (10^{-5} M) + cyanamide (10^{-4} M) + hypoxia–reoxygenation; LA + DAI + hypoxia–reoxygenation: alpha lipoic acid (10^{-5} M) + daidzin (10^{-5} M) + hypoxia–reoxygenation; LA + εV1-2 + hypoxia–reoxygenation: alpha lipoic acid (10^{-5} M) + εV1-2 (10^{-6} M) + hypoxia–reoxygenation; Vehicle + hypoxia–reoxygenation: DMSO + hypoxia–reoxygenation. $^{**}P<0.01$ vs Control; $^{\#\#}P<0.01$ vs HR; $^{+}P<0.05$, $^{++}P<0.01$ vs LA + HR.

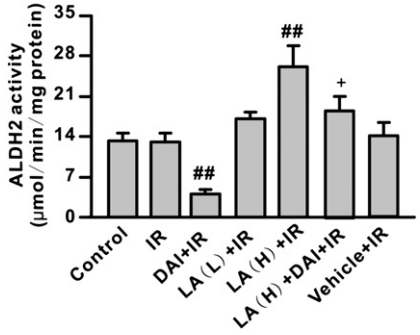

Fig. 3. Effect of alpha lipoic acid on aldehyde dehydrogenase 2 activity in isolated hearts. All values were expressed as means ± S.E.M. (n = 6 in each group). IR: ischemia–reperfusion; DAI + IR: daidzin (10^{-8} M) + ischemia–reperfusion; LA (L) + IR: alpha lipoic acid (10^{-8} M) + ischemia–reperfusion; LA (H) + IR: alpha lipoic acid (5×10^{-8} M) + ischemia–reperfusion; LA (H) + DAI + IR: alpha lipoic acid (5×10^{-8} M) + daidzin (10^{-8} M) + ischemia–reperfusion; Vehicle + IR: DMSO + ischemia–reperfusion. $^{\#\#}P<0.01$ vs IR; $^{+}P<0.05$ vs LA (H) + IR.

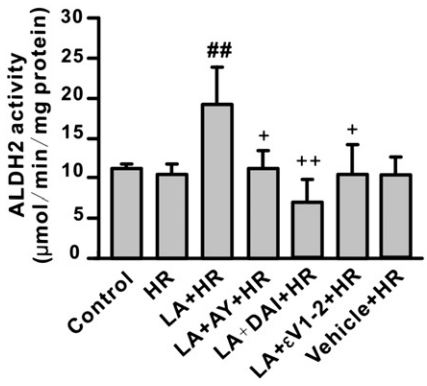

Fig. 4. Effect of alpha lipoic acid on aldehyde dehydrogenase 2 activity in cultured H9c2 cells. All values were expressed as means ± S.E.M. (n = 8 in each group). HR: hypoxia–reoxygenation; LA + HR: alpha lipoic acid (10^{-5} M) + hypoxia–reoxygenation; LA + AY + HR: alpha lipoic acid (10^{-5} M) + cyanamide (10^{-4} M) + hypoxia–reoxygenation; LA + DAI + HR: alpha lipoic acid (10^{-5} M) + daidzin (10^{-5} M) + hypoxia–reoxygenation; LA + εV1-2 + HR: alpha lipoic acid (10^{-5} M) + εV1-2 (10^{-6} M) + hypoxia–reoxygenation; Vehicle + HR: DMSO + hypoxia–reoxygenation. $^{\#\#}P<0.01$ vs HR; $^{+}P<0.05$, $^{++}P<0.01$ vs LA + HR.

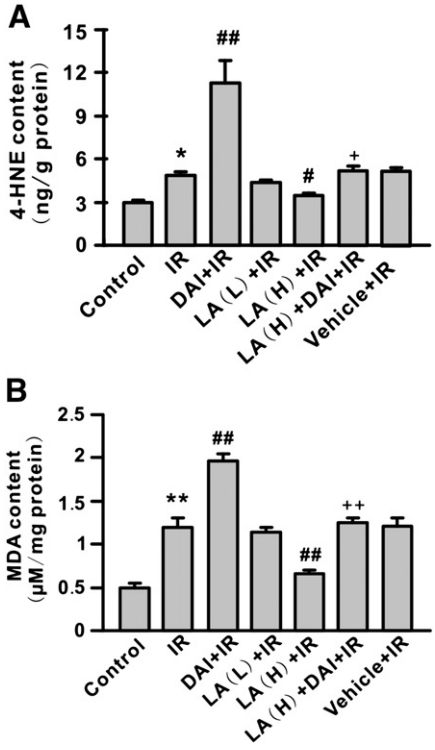

Fig. 5. Effect of alpha lipoic acid on aldehyde production following myocardial ischemia–reperfusion. A. 4-HNE content in cardiac tissue in each group. B. MDA content in cardiac tissue in each group. All values were expressed as means ± S.E.M. (n = 6 in each group). IR: ischemia–reperfusion; DAI + IR: daidzin (10^{-8} M) + ischemia − reperfusion; LA (L) + IR: alpha lipoic acid (10^{-8} M) + ischemia–reperfusion; LA (H) + IR: alpha lipoic acid (5×10^{-8} M) + ischemia–reperfusion; LA (H) + DAI + IR: alpha lipoic acid (5×10^{-8} M) + daidzin (10^{-8} M) + ischemia–reperfusion; Vehicle + IR: DMSO + ischemia–reperfusion. $^{*}P<0.05$, $^{**}P<0.01$ vs Control; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs IR; $^{+}P<0.05$, $^{++}P<0.01$ vs LA (H) + IR.

Alda-1 to rats before an ischemic event could reduce infarct size by as much as 60%, most likely through its inhibitory effect on the production of reactive aldehydes (such as 4-HNE). This study provides compelling evidence that the increase of ALDH2 activity has potential clinical implications in treating ischemia–reperfusion-induced myocardial injury. However, it is not clear so far whether Alda-1 can be used in clinic for treating ischemia–reperfusion injury in the future.

Different from Alda-1, alpha lipoic acid is a natural compound and found in almost all foods. In humans, alpha lipoic acid can be synthesized by the liver and other tissues, and functions as a cofactor for multiple metabolic enzymes, such as pyruvate dehydrogenase and α-keto-glutarate dehydrogenase (Dudek et al., 2008; Ghibu et al., 2009a), alpha lipoic acid and its reduced dithiol form, dihydrolipoic acid, are powerful antioxidants (Ghibu et al., 2009b). Due to its powerful antioxidant property, alpha lipoic acid is found to have potential clinical implications in treating oxidative injury induced by ischemia–reperfusion (Freisleben, 2000; Ghibu et al., 2009b). However, the underlying mechanisms remain poorly understood. Recently, alpha lipoic acid is reported to increase ALDH2 activity by ~60% in isolated rat heart mitochondria (Wenzel et al., 2007). We therefore hypothesize that the cardioprotective effects of alpha lipoic acid on ischemia–reperfusion-induced myocardial injury are through a mechanism involving ALDH2 activation. Our results from the present study showed that, in the ischemia–reperfusion or hypoxia–reoxygenation group, administration of alpha lipoic acid significantly increased ALDH2 activity accompanied by a decrease in 4-HNE and MDA contents, an improvement in cardiac function or a reduction in cardiomyocyte apoptosis, these effects were reversed by ALDH2 inhibitors. These results echoed our hypothesis mentioned above.

Since PKCε signaling pathway is involved in ALDH2 activation (Churchill et al., 2009), we therefore examined whether the effect of alpha lipoic acid on ALDH2 is associated with PKCε pathway. We found that the beneficial effects of alpha lipoic acid on hypoxia–reoxygenation-induced apoptosis were significantly attenuated in the presence of εV1-2, a selective inhibitor of PKCε, concomitantly with the decreased ALDH2 activity, increased 4-HNE and MDA contents. These results confirmed the involvement of PKCε in the cardioprotective effects afforded by alpha lipoic acid.

It is worth mentioning that in addition to ALDH2 a string of other enzymes of dehydrogenase was also reported to involve in cardioprotection. This includes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and muscle form of lactate dehydrogenase (M-LDH) (Jovanovic et al., 2005; Jovanovic et al., 2009a; Jovanovic and Jovanovic, 2005). Interestingly, unlike ALDH2, both GAPDH and M-LDH have been shown to participate in the regulation of the activity

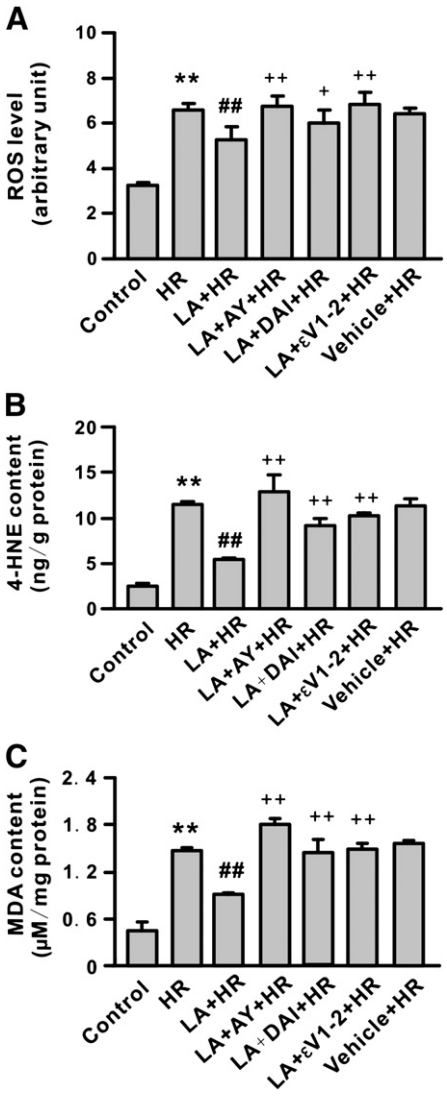

Fig. 6. Effect of alpha lipoic acid on reactive oxygen species and aldehyde production following hypoxia–reoxygenation in cultured H9c2 cells. A. reactive oxygen species level in H9c2 cells in each group; B. 4-HNE content in H9c2 cells in each group. C. MDA content in H9c2 cells in each group. All values were expressed as means ± S.E.M. (n = 8 in each group). HR: hypoxia–reoxygenation; LA + HR: alpha lipoic acid (10^{-5} M) + hypoxia–reoxygenation; LA + AY + HR: alpha lipoic acid (10^{-5} M) + cyanamide (10^{-4} M) + hypoxia–reoxygenation; LA + DAI + HR: alpha lipoic acid (10^{-5} M) + daidzin (10^{-5} M) + hypoxia–reoxygenation; LA + εV1-2 + HR: alpha lipoic acid (10^{-5} M) + εV1-2 (10^{-6} M) + hypoxia–reoxygenation; Vehicle + HR: DMSO + hypoxia–reoxygenation. $^{**}P<0.01$ vs Control, $^{\#\#}P<0.01$ vs HR; $^{+}P<0.05$, $^{++}P<0.01$vs LA + HR.

of sarcolemmal K(ATP) channels, the potential end-effectors of ischemic preconditioning. The cardioprotection afforded by these enzymes was thought to be mediated by K(ATP) channels (Jovanovic et al., 2005; Jovanovic et al., 2009b). Therefore, similar to ALDH2, both GAPDH and M-LDH are also potential targets for development of novel drugs with direct beneficial effects on cardiomyocytes.

In summary, the results presented in this study demonstrate for the first time that the cardioprotective effects of alpha lipoic acid on ischemia–reperfusion injury are through a mechanism involving, at least in part, ALDH2 activation. The regulatory effect of alpha lipoic acid on ALDH2 activity is dependent on PKCε signaling pathway.

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