Downregulation of Na$^+$/Ca^{2+} Exchanger Isoform 1 Protects Isolated Hearts by Sevoflurane Postconditioning but Not by Delayed Remote Ischemic Preconditioning in Rats

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Abstract

Background: Calcium regulatory proteins-L-type Ca^{2+} channels (LTCCs), ryanodine receptor 2 (RyR2), and Na$^+$/Ca^{2+} exchanger isoform 1 (NCX1) have been recognized as important protective mechanisms during myocardial ischemia-reperfusion injury (I/RI). Both sevoflurane postconditioning (SevoPoC) and delayed remote ischemic preconditioning (DRIPC) have been shown to protect the heart against I/RI. In this study, we aimed to compare the effects of SevoPoC and DRIPC on the expression of the three calcium regulatory proteins in an isolated rat heart model.

Methods: After 30-min balanced perfusion, isolated hearts from rats were subjected to 30-min ischemia followed by 60-min reperfusion. Totally 40 isolated hearts were randomly assigned to four groups ($n = 10$/group): time control group, I/RI group, SevoPoC group, and DRIPC group. The effect of SevoPoC (3% v/v) and DRIPC were observed. Myocardial infarct size (IS), cardiac troponin I level, and heart function were measured. The protein and messenger RNA levels of LTCCs, RyR2, and NCX1 were determined.

Results: Both SevoPoC and DRIPC improved the recovery of myocardial function, and reduced cardiac troponin I release after I/RI. The decrease in IS was more significant in the SevoPoC group than that in the DRIPC group (16.50% ± 4.54% in the SevoPoC group [$P = 0.0006$], and 22.34% ± 4.02% in the DRIPC group [$P = 0.0007$] vs. 35.00% ± 5.24% in the I/RI group, respectively). SevoPoC, but not DRIPC significantly inhibited the activity of NCX1 (0.59 ± 0.09 in the I/RI group vs. 0.32 ± 0.16 in the SevoPoC group, $P = 0.006$; vs. 0.57 ± 0.14 in the DRIPC group, $P = 0.072$). No statistical significant differences were observed in the expression of LTCCs and RyR2 between SevoPoC and DRIPC. In addition, subsequent correlation analysis showed a significantly positive relationship between the cardiac troponin I level and the protein expression of NCX1 ($r = 0.505$, $P = 0.023$).

Conclusion: SevoPoC may be more effective in the cardioprotection than DRIPC partly due to the deactivation of NCX1.

Key words: Calcium Regulatory Protein; Delayed Remote Ischemic Preconditioning; Ischemia-reperfusion Injury; Na$^+$/Ca^{2+} Exchanger; Sevoflurane Postconditioning

Introduction

Coronary heart disease, which manifests as myocardial ischemia-reperfusion injury (I/RI), is now the leading cause of death worldwide. The noninvasive protective interventions, including sevoflurane postconditioning (SevoPoC) and remote ischemic preconditioning (RIPC) remain the promising approaches which harness the body's endogenous protective capabilities against the injury elicited by ischemia and reperfusion. Sevoflurane, a commonly used volatile anesthetic, has been demonstrated to protect the heart at the onset of reperfusion in clinical and experimental studies.[1,2] Similar cardiac effect has also been shown in RIPC by applying the protective stimulus at one tissue or organ remote from the heart before a sustained myocardial ischemia.[3] Two

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different phases of protection exerted by RIPC have been proposed: early phase (<4 h) and delayed phase (24–72 h).[4] It has also been successfully applied for cardioprotection in coronary artery bypass grafting[5] and percutaneous coronary intervention (PCI)[6] procedures.

Numerous studies have concerned about the mechanisms of these two noninvasive approaches.[7-10] Currently, it is believed that both SevoPoC and early RIPC could activate reperfusion injury salvage kinase (RISK) pathway, comprising phosphoinositide 3-kinase-protein kinase B/Akt and extracellular signal-regulated kinase 1 and 2, and Survivor Activating Factor Enhancement (SAFE) such as STAT3.[11-14] However, there is little information on the mechanisms responsible for the cardioprotection by delayed remote ischemic preconditioning (DRIPC). Our previous findings in animal hearts have found that DRIPC could activate nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) and Heme oxgenase-1, but not the RISK pathway in SevoPoC.[15,16] As known to all, SevoPoC (after ischemia) and delayed RIPC (before ischemia) could exert similar cardioprotection in different periods. Hence, novel differential underlying mechanisms still need to be further elucidated.

Calcium regulatory proteins are mainly composed of L-type Ca^{2+} channels (LTCCs), ryanodine receptor 2 (RyR2), and Na^+/Ca^{2+} exchanger (NCX). These proteins have been proposed as important mechanisms responsible for myocardial I/RI in recent years.[17,18] However, there have been few studies investigating the effects of SevoPoC and DRIPC on the expression of calcium regulatory proteins. Therefore, we compared the expression of LTCCs, RyR2, and NCX1 between SevoPoC and DRIPC in a Langendorff perfused rat heart model and tried to explore the potential role in cardioprotection.

METHODS

Animals

All experimental procedures were reviewed and approved by the Animal Care and Use Committee of Fuwai Cardiovascular Hospital, and all animals received appropriate care according to the Guide for the Care and the Use of Laboratory Animals published by the US National Institutes of Health (revised in 1996). The researchers who performed the animal experiments, possessed qualified certifications issued by Beijing Association on Laboratory Animal Care (Beijing, China). Adult, male Sprague-Dawley (SD) rats (250–300 g) obtained from Vital River Experimental Animal Company (Beijing, China), were kept in a temperature-controlled room with a 12-h light–dark cycle and had *ad lib* access to food and water.

Langendorff heart preparation, and hemodynamics monitoring

SD rats were heparinized (1000 IU/kg) and anesthetized by intraperitoneal injection of ethyl carbamate. Each rat received the tail-clamp test to make sure that it was pain-free before the operation. After 2% lidocaine was infiltrated into the incision, the hearts were rapidly excised and placed into ice-cold phosphate buffer solution, mounted on a modified noncirculating Langendorff apparatus. Then, hearts were perfused with 95% oxygen oxygenated Krebs-Henseleit (K-H) buffer solution containing (in mmol/L): NaCl 118.5, KCl 4.75, $MgSO_4$ 1.19, $NaHCO_3$ 25.0, KH_2PO_4 1.2, glucose 11.0, HEPES 10.0, and $CaCl_2$ 1.4, at a constant flow rate of 10 ml/min. A small saline-filled latex balloon connected with a pressure transducer was inserted through the left atrium and pushed through the mitral valve into the left ventricle. The balloon volume was adjusted to achieve an end-diastolic pressure of 0-10 mmHg. The cardiac function was then recorded through LabChart 7.0 (AD Instruments Inc., Colorado Springs, CO, USA). Characteristic data derived from left ventricular pressure measurement were left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), maximum LVDP increase (+dp/dt), and decrease (−dp/dt) rate as indices of contractility and relaxation, and heart rate (HR).

Experimental protocols

After a stabilization period of 30 min, 40 isolated hearts were randomly assigned to four groups ($n = 10$/group; Figure 1): (1) time control group: continuous perfusion for 120 min; (2) I/RI group: ischemia for 30 min followed by 60-min reperfusion; (3) SevoPoC group: perfused with 3% (v/v) sevoflurane-bubbled KHBs (Maruishi Pharmaceutical Co., Japan) oxygenated with 95% oxygen for 10 min at the onset of reperfusion and then with normal KHBs for the remaining 50 min. The concentration was monitored by an anesthetic gas monitor (Datex Capnomac Ultima, Division of Instrumentarium Corp., Helsinki, Finland) and a gas chromatograph (GC/MSQP2010PLUS, Shimadzu, Kyoto, Japan): 3% (v/v) sevoflurane (0.51 ± 0.04 mmol/L); (4) DRIPC group: 4 cycles of 5-min occlusion and 5-min reflow at unilateral hind limb once at the day before heart isolation. Ischemia was confirmed using modified pulse oxymetry for the rats with anesthesia by ethyl carbamate (intraperitoneally, 1.0 g/kg).

Cardiac troponin I detection

Coronary effluent (1 ml) was collected at the baseline and at the end of 60-min perfusion for the detection of cardiac troponin I (cTnI) levels using ACS: 180 automated chemiluminescence system with commercial kits (Bayer Corp., Tarrytown, NY, USA). All coronary effluent samples were coded, and the laboratory investigator was blinded to the treatment regimen.

Infarct size determination

Myocardial infarct size (IS) was determined using 2,3,5-triphenyltetrazolium chloride (TTC; Sigma-Aldrich, St. Louis, MO, USA) staining ($n = 5$). At the end of reperfusion, hearts were frozen at −20°C for 1 h, and subsequently sliced into 5 sections of equal thickness (≈1 mm). The slices were incubated in 0.1% TTC solution for 10 min at 37°C and

then fixed overnight in 4% formalin solution. Infarcted myocardial areas (pale color) can be differentiated from viabe ones (brick-red color). The IS was measured by the planimetry using ImageJ 1.43 (Wayne Rasband, Bethesda, MD, USA) and then expressed as a percentage of infarcted area by the total ventricular area.

Western blotting

The left ventricular tissue samples were collected, immediately snap frozen in liquid nitrogen, and stored at −80°C for further analyses. Samples were then powdered under liquid nitrogen and homogenized in lysis buffer (20 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, 1 mmol/L ethylenediamine tetra-acetate, 1 mmol/L EGTA, 1% Triton, 3% sodium doecyl sulfate [SDS], 1 mmol/L phenylmethylsulfonyl fluoride, and 5 mg/ml protease inhibitor cocktail). The homogenates were vortexed for 15 s and then centrifuged at 14,000 $\times g$ for 10 min at 4°C. Protein concentrations were determined by the Bradford method using bovine serum albumin as a standard. Equal amounts of protein were electrophoresed on a 12.5% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane, and probed with polyclonal primary antibodies specific for total LTCCs, the RyR2, and the NCX. Coomassie blue and Ponceau red were used to verify adequate transfer of proteins from the gel to the membrane. β-actin was used to ensure equal protein loading. The membranes were washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Santa Cruz Biotechnology Inc., CA, USA). Proteins were detected using chemiluminescence; bands were visualized by exposure to photographic film (Fuji Protein, Tokyo, Japan). Quantitative analysis of the band densities was performed using Image 1.63 software (National Institute of Health, Bethesda, Maryland, USA).

Real-time polymerase chain reaction

The messenger RNA (mRNA) level of LTCCs, RyR2, NCX1 were analyzed by real-time polymerase chain reaction (PCR). Total RNA was isolated from the LV samples lysed in Trizol solution (Invitrogen, Carlsbad, CA, USA). The RNA samples were then reversely transcribed with first strand complementary DNA (cDNA) synthesis kit (Roche, Indianapolis, IN, USA) in a 20 ml reaction system. The cDNA was then amplified with Universal SYBR Green Master (Rox) in the LightCycle 480 system (Roche, Indianapolis, IN, USA) using the following primers: (1) RYR2 forward, AAACATGTCTGCTGCACTCACT; reverse, CAGGGGCATTCACTTTTATCA; (2) NCX1 forward, TCTGAAAGATTCCGTGACTGC; reverse, GAAGACATTCACAGCGTTGC; (3) LTCCs forward, TCGTGGGTTTCGTCATTGTC; reverse, TGTACTGGTGCTGGTTCTTG. The cDNA was synthesized at 37°C for 15 min in a 10 µl reaction containing 0.5 µg total RNA, 2 µl PrimeScript Buffer, 0.5 µl oligo dT, 0.5 µl random 6 mers, and 0.5 µl PrimeScript RT Enzyme Mix I (TaKaRa, Japan). Real-time PCR reactions included 1 µl of cDNA, 5 µl 2× LightCycler 480 SYBR Green I master mix (Roche), 0.2 µl forward primer, 0.2 µl reverse primer, and 3.6 µl nuclease-free water. All PCR reactions were carried out in triplicate with the following conditions: 95°C for 10 min, followed by 40 cycles of 10 s at 95°C, 30 s at 60°C in the LightCycler 480 II real-time PCR Instrument (Roche). For each selected gene, melting curve analysis was performed to validate the specific generation of the expected PCR product. The expression of each gene was normalized as ΔCt (= Ct of target gene − Ct of internal control gene) using 18SrRNA as the control. Relative quantification using the $\Delta\Delta Ct$ method was applied to compare the amounts of mRNA.

There were 10 isolated rat hearts in each group after the Langendorff protocols. Five of the 10 hearts were sliced to determine the IS, and the remaining 5 ventricular tissue samples were collected to detect the protein and mRNA levels of the three calcium regulatory proteins. We used the correlation analysis to detect the relationship between the cardiac troponin I level and the protein expression of NCX1.

Statistical analysis

The sample size was chosen according to the previous studies by our group.[10,12] Data were expressed as mean ± standard deviation (SD). The variables of LV contractile function were analyzed using two-way analysis of variance (ANOVA). For all other data, one-way ANOVA with *post hoc* Tukey test for multiple comparisons was used. All statistical analyses were performed in SPSS version 13.0 (IBM, Armonk, New York, USA). $P < 0.05$ was considered statistically significant.

Results

Effects on hemodynamic parameters

As shown in Table 1, no significant differences were observed in baseline hemodynamic parameters among all experimental groups ($P = 0.068$). During the reperfusion, a dramatically elevated LVEDP and significant decreases in RPP (the product of HR and LVDP) and ±dp/dt were observed in the I/RI group ($P = 0.036$). Compared with the SevoPoC group, the significant decreases in RPP were observed in the DRIPC group [$P = 0.027$; Table 1].
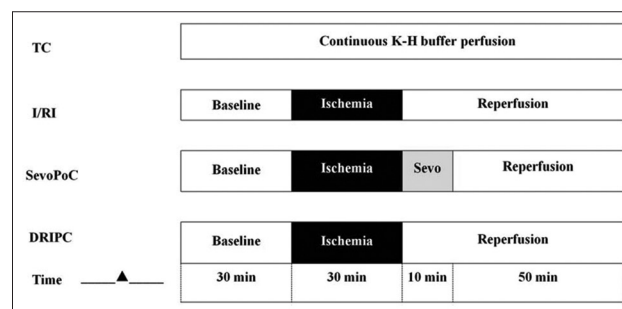

Figure 1: Experimental protocol. Hearts were randomly assigned in one of the above experimental groups ($n = 10$). TC: Time control; I/RI: Ischemia/reperfusion injury; SevoPoC: Sevoflurane postconditioning; DRIPC: Delayed remote ischemic preconditioning; Sevo: Sevoflurane.

Table 1: Hemodynamic parameters in all rat groups

Items	TC group	I/RI group	SevoPoC group	DRIPC group
LVEDP (mmHg)				
Baseline	6.8 ± 2.7	4.7 ± 1.9	5.3 ± 2.5	5.0 ± 3.1
Reperfusion				
30 min	4.3 ± 1.5	31.6 ± 14.4*,†	19.9 ± 5.7*,†,‡	24.9 ± 8.9*,‡,§
60 min	4.7 ± 1.2	34.4 ± 14.3*,†	21.3 ± 7.1*,†,‡	23.3 ± 7.8*,‡
Recovery of RPP (mmHg)				
Baseline	52977.6 ± 3242.8	58567.6 ± 3170.9	53724.0 ± 3649.4	56054.8 ± 3130.8
Reperfusion				
30 min	55997.3 ± 2648.9	43984.3 ± 3221.2*,†	51682.5 ± 3545.8‡	48365.7 ± 2797.7*,‡,§
60 min	54090.1 ± 3019.7	44277.1 ± 3514.1*,†	52327.2 ± 3814.4‡	51642.0 ± 1235.8*,‡,§
Recovery of +dp/dt (mmHg)				
Baseline	5033.5 ± 564.8	4841.0 ± 448.4	5286.4 ± 960.5	4643.1 ± 367.8
Reperfusion				
30 min	5491.5 ± 755.0	3955.1 ± 493.8*,†	5053.8 ± 655.5‡	4285.5 ± 466.2*,‡
60 min	5728.1 ± 664.4	3795.3 ± 522.8*,†	4842.4 ± 798.3‡	4450.5 ± 457.6*,‡
Recovery of −dp/dt (mmHg)				
Baseline	3340.8 ± 322.6	3040.9 ± 380.6	3186.7 ± 343.7	2814.3 ± 222.8
Reperfusion				
30 min	3544.6 ± 273.9	2317.2 ± 243.3*,†	3008.2 ± 328.2‡	2630.1 ± 346.1*,‡
60 min	3484.4 ± 297.3	2359.8 ± 279.8*,†	2984.4 ± 417.0*,†	2831.9 ± 312.4*,†

Data are presented as mean ± SD (n=10). *P<0.05 versus baseline; †P<0.05 versus TC group; ‡P<0.05 versus I/RI group; §P<0.05 versus SevoPoC group. TC: Time control; I/RI: Ischemia/reperfusion injury; SevoPoC: Sevoflurane postconditioning; DRIPC: Delayed remote ischemic preconditioning; LVEDP: Left ventricular end-diastolic pressure; RPP: The product of HR and LVDP; LVDP: Left ventricular developed pressure; ±dp/dt: Maximum LVDP increase (+dp/dt) and decrease (−dp/dt) rate; HR: Heart rate; SD: Standard deviation.

Infarct size and cardiac enzyme release

SevoPoC or DRIPC reduced the IS caused by I/RI (16.50% ± 4.54% in the SevoPoC group [P = 0.0006] and 22.34% ± 4.02% in the DRIPC group [P = 0.0007], vs. 35.00% ± 5.24% in the I/RI group, respectively; Figure 2a and 2b). As a specific marker of myocardial injury, the baseline values of cTnI in all groups were not significantly different. The cTnI levels at the end of reperfusion were largely increased in the I/RI group, whereas less releas was observed both in the SevoPoC group (P = 0.0005) and in the DRIPC group [P = 0.0005; Figure 2c].

Expression of L-type Ca²⁺ channels, ryanodine receptor 2 and Na⁺/Ca²⁺ exchanger isoform 1

Western blotting study showed that NCX1 expression was significantly decreased in the SevoPoC group (0.32 ± 0.16 vs. 0.59 ± 0.09 in the I/RI group, P = 0.006) but not in the DRIPC group [0.57 ± 0.14 vs. 0.59 ± 0.09 in the I/RI group, P = 0.072; Figure 3a and 3b]. No statistical differences were observed in the expression of LTCCs and RyR2 [Figure 3c and 3d]. Despite the transcriptional level of RyR2 in SevoPoC group seemed to be substantially reduced, there was no statistical difference [P = 0.065; Figure 4b]. The difference of mRNA regulation among LTCCs, NCX1 was not observed in both SevoPoC and DRIPC group [P = 0.081; Figure 4a and 4c].

Moreover, the cardiac troponin I level was significantly positively correlated with the protein expression of NCX1 [Pearson correlation: r = 0.505, P = 0.023; Figure 4d].

Discussion

In the current study, we found that SevoPoC and DRIPC offer similar cardioprotection by improving the recovery of myocardial function, reducing cTnI release, and decreasing IS after I/RI. However, SevoPoC may provide more ability to regulate calcium regulatory proteins than DRIPC, especially in the deactivation of NCX1.

It has always been a research focus for comparing the adaptive cardioprotective procedures and relevant mechanisms in different phases. Halkos et al.[19] compared ischemic preconditioning (IPC) and ischemic postconditioning (IPoC), finding that in comparison with IPC, IPoC can only reduce ROS generation during early reperfusion by mechanisms other than those engaged by preconditioning, and explored the mechanism in terms of lipid peroxidation and ROS generation. Deyhimy et al.[20] found that sevoflurane preconditioning (SevoPC) and SevoPoC, were shown to be equally effective in protecting myocardial function, and investigated the mechanisms in terms of intracellular concentrations of Na⁺, H⁺ and Ca²⁺. Both Xin et al.[21] and Tamareille et al.[22] found that remote ischemic perconditioning was as efficacious as local IPoC in protecting myocardium from myocardial I/RI, and explored the involvement of RISK and SAFE signal pathways. Moreover, other researches focused on SevoPC and ischemic late preconditioning,[23] early and late ischemic preconditioning,[24] and SevoPC and SevoPoC,[20] all of which highlighted the importance of adenosine triphosphate-regulated potassium (KATP) channels and mitochondria. Our groups have previously compared the two noninvasive protective approaches, for
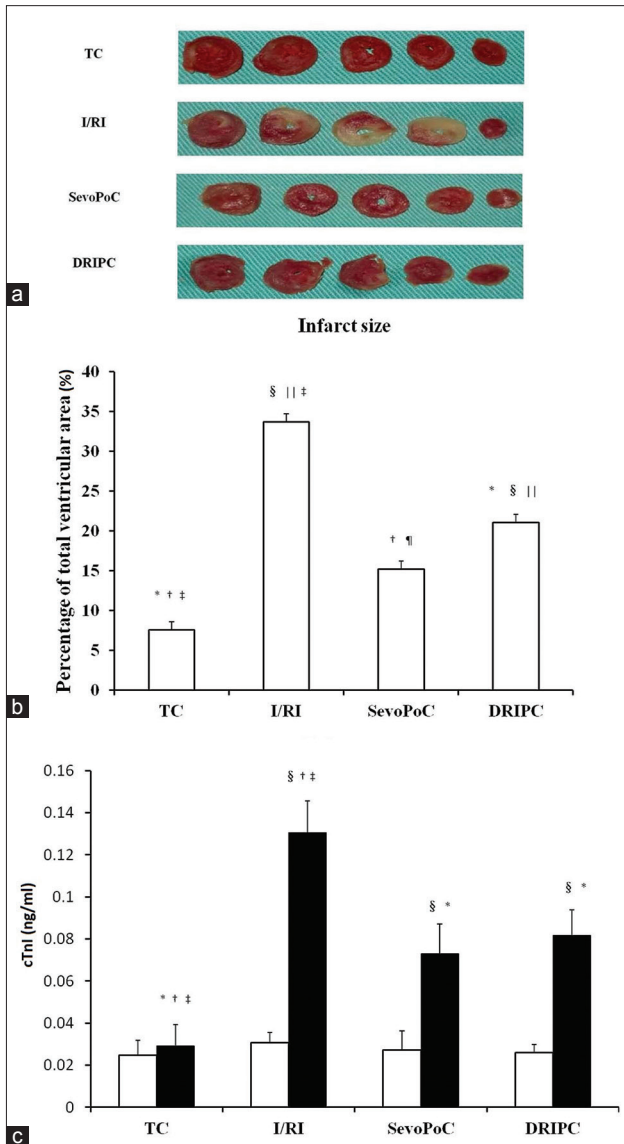
Figure 2: Myocardial injury assessment in the experimental groups. (a) Representative TTC staining of the hearts. (b) Infarct size was expressed as percent of necrotic area over left ventricular area. TTC indicates 2,3,5-triphenyltetrazolium chloride. $*P < 0.05$ versus SevoPoC group, $†P < 0.001$ versus I/RI group, $‡P < 0.001$ versus DRIPC group, $§P < 0.001$ versus TC group, $\|P < 0.001$ versus SevoPoC group, $¶P < 0.05$ versus TC group. (c) cTnI level ($n = 10$) in the coronary effluent. $*P < 0.001$ versus I/RI group, $†P < 0.001$ versus SevoPoC group, $‡P < 0.001$ versus DRIPC group, $§P < 0.001$ versus TC group. TC: Time control; cTnI: Cardiac troponin I; I/RI: Ischemia/reperfusion injury; SevoPoC: Sevoflurane postconditioning; DRIPC: Delayed remote ischemic preconditioning.

the first time, SevoPoC and DRIPC (5-min reflow at the unilateral hindlimb once per day for 3 days before heart isolation), and elucidated the underlying mechanisms in an isolated heart model with focus on the RISK pathway and Nrf2/ARE-HO-1.[16] In the present study, we further compared the cardioprotection elicited by SevoPoC and DRIPC (5-min reflow at unilateral hindlimb once at the day before heart isolation) and focused on expression of calcium regulatory proteins, finding that both SevoPoC and

DRIPC could confer cardioprotection from myocardial I/RI, as manifested by significantly improved cardiac function, reduced cTnI release, and in particular the decreased IS. In addition, there were marked differences in the calcium regulatory protein expression between SevoPoC and DRIPC.

Calcium regulatory protein plays a crucial role in maintaining Ca^{2+} homeostasis in the heart under the physiologic condition, and abnormal Ca^{2+} handling has been considered to be a major downstream effect that ultimately promotes the cardiomyocyte damage. The expression of NCX1 has been known to increase both at mRNA and protein level in failing heart due to ischemic cardiomyopathy.[17] Accumulating evidence indicates that NCX1-mediated Ca^{2+} overload contributes to the death of cardiomyocytes during IR-injury, the inhibition of NCX exerted a protective effect in ischemic ventricular myocytes making NCX1 as an effective therapeutic target for preventing IR-induced cardiomyocyte death.[18,25,26] However, evidence indicates that cellular mechanisms involved could be different in LTCCs and RyR2 activities,[19,20] in our study, we found that only NCX1 was of significant importance in the cardioprotective effect, especially elicited by SPoC. Based on these evidence, the activation of calcium regulatory protein during cardiac IR injury prevention may be with the discrepancy, partly due to the types of IR model, animal species, and conditioning protocols and timing.

Several relevant publications have concerned about the effect of conditioning strategies on the calcium regulatory proteins. Ma *et al.*[27] using neonatal rat heart model found that the cardioprotection by preconditioning with chronic intermittent hypoxia (CIH) was mediated by preserving NCX1 expression. In addition to NCX1 expression, two studies also established the augmentation of RYRs activity in CIH-induced cardioprotection in adult rat heart models.[24,28] Contrary finding from Collins *et al.*[29] revealed that preconditioning-like effect by daily exercise could alter calcium regulatory proteins by decreasing NCX expression in hypertensive rat hearts, indicating that the mechanisms underlying the calcium regulatory protein expression may be conditioning-specific. In this study, the effects of SevoPoC and DRIPC on the three regulatory proteins were systematically investigated both in translational and transcriptional levels. Furthermore, in support of the proposed indication, we did find a differential regulation of NCX1 between SevoPoC and DRIPC.

Although the present data are unable to tell the exact molecular mechanisms underlying the differential expression of NCX1 between SevoPoC and DRIPC, we believe that there are several putative involving signal pathways according to the current publications. First, a previous study using an adult cardiomyocyte model has demonstrated that the inhibition of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity could prevent NCX1 upregulation.[30] Akiko *et al.* demonstrated that sevoflurane exerted the cardioprotection against I/RI through an inhibitory action on CaMKII activity.[31] On the other hand, β-adrenergic pathway has been widely proposed as an important mechanistic
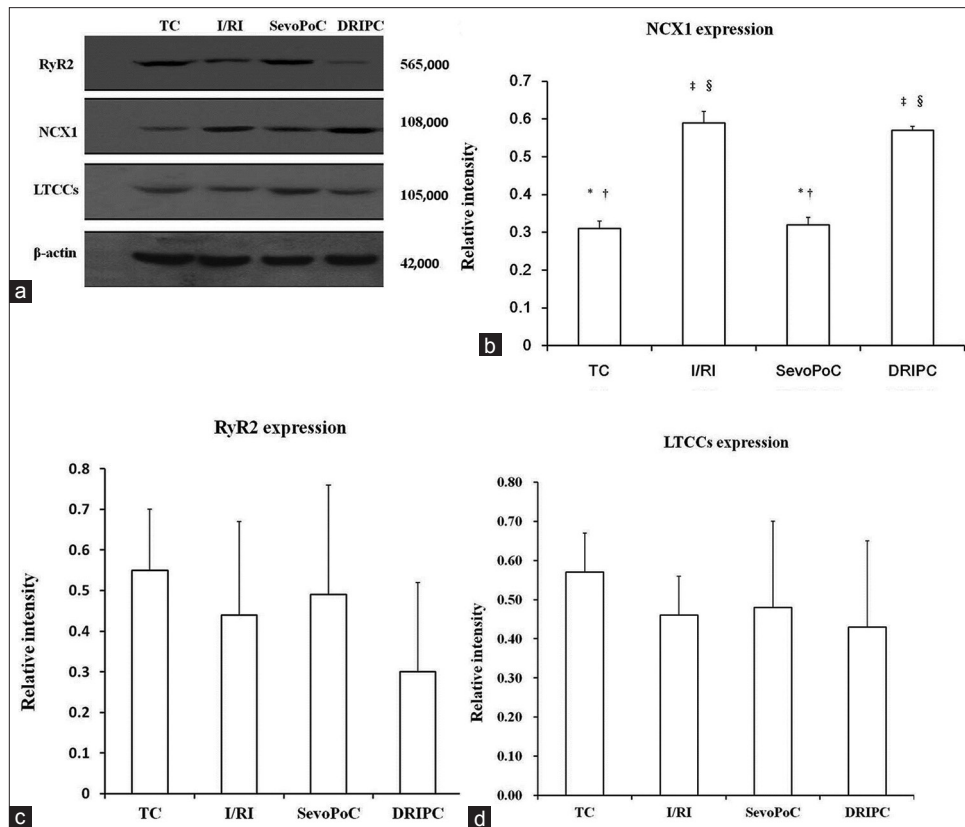
Figure 3: Expression levels of RyR2, NCX1, LTCCs determined by Western blotting analysis ($n = 5$). (a) Representative Western blotting bands of RyR2, NCX1, LTCCs. (b) SevoPoC significantly inhibited the protein expression levels of NCX1 (0.32 ± 0.16 in the SevoPoC group vs. 0.59 ± 0.09 in the I/RI group, $P = 0.006$; 0.57 ± 0.14 in the DRIPC group vs. 0.59 ± 0.09 in the I/RI group, $P = 0.072$). (c and d) No statistical differences were observed in the expression levels of LTCCs and RyR2. *$P < 0.01$ versus I/RI group, †$P < 0.01$ versus DRIPC group, ‡$P < 0.01$ versus TC group, §$P < 0.01$ versus SevoPoC group. TC: Time control; I/RI: Ischemia/reperfusion injury; SevoPoC: Sevoflurane postconditioning; DRIPC: Delayed remote ischemic preconditioning; LTCCs: L-type Ca^{2+} channels; RyR2: Ryanodine receptor 2; NCX1: Na$^+$/Ca^{2+} exchanger isoform 1.

pathway in anesthetic-induced cardioprotection, including SevoPC[32] and desflurane postconditioning.[33] The potential involvement of β-adrenergic pathway in SevoPoC may be rational for the similar mechanisms between anesthetic preconditioning and anesthetic postconditioning. β-adrenergic receptors transduced signals to the second messenger cyclic adenosine monophosphate[34] which has been reported to inhibit NCX.[35] However, there were few relevant publications upon the CaMKII nor β-adrenergic pathway in DRIPC. In the present study, SevoPoC was shown to provide cardioprotection by deactivating NCX1. This mechanism was supported by the correlation analysis revealing a positive relationship between the cardiac troponin I level and the protein expression of NCX1. However, this association should be verified through investigating the influence of ion channels using specific inhibitors in the future. Taken together, these indications may explain the differential regulation in NCX1 between SevoPoC and DRIPC group. The mRNA level was similar between SevoPoC and DRIPC group indicating an involvement of nontranscriptional differential regulation. Hence, the detailed mechanisms should be verified in future studies.

There are several limitations in the current study. First, we evaluated the process of I/RI in the Langendorff model, in which the hearts were denervated and could not reflect the potential role of neurohumor factors. Second, most studies, including ours, investigated the mechanism of I/RI using juvenile and healthy hearts, which is different from clinical setting where patients with various co-morbidities (such as gender[36] and age[37]) and/or co-medications (β-blockers[38]), however, accumulating evidence has shown that unhealthy myocardium may be less amenable to protective interventions.[39-41] Third, we investigated the influence of ion channels without using specific inhibitor, such as KB-R7943[42] (the inhibitor of NCX). Fourth, we did not observe the cytosolic Ca^{2+} concentrations, especially the intracellular diastolic Ca^{2+} concentrations, which may be a direct parameter of the activation of the NCX and contribute to the explanations of the underlying mechanism of I/RI. Finally, the data presented in this paper while suggestive, are insufficient to make a definitive conclusion. Further studies are needed to elucidate the underlying pathways and their detailed interaction and discrepancy.

In conclusion, using a Langendorff perfused rat heart model, the findings from this study suggest that SevoPoc may be more effective in the cardioprotection than DRIPC partly due to the deactivation of NCX1.
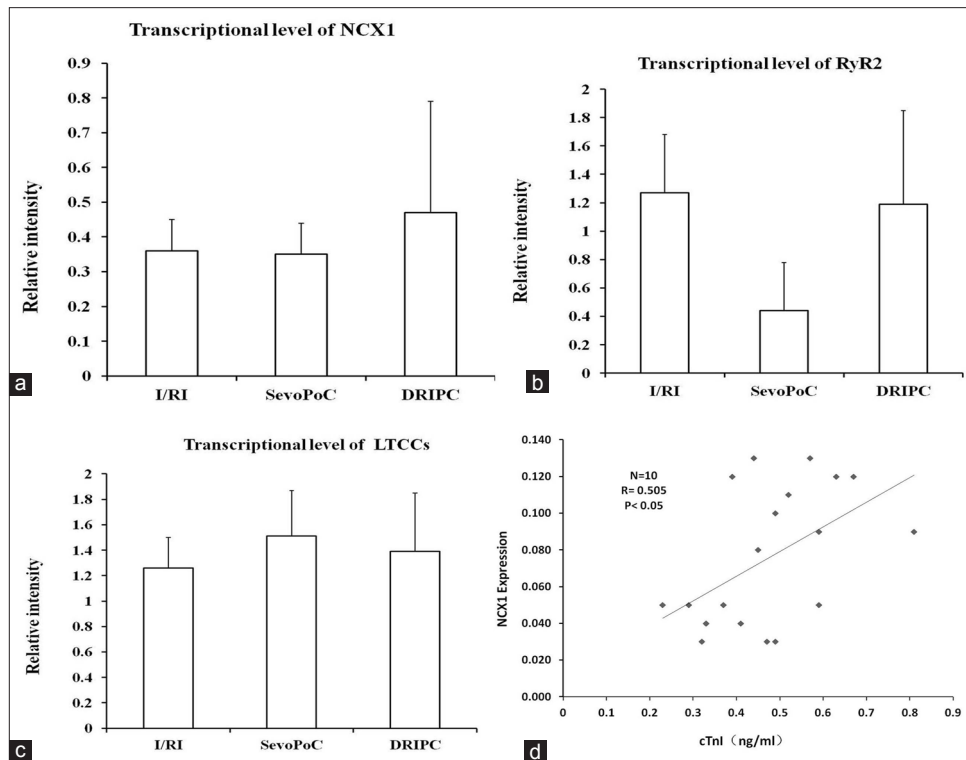
Figure 4: Transcriptional levels of RyR2, NCX1, LTCCs determined by real-time PCR analysis ($n = 5$). (a-c) The difference of mRNA regulation among LTCCs, RyR2, NCX1 was not observed in both SevoPoC and DRIPC group ($P = 0.081$). (d) A significantly positive relationship was observed between the cTnI level and the protein expression of NCX1 ($n = 20$). LTCCs: L-type Ca^{2+} channels; RyR2: Ryanodine receptor 2; NCX1: Na^+/Ca^{2+} exchanger isoform 1; TC: Time control; I/RI: Ischemia/reperfusion injury; mRNA: Messenger RNA; SevoPoC: Sevoflurane postconditioning; DRIPC: Delayed remote ischemic preconditioning; cTnI: Cardiac troponin I.

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Conflicts of interest

There are no conflicts of interest.

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