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Original Article

Sevoflurane alleviates myocardial ischemia/reperfusion injury via actitation of heat shock protein-70 in patients undergoing double valve replacement surgery

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Abstract: This study investigated the cardioprotective effect(s) of sevoflurane in rheumatic heart disease patients undergoing double valve replacement surgery (DVRS) under cardiopulmonary bypass (CPB) and its potential mechanisms (ChiCTR2100051220 on http://www.ChiCTR.org.cn). Forty-six patients were randomly assigned to undergo propofol or sevoflurane anesthesia during surgery. The levels of myocardial injury markers, inflammatory cytokines, heat shock protein-70 (HSP70), and superoxide dismutase (SOD) activity were measured from blood samples. Mean arterial pressure, cardiac index, and stroke volume index were significantly higher in the sevoflurane group than in the propofol group at the end of CPB. However, there were no significant differences in operative duration, length of CPB or aortic cross-clamp time, auto-resuscitation heart rate, drainage within 48 h after surgery, time to extubation, and recovery time after DVRS. The dose of inotropic agents (dopamine and noradrenaline) was significantly lower in the sevoflurane group than in the propofol group. Sevoflurane was associated with smaller increases in the levels of myocardial injury-associated markers (CK-MB and cardiac troponin I [cTnI]) and inflammatory cytokines (interleukin [IL]-6, IL-8, and tumor-necrosis factor-alpha [TNF-α]); however, there was a greater increase in HSP70 levels compared with propofol after surgery. Moreover, SOD activity after surgery was significantly higher in the sevoflurane group than in the propofol group. Increased HSP70 levels in the sevoflurane group were positively correlated with cTnI, IL-6, IL-8, and TNF-α levels, and negatively correlated with SOD activity. These results suggest a cardioprotective effect of sevoflurane during DVRS. Sevoflurane may reduce biomarkers of cardiac injury through its anti-inflammatory effects via upregulation of HSP70.

Keywords: Double valve replacement surgery, cardiopulmonary bypass, sevoflurane, ischemia-reperfusion injury, heat shock protein 70

Introduction

Rheumatic heart disease (RHD) induced by rheumatic fever causes permanent damage to heart valves, and represents a major health problem in low-income and developing countries worldwide [1]. RHD patients may experience valve stenosis and/or regurgitation, leading to valve insufficiency [2]. Generally, for patients with severe valve lesions, double valve replacement surgery (DVRS) is required to improve cardiovascular hemodynamics, achieve a better quality of life, and reduce mortality [3]. However, RHD patients who undergo cardiopulmonary bypass (CPB) surgery often experience abnormal physiological perfusion, thrombosis, and local kidney ischemia or hypoxic injury, which may cause ischemia-reperfusion (I/R) injury to the heart.

A previous review article described that, during cardiac surgery, repeated exposure of the myocardium to a volatile anesthetic agent protects the myocardium against I/R injury [4]. In addition, studies have shown that volatile anesthetics may exert cardioprotective effects, resulting in decreased morbidity and mortality, increased cardiac index (CI), and decreased postoperative elevation(s) in cardiac troponin I (cTnI) levels [5, 6]. Furthermore, because oxidative stress and inflammatory reactions contribute to I/R injury, volatile anesthetic (sevoflurane)-

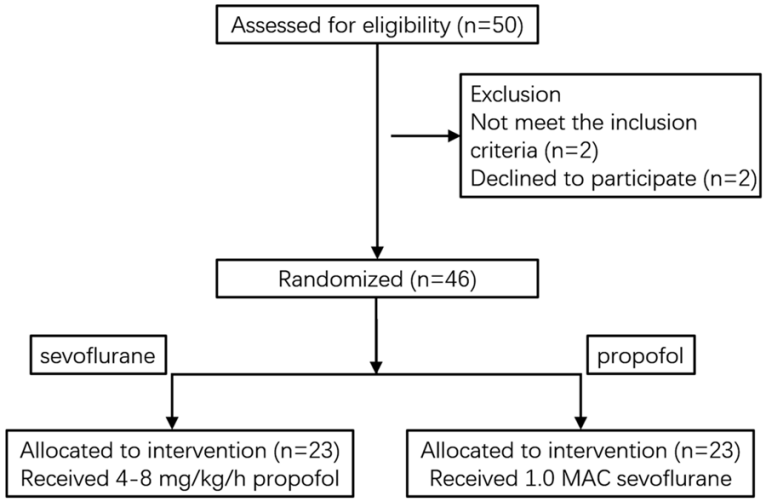

Figure 1. Flow diagram of the present study.

induced preconditioning has been reported to exert inhibitory effects on inflammatory responses during coronary artery bypass graft surgery [7, 8]. A recent study reported that pre-CPB administration of dexmedetomidine can reduce the levels of cTnI and inflammatory cytokines after CPB in valve replacement surgery with sevoflurane postconditioning [9]. Although numerous animal and human studies have explored potential mechanisms and pathways underlying the cardioprotective role of sevoflurane, these mechanisms have only been partly elucidated.

Heat Shock Protein70 (HSP70), as a stress protein, leads to the refolding or degradation of denatured proteins that result from stress including I/R injury.

It was reported that HSP70 levels increased in cardiomyocytes in response to ischemia, and that was connected with cardioprotective effects. In addition, clinical studies of HSP70 have demonstrated elevated HSP70 concentrations in peripheral blood lymphocytes in groups of patients with ischemic stroke and myocardial infarction [10]. Moreover, recent animal experiments also found that sevoflurane postconditioning reduces I/R injury in cardiomyocytes via upregulation of HSP70 [11, 12]. However, there is no clinical research performed to validate the role of HSP70 during the cardio-protective effects of sevoflurane. Consequently, the present study aimed to investigate whether pretreatment of sevoflurane confers cardio-protection against future I/R damage during CPB via induction of HSP70.

Materials and methods

Study population

This single-center randomized controlled study was approved by the Ethics Committee of Shaoxing People's Hospital (Zhejiang, China), and informed written consent was obtained from each patient. From January 2017 to June 2021, data from 50 consecutive patients with RHD, who underwent DVRS under CPB at the Shaoxing People's Hospital were retrieved and analyzed. Patients > 50 years of age and underwent coronary arteriography to rule out coronary heart disease were included. Patients with severe arrhythmia(s), those who underwent previous coronary or valvular cardiac surgery, experienced acute myocardial infarction within the previous 4 weeks, those who exhibited preoperative left ventricular ejection fraction (LVEF) < 40%, and those with severe systemic diseases involving the renal and hepatic systems and respiratory disease were excluded. Ultimately, 46 patients who fulfilled the inclusion criteria were randomly assigned to receive either propofol or sevoflurane anesthesia (**Figure 1**). Data regarding clinical characteristics, preoperative medication(s), and American Society of Anesthesiologists (ASA) class were also collected.

Anesthesia and surgery

Anesthesia was induced using midazolam (0.05-0.1 mg/kg), sufentanil (1 μg/kg), etomidate (0.2 mg/kg), and cisatracurium (0.3 mg/kg) in both groups. Anesthesia in the propofol group was maintained using intravenously administered propofol (2-4 mg/kg/h). In parallel, patients in the sevoflurane group received sevoflurane at 1.0 minimum alveolar concentration. The oxygen flow rate was 5 L/min and depressed to 2 L/min after reaching a predefined concentration of propofol or sevoflurane. In addition, 1 μg/kg/h of sufentanil and 0.15 mg/kg/h of cisatracurium were continuously infused during anesthesia. Body-position

changes, vasoactive drugs (e.g., noradrenaline), epinephrine, and nitroglycerin were administered to maintain mean arterial pressure (MAP) between 70 and 100 mmHg. CPB was established after an activated coagulation time > 480 s. Routine cardioprotective strategies (e.g., 2 g methylprednisolone) were used for each subject. Surgery was performed under standard hypothermic CPB (28-30°C), and all procedures were performed by the same group of anesthesiologists. In the recovery room, indexes of this entity, including respiration time, time to extubation, and time to eye opening (either spontaneous or response to verbal commands) were measured. A modified Aldrete score, including variables of activity, respiration, consciousness, hemodynamic stability, and oxygen saturation, for a total score ranging from 0 to 10 [13], and the time elapsed to reach an Aldrete score of 9 or 10 were assessed. A visual analog scale (VAS) [14] was also administered.

Hemodynamic testing

Hemodynamic variables were registered at six specified time points: before the start of surgery (T0), before the start of CPB (T1), immediately at the end of CPB (T2), immediately after transfer to the intensive care unit (ICU) (T3), and 6 h (T4) and 12 h (T5) after admission to the ICU. Measurements included MAP, heart rate (HR), central venous pressure (CVP), cardiac index (CI), and stroke volume index (SVI).

Measurement of serum myocardial enzyme activity

Before anesthesia, immediately after surgery, and 24 h, 48 h, and 72 h after surgery, arterial blood samples were collected into heparinized and plain tubes. After centrifugation at 3000 rpm for 10 min at 4°C, serum samples were aliquoted into polypropylene tubes and stored at -80°C until further analysis. Serum cTnI levels were measured using a commercially available chemiluminescent immunoassay to detect human cTnI (ADVIA Centaur CP System, Siemens, Tokyo, Japan). The measurement range was 0.006-50.0 ng/ml. Serum CK-MB levels were measured using the double-antibody sandwich ELISA method, in accordance with manufacturer's instructions. The sensitivity of the CK-MB assay was 5 ng/ml.

Determination of inflammatory cytokine levels

Serum inflammatory cytokine levels of interleukin (IL)-6, IL-8, tumor necrosis factor-α (TNF-α), and heat shock protein-70 (HSP70) at the indicated time were measured using commercially available ELISA kits (all obtained from Multi Science, Shanghai, China) in accordance with the manufacturer's instructions. Antioxidant enzyme activity was tested using a commercially available kit (Total Superoxide Dismutase Assay Kit, Beyotime, Jiangsu, China).

Statistical analysis

Data were analyzed using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA). The Kolmogorov-Smirnov test was used to determine whether the continuous variables were normally distributed. The normal distributed data were presented as medians ± standard deviation (SD) and the differences were analyzed using Student's t test between two groups. One-way analysis of variance (ANOVA) was used to compare the difference in the changes of CK-MB, cTnI, HSP70, and serum inflammatory cytokines concentrations (IL-6, IL-8, TNF-α and SOD) before and after surgery in sevoflurane group and propofol group. Non-normally distributed variables were analyzed using the Mann-Whitney U-test for post hoc pairwise comparisons. Categorical variables are expressed as frequencies and analyzed using the χ^2 or Fisher's exact test. Pearson's correlation coefficient was calculated to describe the correlation(s) between HSP70 and cTnI (or changes in inflammatory cytokine levels). Differences with a two-tailed $P < 0.05$ were considered to be statistically significant.

Results

Study population and preoperative characteristics

Forty-six patients were included in the present study; the mean age of the sevoflurane and propofol groups was 54.5 ± 9.7 and 55.9 ± 7.8 years, respectively. The baseline clinical characteristics of all patients and preoperative medication(s) are summarized in **Table 1**. There were no significant differences between the sevoflurane and propofol groups in terms of preoperative data, including concomitant dis-

Table 1. Baseline clinical characteristics of the study groups

Clinical Characteristics	Sevoflurane (n = 23)	Propofol (n = 23)	*P* value
Age (years)	54.5 ± 9.7	55.9 ± 7.8	0.592
Female (n, %)	17 (73.9)	18 (78.2)	1.000
BMI (kg/m^2)	25.8 ± 3.2	26.1 ± 3.1	0.748
Alcohol (n, %)	4 (18.2)	4 (12.9)	1.000
Hypertension (n, %)	11 (47.8)	9 (39.1)	0.766
Diabetes mellitus (n, %)	9 (39.1)	6 (26.1)	0.529
atrial fibrillation (n, %)	10 (43.5)	12 (52.2)	0.768
ASA (II/III)	10/13	12/11	0.768
LVEF (%)	52.1 ± 7.4	53.4 ± 6.6	0.533
Preoperative medication			
beta-blocker (n, %)	7	12	0.231
CCB (n, %)	11	6	0.222
ACEI (n, %)	9	7	0.757
diuretic (n, %)	11	15	0.372

Note: BMI = body mass index; ASA = American association of anesthesiologists; LVEF = left ventricular ejection fraction; CCB = calcium channel blocker; ACEI = angiotensin converting enzyme inhibitors.

ease, ASA classification, cardiac function (e.g., LVEF), and preoperative drug treatment.

Effects of sevoflurane on hemodynamic variables

To investigate the cardiovascular protective effects of sevoflurane in patients undergoing DVRS, hemodynamic variables during surgery and ICU stay were recorded. These included MAP, HR, CVP, CI, and SVI at 6 time points (i.e., T1 to T5), which are summarized in **Table 2**. Results revealed that MAP, CI, and SVI were significantly higher in the sevoflurane group than those in the propofol group at T2 and T3, but demonstrated no difference at the other time points. However, no statistical differences were observed between the groups when comparing the HR and CVP between the two groups at the six specified time points (i.e., T0 to T5).

Effects of sevoflurane on recovery time and postoperative complications

Regarding recovery time after DVRS, data reported in **Table 3** demonstrated that there were no statistically significant differences in respiration time, time to eye-opening, and time to achieve Aldrete 9 or 10 scores between the sevoflurane and propofol groups. There was also no significant difference between VAS scores at 24 h and 48 h after surgery between the two groups. Subsequently, comparison of postoperative characteristics revealed no statistical differences in operative duration, CPB and aortic cross-clamp time, auto-resuscitation HR, amount of drainage within 48 h after surgery, and time to extubation between the two groups. It is noteworthy that dopamine and noradrenaline usages in the sevoflurane group were significantly lower than those in the propofol group. Moreover, ICU monitoring time in the sevoflurane group was also shorter than that in the propofol group (**Table 4**).

Effects of sevoflurane on serum myocardial injury-associated markers

As shown in **Figure 2A-C**, no significant differences were observed in serum CK-MB, cTnI, and HSP70 levels before surgery between the two groups. However, compared with baseline levels, serum levels of CK-MB and cTnI after surgery were markedly increased in the propofol group. For the sevoflurane group, the levels of CK-MB and cTnI at 0 h, 24 h, and 48 h were significantly lower than those in the propofol group. Moreover, expression of HSP70 was significantly increased in both the propofol and sevoflurane groups at 0 h after surgery compared with baseline. HSP70 expression peaked at 24 h after surgery in the sevoflurane group. However, postoperative serum concentrations of HSP70 at 24 h and 48 h were significantly higher in the sevoflurane group than those in the propofol group (**Figure 2D**). The relationship between HSP70 and cTnI after surgery (0 h and 24 h) was analyzed in both the sevoflurane and propofol groups. As illustrated in **Figure 3A** and **3B**, HSP70 levels in the propofol group correlated with cTnI levels at 0 h and 24 h after surgery ($r = 0.839$, $P < 0.001$; $r = 0.553$, $P = 0.009$, respectively). Unexpectedly, no correlation was observed between HSP70 and cTnI in the sevoflurane group, both at 0 h and 24 h after surgery ($r = -0.089$, $P = 0.686$; $r = -0.327$, $P = 0.116$, respectively).

Effects of sevoflurane on serum inflammatory cytokine levels

Alterations in inflammatory cytokine levels among patients undergoing DVRS in the two groups were measured. Results revealed that

Table 2. Hemodynamic status of the two groups at each time point (mean ± SD)

Clinical Characteristics	Sevoflurane (n = 23)	Propofol (n = 23)	*P* value
MAP (mmHg)			
T0	79.8 ± 3.0	80.1 ± 3.1	0.735
T1	75.5 ± 3.3	76.9 ± 2.7	0.119
T2	76.4 ± 2.9	72.6 ± 3.4	< 0.001*
T3	73.9 ± 3.0	70.7 ± 2.6	< 0.001*
T4	74.2 ± 2.8	72.5 ± 3.7	0.086
T5	77.6 ± 5.1	76.4 ± 2.9	0.332
HR (bpm)			
T0	86.0 ± 14.0	90.0 ± 12.0	0.298
T1	82.0 ± 7.0	84.0 ± 9.0	0.391
T2	76.0 ± 8.0	79.0 ± 7.0	0.183
T3	79.0 ± 9.0	83.0 ± 10.0	0.161
T4	83.0 ± 12.0	88.0 ± 11.0	0.148
T5	80.0 ± 9.0	83.0 ± 11.0	0.304
CVP (mmHg)			
T0	9.7 ± 1.2	10.1 ± 0.8	0.189
T1	10.1 ± 0.9	10.5 ± 0.7	0.113
T2	9.5 ± 1.1	9.1 ± 1.4	0.274
T3	8.8 ± 0.9	8.6 ± 2.0	0.649
T4	9.6 ± 1.0	9.3 ± 0.7	0.243
T5	10.1 ± 1.3	9.8 ± 1.2	0.277
CI (L/min/m^2)			
T0	3.3 ± 0.5	3.1 ± 0.6	0.221
T1	2.8 ± 0.4	2.7 ± 0.3	0.339
T2	2.9 ± 0.7	2.5 ± 0.3	0.015*
T3	3.3 ± 0.4	2.9 ± 0.5	0.004*
T4	3.4 ± 0.5	3.2 ± 0.3	0.107
T5	3.7 ± 0.3	3.5 ± 0.9	0.291
SVI (ml/beat/m^2)			
T0	30.9 ± 4.3	31.6 ± 3.7	0.552
T1	27.5 ± 3.5	28.5 ± 2.6	0.274
T2	28.6 ± 2.4	26.2 ± 1.7	0.002*
T3	29.3 ± 3.2	25.3 ± 2.7	< 0.001*
T4	29.8 ± 2.5	27.9 ± 2.8	0.065
T5	31.5 ± 3.7	30.9 ± 4.3	0.606

Note: MAP = mean arterial pressure; HR = heart rate; CVP = central venous pressure; CI = cardiac index; SVI = stroke volume index. T0 = before the start of surgery; T1 = before the start of CPB; T2 = immediately at the end of CPB; T3 = immediately after installation in the ICU; T4 = 6 h after installation in the ICU; T5 = 12 h after installation in the ICU. *P < 0.05 compared with Sevoflurane group obtained with Student t-test.

IL-6, IL-8, and TNF-α levels were dramatically increased after DVRS. Notably, IL-6, IL-8, and TNF-α levels in the sevoflurane group were lower than those in the propofol group at 48 and 72 h after surgery (**Figure 4A-C**). In contrast, decreased SOD activity was observed in both the propofol and sevoflurane groups after surgery. Compared with the propofol group, SOD activity was markedly increased in the sevoflurane group (**Figure 4D**). Furthermore, the relationship between HSP70 and the inflammatory cytokine level was analyzed. As shown in **Table 5**, HSP70 levels were positively correlated with IL-6 (r = 0.498, P < 0.001), IL-8 (r = 0.356, P = 0.029), and TNF-α (r = 0.417, P = 0.002) levels in the sevoflurane group at 0 h after surgery. However, these relationships between HSP70 and inflammatory cytokines (i.e., IL-6, IL-8, and TNF-α) disappeared in the sevoflurane group 24 h after surgery (P > 0.05). No significant relationship was observed between HSP70 and SOD in the sevoflurane group at 0 h (r = 0.159, P = 0.340) and 24 h (r = 0.233, P = 0.179) after surgery. In the propofol group, HSP70 levels were positively correlated with IL-6 (r = 0.469, P < 0.001), IL-8 (r = 0.379, P = 0.015), and TNF-α (r = 0.490, P < 0.001) levels in the sevoflurane group at 0 h after surgery. The relationships between HSP70 and inflammatory cytokines (IL-6, IL-8, and TNF-α) in the propofol group 24 h after surgery persisted (P < 0.05). HSP70 levels were negatively correlated with SOD at 0 h (r = -0.514, P < 0.001) and 24 h (r = -0.318, P = 0.035) after surgery in the propofol group.

Discussion

This study highlighted the cardioprotective effects of sevoflurane on the inflammatory response in RHD patients undergoing DVRS. DVRS requires prolonged aortic cross-clamping and CPB for suture placement for valve fixation [15]. Despite significant advances in surgical techniques, cardiomyocytes may still incur injury from the anesthetics, aortic cross-clamping, I/R, operative wounds, and even myocardial remodeling after valve implantation [16, 17]. Myocardial I/R injury is a common pathophysiological process that occurs during CPB and involves multiple mechanisms, including cardiomyocyte apoptosis, oxidative stress, and inflammatory reactions [18-20]. Recently, volatile anesthet-

Table 3. Comparation of recovery times of patients with rheumatic heart disease undergoing double valve replacement (mean ± SD)

Clinical Characteristics	Sevoflurane (n = 23)	Propofol (n = 23)	*P* value
Respiration time, h	14.1 ± 1.2	13.6 ± 1.3	0.182
Eye-opening time, h	13.6 ± 1.4	13.0 ± 1.5	0.168
Time to Aldrete 9 or 10, h	17.4 ± 2.6	18.1 ± 1.9	0.303
VAS score 24 h	4.4 ± 0.6	4.7 ± 0.5	0.072
VAS score 48 h	1.8 ± 0.4	2.0 ± 0.4	0.097

Note: VAS = Visual analogue scale.

Table 4. Characteristics of postoperative patients with rheumatic heart disease undergoing double valve replacement surgery

Clinical Characteristics	Sevoflurane (n = 23)	Propofol (n = 23)	*P* value
Operation time (min)	249.5 ± 25.4	251.3 ± 23.5	0.801
CBP time (min)	84.7 ± 10.6	86.2 ± 11.4	0.876
Aortic cross-clamp time (min)	53.7 ± 6.8	51.5 ± 6.0	0.425
Epinephrine usage (ng/kg/min)	31.3 ± 7.5	43.1 ± 6.2	0.015*
Noradrenaline usage (ng/kg/min)	28.6 ± 7.6	34.1 ± 6.5	0.010*
Auto-resuscitation heart rate (n, %)	24 (92.3%)	17 (77.3%)	0.223
Drainage within 48 h (mL/kg)	4.7 ± 1.7	4.4 ± 1.5	0.523
Extubation time (h)	5.7 ± 1.0	6.2 ± 0.9	0.103
Use of IABP	0	0	NA
ICU stay (days)	2.3 ± 0.8	3.5 ± 0.7	0.005*
In-hospital death	0	0	NA

Note: CBP=cardiopulmonary bypass; IABP = intra-aortic ballon pump; ICU= intensive care unit. *P < 0.05 compared with sevoflurane group obtained with Student t-test.

ics, such as sevoflurane and desflurane, have been shown to protect the heart from I/R injury in various clinical studies [21]. Due to its wide use in cardiac surgery under CPB, sevoflurane was effective in reducing postoperative cardiac injury [22]. Nevertheless, the cardioprotective effects of sevoflurane on DVRS remain unclear.

Volatile anesthetics tend to be more effective in maintaining hemodynamic stability during cardiac surgery than intravenous anesthesia [23]. A previous study demonstrated that, compared with propofol, sevoflurane could significantly increase CI, but did not influence the CVP or MAP during coronary artery bypass grafting off-pump [24, 25]. However, for patients who underwent DVRS under CPB, we found that MAP, CI, and SVI all remained higher in the sevoflurane group than those in the propofol group, and more stable hemodynamic parameters were observed in the sevoflurane group than in the propofol group. Studies have demonstrated that propofol induces a reduction in MAP, and the inhibition of sympathetic nerve activity may be a major mechanism of hemodynamic depression [14]. The maintenance of MAP, CI, and SVI indicated that sevoflurane had little influence on sympathetic nerve activity during DVRS. Accordingly, we demonstrated that the use of inotropic agents, such as dopamine and noradrenaline, was significantly lower when using sevoflurane anesthesia compared with propofol. Given our results, it may be concluded that the maintenance of anesthesia using sevoflurane in patients undergoing DVRS resulted in more stable hemodynamics than that with propofol. Currently, propofol is frequently used as an intravenous anesthetic due to its rapid recovery profile [26], whereas the most notable side effect of propofol is its depression of cardiovascular and respiratory parameters, particularly for patients with a high ASA classification (i.e., III/IV) [27]. Herein, we demonstrated that sevoflurane was not inferior to propofol in terms of recovery time after surgery. Similar to previous studies [28, 29], the levels of CK-MB and cTnI were significantly elevated after surgery, and sevoflurane anesthesia demonstrated less postoperative increase in serum levels of the above markers of cardiac injury than propofol. Collectively, these data suggest that sevoflurane may be more suitable than propofol for DVRS under CPB.

To date, a series of potential mechanisms, including NF-κB activation [30], PI3K/Akt signaling [31], and activation of the HIF-1/PDK-1 pathway [32], have been reported to be implicated in sevoflurane-mediated protection against I/R injury in cells or in animal models. Nevertheless, clinical evidence supporting these findings remains lacking, and it is difficult to translate experimental results to clinical settings. A recent study by Duan et al. reported

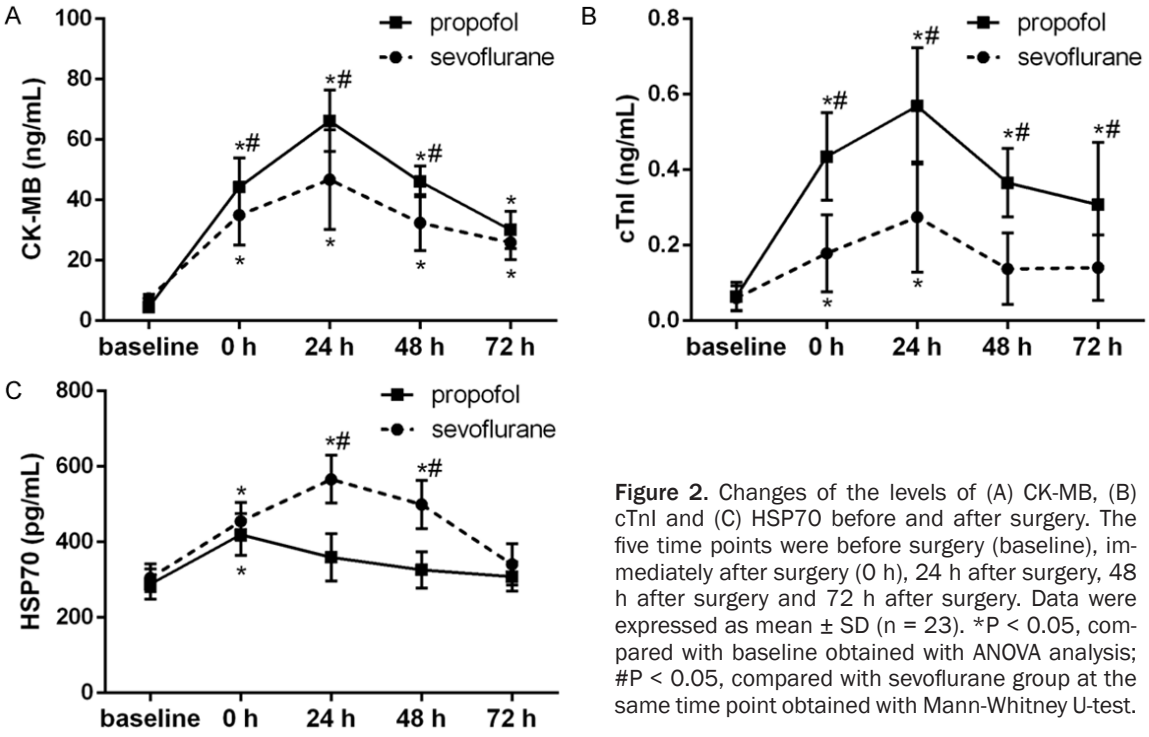

Figure 2. Changes of the levels of (A) CK-MB, (B) cTnI and (C) HSP70 before and after surgery. The five time points were before surgery (baseline), immediately after surgery (0 h), 24 h after surgery, 48 h after surgery and 72 h after surgery. Data were expressed as mean ± SD (n = 23). *P < 0.05, compared with baseline obtained with ANOVA analysis; #P < 0.05, compared with sevoflurane group at the same time point obtained with Mann-Whitney U-test.

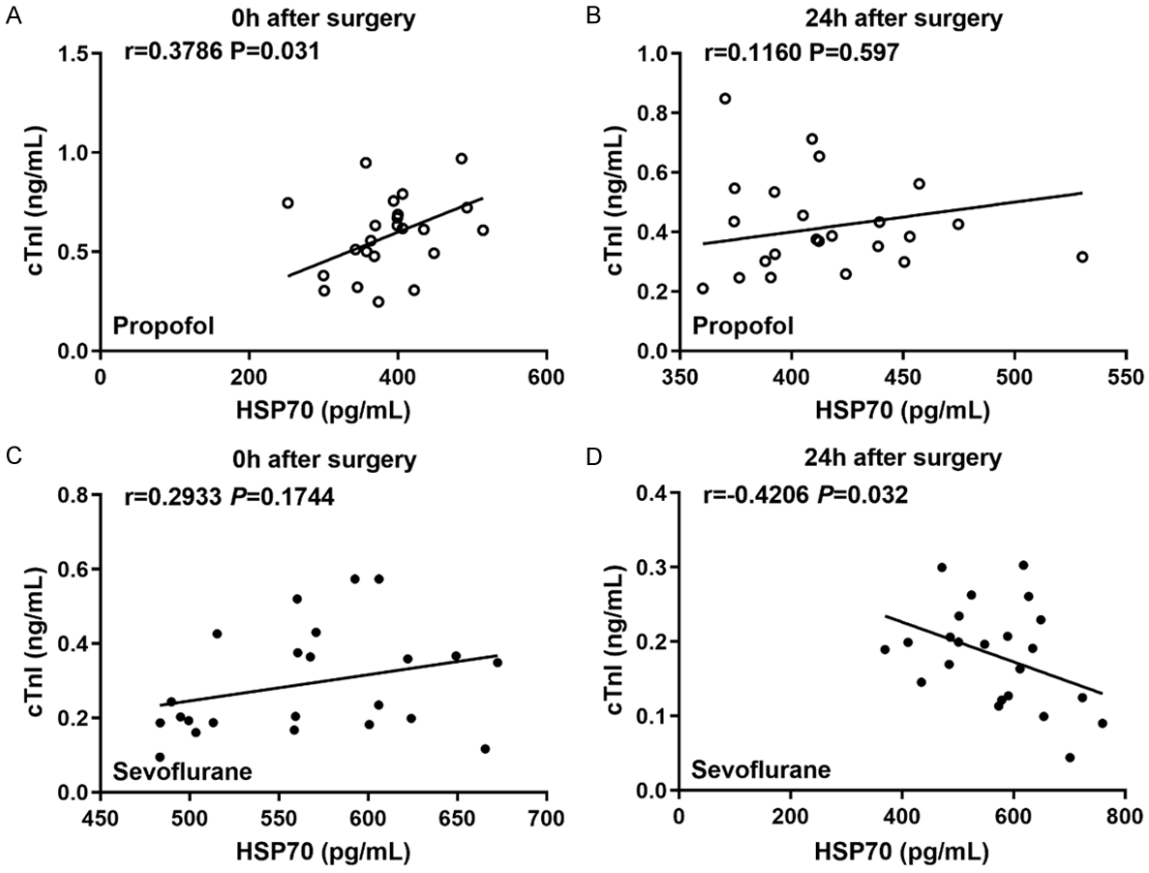

Figure 3. Pearson correlation analysis was performed to reveal the correlation between the serum levels of heat shock protein 70 (HSP70) and cardiac troponin I (cTnI) at 0 h and 24 h after double valve replacement surgery (n = 23).

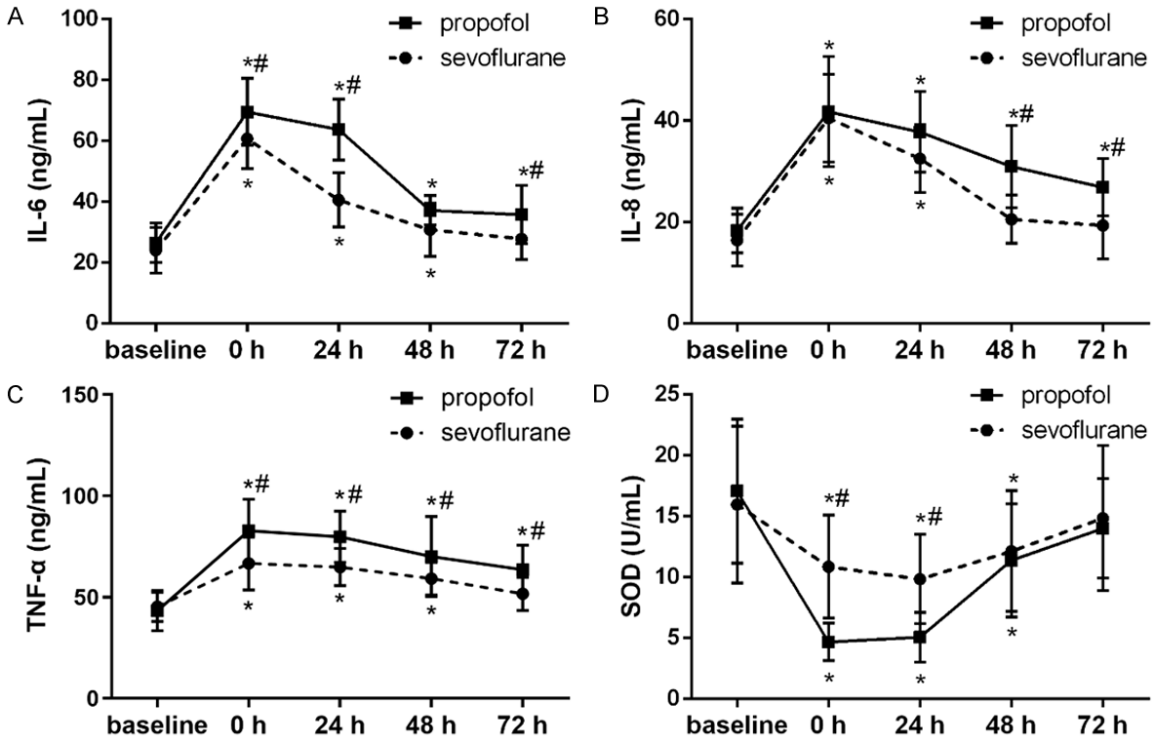

Figure 4. Changes of the levels of (A) IL-6, (B) IL-8, (C) TNF-α and (D) the activity of SOD before and after surgery. The five time points were before surgery (baseline), immediately after surgery (0 h), 24 h after surgery, 48 h after surgery and 72 h after surgery. Data were expressed as mean ± SD (n = 23). *P<0.05, compared with baseline obtained with ANOVA analysis; #P < 0.05, compared with sevoflurane group at the same time point obtained with Mann-Whitney U-test.

Table 5. Results of correlation analysis performed between serum HSP70 and changes of inflammatory cytokines

inflammatory cytokines	Sevoflurane (n = 23)				Propofol (n = 23)			
	0 h		24 h		0 h		24 h	
	r	*P*	r	*P*	r	*P*	r	*P*
IL-6 (ng/ml)	0.498	< 0.001	0.292	0.077	0.469	< 0.001	0.390	0.008
IL-8 (ng/ml)	0.356	0.029	0.291	0.081	0.379	0.015	0.313	0.041
TNF-α (ng/ml)	0.417	0.002	0.320	0.044	0.490	< 0.001	0.447	< 0.001
SOD (U/ml)	0.159	0.340	0.233	0.179	-0.514	< 0.001	-0.318	0.035

Note: IL-6 = interleukin 6; IL-8 = interleukin 8; TNF-α = tumor necrosis factor α; SOD = superoxide dismutase; HSP70 = heat shock protein 70.

that the levels of THAP11 in atrial tissues were positively correlated with cTn-I at 24 h after surgery, and that downregulation of THAP11 may be implicated in the myocardial protective effects of sevoflurane anesthesia [33]. In the current study, we measured the serum levels of HSP70, which serves as a molecular chaperone to refold denatured proteins and promote the degradation of damaged proteins. During acute myocardial I/R, levels of HSP70 are upregulated [34] and enforced overexpression of HSP70 reduces myocardial apoptosis [35]. HSP 70 has been reported to be involved in self-preservation system of the myocardium [36]. The cardioprotective role of HSP70 against myocardial I/R injury has been demonstrated using transgenic animal and gene transfection models [35, 37]. Results of our study revealed that HSP70 levels exhibited a comparable postoperative increase in both the propofol and sevoflurane groups,

whereas the HSP70 levels remained elevated in the sevoflurane group until 48 h after surgery. A positive correlation between HSP70 and cTnI was observed in the propofol group, indicating its potential as a marker of cardiac injury [38]. However, the correlation disappeared in the sevoflurane group; as such, we hypothesized that this may be due to the HSP70 alteration induced by sevoflurane anesthesia.

Cardiac surgery involving CPB disturbs the balance between pro- and anti-inflammatory responses, which can lead to cardiac dysfunction [39]. In this context, increasing attention has been devoted to the influence of anesthetics on inflammatory responses. We found that, compared with propofol, sevoflurane significantly inhibited the inflammatory response, as evidenced by the reduced serum levels of inflammatory cytokines, including IL-6, IL-8, and TNF-α, which are the most important inflammatory cytokines released from injured cardiomyocytes and inflammatory cells after I/R [40, 41]. In the current study, we found that HSP70 levels were positively correlated with the abovementioned inflammatory cytokines in both the propofol and sevoflurane groups at 0 h after surgery. However, these relationships between HSP70 and inflammatory cytokines were abrogated in the sevoflurane group 24 h after surgery. We hypothesized that sevoflurane could increase the expression of HSP70, thereby suppressing inflammation. Due to the anti-inflammatory and cardioprotective properties of HSP70 during cardiac surgery [42], we speculate that the increased HSP70 expression induced by sevoflurane anesthesia may be a mechanism through which sevoflurane regulates the inflammatory response during cardiac surgery.

Upon revascularization and restoration of the myocardial blood flow, oxidative stress may lead to cardiac injury [43]. Herein, we compared the extent of oxidative stress between the groups by measuring antioxidant enzyme activity. SOD is a vital free radical scavenger and an important antioxidant defense mechanism in myocardial cells exposed to oxidative stress [44]. We found that SOD activity in the blood after DVRS was significantly lower in the propofol group and higher in the sevoflurane group. An increase in SOD activity may be one of several mechanisms underlying the protective effects of sevoflurane in DVRS under CPB. We also found that HSP70 was negatively correlated with SOD in the propofol group, whereas it exhibited no relationship with SOD in the sevoflurane group, which may also be due to increased HSP70 by intervention with sevoflurane. Collectively, these results suggest that sevoflurane attenuates CPB-mediated myocardial I/R injury via anti-inflammatory and antioxidative stress properties, which may be mediated by the upregulation of HSP70 expression.

There were limitations to our study that should be addressed, the first of which was its single-center design and small sample size. As such, this study may not have been adequately powered for the measurements performed; thus, further studies are required to confirm our conclusions. Second, we only analyzed the relationship between HSP70 and myocardial injury markers or inflammatory cytokine in the serum sample from patients under CPB. A recent study by Lotz et al. found that sevoflurane as opposed to propofol anesthesia preserved mitochondrial respiration and elicited cardiac protection against I/R-injury [45]. Activation of HSP70 is able to regulate mitochondrial function and cell apoptosis in cardiomyocytes, in vitro [46-48]. These results all supported our hypothesis that sevoflurane exerted its cardioprotective effects via activation of HSP70 during DVRS. Last but not least, due to the limited number of blood samples, we determined the levels of only some crucial mediators. Additional research, therefore, is required to provide further insights into the mechanisms underlying the cardioprotective effects of sevoflurane.

In conclusion, the results of the present study demonstrated that patients who underwent sevoflurane anesthesia during DVRS under CPB exhibited marked improvement in hemodynamic parameters, exhibited less need for inotropic support, experienced a shorter length of ICU stay, and lower postoperative increase in the levels of myocardial injury-associated markers. The underlying molecular mechanisms of sevoflurane-induced protective effects may contribute to the regulation of inflammatory reactions through the increased HSP70 expression.

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Disclosure of conflict of interest

None.

Abbreviations

RHD, Rheumatic heart disease; DVRS, double valve replacement surgery; CPB, cardiopulmonary bypass; I/R, ischemia-reperfusion; cTnI, cardiac troponin I; HSP, heat shock protein; LVEF, left ventricular ejection fraction; ASA, American Society of Anesthesiologists.

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References

[1] Watkins DA, Johnson CO, Colquhoun SM, Karthikeyan G, Beaton A, Bukhman G, Forouzanfar MH, Longenecker CT, Mayosi BM, Mensah GA, Nascimento BR, Ribeiro ALP, Sable CA, Steer AC, Naghavi M, Mokdad AH, Murray CJL, Vos T, Carapetis JR and Roth GA. Global, regional, and national burden of rheumatic heart disease, 1990-2015. N Engl J Med 2017; 377: 713-722.

[2] Fernandes AM, Andrade GM, Oliveira RM, Biscaia GT, Reis FF, Macedo CR, Duraes AR and Aras Junior R. Evaluation of variables responsible for hospital mortality in patients with rheumatic heart disease undergoing double valve replacement. Rev Bras Cir Cardiovasc 2014; 29: 537-542.

[3] Okuyama H, Hashimoto K, Kurosawa H, Tanaka K, Sakamoto Y and Shiratori K. Midterm results of Manouguian double valve replacement: comparison with standard double valve replacement. J Thorac Cardiovasc Surg 2005; 129: 869-874.

[4] De Hert SG. Volatile anesthetics and cardiac function. Semin Cardiothorac Vasc Anesth 2006; 10: 33-42.

[5] Landoni G, Biondi-Zoccai GG, Zangrillo A, Bignami E, D'Avolio S, Marchetti C, Calabro MG, Fochi O, Guarracino F, Tritapepe L, De Hert S and Torri G. Desflurane and sevoflurane in cardiac surgery: a meta-analysis of randomized clinical trials. J Cardiothorac Vasc Anesth 2007; 21: 502-511.

[6] Yu CH and Beattie WS. The effects of volatile anesthetics on cardiac ischemic complications and mortality in CABG: a meta-analysis. Can J Anaesth 2006; 53: 906-918.

[7] Nederlof R, Weber NC, Juffermans NP, de Mol BA, Hollmann MW, Preckel B and Zuurbier CJ. A randomized trial of remote ischemic preconditioning and control treatment for cardioprotection in sevoflurane-anesthetized CABG patients. BMC Anesthesiol 2017; 17: 51.

[8] Wang C, Xie H, Liu X, Qin Q, Wu X, Liu H and Liu C. Role of nuclear factor-kappaB in volatile anaesthetic preconditioning with sevoflurane during myocardial ischaemia/reperfusion. Eur J Anaesthesiol 2010; 27: 747-756.

[9] Zhou H, Zhou D, Lu J, Wu C and Zhu Z. Effects of pre-cardiopulmonary bypass administration of dexmedetomidine on cardiac injuries and the inflammatory response in valve replacement surgery with a sevoflurane postconditioning protocol: a pilot study. J Cardiovasc Pharmacol 2019; 74: 91-97.

[10] Konstantinova EV, Chipigina NS, Shurdumova MH, Kovalenko EI and Sapozhnikov AM. Heat shock protein 70 kDa as a target for diagnostics and therapy of cardiovascular and cerebrovascular diseases. Curr Pharm Des 2019; 25: 710-714.

[11] Zhang J, Wang H and Sun X. Sevoflurane Postconditioning reduces hypoxia/reoxygenation injury in cardiomyocytes via upregulation of heat shock protein 70. J Microbiol Biotechnol 2021; 31: 1069-1078.

[12] Yu F, Tong LJ and Cai DS. Sevoflurane inhibits neuronal apoptosis and expressions of HIF-1 and HSP70 in brain tissues of rats with cerebral ischemia/reperfusion injury. Eur Rev Med Pharmacol Sci 2020; 24: 5082-5090.

[13] Aldrete JA. The post-anesthesia recovery score revisited. J Clin Anesth 1995; 7: 89-91.

[14] Tang L, Liu H, Wu Y, Li M, Li W, Jiang M, Hou J, Jiang Y, Xia Z and Meng Q. Sevoflurane may be more beneficial than propofol in patients receiving endoscopic variceal ligation and endoscopic variceal sclerotherapy: a randomized, double-blind study. Exp Ther Med 2017; 14: 3145-3152.

[15] Davarpasand T, Hosseinsabet A and Jalali A. Concomitant coronary artery bypass graft and aortic and mitral valve replacement for rheumatic heart disease: short- and mid-term outcomes. Interact Cardiovasc Thorac Surg 2015; 21: 322-328.

[16] Oliver WC Jr, Nuttall GA, Orszulak TA, Bamlet WR, Abel MD, Ereth MH and Schaff HV. Hemofiltration but not steroids results in earlier tracheal extubation following cardiopulmo-

nary bypass: a prospective, randomized double-blind trial. Anesthesiology 2004; 101: 327-339.
[17] Meco M, Montisci A, Miceli A, Panisi P, Donatelli F, Cirri S, Ferrarini M, Lio A and Glauber M. Sutureless perceval aortic valve versus conventional stented bioprostheses: meta-analysis of postoperative and midterm results in isolated aortic valve replacement. J Am Heart Assoc 2018; 7: e006091.
[18] Xiong W, Qu Y, Chen H and Qian J. Insight into long noncoding RNA-miRNA-mRNA axes in myocardial ischemia-reperfusion injury: the implications for mechanism and therapy. Epigenomics 2019; 11: 1733-1748.
[19] Chen W, Wang Y, Pan Z, Chen X, Luo D and Wang H. Protective effects of dexmedetomidine on the ischemic myocardium in patients undergoing rheumatic heart valve replacement surgery. Exp Ther Med 2021; 21: 427-427.
[20] Li M, Xu S, Geng Y, Sun L, Wang R, Yan Y, Wang H, Li Y, Yi Q, Zhang Y, Hao J, Deng C, Li W and Xue L. The protective effects of L-carnitine on myocardial ischaemia-reperfusion injury in patients with rheumatic valvular heart disease undergoing CPB surgery are associated with the suppression of NF-κB pathway and the activation of Nrf2 pathway. Clin Exp Pharmacol Physiol 2019; 46: 1001-1012.
[21] Landoni G, Fochi O, Tritapepe L, Guarracino F, Belloni I, Bignami E and Zangrillo A. Cardiac protection by volatile anesthetics. A review. Minerva Anestesiol 2009; 75: 269-273.
[22] Guinot PG, Ellouze O, Grosjean S, Berthoud V, Constandache T, Radhouani M, Anciaux JB, Aho-Glele S, Morgant MC, Girard C, Nguyen M and Bouhemad B. Anaesthesia and ICU sedation with sevoflurane do not reduce myocardial injury in patients undergoing cardiac surgery: a randomized prospective study. Medicine (Baltimore) 2020; 99: e23253.
[23] El Dib R, Guimaraes Pereira JE, Agarwal A, Gomaa H, Ayala AP, Botan AG, Braz LG, de Oliveira LD, Lopes LC and Mathew PJ. Inhalation versus intravenous anaesthesia for adults undergoing on-pump or off-pump coronary artery bypass grafting: a systematic review and meta-analysis of randomized controlled trials. J Clin Anesth 2017; 40: 127-138.
[24] Bein B, Renner J, Caliebe D, Scholz J, Paris A, Fraund S, Zaehle W and Tonner PH. Sevoflurane but not propofol preserves myocardial function during minimally invasive direct coronary artery bypass surgery. Anesth Analg 2005; 100: 610-616.
[25] Huseidzinovic I, Barisin S, Bradic N and Milanovic R. Early cardioprotective effect of sevoflurane on left ventricular performance during coronary artery bypass grafting on a beating heart: randomized controlled study. Croat Med J 2007; 48: 333-340.
[26] Feng AY, Kaye AD, Kaye RJ, Belani K and Urman RD. Novel propofol derivatives and implications for anesthesia practice. J Anaesthesiol Clin Pharmacol 2017; 33: 9-15.
[27] Elgebaly AS and Sabry M. Sedation effects by dexmedetomidine versus propofol in decreasing duration of mechanical ventilation after open heart surgery. Ann Card Anaesth 2018; 21: 235-242.
[28] Amr YM and Yassin IM. Cardiac protection during on-pump coronary artery bypass grafting: ischemic versus isoflurane preconditioning. Semin Cardiothorac Vasc Anesth 2010; 14: 205-211.
[29] Guerrero Orriach JL, Galan Ortega M, Ramirez Aliaga M, Iglesias P, Rubio Navarro M and Cruz Manas J. Prolonged sevoflurane administration in the off-pump coronary artery bypass graft surgery: beneficial effects. J Crit Care 2013; 28: 879, e813-878.
[30] Qiao S, Xie H, Wang C, Wu X, Liu H and Liu C. Delayed anesthetic preconditioning protects against myocardial infarction via activation of nuclear factor-κB and upregulation of autophagy. J Anesth 2013; 27: 251-260.
[31] Liu C, Shen Z, Liu Y, Peng J, Miao L, Zeng W and Li Y. Sevoflurane protects against intestinal ischemia-reperfusion injury partly by phosphatidylinositol 3 kinases/Akt pathway in rats. Surgery 2015; 157: 924-933.
[32] Hou T, Ma H, Wang H, Chen C, Ye J, Ahmed AM and Zheng H. Sevoflurane preconditioning attenuates hypoxia/reoxygenation injury of H9c2 cardiomyocytes by activation of the HIF-1/PDK-1 pathway. PeerJ 2020; 8: e10603.
[33] Duan Z, Zhou X, Chen F, Chen H, Duan G and Li H. THAP11 down-regulation may contribute to cardio-protective effects of sevoflurane anesthesia: evidence from clinical and molecular evidence. Life Sci 2021; 274: 119327.
[34] Rahsepar AA, Mirzaee A, Moodi F, Moohebati M, Tavallaie S, Khorashadizadeh F, Eshraghi A, Alavi MS, Zarrabi L, Sajjadian M, Amini M, Khojasteh R, Paydar R, Mousavi S, Ghayour-Mobarhan M and Ferns GA. Changes in anti-heat shock protein 27 antibody and C-reactive protein levels following cardiac surgery and their association with cardiac function in patients with cardiovascular disease. Cell Stress Chaperones 2013; 18: 65-74.
[35] Suzuki K, Sawa Y, Kagisaki K, Taketani S, Ichikawa H, Kaneda Y and Matsuda H. Reduction in myocardial apoptosis associated with overexpression of heat shock protein 70. Basic Res Cardiol 2000; 95: 397-403.

[36] Sharma M, Ganguly NK, Chaturvedi G, Thingnam SK, Majumdar S and Suri RK. A possible role of HSP70 in mediating cardioprotection in patients undergoing CABG. Mol Cell Biochem 2003; 247: 31-36.
[37] Das DK, Engelman RM and Kimura Y. Molecular adaptation of cellular defences following preconditioning of the heart by repeated ischaemia. Cardiovasc Res 1993; 27: 578-584.
[38] Vicencio JM, Yellon DM, Sivaraman V, Das D, Boi-Doku C, Arjun S, Zheng Y, Riquelme JA, Kearney J, Sharma V, Multhoff G, Hall AR and Davidson SM. Plasma exosomes protect the myocardium from ischemia-reperfusion injury. J Am Coll Cardiol 2015; 65: 1525-1536.
[39] Suzuki H, Sato R, Sato T, Shoji M, Iso Y, Kondo T, Shibata M, Koba S and Katagiri T. Time-course of changes in the levels of interleukin 6 in acutely decompensated heart failure. Int J Cardiol 2005; 100: 415-420.
[40] Zhang M and Chen L. Status of cytokines in ischemia reperfusion induced heart injury. Cardiovasc Hematol Disord Drug Targets 2008; 8: 161-172.
[41] Hu L, Cai N and Jia H. Pterostilbene attenuates myocardial ischemia-reperfusion injury via the phosphatidylinositol 3'-kinase-protein kinase B signaling pathway. Exp Ther Med 2017; 14: 5509-5514.
[42] Hong Y, Wang Z, Rao Z, Wan J, Ling X and Zheng Q. Changes in expressions of HSP27, HSP70, and soluble glycoprotein in heart failure rats complicated with pulmonary edema and correlations with cardiopulmonary functions. Biomed Res Int 2021; 2021: 6733341.
[43] Topcu AC, Bolukcu A, Ozeren K, Kavasoglu T and Kayacioglu I. Normoxic management of cardiopulmonary bypass reduces myocardial oxidative stress in adult patients undergoing coronary artery bypass graft surgery. Perfusion 2021; 36: 261-268.
[44] Escobales N and Crespo MJ. Oxidative-nitrosative stress in hypertension. Curr Vasc Pharmacol 2005; 3: 231-246.
[45] Lotz C, Stumpner J and Smul TM. Sevoflurane as opposed to propofol anesthesia preserves mitochondrial function and alleviates myocardial ischemia/reperfusion injury. Biomed Pharmacother 2020; 129: 110417.
[46] Zhang S, Liu W, Wang P, Hu B, Lv X, Chen S, Wang B and Shao Z. Activation of HSP70 impedes tert-butyl hydroperoxide (t-BHP)-induced apoptosis and senescence of human nucleus pulposus stem cells via inhibiting the JNK/c-Jun pathway. Mol Cell Biochem 2021; 476: 1979-1994.
[47] Boran T, Akyildiz AG, Jannuzzi AT and Alpertunga B. Extended regorafenib treatment can be linked with mitochondrial damage leading to cardiotoxicity. Toxicol Lett 2021; 336: 39-49.
[48] Han Y, Cai Y, Lai X, Wang Z, Wei S, Tan K, Xu M and Xie H. lncRNA RMRP prevents mitochondrial dysfunction and cardiomyocyte apoptosis via the mir-1-5p/hsp70 axis in lps-induced sepsis mice. Inflammation 2020; 43: 605-618.