Synergy of isoflurane preconditioning and propofol postconditioning reduces myocardial reperfusion injury in patients

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ABSTRACT

Either isoflurane preconditioning or high-dose propofol treatment has been shown to attenuate myocardial IRI (ischaemia/reperfusion injury) in patients undergoing CABG (coronary artery bypass graft) surgery. It is unknown whether isoflurane and propofol may synergistically attenuate myocardial injury in patients. The present study investigated the efficacy of IsoPC (isoflurane preconditioning), propofol treatment (postconditioning) and their synergy in attenuating postischaemic myocardial injury in patients undergoing CABG surgery using CPB (cardiopulmonary bypass). Patients (n = 120) selected for CABG surgery were randomly assigned to one of four groups (n = 30 each). After induction, anaesthesia was maintained either with fentanyl and midazolam (control; group C); with propofol at 100 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ before and during CPB followed by propofol at 60 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ for 15 min after aortic declamping (group P); with isoflurane 1–1.5 % end tidal throughout the surgery (group I) or with isoflurane 1–1.5 % end tidal before CPB and switching to propofol at 100 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ during CPB followed by propofol at 60 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ for 15 min after aortic declamping (group IP, i.e. IsoPC plus propofol postconditioning). A joint isoflurane and propofol anaesthesia regimen synergistically reduced plasma levels of cTnI (cardiac troponin I) and CK-MB (creatine kinase MB) and f-FABP (heart-type fatty acid-binding protein) (all P < 0.05 compared with control, group P or group I) and facilitated postoperative myocardial functional recovery. During reperfusion, myocardial tissue eNOS (endothelial NO synthase) protein expression in group IP was significantly higher, whereas nitrotyrosine protein expression was lower than those in the control group. In conclusion, a joint isoflurane preconditioning and propofol anaesthesia regimen synergistically attenuated myocardial reperfusion injury in patients.

Key words: coronary artery bypass graft surgery, isoflurane, myocardial reperfusion injury, postconditioning, preconditioning.

Abbreviations: ACC, aortic cross-clamping; AUC, area under curve; CABG, coronary artery bypass graft; CI, cardiac index; CK-MB, creatine kinase-MB; CPB, cardiopulmonary bypass; CVP, central venous pressure; cTnI, cardiac troponin I; eNOS, endothelial NO synthase; h-FABP, heart-type fatty acid-binding protein; HR, heart rate; ICU, intensive care unit; IL-6, interleukin-6; iNOS, inducible NO synthase; IRI, ischaemia/reperfusion injury; IsoPC, isoflurane preconditioning; MAC, minimum alveolar anesthetic concentration; MAP, mean arterial blood pressure; MDA, malondialdehyde; PI3K, phosphoinositide 3-kinase; PCWP, pulmonary capillary wedge pressure; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumour necrosis factor-α.

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INTRODUCTION

Despite advancement in surgical and anaesthetic techniques, the risk of morbidity and mortality in CABG (coronary artery bypass graft) surgery remains significant, especially since patients are often those with increased age and co-morbidities. Volatile anaesthetic preconditioning, in general, and IsoPC (isoflurane preconditioning) specifically, can provide moderate [1] to minor [2] cardiac protection during CABG. However, there may be limitations to its use given that its duration of action is limited (25–40 min [3]) compared with the typical period of global ischaemia (longer than 60 min) seen during CABG. In addition, aging [4] and diabetes [5] can attenuate IsoPC protection, largely because endogenous superoxide production (a mechanism whereby volatile anaesthetic preconditioning confers its protection [6]) is already increased in aged and/or diabetic patients.

During CABG surgery using CPB (cardiopulmonary bypass), ROS (reactive oxygen species) production is increased [7]. Increased systemic oxidative stress may adversely affect postoperative myocardial functional recovery by increasing lipid peroxidation [8]. The pro-inflammatory cytokine TNF-α (tumour necrosis factor-α) is also increased after CPB [9], which can further increase ROS production. TNF-α plays an important role in the inflammatory processes, which may induce and exacerbate cardiac dysfunction. Experimentally, volatile anaesthetic preconditioning was shown to reduce NF-κB (nuclear factor κB)-dependent inflammatory gene expression and decrease TNF-α production and subsequently attenuate myocardial IRI (ischaemia/reperfusion injury) [10]. On the other hand, the intravenous anaesthetic propofol confers an antioxidant effect [11]. Therefore it is plausible that volatile anaesthetic preconditioning in combination with propofol treatment may synergistically attenuate myocardial IRI in clinical settings. Propofol reduced ROS-induced lipid peroxidation and attenuated IRI in isolated rat hearts [12–16], in a dose-dependent manner [13,16]. We have shown that propofol can prevent hydrogen peroxide-mediated exacerbation of TNF-α cellular toxicity [17], which might be a mechanism whereby propofol attenuates hydrogen peroxide-induced cardiac dysfunction [18]. Recent experimental studies show that propofol postconditioning confers protection against myocardial [19] as well as cerebral [20] IRI, probably through maintaining the activity of the prosurvival PI3K (phosphoinositide 3-kinase)/Akt pathway [20,21]. In addition, experimentally, propofol has been shown to be cardioprotective when used at a relatively high dose solely during ischaemia [16] or when applied in a clinically relevant model of normothermic blood cardioplegic arrest and CPB [22].

We recently demonstrated that high-dose propofol during CPB attenuates postoperative myocardial cellular damage compared with isoflurane or low-dose propofol during CABG [23], whereas the cardioprotective effects of isoflurane did not differ from that of a low-dose propofol regimen. Similarly, more recent clinical trial results show that the use of isoflurane-opioid anaesthesia in comparison with propofol-opioid anaesthesia does not afford additional benefit in terms of postoperative troponin-I release as well as 1-year cardiac morbidity and mortality [24]. However, it is unknown whether or not isoflurane and propofol may have synergy in reducing postischaemic myocardial injury in patients. We hypothesized that synergy of IsoPC and propofol postconditioning may confer superior protection against myocardial IRI in patients compared with an isoflurane or propofol anaesthesia regimen alone and that the mechanism of the synergy is related to promoting or maintaining postischaemic myocardial levels of eNOS (endothelial NO synthase), a critical downstream mediator of the prosurvival PI3K/Akt pathway [25] that plays a key role in isoflurane mediated pre- and postconditioning cardioprotection [26,27].

Part of this work was presented at the American Society of Anesthesiologists Annual Meeting, held in New Orleans on 17–21 October, 2009 and subsequently published in an abstract form [27a].

MATERIALS AND METHODS

Patient population and study design

The clinical trial was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The study protocol was approved by the institutional ethics committee. All subjects gave written informed consent after having been given a full explanation of the purpose, nature and risk of all procedures used.

After obtaining written informed consent, 120 ASA (American Association of Anesthesiologists) class II to III patients, aged 53–76 years, presenting for scheduled primary elective CABG were assigned according to a computer-generated random code to one of four groups: a control group receiving midazolam and fentanyl (group C; n = 30); propofol alone (group P; n = 30); isoflurane alone (group I; n = 30); and a combination of isoflurane and propofol (group IP; n = 30). Subjects were assigned treatment numbers in ascending chronological order of admission in the study. The surgeons, research assistants and medical and nursing staff in the operation room were blinded to the group assignments, facilitated by covering the drug infusion pump and lines and shielding the isoflurane vaporizer from view.

The exclusion criteria were (i) emergency revascularization for unstable angina, (ii) previous coronary or valvular heart surgery, (iii) combined operations (simultaneous valve repair, carotid endarterectomy or left ventricular...
Anaesthesia protocol used in experimental groups

<table>
<thead>
<tr>
<th>Group C</th>
<th>F + Midazolam</th>
<th>F + Midazolam</th>
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<td>F + P 60 μg/kg/min</td>
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<td>Group I</td>
<td>F + Isof 1–1.5 MAC</td>
<td>F + Isof 1–1.5 MAC</td>
<td>F + Isof 1–1.5 MAC (via oxygenator)</td>
</tr>
<tr>
<td>Group IP</td>
<td>F + Isof 1–1.5 MAC</td>
<td>F + P 100 μg/kg/min</td>
<td>F + P 100 μg/kg/min First 15 min</td>
</tr>
</tbody>
</table>

Figure 1  Anaesthesia protocol used in experimental groups

After induction, anaesthesia was maintained either with fentanyl (F) and midazolam (group C); with propofol (P) at 100 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ before and during CPB followed by propofol at 60 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ 15 min after aortic declamping (group P); with isoflurane (Iso) 1–1.5 MAC end-tidal throughout the surgery (group I); or with isoflurane 1–1.5 MAC end-tidal before CPB and switching to propofol at 100 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ during CPB followed by propofol 60 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ 15 min after aortic declamping (group IP). CPB–ACC indicates the onset of CPB to the whole period of ACC. Reperfusion starts at the time of aortic declamping.

Anaesthetic protocols and surgery

All patients received standard premedication of scopalamine at 0.006 mg/kg of body weight and morphine at 0.1 mg/kg of body weight intramuscularly 60 min before surgery. In all groups, anaesthesia was induced with etomidate at 0.3 mg/kg of body weight, fentanyl at 8 μg/kg of body weight and pancuronium bromide at 0.1 mg/kg of body weight given intravenously. After induction, all patients received continuous infusions of fentanyl at 0.6 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ and pancuronium bromide at 15 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ during surgery. The anaesthesia protocol in various groups is illustrated in Figure 1. In brief, anaesthesia was maintained either with fentanyl and midazolam (group C); with propofol at 100 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ before and during CPB followed by propofol at 60 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ for 15 min after aortic declamping (group P); with isoflurane 1–1.5 MAC (minimum alveolar anesthetic concentration) end-tidal throughout the surgery (group I) or with isoflurane 1–1.5 MAC end-tidal before CPB and switched to propofol at 100 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ during CPB followed by propofol 60 μg · kg⁻¹ · min⁻¹ of body weight · min⁻¹ 15 min after aortic declamping (group IP). During anaesthesia, patients were monitored with five-lead ECG, pulse oximetry, capnography, invasive arterial pressure and pulmonary artery pressure during the operation.

Systemic arterial blood pressure was measured via radial artery catheterization. A Swan–Ganz catheter (Edwards Life Sciences) was inserted for central venous pressure, pulmonary artery pressures and cardiac output determinations. Haemodynamics was continuously monitored for 24 h after CPB.

Porcine heparin was administrated (300 units/kg of body weight) and supplemented when needed to maintain an activated coagulation time above 450 s during CPB. Heparin was neutralized with 1 mg of protamine/100 units of heparin administrated after separation from CPB.

Surgery was conducted under conditions of normothermic or tepid CPB (patient oesophageal temperature of 34–37°C) utilizing a non-pulsatile flow rate of 2 l · min⁻¹ · m⁻² and a membrane oxygenator. MAP (mean arterial blood pressure) was maintained between 60 and 75 mmHg during CPB. All patients were treated with intermittent antegrade infusion of warm high-potassium blood cardioplegia during continuous aortic cross-clamping, and the content of the cardioplegic solution was the same for all patients.

The ECG was recorded with a standard 12-lead electrocardiography preoperatively and then daily postoperatively. The ECG diagnosis criteria for perioperative acute myocardial ischaemia were 1 or more of the following: new Q waves of more than 0.04 s in territories other than those identified before surgery; significant ST
segment deviation (elevation at the J point in two or more contiguous leads with cut off point no less than 0.2 mV in men or no less than 0.15 mV in women in leads V2 or V3, and no less than 0.1 mV in other leads with prominent R wave or R/S >1). Postoperative myocardial infarction was considered when new pathological Q wave was accompanied with elevated biomarkers [cTnI (cardiac troponin I) and CK-MB (creatine kinase-MB)] as described in the current expert consensus document regarding the universal definition of myocardial infarction [28].

Postoperative inotropic support was defined as the use of dopamine (≥5 μg·kg⁻¹·min⁻¹) with or without adrenaline (0.1 μg·kg⁻¹·min⁻¹) for a duration of 30 min or longer during the first 12 h postoperatively. Inotropic therapy was standardized to a treatment algorithm to treat a mean arterial pressure less than 60 mmHg despite optimization of preload, afterload and HR (heart rate). Nitroglycerin (0.3–0.5 μg·kg⁻¹·min⁻¹) was administrated intravenously when the values of CVP (central venous pressure) or PCWP (pulmonary capillary wedge pressure) were more than 12 mmHg and levels of pro-inflammatory cytokines TNF-α, IL-6 (interleukin-6). Samples were immediately cooled to 4 °C and centrifuged at 1000 g for 10 min at 4 °C. Plasma was collected and stored at −70 °C until analysed.

**Sample collection**

Central venous blood samples were obtained prior to CPB (pre-CPB), 5 min, 1 h, 4 h, 12 h and 24 h after aortic declamping for the measurements of plasma levels of cTnI and CK-MB (enzyme immunoassay), h-FABP (heart-type fatty acid-binding protein), the lipid peroxidation product MDA (malondialdehyde), the antioxidant enzyme SOD (superoxide dismutase) activity and levels of pro-inflammatory cytokines TNF-α and IL-6 (interleukin-6). Samples were immediately cooled to 4 °C and centrifuged at 1000 g for 10 min at 4 °C. Plasma was collected and stored at −70 °C until analysed.

Samples of right atrial tissue were harvested from 33 patients (n = 8, 7, 9 and 9, respectively, in groups C, P, I and IP) who gave consent to this specific procedure. Samples were harvested with cold sharp dissection and handled in a non-traumatic fashion. Double 3-0 polypropylene purse-string sutures were placed in the atrial appendage. During placement of the venous cannula, the first sample of atrial appendage was harvested (pre-CPB). The superior suture was tightened to secure the venous cannula. The inferior suture remained loose to allow this portion of the atrium to be perfused with blood, exposed to CPB and blood cardioplegia, and reperfused after removal of the aortic cross-clamp. The second sample of atrial appendage (post-CPB) was harvested after CPB during removal of the venous cannula. Part of the tissue samples were frozen and stored at −70 °C until analysed for protein content by Western blotting, and part of the tissue was paraffin-embedded and blocked according to standard procedures to be used for immunohistochemical analysis.

**Nitrotyrosine histological immunostaining and assessment of eNOS and iNOS (inducible NO synthase) by immunostaining and Western blotting**

Atrial tissue was processed with standard methods for immunohistochemical and Western blot analysis as described [29]. In brief, paraffin-embedded atrial tissue blocks were sectioned at 5 μm. The sections were deparaffinized, rehydrated, treated with target retrieval buffer (S1699; Dako), blocked with 3 % hydrogen peroxide, washed with PBS and blocked with 5 % normal goat serum in PBS for 30 min. The slides were then incubated with primary antibodies in PBS containing 1 % normal goat serum overnight at 4 °C.

eNOS and iNOS were stained with monoclonal mouse anti-eNOS and mouse anti-iNOS antibodies (1:300–400 dilutions; Santa Cruz Biotechnology). Secondary antibody used was goat anti-mouse antibody (1:400 dilution; Dako). After incubation with secondary antibody, the sections were dipped in lithium carbonate (to blue for 90 s), washed in running water, dehydrated in increasing grades of alcohol and cleared in xylene before being mounted in resinous mounting medium with Permount (Fischer) coverslips. Some sections were incubated with non-specific mouse IgGs and served as negative controls.

For nitrotyrosine staining, a monoclonal mouse anti-nitrotyrosine antibody (1:400 dilution; Calbiochem) was used. Intensity of tissue nitrotyrosine staining was quantified by an observer blinded to the experimental conditions. A series of three randomly chosen images of equal surface of nitrotyrosine-stained atrial tissue were delineated with an operator-controlled cursor. The sections were analysed with an image analyser (IBAS-2000; Kontron) as described [30]. The density of nitrotyrosine protein expression was expressed in arbitrary units.

In addition, atrial tissue eNOS and iNOS protein contents were quantified by standard Western blotting as described previously [29], with the same antibodies (Santa Cruz Biotechnology) as used for immunostaining. The same blots were stripped and rebotted with antibody to β-actin as internal controls. The images were analysed by an imaging densitometer and quantified by normalizing them with that from β-actin.
Biochemical analysis

h-FABP, a marker of perioperative myocardial damage, has been shown to peak earlier than CK-MB and cTnI during coronary bypass surgery [31]. Plasma h-FABP was measured using ELISA kits (Hycult Biotechnology), and CK-MB and cTnI were measured by using a photometric method (Diasys Diagnostic System) and ELISA (Beckman) respectively.

Plasma TNF-α and IL-6 were measured using ELISA kits (R&D Systems). Plasma SOD activity was determined according to the method described by Sun et al. [32]. Plasma levels of MDA were measured by chemical analysis (commercial kits; Nanjing Jiangzheng Biological Engine Institute) as described previously [33].

Plasma samples used for biochemical assays were coded, and the laboratory investigator was blinded in regard to treatment regimen. All samples were assayed in duplicate. Similarly, all haemodynamic data were collected by trained observers who did not take an authorship in this study and who were blinded to the anaesthetic regimen used.

Sample size estimation

Group sample size was estimated based on differences in cTn I concentration measured at 4 h post-CPB in a pilot study of patients who received propofol (5.21 ± 0.95 ng/ml) and midazolam (4.50 ± 0.82 ng/ml) anaesthesia. The formula [34]: \( n = 15.7/ES^2 + 1 \), where \( ES = \text{effect size} = \text{(difference between groups)/(mean of the S.D. between groups)} \), with \( \alpha = 0.05 \) and power = 0.8, was used to determine that the study would be adequately powered with \( n = 26 \) per group.

Statistical analysis

All continuous data are expressed as means ± S.D. Statistical evaluation of patients’ file and perioperative data was performed by unpaired Student’s t test or \( \chi^2 \) test when appropriate. Between-groups and within-group differences of bioassay data were analysed using two-way ANOVA with repeated measures and Bonferroni’s corrections (GraphPad Prism). Values were considered statistically significant when \( P < 0.05 \).

RESULTS

Preoperative and intraoperative data

Patient characteristics, preoperative haemodynamic data, preoperative medication, dosage of fentanyl used, duration of CPB and ACC (aortic cross-clamping) were similar between groups (Tables 1 and 2). Surgery was completed in all patients. In one patient (male, 69 years, weight 73 kg, from group C), a severe right coronary artery endarterectomy was performed in addition to conventional CABG. This patient suffered from acute perioperative myocardial infarction and died of severe right heart failure on day 2 after surgery. This patient’s data were excluded from statistical analysis.
Table 2  Intra-operative and postoperative procedure characteristics of the patients

Values are means ± S.D. or the number of patients. P values represent the difference among the groups. *Significant difference between the control group and the IP group; CPB, cardiopulmonary bypass; MI, myocardial infarction; NTG, nitroglycerin; SNP, sodium nitroprusside; IABP, intra-aortic balloon pump; NS, not significant.

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<th>C (n = 29)</th>
<th>P (n = 30)</th>
<th>I (n = 30)</th>
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<td>CPB time (min)</td>
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<td>123.6 ± 40.5</td>
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<td>Rectal temperature (°C)</td>
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<td>5</td>
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Postoperative data

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<th>Inotropic drug requirement (n)</th>
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<th>Mild (dopamine ≤ 5 µg·kg⁻¹·min⁻¹ or body weight - 1 of body weight - min⁻¹)</th>
<th>Moderate (dopamine or dobutamine ≤ 5 µg·kg⁻¹·min⁻¹ or adrenaline ≤ 0.1 µg·kg⁻¹·min⁻¹)</th>
<th>Requirement for IABP (n)</th>
<th>ST segment deviation (n)</th>
<th>New pathological Q waves (n)</th>
<th>Duration of ventilation (h)</th>
<th>ICU stay (h)</th>
<th>Hospital stay (day)</th>
<th>Death in hospital (n)</th>
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Postoperative outcome data

Clinical outcome parameters are presented (Tables 2 and 3). On day 1, fewer patients in group IP (n = 4) had signs of myocardial ischaemia evidenced as ST segment deviation and/or new pathological Q waves compared with group C (n = 12), group P (n = 7) and group I (n = 8) (P < 0.05). Significantly fewer patients in the IP group required postoperative inotropic support compared with all other groups.

MAP, HR, CVP and PCWP did not differ among groups throughout the observation interval (Table 3) except that all the values were transiently lower than the corresponding baseline values at the first 5 min of reperfusion. No significant changes of systemic vascular resistance index were observed throughout the observation interval. CI (cardiac index) was significantly increased 1 h after reperfusion and onward in groups P, I and IP compared with baseline or pre-CPB values. By contrast, significant improvement in CI did not occur in group C until after 12 h of reperfusion. During early reperfusion [1 and 4 h post-ACC; Table 3], the values of CI in group IP, but not in groups P or I, were significantly higher than that in group C. Durations of postoperative ventilation and ICU stay in group IP, but not in groups P or I, were significantly shorter than those in group C. All patients (except the one excluded as mentioned above) were discharged from hospital uneventfully. The duration of hospital stay in group IP tended to be shorter than that in group C, but overall, the difference among groups did not reach statistical significance (P = 0.058; Table 2).

Postischaemic myocardial cellular injury and eNOS and nitrotyrosine expression

Baseline and pre-CPB plasma levels of h-FABP, cTnI and CK-MB did not differ among groups, but all significantly increased upon reperfusion at 5 min post-ACC (P < 0.01 compared with baseline, Figure 2). Plasma levels of h-FABP (Figure 2A), cTnI (Figure 2C) and CK-MB (Figure 2B) peaked at 1, 4 and 12 h post-ACC respectively in all groups. At 1 h post-ACC, h-FABP concentration in group IP, but not in groups P or I, was significantly lower than that in group C. The h-FABP level in group IP was also significantly lower than that in groups P and I respectively at 1 h post-ACC. Similarly, at 4 h post-ACC, plasma cTnI in group IP was lower than those in all other groups. The change of CK-MB largely
Table 3  Perioperative haemodynamic data of the patients
Values are means ± S.D., SVRI, systemic vascular resistances index. n = 29 in group C, n = 30 in other groups. *P < 0.05 or P < 0.01 compared with baseline; ^P < 0.05 compared with group C.

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<tr>
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<th>5 min</th>
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<td>MAP (mmHg)</td>
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<td></td>
<td>P</td>
<td>75.2 ± 9.7</td>
<td>77.3 ± 10.2</td>
<td>52.5 ± 11.6 *</td>
<td>68.6 ± 11.9</td>
<td>74.5 ± 10.8</td>
<td>73.6 ± 16.9</td>
<td>76.5 ± 12.3</td>
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<td></td>
<td>I</td>
<td>76.6 ± 11.3</td>
<td>79.4 ± 12.7</td>
<td>56.2 ± 8.4 *</td>
<td>77.2 ± 11.6</td>
<td>76.7 ± 17.6</td>
<td>75.3 ± 13.5</td>
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<td>54.7 ± 9.6 *</td>
<td>73.5 ± 10.7</td>
<td>78.2 ± 19.1</td>
<td>75.8 ± 14.6</td>
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<td>CVP (mmHg)</td>
<td>C</td>
<td>7.8 ± 1.2</td>
<td>8.0 ± 1.2</td>
<td>4.4 ± 0.7 *</td>
<td>6.9 ± 0.7</td>
<td>11.3 ± 3.1</td>
<td>10.7 ± 2.6</td>
<td>11.5 ± 3.6</td>
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<tr>
<td></td>
<td>P</td>
<td>8.1 ± 2.1</td>
<td>8.5 ± 1.8</td>
<td>5.5 ± 0.9 *</td>
<td>7.1 ± 1.1</td>
<td>10.5 ± 2.1</td>
<td>11.8 ± 3.8</td>
<td>12.0 ± 3.2</td>
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<tr>
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<td>8.2 ± 1.7</td>
<td>8.8 ± 1.2</td>
<td>4.8 ± 0.8 *</td>
<td>6.8 ± 0.9</td>
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<td>10.9 ± 3.4</td>
<td>10.7 ± 2.3</td>
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<tr>
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<td>IP</td>
<td>7.9 ± 1.9</td>
<td>8.6 ± 1.7</td>
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<td>7.0 ± 0.6</td>
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<td>PCWP (mmHg)</td>
<td>C</td>
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<tr>
<td></td>
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<td>7.9 ± 1.2</td>
<td>5.0 ± 0.2 *</td>
<td>6.8 ± 0.3</td>
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<td>8.1 ± 1.5</td>
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<td>6.9 ± 0.5</td>
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<td>8.2 ± 1.8</td>
<td>5.1 ± 0.8 *</td>
<td>6.7 ± 0.7</td>
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<td>CI (L·min⁻¹·m⁻²)</td>
<td>C</td>
<td>2.1 ± 0.5</td>
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<td>2.3 ± 0.5</td>
<td>2.3 ± 0.5</td>
<td>2.7 ± 0.7 *</td>
<td>2.5 ± 0.4</td>
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<tr>
<td></td>
<td>P</td>
<td>1.9 ± 0.3</td>
<td>2.1 ± 0.3</td>
<td>2.2 ± 0.3</td>
<td>2.5 ± 0.4 *</td>
<td>2.5 ± 0.4 *</td>
<td>2.8 ± 0.5 *</td>
<td>2.6 ± 0.5 *</td>
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<tr>
<td></td>
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<td>2.2 ± 0.4</td>
<td>2.1 ± 0.5</td>
<td>2.2 ± 0.5</td>
<td>2.4 ± 0.3 *</td>
<td>2.7 ± 0.3 *</td>
<td>2.8 ± 0.4 *</td>
<td>2.7 ± 0.2 *</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>1.9 ± 0.5</td>
<td>2.2 ± 0.4</td>
<td>2.3 ± 0.2</td>
<td>2.8 ± 0.3 *^A</td>
<td>3.1 ± 0.3 *^A</td>
<td>3.0 ± 0.3 *</td>
<td>2.8 ± 0.3 *</td>
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<tr>
<td>SVRI (dyn·s·cm⁻³·m⁻²)</td>
<td>C</td>
<td>2216 ± 472</td>
<td>1857 ± 274</td>
<td>1922 ± 302</td>
<td>2172 ± 226</td>
<td>2371 ± 319</td>
<td>1973 ± 227</td>
<td>1852 ± 218</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>2361 ± 436</td>
<td>2138 ± 263</td>
<td>1875 ± 318</td>
<td>2032 ± 232</td>
<td>1974 ± 318</td>
<td>2051 ± 287</td>
<td>1791 ± 317</td>
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<tr>
<td></td>
<td>IP</td>
<td>2672 ± 328</td>
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<td>2093 ± 274</td>
<td>2148 ± 186</td>
<td>1635 ± 206</td>
<td>1923 ± 281</td>
<td>1882 ± 263</td>
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</table>

Atrial tissue eNOS protein expression did not differ among groups pre-CPB (results not shown). After CPB, eNOS expression in blood vessels could hardly be seen in group C (Figure 3A), but it was visible in group I (Figure 3B) and group P (Figures 3C). Strongest eNOS expressions in vessels were seen in group IP (Figure 3D). Myocardial tissue eNOS expression could be seen in all groups, but it was weakest in group C and was strongest in group IP. The total myocardial eNOS protein content, as measured by Western blotting in group IP, was significantly higher than that in both groups C and P (Figure 3E). Myocardial eNOS protein content in groups I and P were relatively higher than that in group C, and the difference reached statistical significance in group I but not group P. Myocardial nitrotyrosine expression was mirrored that of cTnI during early reperfusion up to 12 h post-ACC. After 24 h post-ACC, plasma levels of h-FABP reduced to baseline values in all groups, whereas plasma cTnI and CK-MB levels remained significantly increased in groups C and P relative to their baseline values and were significantly higher than that in group IP. Plasma values of h-FABP in group P at 12 h post-ACC and values of cTnI in groups P and I as well as values of CK-MB in group I at 24 h post-ACC were respectively lower than that in group C, indicative of marginal cellular protective effects of the propofol or isoflurane anaesthesia regimen used in the current study. At 24 h post-ACC, values of cTnI and CK-MB in group IP were significantly lower than that in group P. The calculated values of AUC (area under curve) for plasma cTnI (Figure 2D) revealed that AUC values in group P (14.1 ± 0.7) and group I (13.7 ± 0.5) were all marginally but significantly lower than that in group C (15.7 ± 1.1) (P < 0.05 or P < 0.01). Of note, the AUC value in group IP (9.2 ± 0.5) was much lower than those in all the other groups (P < 0.001 compared with groups C, P or I). AUC values of cTnI did not differ significantly between groups P and I (P > 0.2). Similarly, AUC for CK-MB in group IP (229.1 ± 10.2) was much lower than those in group C (292.9 ± 20.2), group P (277.5 ± 18.9) and group I (265.7 ± 8.7) (all P < 0.01), while the differences in CK-MB among groups P, I and C were marginal (P > 0.05, group I compared with group C; P > 0.05 group P compared with C).
Figure 2  Perioperative variations of cardiac-specific biomarkers h-FABP (A), CK-MB (B) and cTnI (C) in plasma as well as calculated values of AUC of cTnI (D).

Note that plasma levels of h-FABP, cTnI and CK-MB peaked at 1, 4 and 12 h after reperfusion respectively. Values are means ± S.D., n = 29 in group C, n = 30 in other groups. *P < 0.05 or P < 0.01 compared with baseline; △P < 0.05 and △△P < 0.01 compared with group C; #P < 0.05 and ###P < 0.01 compared with group P; $P < 0.05 and $$P < 0.01 compared with group I.

marginally detectable before CPB but with no apparent difference among groups (results not shown). However, after CPB, protein expression of nitrotyrosine, an index of nitrative stress, was highest in group C (Figure 4A) and was relatively lower in group P (Figure 4B), group I (Figure 4C), and was further lower in group IP (Figures 4D and 4E). The myocardial protein expression of iNOS was not detectable both pre- and post-CPB, when measured by immunostaining or Western blotting.

Pro-inflammatory cytokines TNF, IL-6 and antioxidant enzyme SOD and MDA

Baseline levels of TNF-α, IL-6, SOD and MDA (Table 4) did not differ among groups. Plasma levels of TNF-α and IL-6 significantly increased immediately upon reperfusion at 5 min post-ACC in all groups, which preceded the significant decrease in SOD activity and increase in MDA content that occurred relatively later during reperfusion at 1 h post-ACC and onward. TNF-α levels in group IP were significantly lower than those in all other groups at 1, 4 and 12 h post-ACC (Table 4). At 24 h post-ACC, values of TNF-α in group IP but not in group C or groups P and I returned to baseline values. The combined isoflurane and propofol regimen also significantly reduced plasma levels of IL-6 at 4 and 12 h post-ACC compared with group C or groups P and I. Plasma MDA content in group IP was significantly lower than all other groups at 1 and 4 h post-ACC. During early reperfusion, plasma SOD activities in groups IP, P and I tended to be higher than that in the control group, but the difference did reach statistical significance.
DISCUSSION

The salient finding of the present clinical study is that the application of IsoPC in combination with propofol treatment and postconditioning during ischaemia and early reperfusion was superior to either an isoflurane or propofol anaesthesia regimen alone in reducing indices of oxidant stress, pro-inflammatory cytokines and myocardial injury. Synergy of isoflurane and propofol also reduced the need for inotrope support compared with propofol or isoflurane alone in the present study. The isoflurane or propofol anaesthesia regimen also appeared to be moderately cardioprotective in that we observed lower plasma levels of cTnI and/or CK-MB during late reperfusion in these groups relative to the control group. However, the marginal attenuation of postischaemic myocardial cellular injury conferred by isoflurane or propofol did not translate into immediate clinical and haemodynamic benefit. By contrast, IsoPC and propofol postconditioning significantly enhanced postischaemic cardiac index and shortened ICU stay, which was coincident with profound reductions in plasma levels of TNF-α and MDA content as well as reduction in myocardial levels of nitrotyrosine expression.

Our present finding that volatile anaesthetic pre-conditioning combined with intravenous anaesthetic
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Furthermore, additive benefit by combining volatile anaesthetic pre- and post-conditioning could be achieved in experimental settings when the anaesthetic was used at relatively lower concentrations [42]. This suggests that synergy of anaesthetic pre- and post-conditioning is achievable depending on how it is modulated.

The intravenous anaesthetic propofol has antioxidant properties [11], which may confer cardioprotection via mechanisms that differs from that of volatile anesthetics. Propofol is a scavenger of the potent oxidant ONOO− (peroxynitrite) (a product of superoxide anion and NO reaction) [43,44]. ONOO− at low levels is cardioprotective [45,46] and can serve as a trigger of ischemic preconditioning [47]. However, ONOO− is detrimental to the heart or cardiomyocytes when its production is increased during reperfusion [48–50]. Hypoxic postconditioning-mediated attenuation of cardiomyocyte death following reoxygenation has been shown to be attributable to reduction of ONOO− formation during reoxygenation [48]. Therefore the protection against myocardial [19] as well as cerebral [20] IRI conferred by propofol postconditioning might be attributable to propofol's ONOO−-scavenging property. In the present study, the preconditioning effects of isoflurane (which generates small amount of superoxide anion, NO and ONOO− before ischemia as early triggers that lead to reduced superoxide anion and ONOO− production during reperfusion) and the postconditioning effects of propofol (which scavenges superoxide anion and ONOO−) synergized to produce enhanced cardioprotection. Indeed, during early reperfusion, atrial tissue protein levels of nitrotyrosine,
a footprint of ONOO− production, were much lower in group IP relative to groups I or P. This was accompanied by a profound reduction in plasma levels of MDA, an end product of an oxidant-mediated increase in lipid peroxidation. Synergy of IsoPC and propofol postconditioning may have been achieved via the activation of the PI3K/AKT pathway, indirectly evidenced by the significantly elevated myocardial (atrial) levels of eNOS, a critical downstream mediator of the prosurvival PI3K/Akt pathway [25]. It should be noted, we measured MDA using the conventional spectrophotometric procedure based on absorbance of the thiobarbituric acid–MDA complex at 532 nm. This methodology is not highly specific to MDA and is prone to the interference of other non-lipid-related compounds. Therefore an HPLC-based assay of MDA [51] would be superior and should be applied in future related studies to confirm the finding the present study.

Myocardial necrosis can be recognized by the appearance in the blood of cardiac-specific proteins released into circulation due to cardiomyocyte damage. The biochemical markers cTnI and CK-MB have high, to nearly absolute, myocardial tissue specificity. The significantly lower levels of plasma troponin I and CK-MB seen in group IP are indicative of reduced myocardial cellular injury. In particular, the much significantly lower values of cTnI AUC value in group IP (~40% lower than that in group C) were in contrast with the marginal differences in group P and I (both were about 10% lower than that in group C), which indicates synergistic effects of the treatments in attenuating myocardial cellular damage. The h-FABP is a rapid marker of perioperative myocardial damage and peaks earlier than CK-MB or cTnI [31]. Furthermore, a previous study shows that h-FABP predicts long-term mortality after acute coronary syndrome and identifies high-risk patients across the range of troponin I values [52]. In our present study, plasma h-FABP peaked at the first hour during reperfusion and was significantly reduced by the IsoPC and propofol postconditioning regimen at this early stage of reperfusion. IsoPC and propofol postconditioning significantly reduced cTnI release at a relatively later phase of reperfusion. Our results suggest that h-FABP can serve as an early predictor of the anaesthetic cardioprotective effects during cardiac surgery.

Although there were significant intergroup differences in plasma levels of cTnI and h-FABP as well as in durations of ICU and hospital stay, the patients were not followed up after discharge. Further larger studies are merited to determine the long-term clinical relevance of the changes in biomedical markers observed in the related study. The paucity of the available atrial tissue sample did not allow us to pursue more detailed study regarding the activation status of eNOS, nor the protein levels of PI3K, Akt and nitrotyrosine to identify/confirm the signalling pathway of the protection. Nevertheless, the results from the present study are promising and provide clear clues for future in depth study.

In conclusion, we have shown that IsoPC, combined with propofol postconditioning, act synergistically in attenuating postischaemic myocardial reperfusion injury as determined by the surrogate markers of myocardial injury and function. Further large-scale and long-term studies are required to confirm the clinical benefit of this novel anaesthesia regimen, which may offer a promising therapeutic approach to cardioprotection patients undergoing cardiac surgery.

AUTHOR CONTRIBUTION

Zhengyuan Xia designed the research. Zhiyong Huang, Xingwu Zhong, Shangyi Ji, Gordon Wong, Zhong-yuan Xia and Zhengyuan Xia performed the research. Zhiyong Huang, Michael Irwin, Yanan Liu and Barry Finegan analysed the data, and Zhiyong Huang and Zhengyuan Xia wrote the paper.

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