Sevoflurane preconditions stunned myocardium in septic but not healthy isolated rat hearts


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Background. Recent evidence indicates that sevoflurane treatment before prolonged ischaemia reduces infarct size in normal hearts, mimicking ischaemic preconditioning. We examined whether exposure to sevoflurane before brief ischaemia, inducing a ‘stunned myocardium’, provided such protective effects in an isolated working heart from normal or septic rats.

Methods. With institutional approval, 91 rats were randomly allocated into one of either caecal-ligation and perforation (CLP: n = 50) or sham (Sham: n = 41) procedure groups 24 h before the study. After determination of baseline measurements, including cardiac output (CO), myocardial oxygen consumption (mVO₂) and cardiac efficiency (CE; CO×peak systolic pressure/mVO₂), each isolated heart was perfused with or without 2% sevoflurane for 15 min before global ischaemia (pre-ischaemia). After 15 min ischaemia and 30 min reperfusion, all hearts were assessed for functional recovery of myocardium (post-reperfusion).

Results. During the pre-ischaemia period, 2% sevoflurane caused a significant reduction of CO in the CLP group compared with the Sham group. During the post-reperfusion period, both CO (16.9 vs 11.0 ml min⁻¹) and CE (11.2 vs 7.7 mm Hg ml⁻¹ (μl O₂⁻¹) was higher in the sevoflurane-treated vs -untreated hearts from CLP rats, and was accompanied by lower incidence of reperfusion arrhythmia compared with control hearts (8 vs 32%). In contrast, 2% sevoflurane did not provide cardioprotective effects in normal rats.

Conclusions. The current study demonstrates that pre-treatment with sevoflurane minimizes myocardial dysfunction and the incidence of reperfusion arrhythmia after brief ischaemic insults in septic hearts.

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Several pharmacological agents such as adenosine and opioids have been shown to produce similar properties to ischaemic preconditioning, defined as the concept that repeated short periods of ischaemia render the heart more resistant to a subsequent sustained ischaemic period.¹⁻³ Recently, an increasing amount of evidence indicated that exposure to volatile anaesthetics also displays protective roles on ischaemic myocardium, mimicking such ischaemic preconditioning.⁴⁻⁶ For example, in dogs, sevoflurane treatment before ischaemia reduced infarct size, possibly through the opening of adenosine triphosphate-sensitive potassium (K₂ATP) channels.⁷ Compared with other volatile anaesthetics, sevoflurane is characterized by the preservation of myocardial blood flow through the involvement of collateral circulation, independent of K₂ATP channels.⁸⁻⁹ However, it remains unclear whether such properties of sevoflurane work in stunned myocardium, that is transient myocardial dysfunction after short periods of ischaemia.

Sepsis, characterized by a marked elevation of tissue oxygen demand, is frequently accompanied by multiple organ failure including myocardial dysfunction even at the early stage of the hyperdynamic circulatory state.¹⁰⁻¹¹ A previous study showed that the ultra-structural architecture of the myocardium, including mitochondria and myofibrils, were swollen and damaged in a large animal model of sepsis.¹² Thus, septic myocardium may not be able to utilize oxygen efficiently even under the conditions of supranormal oxygen supply to the myocardium.¹¹ In addition, another
study demonstrated that the flow reserve of the coronary circulation to maximize blood flow and oxygen extraction was limited.\(^\text{13}\) Thus, septic myocardium appears to be more vulnerable to even brief periods of ischaemia and subsequent reperfusion. We, therefore, tested the hypothesis that sevoflurane protected the septic heart from ischaemia to produce a ‘stunned myocardium’. In the present study, we used the caecal-ligation and perforation (CLP) model to develop the early stage of sepsis as described previously.\(^\text{14}\) Using an isolated working heart from CLP-treated rats, we examined whether exposure of 2% sevoflurane served to minimize myocardial dysfunction and the incidence of reperfusion arrhythmia following brief period of ischaemia.

**Materials and methods**

This study procedure was approved by the animal care and utilization committee in Keio University School of Medicine.

**Animal preparation**

Ninety-one male Wistar rats, weighing 310–380 g, were studied after a 3- to 7-day period of acclimatization in our laboratory. Water and laboratory chow were available *ad libitum* after intraperitoneal (i.p.) injection of pentobarbital (30 mg kg\(^{-1}\)) and the femoral vein was cannulated with a catheter (PE50; Intermedic, Sparks, MD, USA) under sterile conditions. The animals were then randomized into either sham-treatment (Sham) or CLP group. In the latter animals, a laparotomy was performed and a ligature was placed around the caecum immediately distal to the ileocaecal valve. The caecum was then punctured twice with an 18-gauge needle. Following this preparatory surgery, normal saline was infused at 3 ml h\(^{-1}\) via the catheter for the next 24 h. This preparation was demonstrated to produce normotensive, hyperdynamic sepsis.\(^\text{14}\) As the laparotomy per se caused septic responses in rats, the Sham animals in this experiment did not receive open laparotomy as described previously.\(^\text{15}\)

![Schematic drawing of experimental procedure](https://academic.oup.com/bja/article-abstract/89/6/896/261650/897)

**Study procedure**

Figure 1 depicts the study procedure. Twenty-four hours after randomization to either the Sham (n=41) or CLP (n=50) group, animals were anaesthetized with sevoflurane in oxygen. After the intravenous (i.v.) injection of heparin (300 IU kg\(^{-1}\)), the abdomen was opened and 1 ml of blood was taken from descending aorta for subsequent analyses of arterial lactate. Thereafter, the hearts were rapidly excised, mounted on a non-recirculating Langendorff apparatus to allow further preparation, and perfused with modified Krebs–Henseleit solution at 37°C (composition in mM: NaCl 120, KCl 4.8, KH\(_2\)PO\(_4\) 1.2, MgSO\(_4\) 1.2, CaCl\(_2\) 1.25, NaHCO\(_3\) 25, and glucose 11) as described previously.\(^\text{5}\) The perfusion buffer was equilibrated with a 95% oxygen/5% carbon dioxide gas mixture, resulting in a buffer PO\(_2\) above 60 kPa.

Spontaneous beating hearts were used throughout the study periods. Following aortic cannulation, the left atrium was cannulated through the pulmonary vein to allow filling and contraction of the left atrium. With the left atrial cannula open, the Langendorff column was closed and the aortic outflow line opened so that buffer entering through
the left atrium could be pumped out of the left ventricle, that is the antegrade working mode. As perfusion of left atrium provided sufficient heart rate in this mode, electrical pacing was obviated to examine the protective effects of sevo-flurane on reperfusion arrhythmia. During this working mode, a left atrial filling pressure of 15 cm H₂O and a mean aortic pressure of 70 cm H₂O were maintained by adjusting the height of the left atrial buffer reservoir above the heart and by a column height equivalent to 70 cm H₂O, respectively. Following ligation of the superior and inferior vena cava in addition to the pulmonary veins to ensure that the coronary effluent passed through the pulmonary artery, the pulmonary artery was cannulated with 16-gauge steel cannula and right ventricular outflow was monitored with transit-time ultrasound flowmeter (T206, Transonic Systems Inc., New York, USA) for the measurement of coronary flow as described previously. The probe of this flowmeter was also attached to the aortic flow root. Simultaneously, a catheter-tip pressure transducer (post-craniotomy subdural pressure monitoring kit; 110-4G, Neuro Care Group Camino, CA, USA) was inserted into the left ventricle through the left atrium.

After 30 min of initial perfusion in working mode, baseline measurements were used. The hearts were further randomized into two subgroups: control or sevo-flurane group. The latter subgroup (Sham/SEVO, n=20 or CLP/SEVO, n=25) was perfused for 15 min with modified Krebs–Henseleit solution, in which sevo-flurane was administered at 2.0% via a calibrated sevo-flurane vaporizer (Datex-Ohmeda, Helsinki, Finland). Control hearts were subjected to the same perfusion procedure without administration of sevo-flurane (Sham/CONT, n=21 or CLP/CONT, n=25). All isolated perfused hearts underwent 15 min global ischaemia at room temperature (approximately 25°C). The hearts were then re-perfused for 5 min in a Langendorff’s perfusion mode, followed by working heart mode at 37°C for 30 min without sevo-flurane. As depicted in Figure 1, measurements described below were repeated during the pre-ischaemic and post-reperfusion periods. Hearts were immediately freeze-clamped between blocks of metal cooled to the temperature of liquid nitrogen and stored at −40°C until extraction for high-energy phosphate analyses.

Specific measurements and calculations
Cardiac output (CO) was defined as the sum of coronary and aortic flows in this experimental setting as described previously. Left-ventricular pressure (LVP) was continuously monitored with a transducer (post-craniotomy subdural pressure monitoring kit; 110-4G, Neuro Care Group Camino, CA, USA and Life Scope II, Nihon Koden, Tokyo, Japan) and recorded in a digital recorder (PC208, Sony, Tokyo, Japan). Using computer software (Acqknowledge v3.0 data acquisition systems, Biopac Systems, Goleta, CA, USA and Microsoft Excel, Microsoft, WA, USA), LV peak systolic pressure (LVPSP), LV end-diastolic pressure (LVEDP), maximum rate of LV pressure rise (dP/dtmax), and heart rate (HR) were calculated, based on the averages of five beats. LV developed pressure (LVDP), which was regarded as a marker of contractility of the isolated rat heart, was calculated by subtracting LVEDP from LVPSP. Reperfusion arrhythmia was defined as the irregular intervals of peak systolic pressure on the pressure monitor 5 min after the initiation of working mode.

PO₂ of the perfusion medium obtained at pre-perfusion (in-flow) and right ventricular outflow (out-flow) was determined using a standard blood gas analyser (Chiron 860 series, Chiron Diagnostics Corp., East Walpole, MA, USA). Oxygen delivery was calculated as in-flow oxygen tension, in millimetres of mercury, multiplied by oxygen solubility (24 µl ml⁻¹ Krebs–Ringer’s solution at 760 mm Hg oxygen and 37°C) and coronary flow (ml min⁻¹). Myocardial oxygen consumption (mVO₂) was calculated as oxygen solubility multiplied by the difference between in-flow and out-flow oxygen tension and coronary flow. CE was determined as the ratio of cardiac work, the product of CO (ml min⁻¹)×LVPSP (mm Hg), to mVO₂ uncorrected for heart weight as described previously.

Biochemical analyses
High-energy phosphates in the myocardium were measured as described previously. Briefly, freeze-clamped left ventricular tissue from CLP and Sham animals were first broken in a frozen sample crusher (TK-CM20S, Yaghi Grassware, Tokyo, Japan) and then homogenized with a teflon-coated homogenizer (Iuchi, Tokyo, Japan) in ice-chilled 0.6 N perchloric acid. The samples were centrifuged (3000 g for 10 min), neutralized with 1 N potassium hydroxide, and re-centrifuged. Myocardial creatine phosphate (CrP), adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) contents were measured using high-performance liquid chromatography with a reverse-phase column (Shim-Pack VP-ODS, Shimazu, Tokyo, Japan). Elution was performed at 1.0 ml min⁻¹, and an ultraviolet/visible spectrophotometer (Shimazu, SPD-10A, Tokyo, Japan) at 210 nm was used to detect these phosphates. Tissue contents of these metabolites were reported in micromoles per gram wet tissue.

Statistical analysis
This experiment utilized a three-factor design with a full 2×2 factorial allotment to (CLP/Sham)×(CONT/SEVO). The third factor, ischaemia-reperfusion, was implemented as a repeated measure. Values are described as mean (sd) unless otherwise specified. To compare and contrast the effects of sepsis across the sevo-flurane treatment groups before the induction of ischaemia-reperfusion, two-way analysis of variance was employed using a statistical
package of SPSS/9.0J for Windows (SPSS Inc., Chicago, IL, USA). After induction of ischaemia-reperfusion, results between baseline and post-reperfusion periods were analysed using repeated-measures analysis of variance. Where appropriate, directed pair-wise comparisons of individual groups were conducted using a Bonferroni-corrected 95% confidence interval. The incidence of arrhythmia was examined using Kruskal-Wallis test. A $P$ value $<0.05$ was regarded as significant.

**Results**

*Sepsis model and myocardial function in vitro*

At 24 h after laparotomy, all CLP rats demonstrated reduced activity, pilo-erection and exudation around eyes and nose. At postmortem, pan-peritonitis with moderate volume of ascites was confirmed in all CLP rats. Arterial lactate levels were not significantly different 24 h after CLP [2.0 (0.5) vs 2.6 (1.1) mmol litre$^{-1}$]. In contrast, sham-treated animals demonstrated an uneventful recovery from anaesthesia and had no marked sign of illness. As no significant differences were found within each major group at baseline, individual data were pooled into either the CLP or Sham group as shown in Table 1. Baseline haemodynamic values, including LVDP, $dP/dt_{\text{max}}$ and CO, along with both HR and stroke volume, were significantly lower in the CLP group than those in the Sham group ($P<0.01$), whereas LVEDP was similar between the groups. Simultaneously, the coronary flow was depressed to approximately 80% of the Sham animals in the CLP group. Figure 2 shows baseline differences of cardiac work, $m$VO$_2$ and cardiac efficiency between the Sham and CLP. Compared with normal, septic hearts from the CLP rats showed not only significantly less work and oxygen consumption but also less efficiency over the baseline study period.

**Effects of sevoflurane in normal or septic heart against ischaemia-reperfusion**

Table 2 illustrates the time course of myocardial function throughout the study periods with or without sevoflurane pre-treatment in the hearts of Sham and CLP rats. In the Sham group, no differences in myocardial function were observed at the baseline between CONT and SEVO subgroups. Although the difference of LVDP at pre-ischaemia was not statistically significant, a reduction of $dP/dt_{\text{max}}$ and approximately a 28% decrease of CO with constant heart rates in the SEVO subgroup were found. Two per cent sevoflurane decreased mainly stroke volume in an isolated working mode of normal rat heart. In contrast, both $m$VO$_2$ and coronary flow did not change during sevoflurane exposure. With ischaemia and reperfusion, all parameters of myocardial function including LVDP, $dP/dt_{\text{max}}$, and CO were significantly depressed, and were accompanied by a reduction of $m$VO$_2$ and coronary flow, indicating that a ‘stunned myocardium’ developed in this experimental setting. Alternatively, sevoflurane exposure before 15 min...
ischaemia caused no significant changes in myocardial function after reperfusion compared with those without sevoflurane treatment. In contrast, in CLP rats, exposure of 2% sevoflurane for 15 min caused a significant depression of CO without changes in LVDP, dP/dtmax, or HR (Table 2). Both coronary blood flow and mV̇O₂ were preserved during sevoflurane exposure as observed in the Sham group. After reperfusion, sevoflurane treatment minimized the depression of CO compared with CLP/CONT group. Other parameters of myocardial function in the SEVO subgroup showed comparable changes with the CONT group. Simultaneously, coronary blood flow and mV̇O₂ were not significantly different between the two subgroups, whereas they were decreased from baseline in both subgroups. Collectively, the data suggest that sevoflurane modified neither mV̇O₂ nor coronary blood flow of septic hearts after reperfusion.

There was no significant difference in the incidence of reperfusion arrhythmia between the sevoflurane-treated and -untreated rats (25 vs 10%) in the Sham group. In contrast, in CLP rats this was significantly reduced with sevoflurane treatment compared with non-treated rats (8 vs 32%, respectively, P = 0.027). Figure 3 shows the differences in cardiac work, mV̇O₂ and cardiac efficiency with or without sevoflurane treatment in the Sham and CLP groups during the post-reperfusion period. All parameters in the CLP group, but not mV̇O₂ with sevoflurane treatment, were significantly lower than those in the Sham group. Both cardiac work and mV̇O₂ were not different irrespective of the presence of sevoflurane in the Sham or CLP groups. However, sevoflurane provided better cardiac efficiency, that is, more efficient oxygen utilization to cardiac work, in the CLP group, whereas this was not significantly different between sevoflurane-treated and -untreated animals in the Sham group.

### Changes in myocardial high-energy phosphates

The effects of sepsis and a brief period of sevoflurane exposure on myocardial high-energy phosphates were examined at the end of the study procedure (Table 3). Myocardial high-energy phosphates such as ATP, ADP, and AMP were not modulated significantly by CLP or sevoflurane exposure during the post-reperfusion period.

### Discussion

The present study indicates that brief exposure to 2% sevoflurane before ischaemic insult to induce ‘myocardial stunning’ provides protective effects in sepsis. Although such effects of sevoflurane were obvious in the preservation of CO, this parameter, consisting of both contractility and heart rate, was likely to represent myocardial performance rather than LVDP or dP/dt per se under a constant afterload. In addition, cardiac efficiency was better preserved after reperfusion in sevoflurane-treated vs -untreated septic hearts.

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**Table 2** Changes of haemodynamic parameters of working heart model in Sham and CLP groups with or without sevoflurane treatment. Data were expressed as mean (SD). Abbreviations: CONT, no treatment; SEVO, sevoflurane treatment. Significance: *P<0.05, **P<0.01 vs CONT group, †P<0.05, ‡P<0.01 vs the baseline period.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Baseline</th>
<th>Pre-ischaemia</th>
<th>Post-reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDP (mm Hg)</td>
<td>Sham</td>
<td>CONT</td>
<td>91.4 (9.2)</td>
<td>86.9 (6.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>88.1 (13.3)</td>
<td>81.4 (13.8)</td>
</tr>
<tr>
<td></td>
<td>CLP</td>
<td>CONT</td>
<td>80.0 (14.1)</td>
<td>77.0 (10.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>75.6 (14.2)</td>
<td>71.1 (13.2)</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>Sham</td>
<td>CONT</td>
<td>7.7 (3.1)</td>
<td>7.7 (3.4)</td>
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<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>7.9 (3.0)</td>
<td>7.9 (2.7)</td>
</tr>
<tr>
<td></td>
<td>CLP</td>
<td>CONT</td>
<td>8.2 (2.9)</td>
<td>8.4 (2.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>7.6 (3.3)</td>
<td>8.1 (3.2)</td>
</tr>
<tr>
<td>dP/dtmax (mm Hg s⁻¹)</td>
<td>Sham</td>
<td>CONT</td>
<td>2570 (520)</td>
<td>2520 (410)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>2650 (440)</td>
<td>2240 (360)*</td>
</tr>
<tr>
<td></td>
<td>CLP</td>
<td>CONT</td>
<td>2090 (420)</td>
<td>2000 (370)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>1970 (560)</td>
<td>1810 (550)</td>
</tr>
<tr>
<td>CO (ml min⁻¹)</td>
<td>Sham</td>
<td>CONT</td>
<td>48.9 (8.4)</td>
<td>46.8 (9.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>48.9 (11.5)</td>
<td>33.7 (12.4)**</td>
</tr>
<tr>
<td></td>
<td>CLP</td>
<td>CONT</td>
<td>31.9 (9.9)</td>
<td>32.1 (8.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>33.2 (12.7)</td>
<td>22.2 (7.8)**</td>
</tr>
<tr>
<td>Heart rate (beats min⁻¹)</td>
<td>Sham</td>
<td>CONT</td>
<td>296 (60)</td>
<td>304 (60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>288 (48)</td>
<td>275 (60)</td>
</tr>
<tr>
<td></td>
<td>CLP</td>
<td>CONT</td>
<td>256 (46)</td>
<td>262 (44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>250 (68)</td>
<td>244 (66)</td>
</tr>
<tr>
<td>mV̇O₂ (μl min⁻¹)</td>
<td>Sham</td>
<td>CONT</td>
<td>200 (36)</td>
<td>190 (36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>198 (48)</td>
<td>174 (45)</td>
</tr>
<tr>
<td></td>
<td>CLP</td>
<td>CONT</td>
<td>160 (39)</td>
<td>155 (39)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>170 (41)</td>
<td>163 (44)</td>
</tr>
<tr>
<td>Coronary flow (ml min⁻¹)</td>
<td>Sham</td>
<td>CONT</td>
<td>13.5 (2.0)</td>
<td>13.3 (2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>13.2 (2.3)</td>
<td>12.3 (2.5)</td>
</tr>
<tr>
<td></td>
<td>CLP</td>
<td>CONT</td>
<td>10.5 (2.1)</td>
<td>10.1 (1.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SEVO</td>
<td>10.9 (2.3)</td>
<td>10.5 (2.4)</td>
</tr>
</tbody>
</table>
Thus, sevoflurane treatment improved oxygen utilization of myocardium to achieve a certain level of cardiac work after brief ischaemia in septic hearts. Another important finding of the present study was that sevoflurane pre-treatment reduced the incidence of reperfusion arrhythmia in septic hearts perfused in a working mode. Therefore, sevoflurane treatment preserves not only contractility but also other profiles of myocardial function including electrical conductance in septic hearts after ischaemia and reperfusion through the improvement of oxygen metabolism.

Whilst previous studies showed that exposure to volatile anaesthetics protected healthy myocardium from prolonged ‘irreversible’ ischaemia by assessing the reduction of infarct size, there were conflicting results as to whether similar profiles could be given to ‘ reversible’ myocardial injury, that is stunned myocardium. Indeed, sevoflurane treatment in this experimental condition did not show any cardioproteective properties against ischaemia-reperfusion injury in healthy hearts. There are several possibilities to account for such a discrepancy between the present and previous studies. First, sevoflurane concentration might not be high enough to obtain its preconditioning-like effects in healthy hearts. Previous studies demonstrated that the minimum alveolar concentration (MAC) of sevoflurane was significantly reduced from 2.4 to 1.35% level in a porcine model of sepsis. Another study using the same CLP-rat model also showed that the MAC required for isoflurane was lowered to a similar extent. 

Given these findings, it is not unreasonable to consider that the potency of anaesthetics on the isolated myocardium is different between the Sham and CLP groups. If equipotent sevoflurane doses were given to each group, this might have protected the healthy heart from ischaemia–reperfusion injury to cause stunned myocardium. Indeed, it should be noted that there are conflicting results, possibly caused by different concentrations of anaesthetic agents exposed at different time periods among previous studies. Secondly, sevoflurane anaesthesia for the excision of hearts might have already provided preconditioning-like effects even in the control hearts. Although sevoflurane is much less likely to remain in situ after reperfusion compared with other anaesthetics because of its lower blood–gas solubility, this possibility could not be fully excluded. On the other hand, the study design to terminate sevoflurane treatment during the reperfusion period could have modified its cardioprotective effects in healthy hearts. Thirdly, ischaemic insults to evoke a stunned myocardium in the present study might not be severe enough to show distinct protective effects of sevoflurane in health where myocardial reserve is more sufficient than in sepsis. In other words, if a longer period of ischaemia was applied to this model, protection might have been unmasked. Furthermore, hypothermic global ischaemia as we applied could be another factor to reduce the severity of the ischaemia–reperfusion injury compared with normothermic ischaemia as described elsewhere.

### Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>CrP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>CONT</td>
<td>0.51 (0.15)</td>
<td>0.90 (0.46)</td>
<td>0.56 (0.35)</td>
<td>0.87 (0.58)</td>
</tr>
<tr>
<td></td>
<td>SEVO</td>
<td>0.54 (0.35)</td>
<td>0.72 (0.20)</td>
<td>0.51 (0.27)</td>
<td>0.70 (0.51)</td>
</tr>
<tr>
<td>CLP</td>
<td>CONT</td>
<td>0.45 (0.17)</td>
<td>0.72 (0.21)</td>
<td>0.54 (0.21)</td>
<td>1.43 (0.94)</td>
</tr>
<tr>
<td></td>
<td>SEVO</td>
<td>0.41 (0.23)</td>
<td>0.65 (0.13)</td>
<td>0.48 (0.18)</td>
<td>0.51 (0.42)</td>
</tr>
</tbody>
</table>

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Fig 3 Effects of sevoflurane treatment on myocardial function in an isolated working heart in both Sham and CLP groups during the post-reperfusion period. Abbreviations: Sham, Sham group; CLP, caecal-ligation and perforation group; mVO₂, myocardial oxygen consumption. Cardiac efficiency, the ratio of cardiac work, the product of CO×LVPSP, to mVO₂. Significance: †P<0.05 vs Sham group, *P<0.05 vs sevoflurane untreated subgroup.
Despite the instability of this septic challenge, the CLP model with fluid resuscitation has been considered most relevant to the early stage of sepsis, concomitantly obviating confounding factors like hypotension.\textsuperscript{12} 14 27 In accordance with these findings, we found no significant elevation of arterial lactate 24 h after CLP, indicating that tissue hypoxia was not evident in this model of sepsis. Even under such conditions, both myocardial contractility, expressed as LVDP, $dP/dr_{\text{max}}$ and CO, and heart rate in the CLP group were significantly lower compared with the Sham group at baseline (Table 1), suggesting that not only inotropic but also chronotropic properties synergistically serve in the development of myocardial dysfunction in sepsis. However, it should also be noted that CO was significantly depressed in the early stage of sepsis under a constant afterload in this isolated preparation. Furthermore, the present study shows that $mV\text{O}_2$ is significantly depressed ex vivo with the reduction in coronary flows and CO (Table 1) whereas it is unknown whether $mV\text{O}_2$ in the hyperdynamic state of sepsis is indeed augmented or not. A previous study demonstrated that pro-inflammatory cytokines, released in sepsis, depressed myocardial function accompanied by reduction of $mV\text{O}_2$ through nitric oxide-dependent mechanisms.\textsuperscript{18} Further investigations are required to clarify this issue.

Neither CLP nor sevoflurane exposure appears to modify myocardial energy levels, indicating that improved high-energy phosphate contents were not singularly important determinants of post-ischaemic ventricular function. Support for this contention was provided by previous studies, which demonstrated the dissociation between ventricular function and ATP levels in post-ischaemic hearts.\textsuperscript{28} 29 Some investigations showed that myocardial high-energy phosphates content correlated neither with myocardial dysfunction in sepsis nor with cardioprotection by ischaemic preconditioning.\textsuperscript{30} 31 Collectively, one possibility could be raised that the significant reduction in CO, accompanied by depressed $mV\text{O}_2$, resulted in stored high-energy phosphates in the CLP group more than those in the Sham group, subsequently preserving the amount of ATP contents in the CLP group. Furthermore, both a reduction of cardiac efficiency and no changes of myocardial high-energy phosphates in the CLP group, indicates a possibility that sepsis reduces the ability of the myocardium to utilize high-energy phosphates for contractile work, but not inhibit mitochondrial respiration. Nevertheless, the current results provide evidence that energy utilization in the septic heart is augmented with treatment of sevoflurane before ischaemia.

There are several limitations to this study. First, despite a physiologically correct direction of perfusion, the isolated working model limits the evaluation of data regarding myocardial function in sepsis. For example, humoral factors like cytokines and circulating inflammatory cells such as leukocytes or platelets, both of which play consequential roles in the development of sepsis, are excluded from the perfusion medium in this study design. Furthermore, because of constant afterload and depressed contractility, the augmentation of CO observed in this early stage of sepsis was not found during the baseline period. Secondly, it could be argued that myocardial function could be evaluated more accurately if the heart rates were kept constant. Contrary to Langendorff mode, however, sufficient heart rate could be obtained in an isolated working heart because of perfusion to left atrium as described previously.\textsuperscript{19} In addition, under pacing conditions this model could lose the effects of sevoflurane on heart rates, subsequently modifying CO. Finally, the isolation procedure per se may be able to ischaemic-precondition the heart, possibly modifying the outcome. However, as we performed the study in the same manner by a single investigator in random, we believe that the data were comparable at least between the groups.

In conclusion, the current study shows that sevoflurane provides protective effects, in septic but not healthy hearts, against brief ischaemic insults through the improvement of myocardial oxygen utilization. Further investigation is warranted to elucidate whether other anaesthetic preconditioning protects the myocardium against brief or prolonged period of ischaemia ‘in diseased hosts’.

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**References**

1 Yao Z, Gross GJ. A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs. Efficacy, time course, and role of $K_{\text{ATP}}$ channels. *Circulation* 1994; 89: 1229–36


4 Cason BA, Gamperl AK, Locum RE, Hickey RF. Anesthetic-induced preconditioning. Previous administration of isoflurane decreases myocardial infarct size in rabbits. *Anesthesiology* 1997; 87: 1182–90


7 Toller WG, Kersten JR, Pagel PS, Hettrick DA, Warttier DC. Sevoflurane reduces myocardial infarct size and decreases the time threshold for ischemic preconditioning in dogs. *Anesthesiology* 1999; 91: 1437–46


9 Kersten JR, Schmeling T, Tessmer J, Hettrick DA, Pagel PS, Warttier DC. Sevoflurane selectively increases coronary...
collateral blood flow independent of K\textsubscript{ATP} channels in vivo. Anesthesiology 1999; 90: 246–56


