Cardioprotection with esmolol cardioplegia: efficacy as a blood-based solution

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Received 29 February 2012; received in revised form 1 May 2012; accepted 7 May 2012

Abstract

OBJECTIVES: Current cardiac surgery patients are older, sicker, with more diffuse disease and hence a reduced tolerance to ischaemia-reperfusion injury. We previously demonstrated that esmolol, an ultra-short-acting \( \beta \)-blocker, can be used as an arresting agent at high (millimolar) concentrations, and that a crystalloid-based esmolol cardioplegia afforded cardioprotection at least equivalent to hyperkalaemic (St Thomas’ Hospital) cardioplegia. Esmolol is rapidly metabolized by blood esterases, so it was important to determine the feasibility of its use in blood-based solutions. This study compared the efficacy of blood-based esmolol cardioplegia with hyperkalaemic cardioplegia in a novel blood-perfused rat heart preparation.

METHODS: Isolated rat hearts were Langendorff blood-perfused with a rat blood/buffer perfusate mixture (flow rate, 3.0 ml/min) and pre-ischaemic baseline function (left ventricular developed pressure) assessed. All values are expressed as mean ± SEM. Three studies were conducted: (i) the efficacy of blood-based vs crystalloid-based esmolol or hyperkalaemic cardioplegia (40 min ischaemia) was evaluated (five groups; six hearts/group); (ii) the effect of the mode of cardioplegia delivery (constant flow/pressure) was assessed (four groups; six hearts/group); (iii) the efficacy of blood-based esmolol compared with hyperkalaemic cardioplegia over extended (60 min) ischaemia duration was evaluated (two groups; six hearts/group). Hearts were reperfused (60 min) and recovery (percent of pre-ischaemic baseline function) measured at the end of reperfusion.

RESULTS: Hearts subjected to blood-based esmolol or hyperkalaemia cardioplegia recovered to 78 ± 4% and 68 ± 6%, whereas crystalloid-based esmolol or hyperkalaemic cardioplegia recovered to 84 ± 1% and 77 ± 2%, respectively [all \( P < 0.05 \) vs control (2 ± 2%)]; there were no differences between cardioplegia groups. When infusion duration was extended, a lower (2 mmol/l) esmolol concentration improved recovery compared with the higher (3 mmol/l) concentration (66 ± 4% vs 29 ± 12%, \( P < 0.05 \)). Extending the ischaemic duration demonstrated enhanced efficacy for blood-based esmolol cardioplegia (70 ± 4%; \( P < 0.05 \)) compared with hyperkalaemic cardioplegia (47 ± 10%).

CONCLUSIONS: Blood-based esmolol cardioplegia improved cardioprotective efficacy compared with hyperkalaemic cardioplegia; the metabolic effects of blood esterase did not appear to influence this efficacy. An esmolol-based cardioplegic solution may be a beneficial alternative to hyperkalaemic solutions.

Keywords: Rat • Blood perfusion • Cardioplegia • Ischaemia-reperfusion • Esmolol • Cardioprotection • Function

INTRODUCTION

Cardioplegia protection of the myocardium during cardiac surgery has improved post-ischaemic haemodynamic function, attenuated the prevalence of perioperative infarction and decreased mortality. The gold standard for myocardial protection for over 30 years has been hyperkalaemic cardioplegia, inducing depolarized arrest. However, depolarized arrest can lead to ionic imbalance, enhanced energy utilization and ischaemia-reperfusion injury [1]. Therefore, many studies have attempted to enhance current intraoperative myocardial protection, as well as examining the potential of using alternatives to hyperkalaemic cardioplegia [1].

Beta-(\( \beta \))-adrenergic antagonists (\( \beta \)-blockers) have been shown to attenuate the extent of myocardial injury during ischaemia and reperfusion [2], but most \( \beta \)-blockers have prolonged (hours) negative inotropic and chronotropic properties, which limits their use during cardiac surgery. Esmolol, an ultra-short-acting and cardioselective \( \beta \)-blocker, has a half-life of around 9 min due to esterase hydrolysis [3], which allows its negative inotropic effects to be abolished rapidly after reduction or cessation of infusion and is thus more amenable for use in acute situations such as cardiac surgery. Esmolol infusion together with maintained perfusion during cardiac surgery has been used as an alternative approach to manage the heart during surgery; several clinical and experimental studies [4–6] have shown that esmolol
can be used to induce 'adequate cardiac surgical conditions' (which manifests as significant slowing of the heart rate). These studies demonstrated similar cardioprotection to that seen with cold crystalloid or blood hyperkalaemic cardioplegia; however, this technique consists of continuous coronary infusion of blood containing esmolol, and does not induce arrest, thereby limiting the options for cardiac surgery.

Previously, we showed [7, 8] that esmolol, at high (mMolar) concentrations, can be used as a cardioprotective (arresting) agent; in isolated Langendorff-perfused rat heart studies, intermittent infusion of an esmolol-based 'crystalloid' cardioprotective solution (1.0 mM esmolol in bicarbonate buffer) provided excellent cardioprotection for extended periods of normothermic global ischaemia. In studies such as these, it has been conventional to use buffer-perfused isolated heart preparations for precise control and manipulation. However, the absence of the blood component has significant disadvantages when investigating drugs such as esmolol, as its efficacy could be influenced by blood metabolism. Therefore, we used our novel blood perfusion system [9], in which rat blood is diluted by 50% to achieve blood metabolism. Therefore, we used our novel blood perfusion system [9], in which rat blood is diluted by 50% to achieve sufficient volume and to mimic the clinical situation of circulatory dilution during cardiopulmonary bypass (CPB). Clinically, most cardiac surgeons use blood cardioplegia; since it is known that esmolol is rapidly hydrolyzed by blood esterases to an inactive metabolite [3], it was important to establish whether esmolol could be an effective cardioplegic arresting agent when used intermittently in a blood-based solution, to compare it with a conventional hyperkalaemic blood-based cardioplegia (St Thomas' Hospital solution) and to compare these with their crystalloid counterparts. Furthermore, we determined whether mode of administration as well as extended ischaemic duration between infusions influenced the protective efficacy.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (240–300 g body weight) were used (Bantin and Kingman, Hull, UK). All animals received humane care in accordance with the 'Guidance on the Operation of the Animals (Scientific Procedure) Act of 1986' published by Her Majesty's Stationary Office, London, UK.

Recirculating blood perfusion system

In this study, our recirculating blood perfusion system [9], designed to mimic a CPB circuit, was modified to use blood from the same animal as the heart, saving both animal usage and experimental cost. Briefly, the circuit comprised a plastic reservoir (containing ~5 ml of blood perfusate and with the base lined with 200-µm nylon gauze (Cadisch Precision Meshes, London, UK) to prevent any particulate debris entering the circuit tubing) inserted into a temperature-controlled (37°C) heart chamber. Silicon tubing (Altesil™, 2.5 mm bore, 1.0-mm wall thickness: Altec, Alton, Hampshire, UK) connected the base of the reservoir through a peristaltic roller pump (Gilson Minipuls 3; Anachem, Luton, Bedfordshire, UK) to a home-made 'membrane oxygenator'. The oxygenator consisted of a coil (of ~10 turns) of thin-walled, gas-permeable silicon tubing (1.47 mm bore, 0.46 mm wall thickness: OsteoTec Ltd., Christchurch, Dorset, UK) in a temperature-controlled (37°C) glass chamber, with a lower inlet into which 95% O2: 5% CO2 was passed in counter current to the perfusate through the tubing. The gas escaped at the top of the chamber through a tube inserted into a tight-fitting silicon bung; this maintained a more physiological pO2 of ~150 mmHg (significantly lower than ~600 mmHg pO2 required for adequate crystalloid perfusate oxygenation for isolated hearts), a pCO2 of ~35 mmHg and physiological perfusate pH. The oxygenator connected, via silicon tubing (Altesil™), to a water-jacketed temperature-controlled (37°C) aortic cannula, through which perfusate returned to the reservoir. When global ischaemia was induced (by stopping flow to the aortic cannula with a three-way tap), blood perfusate was diverted directly into the reservoir to avoid blood stasis; the ischaemic heart was maintained at 37°C throughout the ischaemic duration. The total circuit perfusate volume was ~14 ml.

Perfusion medium

A modified Krebs-Henselit bicarbonate buffer solution (MKH; in mM/l—NaCl, 118.5; NaHCO3, 25.0; KCl, 7.5; MgSO4, 2.4; KH2PO4, 2.4; CaCl2, 5.4; glucose, 22.0), prepared daily and filtered through a 5-µm cellulose nitrate filter, was mixed with Gelofusine® (G; a modified fluid gelatin in saline) in a ratio of 1:1 to give a MKH/G perfusion solution.

Heart isolation and perfusion

The extracorporeal perfusion circuit was primed with MKH/G perfusion solution (14 ml) and continuously recirculated and gassed (95% O2: 5% CO2). Rats were anaesthetized with sodium pentobarbitone (60 mg/kg, i.p.) and anticoagulated with heparin (1000 IU/kg, i.v.). At least 7 ml of blood was rapidly withdrawn from the inferior vena cava into a heparinized (500 IU) 10 ml syringe, and the heart was then excised from the same rat and immersed in cold (4°C) MKH/G solution. The aorta was cannulated (within 30 s of excision) and the heart was perfused at 37°C in the Langendorff recirculating mode with the MKH/G prime solution at an optimal constant flow (CF) of 3.0 ml/min (established from Preliminary Study 1; see below) using a peristaltic pump. The pulmonary artery was incised to allow free drainage of coronary effluent. Rat blood (7 ml) was added to the reservoir to circulate and oxygenate as soon as possible after cannulation, while an equal volume (7 ml) of MKH/G effluent was collected from the perfused heart and discarded, thus, the blood was diluted by 50%

The left atrial appendage was removed and a deflated ultrathin intraventricular balloon, constructed from cling film (Saran Wrap; S.C. Johnson, Racine, WI) over the tip of a 20-gauge cannula and made to match the internal dimensions of the left ventricle, was introduced through the mitral valve into the left ventricle. The balloon catheter was attached to a pressure transducer and the calibrated output recorded on an Apple Macintosh computer (Apple Computer Inc., Cupertino, CA, USA) using the PowerLab system (ADInstruments Ltd., Headington, Oxfordshire, UK). The intraventricular balloon was gradually inflated with water to give a stable left ventricular end-diastolic...
pressure (LVEDP) of 3–8 mmHg, and this isovolumic state was maintained throughout the protocol. During perfusion, heart function [left ventricular systolic pressure (LVSP), LVEDP and heart rate (beats/min)] was measured. Left ventricular developed pressure (LVEDP) was calculated as LVSP minus LVEDP. A second pressure transducer attached to a sidearm of the aortic cannula measured perfusion pressure (PP).

**Exclusion criteria**

Hearts not satisfying pre-assigned exclusion criteria at the time of the baseline readings were excluded from the study. The acceptable values for LVDP and heart rate were >100 mmHg and >150 beats/min, respectively. All acceptable hearts were included in the completed protocols regardless of the final outcome.

**Preparation and administration of esmolol cardioplegia and St Thomas’ cardioplegia**

Esmolol (Brevibloc; Baxter Pharmaceuticals, Crowthorne, Berkshire, UK) vials contained 10 ml of a 250 mg/ml solution. Preliminary Study 2 (see later) was carried out to determine a dose-recovery curve for esmolol. As a result of this study, subsequent esmolol cardioplegic solution was prepared by diluting esmolol with the oxygenated MKH/G perfusate to give the final esmolol concentration. For blood-based esmolol cardioplegia, the oxygenated esmolol solution was mixed in a ratio of 3:1 with blood drawn from another rat (haematocrit of ~40%) just before infusion, giving the final esmolol concentration and a haematocrit of ~10%.

St Thomas’ Hospital cardioplegic solution No. 2 (STH2) was prepared daily with the following composition (in mmol/l): NaCl, 110.0; MgCl₂·2H₂O, 16.0; KCl, 16.0; CaCl₂·2H₂O, 1.2; and NaHCO₃, 10.0. The pH was adjusted to 7.8 at 37°C, and the solution was filtered through a 5-µm cellulose nitrate filter before use. For blood-based STH2 (BSTH2), the hyperkalaemic solution was mixed in a ratio of 3:1 with blood drawn from another rat (haematocrit of ~40%) just before infusion, giving the final esmolol concentration and a haematocrit of ~10%. BSTH2 was oxygenated and administered at 37°C. The final ionic compositions of the cardioplegic solutions are detailed in Table 1.

**Preliminary protocols**

**Preliminary Study 1: determination of optimum CF rate during blood perfusion.** Since the blood perfusion circuit required a small recirculating volume (14 ml) of diluted (50%) rat blood, it was important to determine an adequate flow rate to provide comparable physiological pressure as previous studies using a non-recirculating crystalloid buffer solution. Hearts (n = 3/group) were subjected to one of five different flow-rate groups (2.0, 2.5, 3.0, 3.5 or 4.0 ml/min) and myocardial function (LVEDP, LVDP, heart rate and PP) was monitored for 120 min.

**Preliminary Study 2: determination of dose-recovery curve for different blood-based esmolol arrest concentrations.** The optimal esmolol concentration in crystalloid cardioplegic solution was 1.0 mmol/l [7]; however, infusion volumes were relatively high (at ~14 ml/min), giving an esmolol load of 3–5 mg/min. For blood-based esmolol cardioplegia, these flow rates were not possible, and 1 mmol/l esmolol did not induce arrest (esmolol load ~1 mg/min). Hearts (n = 3/group) were randomly assigned to one of eight different esmolol concentration groups (1.0, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 or 6.0 mmol/l), infused with multidose (2 min infusion every 10 min) esmolol cardioplegia at CF (3.0 ml/min) during 40 min of global ischaemia, and reperfused for 60 min. Recovery of myocardial function was measured.

**Study protocols**

All hearts were subjected to 20 min of aerobic equilibration perfusion, when baseline pre-ischaemic function was measured. During ischaemia, hearts were maintained at 37°C; each infusion of cardioplegic solution was delivered at a constant pressure (CP) adjusted to the individual baseline PP. This was achieved with another peristaltic pump controlled by a calibrated pump controller (ADInstruments Ltd) that could be switched between CF and CP.

**Study 1: comparative efficacy of esmolol blood or crystalloid cardioplegia compared with equivalent STH2.** As esmolol is hydrolyzed by blood esterases, this study determines whether blood-based esmolol cardioplegia has equivalent cardioprotective efficacy to crystalloid esmolol cardioplegia, and compared with blood and crystalloid hyperkalaemic cardioplegia (STH2).

Hearts (n = 6/group) were randomized to one of five groups (Fig. 1): (i) control-global ischaemia (C-Gl), 40 min global ischaemia; (ii) multiple blood esmolol (M-BE), blood-based esmolol (3 mmol/l) cardioplegia; (iii) multiple blood STH2 (M-BS), blood-based STH2 cardioplegia; (iv) multiple crystalloid esmolol (M-CE), crystalloid esmolol (3 mmol/l) cardioplegia; (v) multiple crystalloid STH2 (M-CS), crystalloid STH2 cardioplegia. All cardioplegia solutions were infused for 2 min before and every 10 min during 40 min global ischaemia.

**Study 2: efficacy of blood-based esmolol cardioplegia: effect of mode of infusion and extended intermittent ischaemic duration.** This study examines the optimal mode (CF or CP) of cardioplegic infusion, and whether extended cardioplegic infusion duration associated with prolonged (45 min) ischaemic durations maintains protective efficacy.

Hearts (n = 6/group) were randomized to one of four groups: (i) blood esmolol (3 mmol/l)-constant pressure (BE3-CP), blood-
based esmolol cardioplegia (3 mmol/l for 3 min at CP, adjusted to individual baseline PP); (ii) blood esmolol (2 mmol/l)-constant pressure (BE2-CP), blood-based esmolol cardioplegia (2 mmol/l for 3 min at CP, adjusted to individual baseline PP); (iii) blood esmolol (2 mmol/l)-constant flow (BE2-CF), blood-based esmolol cardioplegia (2 mmol/l for 3 min at CF of 3 ml/min); (iv) blood esmolol (2 mmol/l)-constant pressure+washout (BE2-CP+W), blood-based esmolol cardioplegia (2 mmol/l for 3 min at CP, adjusted to individual baseline PP), followed by discard of original blood perfusate for initial 5 min of reperfusion (washout; W) and subsequent replacement with fresh blood perfusate solution. All cardioplegia solutions were infused for 3 min before and every 15 min during 45 min global ischaemia.

Study 3: comparative efficacy of blood-based esmolol or STH2 cardioplegia for prolonged ischaemia. This study determines the cardioprotective efficacy of blood-based esmolol or STH2 cardioplegia for prolonged ischaemia (60 min).

Hearts (n = 6/group) were randomized into two groups: (i) blood esmolol+washout (BE+W), blood-based esmolol cardioplegia (2 mmol/l); (ii) blood STH2+Washout (BS+W), blood-based STH2. Each cardioplegic solution was infused for 3 min at CP adjusted to individual baseline PP; original blood perfusate discarded over initial 5 min of reperfusion (W) and subsequently replaced with fresh blood perfusate solution.

Hearts in all groups were then subjected to 60 min of reperfusion; recovery of myocardial function (LVDP, LVEDP and PP) was measured throughout. Myocardial injury [creatine kinase (CK) leakage] was also measured following reperfusion.

Expression of results

Contractile function. Post-ischaemic recovery of LVDP was expressed as percent of baseline value; LVEDP and PP were expressed in mmHg.

CK Leakage. After 60 min of reperfusion, the blood perfusate was drained into a cylinder and the volume measured. A sample was centrifuged at 3000 rpm for 5 min and total CK leakage, expressed as IU/g heart wet weight, was assessed (Sigma-Aldrich Diagnostic kits, Poole, Dorset, UK) by a spectrophotometrical analysis of enzyme activity in the sample supernatant.

Statistics

Statistical analysis was performed with GraphPad Prism 5 (GraphPad Software inc., La Jolla, CA, USA) on an Apple Macintosh computer (Apple Computer, Cupertino, CA, USA). All data are reported as mean ± SEM. Comparisons between groups were assessed for significance by analysis of variance with post hoc analysis by the Bonferroni test, allowing for multiple comparisons. The student’s unpaired t-test (or the Mann–Whitney U-test when appropriate) was used to compare between two groups. A value of P < 0.05 was considered statistically significant.

RESULTS

Preliminary Study 1: determination of optimum CF rate during blood perfusion

A flow rate of 3.0 ml/min provided an acceptable LVDP (Fig. 2A) and the highest stable PP (Fig. 2B); hence, 3.0 ml/min was selected for all other studies.

Preliminary Study 2: determination of dose-recovery curve for different blood-based esmolol arrest concentrations

The highest and most consistent recovery of LVDP occurred at an esmolol concentration of 3.0 mmol/l (Fig. 2C), and was hence
selected for use in further studies. Esmolol at 1 mmol/l failed to arrest the heart; 6.0 mmol/l was damaging, with no functional recovery (not shown).

Study 1: comparative efficacy of esmolol blood or crystalloid cardioplegia compared with equivalent STH2

The mean baseline values of LVDP, LVEDP, heart rate and PP at the end of 20 min aerobic perfusion in groups (i–v) are shown in Table 2; there were no significant differences between groups.

Recovery of myocardial function. In control hearts (C-GI), LVDP recovery was only 20% of pre-ischaemia by 10 min and declined thereafter, whereas hearts of all other groups recovered rapidly to significantly higher values than control, reaching a plateau within 10 min. Initial (5 min) recovery in M-CS was significantly \((P < 0.05)\) higher than M-CE or M-BE, but there were no differences between groups at subsequent time points (Fig. 3A).

At the end of ischaemia, LVEDP values were moderately elevated, but there were no differences between groups (Fig. 3B). LVEDP increased further in C-GI hearts, whereas it decreased to near baseline levels (and was significantly \((P < 0.001)\) lower than C-GI at all time points) in all other groups.

The recovery values after 60 min of reperfusion for LVDP, LVEDP, heart rate and PP are shown in Table 2.

Myocardial injury. Total CK leakage (IU/gm dry wt) was significantly \((P < 0.05)\) reduced in all hearts subjected to cardioplegic arrest (Table 2) compared with C-GI; there were no differences between other groups (ii–v).

Study 2: efficacy of blood-based esmolol cardioplegia: effect of mode of infusion and extended intermittent ischaemic duration

The mean baseline values of LVDP, LVEDP, heart rate and PP at the end of 20 min aerobic perfusion for groups (i–iv) are shown in Table 2; there were no significant differences between groups.

Recovery of myocardial function. This study examines whether blood-based esmolol cardioplegia is effective over extended ischaemia durations between infusions, with increased infusion durations (from 2 to 3 min). Since we know that crystalloid esmolol infusions at high concentrations (3 mmol/l) can have a detrimental effect [7] that appears to be related to total esmolol load, we also examined a lower (2 mmol/l) esmolol concentration in the blood-based solution. Additionally, the effect of fresh blood during reperfusion was examined.

Initial recovery of LVDP with the higher esmolol concentration (3 mmol/l: group BE3-CP) was good, reaching a peak at 20 min; subsequently, however, recovery deteriorated rapidly and was significantly \((P < 0.05)\) lower than the other groups at 60 min of reperfusion. Recovery with the 2 mmol/l esmolol concentration groups was slightly (but not significantly) more rapid, and plateaued after 10 min; however, the hearts subjected to CF esmolol infusion (BE2-CF) appeared to deteriorate towards the end of reperfusion (Fig. 4A).

Recovery of LVEDP (Fig. 4B) shows higher initial levels of LVEDP for groups BE3-CP and BE2-CF than the other two groups; they remain higher despite decreasing slightly until 30 min of reperfusion, but subsequently rise significantly by the end of reperfusion. In contrast, groups BE2-CP and BE2-CP+WO are maintained at low and relatively normal levels of LVEDP, although BE2-CP hearts do show an increase at the end of reperfusion. PP for these hearts is shown in Fig. 4C; only hearts from BE2-CP+WO have a maintained and low PP, with the other groups showing an increase in PP as reperfusion progresses.

The recovery values after 60 min of reperfusion for LVDP, LVEDP, heart rate and PP are shown in Table 2.

Myocardial injury. Total CK leakage was lowest in the BE2-CP and BE2-CP+W groups, but they did not reach a significant difference (Table 2).
were not signiﬁcant until 30 min of reperfusion, when STH2-arrested hearts started to show an increase in LVEDP; however, these differences were only signiﬁcant (Fig. 5A); however, the differences were only signiﬁcant (P < 0.05) at 60 min. Interestingly, LVEDP in both groups was not signiﬁcant (Fig. 5B).

Recovery of myocardial function. LVDP recovered rapidly in both groups, but it was sustained and tended to be higher in the blood-based esmolol arrested hearts compared with STH2 hearts (Fig. 5A); however, the differences were only signiﬁcant (P < 0.05) at 60 min. Interestingly, LVEDP in both groups was identical until 30 min of reperfusion, when STH2-arrested hearts started to show an increase in LVEDP; however, these differences were not signiﬁcant (Fig. 5B).

Myocardial injury. Despite a lower CK leakage after esmolol protection (BE2-CP+W) compared with St Thomas’ Hospital protection (BS-CP+W), the difference was not signiﬁcant (Table 2).

DISCUSSION

The present study has demonstrated that: (i) a modiﬁed blood perfusion circuit for rat hearts is suitable to conduct more physiological perfusion studies, saving on animal usage and experimental costs, (ii) blood-based esmolol cardioplegia has an equivalent (at least) efﬁcacy to an established hyperkalaemic cardioplegic solution (St Thomas’ Hospital solution), (iii) blood-based esmolol cardioplegia is effective over prolonged ischaemic periods with an extended duration between intermittent infusions.

<table>
<thead>
<tr>
<th>Study 1</th>
<th>LVDP (mmHg)</th>
<th>Recovery (%)</th>
<th>LVEDP (mmHg)</th>
<th>Recovery (%)</th>
<th>Heart rate (beats/min)</th>
<th>Recovery (%)</th>
<th>PP (mmHg)</th>
<th>Recovery (mmHg)</th>
<th>CK leakage (IU/gm wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-GI</td>
<td>141 ± 6</td>
<td>2 ± 2</td>
<td>4.6 ± 0.3</td>
<td>157 ± 39</td>
<td>205 ± 11</td>
<td>30 ± 17</td>
<td>42 ± 3</td>
<td>198 ± 42</td>
<td>317 ± 40</td>
</tr>
<tr>
<td>M-BE</td>
<td>147 ± 7</td>
<td>78 ± 4</td>
<td>4.2 ± 0.4</td>
<td>9 ± 3</td>
<td>217 ± 13</td>
<td>93 ± 5</td>
<td>46 ± 2</td>
<td>84 ± 11</td>
<td>82 ± 15*</td>
</tr>
<tr>
<td>M-BS</td>
<td>145 ± 4</td>
<td>68 ± 6</td>
<td>4.3 ± 0.4</td>
<td>11 ± 4</td>
<td>216 ± 13</td>
<td>89 ± 5</td>
<td>41 ± 4</td>
<td>93 ± 22</td>
<td>44 ± 6*</td>
</tr>
<tr>
<td>M-CE</td>
<td>141 ± 4</td>
<td>84 ± 1</td>
<td>4.2 ± 0.4</td>
<td>7 ± 2</td>
<td>222 ± 7</td>
<td>93 ± 5</td>
<td>38 ± 1</td>
<td>55 ± 3</td>
<td>71 ± 8*</td>
</tr>
<tr>
<td>M-CS</td>
<td>143 ± 7</td>
<td>77 ± 2</td>
<td>4.2 ± 0.3</td>
<td>4 ± 1</td>
<td>207 ± 9</td>
<td>92 ± 8</td>
<td>45 ± 3</td>
<td>54 ± 6</td>
<td>93 ± 17*</td>
</tr>
</tbody>
</table>

Study 2

BE3-CP: 112 ± 3 29 ± 12 5.4 ± 0.5 49 ± 15 234 ± 9 55 ± 18 40 ± 2 128 ± 13 148 ± 72
BE2-CP: 116 ± 7 66 ± 4 4.8 ± 0.4 10 ± 2 224 ± 8 90 ± 2 42 ± 2 101 ± 17 33 ± 8
BE2-CF: 121 ± 5 48 ± 14 5.2 ± 0.6 28 ± 8 230 ± 5 73 ± 9 41 ± 3 100 ± 19 70 ± 14
BE2-CP+W: 117 ± 4 72 ± 2 5.1 ± 0.4 4 ± 1 217 ± 15 96 ± 5 39 ± 3 47 ± 3 33 ± 6

Study 3

BE+W: 137 ± 8 70 ± 4 4.1 ± 0.4 11 ± 3 202 ± 5 91 ± 8 41 ± 2 66 ± 7 114 ± 55
BS+W: 146 ± 6 47 ± 10 4.1 ± 0.2 30 ± 20 209 ± 10 80 ± 17 44 ± 3 133 ± 30 72 ± 11

Table 2: Baseline values, and recovery at 60 min reperfusion for LVDP, LVEDP, heart rate and perfusion pressure (PP). In addition, total CK leakage at the end of 60 min reperfusion is also shown. *P < 0.05 vs C-GI

Blood perfusion system

Buffer-perfused isolated heart preparations have certain advantages, such as low cost, simplicity, the ease with which experimental conditions can be precisely controlled and manipulated, and avoidance of thrombotic complication [10]. However, the absence of haemoglobin greatly limits the oxygen-carrying capacity of crystalloid solutions and necessitates supranormal coronary flow rates with supranormal pO2 levels in buffer-perfused preparations to support normal cellular respiration, which would elevate capillary hydrostatic pressure. The combination of lower perfusate oncotic pressure, caused by an absence of plasma proteins and elevated capillary hydrostatic pressure will shift the normal equilibrium of Starling forces and lead to increased oedema formation [9].

Therefore, various blood perfusion preparations have been established [9]. Blood perfusion with a support animal for oxygenation offers a more stable preparation, with excellent pressure development, signiﬁcantly less oedema and a coronary flow rate (2–3 ml/min) that is much closer to the physiological range than is the case for a buffer-perfused preparation [10]. However, using whole blood as perfusate can lead to a possible mismatch in blood groups with a consequent thrombus formation and an inability to control biochemical and hormonal variables, as well as side efﬁcts of anaesthetic drugs derived from the support animal. An alternative is to use an erythrocyte-enriched perfusate, but this preparation also includes the possibility of adverse immunological reaction to blood from another species and the lack of leucocytes in the perfusate.

In the present study, we established a blood perfusion system that uses the heart and blood from the same animal, with dilution of the blood by 50% to achieve sufﬁcient volume and also to mimic the situation during CPB. Although the lower haematocrit levels (~20%) may be a concern, studies [11] have shown
that perfusion at a haematocrit of 25% had similar beneficial effects to a haematocrit of 40% regarding coronary flow rate, oxygen consumption, ventricular function and tissue oedema development. It is also possible that perfusate recirculation will result in haemolysis and accumulation of free haemoglobin due to the unphysiological action of the roller pump. In our preliminary studies, potassium levels (which correlate highly with free haemoglobin levels) were monitored throughout the experimental duration and shown to remain <6.0 mmol/l. As a result, this system was able to avoid the complications arising from the use of a support animal, the possibility of immunological interaction between species, and the lack of leucocytes and plasma.

**Myocardial protection with esmolol**

Esmolol is a cardioselective (β₁:β₂ of 33:1) ultra-short-acting β-blocker with a short half-life of around 9 min [12], and this characteristic enables its use in acute situations such as cardiac surgery without prolonged negative inotropic effects. Esmolol attenuates the extent of myocardial injury during ischaemia and reperfusion; possible mechanisms include reduced myocardial oxygen consumption [13], ischaemic anaerobic metabolism [14] and an attenuation of release of oxygen-free radicals [15]. A number of clinical studies [4–6] demonstrated the clinical usefulness of esmolol for cardioprotection during coronary artery bypass surgery. A high-dose bolus of esmolol (equivalent to ~300 mmol/l) was injected into the aortic root perfusion line and a subsequently maintained continuous coronary perfusion with esmolol-enriched (at ~20 µmol/l) blood ensured a reduced heart rate (termed as ‘suitable cardiac surgical conditions’) to allow coronary revascularization. Myocardial protection was slightly improved compared with cold Bretschneider cardioplegia (Custodiol®, Dr F. Kohle Chemie, Germany), with respect to less structural damage (from biopsies) and reduced post-operative inotropic support. This method has the disadvantage that it does not induce cardiac arrest and hence visualization of the operating field is compromised by continuous blood flow. There have also been a few case reports where high-dose esmolol was used to arrest the heart during surgery [16, 17]. Thus, Pirk and Kellovsky [16] used bolus doses of esmolol (of 150–760 mmol/l) into the aortic root to induce arrest for 10–20 min without the application of the crossclamp for distal vein graft anastomoses. These esmolol doses are significantly higher than that required to induce arrest; studies by Ede et al. [18] and from our laboratory [7] have shown that 1.0–1.4 mmol/l esmolol induces complete mechanical arrest in isolated crystalloid-perfused rat hearts. Subsequent characterization studies of the mode of action of esmolol at the cellular level [19] demonstrated that esmolol acts as a blocker of both the fast Na-channels and the L-type Ca-channels (at an EC50 of 0.46 and 0.17 mmol/l, respectively). Our studies [7, 8] have also demonstrated that the use of intermittent infusions of esmolol (at 1 mmol/l) provides excellent
myocardial protection. A limitation of these studies was the crystalloid perfusion solution and its unphysiological effects on the preparation [10]. Consequently, it was important to establish whether intermittent esmolol infusions could be used in the more clinically relevant situation of blood perfusion, and using a blood-based esmolol cardioplegia solution, where increased esmolol metabolism might be expected. Esmolol is rapidly hydrolyzed at its ester link, mediated primarily via blood esterases, with a half-life of ~9 min [20]. The present study demonstrated that there appears to be only limited interaction between esmolol metabolism and its cardioprotective efficacy during blood perfusion, since intermittent blood-based high-dose esmolol infusion induced a similar cardioprotective effect to that of crystalloid esmolol cardioplegia. Studies on species differences in metabolic rate of esterase activity show that the rat is significantly faster than the human [20, 21], suggesting that, in a clinical situation, the duration between intermittent infusions could be extended.

In our previous study [7] using crystalloid-perfused rat hearts (with the associated increased coronary flows), esmolol was shown to have an optimal arrest concentration of 1.0 mmol/l; at the CF infusion rate of 14 ml/min this was equivalent to an esmolol load of 4.6 mg/min to arrest the heart within 1 min; in addition, at a CP infusion (at 45 mmHg, a flow rate of 8 ml/min) 2.7 mg/min were required. Ede et al. [18] showed that, at CF infusion of esmolol of 1.36 mmol/l (~1.8 mg/min) arrest was achieved in 5 min, and for arrest within 60 s, 1.7 mmol/l infusion (~2.8 mg/min) was required. In the blood perfusion preparation of this study, where the flow rate was lower and more physiological, a concentration of 1.0 mmol/l failed to arrest the heart; however, arrest was obtained with an optimal arrest dose of 3.0 mmol/l (3.0 mg/min). It would appear, therefore, that a relatively narrow window of esmolol dose (between ~6 and 9 mg total esmolol/gm of heart tissue) may be required to provide an optimal protective effect of esmolol.

LIMITATIONS

We concede that these studies were conducted in rat hearts perfused throughout by means of CF, a relatively non-physiological perfusion technique that may have affected recovery of function.

It is possible that esmolol may have adverse systemic effects that would not be revealed in our study; this would require investigation in the intact animal. Previous studies have used esmolol clinically during relatively long-term continuous infusions (albeit at lower concentrations), with no reports of any systemic adverse effects. Hence, we speculate that multiple, short-, high-dose infusions are unlikely to have major systemic effects, although this would need verification.

Myocardial ischaemic disease is a multifactorial process; there is a spectrum of injury that affects the method of myocardial protection. Hearts used in this study were taken from healthy rats, and it is likely that any protective effect of esmolol would be different in jeopardized hearts suffering from ischaemic injury or disease. In addition, any such hearts are likely to require prolonged periods of ischaemia to correct the lesion, and the ischaemic duration described in this study is relatively short.

Another problem may relate to the relative inconvenience of regular re-infusions of cardioplegia that could interfere with surgery. It is possible that moderate hypothermia would allow less frequent infusions and maintain or improve the efficacy of esmolol cardioprotection. Further studies are warranted and are currently underway.

In this study, six hearts/group were used; many previous studies have demonstrated the suitability of this group size, which represents a balance between sufficient hearts/group to demonstrate appropriate differences and reducing animal usage.

CONCLUSION

In a physiological blood-perfusion system, either a blood-based or crystalloid esmolol cardioplegia provided myocardial protection that was equivalent to that of St Thomas’ Hospital cardioplegia. Esmolol cardioplegia may provide an efficacious alternative to conventional hyperkalaemic cardioplegic solutions.

Funding

Masahiro Fujii was a visiting Clinical Research Fellow from the Department of Cardiovascular Surgery, Nippon Medical School, Tokyo, Japan. Masahiro Fujii's salary was funded by Nippon Medical School, Japan. Consumable costs were from independent departmental (Cardiac Surgical Research) funds, and a small donation from Senko Medical Instrument Mfg. Co. Ltd, Tokyo, Japan.

Conflict of interest: none declared.
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