Timing of steroid treatment is important for cerebral protection during cardiopulmonary bypass and circulatory arrest: minimal protection of pump prime methylprednisolone

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Received 22 October 2002; received in revised form 28 February 2003; accepted 12 March 2003

Abstract

Objectives: The contact of cardiopulmonary bypass surface and patient’s blood activates systemic inflammatory response which aggravates ischemia-reperfusion injury. This study evaluates the effects of cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) on cerebral protection using different steroid administration protocols. Methods: Eighteen (n = 6/group) 4 week-old piglets were divided in three groups. Methylprednisolone (30 mg/kg) was administered intravenously 4 h prior to CPB in Group I, or added in pump prime in group II. Group III received no steroid. All animals were cooled to 15°C followed by 100 min of DHCA, then rewarmed over 40 min and sacrificed 6 h after CPB. Post-operative weight gain, bioelectrical impedance, colloid oncotic pressure (COP) and interleukin-6 (IL-6) were evaluated. Determination of cerebral trypsin blue and immunohistochemical assays of transforming growth factor (TGF)-β1 and caspase-3 activities were performed. Results: Post-operative % weight gain (13.0 ± 3.8 (I) versus 26.4 ± 9.9 (II) versus 22.6 ± 6.4 (III), P = 0.02); % bioimpedance reduction (14.5 ± 8.0 (I) versus 38.3 ± 13.3 (II) versus 30.5 ± 8.0 (III), P = 0.003); mean COP (mmHg) (14.9 ± 1.8 (I) versus 10.9 ± 2.0 (II) versus 6.5 ± 1.8 (III), P = 0.0001) and systemic IL-6 levels (pg/ml) (208.2 ± 353.0 (I) versus 1562.1 ± 1111.4 (II) versus 1712.3 ± 533.2 (III), P = 0.01) were significantly different between the groups. Spectrophotometric analysis of cerebral trypsin blue (ng/g dry weight) was significantly different between the groups (0.0053 ± 0.0010 (I) versus 0.0096 ± 0.0026 (II) versus 0.0090 ± 0.0019 (III), P = 0.004). TGF-β1 scores were 3.3 ± 0.8 (I) versus 1.5 ± 0.8 (II) versus 1.5 ± 0.5 (III), P < 0.05. Remarkable perivascular caspase-3 activity was observed in groups II and III. Conclusion: Different timing of steroid administration results in different inflammatory mediator response. Steroid in CPB prime is not significantly better than no steroid treatment, while systemic steroid pre-treatment significantly decreases systemic manifestation of inflammatory response and brain damage. © 2003 Elsevier Science B.V. All rights reserved.

Keywords: Cardiopulmonary bypass; Cerebral ischemia; Inflammation; Pediatrics

1. Introduction

Despite significant improvements in the technology of cardiopulmonary bypass (CPB), the contact of blood elements and current synthetic materials still creates intensive inflammatory response [1,2]. While systemic inflammation affects many organ systems, it may not necessarily cause permanent dysfunction in itself. For instance, remarkable swelling of the brain has been documented by early post-operative magnetic resonance imaging in adult patients undergoing coronary artery bypass grafting [3]. Although no obvious associated neurological deficits have been reported in this cohort, a spectrum of neurological injury had been reported following cardiac surgery [4]. Therefore, this seemingly benign inflammatory response may aggravate other mechanisms of brain injury in the presence of ischemia-reperfusion. During pediatric cardiac surgery, the extreme manipulations of temperature and blood flow have been reported to be a major cause of inadequate cerebral perfusion causing perioperative neurological injury, in addition to air embolism and debris [4]. An effective control of the systemic inflammatory response during CPB may greatly ameliorate end-organ damage...
following ischemia-reperfusion injury. Previous study suggested that systemic steroid administration provided superior neuro-protective effects during prolonged deep hypothermic circulatory arrest (DHCA) [5]. Yet, the effects of various timing of steroid administration compared with a control group and the correlation with systemic inflammatory response have not been determined. This piglet study of prolonged DHCA was designed to evaluate the cytokine response, the systemic manifestation of inflammatory reaction and the cerebral effects of two steroid administration protocols used clinically, compared with a control group without steroids.

2. Materials and methods

2.1. Surgical Instrumentation

Four week-old sedated Yorkshire piglets were intubated and mechanically ventilated with pressure-controlled ventilator (ADS1000, Engler Engineering Corporation, Florida, FL) to achieve a normal range pCO2 (∼40 mmHg), pH (∼7.4) and oxygenation (pO2 > 100 mmHg). General anesthesia was maintained by continuous intravenous fentanyl (25 µg/kg per h), midazolam (0.2 mg/kg per h) and pancuronium (0.2 mg/kg per h) throughout the entire experiment, except during the period of DHCA. Esophageal and rectal temperatures were maintained normothermic (37.5–38°C) during pre- and post-operative period.

All animals received humane care in compliance with the European Convention on Animal Care and the institutional guidelines of McGill University. The surgical procedures were carried out in a sterile fashion. A 3 Fr. catheter was inserted through a superficial right femoral artery branch for continuous arterial blood pressure monitoring. The left femoral artery was simultaneously exposed in preparation for arterial cannulation. Central venous pressure (CVP) was monitored with a catheter in the right atrium. A right anterolateral thoracotomy was performed to expose the right atrial appendage. After systemic heparinization (300 IU/kg), an 8 Fr. arterial cannula (Medtronic Bio-Medics, Minneapolis, MN) and a 24 Fr. venous cannula (Stöckert Instrumente Gmbh, Lilienthalalle, Germany) were inserted into the left femoral artery and right atrium, respectively.

2.2. Cardiopulmonary bypass

The CPB circuit consisted of a roller pump and a membrane oxygenator (Lilliput 1, Dideco, Mirandola, Italy) primed with heparinized whole blood obtained from another donor harvested on the same day, cefazolin sodium (25 mg/kg), furosemide (0.25 mg/kg), and sodium bicarbonate (10 mEq). The hematocrit (Hct) in the pump prime was maintained at 25% with Ringer’s Lactate if necessary. Management of CPB was identical in all animals using pH-stat strategy and a flow rate of 100 ml/kg per min. Furosemide (0.2 mg/kg), mannitol (0.5 g/kg), and sodium bicarbonate (10 mEq) were administered into the pump prior to reperfusion.

2.3. Experimental groups

Eighteen (n = 6/group) piglets were divided into three groups. In group I, all animals received a single dose of intravenous methylprednisolone sodium succinate (MPS) (30 mg/kg) 4 h prior to CPB. In group II, MPS (30 mg/kg) was given only in the pump prime. In group III, no steroid was given.

2.4. Experimental conditions and postoperative management

All animals underwent 30 min of CPB cooling (100 ml/kg per min) to an esophageal temperature of 15°C, followed by 100 min of DHCA. Hypothermia was maintained by surface cooling. Each animal was rewarmed over 40 min with a maximal perfusate temperature of 37.5°C to achieve an esophageal and rectal temperatures at least 35°C prior to weaning off CPB. Mechanical ventilation was restarted 10 min before weaning from CPB. Once hemodynamic stability was confirmed, each animal was weaned off CPB, decannulated and intravenous protamine (5 mg/kg) was administered. All incisions were closed in a sterile fashion and the core temperature (37.5–38°C) was maintained by a warming blanket and/or heating lamp.

Post-operatively, all animals were monitored, sedated, paralyzed and mechanically ventilated for 6 h after CPB. Ventilator settings were adjusted to resume normal physiological pCO2, pH and pO2 using FiO2 of 1.0. Post-operative CVP of 10 mmHg with Hct of 27% was maintained by either crystalloid solution or blood transfusion. Dopamine was titrated between 5 and 10 µg/kg per min when indicated in the presence of pulmonary edema, hemodynamic instability and/or blood gas deterioration. Hemodynamic parameters including heart rate, CVP, arterial blood pressure and electrocardiogram were prospectively recorded. All experiments were electrically terminated at 6 h after CPB and the brain was perfusion-fixed in situ with 4% paraformaldehyde for further analysis.

2.5. End point measurements

In addition to the hemodynamic parameters recorded, the manifestation of systemic inflammatory response, and the severity of brain injury from different steroid administration protocols were evaluated.

2.6. Systemic effects

2.6.1. Body weight

Fluid retention was assessed by change in body weight
recorded pre-operatively and repeated post-operatively at 6 h after CPB. It was expressed as % increase from baseline.

2.6.2. Bioelectrical impedance index (BEI)

The BEI was calculated by the resistance and reactance measured by a bioimpedance analyzer (BIA-101Q RJL Systems, Inc., Clinton Twp., MI) at baseline and 6 h after CPB, as previously described [5]. The changes in total body water content (TBWC) were estimated by the % decrease in BEI. Bioimpedance has a reciprocal relation with TBWC (TBWC/height²/BEI) because of the higher conductivity of water in the biological system.

2.6.3. Colloid oncotic pressure (COP)

The COP was measured at baseline, immediately and at 6 h after CPB. The blood COP was measured by a membrane colloid osmometer (Wescor 4420, Wescor, UY).

2.6.4. Systemic interleukin-6 (IL-6) level

The marker for systemic inflammatory reaction was evaluated by immunoassay of systemic IL-6 level measured at baseline and 6 h after weaning CPB. Five milliliter of plasma was frozen at −80°C and batched for analysis in a blinded fashion. Quantitative determination of IL-6 was performed by a ‘sandwich’ enzyme-linked immunosorbent assay using specific porcine monoclonal antibodies (Quantikine P kit, R&D Systems, Minneapolis, MN) [6]. The IL-6 concentration was calculated against a standard curve in pg/ml.

2.7. Cerebral effects

2.7.1. Cerebral trypan blue content

Cerebral trypan blue (=1 ml/g of brain tissue) was infused to evaluate the neurovascular permeability of the blood–brain barrier (BBB). Consistent areas of the cortex were submitted for spectrophotometric analysis (BIO-RAD Microplate Reader model 3550, Baltimore, MA) in a blinded fashion. The mean values in ng/gm of dry weight were used for statistical analysis.

2.7.2. Transforming growth factor (TGF-β1)

The immunohistochemical TGF-β1 expression in the brain was scored in a blinded fashion from 1 to 4 scale (1 = minimal expression, 4 = abundant expression as sham brain specimen).

2.7.3. Caspase-3 assay

The apoptotic index of brain injury was assessed by immunohistochemical caspase-3 fluorescent staining in a blinded fashion [5]. This was carried out using polyclonal antibody recognizing the active 17 kDa caspase-3 fragment (New England BioLabs, cat. # 96615).

2.8. Statistical analysis

All results are expressed as mean or % change from baseline ± standard deviation. Analysis of variance (ANOVA) and Bonferroni correction were used for multiple comparisons of continuous data. Non-parametric Mann–Whitney rank sum test was used when continuous or normal distribution cannot be assumed. A P-value < 0.05 was considered statistically significant.

3. Results

3.1. Operative results

The hemodynamic and blood gas parameters for each group were maintained within physiological range without significant differences throughout the experiment (Table 1). Post-operatively, all group I animals were hemodynamically stable. However, one animal in group II developed rapid deteriorating hypotension, tachycardia despite adequate CVP. Drainage of ascites (400 ml) was performed at a near-arrest state. A second animal in group II developed hypotension, bradycardia and tense abdomen despite Dopamine and required drainage of ascites (450 ml). A similar event occurred in one group III animal requiring ascites drainage and Dopamine. Two other group III animals developed hypotension despite adequate CVP and were not suitable for Dopamine treatment because of severe tachycardia. They were improved by ascites drainage (400–675 ml) post-CPB. One last animal in group III came off CPB hypotensive requiring Dopamine alone.

3.2. Changes in body weight

There were no differences in the pre-operative body weight in all three groups (7.4 ± 1.1 kg (I) versus 6.9 ± 0.8 kg (II) versus 7.5 ± 0.6 kg (III), P = NS). At 6 h after CPB, the % increase in body weight, including the drained ascites, was significantly different between the three groups, P = 0.02 by ANOVA (Fig. 1).

3.3. Total body water content

There were no differences in BEI among the groups pre-operatively (175.2 ± 13.4 Ω (I) versus 169.1 ± 19.0 Ω (II) versus 191.7 ± 20.5 Ω (III), P = NS). At 6 h, the % decrease in BEI was statistically significant among the three groups, P = 0.003 by ANOVA (Fig. 2).

3.4. Colloid oncotic pressure

There were no significant differences in baseline COP among groups. Early changes in the COP immediately and 6 h after CPB were significantly different among the three
groups with \( P = 0.002 \) and \( P = 0.0001 \), respectively by ANOVA (Fig. 3).

### 3.5. Systemic IL-6 level

IL-6 levels were undetectable at baseline in all groups. At 6 h after CPB, there was a significant difference in the IL-6 levels between three groups, \( P = 0.01 \) by ANOVA (Fig. 4).

### 3.6. Cerebral trypan blue content

Quantifications of cerebral trypan blue showed a significant difference between the three groups at 6 h after CPB, \( P = 0.004 \) by ANOVA (Fig. 5).

### 3.7. Cerebral TGF-\( \beta_1 \) score

The mean scores between each individual group were analyzed by Mann–Whitney rank sum test and significant differences were noted between groups I versus II and I versus III (Fig. 6).

### 3.8. Caspase-3 assay

Immunohistochemical analysis of caspase-3 in the brain revealed remarkable fluorescent stainings in the perivascular parenchymal brain tissue in groups II and III (Fig. 7).

Table 1

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>( P )-values</th>
</tr>
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<tbody>
<tr>
<td><strong>MAP (mmHg)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>73.5 ± 7.1</td>
<td>74.8 ± 5.9</td>
<td>74.3 ± 8.9</td>
<td>NS</td>
</tr>
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<td>60.2 ± 15.0</td>
<td>66.2 ± 18.2</td>
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<td>NS</td>
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<td>6 h post-CPB</td>
<td>75.0 ± 8.6</td>
<td>75.2 ± 11.2</td>
<td>73.4 ± 12.6</td>
<td>NS</td>
</tr>
<tr>
<td><strong>CVP (mmHg)</strong></td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
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<td>8.0 ± 1.7</td>
<td>7.2 ± 1.8</td>
<td>NS</td>
</tr>
<tr>
<td>6 h post-CPB</td>
<td>8.8 ± 1.9</td>
<td>10.7 ± 1.8</td>
<td>9.8 ± 2.2</td>
<td>NS</td>
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<tr>
<td><strong>Hct (%)</strong></td>
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<tr>
<td>Baseline</td>
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<td>26.2 ± 1.2</td>
<td>27.2 ± 2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Cooling on CPB</td>
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<td>26.0 ± 0.9</td>
<td>26.0 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Rewarming on CPB</td>
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<td>26.3 ± 1.9</td>
<td>27.3 ± 2.9</td>
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</tr>
<tr>
<td>6 h post-CPB</td>
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<td>26.5 ± 2.0</td>
<td>28.0 ± 2.6</td>
<td>NS</td>
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<tr>
<td><strong>pCO2 (mmHg)</strong></td>
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<tr>
<td>Baseline</td>
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<td>41.3 ± 6.3</td>
<td>43.0 ± 3.7</td>
<td>NS</td>
</tr>
<tr>
<td>6 h post CPB</td>
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<td>40.7 ± 6.1</td>
<td>48.0 ± 11.1</td>
<td>NS</td>
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<tr>
<td><strong>pO2 (mmHg)</strong></td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>552.3 ± 76.2</td>
<td>531.8 ± 67.9</td>
<td>662.0 ± 175.2</td>
<td>NS</td>
</tr>
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<td>6 h post-CBP</td>
<td>468.5 ± 100.3</td>
<td>299.3 ± 120.1</td>
<td>310.0 ± 203.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

CPB = cardiopulmonary bypass; MAP = mean arterial pressure; CVP = central venous pressure; mmHg = millimeter of mercury; Hct = hematocrit; and NS = not statistically significant.
4. Discussion

Interactions between the circulating blood and the artificial synthetic surface of the CPB circuits induce complex humoral and cellular activations [1,2]. These ‘inflammatory reactions’ represent a defensive mechanism against bleeding, thrombosis and invasion by alien organisms and substances. On the other hand, they may cause extensive disturbances in capillary permeability, vascular tone, fluid balance, coagulopathy and end-organ dysfunction. During the early era of CPB, high doses of steroids were administered to improve postoperative hemodynamic stability and subsequent survival [7]. However, with the current refinement of CPB technology, steroid is rarely needed following routine cardiac surgery. This trend attests to the fact that systemic inflammatory response, in itself, is relatively well tolerated clinically. Yet, when a prolonged bypass time is expected and extreme manipulations of blood flow and temperature are used during complex cardiac surgery, especially in the newborn, many centers still use steroids to ameliorate the associated inflammatory reaction. This practice is supported by recent scientific literature that documents the activation of various pro-inflammatory mediators following CPB [8,9]. Indirect evidence further suggested that this inflammatory response was not without potential impurity and may affect all organ systems, including the brain. Magnetic resonance imaging in patients undergoing routine CPB without exposure to profound hypothermia or circulatory arrest revealed substantial diffuse brain swelling, with loss of normal appearance of the sulci and gyri in all patients studied [3]. This was attributed to increased capillary permeability in the BBB following CPB. Most recently, many investigators further demonstrated that different approaches such as aprotinin, heparin-coated CPB circuits and steroids could ameliorate the activation and release of various pro-inflammatory mediators while increase other anti-inflammatory cytokines along with improved clinical outcome [10–12].

While the consequences of isolated inflammatory response can be rather subtle, we hypothesized that it could aggravate the detrimental effect of other ischemia-reperfusion injury induced by DHCA. The molecular mechanisms that are relevant include induction of adhesion receptor expression at the endothelial surface, alterations in the procoagulant-anticoagulant balance promoting thrombus formation, oxidant stress that directly injures cells or indirectly promotes inflammatory upregulation, loss of protective second messenger cyclic nucleotide (cAMP and cGMP) systems and complement activation [13].
common manifestations result in leucocyte infiltration, thrombosis, edema and vasoconstriction.

This study was designed to evaluate the systemic and cerebral effects of 2 steroids administration protocols used clinically compared with a control group in a setting of prolonged DHCA. The systemic manifestations of inflammatory response reflected by the different extent of fluid retention and gradual diminution of COP consistent with capillary leak syndrome were most severe in animals receiving steroids in the pump prime or no steroids. The BEI has been validated and shown to correlate with fluid retention in patients undergoing CPB [14]. This technique measures the resistance of the body to weak alternating current (50 kHz). The change in resistance is inversely proportional to the change in TBW due to the higher conductivity of water in the body. Similarly, the change in permeability of the neurovasculature in the BBB reflected by significantly higher cerebral trypan blue content in groups II and III suggested that steroid in the pump prime had minimal protective effect in the brain compared with systemic steroid pre-treatment. Trypan or Evans blue infusion which normally has minimal extravasation across an intact neurovasculature has been used to demonstrate vasogenic brain edema in various experimental models of brain injury [15,16]. In addition, these manifestations of capillary leak correlated with the inflammatory mediator response which was significantly higher in groups II and III. IL-6 levels were known to peak between 1 and 6 h and remained significantly elevated for up to 48 h post-CPB [12]. This current finding strongly links the role of systemic inflammatory reaction in contributing to the capillary leak syndrome and subsequently translated to severe end-organ damage following ischemia-reperfusion injury. Effective steroid therapy was able to reduce inflammatory response and better protect the brain following prolonged ischemia as suggested by better preservation of the neuroprotective and angiogenic TGF-β3 expression and less activation of the apoptotic caspase-3 in the steroid pre-treated animals.

The reduction of pro-inflammatory mediator release following steroid pre-treatment after CPB was well documented [10,11]. Among these mediators, IL-6, 8 and tumor necrosis factor-α were favorably reduced while IL-10, an anti-inflammatory mediator, was increased by steroid administration. Of interest, in the great majority of such beneficial findings, the steroids were administered into the patients prior to CPB. Nevertheless, how exactly these complex inflammatory mediators affect the organ function and the biological consequences remain unclear. Naturally, one may argue that the change in biochemical mediators may not necessarily have any functional consequence. Data that document the specific organ-protective effects of steroid pre-treatment are much less evident. In a neonatal rat model of hypoxic-ischemic injury created by carotid ligation, almost complete absence of cerebral infarction has been reported if the experimental animals were pre-treated with steroids 6 h prior to injury [17]. In a study using dogs subjected to normothermic CPB, Taylor et al. have shown a consistently lower level of total creatine kinase enzyme and its B-isoenzyme levels in the cerebral spinal fluid if the experimental animals were pre-treated with steroids [18]. Although the difference in biochemical markers did not reach statistical significance when compared with control group, this trend was important considering that no ischemic injury was implemented in the experimental protocol. In a piglet model of DHCA, Langley and co-workers have shown that steroid pre-treatment 8 h prior to CPB was associated with significantly better recovery of global and regional cerebral blood flow as well as cerebral oxygen metabolism compared with no steroids [19]. Aebert et al. further correlated inflammatory response to tissue organ damage at cellular level by culturing human umbilical cord endothelial cells with serum samples obtained from patients undergoing CPB [20]. The authors have demonstrated that the proportion of apoptotic endothelial cells was about six times higher in the post-CPB samples than pre-operative baseline samples obtained from the same individuals. In addition, when serum samples from other patients undergoing lung resections or healthy volunteers were used, no apoptotic activity was found in the cultured endothelial cells. This interesting study suggested that some mediators within the serum of post-CPB patients had a strong apoptosis-inducing activity in the endothelial cells and is responsible for the post-operative increase in capillary permeability. Our results concurrently suggested the contributing role of systemic inflammation after CPB in causing end-organ injury following ischemia-reperfusion.

The mechanisms of the anti-inflammatory effect of steroids, as well as its pharmacokinetic, lend further explanation to the observation that steroids given in the pump prime were much less effective than systemic pre-treatment. First, due to its low water solubility, methylprednisolone is pharmacologically esterified with succinic acid producing a water-soluble produg salt. This is rapidly hydrolyzed by carboxylesterase enzymes once in the circulation, yielding the pharmacologically active methylprednisolone. Among patients undergoing CPB, the peak concentrations of methylprednisolone occurred at 1–2 h after administration.

![Fig. 7. Immunohistochemical assays of caspase-3. A = group I, B = group II, C = group III. Note the remarkable parenchymal fluorescent stains in groups II and III signifying apoptotic caspase-3 activation.](https://academic.oup.com/ejcts/article-abstract/24/1/125/375378)
[21], Second, the complex mechanisms involve interactions at multi-cellular levels that will require time to exert its full therapeutic effect [22,23]. After binding to the endothelial cytoplasmic glucocorticoid receptors, translocation of the receptors to the nucleus inhibits the subsequent transcription of adhesive molecules such as endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1 following inflammatory stimulation. Through this receptors translocation mechanism, steroids also increase the transcription of anti-inflammatory genes. A direct inhibitory interaction between activated glucocorticoid receptors and activated transcription factors such as factor-κB and activator protein-1 may suppress the expression of pro-inflammatory genes upon exposure to inflammatory stimulation. Glucocorticoid receptors also interact with cAMP responsive element-binding protein resulting in deacetylation of histone and tighter coiling of DNA. This reduces the access of various transcription factors to their binding sites thereby suppressing gene expression of pro-inflammatory mediators.

These pharmacokinetic and mechanistic insights of steroids actions suggested that steroids need some time to become available to their corresponding cellular receptors to exert their full pharmacological effects. The dogma to presume that all medications will equally be effective by administrating them in the pump prime is questioned by this current study. Instead, the pharmacokinetics and therapeutic mechanisms of each individual agent should be considered when given in the pump prime.

This study demonstrated an interrelation between the modes of steroid administration, the pro-inflammatory response with its systemic manifestation and cerebral effects in a piglet model of prolonged DHCA. Several inherent limitations remain to be answered. First, the potential side-effects and the minimal effective dose of steroid were not determined. However, there is evidence that very high dose of steroid is important in the context of CPB [24]. Our clinical experience in pediatric cardiac surgery, the transplantation experience worldwide and other reports of steroid administration in the literature did not suggest a prohibitive morbidity associated with short-term steroid use in cardiac patients. Second, the current study only evaluated 4 h pre-treatment versus pump prime and control groups. This by no means represents a universal practice used in all centers. Therefore, the exact timing of steroid pre-treatment required to be effective and dose-response study have not been thoroughly worked out by this current experiment, although the pharmacokinetic literature suggested that few hours were most likely necessary. Third, blood transfusion itself might independently elicit a systemic inflammatory response. Yet, most neonatal and infant patients undergoing CPB will inevitably receive blood transfusion in the pump prime. All animals in this study similarly received blood from the CPB that required a constant priming volume. The need for blood transfusion was rare after CPB in our piglet model and one may assume that the exposure to blood product was similar in all groups. Therefore, the differences in subsequent manifestations of ischemia-reperfusion injury could be attributed to the various degree of inflammatory reaction encountered by the different experimental protocols. Fourth ascitic drainage in some of the animals seemed to have created an unequal treatment in the research design. Yet, the post-operative hemodynamic parameters were set to maintain as close to baseline as possible such that the cerebral effects were minimally altered on the basis of hemodynamic instability alone. Therefore, the decision to drain the ascites was used as a last resort to prevent death that, in fact, was a result of the experimental protocol. Finally, 100 min of DHCA was chosen to create a persistent brain injury in the piglet model, rather than a direct transfer of clinical situation. Our previous study showed that 60 min of DHCA resulted in normalization of neurological examination after 4 days post-operatively with mild histological brain damage [25]. Therefore, the data should be interpreted in the context of significant brain injury rather than direct clinical extrapolation in which the threshold for brain injury is different between human being and piglet.

In conclusion, different timing of steroid administration resulted in different systemic inflammatory mediator response. Steroid administered in the pump prime was not significantly better than no steroid, with respect to the systemic manifestation of inflammatory response and cerebral protection. Systemic steroid pre-treatment, on the other hand, was much more effective in suppressing inflammatory response and subsequent protection of the brain following DHCA.

Acknowledgements

This research was supported by the Heart & Stroke Foundation of Quebec, Andy Collins for Kids Foundation and Jonathan-Ballon Award to Dr Shum-Tim. The authors are grateful to Minh Duong and Dianne Murray for their technical assistance and to Dr Robert Platt for his valuable assistance in the data analysis.

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