1. Introduction

Hypothermic cardiopulmonary bypass (CPB) is routinely employed in many congenital cardiac operations performed in neonates, infants and children. Some procedures, particularly those requiring aortic arch reconstruction, are performed using hypothermic circulatory arrest (HCA). Patients subjected to HCA may manifest neurodevelopmental abnormalities for days, months or even years after their cardiac procedure [6,8]. Such changes have been attributed to the hypoxic–ischaemic injury and the vulnerability of the neonatal blood–brain barrier, cerebral white matter and germinal matrix [10]. Selective antegrade cerebral perfusion (SCP) has been employed to provide cerebral blood flow during HCA in aortic arch operations for adults as well as neonates [11,12,17]. While SCP theoretically affords some degree of neuroprotection to the patient, there are limited data to substantiate this claim [2,3,9,14,15]. This study was designed to determine what specific neurobehavioural abnormalities occur, and what neuropathologic changes occur histologically in neonatal piglets subjected to either continuous hypothermic full flow CPB (FF), HCA or SCP.

Hypothermic extracorporeal circulation in immature swine: a comparison of continuous cardiopulmonary bypass, selective antegrade cerebral perfusion and circulatory arrest

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Abstract

Objective: Selective antegrade cerebral perfusion (SCP) has been widely used during complex congenital heart surgery and theoretically affords some degree of neuroprotection. There are limited data to support this claim, however. This study was designed to compare, at profound hypothermia, continuous cardiopulmonary bypass, SCP and circulatory arrest in a survival model of extracorporeal circulation in immature swine.

Methods: Fifteen piglets (5.9 ± 1.1 kg) were placed on cardiopulmonary bypass (CPB), cooled to a rectal temperature of 15 °C and subjected to 90 min of hypothermic circulatory arrest (HCA), selective cerebral perfusion (30 ml kg⁻¹ min⁻¹) (SCP) or systemic full-flow perfusion (FF; 100 ml kg⁻¹ min⁻¹). Piglets were weaned from CPB and extubated. Daily neurologic assessments were performed for 5 days using neurologic deficit scoring (NDS) and overall performance categories (OPC). On postoperative day (POD) 5, all brains were perfusion-fixed and assigned a total histologic score (THS) of neuronal injury by a neuropathologist blinded to the study groups.

Results: The median POD 1 NDS/OPC was 0 (range 0–115)/1 (range 1–2) for FF, 130 (range 0–195)/2 (range 1–3) for HCA and 0 (range 0–30)/1 for SCP. Although there was a trend for the neurologic status in the HCA group to be worse on POD 1, this did not achieve significance, and both NDS and OPC scores for HCA animals normalised by POD 5.

Conclusions: In this survival model of hypothermic extracorporeal circulatory support in immature swine, histologic brain injury was similar in piglets subjected to FF, SCP or HCA. Although the HCA group tended to have worse early neurologic outcome, any difference clearly disappeared by POD 5. These data raise the possibility that profound hypothermia alone during extracorporeal support may produce this observed brain injury. Additional study is required to define the precise aetiology of the brain injury observed in this animal model.

Keywords: Hypothermia/circulatory arrest; Cardiopulmonary bypass (CPB); Cerebral protection

Abbreviations: SCP, selective antegrade cerebral perfusion; HCA, hypothermic circulatory arrest; FF, full flow; CPB, cardiopulmonary bypass; NDS, neurologic deficit scoring; OPC, overall performance categories; POD, postoperative day; THS, total histologic score.

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2. Methods

All animals received humane care in accordance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised in 1996. All experimental protocols were approved by the Institutional Animal Care and Use Committee at The University of Texas Southwestern Medical Centre.

3. Experimental protocol

Fifteen Yorkshire piglets weighing 5—7 kg and 21—24 days of age were sedated with intramuscular ketamine (20 mg kg\(^{-1}\)) and xylazine (4 mg kg\(^{-1}\)) and intubated. Each animal was ventilated with an inspired oxygen fraction of 0.21 at a respiratory rate of 15—18 breaths per minute to achieve a normal pH and PaCO\(_2\). The piglets were ventilated in a pressure-limited, time-cycled mode (model IV-100B, Sechrist Industries, Inc., Midvale, UT, USA) initially at a peak inspiratory pressure of 20 cm H\(_2\)O, a positive and expiratory pressure of 5 cm H\(_2\)O. After intravenous bolus injections of fentanyl (50 \(\mu\)g kg\(^{-1}\)) and pancuronium (0.5 mg kg\(^{-1}\)), general anaesthesia was maintained by continuous intravenous infusion of fentanyl (25 \(\mu\)g kg\(^{-1}\) h\(^{-1}\)), midazolam (0.2 mg kg\(^{-1}\) h\(^{-1}\)) and pancuronium (0.2 mg kg\(^{-1}\) h\(^{-1}\)) during surgery.

All surgical procedures were performed under sterile conditions. For intra-operative monitoring and blood sampling, arterial and venous lines were placed in the right superficial femoral artery and right femoral vein, respectively. A right anterolateral thoracotomy was performed and the chest was entered in the third intercostal space. Following systemic heparinisation (300 IU kg\(^{-1}\)), an 8—10 F arterial cannula (Medtronic BioMedicus, Eden Prairie, MN, USA) and a 20—24 F angled venous cannula (Research Medical, Inc., Midvale, UT, USA) were inserted into the right chest and the wound was closed and dressed in a sterile fashion.

The CPB circuit consisted of a roller pump (Cardiovascular Instrument Corp, Wakefield, MA, USA), membrane oxygenator (Minimax; Medtronic Inc., Anaheim, CA, USA) and sterile tubing with a 40-\(\mu\)m arterial filter (Olson Medical Sales, Inc., Ashland, MA, USA). Fresh whole blood, from a donor pig drawn on the operating room, was transfused into the prime as required to increase the haematocrit value to 30%. Methylprednisolone (30 mg kg\(^{-1}\), sc) was administered into the pump until a rectal temperature of 37°C was reached. The heart was defibrillated as necessary at a pharyngeal temperature of 30°C. Fresh whole blood was transfused into the prime as required to increase the haematocrit value to 30% in all groups during re-warming. After 45 min of re-warming, animals were weaned from CPB, and the arterial and venous cannulas were removed. Protamine (5 mg kg\(^{-1}\)) was administrated intravenously to reverse the effects of heparin. A 12 F chest tube was inserted into the right chest and the wound was closed and dressed in a sterile fashion.

4. Postoperative care and management

Animals remained sedated and paralysed using a continuous intravenous infusion of fentanyl (25 mcg kg\(^{-1}\) min\(^{-1}\)), midazolam (0.2 mg kg\(^{-1}\) min\(^{-1}\)) and pancuronium (0.2 mg kg\(^{-1}\) min\(^{-1}\)) for 12 h postoperatively, after which time they were extubated and the chest tube was removed in the operating room. Animals were transported to a cage where they were observed, fed and mobilised initially at 2—4-h intervals for the first 24 h, then every 6 h thereafter.

5. Neurological evaluation

Neurological and behavioural evaluations were performed at 24-h intervals by a blinded veterinarian for a total 5 days using NDS and OPC as previously described [18].

6. Histological evaluation

6.1. Brain perfusion—fixation protocol

On postoperative day 5, all animals were sedated with intramuscular Telazol 4.4 mg kg\(^{-1}\) IM and intubated.
animal was ventilated with an inspired oxygen fraction of 0.21 at a respiratory rate of 15—18 breaths per minute and maintained under general anaesthesia with inhaled isoflurane 1—3%. A median sternotomy was performed and the common carotid artery dissected free. The common carotid artery was cannulated with an 8—10 F arterial cannula (Medtronic BioMedicus, Eden Prairie, MN, USA) and the common carotid artery was cross-clamped. One litre of 25°C Normosol was infused at pressure of 100 mmHg and 4 l of 4% paraformaldehyde was infused into cerebral circulation at pressure of 150 mmHg. The inferior vena cava was incised and the animal exsanguinated during the perfusion—fixation. The animal was decapitated and the head submerged in 10% formalin for 24 h. The brain was then removed from cranium and stored submerged in 4% paraformaldehyde.

7. Preparation of brain tissue for microscopic examination

The brain was immersed in 4% paraformaldehyde, 4°C for 7 days. The details were previously described [24]. Although the nomenclature for porcine neuroanatomy was less well documented than that of several other commonly used laboratory animals, the designations described by Yoshikawa were used [25]. A standardised list of 24 of the major grey and white matter structures was examined in each animal and rated on an arbitrary scale (Table 1).

8. Statistical analysis

Blood gas data are presented as mean ± standard deviation. Comparisons among groups were made by analysis of variance (ANOVA) for continuous variables with normal distribution. Data for total and regional histologic scores, NDS and OPC are presented as median values with ranges. The Kruskal—Wallis test was used for comparison of medians among groups for the THS, NDS and OPC on POD1. Bonferroni testing was subsequently used for paired comparisons of experimental groups.

9. Results

A total of 19 animals were used. One FF animal was excluded from the study due to low haematocrit before starting CPB. Two DHCA animals died on POD1 and POD2 due to respiratory acidosis. One SCP animal died on POD 0 due to respiratory acidosis. Fifteen animals survived 5 days. No animals showed any preoperative neurologic deficits. In order to achieve rectal temperature of 15°C, inflow temperature was set below 15°C. During the surgical procedure, there were no statistical differences between groups in arterial blood gas values or rectal/oesophageal temperatures ($p = \text{NS}$) (Table 2). Haematocrit values varied from 34.7 ± 8.1% before CPB, 34 ± 1.6% during 15°C, 41.6 ± 3.8% in re-warming and 39.6 ± 2.1% 5 h after CPB.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before CPB</th>
<th>FF/HCA/SCP</th>
<th>Rewarming</th>
<th>5 h after CPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>7.33 ± 0.10</td>
<td>7.27 ± 0.07</td>
<td>7.46 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>HCA</td>
<td>7.35 ± 0.05</td>
<td>7.44 ± 0.04</td>
<td>7.51 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>SCP</td>
<td>7.39 ± 0.12</td>
<td>7.30 ± 0.10</td>
<td>7.42 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.663</td>
<td>0.644</td>
<td>0.787</td>
<td>0.2</td>
</tr>
<tr>
<td>$p_{O_2}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>375 ± 54</td>
<td>333 ± 181</td>
<td>376 ± 112</td>
<td>572 ± 77.5</td>
</tr>
<tr>
<td>HCA</td>
<td>385 ± 188</td>
<td>281 ± 75.6</td>
<td>455 ± 50.3</td>
<td></td>
</tr>
<tr>
<td>SCP</td>
<td>382 ± 80</td>
<td>397 ± 94</td>
<td>227 ± 66.2</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.994</td>
<td>0.513</td>
<td>0.066</td>
<td>0.057</td>
</tr>
<tr>
<td>$p_{CO_2}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>57 ± 19</td>
<td>70.5 ± 17</td>
<td>41.8 ± 17.4</td>
<td></td>
</tr>
<tr>
<td>HCA</td>
<td>55.8 ± 7.0</td>
<td>35.4 ± 4.2</td>
<td>33.3 ± 7.03</td>
<td></td>
</tr>
<tr>
<td>SCP</td>
<td>48.8 ± 18</td>
<td>65.3 ± 16</td>
<td>41.8 ± 10.9</td>
<td>41.6 ± 6.26</td>
</tr>
<tr>
<td>p value</td>
<td>0.688</td>
<td>0.655</td>
<td>0.623</td>
<td>0.2</td>
</tr>
<tr>
<td>HCO$_3^-$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>29.0 ± 4.3</td>
<td>31.7 ± 7.6</td>
<td>29.5 ± 7.1</td>
<td>31.9 ± 1.65</td>
</tr>
<tr>
<td>HCA</td>
<td>28.4 ± 4.4</td>
<td>24.2 ± 2.17</td>
<td>34.1 ± 3.62</td>
<td></td>
</tr>
<tr>
<td>SCP</td>
<td>31.7 ± 3.3</td>
<td>30.5 ± 2.4</td>
<td>27 ± 1.15</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.496</td>
<td>0.78</td>
<td>0.199</td>
<td>0.686</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. CPB: cardiopulmonary bypass; FF: full flow; HCA: hypothermic circulatory arrest; SCP: selective cerebral perfusion.

Table 2
Blood gas analysis during bypass and recovery.

Table 1
Hypoxic—Ischaemic grading scale.

<table>
<thead>
<tr>
<th>Score</th>
<th>Hypoxic—Ischaemic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Not identified</td>
</tr>
<tr>
<td>1</td>
<td>Isolated, individual neurons</td>
</tr>
<tr>
<td>2</td>
<td>Small clusters of affected neurons</td>
</tr>
<tr>
<td>3</td>
<td>Large clusters of affected neurons or laminar necrosis pattern</td>
</tr>
<tr>
<td>4</td>
<td>Diffuse involvement of all of almost all neurons</td>
</tr>
</tbody>
</table>

Hypoxic—Ischaemic changes: nuclear pyknosis, cytoplasmic eosinophilia, and cytoplasmic retraction.
On POD 1, varying degrees of neurologic deficits were recognised in all groups. The median neurologic deficit score (NDS) for the HCA group was 130 on POD 1, while it was 0 in the FF and SCP groups \((p = 0.03, \text{Kruskal—Wallis}; p = \text{NS, Bonferroni})\). The early neurologic deficits observed in the HCA group included hyperventilation, weakness in stretch reflex, positioning and muscle tone, diminished level of consciousness as well as the absence of normal eating and ambulation patterns. Despite the abnormalities evident in this group, all piglets demonstrated normal neurologic behaviour by POD 5. In the FF and SCP groups, four out of five piglets in each group exhibited completely normal neurologic behaviour on POD 1 while all five piglets in these two groups exhibited normal behaviour by POD 2 as depicted in Fig. 2.

Table 3 depicts the median regional histologic scores, NDS and OPC on POD 1. The median THS was 9 (range 0—11) for the FF group, 12 (range 4—14) for the HCA group and 9 (range 0—11) for the SCP group \((p = \text{NS})\). Histopathologic changes were found in all groups. Major findings found in the FF and SCP groups were isolated or small clusters of affected neurons in all brain sections (grade 0—2). Although in two animals of the HCA group, large clusters of necrotic neurons with nuclear pyknosis were found in the neocortex, in all other HCA sections, the neuronal damage was only grade 0—2. In the hippocampus, necrotic cells presenting pyknotic nuclei, swollen eosinophilic cytoplasm and the presence of an inflammatory reaction secondary to injury were found in all groups.

**Fig. 2.** Neurologic deficit score (NDS). Though there was a trend toward worse neurologic scores in HCA animals on POD 1, all piglets had normal neurologic scores by POD 5.

**Fig. 3.** Representative images to illustrate grades of hypoxic–ischaemic injury identified in HCA animals. H&E stain, 400×.

Serious neurologic injury was evident only in the neocortex of HCA animals.

**10. Discussion**

Neonates and infants with congenital heart disease who undergo repair using HCA are at risk for neurologic injury [6]. Possible factors associated with neurologic injury include the depth of hypothermia, the degree of haemodilution, the rate and duration of core cooling, the duration of circulatory arrest, the type of pH management, genetic predisposition [22] and the presence of pre-existing neurologic disease [6,7]. It is well known that prolonged ischaemic injury leads to cell necrosis caused by adenosine triphosphate (ATP) depletion, and that the injury is characterised histologically by the presence of pyknotic nuclei, swollen eosinophilic cytoplasm and the presence of an inflammatory reaction secondary to injury [10]. In an effort to protect the brain from such ischaemic injury, many investigators have reported their clinical and experimental experiences, attempting to define optimal CPB conditions before and after HCA with respect to such variables as degree of haemodilution, temperature and flow rate [19,20].

Alternatively, others have eliminated HCA, and employed antegrade SCP in aortic arch repair. It remains unclear, however, whether SCP truly protects patients from neurologic injury. Goldberg et al. reported that there were no statistical differences in mental development or psychomotor development scores for patients who were assigned to HCA versus SCP at the time of the Norwood operation [7]. Visconti et al. also reported that neurodevelopmental outcomes at 1 year of age were similar for HCA and SCP patients who underwent the Norwood operation [8]. Given these preliminary results, it remains to be determined...
whether the technique of SCP should be utilised in all patients who undergo aortic arch repair. In addition, optimal conditions for SCP, with regard to variables such as pH strategy, flow rate and temperature should be elucidated.

10.1. pH strategy

In their HCA piglet model, Priestly et al. found that pH-stat strategy may be neuroprotective in terms of neurologic outcome and histopathologic findings by increasing cerebral blood flow and cerebral oxygen delivery during CPB [13]. While it is well known that using pH-stat strategy contributes to rapid and uniform brain cooling, Kurth et al. reported an increase in cerebral emboli in their HCA model with this pH strategy, presumably due to increased cerebral blood flow [5]. Duebener et al. reported that pH-stat management increased tissue oxygenation during deep hypothermic bypass and early after circulatory arrest [21]. It remains unclear whether use of pH-stat strategy is superior to alpha-stat strategy during SCP. It is quite possible that increased cerebral blood flow achieved using a pH-stat strategy may in fact result in cerebral hyperperfusion and flow-induced injury during selective cerebral perfusion. In a recent study, Dahlbacka et al. found no significant differences between pH management strategies in cerebral microcirculation during SCP in their neonatal piglet model [23].

10.2. Flow rate during SCP

Various flow rates (10–50 ml kg\(^{-1}\) min\(^{-1}\)) have been used both clinically, and during SCP in piglet experimental models [1,4,16]. DeCampli et al. reported that 50% of piglets undergoing SCP with a flow rate of 40 ml kg\(^{-1}\) min\(^{-1}\) had significant upper torso oedema, metabolic acidosis, and an unstable recovery period, but that none of piglets perfused with 20 ml kg\(^{-1}\) min\(^{-1}\) had such complications [4] suggesting a deleterious effect of increased flow. In contrast to our study, they used an alpha-stat strategy in which there is relatively less cerebral vasodilation (compared with pH-stat strategy). This may have contributed to their findings. It is unclear from our results whether our flow rate (30 ml kg\(^{-1}\) min\(^{-1}\)) at profound hypothermia is neuroprotective or not.

10.3. Temperature

One possible cause for the neuronal damage observed in our model may be profound hypothermia (15 °C). Though at a low rate, the brain continues to consume oxygen at 15 °C. The injury seen in this model was typical of hypoxic–ischaemic injury seen in human neonates. In human congenital heart surgery patients, this injury is strongly associated with young age. In fact, it is reasonably rare in patients over 30 days of age [24]. We speculate that there may be some deleterious effect, as a result of achieving profoundly hypothermic temperatures, which results in or establishes conditions that produce areas of cerebral ischaemia. These deleterious conditions are yet to be defined. While we do know the certain areas of the neonatal brain are more vulnerable to this observed injury, we do not know whether this is the result of locally increased metabolic demand, intrinsically increased vulnerability of certain immature cell types to ischaemia, or deficiencies in local metabolic 'supply' due to, for example, microvascular events. Defining the intra-operative perfusion strategies that optimise metabolic supply and demand for the neonatal brain during the repair of cardiac defects clearly requires further investigation.

11. Study limitations

All animals were subjected to profound hypothermia. Our conclusion that this hypothermia alone may result in the brain injury observed in this model will have to be additionally tested by adding experimental groups subjected to more moderate hypothermia, and even normothermia. This study examined flow strategy, with all other variables constant. Future studies will compare the same flow strategies at various temperatures.

12. Conclusion

In conclusion, in this survival model of hypothermic extracorporeal circulation in immature swine, there was no statistically significant difference in histologic brain injury between those animals subjected to FF, HCA or SCP. Although there was a trend towards worse early clinical neurologic outcome in the HCA group, this difference disappeared by 5 days postoperatively. These data raise the possibility that profound hypothermia alone during extracorporeal support may produce this brain injury. Further study is required to define the precise aetiology of the brain injury observed in this survival animal model.

References


