Hypoxemic reperfusion exacerbates the neurological injury sustained during neonatal deep hypothermic circulatory arrest: a model of cyanotic surgical repair

Edward J. Hickey, Xiaomang You, Vassil Kaimaktchiev, Ross M. Ungerleider

Objective: Deep hypothermic circulatory arrest (DHCA) is frequently used in infants undergoing the Norwood procedure. These infants are necessarily hypoxic after separation from CPB. Considerable energy has been spent characterizing the physiological and histological consequences of DHCA, but these have largely focused on a normoxic period of reperfusion. Furthermore, evidence has accumulated to suggest that the cerebral vascular autoregulatory mechanisms are dysfunctional following DHCA. In particular, the vasodilatation that elevates cerebral blood flow (CBF) in response to hypoxemia is absent. This study therefore aimed to investigate whether post-CPB hypoxemia exacerbates brain injury resulting from DHCA.

Methods: Twelve neonatal piglets were subjected to 2 h DHCA and then separated from CPB. They were then randomized to either: Group 1, normoxic ventilation (n = 5); or Group 2, hypoxemia (n = 7), in which the arterial PaO2 was reduced to 40–50 mmHg for the duration of reperfusion. Following a 20 h period of warm reperfusion, the animals were perfusion fixed and the brain analyzed for histological evidence of injury. Nine additional animals were studied in one of three control groups.

Results: All animals survived the protocol. Post-operative parameters — including mean arterial pressure, acid–base status, inotrope requirements and arterial PaCO2 — were similar. None of the control animals had any evidence of ischemia. Group 1 animals had moderate injury (total score 7.4 ± 1.6). In Group 2, three animals sustained irretrievable brain injury evidenced by gross edema and early liquefactive necrosis. The remaining four had severe ischemic histological changes (score 14.5 ± 1.6, p < 0.03).

Conclusions: Hypoxemic reperfusion after prolonged DHCA results in increased neuronal loss. The use of platelets, endothelial casts and fibrin may occlude the capillary bed [5], and an imbalance in vasoactive mediators (including endothin-1, nitric oxide and eicosanoids) may contribute to dysfunction of cerebrovascular autoregulation [6].

Cerebral blood flow (CBF) is typically influenced by several powerful mediators. Although neurons are especially vulnerable to ischemia, vasodilatation is predominantly initiated through the accumulation of products of metabolism (carbon dioxide and acidic anaerobic products) rather than by arterial oxygen tensions per se. Under normal circumstances, mild hypoxia has minimal influence on CBF because the hemoglobin–oxygen dissociation ensures that tissue oxygen delivery is adequate. However, when arterial oxygen tensions fall severely low (40–50 mmHg) cerebral vasodilatation is triggered, a response that becomes extremely powerful as oxygen delivery becomes increasingly critical [7, 8]. The combined effect of these cerebrovascular mediators is that near-normal energy states can be
maintained within the brain despite exposure to severe hypoxemia [9].

Reflex cerebral vasodilatation is dysregulated following periods of DHCA [10]. Endothelin-1 [11] and thromboxane A2 [12] levels are elevated and production of NO deficient [13]. Responses to both hypercapnia and acidosis are abnormal [14]. Recently, we have demonstrated the hypoxic cerebral vasodilatation response to be reduced or absent following DHCA [15]. In fact, whereas severe hypoxemia normally precipitates large increases in CBF, following DHCA the exact opposite was true and hypoxemia resulted in progressively depressed CBF. This implies that hypoxemia — intentional or otherwise — following DHCA may potentiate ischemic injury more so than might otherwise be expected [15]. Post-operative hypoxemia is an inexorable component of staged repair of congenital heart lesions (for example single ventricle physiology with parallel circulations): arterial oxygen tensions typically in the range between 30 and 50 mmHg following Norwood palliation for hypoplastic left heart syndrome (HLHS).

Because we have previously demonstrated DHCA to disrupt the CBF response to hypoxemia, we investigated whether post-operative hypoxemia influenced neuronal recovery following DHCA. We hypothesized that histological injury would be exacerbated following periods of prolonged DHCA with superimposed hypoxic reperfusion, and tested this using a novel model of aortal-atrial piglet CPB with myocardial protection and post-operative support in a full intensive care environment.

2. Materials and methods

2.1. Experimental design

A total of 21 neonatal piglets (2–5 kg) were studied. Twelve experimental animals were placed on open-chested CPB and subjected to 2 h DHCA at 18 °C before being re-warmed and separated from CPB. They were then supported anesthetized and ventilated, with full invasive monitoring for a further 20 h. During this warm reperfusion period they were ventilated to either maintain normoxic arterial oxygen (Group 1, n = 5) or hypoxic arterial oxygen tensions (PaO2, 40–50 mmHg) (Group 2, n = 7). Animals were then perfusion-fixed and brain tissue extracted for histological and fluoroscopic (Fluoro-Jade™) scoring of neurological injury in the frontal lobe cortex, basal ganglia, hippocampus and cerebellum. A further nine animals were used to generate histological controls: Group 3 (n = 3), 2 h deep hypothermic full-flow CPB (DHFF) followed by hypoxic ventilation; Group 4 (n = 3), 2 h DHFF with normoxic post-bypass ventilation; and Group 5 (n = 3), experimental shams anesthetized and instrumented but not placed on CPB, euthanized 20 h later. The histological controls were used to reduce false-positive scoring and determine whether hypoxemia in the absence of DHCA was instigating detectable injury.

2.2. Surgical procedures

All animal experiments were conducted with the approval of the institution’s Animal Care and Use Committee. The animals received humane care in compliance with the ‘Guide for the Care and Use of Laboratory Animals’ published by the National Institutes of Health (NIH publication 85-23, revised 1995). Briefly, following premedication with Telozole (8 mg/kg) (Baxter Healthcare, IL) and anesthetic induction (0.5% isoflurane) peripheral access was obtained and used to administer a bolus of fentanyl citrate (25 μg/kg) and infusion thereafter (100 μg/h). Surgical tracheotomy was then performed to allow controlled ventilation, maintaining arterial oxygen and carbon dioxide tensions within normal limits. Rectal and esophageal temperature probes were placed for core temperature monitoring. Catheters were then inserted into the right femoral artery and vein for sampling and pressure monitoring.

The heart was exposed via median sternotomy. After instrumentation and systemic heparinization (500 iu/kg), the aortic root and right atrial appendage were cannulated through purse-string sutures (DLP Inc., MI). Normothermic CPB was established at a rate of 100–150 ml/kg min to maintain mean arterial pressures of 50 mmHg. A left ventricular vent was inserted via the apex to ensure adequate decompression. The animal was then perfusion-cooled using a pH-stat strategy to 18 °C over 30 min. CPB was then ceased and the circulatory volume drained into the reservoir, to induce uninterrupted circulatory arrest for 120 min. During this period, myocardial protection was afforded by slowly perfusing through an isolated coronary circulation. An aortic cross-clamp was applied to the ascending aorta immediately distal to the aortic cannula, and soft clips applied to the inferior and superior vena cavae, thereby isolating the coronary circulation. The extracorporeal circuit was then used to circulate 10 ml/min into the coronary arteries via the aortic root. At the end of the designated strategy, full-flow CPB was re-introduced to allow re-warming, maintaining a mean arterial pressure of at least 50 mmHg and with the use of sodium bicarbonate (8.4%) as necessary. Ventilation was then re-established and the animals separated from CPB in conjunction with a continuous infusion of intravenous norepinephrine (0.01–0.10 μg/ kg min) and volume replacement as necessary.

2.3. Cardiopulmonary bypass circuit

The extracorporeal circuit included the following: a Cobe™ Century (Cobe Cardiovascular, CO) roller pump console; an infant oxygenator-reservoir (Capiox®—Baby RX-05, Terumo, Japan); Cobe™ tubing packs including a 3/16 in. internal diameter arterial line and a 1/4 in. venous line. The circuit was primed with blood harvested from an adult donor animal under sterile conditions and general anesthetic on the day of surgery and stored at 4 °C in citrate—phosphate—dextrose until used.

2.4. Post-operative care

Following wean from CPB, protamine was administered (5 mg/1000 iu heparin) and the animals decannulated and hemostasis ensured. After positioning pleural and pericardial drains the sternum and skin were re-approximated. A bladder catheter was inserted via mini-laparotomy and all peripheral wounds closed. A full laboratory intensive care was used to
support the animals, anesthetized (intravenous propofol 3–5 ml/h) and ventilated, with full invasive alarmed monitoring and continuous veterinary support for the entire duration of the reperfusion period. The arterial pressure was maintained with a mean greater than 50 mmHg.

2.5. Post-bypass ventilation

Animals in Group 1 were ventilated in the usual fashion (rate 20–24 min⁻¹, tidal volume 20 ml/kg, FiO₂ 0.4–0.7) aiming to maintain arterial oxygen tensions between 100 and 250 mmHg, by adjusting the inspired oxygen concentration accordingly. In Group 2, lowering the inspired oxygen concentration towards room air (FiO₂ 0.2) was all that was necessary to achieve target arterial oxygen tensions (40–50 mmHg). Blood gas analysis was performed repeatedly to ensure closely controlled levels. Control Group 3 (DHFF, hypoxemia) typically required single-lung ventilation with room air to achieve the target arterial oxygen tensions. Single-lung ventilation was performed by replacing the endotracheal tube with an under-sized tube and advancing it to enter a main bronchus.

2.6. Tissue processing

Prior to euthanasia, the chest was re-opened to expose the great vessels, which were re-cannulated through purse strings. Perfusion-fixation was performed by initially exsanguinating the animal and concomitantly irrigating with 3 l normal saline and 5 ml euthasol. This was immediately followed by 2 l 4% paraformaldehyde which was re-circulated for 20 min using the circuit. The brain was then harvested intact and immersed in cold formaldehyde for 3 days before making coronal sections through the cortex, basal ganglia, hippocampus and cerebellum and embedding in paraffin blocks. Five micrometer slices were mounted on slides and stained with hematoxylin and eosin for histological interpretation.

2.7. Fluoro-Jade™ staining

Fluoro-Jade™ is an anionic fluorochrome that has green iridescence with excitation peak at 480 nm and an emission peak at 525 nm [16]. It selectively stains irreversibly injured neurons with greater sensitivity and specificity than hematoxylin and eosin. It was identified by Schmued et al. who screened a variety of fluorescent anionic dyes in the pursuit of histochemical tracers to label degenerating neurons. The mechanisms by which injured neurons are stained are not known, but it is believed that they exhibit strongly basic properties and therefore render an affinity for the strongly acidic Fluoro-Jade™ dye. Following de-paraffinization, slides were immersed for 20 min in 0.06% potassium permanganate before washing several times in deionized water. Fluoro-Jade™ crystals were dissolved to a 0.0004% solution in 0.01% acetic acid and the slides were immersed for 20 min before thoroughly air-drying in a warm incubator. After further xylene washes, slides were cover-slipped and viewed using an epifluorescent microscope with blue (450–490 nm) excitation light. A barrier filter allowing passage of all wavelengths longer than 515 nm results in a green emission color from cells stained positively with Fluoro-Jade™.

2.8. Histological scoring

Slides were examined randomly, blinded to the experimental protocol. Morphological criteria used to identify ischemic neurons were a prominent eosinophilic nucleus, with lack of a well-defined nucleolus and Nissl substance, together with nuclear fragmentation and formation of dark apoptotic bodies [17]. These are established signs of irreversible neuronal injury and avoid false-positive identification of hyperchromatic neuronal artifact [17]. Qualitative grading scores were applied according to the cerebral cortex, basal ganglia, hippocampus and cerebellum (where 0 = normal and 4 = confluent, severe injury). The hippocampus is recognized to exhibit regional vulnerability. Anatomical subregions (CA1, CA2, CA3, CA4 and superficial, middle and deep dentate gyrus) were selectively scored as repeated studies using our model have demonstrated reproducible hierarchical injury within these areas. Scores were applied to individual areas in addition to yielding cumulative totals for the hippocampus and all brain regions combined.

3. Results

3.1. General parameters

The peri-operative animal variables for the experimental groups 1 and 2 are shown in Table 1. Pre-operatively the animals were similar other than an inexplicable slightly lower pH in the control group. All other operative and CPB variables were similar with no significant differences between the groups. Post-operatively the animals were continuously monitored to minimize differences at any stage (Table 2). Management of the arterial oxygen tensions proved relatively simple, and was kept close to 50 mmHg at all time points in Group 2. Mean arterial pressure was maintained above 50 mmHg at almost all points and was not different between the experimental groups. The hypoxemic animals (Group 2) were more tachycardic at most points, which may be a reflection of a trend toward a slightly more acidic state induced by the hypoxemia (significance not reached). Because exogenous bicarbonate was not administered following wean from CPB, the normalization of acid–base was a useful indicator of satisfactory systemic perfusion. Total inotrope requirements were similar between the experimental groups (Group 1, 122 ± 18; Group 2, 156 ± 32 μg, p = 0.45). All hemodynamic variables amongst the control groups were similar with no significant differences (data not shown).

Particular attention was taken to reduce differences between variables known to affect cerebral outcome. The carbon dioxide tensions were slightly higher in the hypoxemic group, although within normal limits at almost all time points. It was important to prevent hemodilution, especially in Group 2, which in fact displayed a higher hematocrit at all time points. Post-DHCA hyperthermia is another stimulus known to exacerbate neurological injury and therefore every effort was made to ensure that core temperatures remained...
similar between the experimental groups. No significant differences were noted in the histological controls (data not shown).

3.2. Cerebral viability

During brain harvest, it became apparent that three of the hypoxemic animals had suffered irreversible brain injury. Severe cerebral edema precluded satisfactory perfusion fixation or tissue processing for analysis, and early evidence of liquefactive necrosis was present. Histological scores were therefore not generated by these animals. These animals have not been excluded because we believe their cerebral changes to reflect the most extreme end of the injury spectrum, and are therefore highly significant.

3.3. Histological scores of injury

Histological and Fluoro-Jade™ scores for animals within the representative regions and sub-regions are shown in

Table 2
Parameters for the 20 h warm reperfusion period after separation from CPB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
<th>p value 1 vs 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>49 ± 2.6</td>
<td>61 ± 8.8</td>
<td>55 ± 2.6</td>
<td>55 ± 5.1</td>
<td>58 ± 6.0</td>
<td>52 ± 3.1</td>
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<tr>
<td>HR (min⁻¹)</td>
<td>134 ± 6</td>
<td>153 ± 7.3</td>
<td>143 ± 7.3</td>
<td>140 ± 5.8</td>
<td>149 ± 4.7</td>
<td>168 ± 5.4</td>
</tr>
<tr>
<td>pCO₂ (mmHg)</td>
<td>146 ± 59</td>
<td>227 ± 39</td>
<td>222 ± 24</td>
<td>236 ± 30</td>
<td>193 ± 26</td>
<td>202 ± 29</td>
</tr>
<tr>
<td>pH</td>
<td>7.29 ± 0.03</td>
<td>7.36 ± 0.02</td>
<td>7.47 ± 0.02</td>
<td>7.43 ± 0.03</td>
<td>7.49 ± 0.04</td>
<td>7.44 ± 0.04</td>
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<tr>
<td>BE (mmol/l)</td>
<td>-8.3 ± 0.6</td>
<td>-1.8 ± 1.8</td>
<td>-0.9 ± 0.9</td>
<td>-2.8 ± 1.2</td>
<td>3.8 ± 0.7</td>
<td>2.7 ± 1.2</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>32.0 ± 1.2</td>
<td>32.1 ± 1.1</td>
<td>33.1 ± 1.3</td>
<td>33 ± 1.7</td>
<td>32 ± 1.7</td>
<td>36 ± 1.4</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>35.8 ± 0.18</td>
<td>37.3 ± 0.4</td>
<td>37.3 ± 0.3</td>
<td>36.9 ± 0.27</td>
<td>37.3 ± 0.3</td>
<td>36.9 ± 0.1</td>
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</table>

MAP: mean arterial pressure; HR: heart rate; BE: base excess.
Tables 3 and 4. Importantly, no false-positive scoring for injury occurred in any specimen from a non-DHCA control (groups 3—5). Specimens from all three control groups were indistinguishable from one another. All animals in Group 1 displayed evidence of some ischemic injury in the cerebral cortex, basal ganglia and the hippocampus. The regional vulnerability within the hippocampus is interesting, because conventionally the large CA1 neurons are regarded as the most sensitive to ischemic injury in the mammalian brain. Our laboratory consistently observes the pattern of hippocampal injury seen herein—the superficial region of the dentate gyrus is the most sensitive to injury, and with progressively more severe injury, ischemic neurons are then noted proportionately deeper within the dentate and then into the (adjacent) CA4. Similar dentate gyrus susceptibility has been noted by others [18] 24—48 h post-ischemia. No injury was detected in the cerebellum of any animal in any group, even in the large Purkinje neurons.

Animals managed hypoxemic following DHCA (Group 2) displayed consistently more severe ischemic injury. In addition to the three animals whose brain sustained irretrievable, injury individual light and fluorescent scores of neuronal injury was consistently higher in the remaining Group 2 animals. Differences were especially marked in the superficial region of the dentate gyrus. Fluoro-Jade™ (Fig. 1) proved an extremely useful adjunct to routine light microscopy for easily identifying injured neurons. Scores closely paralleled light microscopy scores, and were significantly worse in the superficial dentate gyrus and cerebral cortex of animals in Group 2. Both the cumulative scores for the hippocampus and all brain regions were significantly worse for animals in Group 2 when compared with Group 1.

4. Discussion

This study confirms that post-operative hypoxemia in the range commonly encountered following staged palliation of complex congenital heart defects significantly exacerbates neuronal loss from ischemic injury sustained during prolonged periods of DHCA. It serves to highlight the notion that the early reperfusion period can critically affect the progression of neuronal injury following DHCA. Furthermore, it implies that procedure-specific patient subpopulations may respond differently to DHCA and that their differential neurological risk should be considered accordingly. The choice of perfusion strategy is presently determined largely by technical considerations, rather than by the implications of post-bypass oxygenation on cerebral recovery.

Cerebral autoregulation is disrupted by periods of ischemia. However controversy exists as to the selective nature of the loss of autoregulation to different stimuli. In the context of normothermic global cerebral ischemia, Leffler et al. discovered that ischemia in piglets abrogates the usual cerebral vasodilation that accompanies hypercapnia and severe hypoxemia [19]. Further studies indicated that the mechanisms were selective in that arteriolar dilatation to
hypotension was absent, but the vasoconstriction response to epinephrine remained intact. The response to hypoxia is less clear, but Armstead et al. reported an intact dilatory response to hypoxemia following normothermic global ischemia [20].

In the context of DHCA, additional variables include the contribution of deep hypothermia and CPB. Although deep hypothermia is deemed to be protective to ischemia by conserving high-energy phosphate consumption and decreasing products of lipid peroxidation and excitatory amino acids, it itself blunts the microvascular responses to both hypercapnia and hypoxemia as determined by near infrared spectroscopy [10]. We have previously examined the CBF response following normothermic CPB, and demonstrated a blunted CBF response to progressive hypoxemia compared to instrumented controls, suggesting that CPB itself deleteriously affects cerebral autoregulation, perhaps through the inflammatory consequences of CPB [15].

Reports detailing the cerebral response to hypoxemia following DHCA are conflicting. Although O’Rourke et al. demonstrated an intact cerebral vasodilatation response to hypoxemia, direct quantification of CBF following DHCA and progressive hypoxemia clearly demonstrated a progressively reduced CBF whilst controlling a constant overall cardiac output [10]. We have previously examined the CBF response following normothermic CPB, and demonstrated a blunted CBF response to progressive hypoxemia compared to instrumented controls, suggesting that CPB itself deleteriously affects cerebral autoregulation, perhaps through the inflammatory consequences of CPB [15].

Reports detailing the cerebral response to hypoxemia following DHCA are conflicting. Although O’Rourke et al. demonstrated an intact cerebral vasodilatation response to hypoxemia, direct quantification of CBF following DHCA and progressive hypoxemia clearly demonstrated a progressively reduced CBF whilst controlling a constant overall cardiac output [10]. Furthermore, using the microsphere technique, we have demonstrated that although progressive hypoxemia yields a blunted elevation in CBF following continuous CPB, CBF progressively falls with worsening hypoxemia after DHCA [15]. This observed inability of the cerebral vasculature to augment its blood flow is consistent with the repeated demonstration of the cerebral no-reflow phenomenon following DHCA [21,22].

Although lengthy DHCA durations are necessary to generate useful histological models of brain injury, we have chosen this experimental strategy because histopathological change is still one of the most reliable and important parameters by which ischemic damage and therapeutic effects can be evaluated. All animals exposed to DHCA exhibited injury and the hippocampus (dentate gyrus) displayed particular vulnerability. All animals exposed to post-CPB hypoxemia in the range frequently encountered following the Norwood procedure sustained worse brain injury. In fact, three of the animals sustained such severe injury that irretrievable decompensated cerebral edema ensued. Despite the inability to tissue process these subjects, they are significant because they represent injury at the most extreme end of the spectrum. We have not observed a similar cerebral response in animals in any other histological study within our program.

The lengthy durations of ischemia required to generate reproducible and quantifiable ischemic histological injury are a significant limitation common to all models histological injury. Uninterrupted DHCA for 90, 100 or 120 min are typical [23–25] and therefore represent a significant departure from clinical practice. Nevertheless, we and others investigating methods of neuroprotection against ischemic insults adopt the premise that protection observed in severely ischemic models may translate into reduced injury in (clinical) situations where only extremely subtle neuronal loss occurs.

### Table 4

<table>
<thead>
<tr>
<th>Animal</th>
<th>Cortex</th>
<th>Basal Ganglia</th>
<th>Cerebellum</th>
<th>CA1</th>
<th>CA2</th>
<th>CA3</th>
<th>Hippocampus</th>
<th>DG sup</th>
<th>DG med</th>
<th>DG deep</th>
<th>Hippocampus Total</th>
<th>Total Animal Score</th>
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<td>CA4</td>
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<td>4</td>
<td>2.8±.9</td>
<td>0</td>
<td>9.3±.6</td>
<td>14.3±.9</td>
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</table>

P 1 vs 2 .03 .29 - - - .09 .15 .02 .07 - .03 .03

Controls

| Group 3 | 0 0 0 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Group 4 | 0 0 0 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Group 5 | 0 0 0 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

CA: cornus ammonis subregions of hippocampus; DG: dentate gyrus region of hippocampus.
An additional problematic feature of cyanotic animal models is that the hypoxemia is abrupt and therefore precludes the contribution of compensatory mechanisms (polycythemia, neovascularization, intra-cellular augmentation of metabolic pathways). This is difficult to overcome without complex pre-operative incubation of animals (perhaps from birth) in enclosed chambers of sub-physiological oxygen. This has been achieved in rodent studies [26] which suggest that the neonatal brain is capable of adapting to profound cyanosis in the post-natal window. Similarly, functional and magnetic resonance imaging studies have failed to demonstrate cerebral differences between cyanotic and acyanotic infants, which implies that humans can tolerate severe hypoxemia, even occurring abruptly (for example at birth). Control animals exposed to severe hypoxemia following DHFF were indistinguishable from normoxemic controls (groups 4 and 5); supporting but not proving the notion that hypoxemia itself can be tolerated without injury. We instead believe post-ischemic oxygen debt, cerebrovascular dysregulation and hypoxemia to be the harmful combination.

Perhaps the most pertinent question that is raised by this study is whether a particular window exists in which augmenting cerebral oxygen delivery has a protective effect in reducing the progression of neuronal loss. If cerebrovascular dysregulation, no-reflow and the exaggerated injury observed in this study are indeed linked as we suggest, then the window of the no-reflow phenomenon — perhaps only the first 8 h [27] — may be a period during which artificial enhancement of oxygen delivery could limit injury induced by post-DHCA hypoxemia. Techniques to augment oxygen delivery might include extra-corporeal membrane oxygenation (ECMO), or boosting cardiac output using ventricular assist devices.

Overall, this study serves to emphasize that the period of reperfusion following DHCA is crucial to the progression of neurological injury sustained. The contribution of the early period of reperfusion has been seriously overlooked until very
recently [28]. Decisions regarding the use of DHCA typically relate to technical considerations, rather than patient-specific ones. It may be that either DHCA should preferentially be avoided in staged cyanotic congenital repairs, or used in the knowledge that the ischemic duration may have greater significance than in other patient populations. Furthermore, attempts to augment cerebral oxygen delivery by ensuring optimum perfusion pressures, above normal hematocrits and perhaps temporary methods of artificial oxygenation may need to be aggressively pursued in order to minimize neurological consequences.

Acknowledgements

Our continued pursuit of the study of cerebral protection has only been possible through the support of generous grants from the Medical Research Foundation of Oregon and the Children’s Heart Foundation, who have funded this and related preliminary work in our laboratory.

References


Appendix A. Conference discussion

Dr A. Caruso (Liverpool, United Kingdom): If I’m not wrong, the animals that you have used were all normoxic before the experiment, right?

Dr Hickey: That’s correct, yes.

Dr Caruso: At this point I would like to ask you what is the implication of this parameter? Because among the patients undergoing circulatory arrest, whatever kind of cardiopulmonary bypass policy you have, the vast majority of neonates are hypoxic before surgery, not normoxic. We have done an experimental study on animals, using more or less the same approach of having cardiopulmonary bypass with a period of reoxygenation at beginning of bypass, followed by ischemia and then reperfusion, but we have used all animals previously exposed to a period with chronic hypoxia. And what we have found is that there was already cerebral damage before the cardiopulmonary bypass in the chronic hypoxic group versus the normoxic. This pre-operative damage can have some influence in the post-operative management.

Second point, the reactivity of the brain of a normoxic animal is totally different from the reactivity in a chronic hypoxic animal. And by the way, it’s
the effects of no reflow have been done on animals involving 90 min to 2 h of clinical threshold of 45 min as the harmful threshold, yet studies delineating trying to learn about by the amplification of injury. For example, yes, we see a to amplify the experimental conditions to generate useful models of 90 min of DHCA rather than 120, we see no histological injury at 24 h. You have the manuscript we have recently submitted — if you subject neonatal piglets to hypothermic circulatory arrest’ this is what we have observed.

However, I don’t think that detracts from the fact that we have confirmed that the histological outcome is reflected by these physiological effects that we have seen previously.

Dr C. Pizarro (Wilmington, Delaware, USA): The problem I have is that although you provided proof of concept, you’ve used an extremely prolonged period of deep hypothermic circulatory arrest, which is probably clinically not relevant. I don’t know of anybody, unless in a very special circumstance, would use 2 h of circulatory arrest without doing anything, particularly knowing that you could use intermittent reperfusion or regional cerebral perfusion.

In any event, are you suggesting that based on this data for example in a patient who undergoes a Norwood operation with a period of circulatory arrest of, let’s say, 45 min, we should take the message that we should observe the same phenomenon?

I think that in your conclusions you should probably add the word ‘prolonged,’ so the statement is that ‘after a period of prolonged deep hypothermic circulatory arrest’ this is what we have observed.

Dr Hickey: You’re absolutely right again. The study of DHCA, across all experimental animals in all international research departments, necessarily involves prolonged DHCA. Believe it or not — it is the subject of another manuscript we have recently submitted — if you subject neonatal piglets to 90 min of DHCA rather than 120, we see no histological injury at 24 h. You have to amplify the experimental conditions to generate useful models of histological injury.

Nevertheless, that does not detract from the general concepts that we’re trying to learn about by the amplification of injury. For example, yes, we see a clinical threshold of 45 min as the harmful threshold, yet studies delineating the effects of no reflow have been done on animals involving 90 min to 2 h of DHCA. We know from xenon clearance studies that DHCA exists in clinically relevant durations of DHCA.

So I think the message from the animal studies, where we have to amplify the conditions, have in the past been applicable to the clinical scenario. And the general concept of no reflow occurring, it holds true. And yes, we would suspect that post-operative cyanosis will have a negative impact on the recovery of the brain following bypass using DHCA.

So I think the take-home message that you asked for would be that when you choose to use DHCA in a patient that’s going to be cyanotic post-operatively, you should recognize that this is an at-risk subpopulation for which optimizing the cardiac output and cerebral delivery of oxygen is critical afterwards, and that may be through the use of ECMO.

Dr F. Lacour-Gayet (Denver, Colorado, USA): I would like to ask you, what is your real message? Is your real message, don’t do hypoxemia following a Norwood operation, or don’t do deep circulatory arrest?

I just want to mention that today there is a great majority of surgeons who don’t do deep circulatory arrest even in the Norwood operation. Using a different technique, including an arterial canula placed in a shunt in the brachiocephalic artery, you can achieve not a low-flow brain perfusion but a full-flow brain perfusion in normothermia or in hypothermia. And some surgeons are now starting to perfuse the descending aorta. Therefore, deep circulatory arrest has become today, I believe, a technique that is used by a minority of surgeons.

My question to you is, what is your real message—no circulatory arrest or no hypoxemia? Because no hypoxemia after a Norwood is very difficult to achieve.

Dr Hickey: You’re absolutely right that the majority of currently practicing congenital surgeons avoid the use of DHCA, but the vast majority still include it in their armamentarium and I would quote audience response session from the STS in 2004 and the Congenital Heart Surgeons Society of North America of 2004, where exactly these questions were asked. The majority of surgeons still include it as a perfusion technique from time to time.

Secondly, the alternatives, including low-flow perfusion, still display some of the physiological anomalies of complete deep hypothermic circulatory arrest. We know low flow, for example, still harbors some of the perfusion anomalies once flow is reintroduced. And I would suspect, we’ve again had to look at extremes here, I suspect if we looked at cyanosis following extremely low-flow cerebral perfusion, we would see the same consequence although blunted following low-flow perfusion.

Dr Lacour-Gayet: Let me just add a word. When you perfuse the brain with a flow of 50–80 ml/kg min, you’re not at low-flow brain perfusion, you have full flow. I mean, the flow that is delivered to the brain is strictly normal at this rate, so we cannot talk about low-flow brain perfusion when you perfuse the brain through the circle of Willis with a flow that is 50–80 ml/min. It’s not low-flow, it’s full-flow brain perfusion.