Avoidance of hemodilution during selective cerebral perfusion enhances neurobehavioral outcome in a survival porcine model

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Abstract

Introduction: The ideal hematocrit (HCT) level during hypothermic selective cerebral perfusion (SCP) — to ensure adequate oxygen delivery without excessive perfusion — has not yet been determined. Methods: Twenty pigs (26.0 ± 2.6 kg) were randomized to low or high HCT management. The cardiopulmonary bypass (CPB) circuit was primed with crystalloid in the low HCT group (21 ± 1%), and with donor blood in the high HCT group (30 ± 1%). Pigs were cooled to 20°C and SCP was carried out for 90 min. During rewarming, whole blood was added in the low HCT group and crystalloid in the high HCT group to produce equivalent HCT levels by the end of the procedure. Using fluorescent microspheres and sagittal sinus sampling, cerebral blood flow (CBF) and oxygen metabolism (CMRO 2) were assessed at baseline, after cooling, at two points during SCP (30 and 90 min), and at 15 min and 2 h post-CPB. In addition, a range of physiological and metabolic parameters, including intracranial pressure (ICP), were recorded throughout the procedure. The animals’ behavior was videotaped and assessed blindly for 7 days postoperatively (maximum score = 5). Results: HCT levels were equivalent at baseline, 2 h post-CPB, and at sacrifice, but significantly different (p < 0.0001) during cooling and SCP. Mean arterial pressure, pH and pCO 2, and CMRO 2 were equivalent between groups throughout. ICP was similar in the two groups throughout cooling, SCP, and rewarming, but was significantly higher in the low HCT animals after the termination of CPB. CBF was similar at baseline, but thereafter markedly higher in the low HCT group. Neurobehavioral performance was significantly better in the high HCT animals (median score 3.5 vs 4.5 on day 3, and 4.5 vs 4.75 on day 7, p = 0.003). Conclusions: Higher HCT levels for SCP produced a significantly superior functional outcome, suggesting that the higher CBF with a lower HCT may be injurious, possibly because of an increased embolic load.

Keywords: Great vessels; Cerebral protection; Hematocrit

1. Introduction

Despite advances in the practice of cardiovascular surgery that have reduced risk-adjusted mortality, significant neurological morbidity still occurs relatively frequently, especially after prolonged procedures involving hypothermic cardiopulmonary bypass (CPB) and circulatory arrest (HCA). However, several modifiable factors do impact on the cerebral outcome after HCA, including hematocrit (HCT) level, pH-management strategy, and the temperature at which arrest is conducted.

Hemodilution has long been advocated for hypothermic CPB to avoid the use of blood products, and in the hope of improving microcirculatory flow by lowering blood viscosity. Recent work, largely from the Children’s Hospital and Harvard Medical School, Boston, has shown that this practice may be misguided. Through both experimental work and clinical trials, they have demonstrated that lower HCT levels during CPB cooling and rewarming in association with HCA compromise cerebral oxygenation, decrease intracellular high-energy phosphates, and yield poorer histological and functional neurological outcomes despite higher cerebral blood flow (CBF) [1—6]. They have postulated that the increased cerebral embolic load from higher CBF may worsen outcome, and that the higher CMRO 2 they observe with a lower HCT is a reflection of intracellular acidosis or increased substrate transport requirements.

Recent trends have shown an increase in the use of low flow CPB in both adult and pediatric cardiac surgical practice in conjunction with or instead of HCA. Care must be taken in applying results from studies of prolonged HCA to circumstances where arrest of the circulation is being minimized or avoided. The clinical superiority of antegrade selective
cerebral perfusion (SCP) over either HCA alone or retrograde cerebral perfusion for the surgery of aortic arch aneurysms is becoming clear, and there is consequently a need to investigate the influence of different hematocrit levels on neurological outcome following a period of hypothermic SCP, since several of the mechanisms by which a low HCT is postulated to cause cerebral injury during HCA may be modified by on-going perfusion. We addressed this question with a study focused on intraoperative cerebral physiology and functional neurobehavioral outcome following SCP with different HCT levels in a survival pig model.

2. Materials and methods

2.1. Study design

Twenty juvenile female Yorkshire pigs (approximately 3 months of age) with a mean weight of 26.0 ± 2.6 kg were studied (Animal Biotech Industries Inc., Danboro, PA). Computer-generated randomization was undertaken allocating the animals to either Group A (low HCT, n = 10) or Group B (high HCT, n = 10). Group A animals had their cardiopulmonary bypass circuit primed with 800 ml 0.9% saline; those in Group B had initial rinsing of the circuit with 800 ml 0.9% saline, but this was then replaced with 800 ml donor pig whole blood. Thereafter, all animals were placed on CPB and cooled to 20 °C; this was followed by 90 min of SCP at a mean pressure of 50 mmHg. After the SCP period, the animals were rewarmed with full CPB. During rewarmin, the Group A animals received 800 ml donor pig whole blood, and the Group B animals 800 ml 0.9% saline, so as to equalize their hematocrits and focus attention on the effects of HCT level during SCP.

All animals received humane care in accordance with the guidelines from Principles of Laboratory Animal Care formulated by the National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication No. 86—23, revised in 1985). The Mount Sinai Institutional Animal Care and Use Committee approved the protocol for this experiment.

2.2. Perioperative management and anesthesia

The animals were premedicated with intramuscular ketamine (15 mg/kg) and atropine (0.03 mg/kg) to induce deep sedation and facilitate endotracheal intubation. The pigs were mechanically ventilated with an inspired oxygen fraction of 0.7. During normothermia, the minute volume was adjusted to produce an arterial carbon dioxide tension of 35—45 mmHg. Anesthesia was maintained with isoflurane at 1.5% and paralysis was achieved with intravenous pancuronium (0.1 mg/kg). Arterial oxygen tension was maintained above 100 mmHg at all times.

An 8F Foley bladder catheter was placed for continuous assessment of urine output. A 14G arterial line was placed in the right brachial artery for pressure monitoring, arterial blood gas sampling (Ciba Corning 865, Chiron Diagnostics, Norwood, MA), and the withdrawal of reference samples for regional blood flow determinations.

2.3. Intracranial monitoring

Before heparinization, a midline scalp incision was made and carried down to the periosteum to reveal the intersection of the sagittal and coronal sutures. A 2 mm cutting tool was used to create a 1 cm diameter burr hole through which the superior sagittal sinus was visualized. The sinus was cannulated with a 24G catheter and used for blood gas analyses and sinus pressure monitoring. An intracranial pressure (ICP) monitoring probe was passed extradurally through this burr hole to allow continuous assessment (Codman ICP Express, Johnson and Johnson Proinf Inc., Raynham, MA), and a temperature probe was inserted into the cerebral parenchyma.

2.4. Operative technique

The chest was opened via a small left thoracotomy in the fourth intercostal space. The pericardium was opened and the heart and great vessels were identified. After heparinization (300 IU/kg), the right atrium was cannulated with a 26F single-stage cannula and the aortic arch with a 16F arterial cannula. CPB was initiated at a flow rate of 80—100 ml/kg min and thereafter adjusted to produce a minimum mean arterial pressure of 45 mmHg. A 10F left atrial cannula was inserted for venting the left heart and injecting fluorescent microspheres.

The CPB circuit consisted of non-pulsatile roller heads, a membrane oxygenator (VPCML Plus, Cobe Cardiovascular Inc., Arvada, CO) and heat exchanger (Hemotherm Cooler/Heater, Cincinnati Sub-Zero, Cincinnati, OH); cardiomya suction was not used. The circuit was primed with 1000 ml 0.9% saline and 4000 IU heparin. Once stable CPB was established and cooling to 20 °C was undertaken (alpha-stat pH management). CPB was continued for a minimum of 30 min after initiation to ensure thorough cooling, and the operating room was kept between 18 and 20 °C to prevent an upward temperature drift.

Just prior to the commencement of SCP, diastolic cardiac arrest was achieved by adding 1 meq./kg potassium chloride to the venous reservoir. Clamps were placed across the ascending aorta and the proximal descending aorta to isolate the arch, and SCP was initiated and maintained at a mean pressure of 50 mmHg. Myocardial protection was supplemented by the irrigation of the pericardium with iced saline (−4 °C).

Following the 90-min SCP interval, the clamps were removed and CPB with whole-body perfusion reinstituted. Rewarming was undertaken and carried through to a brain temperature of 36.5 °C. Care was taken to avoid a temperature difference of more than 10 °C between the perfusate and brain/rectal measurements. Cardiac defibrillation was achieved electrically without the need for pharmacological adjuncts.

2.5. Cerebral blood flow and metabolism determination

Fluorescent microspheres were used to determine cerebral blood flow, as detailed in previous studies [7]. This study utilized six colors, with each one injected at a specific time point: at baseline; after 30 min of cooling (20 °C); at 30 min of SCP; at 90 min of SCP; 15 min post-CPB, and 2 h post-CPB.
For each injection, 2.5 million microspheres (15 μm diameter, Interactive Medical Technologies Ltd., Irvine, CA) were administered, into the left atrial catheter at baseline post-CPB, and delivered directly into the arterial catheter for the measurements after cooling, and during SCP. To allow calculation of regional blood flow rates, a reference sample was withdrawn from the axillary catheter at a rate of 2.91 ml/min with a Harvard pump (Harvard Bioscience Inc., Holliston, MA).

After the 1-week period of neurobehavioral assessment, the animals were sacrificed by exsanguination under anesthesia and their brains were removed. The two hemispheres were divided and the samples (1–3 g in weight) were taken from the right hemisphere at four locations: hippocampus, neocortex, cerebellum, and brainstem. Microspheres were recovered from the samples by sedimentation and counted using a fluorescent spectrophotometer. CBF was then determined from the fluorescent intensities of the tissue and blood reference samples using the formula:

\[
\text{CBF (ml/100g/min)} = 100 \times \left( \frac{R_l}{I_{br} W_t} \right)
\]

where R is blood reference withdrawal rate (2.91 ml/min), \(I_l\) and \(I_{br}\) are the tissue and blood reference samples' fluorescent intensities, and \(W_t\) is the weight of the tissue sample (g). From this the cerebral metabolic rate for oxygen (CMRO₂) can be derived:

\[
\text{CMRO}_2 \text{ (ml/100g/min)} = \frac{\text{CBF} \times (\text{arterial O}_2 \text{ content} - \text{sagittal sinus O}_2 \text{ content})}{100}
\]

2.6. Hemodynamic and blood sample analyses

In addition to the injection of microspheres detailed above, various hemodynamic, and arterial and sagittal sinus blood gas data were collected. These were taken for the following time points: baseline, after 15 min of cooling, after 30 min of cooling, 30 min SCP, 60 min SCP, 90 min SCP, after 15 min of rewarming, 30 min of rewarming, 15 min post-CPB, and 2 h post-CPB. The following data were recorded: brain temperature, mean arterial pressure (MAP), ICP, carotid arterial oxygen saturation, sagittal sinus O₂ content, and hematocrit.

2.7. Visual-evoked potential (VEP) recording

Through the midline scalp incision, two sterile stainless steel screw electrodes were attached to the skull. They were placed bilaterally, 10 mm lateral, and 10 mm posterior to the intersection of the sutures, and secured to the skull close to the underlying occipital cortex. A supramaximal visual stimulus was delivered to the retina from a photo stimulator (model PS 22A, Grass, Quincy, MA). Each VEP consisted of the averaged response from 150 flashes. These responses were amplified, bandpass filtered, digitized, and stored on an optical disk for subsequent analysis (Spectrum 32 neurophysiological recording system, Cadwell Laboratories Inc., Kennewick, WA, USA). At each time point, three averaged VEPs were recorded. The first cortical wave was analyzed, which has a peak latency of approximately 60 ms. VEPs were assessed at baseline, 15 min post-CPB, and 2 h post-CPB.

2.8. Behavior and postoperative neurological outcome

In the early recovery phase (defined as the first 3 h after extubation), the animals were scored according to a six-point scale reflecting both early mental alertness and activity [8]. On a daily basis, the animals were taken from their holding areas and allowed to explore a larger environment in a specially designed room baited with strategically placed apple pieces. Animals were videotaped and their performance scored in a blinded manner (5 = normal score, 0 = dead) by a neuroscientist.

2.9. Statistical methods

A member of the biomathematics department prepared the randomization scheme; the group assignment was revealed just prior to the institution of CPB. Hemodynamic and intraoperative variables were compared at baseline using ANOVA between groups. Later comparisons were based on absolute values, or on changes from baseline if deemed more relevant. For data that were consistent with the requisite assumptions, groups were compared by ANOVAs separately for periods of cooling, SCP, rewarming, and recovery post-CPB. Pairwise comparisons, using the Bonferroni multiple testing correction to control for an overall 0.05 significance level, were conducted if the corresponding average difference or time by group interaction was statistically significant. Other variables were compared by Wilcoxon tests at each time point. Analyses were performed using SAS software (SAS Institute, Cary, NC).

3. Results

3.1. Comparability of experimental groups

The mean (±SD) preoperative weights of the animals in the two groups were similar (Group A 27 ± 3, Group B 25 ± 2, p = ns). In addition, all animals were examined daily by a veterinary team and found to be in normal health prior to surgery.

3.2. Hemodynamic and CPB-related data

The baseline mean arterial pressure was not statistically significantly different between groups at baseline, or during the subsequent time course of the experiment (Table 1). An α-stat strategy was employed, and the resulting pH and pCO₂ values were in close agreement between the groups.

Baseline brain temperature was in close agreement between the groups (Table 1). During cooling, we observed slightly faster cooling in Group A, although this did not reach statistical significance. Likewise, marginally faster rewarming was also seen in Group A. These patterns probably reflect the higher flow rates associated with a lower HCT level.

Hematocrit values were similar at baseline. Thereafter, in accordance with the experimental design, the levels were
markedly different, averaging 21 ± 1% in Group A and 3 ± 1% in Group B during cooling and SCP, \( p < 0.0001 \) (Table 1). During rewarming, as further additions (detailed in Section 2) were made to the circuit, the HCT levels converged. There was significant hemoconcentration in both groups. By the end of the post-CPB observation period at 2 h, the HCT levels were identical in both groups (33 ± 1%). Similarly, when the animals were sacrificed 1 week after surgery, the HCT levels were identical in both groups (33 ± 1%).

All values are shown as mean ± standard error, unless otherwise indicated. Temperature values shown are those recorded from the brain parenchyma. MAP denotes mean arterial pressure. \( \text{pH} \) and \( \text{pCO}_2 \) values are for arterial blood, likewise Art. \( \text{O}_2 \) sat. is the arterial oxygen saturation.

### 3.3. Intracranial pressure

Values at baseline were low in both groups (median 3.0 and 2.5 mmHg, respectively), and remained so during cooling and SCP, with no important differences (Fig. 1). The ICP increased in both groups during rewarming, but with no significant between-group differences. After the end of CPB, the ICP continued to rise in Group A, but fell in Group B, resulting in a statistically significant difference for this period as a whole (\( p = 0.04 \)).

### 3.4. Cerebral blood flow

The mean values for CBF over the time course of the experiment are shown in Fig. 2. The baseline CBF values of the groups were very similar. During cooling, there was a marginal fall in CBF in Group A; Group B experienced a greater fall (\( p = 0.005 \)). Thereafter, once SCP was initiated, the levels of CBF increased in both groups, but to a far greater degree in Group A (\( p = 0.06 \)). After termination of CPB, CBF was found to be above baseline in both groups at both time points (15 min and 2 h); however, there were far more marked elevations in CBF in Group A (\( p = 0.005 \) and \( p = 0.002 \) for the between-group comparisons at the two time points).
3.5. Cerebral metabolic rate for oxygen (CMRO₂)

The baseline CMRO₂ values (Fig. 3) were in close agreement between the groups, and similar to values observed previously [7]. During cooling, there was a marked decrease in CMRO₂ in both groups. Thereafter during SCP, an initial slight increase compared with the observation taken at the end of cooling was seen in both groups, without between-group differences. By the end of SCP, the values had fallen again to levels similar to those seen at the end of cooling. Post-CPB, the CMRO₂ was elevated just above baseline in both groups, both at the 15-min and 2-h observations, with no statistically significant between-group differences.

3.6. Visual-evoked potentials

The results are shown in Table 2. The median cortical wave amplitudes were close to baseline by 15 min post-CPB in both groups. Thereafter, the responses diminished marginally at the 2-h observation point. There were no statistically significant differences between the groups (p = 0.66).

3.7. Early recovery scores and neurobehavioral assessment

The median early recovery scores were different between the groups (Group A: 3.5, Group B: 6) for each of the first 3 h post-extubation. These differences were statistically significant (p = 0.001).

4. Discussion

The data from this experiment show higher levels of cerebral blood flow with hemodilution despite identical perfusion pressures and corroborating clinical findings [9]. Moreover, animals undergoing SCP under conditions of hemodilution had a poorer neurological outcome, as evidenced by significantly worse functional scores on early awakening and daily neurobehavioral analyses. Although inadequate cerebral oxygen delivery with hemodilution could possibly be evoked to explain the findings, ischemic injury in these animals does not seem likely in the absence of significant differences from the high HCT group in the cerebral metabolic rate for oxygen.

Others have demonstrated the superiority of a higher hematocrit during CPB for cooling before and rewarming after a period of HCA. Sakamoto and co-workers [10] have shown in their piglet model that a hematocrit of 30% relative to 20% confers histological benefit, most importantly in the hippocampus, an area of the brain notoriously sensitive to the effects of ischemia. Also, a randomized clinical trial has shown that psychomotor outcomes following low flow hypothermic CPB — and HCA in some cases — were superior with a mean hematocrit of 27.8% compared with 21.5% [6].
The benefits of higher HCT levels do not seem to be confined to the conduct of deeply hypothermic CPB. Two large retrospective studies of coronary artery bypass graft patients have shown that lower HCT levels increase the risk of perioperative death, the need for intra-aortic balloon pump support, and difficulty in weaning from CPB [11,12]. The present study demonstrates that the advantages of undertaking extracorporeal perfusion with higher hematocrit levels are likely to include the conduct of selective cerebral perfusion. However, a note of caution must be sounded against injudicious use of allogeneic blood products to support HCT during CPB or SCP. Clearly there are risks of both short- and long-term sequelae [13], and, as such, maintenance of a more physiological HCT through ultrafiltration, miniaturization of CPB circuits, cell-saving devices, and the use of autologous blood prime may be preferable.

4.1. Cerebral physiology

The higher levels of cerebral blood flow detectable with low hematocrit perfusion have been noted before [14], although in their model Sakamoto and co-workers used flow-driven CPB at 150 or 100 ml/kg/min. We chose to adopt a primarily pressure-driven approach, which is what many surgeons in clinical practice use also. It is likely that hemodilution has an intrinsic tendency to increase cerebral blood flow under hypothermic conditions because it reduces blood viscosity. But if autoregulation is present, one might also expect—in the face of equivalent demand—an increase in flow at a lower hematocrit in order to maintain oxygen delivery.

Even when hematocrit levels were equal in both groups 2 h post-CPB, CBF was still significantly higher in Group A (low HCT animals). If one accepts the likelihood that autoregulation is reasonably intact, this may indicate repayment of some form of oxygen debt, or attempted recovery from cerebral injury. Clearly, in spite of the luxuriant levels of perfusion seen in Group A, significant neurological dysfunction was seen postoperatively in both behavioral assays. Our impression that the elevated CBF during recovery from SCP is a reflection of cerebral injury is also supported by the finding of a significantly higher ICP in the Group A animals, since elevated intracranial pressure has previously been shown in this model to correlate with adverse neurological outcome [15]. Reduced colloid oncotic pressure associated with low hematocrit levels has been shown to produce greater perioperative weight gain and cerebral edema in another porcine model [16].

In addition to the possibility of ischemia and cerebral edema, it is also conceivable that an increase in embolization could be responsible for cerebral injury in a situation that involves significantly increased cerebral blood flow. The higher levels of flow during low HCT SCP are especially worrisome in this regard in the clinical situation, since patients often have extensive atherosclerosis in conjunction with an arch aneurysm. Unfortunately our experimental model does not include the capability to undertake transcranial Doppler ultrasonography, which can be used to assess cerebral blood flow velocity and detect embolic events. Clearly this area of potential future study would interrogate the extent to which raised embolic loads to the brain explain the different outcomes observed. Moreover, heightened inflammatory activation in the lower HCT group is another possible mechanism of cerebral injury: elevated levels of neutrophil CD11b, a marker of their activation, are associated with hemodilution [17].

The CMRO₂ levels we observed during cooling and SCP were not significantly different between the groups, although the low HCT animals (Group A) did have slightly higher values. This fails to corroborate findings from experimental HCA studies, in which low HCT animals displayed significantly higher CMRO₂ levels both during cooling and rewarming [14]. It is possible that the ongoing perfusion in our study may have attenuated the intracellular acidosis cited as a potential explanation for the postoperative hyperemia in the HCA study. Furthermore, the Boston group used jugular venous sampling to determine cerebral venous oxygen content, which may include blood draining from the huge mass of neck and facial musculature in the pig, whereas the sagittal sinus, which we use, drains only the brain.

4.2. Neurobehavioral assessment

We have struggled to devise the best possible way of obtaining a reliable assessment of neurological outcome, especially in evaluating subtle findings in animal models. Whilst large mammalian species such as the pig can demonstrate sophisticated cerebral function—their sense of smell is profoundly acute—investigators’ ability to interrogate this, with a view to determining the superiority of one operative approach over another, is limited. The use of daily neurological examination by an observer blinded to the surgical group is useful and relevant, but may be susceptible to a lack of independence from 1 day’s observations to the next. We have supplemented our early recovery score with daily, videotaped sessions in a specifically designed room baited with apples in order to assess functional outcome. The animals explore the environment, moving between sectors to gather the apple pieces, which are standardized for size and location. Recordings are made onto several videotapes concurrently during the experimental series, and when they are subsequently reviewed blind, there should be no potential source of bias. We think this is the best method we have thus far devised for standardized functional assessment in this experimental model, and thus we have confidence in the conclusion that a higher hematocrit results in optimal flow during SCP.

References


