Effects of Phentolamine Infusion During Selective Cerebral Perfusion in Neonatal Piglets

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Background. An optimal selective cerebral perfusion protocol in pediatric cardiac surgery is unknown. Phentolamine is frequently used in pediatric cardiopulmonary bypass. We sought to determine the effects of continuous phentolamine infusion during selective cerebral perfusion.

Methods. Twenty-seven neonatal piglets (3.38 ± 0.32 kg) were randomly assigned to 3 groups; sham (n = 7, anesthesia alone, no surgery or bypass), control (n = 10, saline infusion), or experimental (n = 10, phentolamine infusion 0.1 mg/kg per hour). Animals underwent 90 minutes of selective cerebral perfusion. Cerebral vascular resistance index (CVRI) and metabolic rate of oxygen (CMRO2) were determined every 15 minutes. Standardized sections of hippocampus, basal ganglia, and neocortex were obtained. Tissue samples were stained for caspase-3 and analyzed for positive apoptotic cell count. Data were analyzed with repeated measures and one-way analysis of variance.

Results. The CVRI tended to increase over time in the control group and decrease over time in the experimental group, but difference was not statically significant (0.46 ± 0.24 vs 0.39 ± 0.10 mm Hg × min × kg−2/3/mL, p = 0.15). Mean CMRO2 was higher in the control group compared with the experimental group (0.90 ± 0.27 vs 0.59 ± 0.12 mLO2/min × kg−2/3, p = 0.005) and decreased over time in both groups. The percentage of caspase-3 positive cells was significantly different among regions (hippocampus = 16.9 ± 8.8; basal ganglia = 14.6 ± 7.5; neocortex = 10.8 ± 6.3; p < 0.0001) but not significantly different among sham (11.8% ± 2.68%), control (14.4% ± 2.24%), and experimental (15.5% ± 2.24%) groups.

Conclusions. A continuous infusion of phentolamine during selective cerebral perfusion significantly decreases CMRO2 and tends to decrease CVRI when compared with control. At the dose studied and at the time of tissue sampling, phentolamine does not appear to decrease apoptosis during or early after selective cerebral perfusion.

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Material and Methods

Experimental Setup

Twenty-seven 5- to 8-day-old neonatal piglets weighing 3.38 ± 0.32 kg were studied according to a protocol reconstruction [1, 4]. This technique is considered to be neuroprotective compared with DHCA but variations in its application exist among institutions and the optimal protocol is unknown.

Phentolamine is a vasodilator used in pediatric hypothermic CPB. Vasodilation is associated with a more rapid and uniform body cooling and rewarming, characteristics that have been proposed to enhance both peripheral and cerebral perfusion during cardiopulmonary bypass. However, controversy still exists regarding the neuroprotective benefits of phentolamine administration in pediatric open-heart surgery [5–7]. We sought to determine the effects of a continuous infusion of phentolamine during SCP on cerebral vascular resistance, oxygen metabolism, and neuronal integrity.

L ong-term morbidity associated with the surgical correction of congenital heart disease has become more evident as surgical results and survival of patients have improved significantly [1]. Neurodevelopmental abnormalities in these patients can be attributed to non-modifiable factors such as genetic syndromes and specific polymorphisms, and to factors that can be modified such as intraoperative perfusion strategies [2].

Cardiopulmonary bypass (CPB) with deep hypothermic circulatory arrest (DHCA) has been associated with immediate and late neurodevelopmental morbidities [3]. Alternative techniques, including selective cerebral perfusion (SCP), have been developed in an attempt to limit brain injury. SCP has been shown to provide adequate circulatory support to the central nervous system and somatic circulation throughout aortic arch reconstruction [1, 4]. This technique is considered to be neuroprotective compared with DHCA but variations in its application exist among institutions and the optimal protocol is unknown.
approved by the Institutional Animal Care and Use Committee of the Orlando Health Medical Education Research and Training Center in compliance with the Guide for the Care and Use of Laboratory Animals (NIH publication #96-03, revised 1996). Figure 1 displays a representative timeline of our protocol. The animals were fasted overnight but had free access to water. After a standardized induction of anesthesia, the trachea was intubated and mechanical ventilation initiated. Intravenous access was secured using the femoral vein while arterial access utilized the femoral artery. Anesthesia was maintained with isoflurane, fentanyl 0.1 mg/kg per hour and pancuronium 0.2 mg/kg per hour. Noninvasive monitoring included pulse oximetry, electrocardiogram, and rectal temperature. The animals were randomly assigned to sham, experimental, or control group. Subjects in the sham group were anesthetized and underwent minimal surgical manipulation without CPB.

Surgical Protocol
The external jugular vein and carotid artery were accessed through a right neck incision and the heart and great vessels were accessed through a left thoracotomy. A ligature was placed on the ductus arteriosus. After heparinization (heparin 500 UI/kg) the descending aorta was cannulated. The aortic cannula had a stopcock on the side port which was used to connect the cardioplegia tubing and SCP cannula. The external jugular vein was cannulated through the right cervical incision. An arterial cannula was placed in the carotid artery in the cephalad direction for cerebral perfusion and monitoring of perfusion pressure (Fig 2).

Perfusion and Experimental Protocol
All animals were administered an intravenous dose of dexamethasone (1 mg/kg) before initiating CPB. The CPB circuit consisted of 1/4" non-coated polyvinyl chloride tubing, Capiox AF02X pediatric arterial filter (Terumo, Ann Arbor, MI), Capiox RX05 oxygenator with XCoating (Terumo), and venous saturation cuvette for CDI 100 monitoring system (Terumo). The pump was primed with 200 mL of whole blood from a donor adult pig (Sierra Medical, Inc, Whittier, CA). After cannulation, the animal was placed on CPB at 100 mL × kg⁻¹ × min⁻¹ and a hematocrit of 24% was maintained. Principles of pH-stat were used for blood gas management. The animals were cooled to a core temperature of 18°C over 30 minutes. At the beginning of the arrest period, the arch vessels and descending aorta were occluded with tourniquets and cardioplegia solution was administered (20 mL/kg). Maintenance doses (10 mL/kg) were given every 20 minutes. The animals then underwent SCP (20 mL × kg⁻¹ × min⁻¹) remaining arrested for 90 minutes. Subjects in the control group received a continuous infusion of normal saline and those in the experimental group received a continuous infusion of phentolamine (0.1 mg/kg per hour).

After the SCP period, the animals were rewarmed to 35°C over 30 minutes. Mannitol (0.5 g/kg) and furosemide (0.25 mg/kg) were administered upon rewarming. All animals received a bolus of phentolamine (0.1 mg/kg) at the beginning of cooling and rewarming. The animals were weaned from CPB and mechanically ventilated for 1 hour prior to being euthanized. The brain was fixed in situ by injecting 1000 mL of formalin through the carotid artery cannula. A skull necropsy was performed and the brain collected as a whole for histologic study.

Hemodynamic, Metabolic and Neurohistologic Assessment
Arterial and venous blood pressures during SCP were monitored through a 4F catheter placed in cephalad direction distal to the tourniquet in the brachiocephalic trunk, and a 5F catheter placed in cephalad direction in the external jugular vein, respectively. These measurements were used to calculate the cerebral vascular resistance index (CVRI) before initiating the phentolamine infusion, then every 15 minutes and 20 minutes after discontinuation of the phentolamine infusion. Stopcocks allowed sampling of arterial and venous blood to determine oxygen content and cerebral oxygen extraction. The cerebral metabolic rate of oxygen (CMRO₂) was then calculated. Samples were collected at the beginning of the circulatory arrest period (18°C) and every 15 minutes thereafter. The final sample

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Fig 1. Protocol timeline. (CPB = cardiopulmonary bypass; DHCA = deep hypothermic circulatory arrest; SCP = selective cerebral perfusion.)
was collected after rewarming (35°C). Blood samples were analyzed using the i-STAT system (Abbott Laboratories, Abbott Park, IL). Raw values were corrected for body surface area, defined as the body weight in kg to the 2/3 power. The quantities were calculated as follows: CVRI = (mean cerebral arterial pressure – mean cerebral venous pressure)/(cerebral blood flow/body surface area); O₂ content = [hemoglobin × 1.36 × (O₂ saturation/100)] + (0.0031 × O₂ partial pressure); cerebral O₂ extraction = (arterial O₂ content – venous O₂ content); and CMRO₂ = [cerebral O₂ extraction × (cerebral blood flow/body surface area)]/100.

The brain was dissected and standardized samples of the neocortex, hippocampus, and basal ganglia obtained. Tissue samples were stained for apoptosis marker caspase-3 (Sandford-Burnham Medical Research Institute, Orlando, FL), scanned with ScanScope (Aperio Technologies Inc, Vista, CA), and analyzed using the Aperio Nuclear v9 algorithm (Aperio Technologies Inc) for positive cell count.

Statistical Analysis
Statistical analysis was performed with repeated measures and one-way analysis of variance. The Student t test was used when indicated and a p value less than 0.05 was considered statistically significant.

Results
The results for the hemodynamic, metabolic, and histologic data are displayed in Table 1. The CVRI tended to increase over time in the control group and decrease over time in the experimental group, although this difference was not statistically significant. Mean CMRO₂ was significantly higher in the control group compared with the experimental group and decreased over time in both groups. The percentage of caspase-3 positive cells was not significantly different among groups.

The baseline value of CVRI was slightly lower in the control group than in the experimental group (0.57 vs 0.62 mm Hg × min × kg⁻²/³/mL, respectively). In both groups, CVRI decreased to similar values at 15 minutes of SCP (control = 0.42, experimental = 0.41 mmHg × min × kg⁻²/³/mL). The CVRI remained at comparable values during the first 45 minutes of SCP, but thereafter values in the control group started to increase whereas in the experimental group they decreased (Fig 3). A t test comparing the mean values of CVRI at 90 minutes of SCP was significantly different (control = 0.54, experimental = 0.31 mm Hg × min × kg⁻²/³/mL, p = 0.0002).

Analogously, the baseline CMRO₂ was slightly lower in the control group than in the experimental group (0.53 vs 0.62 mLO₂/min × kg⁻²/³, respectively). At 15 minutes of SCP, the control group showed an increase in CMRO₂ while it was almost unchanged in the experimental group (control = 0.93, experimental = 0.57 mLO₂/min × kg⁻²/³, respectively). The CMRO₂ remained at higher values in the control group than in the experimental group throughout the 90 minutes of SCP. The CMRO₂ values were more stable in the experimental group throughout the 90 minutes of SCP. Both groups showed a trend toward lower CMRO₂ over time. However, in the control group CMRO₂ values remained above baseline while in the experimental group reached values below baseline (Fig 4).

The percentage of caspase-3 positive cells was significantly different among regions (hippocampus = 16.9 ± 8.8; basal ganglia = 14.6 ± 7.5; neocortex = 10.8 ± 6.3; p < 0.0001). Representative slides of the histologic sections stained for caspase-3 and the positive-cell-count filter are shown in Figure 5.

Table 1. Hemodynamic, Metabolic, and Histologic Results (Mean ± Standard Deviation)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>Control</th>
<th>Experimental</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVRI (mm Hg × min × kg⁻²/³/mL)</td>
<td>N/A</td>
<td>0.46 ± 0.24</td>
<td>0.39 ± 0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>CMRO₂ (mLO₂/min × kg⁻²/³)</td>
<td>N/A</td>
<td>0.90 ± 0.27</td>
<td>0.59 ± 0.12</td>
<td>0.005</td>
</tr>
<tr>
<td>% apoptosis positive cells (caspase-3)</td>
<td>11.80 ± 2.68</td>
<td>14.40 ± 2.24</td>
<td>15.50 ± 2.24</td>
<td>0.15</td>
</tr>
</tbody>
</table>

CMRO₂ = cerebral metabolic rate of oxygen; CVRI = cerebral vascular resistance index; N/A = not applicable.
Comment

A recent survey of 140 congenital heart surgeons in North America regarding the use of various perfusion techniques found that 83% of surgeons use SCP in an exclusive, routine, or selective manner, while only 17% do not use this technique [8]. Selective cerebral perfusion has become a therapeutic intervention to minimize neurologic injury associated with deep hypothermic circulatory arrest. The degree of neurologic injury in these patients depends on specific factors that cannot be controlled, coupled with perioperative factors that can be optimized [2, 9, 10]. Although implementation of SCP has shown improvement of neurologic outcomes, the optimal conditions to conduct SCP remain to be determined [11–15].

The application of SCP varies among institutions. The differences in the technique are related to pump flow rate, temperature, and perfusion pressure. Animal studies have reported the effects of these parameters on cerebral blood flow, metabolism, and vascular resistance [16–20]. Haldenwang and colleagues [17, 18], using adult pigs, reported that higher perfusion pressure and pump flow rate carries the risk of cerebral edema and increased cerebral vascular resistance over time. In their studies, lower temperature, perfusion pressure, and pump flow rates decrease cerebral vascular resistance and metabolism [17–20]. Similarly, Decampli and colleagues [21] reported better recovery of neonatal piglets with lower flow rates during SCP. This suggests improved neuroprotection despite less cerebral blood flow as

Fig 3. Cerebral vascular resistance index (CVRI) values during selective cerebral perfusion (SCP) (p = 0.15).

Fig 4. Cerebral metabolic rate of oxygen (CMRO2) values during selective cerebral perfusion (SCP) (p = 0.005).
observed by lower perfusion pressure and moderate hypothermia (25°C) [19, 21].

Cerebral vascular resistance is a variable that can be manipulated with the use of a vasodilator and blood gas management strategies. Phentolamine is a nonselective competitive alpha adrenergic receptor blocker with a half-life of 19 minutes and predominantly renal elimination [7]. Its vasodilator effect allows more uniform temperature control during CPB cooling and rewarming, as well as shorter duration of these periods [5, 6]. Although the use of phentolamine seems to benefit CPB management, Gazzolo and colleagues [7] reported that its administration during pediatric open heart surgery correlated with increased cerebral vascular resistance and brain damage. The effects of a continuous infusion of phentolamine during SCP on cerebral hemodynamics have not yet been reported.

In our study, CVRI during SCP was not significantly affected by phentolamine. The control and experimental groups showed similar values during the first half of regional perfusion. This phenomenon may be due to cerebral autoregulation being maintained by alternative mechanisms despite sympathetic blockade [22]. After 45 minutes of SCP, the CVRI values in the control and experimental groups started showing opposite changes, increasing in the control group and decreasing in the experimental group, eventually becoming statistically significantly different. We hypothesize that at this point cerebral autoregulation failed, allowing the effect of sympathetic blockade to become evident. Greely and colleagues [23] concluded that during deep hypothermic temperatures, cerebral vascular resistance increases with temperature reduction and that severe temperature reductions impair vascular relaxation, described as a cold-induced “cerebrovasoparesis.” In addition, our finding corresponds with the observation by Strauch and colleagues [19, 20] in adult pigs, that cerebral vascular resistance increased after 60 minutes regardless of the temperature management during SCP (25°C vs 30°C), and this change correlated with a decrease in cerebral blood flow. Therefore it is possible that the lower CVRI induced by phentolamine may sustain cerebral blood flow during the latter part of SCP.

There was a significant effect of sympathetic blockade on cerebral metabolism. The CMRO₂ increased in the control group and this observation could be expected; it has been previously described that there is a mild increase in CMRO₂ after starting SCP at deep hypothermia [17–20]. The CMRO₂ increased soon after initiation of SCP and remained above baseline throughout the regional perfusion period. As oxygen consumption is reflective of cerebral metabolism, any increase in CMRO₂ during SCP is intuitively undesirable. Cerebral metabolic rate of oxygen in the experimental group did not increase after initiating SCP and showed a continuous decrease over time, reaching values below baseline. The lower CMRO₂ in the experimental group can be attributed to the α₁ sympathetic blocking effect of phentolamine, resulting in vasodilation and more uniform cooling, consequently further decreasing in CMRO₂. Strauch and colleagues [20] proposed that because blood brain barrier permeability is altered by hypothermia, during SCP catecholamines released from the ischemic lower body cross the blood brain barrier and increase cerebral metabolism.

Fig 5. Representative slides of (A) hippocampus, (B) basal ganglia, and (C) neocortex, stained for caspase-3 (top row) and color filter display identifying positive cells (bottom row) at 20× magnification.
This theory corroborates the metabolic changes observed in our study. The lower CMRO₂ found in the experimental group over time may improve cerebral protection and therefore outcomes. This could be investigated using a recovery model with delayed brain tissue sampling. Lower cerebral metabolism during SCP may be beneficial and in this model it could be due to the combination of achieving more uniform cerebral cooling and blocking catecholamine action.

Several studies have demonstrated the activation of apoptosis after CPB, especially with DHCA [24, 25]. The apoptotic cascade is activated within 1 hour of initiation of extracorporeal circulation and continues to occur for several days postoperatively [3, 25]. Caspase-3 is a cysteine protease central to the execution of apoptosis and its presence is indicative of cellular death [25, 26]. We found that the presence of caspase-3 was similar between the control and experimental groups despite the presumably beneficial effects of phentolamine. In this study, the absence of a significant difference in caspase-3 activation may be due to the relatively short time interval (1 hour) between weaning CPB and harvesting the tissue. Most studies in the literature, however, report significant differences of caspase-3 activity after a monitoring period of a minimum of 2 hours [24–29]. In addition, some investigators have reported that SCP may attenuate caspase-3 activation [27–29]. The percentage of caspase-3 positive cells was significantly different among the regions studied. The hippocampus was the most injured area, followed by the basal ganglia and neocortex, respectively. It has been reported that these areas of the brain are the most susceptible to activation of CBP-related apoptosis [9, 10, 24, 25, 27–29]. Furthermore, in correlation with our results, the hippocampus has been found to be the most sensitive region for this type of injury [19].

In conclusion, in our protocol and at the dose studied, a continuous infusion of phentolamine during SCP does not appear to decrease neuronal apoptosis during or early after CPB. Phentolamine infusion shows a trend toward lower cerebral vascular resistance and is associated with a more stable metabolic state. Our findings suggest more uniform cerebral perfusion and cooling with a continuous infusion of phentolamine during SCP. We believe that the observed effects of phentolamine may be beneficial if optimized and that further studies using higher doses are warranted.

As an animal model, our study is subject to several limitations. We did not directly monitor cerebral temperature and therefore we cannot ascertain a more uniform decrease in cerebral temperature in the experimental group than in the control group. This can only be inferred based on a lower cerebral metabolic state. Also, we did not measure cerebral blood flow (ie, near infrared spectroscopy), thus we can only speculate that cerebral blood flow increased when the CVRI decreased. Finally, we only monitored the animal for 1 hour after weaning CPB. As apoptosis is known to continue for 8 hours or more after injury, it is possible that our assay at 1 hour post-SCP reflected only the earliest apoptotic changes. Assays at 2 to 8 hours post injury may or may not have yielded larger differences between the groups.

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References


