Desflurane Confers Neurologic Protection for Deep Hypothermic Circulatory Arrest in Newborn Pigs

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Background: Cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA), as used for infant heart surgery, carry a risk of ischemic neurologic injury. Volatile anesthetics have neuroprotective properties against both global and focal ischemia at normothermia. The authors examined the hemodynamic and neuroprotective effects of desflurane in a piglet CPB–DHCA model.

Methods: Twenty piglets aged 5–10 days received a desflurane- (6–9% expired) or fentanyl-based anesthetic before and during CPB (before and after DHCA). DHCA lasted 90 min at 19°C brain. Cardiovascular variables (heart rate, arterial pressure, blood gases, glucose, brain temperature) were monitored. On postoperative day 2, neurologic and histologic outcomes were determined.

Results: Cardiovascular variables before, during, and after CPB were physiologically similar between groups. The desflurane group had better neurologic performance (P = 0.023) and greater postoperative weight gain (P = 0.04) than the fentanyl group. In necortex, the desflurane group had less tissue damage (P = 0.0015) and fewer dead neurons (P = 0.0015) than the fentanyl group. Hippocampal tissue damage was less in the desflurane group (P = 0.05), but overall, neuronal cell counts in the CA1 sector of the right hippocampus were similar to those in the fentanyl group.

Conclusions: Desflurane-based anesthesia yields hemodynamics during CPB with DHCA that are similar to those with fentanyl-based anesthesia. However, desflurane-based anesthesia improves neurologic and histologic outcomes of CPB–DHCA in comparison with outcomes with fentanyl-based anesthesia.

DURING the past 20 yr, survival among children born with complex congenital heart disease has improved greatly. One factor contributing to the improved survival is the ability to surgically intervene in early infancy, before secondary cardiovascular disease ensues. Cardiopulmonary bypass (CPB) and deep hypothermic circulatory arrest (DHCA) are often used in the repair of complex cardiovascular defects in young infants. Although CPB–DHCA enables surgical repair, it carries a risk of neurologic injury. Manifestations of this injury include seizures, diminished cognition, cerebral palsy, impaired coordination, and attention deficit hyperactivity disorder in 5–40% of survivors.1,2 Thus, development of neuroprotective therapies without long-lasting adverse cardiovascular side effects would improve neurologic outcome for infants requiring heart surgery with CPB–DHCA.

Recent studies indicate that volatile anesthetics confer neurologic protection for both global and focal cerebral ischemia at normothermia.3–10 Halothane, isoflurane, desflurane, and sevoflurane all appear to confer neuroprotective protection at approximately one minimum alveolar concentration. They seem to protect via several mechanisms, including inhibition of excitotoxicity,11–14 depression of metabolic demand,15 and improvement of brain tissue oxygenation.10 Hypothermia also protects via some of these mechanisms, as well as others.5,14 Whether volatile anesthetics confer neurologic protection in the presence of deep hypothermia is unknown.

Of the volatile anesthetics, desflurane is particularly attractive for application to infant heart surgery because of its physicochemical and cardiovascular properties. Desflurane decreases systemic vascular resistance and arterial pressure while it maintains cardiac output and brain, liver, kidney, and coronary blood flow.16,17,18 This relative lack of cardiovascular depression may be advantageous to infants with limited cardiovascular reserve. For infant heart surgery with DHCA, CPB is usually brief, which limits the time the drug can be administered and cleared. The relative insolubility of desflurane may be advantageous in uptake to achieve neuroprotective tissue concentrations before DHCA and in elimination after CPB to remove unwanted cardiovascular depression. The current study examined, in a piglet CPB–DHCA model, the uptake and elimination of desflurane as well as the hemodynamic and neuroprotective effect of desflurane.

Methods

Animal Care

After approval of the Institutional Animal Care and Use Committee, 20 piglets aged 5–10 days were randomly assigned to a fentanyl-based anesthetic (fentanyl group) or desflurane-based anesthetic (desflurane group) during the study. For anesthetic induction, intramuscular ketamine (33 mg/kg) and acepromazine (3.3 mg/kg) were
administered to all animals, followed by orotracheal intubation and mechanical ventilation. After insertion of an intravenous catheter, the fentanyl group received intravenous fentanyl (25 µg · kg⁻¹ · h⁻¹) and droperidol (0.25 mg · kg⁻¹ · h⁻¹); the desflurane group received desflurane 6% (expired) and one dose of intravenous fentanyl (25 µg/kg). Expired CO₂ and electrocardiographic values were monitored (Gould Instrument Systems, Valley View, OH). A catheter was inserted into an extremity artery to monitor pressure, glucose concentrations (Surestep monitor; Lifescan, Milpitas, CA), blood gases, pH, and hemoglobin concentrations (iSTAT Co, East Windsor, NJ). Thermistors (Yellow Springs Instruments, Yellow Springs, OH) were inserted into the cranial epidural space, rectum, and esophagus to monitor brain and core temperatures. Cefazolin (25 mg/kg) was given intravenously.

From a right-neck incision, the carotid artery and external jugular vein were exposed, through which 8-French cannulae (Medronic, Minneapolis, MN) were advanced to the ascending aorta and right atrium for CPB. Heparin (200 U/kg) was administered intravenously. Blood (10 ml/kg) was withdrawn before CPB for CPB. Heparin (200 U/kg) was administered intravenously. Blood (10 ml/kg) was withdrawn before CPB for transfusion postoperatively. The CPB circuit used a bubble oxygenator (Bio-2; Baxter Healthcare, Deerfield, IL), a 40-µm arterial filter (LPE 1440; PALL Biomedical, East Hills, NY), and a nonpulsatile roller pump (RS 7800; Renal Systems, Minneapolis, MN) flowing at 100 ml · kg⁻¹ · min⁻¹. Arterial blood gas management followed α-stat principles.

In the desflurane group, the oxygenator received desflurane 9–12% in combination with O₂ adjusted to obtain 9% arterial concentration, measured from the oxygenator exhaust port gas (POET; Criticare Systems, Waukesha, WI).¹⁹ The oxygenator in the fentanyl group received only O₂. The oxygenator prime (approximately 400 ml) contained pig whole blood, heparin (2,000 U), fentanyl (50 µg), pancuronium (1 mg), calcium chloride (500 mg), dexamethasone (30 mg), cefazolin (500 mg), furosemide (3 mg), and sodium bicarbonate (25 meq). Plasma-lyte A (Baxter Healthcare) was added to yield a hematocrit of 20–25% during CPB.

During CPB cooling, the temperature of arterial perfusate was 5–10°C less than all body temperatures. Ice bags around the head and body facilitated cooling. DHCA was induced at 19°C (brain), as confirmed by asystole and no arterial pressure. Constant brain temperature was maintained. After 90 min of DHCA, CPB was resumed, with the temperature of arterial perfusate 5–10°C greater than all body temperatures (maximum, 38°C). A radiant heating lamp and circulating water blanket facilitated rewarming. After 10 min of reperfusion, the heart was defibrillated and mechanical ventilation was resumed. At 28°C (brain), mannitol (0.5 g/kg) was administered intravenously. After 30 min of CPB reperfusion, desflurane was withdrawn in the desflurane group and fentanyl–droperidol was withdrawn in the fentanyl group. CPB was discontinued when all body temperatures were greater than 34°C (after about 40 min of CPB reperfusion). CPB cannulae were removed, propramine (4 mg/kg) was administered intravenously, and the neck incision was closed.

Postoperatively, inspired O₂ concentration and minute ventilation were adjusted to maintain arterial partial pressure of CO₂ (Paco₂) of 35–45 mmHg and arterial partial pressure of O₂ (Pao₂) of > 75 mmHg. The blood collected before CPB was transfused over 1–2 h at a rate maintaining a mean arterial pressure > 50 mmHg, after which dextrans (5%) in lactated Ringer’s solution was infused intravenously (4 ml · kg⁻¹ · h⁻¹). Brain temperature was controlled between groups until 2 h after CPB, at which point the thermistor probe was removed. The trachea was extubated when purposeful movements, airway reflexes, strength, and regular breathing had returned. Animals were inspected hourly for the initial 4 h and then every 6–8 h. Those unable to ambulate and feed were bottle-fed, or intravenous fluids were administered.

Neurologic Outcome

Both behavioral and neuropathologic criteria were used to assess neurologic outcome. Behavioral criteria included postoperative weight gain and neurologic examination. In the neurologic examination, scored by a blinded observer, points were recorded for deficits in level of consciousness (range, 0–25), cranial nerve function (range, 0–6), sensory function (range, 0–14), gait (range, 0–25), and behavior (range, 0–20).²⁰ The scores from each category were summed. The minimum score (0) represented no deficits (normal findings), whereas the maximum score (95) indicated severe impairment (brain death).²⁰ Gait and behavior were scored by observation, and level of consciousness and cranial nerves were scored by physical examination.

After 2 days’ survival, piglets received intramuscular ketamine and acepromazine, as well as intravenous heparin 300 U/kg and pentobarbital 100 mg/kg (euthanasia). One liter chilled saline, 0.9%, and then 4% paraformaldehyde in 11 phosphate buffered saline (pH 7.4; 0.15 M) were infused into the carotid artery to fix the brain in situ. After the brain was removed in toto, a superficial cut was made along the undersurface of the right hemisphere to identify it from the left. The whole brain was then cut coronally into 5-mm blocks, which were dehydrated in ethanol and xylene (Citadel 2000; Shandon Lipshaw, Pittsburgh, PA) and embedded in paraffin (Histoclear 1160; Leica Instruments, Nussloch, Germany). One 8-µm section from each tissue block was cut (Microtome 2155; Leica Instruments), put on a slide, and stained with hematoxylin and eosin to determine cell damage.

A neuropathologist blinded to group assignment evaluated the slides containing neocortex and hippocampus,
the regions damaged in this DHCA model.20 Slides were examined for neuronal cell death, inflammation, hemorrhage, and infarction, scored semiquantitatively for the region. The semiquantitative scores (0–5) were as follows: 0 = none (normal structure); 1 = rare damage (<1% neurons dead; no inflammation or infarction); 2 = mild (1–5% neurons dead; no inflammation or infarction); 3 = moderate (6–15% neurons dead; no inflammation or infarction); 4 = severe (16–30% neurons dead, with or without inflammation or infarction); 5 = very severe (>30% of all neurons dead; inflammation and infarction). The score for the region represents the average score of the slides. The number of dead and viable-appearing neurons were also counted on one slide in predetermined areas (2.5 mm²) of the neocortex (right hemibrain, about 0.5 mm posterior to bregma) and hippocampus (CA1 region, right hemibrain).

Protocol

Hemodynamic data and desflurane concentrations were recorded before CPB, every 5 min during CPB, and every 15–30 min after CPB until extubation. Arterial blood gases, pH, hemoglobin, and glucose concentrations were recorded before CPB, at the end of CPB cooling, and near the end of CPB rewarming, as well as 15 min and 2 h after CPB. Duration of CPB and time to extubation (from onset of CPB reperfusion) were noted. Neurologic performance score and body weight were assessed 24 and 48 h after onset of CPB reperfusion.

Statistical Analysis

Normally and non-normally distributed data are presented as mean (±SD) and median (25th–75th percentiles), respectively. Groups were compared with unpaired t tests and Mann–Whitney U tests for normally and non-normally distributed data, respectively, with use of Bonferroni correction for multiple comparisons. Statistical significance was set at P < 0.05.

Results

Table 1 displays the physiologic data obtained during the study. Before CPB, heart rate was significantly greater (P = 0.005) in the fentanyl group. At the end of CPB cooling, the fentanyl group had significantly greater arterial PO2 values (P = 0.005) and glucose concentrations (P = 0.002). All other values before, during, and after CPB were not statistically different between groups. In all animals, sinus rhythm was present during CPB cooling and rewarming; on reperfusion, ventricular fibrillation was easily converted to sinus rhythm.

The desflurane and fentanyl groups had similar durations of CPB cooling (23 ± 3 vs. 22 ± 4 min), CPB reperfusion (37 ± 6 vs. 40 ± 5 min), and total CPB (60 ± 7 vs. 62 ± 8 min). Brain temperature during DHCA was similar in the desflurane versus fentanyl group (19.0 ± 0.9 vs. 19.1 ± 0.6°C).

Arterial desflurane concentrations (fig. 1) increased rapidly during CPB cooling and reperfusion, reaching nearly 9% concentration by 10 min and increasing slowly thereafter. Desflurane concentration decreased rapidly after withdrawal of the drug during CPB rewarming, reaching approximately 2% by 10 min (the end of CPB).

Figures 2–4 display neurologic and histologic outcomes. Overall, both were better in the desflurane group. The neurologic performance score (fig. 2) was less in the desflurane group than in the fentanyl group, reflecting fewer neurologic deficits. The scores for the desflurane versus fentanyl group on postoperative day 1 were 5 (5–10) versus 10 (5–40) (P = 0.14), and those on postoperative day 2 were 0 (0–0) versus 5 (5–10) (P = 0.023). Weight gain, reflecting the ability to feed, was not statistically significant for the desflurane versus fentanyl group on postoperative day 1 (100 [–50 to 250] g vs. 0 [–50 to 0] g; P = 0.16) but was so by postoperative day 2 (150 [50–250] g vs. 25 [–50 to 0] g; P = 0.04). Time to extubation was not significantly different in the desflurane versus fentanyl group (283 ± 51 vs. 321 ± 69 min; P = 0.18).

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Histologic outcome in the neocortex was significantly better for the desflurane group than for the fentanyl group by all measures, whereas findings in the hippocampus were mixed. Tissue damage (fig. 3) in the neocortex was moderate for the fentanyl group (3 [2.5–4.5]) and rare for the desflurane group (1 [0–1]), a statistically significant difference ($P = 0.0015$). The score for tissue damage in the hippocampus was moderate (3 [1.5–4]) for the fentanyl group and mild (2 [1–2]) for the desflurane group ($P = 0.05$). The percentage of neurons that were dead (fig. 4) in the neocortex was significantly less ($P = 0.0015$) in the desflurane group (1 [0–1]%) than in the fentanyl group (9 [5–28]%). However, the percentage of neurons that were dead in the CA1 region of the hippocampus was similar ($P = 0.41$) in the desflurane group (6 [1–17]%) and the fentanyl group (10 [1–29]%). Cell counts for viable-appearing neurons in the desflurane versus fentanyl group were as follows: neocortex, 1,093 ± 386 versus 779 ± 962.
The current study used desflurane at a 6–9% concentration, which is anesthetic and potentially neuroprotective, with fentanyl for postoperative analgesia.

Given this study design, it is possible to attribute the difference in outcome between groups to the desflurane, the dose of fentanyl, or the droperidol. However, previous work suggests the latter two possibilities are unlikely. Desflurane might have been neuroprotective through direct or indirect effects on one or more of the many mechanisms contributing to ischemic–reperfusion damage. Potential direct protective effects include blockade of excitotoxicity, as volatile anesthetics inhibit glutamate release and binding to N-methyl-D-aspartate and \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionic receptors and upregulate \(\gamma\)aminobutyric acid receptors. 

Other potential direct protective effects include desflurane inhibition of voltage-gated calcium channels; depression of cerebral metabolic rate; improved brain tissue oxygenation; stimulation of adenosine triphosphate-sensitive potassium channels; and upregulation of second messenger systems. Indirect protective effects of desflurane relate mainly to inhibition of the stress response, as the desflurane group had lower heart rates before CPB and lower concentrations of serum glucose at the end of CPB cooling, both possible manifestations of less stress. However, these differences were transient and of a magnitude not likely to be physiologically significant, although other stress hormonal responses that were not measured in this study cannot be excluded. Brain temperature was not responsible for the outcome differences, because temperature over the neocortex, the region where protection was observed, was identical between groups, and regional temperature differences were not present in our model under the conditions of the study.

Desflurane improved histologic outcome by all measures in the neocortex and by some and not other measures in the hippocampus. In our CPB–DHCA model, neuronal death in neocortex occurs mainly by apoptosis, whereas neuronal death in hippocampus involves both apoptosis and necrosis. Desflurane might have been more protective in the neocortex than in the hippocampus because it was more effective against apoptosis than necrosis. The tissue damage score reflects the pathologist’s view of all sectors of hippocampus on many slides, whereas the cell counts represent part of one sector (CA1) on one slide. The hippocampal damage score might have been improved without improvement of the cell counts because desflurane might have been more effective in protecting one of the other hippocampal sectors or the cell-counting relative to the score was underpowered to detect a protective effect.

Our results add to a growing body of evidence that volatile anesthetics confer protection against cerebral ischemia–reperfusion injury.
property. Other work has shown volatile anesthetics to protect the heart, lung, and liver against ischemia–reperfusion damage at clinically relevant concentrations.\textsuperscript{25–28} Despite the number of favorable studies, uncertainties persist about the potency of neuroprotection. Volatile anesthetics fail to protect against infarction (severe ischemia) yet protect against selective neuronal death (mild to moderate ischemia).\textsuperscript{10} Similarly, desflurane might not protect against DHCA longer than in the current study, as ischemic damage would be more severe.

Several issues remain to be resolved before desflurane is adopted for infant heart surgery. Low-flow CPB is replacing DHCA for many complex heart operations. Neuroprotective effectiveness of desflurane may differ in low-flow CPB and DHCA. Membrane oxygenators have largely replaced bubble oxygenators in cardiac surgery. We used bubble oxygenators because the arterial anesthetic concentration can be monitored on-line.\textsuperscript{19} It would have been preferable to monitor brain tissue concentrations rather than arterial blood concentrations. However, of the volatile anesthetics, desflurane reaches tissue–blood equilibrium the fastest. The near-steady state arterial concentrations before and after DHCA suggest that comparable brain tissue levels were achieved.

References

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