The effect of blood pressure on cerebral outcome in a rat model of cerebral air embolism during cardiopulmonary bypass

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Objective: Higher mean arterial pressure during cardiopulmonary bypass may improve cerebral outcome associated with cerebral air embolism by increasing emboli clearance and collateral flow to salvage the ischemic penumbra. However, this may come at the expense of increased delivery of embolic load. This study was designed to investigate the influence of mean arterial pressures on cerebral functional and histologic outcome after cerebral air embolism during cardiopulmonary bypass in an established rat model.

Methods: Male Sprague–Dawley rats were exposed to 90 minutes of normothermic cardiopulmonary bypass with 10 cerebral air embolisms (0.3 μL/bolus) injected repetitively. Rats were randomized to 3 groups (n = 10, each) that differed in mean arterial pressure management during cardiopulmonary bypass: 50 mm Hg (low mean arterial pressure), 60 to 70 mm Hg (standard mean arterial pressure), and 80 mm Hg (high mean arterial pressure). Neurologic score was assessed on postoperative days 3 and 7 when cerebral infarct volumes were determined. Cognitive function was determined with the Morris water maze test beginning on postoperative day 3 and continuing to postoperative day 7.

Results: Neurologic score was better in high and standard mean arterial pressure groups versus low mean arterial pressure groups. High mean arterial pressure resulted in shorter water maze latencies compared with standard and low mean arterial pressure on postoperative days 6 and 7. Total infarct volume and number of infarct areas were not different among groups.

Conclusions: The use of higher mean arterial pressure during cardiopulmonary bypass in a rat model of cerebral air embolism conveyed beneficial effects on functional cerebral outcome with no apparent disadvantage of increased delivery of embolic load. Maintaining higher perfusion pressures in situations of increased cerebral embolic load may be considered as a collateral therapeutic strategy. (J Thorac Cardiovasc Surg 2011;142:424-9)

Cerebral injury, ranging from neurocognitive dysfunction to overt stroke, remains as a significant cause of morbidity and mortality after cardiac surgery using cardiopulmonary bypass (CPB).[1] Although considered multifactorial, the most importantly cited etiologic factors are cerebral macro- and microembolism and hypoperfusion.[2,3] Among them, the principal cause of neurocognitive impairment is believed to be cerebral microembolization with the majority being gaseous, which invariably occurs during CPB.[4,5] Accordingly, both experimental and human studies have demonstrated a significant correlation between the number, as well as the volume, of cerebral air emboli (CAE) and adverse neurologic outcome after CPB.[3,5-7]

With the view to provide neuroprotection during CPB, a generally accepted definition of optimal mean arterial pressure (MAP) to ensure adequate tissue perfusion has not been established. Although cerebral perfusion is assumed to remain constant over a wide range of MAPs, it is generally regulated by MAP rather than the pump flow rate during CPB.[8,9] In the presence of pathologic CAE impairing tissue perfusion, maintaining higher MAP on CPB has the theoretic advantage of enhancing collateral blood flow and facilitating emboli clearance. Yet, this may also lead to cerebral edema or increased delivery of embolic load to the brain.[2,8] In conjunction, the results of the limited observational clinical studies are contradictory, and no comprehensive data exist regarding the contribution of MAP to cerebral outcome after CPB.[10-12]

Therefore, the purpose of the present study was to investigate the influence of MAP during CPB on CAE-induced functional (neurologic and cognitive) and histologic cerebral injury in a randomized and controlled experiment using an established rodent model of CPB combined with CAE.[7]

MATERIALS AND METHODS

The Duke University Institutional Animal Care and Use Committee approved this study, and all procedures met the National Institutes of Health guidelines for animal care (Guide for the Care and Use of Laboratory

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This study was funded by departmental funds.

Disclosures: Authors have nothing to disclose with regard to commercial support.

Received for publication Aug 11, 2010; revisions received Nov 9, 2010; accepted for publication Nov 25, 2010; available ahead of print Jan 31, 2011.

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0022-5223/$36.00

doi:10.1016/j.jtcvs.2010.11.036

Abbreviations and Acronyms
CAE = cerebral air emboli
CPB = cardiopulmonary bypass
IV = intravenously
MAP = mean arterial pressure
POD = postoperative day
ROI = regions of interest

Animals, available at: www.nap.edu/catalog/5140.html). Thirty male Sprague–Dawley rats (age, 12–14 weeks; weight, 375–400 g; Charles River Laboratories, Inc, Wilmington, Mass) were studied. Animals were randomly assigned to 1 of 3 groups with repetitively administered CAEs (n = 10 each) that differed in the management of MAP during 90 minutes of normothermic CPB. MAP was maintained at 80, 60–70, or 50 mm Hg in the high, standard, and low MAP groups, respectively. MAP was adjusted by varying the inhalational concentration of isoflurane (range, 0.5%–2.5%) or using phenylephrine (not exceeding 2–3 μg intravenously [IV] per bolus).

Surgical Preparation and Cardiopulmonary Bypass

Fasted rats were anesthetized with 5% isoflurane in oxygen in a plastic induction box. After induction of anesthesia, the trachea was intubated and the lungs were mechanically ventilated (Harvard Rodent Respirator; Boston, Mass) with a tidal volume of 10 mL/kg and respiratory rate of 50 to 55 beats/min maintaining arterial P_{CO_2} between 36 and 42 mm Hg. During surgery, anesthesia was maintained with 2.0% to 2.5% isoflurane and fentanyl (25 μg/kg, intravenous, as a bolus injection).

Animals were cannulated for CPB as previously reported.[7,13] Briefly, the tail artery was cannulated for aortic inflow, a multistaged venous return cannula was placed in the heart through the right external jugular vein, and the right superficial caudal epigastric artery was cannulated for monitoring of MAP (model 90603A; SpaceLabs, Inc, Redmond, Wash). After the cannulation of the tail artery, animals received 150 units of heparin. During CPB, anesthesia was maintained using 0.5% to 1.2% isoflurane and fentanyl (150 μg/kg IV). No paralytics were administered until just before CPB when pancuronium (0.1 mg/kg) was administered to prevent spontaneous breathing during CPB. Fentanyl and pancuronium were repeated (at half the dose) at 30-minute intervals, as necessary. Pericranial temperature was monitored with CSC 32 (Omega Engineering, Inc, Stamford, Conn) and maintained at 37.5°C ± 0.1°C using a heating blanket and convective forced-air heating system.

The CPB circuit consisted of a venous reservoir, a peristaltic pump (Tygon; Cole-Patmer Instrument, Vernon Hills, Ill), a membrane oxygenator, and an arterial inflow cannula. An in-line flow probe (2N806 flow probe and T208 volume flowmeter; Transonics Systems, Inc, Ithaca, NY) was used to continuously measure CPB flow. To avoid excessive hemodilution, the bypass circuit was primed with 14 mL whole blood obtained from a heparinized (150 units IV heparin per rat) donor rat. In addition, 6% hetastarch (4 mL) was added to the circuit, as needed. One hundred units of heparin were added to the prime. Arterial line inflow temperature was maintained at 37.5°C using a circulating water bath system. Arterial blood gases were analyzed using a GEM Premier 3000 blood gas/electrolytic analyzer (model 5700; Instrument Laboratories, Inc, Lexington, Mass). Basic physiologic data, including MAP, temperature, and blood gases, were collected 15 minutes before commencement of CPB. All animals were subjected to 90 minutes of normothermic and nonpulsatile CPB with flow rates of 160 to 180 mL/min/kg corresponding to a normal cardiac output in the rat.[12] For the entire CPB period, ventilation of the lungs was discontinued. After 90 minutes of CPB, the animals were weaned from CPB without the need for inotropic support. Heparin-induced anticoagulation was allowed to dissipate spontaneously without supplemental administration of protamine. After decannulation, rats were maintained anesthetized with 0.5% to 1.2% isoflurane, intubated, and ventilated for 1 hour. When adequate spontaneous breathing resumed, the animals were extubated and recovered in an oxygen-enriched box for 24 hours with free access to water and food. During the first 6 hours of recovery, they were continuously observed to identify signs of immediate cerebral death and severe neurologic dysfunction (fixed pupils, absence of spontaneous breathing, seizures, and inability to ambulate). Animals demonstrating signs of severe neurologic dysfunction were sacrificed. All others were returned to their cages and housed individually.

Cerebral Air Embolism

The methodology of the CAE model used in the current study has been reported.[7] Briefly, rats subjected to 90 minutes of normothermic CPB received 10 equally sized CAEs (0.3 μL/single bolus). The choice of 0.3 μL per bolus was based on preliminary work showing that this size of CAE during CPB is associated with a mortality rate of 1% (95% confidence interval, 0.1–14.5) and an incidence for neurologic deficits of 85.8% (95% confidence interval, 40.7–98.2).[7] For the injection of CAEs, a PE-10 catheter (Intramedic; Becton-Dickinson, Sparks, Md) was inserted via the stump of the right external carotid artery and advanced into the right internal carotid artery beyond the pterygopalatine branch (0.8 cm distance) that was ligated. The catheter was connected to a syringe pump (KDS100; KD Scientific Inc, Holliston, Mass). The first embolus was administered at 15 minutes of CPB, and the last embolus was administered at 75 minutes of CPB. By using a Hamilton syringe with a 1.2-cm long needle (5 μL SYR, 75N; Hamilton Co, Reno, Nev), the size of the air bubble could be exactly determined by the placement of the air between 2 μL saline aliquots. After the delivery of the last CAE, 10 μL saline were injected to flush the last air embolus into the cerebral circulation.

Neurologic and Neurocognitive Testing

On the third and seventh postoperative days (PODs), animals underwent standardized functional neurologic testing using an established neurologic scoring system that evaluates 4 different functions, including general status, simple motor deficit, complex motor deficit, and sensory deficit.[14] The score given to each animal at the completion of the testing (by an observer blinded to group assignment) was the sum of all 4 individual scores: 0 was the minimum (best) score and 48 was the maximum (worst) score.

In addition to the neurologic evaluation, neurocognitive outcome was evaluated daily (starting on POD 3) in the Morris water maze using a computerized video tracking system (EthoVision; Noldus, Wageningen, The Netherlands).[15] The Morris water maze consisted of a 1.5-m diameter, 30-cm deep pool of water (27°C) with a hidden submerged (3 cm below surface) platform in 1 quadrant. Rats were placed in the water in a dimly lit room with various visual clues around the maze. The time to locate the submerged platform (defined as the latency) was measured to test for impairment in visuospatial learning and memory components of neurocognition. Rats underwent daily testing in the water maze with 4 trials per testing period, each limited to a 90-second water exposure. Each of the trials was begun from a separate quadrant. Testing was performed for 5 consecutive days until POD 7.

Histologic Examination

After completion of the testing on the final day, the animals were anesthetized with 5% isoflurane and decapitated. The brains were removed, snap-frozen at –40°C in 2-methyl-butane, and stored at –80°C for later analysis.

Infarct volume was measured by using previously published methods.[14] Serial quadruplicate 20-μm–thick coronal sections were taken by using a cryotome at 660-μm intervals over the rostrocaudal extent of the infarct. The sections were dried and stained with hematoxylin–eosin. A representative section from each 660-μm interval was digitized with a video camera controlled by an image analyzer (M2 Turnkey System; Imaging Research,