Rat model of cardiopulmonary bypass for deep hypothermic circulatory arrest

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Large animal cardiopulmonary bypass (CPB) models have disadvantages, such as cost, lack of methods for assessment of neurocognitive function, and difficulties with long-term recovery. As a result, rodent models have been developed.¹⁻⁴ Previous rodent models have certain drawbacks, including irrelevance to human flows used clinically. We established a clinically relevant model of CPB with deep hypothermic circulatory arrest (DHCA) in the rat. Our CPB circuit has several novel features, including a small priming volume, active cooling/rewarming processes, vacuum-assisted venous drainage, peripheral cannulation without thoracotomy or sternotomy, and an accurate means of monitoring peripheral tissue oxygenation. This small animal model provides a simple experimental system that could be used in future studies that use CPB and DHCA.

MATERIALS AND METHODS

Animal Model

The University of Iowa Institutional Animal Care and Use Committee approved this study. Fifteen Sprague-Dawley rats (350–450 g) from Charles River Laboratories (Wilmington, Mass) were housed under standard conditions. Nonfasted rats were anesthetized with chloral hydrate and isoflurane in 60% oxygen. During surgical preparation and recovery, anesthesia was maintained with isoflurane. Rats were allowed to spontaneously breathe air with an inspired oxygen fraction of 100%. After the start of CPB, the oxygenator gas flow rate was held between 1 and 2.5 L/min to maintain blood gases within the physiologic range. Animals were not intubated.

Surgical preparation consisted of cannulation of the left femoral artery for arterial inflow with a 20-gauge Angiocath catheter (Becton Dickinson, Franklin Lakes, NJ) and the right femoral artery for blood pressure monitoring with a 22-gauge Angiocath catheter via a cutdown. An injection of 400 IU/kg of porcine heparin was given after placement of the first catheter, and activated clotting time was maintained above 500 during CPB. The right common jugular vein was cannulated with a modified 4-hole 16-gauge Angiocath catheter that was advanced into the right atrium for venous drainage. A vacuum regulator with an applied pressure of –30 mm H₂O was developed to facilitate increased venous drainage.

A rectal temperature of 18°C was achieved over 40 minutes, using CPB-assisted cooling. At this temperature, rats still had a heart rate in the 30s. If the heart rate was not 0, we further cooled to cardiac arrest by echocardiogram (typically 14°C). After reaching arrest, the circuit was turned off and rats were left in a DHCA state for 15 minutes. Next, rats were rewarmed to

34°C to 35°C over a period of 36 to 42 minutes using CPB-assisted re-warming, a heating blanket, and a heating lamp. The temperature gradient between the CPB circuit and body core did not exceed 10°C. Simultaneously, 0.1 mEq of sodium bicarbonate and 0.14 mEq of calcium chloride were administered. After full rewarming, the remaining priming volume was reinfused and animals were weaned from CPB.

Physiologic Parameter Measurement and Analysis

The core and circuit temperature were continuously monitored with a dual temperature monitor (Figure 1, A) (DeBusk Temperature Systems; DeRoyal Technologies, Inc, New Tazewell, Tenn). An iStat Analyzer (Abbott Point of Care; Abbott Laboratories, Abbott Park, Ill) was used to analyze arterial blood gases (α -stat method), hemoglobin, and hematocrit. Samples were taken at baseline, 27°C, and 18°C during the cooling stage (approximately 20 and 40 minutes, respectively) and at 27°C and 34°C during the rewarming stage (approximately 60 and 80 minutes, respectively). The DHCA duration was excluded from aforementioned time. During CPB, the blood flow and venous saturation were continuously monitored. Targeted CPB flow was 100 to 120 mL · kg⁻¹ · min⁻¹, corresponding to about 70% of normal cardiac output.

Experimental Circuitry

The CPB circuit (shown in entirety in Figure 1) was primed with 12 mL of Plasma-Lyte solution (Baxter Healthcare, Deerfield, Ill) and consisted of the following components:

- Venous reservoir (Figure 1, F), connected to vacuum regulator (Ohmeda; Ohio Medical Corporation, Gurnee, Ill)
- Peristaltic pump (Figure 1, C) (Masterflex; Cole-Parmer, Vernon Hills, Ill)
- Heat exchanger (Figure 1, E) (Sarns cardioplegic set; Terumo Cardiovascular Systems, Ann Arbor, Mich)
- Specifically designed rodent membrane oxygenator (Figure 1, D) (Arterocyte, Cleveland, Ohio). The membrane oxygenator is constructed from 2 Plexiglas shells (Rohm and Haas Company, Philadelphia, Pa), which carry the diffusion membrane. The diffusion membrane is composed of 3 layers of polypropylene hollow-fiber mats (Jostra AG, Hirrlingen, Germany) fused in a crosswise fashion to improve oxygenation. The membrane has a gas exchange area of 558 cm² and a priming volume of 4 mL.
- Ultrasound flowmeter (Figure 1, B) (2N806 flow probe and T208 volume flowmeter; Transonic Systems, Ithaca, NY)
- Mixed venous oximeter (Oximetrix 3, Abbott Critical Care Systems)
- Silicone tubing with a 1.6-mm internal diameter (Cole-Parmer Instruments)

RESULTS

Fifteen rats were used for analysis. The average values of physiologically relevant parameters, taken immediately before the initiation of the cooling process (baseline) and at various stages of the procedure (ie, during cooling at 20 and 40 minutes and during rewarming at 60 and 80 minutes) are shown in Table 1.

Rats were typically cooled to 14°C to 18°C until complete arrest. The duration of DHCA was excluded from

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FIGURE 1. Cardiopulmonary bypass circuit: A, temperature monitor; B, ultrasonic flowmeter; C, roller pump; D, membrane oxygenator; E, heat exchanger; F, venous reservoir; G, arterial line; and H, venous line.

data analysis (15 minutes). Owing to hemodilution, some rats demonstrated a low mean arterial pressure and hemoglobin concentration as compared with baseline values. The mean arterial pressure dropped 20 points (on average) at the beginning of CPB but was subsequently maintained above 50 mm Hg via epinephrine injection. Typically, during rewarming, mean arterial pressure remained above 50 mm Hg without the use of inotropic agents. Lidocaine 2 mg and calcium chloride 0.14 mEq were routinely administered during rewarming. If animals had a pH less than 7.0, they received sodium bicarbonate 0.1 mEq.

DISCUSSION

We established a small animal model of CPB with DHCA in the rat. Our circuit has several innovative elements, including vacuum-assisted venous drainage, peripheral cannulation (without thoracotomy or sternotomy), active heating and cooling with a heat exchanger, a novel small membrane oxygenator, lack of need for intubation, a venous oximeter, and ultrasound flowmeter. With all these modifications, we were able to achieve 100 to 150-mL · kg⁻¹ · min⁻¹ flow, which is relevant to clinically used CPB circuits.

TABLE 1. Physiologically relevant parameters of rats undergoing CPB with DHCA (n = 15)

Time	Baseline	Cooling	Rewarming		
	0 min	20 min	40 min	60 min	80 min
Hemoglobin (g/dL)	13.3 (1.4)	7.3 (1.3)	6.6 (0.9)	6.3 (1.1)	6.3 (0.8)
Hematocrit	39.7 (3.9)	21.8 (3.7)	19.6 (3.2)	18.9 (3.6)	18.6 (3.2)
Flow (mL/min)	49.1 (7.5)	40.6 (12.5)	39.7 (14.7)	51.7 (14.8)	46.6 (11.7)
Temperature (°C)	33.8 (0.8)	27.1 (1.6)	17.9 (0.7)	27.8 (3.2)	35.5 (0.8)
Heart rate	185.4 (43.3)	56.5 (18.7)	23 (4.8)	149.8 (42.9)	190.9 (41.3)
MAP	69.9 (20.1)	49.8 (18.3)	51 (16.1)	61.8 (15.3)	59.8 (7.4)
pH	7.15 (0.12)	7.12 (0.11)	7.14 (0.08)	7.18 (0.14)	7.15 (0.14)
Pco ₂	52.3 (20.0)	46.9 (9.9)	43.0 (7.1)	44.25 (17.2)	43.3 (15.4)
Po ₂	184.8 (128.0)	111.2 (73.2)	187.5 (103.7)	98 (32.9)	88.41 (31.3)
HCO ₃ ⁻	18.7 (4.8)	15.8 (2.9)	15.5 (3.5)	17.9 (7.6)	15.6 (6.9)
Base excess	-11.4 (3.8)	-12.3 (4.2)	-13.4 (5.3)	-9.1 (8.4)	-11.7 (8.8)
Sao ₂	95.3 (5.4)	87.6 (11.8)	96.9 (2.6)	92.1 (3.9)	90.2 (6.1)
MVO ₂	64.8 (18.5)	61.9 (15.7)	72 (12.3)	56.1 (13.9)	50.6 (13.1)

CPB, Cardiopulmonary bypass; DHCA, deep hypothermic circulatory arrest. Mean and (standard deviation) for Hemoglobin, hematocrit, flow through the pump, rectal temperature, heart rate, mean arterial pressure (MAP), pH, carbon dioxide tension (Pco₂), oxygen tension (Po₂), and bicarbonate (HCO₃⁻), as well as base excess, arterial oxygen saturation (Sao₂), and mixed venous (MVO₂) oxygen saturation. These were measured at time 0, as well as 20 and 40 minutes into cooling, which corresponded with 37°C, 27°C, and 17°C. DHCA duration was 15 minutes, then after 20 and 40 minutes (labeled 60 min and 80 min, respectively).

Miniaturization of the membrane oxygenator represents the major limiting factor in the creation of a successful CPB model in small animals. Previous models have required high priming volumes to achieve acceptable hematocrit concentrations during the experiment. Our study uses a miniature novel oxygenator, modified venous cannula design, and reduction in the length of connection tubing, which allowed minimization of the priming volume, thus improving postoperative outcomes.

This model was developed to study neurocognitive models after DHCA. Thus, peripheral cannulation instead of thoracotomy or sternotomy is desired. Endotracheal intubation with mechanical ventilation was not performed. The absence of mechanical ventilation helped prevent the hypotension and bradycardia commonly observed at cessation of ventilation during CPB. Vacuum-assisted venous drainage, along with careful positioning of the venous outflow catheter, allowed an optimal venous drainage and provided an adequate perfusion flow rate of 100 to 120 mL · kg⁻¹ · min⁻¹, which is comparable with human flows. Ready access to femoral arteries reduced surgical trauma and resulted in decreased blood loss, in addition

to better perfusion flow as compared with cannulation of the tail artery.

Full cardiac arrest was achieved at 14°C in all animals. Whereas others have used 9 mg of esmolol and 0.2 mEq of potassium chloride to reach cardiac arrest,⁴ we managed to achieve cardiac arrest with cooling alone (at a temperature of 15.7°C ± 0.4°C).

Certain limitations to this study remain, such as the absence of monitoring of pericranial temperature. Future modifications include the use of a hemoconcentrator to improve hematocrit. We will use this model in future studies to assess the neuroprotective effects of small molecules during DHCA.

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