Neurologic outcome after cardiopulmonary bypass with deep hypothermic circulatory arrest in rats: Description of a new model

Bettina Jungwirth, MD,[a] G. Burkhard Mackensen, MD,[a,b] Manfred Blobner, MD,[a] Frauke Neff, MD,[d] Bruno Reichart, MD,[c] Eberhard F. Kochs, MD,[a] and Georg Nollert, MD, FAHA[c]

Drs B. Jungwirth and G. B. Mackensen (*left to right*)

Objective: Neurodevelopmental impairments after repair of congenital heart disease with cardiopulmonary bypass and deep hypothermic circulatory arrest continue to affect the lives of children. To date, the preclinical investigation of cerebral injury mechanisms related to deep hypothermic circulatory arrest has been restricted to expensive, personnel-demanding, and cumbersome large-animal models without validated neuropsychologic assessment. We aimed to establish a rodent recovery model of deep hypothermic circulatory arrest to overcome these disadvantages.

Methods: Male rats (n = 34) were cannulated for cardiopulmonary bypass, cooled to a rectal temperature of 16°C to 18°C within 30 minutes, and assigned to deep hypothermic circulatory arrest durations of 0, 45, 60, 75, 90 (n = 6, respectively), or 105 (n = 4) minutes. After rewarming within 40 minutes, animals were weaned from cardiopulmonary bypass at 35.5°C. Neurologic and cognitive performance was assessed with the modified hole board test until postoperative day 14. Thereafter, brains were perfusion fixed and histologically analyzed.

Results: Logistic regression analyses identified dose-dependent associations between survival, neurologic or cognitive function, and duration of deep hypothermic circulatory arrest. Functional and histologic deficits were detectable after clinically relevant deep hypothermic circulatory arrest durations. The overall neurologic function did not correlate with histologic outcome ($r = 0.51$, $P > .05$).

Conclusions: The current study presents a novel recovery model of cardiopulmonary bypass with deep hypothermic circulatory arrest in the rat. In contrast to studies in large animals, even clinically relevant deep hypothermic circulatory arrest durations up to 60 minutes resulted in detectable deficits. Consequently, this experimental model appears to be suitable to further elucidate the mechanisms associated with adverse cerebral outcome after cardiac surgery and deep hypothermic circulatory arrest and to investigate potential neuroprotective strategies.

From Klinik für Anaesthesiologie[a] and Institut für Allgemeine Pathologie und Pathologische Anatomie,[d] Technische Universität München, Klinikum rechts der Isar, Munich, Germany; the Department of Anesthesiology, Duke University Medical Center,[b] Durham, NC; and Herzchirurgische Klinik im Klinikum Großhadern, Ludwigs-Maximilian Universität München,[c] Munich, Germany.

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Address for reprints: G. Burkhard Mackensen, MD, Department of Anesthesiology, Division of Cardiothoracic Anesthesiology and Critical Care Medicine, Duke University Medical Center, Durham, NC 27710 (E-mail: b.mackensen@duke.edu).

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Although overall morbidity and mortality of children after repair of congenital heart disease with deep hypothermic circulatory arrest (DHCA) have been substantially improved, considerable neurologic and neurodevelopmental sequelae still affect this patient population. Preventing cerebral injury remains difficult because the underlying mechanisms are incompletely understood. Although several preoperative and intraoperative factors, including the use of DHCA, have been identified as risk factors for postoperative cerebral impairments,[1-3] clinical studies have only recently contributed to reveal the underlying injury mechanisms.[4] Therefore, an appropriate disease model of cardiopulmonary bypass (CPB) and DHCA is needed.[5] To date, preclinical investigations of cerebral injury after DHCA have been restricted to large-animal models. These models all have certain disadvantages, drawbacks, and limitations, including significant costs,

Abbreviations and Acronyms
CPB = cardiopulmonary bypass
DHCA = deep hypothermic circulatory arrest
MAP = mean arterial blood pressure
mHBT = modified hole board test

logistic requirements, lack of suitable tests for the assessment of neurologic and neurocognitive function, and difficulties with long-term recovery. The rat is the most widely accepted animal to study cerebral injury, with various behavioral and cognitive tests available. Because a long-term recovery model of CPB in this species has recently been introduced,[6] we sought to assess whether a clinically relevant recovery model of CPB and DHCA in the rat could be established to investigate basic mechanisms of cerebral injury.

The aims of the present study were to determine (1) whether DHCA in the rat could be performed mimicking current clinical standards and whether survival after different durations of DHCA is feasible; (2) whether exposure to different durations of DHCA affects postoperative neurologic, neurocognitive, or behavioral performance; and (3) whether these impairments are associated with histologic alterations.

Methods

All animals were treated in compliance with the "Principles of Laboratory Animal Care" formulated for the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Science (National Institutes of Health publication no. 86-23, revised 1985). Experimental protocols were approved by the institutional animal care committee.

Male Sprague Dawley rats from Charles River Laboratories (Sulzfeld, Germany) were housed under standard laboratory conditions (12 hours light/12 hours dark, lights on at 12:30 AM, 22°C, 60% humidity, and free access to water and standard rat chow) 3 weeks before the experiments to acclimate to the changed day-night rhythm. Nine days before surgical preparation, animals were housed in the modified hole board environment for habituation.

Rats were randomly assigned to 1 of 6 groups with different durations of DHCA. In the 0-minute DHCA group (n = 6) rats were cooled to a rectal temperature of 16°C to 18°C and immediately rewarmed over 40 minutes without any arrest time. Animals in the other 5 groups were cooled accordingly but subjected to either 45 minutes (n = 6), 60 minutes (n = 6), 75 minutes (n = 6), 90 minutes (n = 6), or 105 minutes (n = 4) of DHCA.

Surgical Preparation

Nonfasted rats (356 ± 19 g, 10 weeks old) were cannulated for CPB, as previously reported.[6] In brief, surgical intervention was performed in anesthetized (2-2.5 Vol% isoflurane), endotracheally intubated (14-gauge intravenous catheter), and mechanically ventilated (45% O_2/balance N_2, $PaCO_2$ of 35-45 mm Hg) rats by using aseptic techniques. The tail artery was cannulated with a 20-gauge catheter for aortic inflow. After the placement of the arterial catheter, rats were given 150 IU of heparin and 5 μg of fentanyl. Through the right external jugular vein, a 4.5F multiorifice cannula was advanced into the right atrium for venous return. Mean arterial blood pressure (MAP) was monitored through the right superficial caudal epigastric artery. Baseline physiologic measurements, including MAP, pericranial and rectal temperature, and blood gases (Rapidlab 860 blood gas analyzer; Diamond Diagnostics, Holliston, Mass), were recorded 10 minutes before commencement of CPB. During surgical preparation, the temperature was allowed to decrease spontaneously; however, rectal temperatures of less than 34°C were avoided by warming the animals with heating blankets and a convective forced-air heating system (Warmtouch 5200; Nellcor, Hazelwood, Mo).

CPB and DHCA

The CPB circuit consisted of a venous reservoir, a peristaltic pump (Masterflex; Cole-Parmer Instrument Co, Vernon Hills, Ill), a specifically developed membrane oxygenator (prime volume of 4 mL, gas exchange area of 558 cm^2), an inline flow probe (2N806 flow probe and T208 volume flowmeter; Transonics Systems, Inc, Ithaca, NY), and an arterial inflow cannula, all of which were connected through 1.6-mm internal diameter silicone tubing (Tygon, Cole-Parmer Instrument Co). The CPB circuit was primed with 10 mL of 6% hetastarch. The small-volume oxygenator developed specifically for the use in rats is built of 2 Plexiglas shells (12.8 cm × 12.8 cm × 2.7 cm) carrying the diffusion membrane. The membrane consists of 3 layers of polypropylene hollow-fiber mats (Jostra AG, Hirrlingen, Germany) glued together in a crosswise fashion to improve oxygenation. The provided gas exchange area is 558 cm^2. CPB was instituted at a flow rate of 160 to 180 $mL \cdot kg^{-1} \cdot min^{-1}$ and was consecutively decreased by half during the cooling period. During CPB, the oxygenator received a gas mixture of O_2 and variable concentrations of CO_2 as arterial blood gases were controlled with the pH-strategy ($PaCO_2$ of 31-40 mm Hg, temperature adjusted). The animals were cooled to a rectal temperature of 16°C to 18°C over 30 minutes by using a heat exchanger and topical cooling with ice bags and a cooling blanket. CPB was discontinued, and venous blood was drained to the reservoir. DHCA confirmed by asystole was maintained for 45, 60, 75, 90, or 105 minutes at 16°C to 18°C. Animals subjected to 0 minutes of DHCA were immediately rewarmed without arrest. After DHCA, CPB was reinstituted, and rats were rewarmed to rectal temperatures of at least 35.5°C over 40 minutes; subsequently, CPB was terminated. During CPB, the rats were anesthetized with 0.8% to 1% isoflurane, cisatracurium (1.6 mg/h), and repetitive boluses of 5 μg of fentanyl, while anesthesia was discontinued during DHCA. During DHCA, animals were not ventilated, but during CPB, a continuous positive airway pressure mode (5 cm H_2O) with a fraction of inspired oxygen of 0.21 was applied to avoid atelectasis. After DHCA, bicarbonate was administered to correct acidosis. During rewarming, MAP was kept at greater than 50 mm Hg with norepinephrine as soon as rectal temperatures of at least 30°C and blood flows of at least 150 $mL \cdot min^{-1} \cdot kg^{-1}$ were achieved.