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

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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.

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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 ven-

tilated (45% O₂/balance N₂, PaCO₂ 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 μ g 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 cm²), 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 $\times$ 12.8 cm $\times$ 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 cm². 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 O₂ and variable concentrations of CO₂ as arterial blood gases were controlled with the pH-strategy (PaCO₂ 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 μ g 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 H₂O) 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.

Recovery and Survival

After decannulation, rats were maintained anesthetized with 1.0% to 1.5% isoflurane, intubated, and ventilated for 1 hour (rectal temperature of 36.5°C). To increase the hematocrit value to greater than 30%, the remaining blood in the CPB circuit was collected, centrifuged (2000 rpm for 10 minutes), and transfused to the animals. Bicarbonate, calcium, and glucose levels were monitored and administered as needed. Heparin-induced anticoagulation was not reversed by protamine. After spontaneous ventilation had resumed, animals were extubated and allowed to recover in an oxygen-enriched environment for 12 hours, with free access to water and food. During the first 6 hours of recovery, they were continuously observed to identify severe neurologic dysfunction, such as immediate signs of cerebral death (fixed pupils, no reaction to pain, and absence of spontaneous breathing), seizures, and inability to ambulate. Animals demonstrating signs of severe neurologic dysfunction were killed. All others were returned to their home board cages at the first postoperative day and housed in familiar groups.

Neurologic, Cognitive, and Behavioral Testing

All neurologic, cognitive, and behavioral tests were performed by an investigator blinded to group assignment. On the preoperative, as well as on the first, second, third, and twelfth postoperative days, animals underwent standardized functional neurologic testing, as previously described (Table 1).⁷ In addition, cognitive and behavioral functions were assessed by the modified hole board test (mHBT), according to an established protocol.^{8,9} In brief, the rats were housed in an area divided into a home cage (80 × 60 × 50 cm) and a test arena (40 × 60 × 50 cm) with the centrally placed hole board (20 × 40 cm). Fifteen holes covered by lids were staggered on the board. After being opened, coil springs force the lids back to their original position. Three holes baited with puffed rice were marked with white tape. All holes were flavored with the aroma of black currants to cover the odor and smell of the puffed rice.

After habituation to the mHBT on postoperative days 2 and 3, rats learned the test procedure over 6 consecutive days (learning period). Subsequent to a resting period of 4 days, memory function was assessed on postoperative day 14 (retest). Animals were tested in the mHBT for 3 trials daily, and the sequence of marked holes was randomly changed every day. Various cognitive, motor, and behavioral parameters were studied with the mHBT (Table 1).

Histologic Examination

After completion of the neurocognitive testing on the 14th postoperative day, animals were anesthetized with 5% isoflurane and underwent in situ brain fixation by a standardized intracardiac injection of 20 mL of saline, followed by 20 mL of paraformaldehyde (4%). The brain was removed in toto, cut in 2-mm slices, and then embedded in paraffin. The first section (10 μm) of each block was harvested and stained with hematoxylin and eosin to determine cell damage. All slides were scored by a neuropathologist blinded to the experimental groups. Five brain regions of each hemisphere were chosen for evaluation of neuronal cell damage: hippocampus, frontal cortex (motor area), cerebellar vermis, cingulate cortex (posterior part), and striatum (caudate putamen). Five high-magnification (440×) fields per region were ex-

amined by use of the following scores: 0, fewer than 5 damaged neurons; 1, 5 to 10 damaged neurons; 2, more than 10 damaged neurons; and 3, infarction. The score values for the single regions were summed to a total histologic score for each animal, with a maximum score of 150 (5 high-magnification fields × 3 as worst score × 10 brain regions) and a normal range of 0 to 12 taken from 6 healthy untreated rats (mean ± 2 standard deviations).

Statistics and Data Management

Physiologic values were analyzed by use of general linear models with the between-groups factor DHCA duration, the within-groups factor time², and their interaction term (time² × DHCA duration). Effects of time levels were analyzed quadratically, focusing on biphasic changes of all variables during the observation period. Once time² × DHCA duration was significant ($P < .05$), post-hoc values at certain times were analyzed by factorial analysis of variance, followed by Bonferroni *t* tests.

Logistic regression analyses were performed with DHCA duration as the independent variable and survival, survival without motor deficit, or survival without cognitive deficits as dependent variables to analyze the effect of DHCA duration on outcome. Survival without the respective deficit was assumed if none of the tested qualities was out of its normal range (Table 1).

Additionally, functional outcome of surviving rats was correlated with the histologic outcome by Spearman rank correlation. Therefore, individual functional parameters were recorded as score values (maximum scores being the worst outcome) and summed to a total neurologic score (0-85) for each animal (Table 1).

Results

Three animals were excluded from further data analysis because of either insufficient venous return (1 animal in the 45-minute DHCA group) or cannulation problems (1 rat in the 60-minute and 1 rat in the 75-minute DHCA groups). These rats were replaced to keep sample size equal. Hemodynamic and physiologic values of rats are summarized in Table 2. Priming the circuit with 6% hetastarch led to a decrease in hemoglobin concentration from 14.7 ± 0.3 g/dL at baseline to 7.1 ± 0.3 g/dL during CPB. Retransfusion of red cells after CPB restored the hemoglobin concentration to 10.7 ± 0.3 g/dL.

Survival was related to DHCA duration in a dose-dependent manner, with 50% survival at 83 minutes (95% confidence interval, 71-99 minutes; Figure 1, A). All animals exposed to 105 minutes of DHCA showed immediate signs of brain death. In the 90-minute group, 2 rats were killed within the first postoperative hours because of severe cerebral injury, and 1 rat died during the first postoperative day because of gastrointestinal paresis. After initial survival, 3 animals in the 75-minute DHCA group died of intestinal dysfunction on the first, second, and fourth postoperative days, respectively. All remaining rats survived the entire recovery period until brain fixation at postoperative day 14.

Motor function and neurocognitive outcome were affected by DHCA duration. With increasing DHCA durations, the percentage of animals without motor and cogni-

TABLE 1. The whole test battery used in the current study for the assessment of motor, cognitive, behavioral, and sensory function after deep hypothermic circulatory arrest

Quality	Test	Level of impairment							Score	Normal
		0	1	2	3	4	5	6		
Motor function										
Balance	Balance time on beam	50-60 s	30-49 s	10-29 s	1-10 s	unable	—	—	0-4	0-1
Coordination	Missteps on beam	1-3	4-10	11-20	>20	unable	—	—	0-4	0
Physical strength	Time gripping a rope	11-15 s	6-10 s	1-5 s	unable	—	—	—	0-3	0
Physical strength	Time on a rotating grid	>5 s at a 180° turn	<5 s at a 180° turn	Only 90° turn	unable	—	—	—	0-3	0
Movement symmetry	Observation of active movement	Symmetric	One-sided weakness	Hemiplegia	—	—	—	—	0-2	0
Gait	Observation of gait	Normal	Ataxia	Circular motion	—	—	—	—	0-2	0
Fine-motor skill	time needed for 1 food intake (mHBT)	<20 s	20-30 s	30-45 s	>45 s	—	—	—	0-3	0
Dexterity	Futile efforts to open a baited hole (mHBT)	0	1	2	>2	—	—	—	0-3	0
Cognitive function (assessed with the mHBT)										
Declarative memory	Wrong choices of 3 trials	0	1	2	3	4	5	>5	0-6	0-1
	Omission errors of 3 trials	0	1	2	3	4	5	>5	0-6	0
Working memory	Repeated choices of 3 trials	0	1	2	3	4	5	>5	0-6	0-1
Cognitive performance	Time needed to complete 3 trials	<200 s	200-399 s	400-599 s	600-799 s	800-999 s	1000-1199 s	>2000 s	0-6	0-1
Behavior (assessed with the mHBT)										
Anxiety	Time spent on board per trial	>15%	6%-15%	<6%	—	—	—	—	0-2	0
	Latency to first enter the board	<10 s	>10 s	—	—	—	—	—	0-1	0
	No. of board entries	>1	No entries	—	—	—	—	—	0-1	0
Welfare	Immobility per trial	<5%	5%-10%	10%-30%	>30%	—	—	—	0-3	0
Directed exploration	Latency to first visit a baited hole	<30 s	>30 s	—	—	—	—	—	0-1	0
	Visits of baited holes	>2	<2	—	—	—	—	—	0-1	0
General exploration	No. of rearings	<3	3-5	5-10	>10	—	—	—	0-3	0
Arousal	No. of groomings	<2	2-3	3-4	>4	—	—	—	0-3	0
	No. of defecations	None	>1	—	—	—	—	—	0-1	0
Social affinity	No. of group contacts	<4	4-6	6-8	>8	—	—	—	0-3	0
Locomotor activity	No. of line crossings	<3	3-4	4-5	>5	—	—	—	0-3	0
Sensory function										
Vision	Reaction to a falling cotton wool	Both sides	Only one side	No reaction	—	—	—	—	0-2	0
Hearing	Reaction to a whistle	Yes	No	—	—	—	—	—	0-1	0
Body proprioception	Reaction to a stimulus on each body side	Normal on both sides	Slowly on one side	Slowly on both sides	No on one side	No on both sides	—	—	0-4	0
Vibrissae proprioception	Turning head after touching vibrissae	Equally on both sides	Slowly on one side	Slowly on both sides	None on one side	None on both sides	—	—	0-4	0
Consciousness	Observation of activity	Normal	—	Clouded	—	Lethargic	—	—	0-4	0
Total neuroscore									0-85	0-4

Normal ranges were defined as the mean $\pm$ 2 standard deviations of 96 healthy untreated rats. *mHBT*, Modified hole board test.

tive deficits decreased. Fifty percent of the animals survived without any deficits in motor function after 48 minutes of DHCA (95% confidence interval, 19-63 minutes; [Figure 1, B](#)) and without any cognitive impairment after 40 minutes

of DHCA (95% confidence interval, 0-60 minutes; [Figure 1, C](#)). With the exception of 3 animals (1 in the 60-minute, 1 in the 75-minute, and 1 in the 90-minute DHCA groups), rats demonstrated no alterations in behavior 14 days after

TABLE 2. Physiologic data during the operative procedure in 6 groups of escalating durations of deep hypothermic circulatory arrest

	DHCA duration	Before CPB	Before DHCA	After DHCA	CPB end	After CPB (1 h)	Time ² × DHCA	Time ²
Pericranial temperature (°C)	0 min	35.8 ± 0.3	13.9 ± 0.7	19.1 ± 1.1	35.8 ± 0.1	36.7 ± 0.2	<i>P</i> < .02	<i>P</i> < .01
	45 min	36.0 ± 0.4	14.9 ± 0.3	19.5 ± 0.8	35.9 ± 0.2	36.4 ± 0.3		<i>P</i> < .01
	60 min	35.9 ± 0.7	13.9 ± 1.2	18.9 ± 2.9	35.4 ± 0.6	36.4 ± 0.3		<i>P</i> < .01
	75 min	35.1 ± 0.4	15.5 ± 0.9	21.2 ± 0.9	35.8 ± 0.0	36.6 ± 0.2		<i>P</i> < .01
	90 min	35.2 ± 0.3	14.3 ± 0.5	23.1 ± 0.3	35.8 ± 0.2	36.7 ± 0.2		<i>P</i> < .01
	105 min	36.5 ± 0.4	16.2 ± 0.3	21.5 ± 1.0	36.1 ± 0.2	38.4 ± 0.8*		<i>P</i> < .01
Mean arterial pressure (mm Hg)	0 min	84 ± 2	35 ± 3	32 ± 2	<i>I</i>	78 ± 4	<i>P</i> < .03	<i>P</i> < .01
	45 min	79 ± 4	32 ± 1	53 ± 8		77 ± 6		<i>P</i> < .01
	60 min	81 ± 4	38 ± 4	45 ± 10		78 ± 10		<i>P</i> < .01
	75 min	84 ± 5	34 ± 2	45 ± 4		81 ± 4		<i>P</i> < .01
	90 min	89 ± 3	33 ± 2	45 ± 4		86 ± 6		<i>P</i> < .01
	105 min	87 ± 3	37 ± 2	71 ± 9†		71 ± 2		<i>P</i> < .02
Glucose concentration (mg/dL)	0 min	125 ± 23	143 ± 25	187 ± 38	289 ± 51	208 ± 34	<i>P</i> < .01	NS
	45 min	126 ± 15	139 ± 12	199 ± 28	237 ± 50	158 ± 18		<i>P</i> < .04
	60 min	140 ± 34	143 ± 45	252 ± 39	315 ± 73	190 ± 66		<i>P</i> < .01
	75 min	135 ± 16	129 ± 7	226 ± 17	307 ± 32	181 ± 23		<i>P</i> < .01
	90 min	124 ± 10	138 ± 8	284 ± 24	344 ± 19	141 ± 18		<i>P</i> < .01
	105 min	103 ± 7	160 ± 9	291 ± 48	291 ± 33	77 ± 10†, //		<i>P</i> < .01
PaO ₂ (mm Hg)	0 min	215 ± 20	499 ± 18	426 ± 23	338 ± 25	189 ± 32	NS	<i>P</i> < .01
	45 min	161 ± 14	486 ± 16	401 ± 22	357 ± 15	188 ± 26		<i>P</i> < .01
	60 min	194 ± 42	512 ± 62	453 ± 51	329 ± 42	157 ± 41		<i>P</i> < .01
	75 min	171 ± 20	496 ± 35	429 ± 28	345 ± 22	171 ± 15		<i>P</i> < .01
	90 min	192 ± 17	463 ± 37	407 ± 20	346 ± 22	215 ± 34		<i>P</i> < .01
	105 min	237 ± 32	462 ± 27	423 ± 17	398 ± 27	112 ± 9		<i>P</i> < .01

Some values were controlled within defined physiologic ranges at certain times, and therefore they are not presented and statistically analyzed. *I*, Mean arterial pressure was controlled above 50 mm Hg by means of norepinephrine infusion at mean infusion rates of $2 \pm 1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (0-minute DHCA group), $2 \pm 1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (45-minute DHCA group), $3 \pm 2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (60-minute DHCA group), $3 \pm 1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (75-minute DHCA group), $6 \pm 1 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (90-minute DHCA group), and $16 \pm 4 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (*105-minute DHCA group). *//*, serum glucose concentration was controlled above 60 mg/dL by means of glucose infusion of $100 \pm 58 \text{ mg per rat}$; (*105-minute DHCA group). After DHCA, base excess was controlled between -3 and 3 mmol/L by using total doses of NaHCO_3 of $0 \pm 0 \text{ mmol/L}$ (0-minute DHCA group), $28 \pm 8 \text{ mmol/L}$ (†45-minute DHCA group), $53 \pm 29 \text{ mmol/L}$ (†60-minute DHCA group), $80 \pm 12 \text{ mmol/L}$ (†75-minute DHCA group), $99 \pm 3 \text{ mmol/L}$ (†90-minute DHCA group), and $265 \pm 21 \text{ mmol/L}$ (*105-minute DHCA group). Data are presented as means $\pm$ SEM. DHCA, Deep hypothermic circulatory arrest; CPB, cardiopulmonary bypass; NS, not significant. **P* < .05 compared with 0, 45, 60, 75, and 90 minutes; †*P* < .05 compared with 0-minute DHCA group.

DHCA. Sensory function was not impaired in any of the surviving rats at postoperative day 12.

Neurologic outcome, including motor, cognitive, sensory, and behavioral function, at postoperative days 12 or 14 was not correlated with the histologic score ($r = 0.51$, Figure 2). However, distinct histologic changes were evident in the hippocampus and the cerebellar vermis (Figure 3).

Discussion

This study presents a novel long-term recovery model of DHCA in the rat on the basis of a recently introduced and improved rodent model of complete CPB.^{6,10} Long-term recovery of rats exposed to DHCA was accomplished, with survival depending on DHCA duration. Motor, as well as neurocognitive, performance was impaired after clinically relevant durations of DHCA up to 60 minutes and persisted for at least 14 days. At this time, the overall functional outcome was not correlated to the histopathologic findings.

The current model does not represent the primary description of DHCA in the rat. Mendler and colleagues¹¹ pioneered in this field some 30 years ago with the original report of partial CPB in deep hypothermia in the rat. Because of marked improvement of technology, this original work appears to be less applicable to the current clinical situation. Thirty years later, Kawaguchi and associates¹² described a technically advanced rat model of DHCA. This work represents a major step toward a clinically relevant long-term recovery model of DHCA in rodents. However, CPB flows of 30 to $36 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ did not exceed one fifth of the normal cardiac output in the rat, and continued ventilation was required to sustain sufficient oxygenation. In the current study long-term survival was accomplished by miniaturization of the CPB circuit and incorporation of a recently developed rat oxygenator. The proportion of prime volume (10 mL) to rat total blood volume (25 mL) is similar to that commonly used in human subjects (2 L of prime

Figure 1. Survival, motor, and neurocognitive function after escalating durations of deep hypothermic circulatory arrest (DHCA). Logistic regressions demonstrate a reciprocal relation between survival (A), motor (B), or neurocognitive (C) outcome and the duration of DHCA (mean value $\pm$ 95% confidence interval).

vs 5 L of blood volume). Reducing the prime to 10 mL allowed the conduct of CPB and DHCA without blood transfusion at hematocrit values commonly seen in clinical practice. Furthermore, the model allows CPB flows equivalent to normal cardiac output in the rat ($160\text{--}180\text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)¹³ and clinically used CPB flows ($100\text{--}150\text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), resulting in sufficient oxygenation of the nonventilated animals.

Figure 2. Correlation between cerebral functional outcome and histopathologic findings. Spearman rank correlation revealed no correlation between the functional and the histologic score ($r = 0.51$). The normal range for the total neuroscore (Table 1) is between 0 and 4 (horizontal line), and the normal range for the histopathology is between 0 and 12 (vertical line). Therefore 2 of 24 animals show normal values for both functional and histologic score. Ten animals demonstrate impaired cerebral function with a histologic score beyond normal ranges. Twelve animals show pathologic findings in histology, although the functional outcome was within normal ranges.

In the current study, extended DHCA times (90–105 minutes) beyond the duration of what is commonly required in clinical practice caused severe cerebral injury. Reducing DHCA duration to 75 minutes allowed survival of the animals, initially without signs of severe cerebral injury. However, these rats later had end-organ dysfunction. Further reduction of the DHCA to 60 minutes or less resulted in excellent long-term survival, with morbidity confined to neurologic deficits.

In human subjects the duration of DHCA is considered to be an important risk factor for adverse cerebral outcomes after the repair of congenital heart disease. However, the upper safe limit of DHCA is still discussed controversially or might not even exist.^{14–17} Recent large-animal studies had to use DHCA durations of at least 80 to 120 minutes to demonstrate neurologic deficits. Because these DHCA durations are beyond customary clinical practice,^{12,18,19} the results of those studies might not be applicable to the clinical scenario. In contrast, the current study demonstrates that clinically relevant DHCA durations of up to 60 minutes result in detectable motor, as well as neurocognitive, impairments that remain for at least 2 weeks.

Cerebral function was evaluated by a large test battery, including commonly used motor and sensory tests, as well

Figure 3. Histologic findings: representative coronal brain sections of the hippocampus and the cerebellar vermis that were stained with hematoxylin and eosin and visualized at a high-field magnification of 440 times. Both areas contain neurons that are highly susceptible for hypoxia (images of hippocampus area CA1, A-C; images of the cerebellar vermis, D-E). A, Sham-operated animal with a normal histologic score. One incidental shrunken neuron with pyknotic nucleus and highly eosinophilic cytoplasm is visible (dark neuron; *small arrow*). B, Animal after 45 minutes of deep hypothermic circulatory arrest with a histologic score of 30. Three damaged neurons were detected, 2 resembling dark neurons (see above) and 1 with moderate eosinophilic cytoplasm and condensed substance of nissl (chromatolysis; *large arrow*). C, Animal after 90 minutes of deep hypothermic circulatory arrest and a histologic score of 86. The image shows an increased number of injured neurons, with a mixture of irreversible damaged dark neurons (*small arrows*) and swollen cells with marked eosinophilic cytoplasm and chromatolysis (*large arrows*). D, Same animal as in panel A. The Purkinje cells (*white arrow*) appear to be normal and without any signs of hypoxic alteration. E, Same animal as in panel B. The *arrow* points toward a single Purkinje cell with chromatolysis and eosinophilic cytoplasm. F, Same animal as in panel C. The image shows marked hypoxic changes of most Purkinje cells (*large arrow*).

as the mHBT.^{7-9,20,21} The latter represents a unique task to investigate a variety of cognitive, behavioral, and fine-motor parameters in a single test. It allows a comprehensive and detailed evaluation of cerebral outcome in rats. Of note, the functional score obtained by using this test battery did not correlate with the histologic findings 14 days after DHCA. This is in accordance with other studies of cerebral injury showing discrepancies between functional and histologic outcomes when other frequently used neurologic and cognitive tests were applied.²²⁻²⁴ Of particular interest, several animals in the current study showed severe histologic damage without demonstrating functional deficits 14 days after DHCA. These results deserve further discussion. Enhanced neurogenesis as a response to cerebral injury with an improved functional outcome in animals with stimulated neurogenesis has been introduced as a potential mechanism to allow for functional recovery.^{25,26} Alternatively, the training effect of the animals from repetitive testing might have improved function over time. This could explain why overall functional outcome on day 12 was considerably better than expected given the histologic damage detected. Comparable findings were recently published, with improved performance of animals on the rotarod during the first days after cerebral ischemia without correlation to infarct volume.²⁴

Even though this rodent model of CPB and DHCA was established to mimic clinical standards as closely as possible, some important limitations remain. Median sternotomy, direct cardiac cannulation, and surgical intervention were not performed to allow long-term survival of the animals.

There are several reasons why an appropriate disease model of DHCA in the rat might be considered a realistic alternative to use of large-animal models. First, the current model is far less expensive and more accessible to many potential investigators. Second, the current model might be used to specifically examine the effect of various flow patterns, different temperature regimens, and anesthetics on cerebral and other organ dysfunction after DHCA. Furthermore, because molecular pathways are suspected mechanisms behind cerebral dysfunction associated with CPB and DHCA, the availability of various techniques of cellular and molecular biology significantly add to the value of this model.

In summary, this study presents a novel long-term recovery model of DHCA in the rat. Neurologic, cognitive, and histologic deficits were detected after clinically relevant DHCA durations. This experimental setup appears to be a suitable disease model to further elucidate mechanisms associated with adverse cerebral outcome after cardiac surgery and DHCA and to investigate potential neuroprotective strategies.

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