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Cerebral Air Emboli Differentially Alter Outcome after Cardiopulmonary Bypass in Rats Compared with Normal Circulation

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Background: **Cerebral air emboli (CAE) are thought to contribute to adverse cerebral outcomes following cardiac surgery with cardiopulmonary bypass (CPB). This study was designed to investigate the effect of escalating volumes of CAE on survival and neurologic and histologic outcomes. In addition, the effect of xenon administration during CAE on these outcomes was determined.**

Methods: **With institutional review board approval, four groups were studied (n = 15). In two CPB–CAE groups, rats were subjected to 90 min CPB with 10 repetitively administered CAE. Rats in two sham–CAE groups were also exposed to CAE but not to CPB. Rats were randomly assigned to sequential dose cohorts receiving CAE ranging from 0.2 to 10 μl in a dose-escalating fashion. Groups were further subdivided into xenon (56%) and nitrogen groups. Rats with severe neurologic damage were killed; others were neurologically tested until postoperative day 7, when infarct volumes were determined. Survival and neurologic and histologic outcomes were tested with logistic regression analyses ($P < 0.05$).**

Results: **This study demonstrates a dose-dependent relation between CAE volumes and survival, neurologic outcome, and histologic outcome. For all outcomes, CPB adversely affected the dose–effect curves compared with sham–CAE groups ($P < 0.05$). Xenon demonstrated no impact on either outcome.**

Conclusions: **This study describes the successful incorporation of CAE in a rodent CPB model and allows identifying suitable CAE volumes for subsequent studies. CAE exhibit a differential effect on outcome in rats undergoing CPB *versus* those not exposed to CPB. Perioperative administration of xenon remained without any effect on outcome.**

NEUROLOGIC and neurocognitive deficits after cardiac surgery using cardiopulmonary bypass (CPB) remain common and severe complications with impact to patients' quality of life.[1–3] The etiology of these adverse outcomes is most likely multifactorial, including mainly the effects of cerebral emboli and generalized cerebral hypoperfusion next to inflammatory reactions.[4,5] Cerebral emboli during CPB may present as particulate emboli released from atheromatous plaques during manipulation of the thoracic aorta or as gaseous emboli mainly entrained during open-chamber procedures or generated within the CPB circuit.[6,7] To further elucidate the effects of cerebral air emboli (CAE) during CPB on cerebral outcome, an appropriate disease model of CPB combined with CAE is required.[8]

Such a disease model would allow investigation of potential neuroprotective strategies and compounds, such as the noble gas xenon in a preclinical setting. Xenon has experimentally been shown to provide neuroprotective properties, most likely *via* *N*-methyl-D-aspartate receptor antagonism.[9–12] Besides its safety and efficacy profile described in noncardiac surgery,[13] xenon offers cardiovascular stability[14–16] and pharmacokinetic benefits with rapid induction and recovery of anesthesia and profound analgesia.[17,18] The neuroprotective properties of xenon have not been tested clinically, although it would be of particular interest to administer xenon to patients at risk for perioperative cerebral injuries, such as cardiac surgery patients undergoing CPB.[1,2] A recent phase I trial studied the feasibility and safety of xenon delivery to 16 patients undergoing cardiac surgery while on CPB and concluded that its application is safe during CPB.[19] However, xenon's disposition to expand air bubbles that are present during CPB as CAE[20–22] may abolish any neuroprotective effects after CPB with CAE. This was recently demonstrated in a rat model of combined CPB and CAE using predefined CAE volumes of 0.3 μl that allowed for long-term survival of the animals.[23]

The aims of the current study were (1) to investigate the effect of escalating volumes of CAE on survival as the primary endpoint, and on neurologic, cognitive, and histologic outcome as secondary endpoints after CAE during CPB or sham operation and to determine dose–effect curves for these outcomes; and (2) to study the effect of xenon administration on these dose–effect curves by using the new model as a preclinical screen for potential neuroprotective drugs.

Materials and Methods

The following experimental protocols were approved by the institutional animal care committee (Regierung von Oberbayern, München, Germany). 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 the Use of Laboratory Animals* prepared by the National

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Academy of Science (NIH Publication No. 86-23, revised 1985).

In a randomized, dose-escalation, three-stage study, four experimental groups of animals were investigated (n = 15 per group): Rats in the CPB–CAE groups were subjected to 90 min of normothermic CPB with 10 repetitively administered CAE. Rats in the sham-operated (sham–CAE) groups were also exposed to CAE as well as to the same surgical preparation and anesthesia but were not connected to CPB. Groups were further subdivided into xenon (56% xenon, 5% N_2, 34% O_2, 5% CO_2) and nitrogen (61% N_2, 34% O_2, 5% CO_2) groups with the accordant mixture inhaled 20 min before CPB, during CPB, and 30 min after CPB or at equivalent times in the sham-operated groups.

Surgical Preparation and Cardiopulmonary Bypass

Nonfasted male Sprague-Dawley rats (356 ± 17 g, 10 weeks old) from Charles River Laboratories (Sulzfeld, Germany) were cannulated for CPB as previously reported.[24] Briefly, the tail artery was cannulated for aortic inflow, the right external jugular vein was cannulated for venous return, and the right superficial caudal epigastric artery was cannulated for monitoring of mean arterial blood pressure (MAP). The corresponding vein was cannulated for drug administration. After the cannulation of the tail artery, animals received 150 U heparin. For the injection of CAE, a PE-10 catheter (Intramedic; Becton Dickinson GmbH, Heidelberg, Germany) was inserted *via* the stump of the external carotid artery and advanced into the internal carotid artery just beyond the pterygopalatine branch, which was ligated.[25] Rats were ventilated with a custom-designed, closed-circuit gas-delivery system, and inspiratory xenon concentrations were measured using a thermal conductivity sensor (provided by AGA AB, Lidingö, Sweden). During surgical preparation, anesthesia was maintained with 1.5–2.0% isoflurane and repetitive doses of fentanyl (5-μg boluses). Pericranial temperature was monitored and controlled to 37.5°C with heating blankets (HYP-1; Newport, Santa Ana, CA).

After completion of surgical preparation, animals were denitrogenated, and the gas mixture was adjusted according to group assignment (56% xenon, 5% N_2, 34% O_2, and 5% CO_2 in xenon groups and 61% N_2, 34% O_2, and 5% CO_2 in nitrogen groups). Concomitantly, the anesthetic regimen was changed to midazolam (0.4 mg/kg intravenous), fentanyl (30 μg/kg intravenous), and atracurium (0.5 mg/kg intravenous) as a bolus injection, followed by a mixed continuous infusion (0.03 mg · kg^{-1} · min^{-1} intravenous midazolam, 2.5 μg · kg^{-1} · min^{-1} intravenous fentanyl, and 0.084 mg · kg^{-1} · min^{-1} intravenous atracurium) and was sustained until the end of the CPB period. The gas mixture as described above was applied 20 min before CPB, during CPB, and 30 min after CPB and at equivalent times in the sham-operated animals.

The CPB circuit consisted of a venous reservoir, a peristaltic pump (Masterflex®; Cole-Parmer Instrument Co., Vernon Hills, IL), a novel membrane oxygenator, an in-line flow probe (2N806 flow probe and T208 volume flowmeter; Transonics Systems, Inc., Ithaca, NY), and an arterial inflow cannula. The oxygenator was specifically designed for this study to deliver xenon during CPB and to provide sufficient oxygenation with an inspiratory oxygen concentration of 34%, despite a small prime volume of only 10 ml. The CPB circuit was primed with 14 ml whole blood obtained before the start of the experiment from one heparinized (150 U/rat) donor rat and 2 ml hetastarch, 6%. One hundred units heparin was added to the prime. CPB–CAE animals were subjected to 90 min of normothermic nonpulsatile CPB with flow rates of 160–180 ml · min^{-1} · kg^{-1}. For the entire CPB period, ventilation of the lungs was discontinued. After 90 min of CPB, the animals were weaned from CPB without the need for inotropes or vasopressors. The heparin anticoagulation was allowed to dissipate spontaneously without supplemental administration of protamine. Glucose, bicarbonate, and calcium were administered if required. After decannulation, rats remained anesthetized (continuous infusion of 0.048 mg · kg^{-1} · min^{-1} midazolam, 3.9 μg · kg^{-1} · min^{-1} fentanyl, and 0.129 mg · kg^{-1} · min^{-1} atracurium), intubated, and ventilated for 1 h. When the animals resumed spontaneous ventilation, the tracheas were extubated, and the animals recovered in an oxygen-enriched environment for 12 h with free access to water and food. During the first 6 h of recovery, they were continuously observed to identify signs of immediate cerebral death and severe neurologic dysfunction (fixed pupils, no reaction to pain, absence of spontaneous breathing, seizures, and inability to ambulate). Animals demonstrating signs of severe neurologic dysfunction were killed. All others were returned to their cages on the first postoperative day and were housed in familiar groups.

The sham–CAE groups had all CPB catheters left in place for 90 min (thereby mimicking the surgical procedures for CPB) but were not connected to the external CPB circuit itself. The animals were decannulated and recovered in the same fashion as the animals in the CPB–CAE groups.

Cerebral Air Embolism

During 90 min of CPB or equivalent times in sham-operated rats, 10 equally sized CAE were injected into the right internal carotid artery. The first embolus was administered at 15 min of CPB, and the last embolus was administered at 75 min of CPB. Using a Hamilton syringe with precise scale markings (10-μl Gastight® No. 1701N or 50-μl Gastight® No. 1705N; Hamilton Bonaduz AG, Bonaduz, Switzerland), the size of the air bubble could be exactly determined by the placement of the air between 5-μl saline aliquots. Ten microliters saline was

injected to flush the last CAE into the cerebral circulation. In addition, the use of a Harvard precision pump allowed the administration of the air bubble–saline mixture with a constant velocity (0.15 ml/h) and within a predictable time period. Rats were randomly assigned to sequential dose cohorts receiving emboli volumes ranging from 0.2 to 10 μl in a dose-escalating fashion. This range of emboli volumes was selected based on previous studies investigating the effect of single CAE on the brain in rats and rabbits.[25,26] To establish dose–effect curves with a survival rate of 50% in each group, the number of animals receiving defined CAE volumes were selected in a three-stage approach: In the first stage, seven rats per group received 10 CAE with a volume of 0.2, 0.5, 1, 1.6, 2.5, 5, or 10 μl per single bolus. In the second stage, additional animals were studied with the injected CAE volume based on the survival rate in stage 1. The lowest applied volume was selected two steps below the CAE volume that allowed for survival of the rats, and the highest volume was selected two steps above the CAE volume that was not compatible with survival in stage 1. Accordingly, in the third stage, CAE volumes were studied based on the results of the second stage with one step up and down. This approach allowed optimization of the dose selection for each group (median of the dose interval produces a 50% effect). Although the relevant volume range for CAE could only be assumed from the literature, this approach also enabled us to avoid unnecessary experiments at the upper and lower limit of the tested CAE range.

Neurologic and Cognitive Testing

To define normal ranges of neurologic and cognitive function in healthy untreated rats, the following tests were performed in 96 additional animals. Standardized functional neurologic testing as previously described included assays of prehensile traction, balance beam performance, and movement symmetry (table 1).[27] Sensory function was evaluated using an established protocol that included body proprioception and response to vibrissae touch.[28] Cognitive function was assessed using the modified hole board test according to an established protocol.[29,30] Briefly, 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 hole board (20 × 40 cm) placed in the middle of the test arena. Fifteen holes covered by lids were staggered on the board. After opening, 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. Rats learned the test procedure over 7 consecutive days (learning period). Animals were tested in the modified hole board test for four trials daily. The sequence of marked holes was randomly changed every day. Cognitive parameters assessed with the modified

Table 1. Tests Used in the Current Study for the Assessment of Motor, Cognitive, and Sensory Function after Cardiopulmonary Bypass Combined with Cerebral Air Emboli

		Level of Impairment					
Quality	Test	3	2	1	0	Score	Normal
Motor function							
Balance	Balance time on beam	21–30 s	11–20 s	1–10 s	Not able	0–3	3
Physical strength	Prehensile traction/ time gripping a rope	5 s with a third limb to rope	5 s without a third limb to rope	3–4 s	0–2 s	0–3	3
Physical strength	Time on a screen	15 s	11–14 s	6–10 s	0–5 s	0–3	3
Movement symmetry	Active movement	Symmetric movement	One-sided weakness	Hemiplegia	Not able	0–3	3
Cognitive function (assessed with the modified hole board test)							
Declarative memory	Wrong choices of 3 trials	0–1	2–3	3–4	>5	0–3	3
	Omission errors of 3 trials	0	1–3	3–4	>5	0–3	3
Working memory	Repeated choices of 3 trials	0–1	2–3	3–4	>5	0–3	3
Cognitive performance	Time needed to complete 3 trials	<135 s	135–300 s	300–599 s	≥600 s	0–3	3
Sensory function							
Body proprioception	Reaction to a stimulus	Normal on both sides	Slowly on one side	None on one side	Not able	0–3	3
Vibrissae proprioception	Reaction to stimulus on the vibrissae	Equally on both sides	Slowly on one side	None on one side	Not able	0–3	3
Total neurologic score						0–30	30

Normal ranges are defined as the mean ± 2 SDs of 96 healthy untreated rats. The score is a simplified version of a previously published neurologic score.[44]

hole board test included working memory, declarative memory, and cognitive performance (table 1).

Neurologic and cognitive testing in the study animals was performed by an investigator blinded to group assignment. On the preoperative day as well as on the first, third, and seventh postoperative days, surviving rats underwent neurologic and sensory testing as described above. Starting on the first postoperative day, rats were also tested in the modified hole board test.

Histologic Examination

On postoperative day 7, animals were anesthetized with 5% isoflurane and underwent *in situ* brain fixation using intracardiac injection of normal saline followed by buffered 4% formalin. The brains were removed *in toto*, serially cut (10-μm sections) at 150-μm intervals, and stained with hematoxylin and eosin. Infarct volume was determined by digitally sampling stained sections with a video camera controlled by an image analyzer. With the observer blinded to experimental conditions, infarct borders in the cortex and the subcortex were individually outlined using an operator-controlled cursor, and consequently, infarct area was calculated automatically. Infarct volumes were computed as running sums of infarct area multiplied by the known interval (*e.g.*, 150 μm) between sections over the extent of the infarct expressed as an orthogonal projection. In addition, brains of animals killed before postoperative day 7 were serially cut at 2-mm intervals and immediately stained with 2,3,5-triphenyl-tetra-zolium-chlorid (TTC) to confirm that cerebral injury was too severe to allow recovery.

Statistical Analysis

Multiple logistic regression analyses for the respective groups were performed with CAE volume, preparation (CPB or sham), and inhalation agent (xenon or nitrogen) as independent variables and survival, survival without motor deficit, survival without cognitive deficits, or survival without sensory deficits as dependent variables. Survival without the respective deficit was assumed if none of the tested qualities were out of their normal range (table 1). Normal ranges were defined as the mean $\pm$ 2 SDs of 96 healthy untreated rats. The independent variables were stepwise included in the model. The CAE volume, the inhalation agent, and preparation were correlated with the infarct volume on postoperative day 7 using linear regression analysis. Again, the factors were stepwise included in the model (exclusion: $P > 0.1$; inclusion: $P < 0.05$).

Results

Five animals were excluded from further data analysis because of insufficient venous return (one animal in the CPB–CAE–xenon group), cannulation problems (one rat in the CPB–CAE–nitrogen group and one rat in the sham–CAE–nitrogen group), or postoperative development of cervical hematoma with inspiratory stridor (one animal in the CPB–CAE–nitrogen group and one animal in the sham–CAE–xenon group). These rats were replaced to keep sample size equal.

Table 2 displays the physiologic parameters of the four groups. Because of the nature of CPB, rats subjected to CPB demonstrated lower MAP, lower hemoglobin concentrations, and higher partial pressure of oxygen during CPB than sham groups.

The 96 healthy untreated rats demonstrated all maximum scores for motor, cognitive and sensory function as displayed in table 1.

Table 2. Physiologic Data during the Operative Procedure in the Four Groups Studied

	Preparation	Treatment	Pre-CPB	45 min CPB	90 min CPB	1 h Post-CPB
Mean arterial pressure, mm Hg	Sham–CAE	Nitrogen	97 ± 14	118 ± 22	126 ± 20	120 ± 25
	Sham–CAE	Xenon	92 ± 12	105 ± 18	120 ± 27	118 ± 16
	CPB–CAE	Nitrogen	87 ± 18	89 ± 18*	80 ± 11*†	119 ± 21
	CPB–CAE	Xenon	92 ± 13	81 ± 16*†	86 ± 20*†	117 ± 19
Hemoglobin concentration, mg/dl	Sham–CAE	Nitrogen	13.7 ± 0.8	13.4 ± 0.9	13.2 ± 1.0	12.8 ± 1.1
	Sham–CAE	Xenon	13.6 ± 1.3	12.9 ± 1.0	13.1 ± 1.2	12.3 ± 1.3
	CPB–CAE	Nitrogen	13.6 ± 0.9	12.1 ± 0.5*†	11.8 ± 0.8*†	11.5 ± 0.9*
	CPB–CAE	Xenon	14.0 ± 0.9	11.8 ± 0.3*†	11.9 ± 0.7*†	11.5 ± 1.0*
$Paco_2$, mm Hg	Sham–CAE	Nitrogen	36.1 ± 3.6	36.1 ± 2.8	34.7 ± 6.9	37.2 ± 4.6
	Sham–CAE	Xenon	39.0 ± 3.8	40.3 ± 3.1	38.7 ± 3.7	35.4 ± 5.8
	CPB–CAE	Nitrogen	35.7 ± 3.7	35.5 ± 6.5	37.2 ± 3.4	36.8 ± 3.6
	CPB–CAE	Xenon	39.1 ± 4.6	38.5 ± 5.5	38.0 ± 4.7	35.2 ± 2.4
Pao_2, mm Hg	Sham–CAE	Nitrogen	129 ± 22	131 ± 9	121 ± 27	211 ± 37
	Sham–CAE	Xenon	119 ± 20	119 ± 20	119 ± 24	224 ± 45
	CPB–CAE	Nitrogen	116 ± 30	178 ± 21*†	170 ± 28*†	180 ± 49
	CPB–CAE	Xenon	120 ± 38	191 ± 27*†	181 ± 24*†	175 ± 42†

Variables were obtained before cardiopulmonary bypass (pre-CPB), at 45 min of CPB (45 min CPB), at 90 min of CPB (90 min CPB), and 1 h after CPB (1 h post-CPB) or at equivalent times in the sham-operated groups (sham–cerebral air emboli [CAE]). Values are presented as mean ± SD.

* $P < 0.05$ compared with sham–CAE–nitrogen group. † $P < 0.05$ compared with sham–CAE–xenon group.

$Paco_2$ = partial pressure of carbon dioxide; Pao_2 = partial pressure of oxygen.

A total of 26 animals out of 60 survived the entire observation period of 7 days: 6 animals in the CPB–CAE–xenon group, 6 in the CPB–CAE–nitrogen group, 6 in the sham–CAE–xenon group, and 8 in the sham–CAE–nitrogen group. In all rats killed before postoperative day 7, TTC staining revealed large cerebral infarcts consistent with the observed severe neurologic deficits that triggered the decision to kill these animals.

All tested outcome variables were significantly correlated with the volume of the CAE showing worse outcome with increasing CAE volumes. Therefore, survival rate (fig. 1), survival rate without any motor deficit (fig. 2), survival rate without any cognitive deficit (fig. 3), and survival rate without any sensory deficit (fig. 4) were all decreased with increasing CAE volumes. Linear regression demonstrated a relation between infarct volumes and logarithm of CAE volumes of the surviving animals ($r = 0.652$; fig. 5). All tested outcome variables with the exception of the cognitive one were significantly correlated with the preparation, demonstrating that CPB shifted the volume–effect curves significantly leftward. Xenon did not effect these relations in any tested outcome variable regardless of the preparation.

Discussion

Although CAE are discussed as one important contributor to adverse cerebral outcome after cardiac surgery with CPB, to date no appropriate preclinical disease model exists that allows for the investigation of the effects of CAE during CPB on long-term cerebral outcome. The current description of the successful incorporation of CAE in a preexisting long-term recovery model of CPB in rats represents an important step forward and allows identifying suitable CAE volumes for subsequent studies using the obtained dose–effect curves. In the past, several animal models of CAE were established and provided important insights into the kinetics of CAE with respect to variation in pial arteriole diameters, changes in cerebral blood flow, and alterations in somatosensory evoked potentials after different volumes of CAE.[25,31] The combination of CAE and CPB for the investigation of the effects of CAE on cerebral outcome after CPB was first described by Hindman

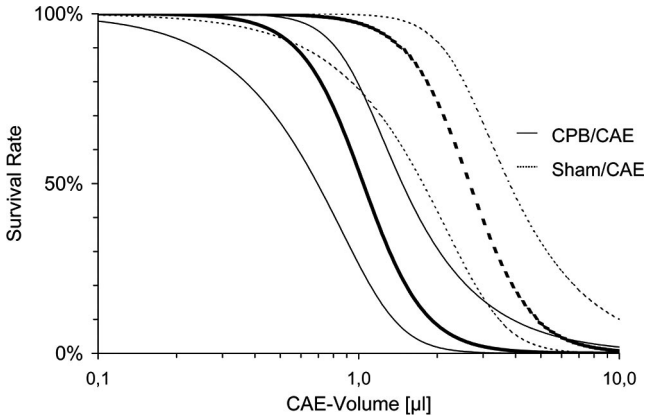

Fig. 1. Survival after escalating volumes of cerebral air emboli (CAE) during cardiopulmonary bypass (CPB) or sham operation (sham). Logistic regression demonstrates a relation between survival rate and the logarithm of CAE volumes (mean value ± 95% confidence interval) with dose–effect curves shifted significantly leftward for CPB–CAE groups (ED_{50} = 1.0 µl; 95% confidence interval, 0.7–1.5 µl) compared with the sham–CAE groups (ED_{50} = 2.7 µl; 95% confidence interval, 1.8–3.6 µl; P = 0.006). Treatment (xenon *vs.* nitrogen) showed no impact on survival after CPB or sham operation. Therefore, dose–effect curves for these subgroups (xenon and nitrogen groups) are not displayed.

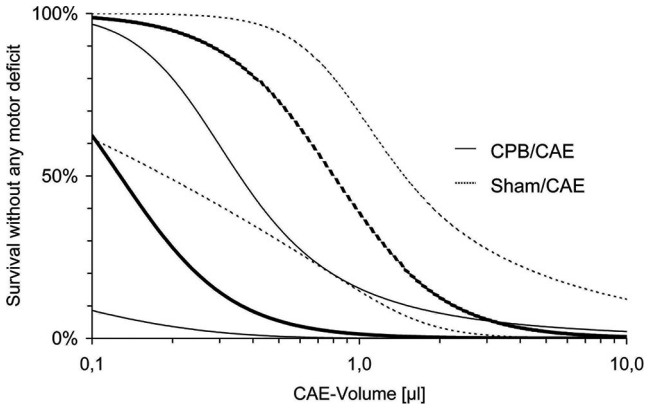

Fig. 2. Motor function after escalating volumes of cerebral air emboli (CAE) during cardiopulmonary bypass (CPB) or sham operation (sham). Logistic regression demonstrates a relation between the survival without any motor deficits and the logarithm of CAE volumes (mean value ± 95% confidence interval) with dose–effect curves shifted significantly leftward for CPB–CAE groups (ED_{50} = 0.1 µl; 95% confidence interval, 0.0–0.4 µl) compared with the sham–CAE groups (ED_{50} = 0.8 µl; 95% confidence interval, 0.2–1.5 µl; P = 0.014). Treatment (xenon *vs.* nitrogen) showed no impact on motor outcome after CPB or sham operation. Therefore, dose–effect curves for the subgroups (xenon and nitrogen groups) are not displayed.

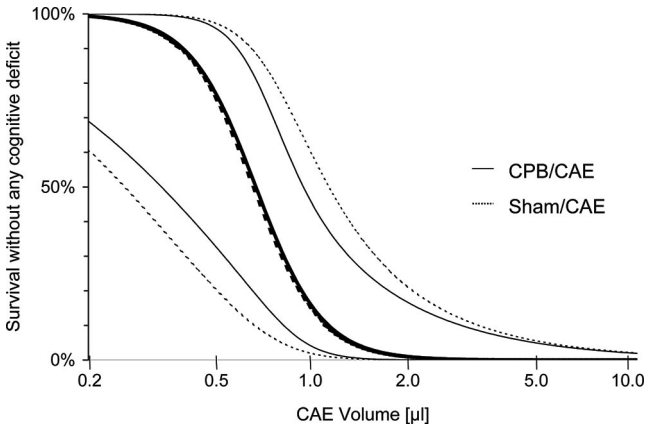

Fig. 3. Cognitive function after escalating volumes of cerebral air emboli (CAE) during cardiopulmonary bypass (CPB) or sham operation (sham). Logistic regression demonstrates a relation between the survival without any cognitive deficits and the logarithm of CAE volumes (mean value ± 95% confidence interval). Preparation (CPB *vs.* sham) as well as treatment (xenon *vs.* nitrogen) showed no impact on cognitive outcome. Dose–effect curves for the subgroups (xenon and nitrogen groups) are not displayed.

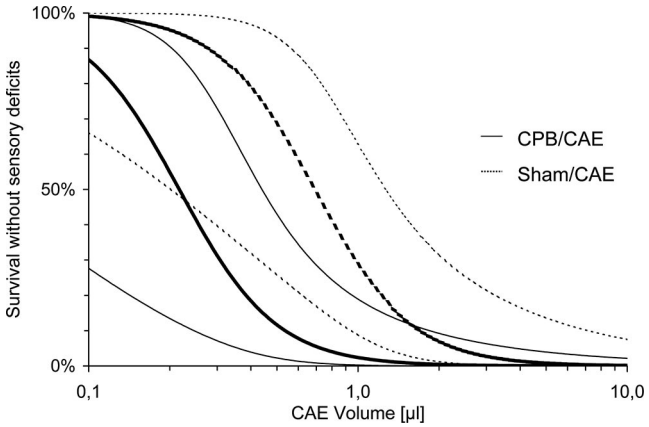

Fig. 4. Sensory function after escalating volumes of cerebral air emboli (CAE) during cardiopulmonary bypass (CPB) or sham operation (sham). Logistic regression demonstrates a relation between survival without any sensory deficits and the logarithm of CAE volumes (mean value ± 95% confidence interval) with dose–effect curves shifted significantly leftward for CPB–CAE groups (ED_{50} = 0.2 µl; 95% confidence interval, 0.0–0.4 µl) compared with the sham–CAE groups (ED_{50} = 0.7 µl; 95% confidence interval, 0.2–1.3 µl; P = 0.04). Treatment (xenon *vs.* nitrogen) showed no impact on motor outcome after CPB or sham operation. Therefore, dose–effect curves for the subgroups (xenon and nitrogen groups) are not displayed.

et al.[32] Although their model in rabbits offered a valid approach, certain limitations remained, such as the lack of suitable tests for neurocognitive assessment and difficulties with long-term survival of these animals. Combining a previously described technique of selective cerebroarterial injection of air emboli with an established model of CPB in rats, we aimed to overcome these limitations.[24,25] However, previous studies used a wide variety of volumes for CAE dependent on the outcome selected and on the animal species used. Further, most studies analyzed the effect of only a single embolus and not of repetitive emboli on cerebral outcome. Therefore, the current experiment was designed as a dose-finding study investigating a wide range of different CAE volumes administered in a repetitive manner and their effects on cerebral outcome.

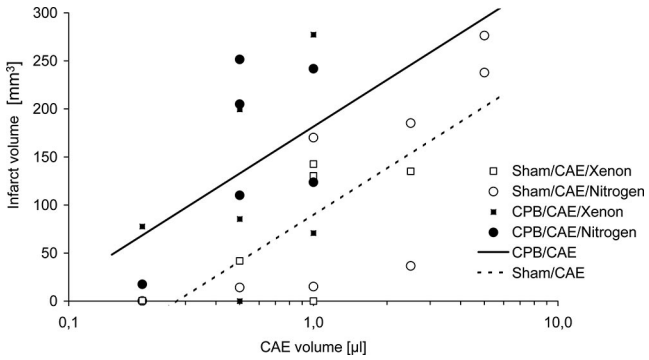

Fig. 5. Histologic outcome 7 days after escalating volumes of cerebral air emboli (CAE) during cardiopulmonary bypass (CPB) or sham operation (sham). Linear regression demonstrates a relation between infarct volumes and logarithm (ln) of CAE volumes of the surviving animals (infarct volume = 273 + 70 × ln CAE [µl] − 92 × preparation [CPB = 1; sham = 2]; ln CAE: P < 0.001; preparation: P < 0.01; r = 0.652). The dose–effect curves are significantly shifted leftward for CPB–CAE groups compared with the sham–CAE groups. Treatment (xenon *vs.* nitrogen) showed no impact on histologic outcome 7 days after CPB or sham operation. Therefore, dose–effect curves for the subgroups (xenon and nitrogen groups) are not displayed individually.

Using this approach, we obtained the dose–effect curves for a variety of outcomes such as survival and motor, cognitive, and sensory function as well as histology, allowing subsequent studies to select a specific bubble size dependent on the desired survival rate and the sensitivity of neurologic and cognitive tests. Interestingly, the injection of CAE differentially affected all outcomes, with the exception of cognitive function in rats undergoing CPB *versus* those not exposed to CPB. Significantly larger volumes of CAE were tolerated in the absence of CPB. These findings are in accordance with a study by Hindman *et al.* demonstrating an impaired recovery of somatosensory-evoked potential (with a good correlation to subsequent neurologic outcome) after CAE with CPB compared with CAE alone.[32,33] Several potential factors may have contributed to the accentuated adverse effects of CAE during CPB. First, CPB is associated with a systemic inflammatory reaction that is amplified by the foreign surface of the circuit, gut hypoperfusion followed by exposure to endotoxin,[5] and the effects of blood transfusion.[34] The resulting whole body inflammation may add to an already present cerebral inflammatory reaction to the air bubble itself.[35] Another mechanism by which enhanced inflammation may have an impact relates to the susceptibility of the penumbra area to damage by inflammatory cells.[36] After focal ischemia, an influx of inflammatory cells to the penumbra area is known to occur.[37] These cells migrate to this region and infiltrate the brain parenchyma after mediation from adhesion molecules that are, at least in part, affected by proinflammatory cytokines.[38] One can speculate that CPB-induced cytokine production might amplify this initial response. Second, we cannot exclude that the physiologic characteristics of CPB (*e.g.*, lower hemoglobin concentrations and lower MAP compared with the sham-operated groups) may have augmented the effects of CAE. Clinically, hypotension during CPB does not seem to impact cognitive or neurologic function after cardiac surgery,[39,40] and autoregulation remains intact within a wide normal range of MAP (50–100 mmHg) as long as pH and arterial carbon dioxide are kept constant.[41,42] Experimentally, we have recently shown that higher MAP of 75–85 mmHg compared with MAP of 40–50 mmHg results in improved postoperative cognitive function after CAE and CPB in rodents.[43] However, in light of relatively high hemoglobin concentrations and MAP values of 80–90 mmHg during CPB in the current study and because these differences mimic the clinical scenario of CPB, no interventions to adapt the respective values to the sham-operated animals were initiated. Third,

nonpulsatile blood flow associated with CPB may have influenced the impact of CAE on cerebral outcome by compromising absorption kinetics and collateral blood flow itself.

The inert gas xenon did not affect survival or neurologic, cognitive, or histologic outcomes after CAE during CPB or sham operation in this study. The effects of xenon in the context of CPB and CAE were studied for several reasons. Xenon was shown to provide neuroprotective properties in both *in vitro* and *in vivo* studies. The anesthetic gas was shown to improve neurologic and histologic outcome after transient focal cerebral ischemia in mice and attenuated CPB-induced neurocognitive dysfunction in rats.[10,11] On the other hand, *in vitro* and more recently *in vivo* studies demonstrated xenon's disposition to expand air bubbles mainly because of its low solubility.[20,22] Air bubbles present during CPB as CAE are discussed as one potential contributor to cerebral injury. Any augmentation of the effects of CAE by xenon may increase the risk of cerebral injury and must be weighed against its previously well-documented neuroprotective effects. Despite recent clinical evidence for the feasibility and safety of xenon administration during CPB, further investigation of this safety aspect seems to be particularly important before considering xenon in those cardiac surgery settings that are known to expose patients to higher numbers of CAE such as open-chamber procedures.[19] Interestingly, any previously described neuroprotective properties of xenon were most probably abolished by the effect of xenon on CAE in the current study.

Of note, this experiment did not reveal any adverse effects of xenon on survival or cognitive, sensory, and gross neurologic function in the presence of escalating doses of CAE. Therefore, the current study does not corroborate our previous finding that xenon demonstrates a deleterious effect on long-term cognitive outcome after CPB and CAE.[23] Possible reasons for this difference include (1) a small number of only 26 animals available for cognitive testing, (2) substantial variations with respect to individual CAE volumes in surviving animals of the current study as opposed to small and predefined CAE volumes resulting in more consistent cerebral injuries in our previous study, (3) delayed commencement of the modified hole board test after an initial recovery phase on postoperative day 4 in the previous study, and (4) a longer test period (until postoperative day 14) in the previous study. These reasons may also help to explain why the current study did not even reveal an effect of CPB on cognitive outcome assessed with the modified hole board test.[23]

Although this model was established to mimic clinical standards as close as possible, some important limitations remain. First, we chose to inject the CAE directly into the cerebral circulation to ensure standardized and controlled CAE. Clinically, CAE are generated within the CPB circuitry or are entrained during open-chamber procedures and reach the cerebral circulation *via* the aortic cannula. Second, the generation of a shower of CAE as detected in the operating room by means of transcranial Doppler techniques would have been preferable, but because of technical limitations and the miniaturization of this model, a maximum of 10 equally sized CAE were administered. Third, the experimental protocol did not allow performing the injection of CAE in a blinded fashion. Although we are not in a position to exclude any effect this may have had on outcome, we strongly believe that keeping the investigators responsible for neurologic testing and histology blinded was essential to avoid any major impact. Last, median sternotomy, direct cardiac cannulation, and surgery were not performed to allow long-term survival of the animals.

In summary, this study describes the successful incorporation of CAE in a long-term recovery model of CPB in rats and allows identifying suitable CAE volumes for subsequent studies using the dose–effect curves. The injection of CAE exhibits a differential effect on outcome in rats undergoing CPB *versus* those not exposed to CPB. Potentially, significantly larger volumes of CAE are tolerated in the absence of CPB.

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