Xenon Impairs Neurocognitive and Histologic Outcome after Cardiopulmonary Bypass Combined with Cerebral Air Embolism in Rats

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**Background:** The neuroprotective properties of xenon may improve cerebral outcome after cardiac surgery using cardiopulmonary bypass (CPB). However, its disposition to expand gaseous bubbles that during CPB present as cerebral air emboli (CAE) could abolish any beneficial effect or even worsen cerebral outcome. Therefore, the authors studied the impact of xenon on neurologic, cognitive, and histologic outcome after CPB combined with CAE in rats.

**Methods:** With institutional review board approval, 40 rats were assigned to four groups (n = 10). In two CPB–CAE groups, rats were subjected to 90 min of normothermic CPB with 10 repetitively administered CAEs (0.3 μl/bolus). Rats in two sham groups were not exposed to CPB and CAE. Groups were further subdivided into xenon (56%; 20 min before, during, and 30 min after CPB) and nitrogen groups. Neurologic and cognitive function was tested until postoperative day 14, when cerebral infarct volumes were determined.

**Results:** Animals of the CPB–CAE groups showed transient deficits in gross neurologic function. Further, rats of the CPB–CAE–xenon group demonstrated impaired fine motor and cognitive performance persisting until postoperative day 14. Consistently, infarct volumes were larger in the CPB–CAE–xenon group compared with the CPB–CAE–nitrogen group (P = 0.03).

**Conclusions:** This is the first demonstration in which the neurologic effects of CAE have been examined in a rat model of CPB. Xenon exposure aggravated the neurologic dysfunction that is produced by CAE during CPB; potential neuroprotective effects of xenon may have been masked by the effects of xenon on CAE.

**Since** the anesthetic properties of the noble gas xenon have been recognized in the middle of the past century,¹ xenon has been characterized as an almost ideal anesthetic. Besides its safety and efficacy profile described in noncardiac surgery,² xenon offers cardiovascular stability,³–⁵ pharmacokinetic benefits with rapid induction and recovery of anesthesia, and profound analgesia.⁶–⁷ Recently, xenon has been shown to provide neuroprotective properties in both in vitro as well as in vivo studies, most likely *via* N-methyl-D-aspartate receptor antagonism.⁸–¹¹ Clinically, these properties have not been tested, although it would be of particular interest to administer xenon to patients at risk for perioperative cerebral injuries such as cardiac surgery patients undergoing cardiopulmonary bypass (CPB).¹²,¹³ However, recent *in vitro* and *in vivo* studies have shown that xenon may expand enclosed gas spaces such as the microbubbles that are both entrained as well as generated within the CPB circuit.¹⁴–¹⁶ Therefore, it cannot be excluded that xenon may worsen neurologic injury from cerebral air embolism (CAE). High numbers of CAEs are known to be present in most cardiac surgery procedures and have been implicated as one of several contributors (hypoperfusion, atheromatous emboli, and others) to postoperative cognitive impairment.¹⁷ Consequently, xenon’s disposition to expand air bubbles may abolish any neuroprotective effect or even amplify neurologic injury after CPB with CAE. The current study was designed to investigate the safety of xenon application in the presence of CAE using a long-term recovery model of CPB in rats. In preparation for this study, we identified a suitable volume for CAE during CPB that allows long-term recovery of rats in the presence of neurologic deficits.¹⁸

The aims of the current study were to determine (1) whether xenon anesthesia during CPB combined with CAE influences neurologic, cognitive, and behavioral long-term outcome and (2) whether xenon alters histologic outcome in this context.

**Materials and Methods**

All animals were treated in compliance with the *Principles of Laboratory Animal Care* formulated for the National Society for Medical Research¹⁹ and the *Guide for the Care and the Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources.²⁰ Experimental protocols were approved by the institutional animal care committee (Regierung von Oberbayern, Munich, Germany).

Four experimental groups were investigated (n = 10 per group) in a randomized fashion: Rats in the CPB–CAE
groups were subjected to 90 min of normothermic CPB with 10 repetitively administered CAEs, whereas rats in the sham-operated groups were exposed to the same surgical preparation and anesthesia but were neither connected to CPB nor received CAEs. Groups were further subdivided into xenon (56% xenon, 5% N2, 34% O2, and 5% CO2) and nitrogen (61% N2, 34% O2, and 5% CO2) groups with the accordant mixture inhaled 20 min before CPB, during, and 30 min after CPB or to equivalent times in the sham-operated groups.

**Surgical Preparation and Cardiopulmonary Bypass**

Nonfasted male Sprague-Dawley rats from Charles River Laboratories (363 ± 17 g, 10 weeks old; Sulzfeld, Germany) were cannulated for CPB as previously reported.21 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. The corresponding vein was cannulated for drug administration. All rats were ventilated with the same closed-circuit gas-delivery system, and inspiratory xenon concentrations were measured using a thermal conductivity sensor (provided by AGA AB, Lidingö, Sweden). For the injection of CAE, a PE-10 catheter (PE-10, Intramedic; Becton Dickinson GmbH, Heidelberg, Germany) was inserted via the stump of the right external carotid artery and advanced into the internal carotid artery just beyond the pterygopalatine branch, which was ligated.22 During surgery, anesthesia was maintained with 1.5–2.0% isoflurane and repetitive fentanyl boluses (5 µg). Pericranial temperature was monitored and controlled to 37.5°C (HYP-1 Newport, Santa Ana, CA). This temperature was selected based on previous work examining xenon during experimental CPB in rats.9

After completion of surgery, animals were denitrogenated, and the gas mixture was adjusted according to group assignment (56% xenon, 5% N2, 34% O2, and 5% CO2 in xenon groups and 61% N2, 34% O2, and 5% CO2 in nitrogen groups). This gas mixture was applied 20 min before CPB, during CPB, and 30 min after CPB and for equivalent times in the sham groups. Concomitantly, the anesthetic regimen was changed to intravenous midazolam (0.4 mg/kg), fentanyl (30 µg/kg) and atracurium (0.5 mg/kg) as a bolus injection, followed by a mixed continuous infusion (2.5 µg·kg⁻¹·min⁻¹ fentanyl, 0.03 µg·kg⁻¹·min⁻¹ midazolam, and 0.084 µg·kg⁻¹·min⁻¹ atracurium) and was sustained until the end of the CPB.

The CPB circuit consisted of a venous reservoir, a peristaltic pump (Masterflex®; Cole-Parmer Instrument Co., Vernon Hills, IL), a custom-designed membrane oxygenator, an in-line flow probe (2N806 probe and T208 flowmeter; Transsonics Systems, Inc., Ithaca, NY), and an arterial inflow cannula. The CPB circuit was primed with 14 ml whole blood obtained from one heparinized (150 U/rat) donor rat and 2 ml hetastarch, 6%. One hundred units of 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 inotropic support. The heparin anticoagulation was allowed to dissipate spontaneously without administration of protamine. Glucose, bicarbonate, and calcium were administered if required. After decannulation, rats remained anesthetized (continuous infusion of 3.9 µg·kg⁻¹·min⁻¹ fentanyl, 0.048 mg·kg⁻¹·min⁻¹ midazolam, and 0.129 µg·kg⁻¹·min⁻¹ atracurium), intubated, and ventilated for 1 h. After resuming spontaneous ventilation and extubation, the animals were recovered in an oxygen-enriched environment for 12 h with free access to water and food. On the first postoperative day, rats were returned to their hole-board cages and housed in familiar groups. The sham-operated groups had all CPB catheters left in place for 90 min (thereby mimicking the CPB protocol) but were not connected to the CPB circuit itself. Sham animals were decannulated and recovered in the same fashion as the CPB–CAE animals.

**Cerebral Air Embolism**

Rats subjected to 90 min of normothermic CPB received 10 equally sized CAEs (0.3 µL/single bolus) via the right internal carotid artery. 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 of neurologic deficits of 85.8% (95% confidence interval, 40.7–98.2%).18 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 (10-µL Gastight® No. 1701N; 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. At the end, 10 µL saline was injected to flush the last air bubble into the cerebral circulation. The sham-operated animals received saline (10 µL) instead of CAE.

**Neurologic and Neurocognitive Testing**

All neurologic, cognitive, and behavioral tests were performed by an investigator blinded to treatment. Rats were housed under standard laboratory conditions (12 h light–12 h dark, lights on at 0:30 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.

On the preoperative as well as on the 1st, 2nd, 3rd, and 12th postoperative days, animals underwent standardized functional neurologic testing performed as previ-
PaCO₂, mmHg

Sham Air 35.5
Sham Xenon 35.5
CPB Air 33.2
CPB Xenon 34.2

Hemoglobin concentration, mg/dl

Sham Air 13.5
Sham Xenon 13.2
CPB Air 13.5
CPB Xenon 13.2

Paco₂, mmHg

Sham Air 35.5
Sham Xenon 36.2
CPB Air 33.2
CPB Xenon 34.2

Pao₂, mmHg

Sham Air 126.12
Sham Xenon 117.14
CPB Air 126.12
CPB Xenon 124.23

Variables were obtained before cardiopulmonary bypass (before CPB), at 45 min of CPB (45 min CPB), at 90 min of CPB (90 min CPB), and 1 h after CPB (1 h after CPB) or at equivalent times in the sham-operated groups (sham). Values are presented as mean ± SD. Rats subjected to CPB demonstrate lower mean arterial pressure, lower hemoglobin concentrations, and higher arterial oxygen tension (PaO₂) during CPB than sham groups. Before CPB, mean arterial pressure in the two xenon groups was lower compared with the sham–nitrogen group, probably because of the additional anesthesia effect of xenon. Higher arterial carbon dioxide tension (PaCO₂) levels in the two xenon groups compared with the nitrogen groups before CPB and lower PaCO₂ levels in the sham–nitrogen group compared with the two xenon groups were extended. Three cognitive parameters were evaluated: (1) Deficits in overall cognitive performance were assumed if the time required to complete one trial was extended; (2) deficits within the declarative memory were assumed if rats visited nonbaited holes or did not visit baited holes, referred to as wrong choice; and (3) deficits within the working memory were assumed if rats revisited a baited hole, referred to as repeated choice. In addition, rat behavior, such as motivation, anxiety, and exploration, was assessed with the modified hole-board test.

Histologic Examination

On postoperative day 14, animals were anesthetized with 5% isoflurane and subjected to in situ brain fixation. The brains were removed in toto, were serially cut (10-μm sections) at 150-μm intervals, and were 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, the number of single infarcts was recorded.

Statistical Analysis

Physiologic, neurologic, cognitive, and behavioral values were analyzed using general linear models with the
between-group factors preparation (CPB–CAE vs. sham) and treatment (xenon vs. nitrogen), the within-group factor time, and their interaction terms. Values were analyzed post hoc using factorial analysis of variance followed by Bonferroni t tests. Physiologic values were post hoc analyzed at each time; neurologic, cognitive, and behavioral variables were analyzed on the final postoperative day (day 12 for neurologic and day 14 for cognitive and behavioral values). Histologic values were compared using the Student t test.

**Results**

Two animals were excluded from further data analysis because of postoperative development of cervical hematoma with inspiratory stridor (one animal in the CPB–CAE–nitrogen group and one animal in the CPB–CAE–xenon group). These two rats were replaced to keep sample size equal.

**Hemodynamic and Physiologic Data**

Table 1 displays the physiologic values of rats after CPB with CAE or sham operation. Rats subjected to CPB and CAE demonstrated lower mean arterial pressure, lower hemoglobin concentrations, and higher arterial oxygen tension during CPB than sham groups, most likely because of the nature of CPB. During CPB and CAE, there were no differences in physiologic values between the CPB–CAE–xenon group and the CPB–CAE–nitrogen group. The rats’ body weight decreased more in the CPB–CAE groups compared with the sham groups, with a nadir on postoperative day 6 (P = 0.007).
Neurologic, Cognitive, and Behavioral Assessment

Gross neurologic function was impaired in all animals subjected to CPB and CAE on the first postoperative days. Further, animals exposed to xenon during CPB and CAE demonstrated sensorimotor and fine motor deficits that persisted for at least 2 weeks (figs. 1 and 2). Rats of the CPB–CAE–xenon group demonstrated a worse neurocognitive outcome compared with the remaining groups until postoperative day 14 (fig. 3). No differences in behavior were detected during the observation period. Therefore, differences in neurologic and neurocognitive outcomes cannot be attributed to altered behavior.

Histologic Results

None of the sham-operated rats demonstrated any infarct. All of the rats subjected to CPB and CAE showed at least one infarct in the cortex of the right hemisphere. Larger infarcts involved the striatum and hippocampus, and one rat (CPB–CAE–xenon group) demonstrated an infarct that extended into the left hemisphere. In animals exposed to CPB and CAE, xenon resulted in larger infarct volumes and a higher number of infarcts (fig. 4).

Discussion

The “inert” gaseous anesthetic xenon caused a significant increase in ischemic brain damage when administered perioperatively in a combined model of CPB and CAE in rats. This effect was evident both histologically and functionally in rats allowed to survive 14 days after CPB and CAE. Xenon increased cerebral infarct size and worsened motor as well as neurocognitive performance. Although theoretical models and in vitro studies in aqueous solution have outlined xenon’s potential to expand air bubbles, this study describes the first in vivo setting that allowed investigation of the safety profile of xenon inhaled during CPB in the presence of CAE.14,15

The effects of xenon in the context of CPB and CAE were studied for several reasons. First, xenon’s use in patients undergoing CPB for cardiac surgery has been suggested because of its anesthetic qualities and its attractive hemodynamic profile. Second, it has been demonstrated that xenon possesses protective properties in both experimental myocardial and cerebral injury.10,27 More recently, xenon has also been shown to provide neuroprotection in a rat model of CPB.9 Third, despite xenon’s abundant advantages, it may also have some significant disadvantages in the setting of cardiac surgery. Xenon’s low solubility makes it theoretically more likely to augment gaseous microbubbles that, during cardiac surgery with CPB, present as CAEs.17 Any augmentation by xenon may increase the risk of cerebral injury and must be weighed against its previously well-documented neuroprotective effects. The 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 CAEs, such as open-chamber procedures.

In the past, several animal models of CAE have been established and provide 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.22,28 The combination of CPB and CAE for the investigation of the effects of CAE on cerebral outcome was first described by Hindman et al.29 Al-
though 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 the 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. Therefore, the current study presents the primary description of a long-term recovery model of CPB combined with CAE in rats that allowed us to investigate the effect of xenon on long-term neurologic and neurocognitive outcome in this context.

Recently, xenon has been shown to attenuate CPB-induced neurologic and neurocognitive deficits in rats.9 These neuroprotective properties seem to be offset by the effects of xenon on CAE in the current study. Larger infarct volumes, together with a worsened functional outcome in animals exposed to xenon, may foster safety concerns regarding the disposition of xenon to expand air bubbles. Of interest, xenon anesthesia also resulted in a higher number of detected infarcts. One can only speculate that xenon, by altering the bubble volume or absorption kinetics of CAE, may have led to prolonged occlusion of a given brain territory, which then resulted in the redirection of blood flow into adjacent brain regions. This may then have produced further infarction due to the redistribution of CAE. Neurologic and neurocognitive performance were assessed using the modified hole-board test, a unique test battery that allows a comprehensive and detailed evaluation of cerebral outcome in rats. This test offers a sensitive testing environment allowing the assessment of a variety of cognitive, fine motor, and behavioral parameters in only one test.26 Further, this test battery allowed a complete evaluation of neurologic performance from the first postoperative day until day 14. In addition, behavior aspects such as anxiety and motivation were determined, because it is well known that these parameters can influence cognitive function.25

Even though this model was established to mimic clinical standards as closely 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, CAEs 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 CAEs 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, only 10 equally sized CAEs were administered. Third, the study was restricted to the investigation of a certain embolus size (0.3 μL) that was shown to generate neurologic deficits in the major-
ity of rats as evaluated in a pilot study. It remains unclear whether the CAE size/damage ratio described herein is relevant for the clinical setting. That is, it is uncertain whether xenon would exert similar effects on cerebral outcomes in humans undergoing CPB for cardiac surgery. Last, median sternotomy, direct cardiac cannulation, and surgery were not performed, to allow long-term survival of the animals.

In summary, if CAE occurs during xenon exposure in a rat model of CPB, fine motor, cognitive, and histologic outcomes are impaired. Potential neuroprotective properties of xenon, as recently revealed in a rat model of CPB, may have been masked by the effects of xenon on CAE. Use of xenon for its neuroprotective potential may have to be restricted in cardiac surgical procedures where similar CAE size/damage ratio occurs.

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