Xenon Ventilation During Therapeutic Hypothermia in Neonatal Encephalopathy: A Feasibility Study

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KEY WORDS
hypoxic-ischemic encephalopathy, hypothermia, neonatal encephalopathy, newborn, quality of life, sedation, ventilation, xenon

ABBREVIATIONS
aEEG—amplitude-integrated EEG
BS—burst suppression
CRP—C-reactive protein
ETCO2—end-tidal carbon dioxide
FiO2—fraction of inspired oxygen
HIE—hypoxic-ischemic encephalopathy
HI—hypoxic-ischemic
MAP—mean arterial blood pressure
MDI—mental developmental index
NE—neonatal encephalopathy
PDI—psychomotor developmental index
TcSO2—transcutaneous oxygen saturation
TH—therapeutic hypothermia
Xe—xenon

Dr Dingley was responsible for xenon administration and technical Medicines and Healthcare Products Regulatory Agency documentation, and contributed to drafting and reviewing of the manuscript; Dr Tooley was responsible for organizing outborn transport and early treatment of the infants, was clinically responsible and contributed to administering the treatment, and contributed to drafting of the manuscript; Dr Liu administered the study, collected and analyzed data, contributed to drafting and manuscript revisions, and created the figures; Mrs Scull-Brown organized the administration of the study and collected data; Dr Elstad collected and analyzed data and contributed to manuscript revisions; Dr Chakkarapani treated the infants, collected and analyzed data, and contributed to the manuscript; Dr Sabir treated the infants, entered and analyzed data, created figures, and contributed to the manuscript; and Dr Thoresen was the Principal Investigator of the study who planned the study protocol with Dr Dingley and secured the funding for the study. Dr Thoresen also treated and organized follow-up of patients, supervised the data collection and analysis, and participated in data analysis, manuscript drafting, and revision. All authors approved the final manuscript as submitted.

This trial has been registered with the ISRCTN Register (ISRCTN75602528).

WHAT’S KNOWN ON THIS SUBJECT: Hypothermia treatment of neonatal encephalopathy reduces death and disability from 66% to 50%; additional neuroprotective therapies are needed. We previously found in animal models that adding 50% xenon to the breathing gas during cooling doubled neuroprotection.

WHAT THIS STUDY ADDS: This clinical feasibility study used 50% xenon for 3 to 18 hours in 14 cooled infants with cardiovascular, respiratory, and amplitude-integrated EEG monitoring. This depressed seizures, with no blood pressure reduction. Xenon is ready for randomized clinical trials in newborns.

BACKGROUND AND OBJECTIVES: Therapeutic hypothermia has become standard of care in newborns with moderate and severe neonatal encephalopathy; however, additional interventions are needed. In experimental models, breathing xenon gas during cooling offers long-term additive neuroprotection. This is the first xenon feasibility study in cooled infants. Xenon is expensive, requiring a closed-circuit delivery system.

METHODS: Cooled newborns with neonatal encephalopathy were eligible for this single-arm, dose-escalation study if clinically stable, under 18 hours of age and requiring less than 35% oxygen. Xenon duration increased stepwise from 3 to 18 hours in 14 subjects; 1 received 25% xenon and 13 received 50%. Respiratory, cardiovascular, neurologic (ie, amplitude-integrated EEG, seizures), and inflammatory (C-reactive protein) effects were examined. The effects of starting or stopping xenon rapidly or slowly were studied. Three matched control subjects per xenon treated subject were selected from our cooling database. Follow-up was at 18 months using mental developmental and physical developmental indexes of the Bayley Scales of Infant Development II.

RESULTS: No adverse respiratory or cardiovascular effects, including post-extubation stridor, were seen. Xenon increased sedation and suppressed seizures and background electroencephalographic activity. Seizures sometimes occurred during rapid weaning of xenon but not during slow weaning. C-reactive protein levels were similar between groups. Hourly xenon consumption was 0.52 L. Three died, and 7 of 11 survivors expressed follow-up.

CONCLUSIONS: Breathing 50% xenon for up to 18 hours with 72 hours of cooling was feasible, with no adverse effects seen with 18 months’ follow-up. Pediatrics 2014;133:809–818

abstract

(Continued on last page)
Severe neonatal encephalopathy (NE), mostly of hypoxic-ischemic (HI) origin, leads to brain injury in term newborns, often causing lifelong motor and cognitive impairment. The primary insult is followed by a self-sustaining destructive cascade that postinsult therapies might limit. No clinical intervention except therapeutic hypothermia (TH) is known to improve the neurologic outcome. A meta-analysis of 7 large randomized controlled cooling trials has shown that poor outcome is reduced from 66% to 50% in cooled infants. Adding the anesthetic gas xenon (Xe) to TH for additional neuroprotection has been investigated in small and large experimental in vivo preclinical outcome studies by us as well as other researchers. Adding 50% Xe to cooling immediately post-insult doubles the neuroprotective effect as measured by using histologic neuropathology scores, and Xe is effective even after a 2-hour delay. After HI, reperfusion leads to excessive glutamate release, N-methyl-D-aspartate receptor overactivation, and calcium influx-mediated apoptotic neuronal death. Xe partially inhibits the N-methyl-D-aspartate receptor, and Xe pretreatment up-regulates the prosurvival proteins Bcl-2 and brain-derived neurotrophic factor in rats. Xe is an almost inert elemental gas, 4 times heavier than air and free of adverse physiologic effects or toxicity. It is eliminated unchanged via the lungs within minutes when delivery is stopped and therefore is a potentially reversible intervention. Xe is licensed for adult anesthesia in Europe (LENOxe TM, Air Liquide, Paris, France) and Russia (KseMed, Akela-P Medical Gases Pvt. Ltd, Moscow, Russia; Russian Federation license 64/0125-JP/02 of 19.12.2002), but the high cost ($30 per liter) and fixed annual production limit its use. The only previous neonatal experience with Xe was from 133Xe isotope studies. An economical Xe delivery system was developed that recirculates exhaled gases, removes carbon dioxide, and adds oxygen/Xe to replace patient uptake. In preclinical pig studies, 0.25 L/h of Xe maintained a steady 50% Xe concentration after initial circuit priming, minimizing costs compared with an open ventilator using up to 180 L/h of Xe. Additional costs included $200 for the single-use ventilation circuit and $80 for theuffed endotracheal tube.

Adding 50% Xe to TH in both short-term and long-term neonatal brain injury models doubles the neuroprotection compared with TH alone. Moving from a rodent to a larger whole-body HI pig survival model confirmed an additive effect between Xe and TH, resulting in 75% protection in neuropathology. In a newborn pig brain ischemia model, metabolic markers favored the Xe + TH group compared with Xe or TH alone. Xe + TH improved cardiovascular stability and reduced inotropic requirements. There was no increased oxygen requirement, no cuffed tracheal tube complications, and no stridor or extubation delays in either our experimental pig study (n = 120) or this clinical study. There are ongoing concerns regarding neuroapoptosis in the immature brain with inhalation anesthetic agents. Although atypical in mode of action, Xe is an inhalation anesthetic. Recently, we ventilated newborn pigs for 24 hours with 50% Xe, finding no increase in neuroapoptosis over normal controls unlike those ventilated with 2% isoflurane, in which there was a >10-fold increase. A study of 120 newborn pigs provided safety data for all organ systems and supported our use of 50% Xe in this clinical feasibility study in term infants undergoing TH.

METHODS
This was a single-arm, dose-escalation feasibility study adding Xe ventilation to 14 cooled infants with NE. We used the Sarnat clinical severity scoring system of hypoxic-ischemic encephalopathy (HIE) (1: mild; 2: moderate, and 3: severe) and included grade 2 or 3 infants. The setting was a single tertiary center treating in- and outborn patients fulfilling standard TH criteria. Approvals from the Medicines and Healthcare Products Regulatory Agency for the Xe delivery system (C1/2009/0043) as well as for off-license Xe use (LenoXe, Air Liquide, Dusseldorf, Germany) (12893/0223/001-0001) were obtained, as was ethics approval from Bristol University, United Kingdom (UK09/H0106/4). Recruitment was from March 2010 until April 2011.

Primary outcomes were technical feasibility and physiologic stability, particularly with respect to cardiorespiratory parameters and EEG. The regional ethical committee requested follow-up as in the ongoing cooling program, with a Bayley Scales of Infant Development II examination performed at 18 to 20 months as a secondary safety analysis. The mental developmental index (MDI) and the psychomotor developmental index (PDI) are classified as mild delay (<1 SD below the mean) if values are <85 and as major delay if <70. The regional ethical committee also requested a limited data set of historical controls from our institution; 3 cooled patients per Xe-treated patient were matched for oxygen requirement and severity of NE at entry. Signed informed parental consent was obtained for adding Xe to cooling, including replacement of the standard uncuffed endotracheal tube with auffed tube (code 3511; Kimberly-Clark Health Care, Roswell, Georgia) of the same or 0.5 mm smaller diameter. Minimum cuff pressures for a complete seal were monitored hourly (Portex, Hythe, UK) (Fig 1D). The number of deaths that statistically would require trial suspension, pending review, as calculated for patients 1
through 14 based on a retrospective cooling mortality of 25% is shown in Table 1. This stopping rule is such that the trial is stopped if the binomial probability for the observed number of deaths is <0.05 but continued if this value is >0.05. These calculations were made by our study statistician based on this principle and our cooling mortality data over the preceding 6 years.

After initial resuscitation and medical management following usual guidelines for NE and cooling therapy, additional inclusion criteria for Xe were applied: <18 hours old, weight greater than the second percentile for gestational age, intubated and ventilated, sedated, being cooled, seizures under control, stable cardiovascular parameters, mean arterial blood pressure (MABP) >40 mm Hg, oxygen requirement via mechanical ventilator <0.35, positive end-expiratory pressure ≤8 cm H₂O, arterial/capillary/venous PO₂ <7.0 kPa (ideally arterial), and absence of major congenital abnormalities (in particular, imperforate anus or any bowel obstruction).4,6 Thirteen infants were outborn and 1 was inborn. All started “passive” cooling followed by active servo-controlled body cooling by using Criticool (MTRE Advanced Technologies Ltd, Rehovot, Israel) (n = 12) or Tecotherm Neo (Inspiration Healthcare Ltd, Leicester, UK) (n = 2) machines. Umbilical arterial/venous catheters were inserted for arterial pressure, blood gas measurements, fluid infusions of dextrose with electrolytes (n = 12), or total parenteral nutrition (n = 2), sedatives, anticonvulsants, inotropes, and antibiotics. Routine sedation for ventilated infants was 20 μg/kg per hour of morphine. Hourly neonatal facial coding scoring for pain evaluation was performed, and the dose adjusted accordingly.29 Clinical or subclinical seizures recognized on an amplitude-integrated EEG (aEEG) and an EEG (Olympic CFM 6000, Natus Medical Incorporated, San Carlos, California) lasting >3 minutes were treated with anticonvulsants. The first infant who received Xe during cooling was given 25% Xe for 3 hours; the following infants received 50% Xe for 3, 6, 12, or 18 hours (Table 2). Because no human neonate had previously received Xe, we gradually increased the treatment duration to our target of 18 hours, which is the amount shown to be neuroprotective in the preclinical study.12

Figure 1 shows the monitoring and breathing system assembly (see Supplemental Video). The closed ventilator circuit was partially flushed with fresh gas every 2 hours as a precaution. Cardiovascular, respiratory,
TABLE 1 Number of Deaths That Would Require Trial Suspension and Review of Recruitment Relative to Infants Included

<table>
<thead>
<tr>
<th>No. of Infants Included in the Study</th>
<th>Stopping Rule for No. of Deaths</th>
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<td>2</td>
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* Based on 25% mortality in the cooled cohort, it is not unlikely that the first 2 consecutive infants might die ($P = .06$). If the third infant also dies, the probability that this is a random event is very small ($P = .016$); thus, the study should stop and review after 3 deaths.

RESULTS

The cohort recruited was typical, with 4 infants assessed as grade 2 HIE and 9 as grade 3 HIE on day 1 (Table 3).\textsuperscript{27} Cooling commenced pretransfer, and the target temperature was reached between 2.4 and 9.7 hours of age (Table 2). Xe delivery started at a median of 11 hours (range: 5–18 hours). Xe was stopped prematurely after 8 hours in 1 infant due to a fraction of inspired oxygen ($F_{IO2}$) requirement $>0.35$. Stopping Xe in this patient did not change the $F_{IO2}$ requirement, which continued to increase to 0.50. Surfactant treatment (Curosurf, Chiesi Farmaceutici, SpA, Parma, Italy) was administered, and the $F_{IO2}$ was reduced to 0.28 in this 36-week gestation infant who was delivered by cesarean delivery without onset of labor.

The mean values for cardiovascular and respiratory variables recorded every 5 minutes during 3 time periods (from 4–6 hours before Xe, during the whole Xe period [3, 6, 12, or 18 hours], and for up to 6 hours after stopping Xe) are shown in Fig 2. There were no significant changes over time. The mean $\pm$ SD Xe concentration was stable at 50 $\pm$ 2% when administered, and it was never interrupted during each delivery period, despite circuit flushes or $F_{IO2}$ adjustments. The net Xe volume required to maintain a steady 50% Xe concentration (ie, excluding circuit priming of 1.64 L of Xe and flushes) was 0.29 $\pm$ 0.129 L/h. Overall Xe usage, including priming and subsequent flushes, was 0.52 $\pm$ 0.179 L/h. Patient 1 received a precautionary steroid dose and exchange to an uncuffed endotracheal tube after Xe administration. The cuff was deflated after Xe administration until extubation in all subsequent infants. There was no postextubation stridor or delay in extubation. When Xe was commenced in stable newborns, transcutaneous oxygen saturation ($TcSO_2$) mean on pulse oximetry was 95 $\pm$ 2.5%. Carbon dioxide removal was adequate, with mean end-tidal carbon dioxide ($ETCO_2$) on capnography of 31 $\pm$ 3.5 mm Hg, and $F_{IO2}$ was 0.24 $\pm$ 0.08.

Thirty minutes after commencing Xe, mean $TcSO_2$ was 95 $\pm$ 3.1%, $ETCO_2$ was 34 $\pm$ 3.6 mm Hg, and $F_{IO2}$ was 0.28 $\pm$ 0.06. The mean $TcSO_2$ during the entire Xe administration was 96 $\pm$ 2.5%, $F_{IO2}$ was 0.28 $\pm$ 0.07, and the $ETCO_2$ was 32 $\pm$ 3.2 mm Hg. Figure 3 demonstrates that carbon dioxide levels, seizures, reinitubation, and extubation followed standard clinical practice.

TABLE 2 Patient Characteristics, Cooling, and Xe Details for the 14 Study Infants

<table>
<thead>
<tr>
<th>Infant No.</th>
<th>Gestation (weeks + days)</th>
<th>Birth Weight (percentile %)</th>
<th>10-Minute Apgar Score</th>
<th>Worst pH (1st hour)</th>
<th>Age to Target Temperature 33.5°C (h)</th>
<th>Xe Delivery Period (h)</th>
<th>Percentage Xe in Breathing Gas</th>
<th>Age at Start of Xe (h)</th>
<th>$F_{IO2}$ at Start of Xe</th>
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\* All received the planned concentration of Xe and 13 received the planned duration (3–18 hours) of Xe ventilation except patient 14.
Xe did not reduce MABP below critical values.

A severely depressed aEEG with either burst suppression (BS), low amplitude, or flat tracing (Table 3), both before cooling and before Xe, occurred in 10 infants; these infants were also classified as grade 3 HIE on day 1. Eight infants had documented clinical or EEG seizures before Xe (6 had received phenobarbital); however, only 1 of 14 had a seizure during Xe administration. Figure 4 illustrates the depressant effect of Xe on aEEG at Xe onset in 2 infants. In Fig 4A, the pattern changed from discontinuous normal voltage to BS; 12 hours later, Xe was weaned slowly over 40 minutes, and the background pattern was continuous normal voltage. In the second patient (Fig 4B), starting Xe severely depressed the aEEG to BS; 12 hours later, Xe was stopped rapidly over <10 minutes, and seizures started within 5 minutes. Four of 8 infants who had seizures before but not during Xe delivery had seizures soon after rapid Xe cessation. Five infants with discontinuous normal voltage before Xe had a depressed aEEG during Xe, and 4 infants with flat tracing or low amplitude tracings showed no change in aEEG pattern (the aEEG was already flat). Of the 5 starting with a BS pattern, 3 developed an even more depressed aEEG, and 2 did not change.

At 18 to 20 months of age, 7 infants had normal (MDI and PDI >70) or mildly delayed (MDI and PDI >70) outcomes. One had unilateral severe hearing loss caused by a further reduction; however, MABP was maintained (Figs 5B, 5C, and 5D).

All infants received intravenous antibiotics after birth for ≥2 days because infection could not be initially excluded. No infant had a positive blood culture result. One mother had an elevated C-reactive protein (CRP) level >100 mg/L and a positive blood culture result. In addition, 6 mothers had cultures taken (the results of which were all later confirmed as negative) and were treated with intravenous antibiotics during delivery. Data were collected from 3 cooled patients with similar baseline variables (n = 42) matched to each Xe-treated patient. All had routine CRP measured 1 to 2 times daily. We compared the time course and peak CRP value during the first 3 days of life between the Xe group and the 42 matched control subjects. There was evidence that Xe did not reduce MABP below critical values.
no difference between the 2 groups in either the peak CRP value (median [total range]) between the matched controls ($n = 42$; 16 mg/L [1–27 mg/L]) and Xe ($n = 14$; 18 mg/L [1–32 mg/L]) or the time from birth to peak CRP (controls: 30 hours [1–52 hours]; Xe: 24 hours [1–58 hours]).

**DISCUSSION**

This feasibility study describes the use of Xe as a second neuroprotectant in human term infants undergoing hypothermia. Xe is an expensive and scarce anesthetic gas; either of these factors may limit its use as a neuroprotectant. We addressed this limitation by developing a closed-circuit Xe delivery system.
system\textsuperscript{21} in which the net use of Xe gas to maintain a steady 50% concentration was only 0.29 L/h. As a precaution, we followed anesthesia clinical practice by periodically flushing the circuit with fresh gas. The overall Xe requirement, incorporating these flushes and an initial bolus to prime the circuit, was 0.52 ± 0.179 L/h. At approximately $30 per liter, this amount represents a clinically acceptable cost of $15.60 per hour, in addition to $200 for the ventilator circuit and $80 for the cuffed endotracheal tube. Concurrent nitric oxide delivery is a possibility (currently under laboratory investigation).

Permission to study humans was given in view of the additive neuroprotective effect of Xe plus TH in both rodents\textsuperscript{9,11,13} and pigs,\textsuperscript{12} near-inert chemistry, acceptable cost if delivered efficiently, and the reversibility of the treatment. Another advantage is that Xe is known to be relatively free of hemodynamic adverse effects.\textsuperscript{22} The optimal duration of Xe plus TH treatment is unknown. We chose a maximum of 18 hours of 50% Xe because this combination doubled histologic neuroprotection compared with hypothermia alone in a global newborn piglet model.\textsuperscript{12} In rodents, as little as 1 hour of 50% Xe during TH increased neuroprotection.\textsuperscript{13} There are several important safety issues regarding inhalation anesthetics and the immature brain. Isoflurane applied for a few hours can induce neuroapoptosis in rodents.\textsuperscript{30} Recently, we were reassured that 24 hours of 50% Xe did not increase neuroapoptosis in newborn healthy pigs; in contrast, 2% isoflurane increased apoptosis >10-fold.\textsuperscript{25} The neuroapoptotic effect is therefore not shared by all inhalation anesthetic agents, and Xe is more likely than others to be safe for the immature brain.

The sedative effect of Xe per se cannot be assessed reliably in infants with varying degrees of cerebral depression. Some were comatose and unresponsive; others needed increased morphine sedation during hypothermia. However, it was apparent clinically and on EEG that 50% Xe increased sedation (Fig 4). Rapid reversal of clinical and EEG depression was clearly seen when stopping Xe; infants opened their eyes and began moving within 1 to 2 minutes (see Supplemental Video). The minimal alveolar concentration Xe sedation value is unknown for newborns. We recently defined the minimal alveolar concentration for Xe in <24-hour-old pigs,\textsuperscript{31} finding a wide interindividual range (median: 120% [range: 60%–140%]). This value is not known for newborn humans; however, if similar to pigs, 50% Xe would sedate infants with great variability. It is unknown whether individual neuroprotection is linked to sedative potency. Early in the study, we reduced the morphine sedation during Xe inhalation but observed that some infants became agitated during the subsequent rapid Xe weaning. Thereafter, we maintained our standard morphine infusion rate of 20 μg/kg per hour throughout. We also observed that infants rarely had seizures during Xe treatment; however, on rapid cessation, 4 infants started to experience seizures, which was another reason to slowly wean Xe.

Although Xe is 4 times denser than oxygen, at 50%, it only minimally changes overall viscosity.\textsuperscript{32} High Xe concentrations in pigs produce only a modest airway pressure increase.\textsuperscript{33,34} In dogs, high concentrations only modestly increase airway resistance, even after drug-induced bronchoconstriction.\textsuperscript{35} In our study, ETCO\textsubscript{2} was unchanged in the presence of Xe. Cuffed tubes were used to prevent Xe loss and are necessary for a closed breathing system. We acknowledge previous concerns regarding the cuffed endotracheal tubes but observed no stridor or extubation problems in 120 pigs\textsuperscript{12} or these 14 study infants. In an anesthesia study in 2246 children, 326 of whom were <8 months old, extubation stridor occurred in 4.4% of those with cuffed tubes and 4.7% of those without.\textsuperscript{36} Xe diffusion into tube cuffs has been investigated by others and is likely to be slow.\textsuperscript{37} As a precaution, we monitored the cuff pressure hourly. It has been suggested that Xe interacts with the immune system by preserving neutrophil and monocyte antibacterial capacity and modulation of inflammatory cytokines such as tumor necrosis factor–α and interleukin-6 in monocytes.\textsuperscript{38,39}

In the present study, Xe had no effect on the commonly monitored inflammatory marker CRP. We compared degree and timing of the individual CRP responses with data from matched children.
undergoing cooling only. Modulation of inflammatory responses or immune tissue damage by Xe may in the future reveal additional protective responses with respect to other organ systems, for which there is already some evidence from animal studies.\(^{40-42}\)

**CONCLUSIONS**

Breathing 50% Xe for up to 18 hours in conjunction with 72-hour cooling in term and near-term infants with NE at birth by using a closed Xe breathing system and cuffed endotracheal tube was feasible with no adverse effects. No significant cardiovascular or respiratory changes during or after Xe administration were seen in these study patients. Cuffed endotracheal tubes (with cuff pressure monitoring) did not adversely affect extubation. Xe did not influence the peak CRP or time course for the first 3 days after birth. Xe depressed EEG in those who were not comatose and also depressed seizures because some infants started to experience seizures when Xe was discontinued. Attention to slow weaning and the level of sedation are required when Xe is stopped. Outcome at 18 months, reported for safety purposes, showed no delay or mild delay in 50% of these patients, which is similar to and no worse than expected from cooling alone. This study confirmed that it is feasible and safe to do a Phase II randomized outcome study combining TH with 50% Xe, and that study is now underway.

**ACKNOWLEDGMENTS**

The authors thank Professor Lars Walløe, University of Oslo, for statistical advice; developmental physiotherapist Sally Jary, University of Bristol, for the Bayley examinations; and Professor Krisa van Meurs, Stanford University, for manuscript revisions.
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Pediatrics 2014;133;809
DOI: 10.1542/peds.2013-0787 originally published online April 28, 2014;
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Pediatrics 2014;133;809
DOI: 10.1542/peds.2013-0787 originally published online April 28, 2014;

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