British Journal of Anaesthesia **98 (2):** 236–40 (2007)
doi:10.1093/bja/ael340 Advance Access publication January 8, 2007

BJA

Asynchronous administration of xenon and hypothermia significantly reduces brain infarction in the neonatal rat

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Background. Neonatal asphyxia causes long-term neurological and behavioural impairment in the developing brain. Concurrent administration of xenon and hypothermia synergistically reduces long-term damage in a rat model of neonatal asphyxia. This study sought to investigate whether *asynchronous* administration of xenon and hypothermia is capable of combining synergistically to provide neuroprotection.

Methods. Seven-day-old rats were subjected to right common carotid artery occlusion followed by 90 min hypoxia with 8% oxygen. After a 1 h recovery period, rats received asynchronous administration of mild hypothermia (35°C) and xenon (20%) with a 1 or 5 h gap between interventions, xenon (20%) alone, or mild hypothermia (35°C) alone. Infarct volume in the brain was measured 4 days after injury.

Results. Administration of hypothermia or xenon alone, 1 and 6 h after the hypoxic ischaemic insult, respectively, provided no neuroprotection. Asynchronous administration of xenon and hypothermia at a 1 h interval produced a significant reduction in infarct volume [93 (7) *vs* 74 (8); $P<0.05$]. Reduction in infarct volume was also present when hypothermia and xenon were asynchronously administered with an intervening gap of 5 h [97 (5) *vs* 83 (3); $P<0.05$].

Conclusions. This finding provides a rationale for investigating the combined use of hypothermia and xenon in a progressive manner for the management of neonatal asphyxia. Thus, hypothermia can be administrated at the site of delivery and xenon can be administered later.

Br J Anaesth 2007; **98**: 236–40

Keywords: anaesthetics gases, xenon; hypothermia, asynchronous; hypoxia-ischaemia; neonate; rat

Accepted for publication: October 28, 2006

Perinatal hypoxic ischaemia (HI) is a devastating complication of childbirth; estimates suggest that 2–4 per 1000 term infants will suffer such an episode during labour.[1] Of the infants that develop HI encephalopathy, 15–20% will die and of the survivors, a quarter will develop chronic neurological deficits such as cerebral palsy.[2 3] Although significant progress has been made in understanding the pathogenesis of the neuronal damage, the development of neuroprotective strategies has proved largely unsuccessful. However, subgroup analysis from the *Cool Cap Study* showed significant benefit of mild hypothermia (34–35°C) initiated within 6 h and administered for 72 h in mild to moderately injured sufferers of perinatal HI.[4] Furthermore, a second large randomized control trial of neonates with HI encephalopathy due to acute perinatal asphyxia demonstrated that systemic hypothermia (33.5°C) for 72 h reduces death and moderate or severe disability by 18% when followed-up at 18 to 22 months of age.[5] Any intervention that could augment the protection afforded by hypothermia would be of great significance.

We have recently demonstrated that xenon potentiates hypothermic neuroprotection in *in vitro* and *in vivo* models of perinatal HI injury in a synergistic manner[6] with efficacy present even when therapy was delayed up to 6 h after the insult. However, as xenon administration is

[†]*Declaration of interest*. Professors Maze and Franks are paid consultants to Air Products, a company that is interested in developing clinical applications for medical gases, including xenon. In addition, Air Products have funded, and continue to fund, work in the authors' laboratories that bears on the actions of xenon as an anaesthetic and neuroprotectant.

currently expensive and the delivery systems capable of mitigating the costs not likely to be widely available, clinical translation of xenon-hypothermia combination therapy may be more feasible if the therapies are started asynchronously with hypothermic therapy initiated at the primary care centre and maintained during transport to a tertiary referral centre where xenon can be delivered. Herein, we report that in *in vivo* models of neonatal HI, xenon and hypothermia combine synergistically to afford neuroprotection even when administered asynchronously.

Methods

This study conformed to the United Kingdom Animals (Scientific Procedures) Act of 1986 and was approved by the Home Office (UK). Every effort was made to minimize animal suffering and the number of animals used. Twelve pups of either sex per dam were used and housed with a 12 h light/12 h dark schedule in a temperature- and humidity-controlled colony room.

Animal model of hypoxic-ischaemic injury

Seven-day-old Sprague-Dawley rat pups of either sex, weighing between 10 and 14 g were subjected to a model of HI injury as previously described by Rice and colleagues.[7 8] In brief, rat pups were anaesthetized with isoflurane 2%, unilateral right common carotid artery ligation was then performed through a midline neck incision using 5.0 silk suture. After completion of surgery, pups recovered from the anaesthesia before being returned to their dams for 1 h. The environment was maintained at a constant temperature (23°C) and humidity (48%) for 1 h.

After the 1 h recovery period, pups were exposed to hypoxia by placing them in purpose-built chambers, partially submerged in a 37°C water bath. The animals were exposed to systemic hypoxia by a continuous flow of 8% oxygen balanced with nitrogen for 90 min. This mixture was monitored every 15 min using a Datex Ohmeda (Bradford, UK) analyser. The duration of hypoxia was determined by preliminary experiments which showed that such a period of hypoxia produced maximal HI damage, as measured by hemispheric weight.[6]

Following HI, pups were returned to their dam for a second 1 h recovery period. Littermates were then divided randomly into control and treatment groups. Treatment groups were subjected to one of the following treatment strategies; asynchronous administration of mild hypothermia (35°C) and xenon (20%) with a 1 or 5 h interval between the completion of mild hypothermia and the initiation of xenon, xenon (20%) alone, or mild hypothermia (35°C) alone (Fig. 1). Each treatment strategy was performed on a separate litter with littermates randomly divided into control and treatment groups.

Treatment strategies

Pups subjected to the asynchronous treatment strategy of hypothermia and xenon initially underwent 2 h of mild hypothermia (35°C). A pup was selected at random, and under isoflurane and local anaesthesia, a temperature probe was inserted into the cortex and held in place with superglue. Pups were placed in chambers (as before) with a continuous flow of 30% oxygen balanced with nitrogen. The water bath was maintained at a temperature that maintained the cortical temperature at 35°C, as measured with a telemetry system (VitalView, Mini-Mitter, OR, USA). (The pup with the cortical temperature probe was killed upon completion of hypothermia and was not used for analysis.) After treatment, the pups were returned to their dam for an interval of 1 or 5 h. Pups were subsequently placed into the chambers (as before), with the water bath maintained at 37°C, for 90 min. The gas mixture was maintained at 20% xenon, 30% oxygen, and 50% nitrogen via a purpose built closed system which minimized

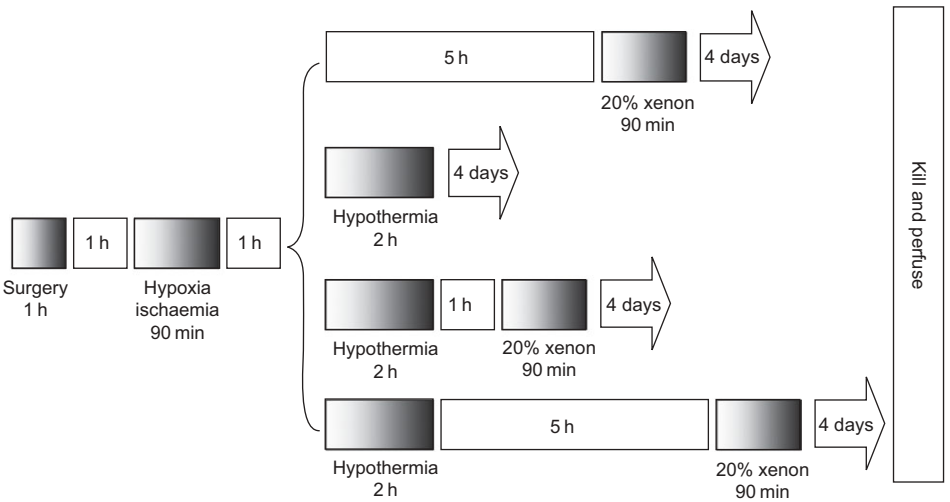

Fig 1 Experimental protocol for the different treatment strategies.