British Journal of Anaesthesia **113** (1): 168–76 (2014)
Advance Access publication 24 November 2013 · doi:10.1093/bja/aet352

BJA

Timing of xenon-induced delayed postconditioning to protect against spinal cord ischaemia–reperfusion injury in rats

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Editor's key points

- Neurological deficits due to spinal cord injury remain a significant devastating complication after aortic surgery.
- A rat model of temporary aortic occlusion was used to study the neuroprotective effects of xenon postconditioning.
- Xenon administered after ischaemia reduced spinal cord injury, with a peak effect when initiated 1 h after ischaemia.

Background. This study was designed to assess the neuroprotective effect of xenon-induced delayed postconditioning on spinal cord ischaemia–reperfusion injury (IRI) and to determine the time of administration for best neuroprotection in a rat model of spinal cord IRI.

Methods. Fifty male rats were randomly divided equally into a sham group, control group, and three xenon postconditioning groups ($n=10$ per group). The control group underwent spinal cord IRI and immediately inhaled 50% nitrogen/50% oxygen for 3 h at the initiation of reperfusion. The three xenon postconditioning groups underwent the same surgical procedure and immediately inhaled 50% xenon/50% oxygen for 3 h at the initiation of reperfusion or 1 and 2 h after reperfusion. The sham operation group underwent the same surgical procedure without aortic occlusion, and inhaled 50% nitrogen/50% oxygen. Neurological function was assessed using the Basso, Beattie, and Bresnahan score at 4, 24, and 48 h of reperfusion. Histological examination was performed using Nissl staining and immunohistochemistry, and apoptosis was detected by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labelling staining.

Results. Compared with the control group, the three xenon postconditioning groups showed improvements in neurological outcomes, and had more morphologically normal neurones at 48 h of reperfusion. Apoptotic cell death was reduced and the ratio of Bcl-2/Bax immunoreactivity increased in xenon-treated rats compared with controls.

Conclusions. Xenon postconditioning up to 2 h after reperfusion provided protection against spinal cord IRI in rats, but the greatest neuroprotection occurred with administration of xenon for 1 h at reperfusion.

Keywords: neuroprotectant; spinal cord, ischaemia–reperfusion injury; xenon, delayed postconditioning

Accepted for publication: 11 July 2013

Spinal cord injury after aortic surgery, such as for ascending aortic aneurysms or aortic dissection, remains a major cause of acute and delayed paraplegia.[1] Given the largely ineffective current therapeutic strategies, new therapeutic measures are needed to mitigate the severity of disability for patients and subsequent financial cost for society.

Ischaemic postconditioning was first described by Zhao and colleagues[2] in a dog model of myocardial ischaemia–reperfusion injury (IRI), and similar beneficial effects were recently demonstrated in other animal species, including humans.[3–6] Some drugs have been reported to induce postconditioning neuroprotective effects in rabbits and mice in the brain and spinal cord.[7–9] A new strategy, termed delayed ischaemic postconditioning, has shown improved outcomes in acute myocardial and brain infarction.[10 11] Similarly, pharmacological postconditioning after reperfusion has been shown to reduce spinal cord IRI in rat and rabbit models.[12 13]

Xenon is an inert gas with anaesthetic properties,[14] and has been shown to provide neuroprotection when applied 1 h after resuscitation in pigs or when administered up to 2 h after brain ischaemia in rats.[8 15] Moreover, xenon postconditioning at the initiation of reperfusion produced a neuroprotective effect on spinal cord IRI in rats.[16] However, there are no available data regarding the effect of xenon-induced delayed postconditioning on spinal cord IRI. This experiment was designed to assess the neuroprotective effect of xenon postconditioning on spinal cord IRI and to determine the optimal time of administration for protection in an *in vivo* rat model.

Methods

Animal procedures were approved by the animal care and use committee of the Capital Medical University. Eight-week-old male Sprague–Dawley rats (300–350 g) provided by the

animal centre of the Capital Medical University were used. Rats received humane care in compliance with the 'Guide for the care and use of laboratory animals' established by NIH Publication No. 85-23, revised 1996, and in compliance with ARRIVE guidelines.

Rats were neurologically intact before anaesthesia and instrumentation. Rats were anaesthetized by intraperitoneal injection of 3% (v/v) sodium pentobarbital, 30–50 mg kg^{-1}, with a supplement of 25% of the first dose when rats appeared restless until the end of the surgical procedure. Adequate depth of anaesthesia was ascertained by confirming absence of response to paw pinch. Rectal temperature was monitored and maintained between 36.5 and 37.5°C with an infrared heat lamp and a heating pad. The left carotid artery was cannulated with a 24 G catheter (B. Braun Medical Inc., Bethlehem, PA, USA) for measurement of mean proximal aortic pressure (MPAP), which was maintained at 90–110 mm Hg throughout the procedure by postural adjustment. A 24 G catheter was inserted into the tail artery for monitoring mean distal arterial pressure (MDAP). The carotid artery cannula was connected to a heated blood collection circuit at 37.5°C that was primed with heparinized normal saline (4 units ml^{-1}). MPAP, MDAP, and temperature were recorded by a Powerlab 8SP Polygraph (AD Instruments Pty, Australia). Spinal cord ischaemia was induced by inserting a 2 F Dogarty balloon catheter (Edwards Lifesciences, Irvine, CA, USA) via the left femoral artery retrograde into the descending thoracic aorta, 10 cm from the femoral arteriotomy so that the tip of the catheter balloon lay 3–4 mm caudal to the left subclavian artery. After instrumentation, rats received 200 units of heparin sodium through the carotid artery cannula. The catheter was inflated with 0.05 ml of distilled water, and aortic occlusion was confirmed by an immediate and sustained loss of detectable pulse pressure and a decrease in MDAP. At the end of the 25 min ischaemic period, the balloon was deflated, animals received 200 units of protamine sulphate, catheters were removed, spinal cord blood flow was restored, and surgical incisions were closed. Arterial blood samples were obtained from the tail artery 10 min before aortic clamping and 15 min after reperfusion. Arterial blood gases were measured by a blood gas and pH analyzer (Ciba-Corning Diagnostics, East Walpole, MA, USA).

Rats were randomized, using a random number table, into five groups (n=10 in each group) (Fig. 1). The sham group (Group S) underwent the surgical procedure but did not undergo aortic occlusion, and inhaled 50% nitrogen/50% oxygen immediately at the initiation of reperfusion for 3 h. Rats in the control group (Group C) underwent the same intervention as the sham group and experienced 25 min ischaemia with aortic occlusion. Rats in the three xenon postconditioning groups (P0, P1, and P2) underwent 25 min ischaemia with aortic occlusion and inhaled 50% xenon/50% oxygen (v/v) at the initiation of reperfusion or, 1 and 2 h after. Rats in Group P0 continued to inhale 50% nitrogen/50% oxygen for 2 h after xenon treatment, rats in Group P1 inhaled 50% nitrogen/50% oxygen before and after xenon treatment for 1 h, and rats in Group P2 continued to inhale 50% nitrogen/50% oxygen for 2 h before xenon treatment. Oxygen, nitrogen, and xenon of medical grade were purchased from Wuhan Gases Group (Wuhan, China). Gas mixtures containing 50% nitrogen/50% oxygen or 50% xenon/50% oxygen were obtained using calibrated flowmeters and gas analyzers. Rats were placed into a double-layer chamber design with CO_2 scavenging. A closed-loop gas recirculation system was used to conserve xenon, with a maintenance of CO_2 levels at $<$0.03% and xenon between 49.5% and 50.5%.

The trachea was extubated at 3 h after reperfusion and rats were allowed to recover from the anaesthetic. For analgesia,

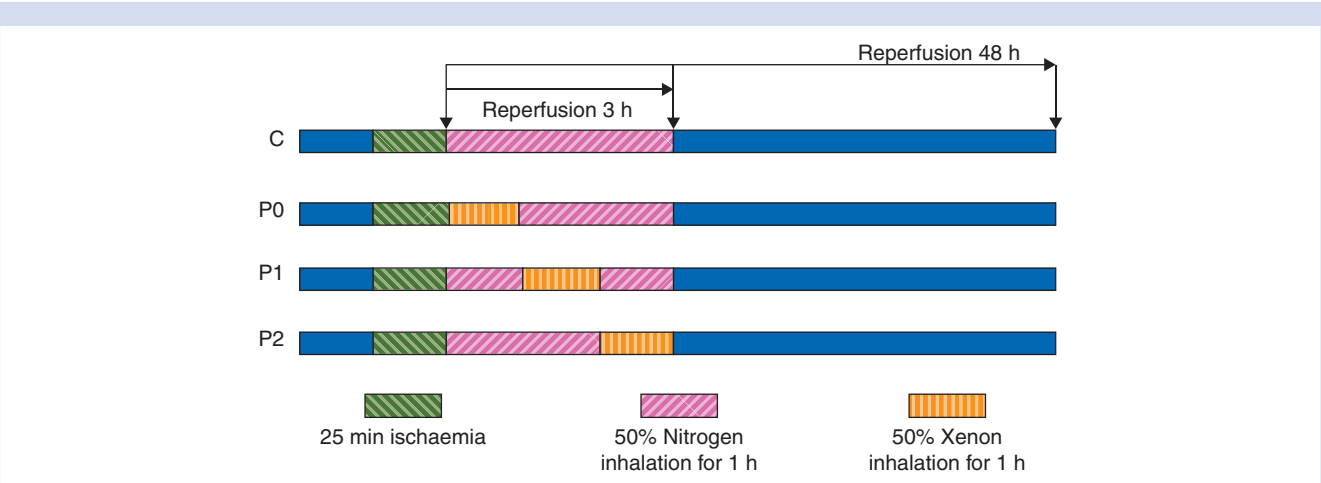

Fig 1 Experimental protocols. Sprague–Dawley rats underwent a surgical protocol of spinal cord ischaemia/reperfusion. The green box represents the period of ischaemia. Cellular degeneration and necrosis were performed at 48 h after reperfusion. The rats of Group C underwent a 25 min ischaemia followed by a 48 h reperfusion and immediately inhaled 50% (v/v) nitrogen at 60 min of reperfusion. The rats of Groups P0, P1, and P2 were subjected to a 25 min ischaemia followed by a 48 h reperfusion and inhaled 50% (v/v) xenon continuously for 1 h at different time durations (Δt) corresponding to Δt=0, 1, 2 h.