Argon augments Hypothermic Neuroprotection in a Perinatal Asphyxia Piglet Model: Evaluation by 31P and 1H MRS

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Introduction: Hypoxic-ischaemic neonatal encephalopathy (NE) accounts for approximately one million annual deaths world-wide. A similar number of survivors develop life-long neuro-disabilities. Therapeutic hypothermia[1] has become the standard of care in the developed world however, around 50% of treated infants still suffer from adverse outcomes. Current research focuses on treatments that can augment therapeutic hypothermia. Noble gases offer potent neuroprotection without toxicity and a phase IIA study is underway in babies of Xenon-augmented hypothermia[2]. Unfortunately, Xenon is expensive and necessitates specialist ventilators. Recent data indicates that another noble gas, Argon, provides similar neuroprotection to Xenon with no known toxicity[3]. Argon is 100-200 times cheaper than Xenon, does not require a recirculating ventilator and unlike Xenon does not induce anaesthesia/sedation. Using a piglet model of perinatal asphyxia, the aim of this study was to evaluate whether Argon after transient hypoxia-ischaemia (HI) is neuroprotective based on 31P MRS and 1H cerebral biomarkers.

Methods: Experiments were performed under UK Home Office guidelines. Eighteen healthy piglets (aged <48hrs) were anaesthetised and physiologically monitored with intensive life support. Transient cerebral HI was induced by inflating bilateral carotid artery occluders and reducing the inspired oxygen fraction to 6% as previously described[4]. At the end of transient HI subjects were randomised to 2 groups: i) **Control** (hypothermia alone, 2-26 hrs at 33.5°C) (n = 8); ii) **Argon** (hypothermia plus 50% argon, 2-26 hrs) (n=10). Argon was delivered through a standard neonatal ventilator. 31P MRS and 1H and were acquired at baseline, 24 hrs and 48 hrs after HI using a 9.4 Tesla Agilent spectrometer. 31P data was also acquired sequentially during HI and for 1 hr afterwards. Whole-brain 31P MRS data were acquired using a single-pulse acquisition (TR = 10 s, 60 averages; 6 averages during HI). 1H MRS data were acquired from voxels positioned in the deep grey matter centred on both lateral thalami (DGM voxel, 15x15x10mm), and dorsal right subcortical white matter (WM voxel, 8x8x15mm) as shown in figure 1, using a LASER pulse sequence (TR = 5000ms, TE =288ms, 128 averages). Spectra were analysed using the AMARES[5] algorithm implemented within the jMRUI software[6]. The metabolite ratio nucleotide triphosphate (NTP)/epp (epp = exchangeable phosphate pool = Inorganic phosphate + Phosphocreatine + 2γ-NTP + β-NTP) was calculated from 31P MRS data and the ratio Lactate (Lac) / N-acetyl aspartate (Naa) from 1H MRS data. Insult severity was estimated by calculating the time integral of the change in NTP/epp during HI and the first 60 min of resuscitation, as previously described[4]. MRS data were analysed using a non-linear mixed effects model. Grouped data are given as mean (standard deviation).

Results: There were no physiological differences between groups (Table 1). Table 2 shows the results from the mixed effects model. Predicted values for NTP/epp are significantly higher in the argon group than in the control group. Predicted WM Lac/Naa ratios are significantly lower at both 24hrs and 48 hrs post HI in the argon group compared to the control group. Figure 2 shows the predictions from the model for WM Lac/Naa and NTP/epp adjusted for time and treatment group. DGM Lac/Naa showed no significant differences between argon and control groups.

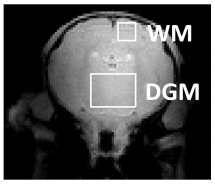


Figure 1: Voxel positions

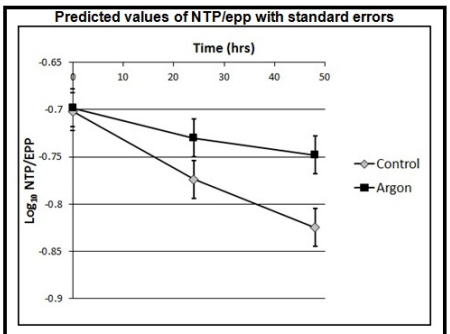
Mean (SD)	Control	Argon	p-value
Age (h)	27 (5.4)	27.6 (5.9)	0.92
Body weight (kg)	1978 (109)	1925 (89)	0.19
Duration of HI (min)	24.2 (2.8)	23.0 (1.9)	0.30
Insult severity (x 10^{-2} min)	10.0 (3.3)	8.8 (2.5)	0.77

Table 1: Physiology

Discussion: This study demonstrated a significant preservation of the whole brain NTP/Epp at 48 hrs post HI in the group exposed to 50% Argon plus therapeutic hypothermia versus therapeutic hypothermia alone. This neuroprotection appears to occur predominantly in the WM as significantly reduced WM Lac/NAA is observed at 24hrs and 48hrs post HI. Argon may provide a cheaper and more practical alternative neuroprotective therapy to Xenon as an augmentation to therapeutic hypothermia.

	Estimated difference in group mean WM Lac/Naa (Control- Argon) [95% confidence interval]	p-value	Estimated difference in group mean NTP/epp (Control- Argon) [95% confidence interval]	p-value
Baseline	0.017[-0.45 to 0.48]	0.94	-0.004 [-0.06 to 0.05]	0.88
24 hrs. post HI	0.514[0.04 to 0.99]	**0.03**	-0.043[-0.1 to 0.01]	0.12
48 hrs. post HI	0.490[0.02 to 0.96]	**0.04**	-0.077[-0.13 to -0.02]	**0.01**

Table 2: Predicted values from mixed effects model, differences between groups



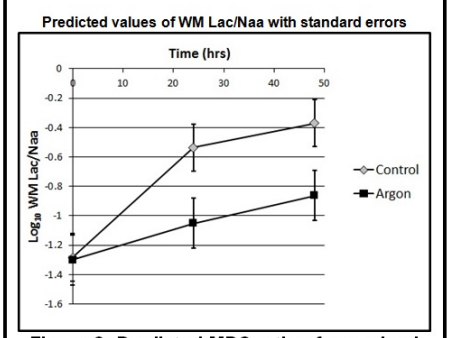


Figure 2: Predicted MRS ratios from mixed effect model (log scale)

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