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SHORT REPORT

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The effects of xenon on sevoflurane anesthesia-induced acidosis and brain cell apoptosis in immature rats

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1 | INTRODUCTION

Anesthesia-induced neurodegeneration (AIN) occurs in newborn large animal models where physiological homeostasis is maintained. However, most immature rodent models of AIN report mortality rates, and hypercarbia alone, mimicking that produced by anesthesia, increases neuro-apoptosis.[1] The potential influence of physiological derangement is one reason why rodent models of AIN are not ideal for predicting clinical effect. However, they remain necessary for the initial pre-clinical investigation of toxicity or treatment.

Xenon, a cardio-stable sedative with analgesic properties and shown to reduce isoflurane-AIN in an immature rodent model,[2] was recently administered to critically ill babies in UK research trials and was shown to reduce the requirement for sevoflurane during anesthesia in another European trial. Co-administration during anesthesia for babies and young children potentially offers improved hemodynamics and neuroprotection.

We aimed to compare the effect of co-administering Xenon with sevoflurane on neuroapoptosis and acid–base homeostasis in immature rats.

2 | METHOD

These experiments were carried out under Home Office License, with University ethical approval. Mixed sex, Wistar rats were randomly assigned on postnatal day 8 to 6-hour exposures to equipotent mixtures of sevoflurane ± xenon in 30% oxygen (equivalent to thrice the effective inhaled concentration of sevoflurane or xenon preventing cold-stimulated vocalization in 95% [EiC95 CSV][3]): 2.7% sevoflurane alone (Sevo), 1.8% sevoflurane in 35% xenon (SevoXe35) or 0.9% sevoflurane in 70% xenon (SevoXe70) (n = 9 per group). Two control groups were used (Naïve: anesthetized and culled on removal from the home cage, and Sham: expose to 30% oxygen alone [n = 6 per group]). Gasses were delivered using calibrated, low flow, rotameters and monitoring ensured that CO2 rebreathing was limited to 2%. Immediate decapitation allowed collection of mixed arteriovenous blood for analysis.

Brains were harvested, drop fixed, cryoprotected, and coronal blocks were frozen. 25 μm section were cut on a cryostat (Leica 3050), mounted on slides, and stained for cleaved caspase-3 (CC3) immunofluorescence. CC3-positive cells were counted by a researcher blinded to group allocation in four brain areas: Pre-frontal

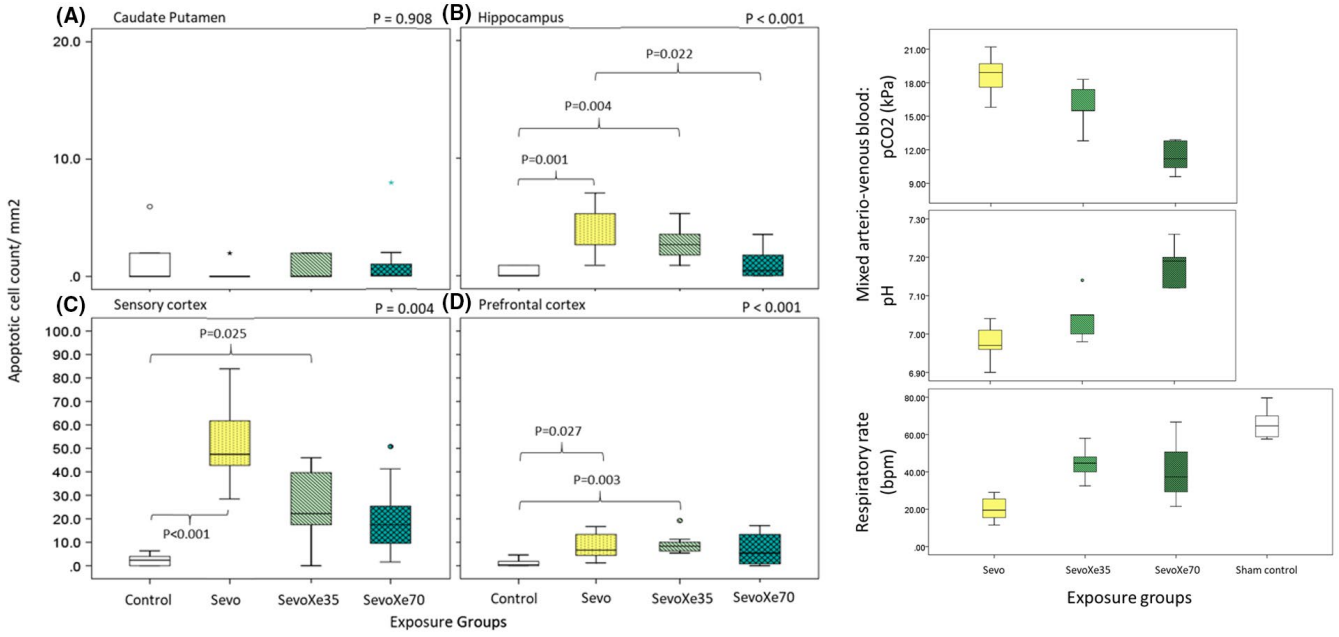

FIGURE 1 Median (IQR) apoptotic cell count in four brain areas according to treatment group (Panels, A-D): There were few apoptotic cells in any control animals (<8/mm^2)(A-D). Compared to controls, there were more apoptotic cells in the Hippocampus, Sensory Barrel Cortex and Pre-frontal Cortex in the Sevo and SevoXe35 groups, but not SevoXe70 (B-D). In the Hippocampus there were fewer apoptotic cells in SevoXe70 compared to Sevo group (B). In contrast, in the Caudate Putamen there were very few apoptotic cells seen and no difference between groups (A). Differences were tested using Kruskall-Wallis (*P* value above each panel) with Bonferroni correction for multiple comparisons (bracketed *P* values within panels). Median (IQR) Respiratory rate, PCO2 and pH of mixed arterio-venous blood taken immediately after 6-hour exposures (right sided panels): All animals exposed to sevoflurane alone or combined with xenon had lower respiratory rates compared to sham animals and were acidotic (pH < 7.35) [Colour figure can be viewed at wileyonlinelibrary.com]

cortex (comprising Sensory Cortex [S1], Motor cortex [M1] and Cingulate Gyrus [Cg]), Caudate Putamen [Cpu], Somatosensory 1 Barrel Field [S1BF] and hippocampus comprising CornuAmmonis 1 and 3 [CA1, CA3] and Dentate Gyrus [DG]).

Sample size was calculated using a conservative interpretation of data from Ma et al.[2] A two-way ANOVA was used to compare apoptotic cell count by brain area and control group (Naive or Sham). There was no effect of control group ($P = .645$), therefore, differences in apoptotic cell count between Control, Sevo, SevoXe35, and SevoXe70 were tested using Kruskal–Wallis with Bonferroni correction for multiple comparisons. The relationships between RR and PCO2, and pH and induced apoptosis were estimated using Spearman's Rank correlation coefficient (values between 0.4 and 0.59, 0.6 and 0.79 or 0.8 and 1.0 indicate moderate, strong or very strong correlation).

3 | RESULTS

No animals died during the six-hour exposures. The results are shown in the Figure 1. Sevoflurane induced severe acidosis and significant apoptosis in the Sensory Cortex, Pre-frontal Cortex, and Hippocampus. Co-administration of Xenon, reducing the exposure to sevoflurane, significantly lessened the acidosis in a dose-dependent manner and, at the higher Xenon concentration (70%), prevented induced apoptosis.

Lactate remained normal in our animals suggesting severe circulatory failure was not present, and the pH we report is in keeping with a recent study showing induced apoptosis in rats exposed to six hours of 2.5% sevoflurane (mean arterial blood pH: 7.04).[4]

The relationships between RR and PCO2, and pH and induced apoptosis were estimated using Spearman's Rank correlation coefficient. There was a very strong negative correlation between respiratory rate and pCO2 of mixed arterio-venous blood (−0.99, $P < .001$). There was less acidosis with increased xenon/decreased sevoflurane ($P < .001$). Lactate was not raised in any animal (<3 mmol/L), and there was no difference between the three groups ($P = .282$).

There was a moderate negative correlation between pH and apoptotic cell count in the sensory cortex (−0.50, $P = .028$), but not in the hippocampus or the pre-frontal cortex ($P = .092$ and $P = .342$, respectively).

4 | CONCLUSIONS

Our study shows that xenon-co-administration in neonatal rats, used to reduce sevoflurane exposure, lessened physiological disturbances and this was accompanied by reduced apoptosis in the brain. Further pre-clinical studies are needed where cardiorespiratory derangement is minimized and equal between groups.

CONFLICT OF INTEREST

HG and AEP have no conflicts of interest.

PRIOR PRESENTATION

This work was presented at The Anaesthetic Research Society/British Journal of Anaesthesia Research Forum, Royal College of Anaesthetists, London, May 2019. The abstract was published in the BJA.

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