BJA

British Journal of Anaesthesia, 0 (0): 1–14 (2017)

doi: 10.1093/bja/aex219
Laboratory Investigation

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Alternative technique or mitigating strategy for sevoflurane-induced neurodegeneration: a randomized controlled dose-escalation study of dexmedetomidine in neonatal rats

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Abstract

Background. Brain injury in newborn animals from prolonged anaesthetic exposure has raised concerns for millions of children undergoing anaesthesia every yr. Alternative anaesthetic techniques or mitigating strategies are urgently needed to ameliorate potentially harmful effects. We tested dexmedetomidine, both as a single agent alternative technique and as a mitigating adjuvant for sevoflurane anaesthesia.

Methods. Neonatal rats were randomized to three injections of dexmedetomidine (5, 25, 50, or 100 μg kg^{-1} every 2 h), or 6 h of 2.5% sevoflurane as a single agent without or with dexmedetomidine (1, 5, 10, or 20 μg kg^{-1} every 2 h). Heart rate, oxygen saturation, level of consciousness, and response to pain were assessed. Cell death was quantified in several brain regions.

Results. Dexmedetomidine provided lower levels of sedation and pain control than sevoflurane. Exposure to either sevoflurane or dexmedetomidine alone did not cause mortality, but the combination of 2.5% sevoflurane and dexmedetomidine in doses exceeding 1 μg kg^{-1} did. Sevoflurane increased apoptosis in all brain regions; supplementation with dexmedetomidine exacerbated neuronal injury, potentially as a result of ventilatory or haemodynamic compromise. Dexmedetomidine by itself increased apoptosis only in CA2/3 and the ventral posterior nucleus, but not in prefrontal cortex, retrosplenial cortex, somatosensory cortex, subiculum, lateral dorsal thalamic nucleaus, or hippocampal CA1.

Conclusions. We confirm previous findings of sevoflurane-induced neuronal injury. Dexmedetomidine, even in the highest dose, did not cause similar injury, but provided lesser degrees of anaesthesia and pain control. No mitigation of sevoflurane-induced injury was observed with dexmedetomidine supplementation, suggesting that future studies should focus on anaesthetic-sparing effects of dexmedetomidine, rather than injury-preventing effects.

Editorial decision: May 31, 2017; **Accepted:** June 17, 2017

Key words: anaesthetics, inhalation, sevoflurane; apoptosis; brain injury; dexmedetomidine; neuroprotection, safety, toxicity

Editor's key points

- Sevoflurane and other volatile anaesthetics cause widespread neuronal death in young animals of multiple species.
- Treatment of neonatal rats with sevoflurane alone increased neuroapoptosis, while dexmedetomidine alone produced restricted neuroapoptosis at high doses.
- However, dexmedetomidine dose-dependently enhanced sevoflurane-induced neuroapoptosis, and increased mortality.
- Dexmedetomidine enhanced anaesthetic neurotoxicity, perhaps as a result of physiological derangement or enhanced anaesthetic depth.

Before human application, new medical products, devices, and drugs are typically rigorously tested in animal models to identify potential toxicity and safety concerns. As preclinical and even clinical testing specifically for paediatrics is often lacking, much of paediatric medicine relies on off-label use of conventional therapies that have been previously approved for adult applications. Thus, the growing number of animal studies over the past two decades, demonstrating brain structural and cognitive abnormalities in a wide variety of species after prolonged anaesthetic exposure early in life, have raised substantial concerns regarding the safe use of these drugs during paediatric anaesthesia, particularly in infants and young children undergoing life-saving surgeries (recently reviewed in[1]). However, the immediate clinical translatability of these laboratory findings remains undetermined.[2 3] All commonly used general anaesthetics, including sevoflurane, the most frequently used anaesthetic in paediatric practice, have been found to cause widespread apoptotic neuronal death and to alter dendritic architecture, and deficits in long-term cognitive and behavioural function in animals, including small rodents and non-human primates, after exposures of up to six h early in life.[4–10] Structural brain injury in these animal studies was found after prolonged exposures to sevoflurane in doses of 2.5% to 4%. Clinical investigations, on the other hand, have been equivocal. The only clinical trial specifically focusing on sevoflurane has not demonstrated any detrimental effects on interim cognitive outcomes two yr after a one-h exposure to sevoflurane for inguinal hernia repair in infancy.[11] While some epidemiological studies examining early life exposures to a variety of anaesthetic drugs, including sevoflurane, have associated anaesthetic exposures and subsequent abnormalities in learning, cognition, or brain structure,[12 13] others have not found demonstrable cognitive impairment.[14]

Despite a lack of strong confirmatory findings in human studies, additional research into better understanding developmental anaesthetic neurotoxicity are urgently needed, because of several concerns. Firstly, the developing brain is exquisitely sensitive to deleterious effects during prolonged or repeated exposures, yet anaesthetics and sedatives are used in millions of young children for surgeries that cannot be postponed until a potentially less vulnerable older age. Secondly, no general anaesthetic has been identified in animal studies to be unequivocally non-injurious, leaving paediatric clinicians no alternative agents. Lastly, no biological tenet exists that would exempt humans from the structural and cognitive abnormalities observed after prolonged anaesthetic exposures in all animal species that have been tested, including non-human primates. Accordingly, in 2008 the US Food and Drug Administration (FDA) and the International Anesthesia Research Society (IARS) formed SmartTots, a public-private partnership to coordinate and fund research into this growing paediatric health concern. Moreover, in December 2016 the FDA published a warning that repeated or lengthy exposures to general anaesthetics or sedative drugs in children $<$ three yr of age might adversely affect their brain development, underscoring the need for additional studies. Given the strong evidence and serious potential ramifications of paediatric exposures to anaesthetics or sedatives for patients, care providers, and society, it seems prudent to not only attempt to identify safer, alternative anaesthetic techniques in animals, but also to develop mitigating strategies or adjuvants to ameliorate potentially harmful anaesthetic effects. If experiments in model species found strong evidence for safe alternative strategies, human trials could start promptly. Among several potential pharmacological targets, dexmedetomidine is a promising candidate, because it provides sedation via α_2-receptor agonism and therefore avoids NMDA-receptor antagonism or $GABA_A$-receptor potentiation characteristic of drugs implicated in deleterious anaesthetic effects. Moreover, dexmedetomidine has been found to protect the brain during hypoxic-ischaemic insults and to alleviate some of the toxic effects observed during isoflurane exposure.[15–19]

Several important uncertainties remain regarding dexmedetomidine's widespread use, including its limited effectiveness as a sedative when utilized in isolation. Furthermore, its interactions with sevoflurane, the most frequently used inhaled anaesthetic in paediatric anaesthesia, have not been fully elucidated. We therefore tested the hypothesis that dexmedetomidine as the sole sedative agent during a six-h exposure in neonatal rodents, even in high doses, would not affect structural integrity in several brain regions previously identified to be vulnerable to anaesthesia-induced abnormalities, while providing adequate anaesthetic depth and stable vital signs. We further expected dexmedetomidine co-administration to protect from the deleterious effects of 2.5% sevoflurane. This dose was selected as a clinically applicable dose, that has previously been shown to induce injury in order to test dexmedetomidine's protective effects as a mitigating adjuvant, independent of its sevoflurane-sparing properties.

In order to address recently raised concerns regarding proper reporting of animal studies, difficulties in replicating results, and their questionable translational value for clinical practice, this study followed the Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines.[20] We also took the unique approach to concurrently plan and conduct two parallel studies with the same goals in two separate animal laboratories, utilizing the respective methodologies available to each research