

Selective induction of IL-1 β after a brief isoflurane anesthetic in children undergoing MRI examination

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Abstract

Purpose To determine if isoflurane anesthesia without surgery causes systemic inflammation in children. Inflammation is targeted as responsible for the development of many neurologic pathologies. The effect will be evaluated by measuring serum cytokine levels before and after isoflurane anesthesia. The possible neurotoxic effect of anesthetic agents is a concern in pediatric anesthesia. Questions remain as to the true effects of anesthesia alone on systemic inflammation. The current study assesses systemic inflammatory response to general anesthesia in children not exposed to surgical stress.

Methods Twenty-five patients, aged 6 months to 11 years undergoing MRI scanning were recruited. Patients with ASA Physical Status Classification >II, known neurologic disease, prematurity, recent infection, or current treatment with anti-inflammatory medications were excluded. Each patient received a sevoflurane induction, peripheral intravenous catheterization, and laryngeal mask airway placement. Isoflurane was titrated to ensure adequate depth of anesthesia. Two peripheral blood samples were obtained: one immediately after placement of the PIV and one upon

arrival to the post-anesthesia care unit. Serum cytokine levels were compared between pre- and post-isoflurane time points using paired *t* tests.

Results For all patients, interleukin-1 β increased after isoflurane when compared to pre-isoflurane samples (pre = 25.97 ± 9.01 , post = 38.53 ± 16.56 , $p = 0.0002$). Serum levels of IL-6 (pre = 2.28 ± 2.27 , post = 2.04 ± 2.15 , $p = 0.146$) and tumor necrosis factor- α (pre = 94.26 ± 18.07 , post = 85.84 ± 12.12 , $p = 0.057$) were not significantly changed. Interleukin-10 and vascular endothelial growth factor were undetectable in pre- and post-isoflurane samples at a minimum detection threshold of 6.6 and 10 pg/ml, respectively.

Conclusions A brief (approximately 60 min) exposure to isoflurane general anesthesia, without induced surgical stress, significantly increased serum IL-1 β , a selective activation marker of systemic inflammation (IL-1 β pathway).

Keywords Pediatric anesthesia · Anesthesia-induced developmental neurotoxicity (AIDN) · Neuroinflammation · Isoflurane

Introduction

Anesthesia-induced developmental neurotoxicity (AIDN) continues to generate much attention in the pediatric anesthesia scientific community. Recent studies in humans are reassuring, suggesting a lack of measurable clinical effect in children under specific clinical circumstances, particularly during brief general anesthesia with sevoflurane [1, 2]. These were prospective studies that were designed to specifically assess neurocognitive outcomes after anesthesia, in contrast to previous, retrospective studies that were not controlled or randomized. Yet, these findings cannot rule

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out that the pathologic process underlying AIDN occurs in children, particularly those especially vulnerable to this effect. Of course, the aforementioned studies were performed in the setting of a 60-min, standardized general anesthetic, which could be considered to be at odds with retrospective studies, which were extremely heterogeneous with regard to anesthetic regimen.

Numerous animal studies [3], including our own study in a preclinical piglet model [4], have demonstrated marked cellular and/or behavioral changes after anesthesia exposure. Despite this, the true molecular and behavioral phenotype of AIDN remains to be identified. It remains to be seen if apoptosis contributes significantly to the development of neurocognitive deficits after anesthesia exposure. A “cause-and-effect” relationship between apoptosis and subsequent derangements in neurocognitive development is difficult to infer from available data. Therefore, it is imperative that other potential mechanisms involved in AIDN be elucidated. While the majority of animal studies have used apoptosis as a neurotoxic endpoint, other studies have investigated alternate mechanisms of AIDN, including defects in neurogenesis, synaptogenesis [5], growth factor signaling systems, calcium dysregulation, receptor activation, gene expression, and miRNA expressions [6]. Finally, while relatively understudied, systemic inflammation is considered a potential mechanism of other neurodegenerative conditions, making it a relevant target for the current study [5, 7, 8].

Inflammation has been linked to a myriad of neurodevelopmental and neurocognitive disorders, including neuropsychiatric disorders [9, 10] and autism [11]. Neuroinflammation influences the development of postoperative cognitive dysfunction (POCD), which is seen primarily in elderly patients after anesthesia and surgery [12, 13]. Surgery and trauma are known to induce a systemic inflammatory response. This response is comprised of immunologic, hormonal, and metabolic elements. It is reasonable to surmise that systemic inflammation may play a role in the development of AIDN. Profound inflammatory states are seen after surgery in both adults and children [14, 15]. One criticism of research investigating AIDN is that the potential effect of surgery, and its inflammatory response, has been largely ignored. Thus, it is important to evaluate the effect of anesthesia with and without surgical stress on the inflammatory response.

Anesthetic exposure with and without surgery has been reported to be immunosuppressant in adult models [16]. Very little work has explored the inflammatory effects of anesthesia, particularly in children. Anesthetic agents induce neuroinflammation in juvenile animal models in the absence of surgical stress [17]. However, the effects of anesthesia alone on systemic inflammation in children have not been examined. We hypothesize that

anesthesia exposure alone triggers a systemic inflammatory response that can be identified by release of inflammatory biomarkers into the serum of children undergoing MRI examinations.

Methods

Study design and subjects

The study was approved by the Nationwide Children’s Hospital Institutional Review Board. This was a prospective, single-blinded study in 25 children undergoing elective MRI scanning under general anesthesia. Written informed consent was obtained from each subject’s parents or legal guardian prior to enrollment. The patients were aged between 6 months and 11 years and had an American Society of Anesthesiologists (ASA) Physical Status Classification of I or II. Children with a history of known central nervous system disease, fever or infection in the 2 weeks prior to the scan, a history of prematurity, and those who had undergone treatment with any drug known to suppress or modulate inflammation in the 2 weeks prior to the scan were not enrolled in the study.

Standardized anesthetic regimen

After written informed consent was obtained, patients underwent an inhalational induction with 8% sevoflurane in 100% oxygen. After ensuring an adequate plane of anesthesia, an intravenous catheter was placed, and the first blood sample was drawn (called “pre-isoflurane” sample). Each sample was collected in a serum blood collection tube (Becton-Dickson, Franklin Lakes, NJ, USA). Thereafter, an appropriately sized laryngeal mask airway (LMA) was placed. Isoflurane in 40% O₂ and air was used to maintain anesthesia. Isoflurane was titrated to ensure an appropriate depth of anesthesia, delivering up to 2% inspired end-tidal concentration. We selected isoflurane because while it is not the most common inhaled anesthetic used in the United States, it is the most commonly used anesthetic worldwide, making our results more broadly applicable. Each patient received 20 ml/kg of Ringer’s lactate without other medications. At the conclusion of the procedure, each patient was taken to the post-anesthesia care unit (PACU) with the LMA in place. An additional blood sample was drawn at this time before the LMA was removed (“post-isoflurane” sample).

Sample analysis

Samples were immediately transported to the laboratory and were centrifuged at 4 °C to obtain the serum. They

were frozen at $-80\text{ }^{\circ}\text{C}$ for later analysis. Pro-inflammatory cytokines and Interleukins (IL) were subsequently measured. Samples were analyzed for tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), interleukin-1 β (IL-1 β), vascular endothelial growth factor (VEGF), and interleukin-10 (IL-10) using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Abcam, Cambridge, UK).

Data analysis and statistics

Sample size was calculated based upon known values for serum cytokines in children. We determined that in order to detect a 50% difference in serum cytokines at 80% power and a type I error of 0.05, 25 patients would be needed. Since this sort of study has not been done before, the benchmark of 50% difference was selected based on levels of known biologic relevance in prior studies [18]. All parametric data are presented as mean \pm standard deviation. Cytokine data were analyzed using GraphPad Prism Statistical Analysis software (La Jolla, CA, USA). Paired Student’s *t* tests were used to compare the pre- and post-isoflurane samples for each cytokine. A $p < 0.05$ was considered statistically significant.

Results

All patients completed the study successfully, and there were no complications at any time during the study period. Demographic and anesthesia characteristics are given in Table 1. The mean anesthetic time was 54.7 ± 24.4 min. Results from ELISA analysis are summarized in Table 2. A statistically significant increase in IL-1 β was found post-isoflurane ($p < 0.0002$) (Fig. 1). Of note, VEGF and IL-10 were not detectable at the lower limit of detection for the test, established at 6.6 and 10 pg/ml, respectively. There

Table 1 Summary of demographic information

	Weight (kg, mean \pm SD)	17.4 \pm 8.30
	Age (years, mean \pm SD)	3.73 \pm 2.49
	Anesthetic time (min, mean \pm SD)	54.7 \pm 24.7
Sex	Male (%)	15/25 (60)
	Female (%)	10/25 (40)
Race	African American (%)	1/25 (4)
	Asian (%)	3/25 (12)
	Caucasian (%)	21/25 (84)
Previous anesthesia	Yes (%)	12/25 (48)
	No (%)	13/25 (52)
ASA classification	I	8/25 (32)
	II	17/25 (68)

Table 2 Cytokine concentration pre- and post-isoflurane anesthesia in children

	Pre-isoflurane	Post-isoflurane	<i>p</i> value
IL-1 β (pg/ml)	25.97 \pm 9.01	38.53 \pm 16.56	0.0002
TNF- α (pg/ml)	94.26 \pm 18.07	85.84 \pm 12.12	0.057
IL-6 (pg/ml)	2.28 \pm 2.27	2.04 \pm 2.15	0.146
IL-10 (pg/ml)	ND*	ND*	N/A
VEGF (pg/ml)	ND*	ND*	N/A

* ND not detected, N/A not applicable

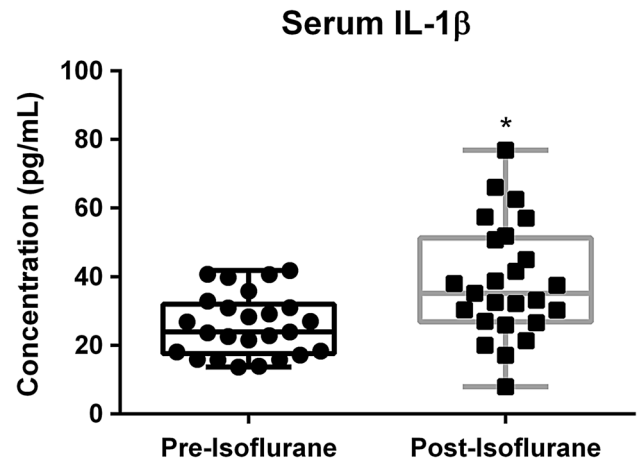


Fig. 1 Serum IL-1 β pre- and post-isoflurane anesthesia in children. The asterisk represents a statistically significant difference between the post- and pre-isoflurane measurement ($p < 0.05$)

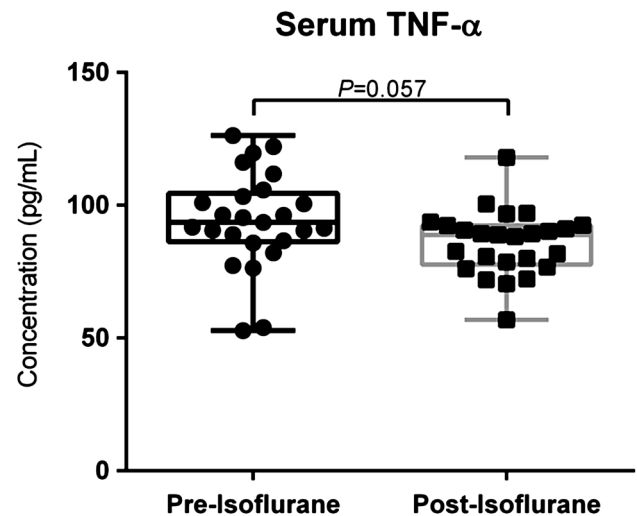


Fig. 2 Serum TNF- α pre- and post-isoflurane in children

was no significant increase in TNF- α or IL-6 when post-isoflurane measurements were compared to pre-isoflurane values (Figs. 2, 3).

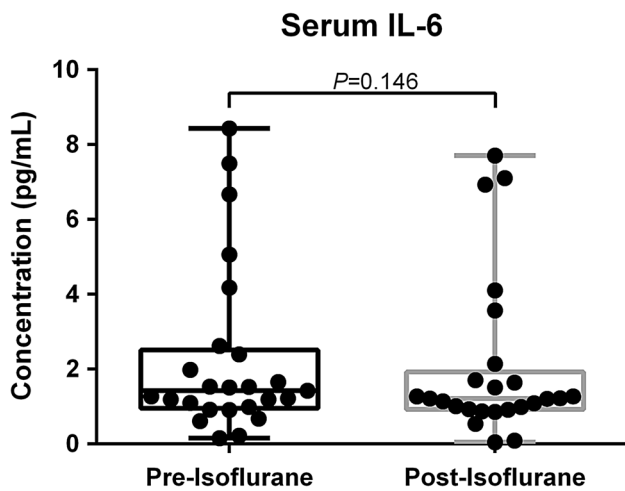


Fig. 3 Serum IL-6 pre- and post-isoflurane in children

Discussion

Changes in circulating cytokine levels were examined before and after brief exposure to isoflurane anesthesia in children undergoing MRI without any surgical stress or procedure. Four proinflammatory cytokines (IL-1 β , TNF- α , IL-6, and VEGF) previously reported to be elevated in the cerebrospinal fluid and blood after anesthesia and/or surgery or in children affected with neuroinflammatory disorders were studied [19–21]. Since anesthetic agents are generally assumed to attenuate the inflammatory response, the IL-10 anti-inflammatory cytokine was also measured. The increase in IL-1 β found in the present study is suggestive of a selective, yet significant activation of systemic inflammation after isoflurane anesthesia alone.

In both animal and human studies, systemic inflammation has been demonstrated to cause measurable neuroinflammatory effects and lasting neurodegenerative changes in both adults and juveniles [22–28]. Systemic inflammation and the resultant neuroinflammation have been linked to worsened neurocognitive outcomes in children in certain populations [29]. Furthermore, IL-1 β has been specifically implicated in the development of a number of neurodegenerative disorders, including Parkinson's disease [30], ALS [31], and seizure disorders [32]. Therefore, surgery-induced or anesthesia-induced systemic inflammation is likely to be reflected in the central nervous system. Unfortunately, it is difficult, if not impossible, to obtain biological samples (brain tissue, CSF) from children that can be analyzed for signs of neuroinflammation. Hence, peripheral blood was used to assess systemic inflammation as a surrogate for neuroinflammation, as inflammatory changes seen in the blood are likely to be present in the central nervous system.

Interestingly, neither pre- nor post-isoflurane blood samples yielded detectable levels of VEGF or IL-10 in the present study. VEGF, as its name implies, is primarily involved in the genesis of new blood vessels. It is a hallmark of inflammation when secreted by cells in response to injury (trauma, presence of immune mediators, hypoxia). In turn, it can induce or exacerbate neuroinflammation by activating microglia [33] and increasing blood–brain barrier (BBB) permeability. In contrast, IL-10 is a known inhibitor of inflammatory cytokines. Our data suggest that if isoflurane does have anti-inflammatory effects, they are not mediated by an increased IL-10 expression. Though the increase in TNF- α approached significance, because of the relatively small sample size, this result is difficult to interpret.

We report a greater than 1.5-fold increase in serum IL-1 β in children after approximately 1-h of isoflurane anesthesia. IL-1 β is a very potent pro-inflammatory cytokine protein produced primarily by macrophages as a pro-cytokine that is proteolytically cleaved by caspase-1 to the active protein. IL-1 β has a key role in the normal inflammatory response to illness or injury, and is known to be involved in cell differentiation, proliferation, and induction of apoptosis. In the brain, IL-1 β possesses diverse functions. In the normal brain, IL-1 β is expressed at low levels, but can be up-regulated quickly in response to CNS or peripheral insults [34]. Interleukin receptors are found throughout the mammalian brain, but are most predominant in neuron-rich areas. They are found with particular density on neuronal cell bodies in the granule cell layer of the dentate gyrus, the pyramidal cell layers of the hippocampus, and the granule cell layer of the cerebellum. Localization of IL-1 β receptors in the developing brain is also relevant. For instance, the dentate gyrus is a late-developing structure, making it a particularly interesting target for AIDN studies.

Binding of IL-1 β to IL receptors can elicit responses even in cells with low numbers of receptors because activation of these receptors leads to a complex signal transduction that results in signal amplification. In addition, the same process that leads to an increase in systemic IL-1 β is likely to also occur in the CNS. IL-1 β has been shown to cross the blood–brain barrier via saturable transport systems [35], so even a small up-regulation in IL-1 β levels could potentially be clinically significant.

Though the IL-1 β signal transduction pathway is extremely complex, there are a number of effects that have the potential to contribute to the development of AIDN. Firstly, and most importantly, IL-1 β has been implicated in the induction of apoptosis, likely via the p38 mitogen-activated protein kinase pathway [36]. It is possible that IL-1 β may contribute to the production of the apoptotic

phenotype seen in animal models of AIDN. Secondly, IL-1 β is implicated in microglial activation. Microglia are known to secrete IL-1 β when activated. This represents a possible “vicious cycle” in which exceptionally high levels of IL-1 β are present, leading to worsened neurodegeneration. Further, anesthesia itself has been postulated to cause microglial activation, which could further exacerbate this process [37].

The present study is not without limitations. The short anesthetic exposure may not have allowed sufficient time for up-regulation of all cytokines studied. However, this observation may also imply that the reported increase in IL-1 β could have been significantly more important with a longer anesthesia exposure. Also, one may speculate that an up-regulated kinetic curve may have been seen if blood samples had been obtained later after exposure. The patients in this study presented for outpatient MRI scanning, which prevented the establishment of the inflammatory response curve since collections of blood samples at later time points were not ethically possible. The significant increase in IL-1 β recorded after this short period of time may suggest a more significant anesthesia-induced inflammatory response.

This study reveals a selective but significant induction of inflammation in children after approximately 1 h of isoflurane anesthesia without surgical stress. Based on our exclusion criteria, it seems less likely that the effect observed is associated with factors other than the isoflurane anesthesia. Therefore, significant up-regulation of IL-1 β raises the concern that anesthesia alone might contribute directly to AIDN. Given mounting experimental evidence supporting a complex phenotype for AIDN, the role of IL-1 β signaling merits additional investigation.

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Compliance with ethical standards

Ethics approval This study was approved by the Nationwide Children’s Hospital IRB on 1/20/2015 (#IRB14-00625, NCT02512809).

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Conflict of interest The authors have no conflicts of interest to report.

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