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Lipidomics reveals a systemic energy deficient state that precedes neurotoxicity in neonatal monkeys after sevoflurane exposure

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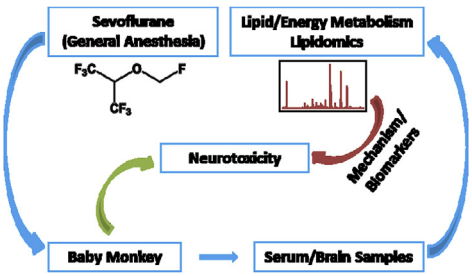
GRAPHICAL ABSTRACT



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ABSTRACT

Although numerous studies have raised public concerns regarding the safety of anesthetics including sevoflurane in children, the biochemical mechanisms leading to anesthetics-induced neurotoxicity remain elusive. Moreover, potential biomarker(s) for early detection of general anesthetics-induced brain injury are urgent for public health. We employed an enabling technology of shotgun lipidomics and analyzed nearly 20 classes and subclasses of lipids present in the blood serum of postnatal day (PND) 5 or 6 rhesus monkeys temporally collected after exposure to sevoflurane at a clinically relevant concentration or room-air as control. Lipidomics analysis revealed numerous significant anesthetic-induced changes of serum lipids and their metabolites as well as short chain acylcarnitines in the brain and cerebrospinal fluid after anesthetic exposure. These include decreased carnitine and acylcarnitines, unchanged triacylglycerol mass but accumulation of 16:0 and 18:1 fatty acyl chains in the triacylglycerol pool, losses of polyunsaturated fatty acids in both non-esterified fatty acid and phospholipid pools, and increased 4-hydroxynonenal content as early as 2 h after sevoflurane exposure. Importantly, the amounts of short chain acylcarnitines in the brain and cerebrospinal fluid were also significantly reduced after anesthetic exposure. We propose that this serum lipidomic profile can serve as indicative of neuronal damage. Our results reveal that sevoflurane exposure induces an energy deficient state in the brain

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evidenced by reduced free and acyl carnitine contents, as well as the presence of a pro-inflammatory state in the exposed animals, providing deep insights into the underlying mechanisms responsible for anesthetic-induced neurotoxicity.

1. Introduction

Numerous studies using both *in vitro* and *in vivo* preclinical models including nonhuman primate models have unraveled that prolonged and/or high-dose treatment with general anesthetics leads to neuronal injury in the developing brain [1–9]. Although these studies have raised public concerns regarding the safety of anesthetics in children, the biochemical mechanisms leading to the neurotoxicity remain elusive. Moreover, potential biomarker(s) for early detection of general anesthetics-induced brain injury are urgent for public health.

Sevoflurane, a volatile anesthetic of the ether group, is a commonly used anesthetic because of its various advantages over other intravenous or inhalation anesthetics. These advantages include (1) less discomfort, (2) less irritation to airways and a more pleasant smell, (3) lower blood-gas partition coefficient facilitating rapid induction and recovery, (4) more hemodynamic stability than other volatile anesthetics such as isoflurane or halothane [10], and (5) possibly less side effects on the developing brains [11]. However, large amounts of pre-clinical evidence indicate that sevoflurane can cause neuronal apoptosis and behavioral dysfunction [12–16]. In a recent study, we have applied a variety of techniques including genomics, lipidomics, and protein and histological assays for characterizing the adverse effects of sevoflurane exposure on brain structure and function [6]. We have demonstrated that a 9-h exposure of sevoflurane at a clinically-relevant concentration resulted in profound changes in brain gene expression, cytokine levels, lipid metabolism, and subsequently, neuronal damage [6].

Currently, there has been limited research evaluating whether and how anesthetic agents affect cellular lipids, the most abundant components of the brain other than water. Given that blood samples can be collected at any time point during the anesthesia, specific lipid changes in serum could be used as potential biomarkers to indicate if anesthetics-induced neuronal damage is present or will occur. The observations in our recent study led us to hypothesize that the alterations in brain lipid metabolism induced by sevoflurane exposure might also occur in the periphery and lead to changes in serum lipid content and composition. If changes in peripheral lipid profiles precede the neuronal damage induced by anesthetics then they could serve as early markers of brain injury. Moreover, altered peripheral lipid metabolism may provide insight into the biochemical mechanisms leading to sevoflurane-induced neurotoxicity. To this end, lipidomics should facilitate this kind of studies.

In the current lipidomics practice, there is an accurate and sensitive platform, termed multi-dimensional mass spectrometry-based shotgun lipidomics (MDMS-SL) [17–19], which can be applied for studying lipid metabolism comprehensively [20,21]. This platform allows scientists to quantitatively analyze nearly 50 lipid classes, hundreds to thousands of lipid species, and over 95% of lipid mass content from a small amount of biological resource materials. This is critical for uncovering the mechanisms responsible for alterations in lipid metabolism in biology systems since comprehensive analysis of lipid classes and individual lipid molecular species could provide complete mapping of all involved pathways [22]. As an example, MDMS-SL has recently been used for analysis of serum lipidomes from patients with systemic lupus erythematosus and oxidative stress as the molecular mechanism responsible for the reduced levels of plasmalogen species has been identified from the comprehensive study [23]. In another recent study, diverging lipogenesis pathways as demonstrated with the presence of different profiles of fatty acyl chains in *ob/ob* mouse liver and skeletal muscle have been revealed from the lipidomics analysis of these organs by MDMS-SL [24].

Herein, we conducted lipidomics analyses of nearly 20 classes and subclasses of lipids present in sera temporally collected from eight postnatal day (PND) 5 or 6 rhesus monkeys with and without exposure to sevoflurane using MDMS-SL. The lipids analyzed included all subclasses of choline glycerophospholipid (PC), lysoPC (LPC), all subclasses of ethanolamine glycerophospholipid (PE), lysoPE (LPE), phosphatidylinositol (PI), lysoPI (LPI), phosphatidylserine (PS), lysoPS (LPS), sphingomyelin, ceramide, carnitine/acylcarnitine, lysophosphatidylglycerol (LPG), triacylglycerol (TAG), non-esterified fatty acid (NEFA), and 4-hydroxyalkenol. Lipidomics analysis of serum samples revealed remarkable changes of numerous lipid classes and molecular species at the earliest time points when serum samples were collected. An energy deficient and pro-inflammatory state in the exposed animals was uncovered from the analysis. Such a state could sufficiently lead to brain injury. Collectively, assessing lipid profiles and better-understanding lipid metabolism may not only assist in the early detection of the neurotoxic effects associated with general anesthesia, but also provide deep insights into the underlying mechanisms [21] responsible for sevoflurane-induced neurotoxicity [6,7].

2. Materials and methods

2.1. Materials

All internal standards including phospholipids, lysophospholipids, N-lauroryl sphingomyelin, N-heptadecanoyl ceramide, and triheptadecenoyl glycerol (T17:1 TAG) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). D_3-4-hydroxynonenal (d_3-HNE) was purchased from Cayman Chemical Co. (Ann Arbor, MI), and 1,2,3,4-$^{13}C_4$-palmitoyl-L-carnitine hydrochloride ($^{13}C_4$-16:0 CN) and d_3-acetyl-L-carnitine hydrochloride (d_3-2:0 CN) were purchased from Sigma-Aldrich (St. Louis, MO). 7,7,8,8-d_4-Palmitic acid used as an internal standard for quantification of non-esterified FAs was obtained from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA). Sevoflurane and glycopyrrolate were purchased from Tec 7 (Baxter, Dallas, TX) and American Reagent (Shirley, NY), respectively. All solvents were obtained from Burdick and Jackson (Honeywell International Inc., Muskegon, MI). All other reagents were at least analytical grade and purchased from Fisher Scientific (Pittsburgh, PA), Sigma-Aldrich (St. Louis, MO), or as specified.

2.2. Animals

This is a follow-up study on sevoflurane-induced neurotoxicity with the same set of infant nonhuman primates as previously described [6]. Briefly, eight rhesus monkeys (PND 5 or 6) were used. All monkeys were born and housed in the nonhuman primate

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