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Biomarkers, Genetics and Epigenetic Studies to Explore the Neurocognitive Effects of Anesthesia in Children

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Abstract

Exposure to commonly used anesthetic agents causes widespread neuronal degeneration in the developing mammalian brain and has been shown to impair neurodevelopment in a variety of newborn vertebrate animal species. Although retrospective studies have suggested an association between anesthesia exposure in childhood and subsequent neurodevelopmental abnormalities, a causal relationship in humans has yet to be demonstrated. Unfortunately, translation of findings from bench to bedside is limited by several factors and histologic assessment in healthy children following exposure to anesthesia is not possible. Therefore, in order to prove that anesthesia-induced neurotoxicity occurs in humans, alternative approaches are necessary. Here we present the summary of a focus group discussion regarding the utility of biomarkers in translational studies of anesthetic neurotoxicity as part of The 2016 Pediatric Anesthesia NeuroDevelopmental Assessment (PANDA) Symposium at Columbia University Medical Center. The experts agreed that defining intermediate phenotypes using advanced neuroimaging as a biomarker is a highly feasible and reasonable modality to provide new insights into the deleterious effects of anesthetic exposure in the developing human brain and could illuminate a viable investigative path forward. Ultimately, well-defined intermediate phenotypes may allow us to fully understand the neurodevelopmental impact of anesthesia-induced neurotoxicity and permit us to develop the safest and most effective anesthetic strategies for the infants and children we care for.

Keywords

Anesthesia-induced neurotoxicity; biomarkers; children; developing brain; phenotype; imaging

Introduction

Exposure to the most commonly used anesthetic and sedative agents in the early postnatal period has been shown to cause widespread neuronal degeneration in the developing mammalian brain and result in cognitive impairments and behavioral abnormalities later in life (1–9). These effects have been demonstrated in numerous preclinical studies using a variety of newborn vertebrate animal species including non-human primates (10). Putative developmental processes targeted by anesthetics include neuronal apoptosis, dendritic branching, synaptogenesis, and neurogenesis, while underlying molecular mechanisms have been proposed to include abnormalities of N-Methyl-D-aspartate (NMDA) and/or γ -aminobutyric acid (GABA) receptor expression, activity, and signaling, alterations in brain trophic and growth factor (e.g., BDNF) production and signaling, stimulation of neuroinflammatory pathways, increased mitochondrial reactive oxygen species production, and dysregulation of other growth and survival pathways such as phosphatidylinositol-3 kinase/Akt and the mammalian target of rapamycin (mTOR) (11–18). Several retrospective observational studies have also suggested an association between anesthesia exposure in young children and subsequent neurodevelopmental abnormalities, such as deficits in learning and scholastic performance (5, 19–21). However, a causal relationship in humans has yet to be demonstrated. Because millions of children in the United States alone are exposed to anesthetic agents annually, confirming whether or not anesthesia-induced neurotoxicity occurs in the developing human brain has become critically important (22).

It is generally recognized that attempts to translate findings from investigations of anesthesia-related neurotoxicity in the immature animal brain to the human state are limited by several factors (23). These include the following: 1) anesthetic dose and duration of exposure in animal studies often exceed clinically relevant concentrations and length of time; 2) the differential timing and rate of brain maturation across species makes comparisons to human development difficult; and 3) the assessment of neurodevelopmental outcome in other animal species fails to address the complexities of higher-order cognitive function in children (23–26). In addition, the human scenario is confounded by the indication for anesthetic exposure, whereby it is impossible to isolate the effects of the anesthetic agent from the underlying disease condition (that led to the need for anesthesia and surgery) (27). Because histologic assessment of the developing brain in healthy children following exposure to anesthesia is not possible, investigators have, thus far, been unable to prove that anesthesia-induced neuronal cell death (the most widely accepted mechanism) actually occurs in humans (23). Therefore, alternative approaches are necessary to explore the neurocognitive effects of anesthetics in infants and children.

The 2016 Pediatric Anesthesia NeuroDevelopmental Assessment (PANDA) Symposium organized by the Department of Anesthesiology of Columbia University Medical Center convened in New York on April 16th and 17th, 2016. As part of small group sessions focused on discussing the next phases of anesthetic neurotoxicity research, a collection of experts was asked to address the utility of biomarkers, genetic, and epigenetic factors with regard to translational studies. The following is a summary of the ensuing discussion.

Biomarkers

The National Institutes of Health Biomarkers Definitions Working Group defined the term, “biomarker”, as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (28). Biomarkers are often used as surrogate endpoints in clinical trials because they offer many advantages (28). For example, using biomarkers as an investigative tool permits more efficient study design with a reduced number of subjects. Biomarkers also enhance the ability to obtain interim data prior to establishing long-term primary outcomes and can function as surrogate endpoints in lieu of clinical outcomes that may occur infrequently or may be unethical to confirm (28). Thus, biomarkers seem appropriate and well-suited for translational studies of anesthetic neurotoxicity. However, in order to be considered as a surrogate endpoint, a biomarker must be consistent and accurate in predicting a specific outcome and be clinically relevant (28).

Considering other neurologic disease processes for which biomarkers have been developed serves as a useful starting point in assessing their utility to detect potential toxic effects of anesthetic exposure in the developing human brain. For example, in Alzheimer’s disease, investigators currently use biomarkers for diagnostic purposes and to identify asymptomatic patients who are at risk (29). Specifically, cerebrospinal fluid (CSF) sampling and positron emission tomography (PET) imaging can be used to quantify tau and amyloid protein as pathophysiologic biomarkers for disease in asymptomatic patients, while magnetic resonance imaging (MRI) detection of morphologic changes and fluorodeoxyglucose-PET identification of hypometabolic regions are used as topographical biomarkers to screen patients at risk (29). In adult and pediatric traumatic brain injury, S100 β , neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), alpha II-spectrin breakdown products (SBDPs), myelin basic protein, ubiquitin carboxyl-terminal hydrolase isozyme L1, and apolipoprotein E (ApoE) have been studied as potential biomarkers to assess injury severity and predict outcome (30). In addition, many of these proteins have also been examined for their ability to provide prognosis in other conditions such as neonatal hypoxic-ischemic encephalopathy and adult stroke (31, 32).

There is general consensus that in order for biomarkers to be ideal for neurologic disease processes, they must be detectable in blood or CSF in a well-defined temporal period after insult or exposure, be sensitive to the magnitude of injury, and be specific to central nervous system (CNS) tissue (30). Furthermore, biomarkers should permit the detection of influences of sex and age in a predictable manner (30). Ideally, they should also indicate the specific cell population of origin that has been injured (i.e., neurons, astrocytes, or oligodendrocytes) (30). However, many of the potential biomarkers that have been studied in the context of neurologic disease have failed because they lack specificity to the nervous system and their biokinetics (time to peak concentration and half-life) make them less suitable for detecting injury in a timely manner (33). Because of these limitations, some have advocated for using multiple biomarkers in a combined, simultaneous assessment (33).

With regard to anesthesia-induced neurotoxicity, the focus group stressed the importance of distinguishing between using biomarkers for detection of injury (i.e., demonstrating

neurotoxicity occurred), prognostication (i.e., quantifying the magnitude of injury and predicting outcome), assessment of susceptibility (identifying patients and cohorts at highest risk of developing anesthetic neurotoxicity), and for the study of underlying mechanisms. There was general agreement that, at this stage, serum or CSF biomarkers would provide limited utility in detecting pathology related to anesthetic exposure given that the exact mechanism(s) of anesthetic toxicity in the developing brain remain unknown. Although early work implicated the activation of the oxidative stress-associated mitochondrial pathway of apoptosis following anesthetic exposure, the experts agreed that it has not been unequivocally established that anesthesia-induced neuron loss represents the central mechanism of long-term neurological impairment (14, 17, 34–36). Thus, biomarkers aimed at detecting neuronal apoptosis or increased mitochondrial oxidative stress, for example, may not be relevant for identifying patients at risk for neurological impairment or those who have suffered an anesthetic-related neurotoxic event. Moreover, unlike traumatic brain injury and cerebral infarction that result in extensive injury and impact massive numbers of neurons, the magnitude of anesthesia-induced neuronal cell death may only affect a small percentage of the total cell population and may not lead to a measurable biomarker signal (37).

Strategies Going Forward

A common theme identified throughout the Symposium was the substantial challenges involved in investigating the effects of anesthetics on the developing brain. The most significant obstacle the field currently faces is the lack of a well-defined phenotype in children. The inability to define and detect a significant and specific acquired injury prevents development of clinical trials designed to test mitigation strategies. The group agreed that animal studies will be important in identifying specific biochemical, radiologic, and clinically-relevant outcome measures to be tested in humans and in defining the exact mechanism(s) of toxicity. Furthermore, if the response in the developing brain to anesthetics is that of classic toxicology, then a dose-response relationship should be established. In addition, periods of vulnerability should be defined for identified candidate mechanisms. The group also stressed that current and future animal studies should include assessment of substances in blood (either known [as with other types of neurological injury] or exploratory [i.e., microRNAs]) and use advanced neuroimaging modalities (see below).

With regard to translational investigation, commentary was made regarding future study populations. Focusing on pediatric cohorts undergoing multiple and prolonged anesthetic exposures would build on previously established associations (20, 21). Studying populations already at high risk for neurocognitive impairment (e.g. premature infants, children with congenital heart disease, children with mitochondrial cytopathies) could potentially be valuable since the “two-hit” aspects of such a model might generate a more robust signal or could identify children who are more susceptible to neurotoxic effects of anesthesia exposure. The major limitations of such approaches, however, include the high prevalence of confounding factors in these patient populations, which will make establishing a causal link more difficult. In addition, it was recognized that scientific findings in these vulnerable cohorts may not be relevant to the general population. Thus, the path forward is not clear-cut. Given the challenges in translating findings from immature animal models to young

children, novel and alternative approaches will be required to define the neurodevelopmental impact of anesthetics in infants and children.

Defining an Intermediate Phenotype

The notion of defining an intermediate phenotype using imaging studies as a biomarker was raised. This is an intriguing idea that should be considered for this disease process (38). MR imaging, for example, provides a reliable non-invasive technique that allows determination of brain morphology and quantification of neurochemical compounds *in vivo* (39). Thus, it is possible that such technology could be used to permit identification of imaged-based phenotypes in immature animals and children following anesthetic exposure prior to manifestation of neurological symptoms.

Moreover, MR spectroscopy permits measurement and detection of brain metabolic signatures and neurometabolites such as alanine, aspartate, creatine, GABA, glucose, glutamate, glutamine, glycerol-phosphoryl-choline, guanidoacetate, phosphoryl-choline, myo-inositol, lactate, *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate, phospho-creatine, scyllo-inositol, and taurine (39). This imaging modality has been used to detect alterations in neurometabolites within several brain regions in children with attention deficit/hyperactivity disorder, for example (39). It was suggested that NAA could be of particular interest in the context of anesthesia-induced neurotoxicity given that a decrease in its signal indicates neuron loss or significant neuronal dysfunction (40, 41). Furthermore, MR spectroscopy would also permit creation of lactate maps that may identify regions of abnormal metabolism within the brain following anesthetic exposure (42).

With regard to brain connectivity, functional MRI (fMRI) would allow for assessment of the interaction between different neuronal networks during performance of a task (43). fMRI can utilize blood-oxygen level dependent (BOLD) signals as an index of neuronal activity and provides greater three-dimensional spatial resolution than electroencephalogram (EEG) and near-infrared spectroscopy (NIRS) (44). Thus, fMRI could potentially detect abnormalities in functional brain connectivity in children previously exposed to anesthesia independent of or in the absence of morphologic changes and can be performed at earlier time points (prior to those necessary for accurate neurodevelopmental testing) (43). Furthermore, small animals such as rodents can be imaged using fMRI such that findings may be translatable from bench to bedside (44).

Given the nuances and expense of such technology, the group discussed the pros and cons of imaging children who are current study subjects within the PANDA, Mayo Anesthesia Safety in Kids (MASK), or General Anesthesia compared to Spinal anesthesia (GAS) cohorts. It was suggested that investigators consider such imaging modalities as a component of any future prospective clinical studies in an effort to identify intermediate phenotypes using these techniques. However, it was recognized that young children often require an anesthetic for these imaging procedures. This raises ethical concerns regarding the need for a subsequent anesthetic exposure, introduces confounders that could complicate interpretation of results, and creates challenges with regard to study design. In lieu of this, ongoing studies are encouraged to prospectively collect serial biosamples from study

participants, both before and after surgery, in anticipation of future testing and validation of candidate biomarkers. Finally, the experts agreed that investigation of genetic or epigenetic effects would be premature at this point but that epigenetic influences on susceptibility and injury severity may be important components of the problem (45).

Conclusions

Despite compelling and extensive data in animal models, lack of clear evidence of an injury phenotype in infants and children will soon subvert the claim that anesthetic-related neurotoxicity is a clinical problem and that there is a significant risk to public health that warrants the costs and effort of additional research on this topic. In addition, failure to define and identify a robust phenotype will eventually undermine the basis for potential trials of alternative anesthetic regimens and other mitigation strategies. Therefore, our first task is to identify novel approaches, innovative experimental paradigms, and expert input that will be necessary to fill this void in both preclinical and clinical investigation. Defining valid intermediate phenotypes using advanced neuroimaging as a biomarker, for example, appears to be a highly feasible and a reasonable modality for current and planned animal and human studies. Identification of such phenotypes could provide new insights into the deleterious effects of anesthetic exposure in the developing human brain and illuminate a viable investigative path forward. Ultimately, well-defined intermediate phenotypes may allow us to fully understand the neurodevelopmental impact of anesthesia-induced neurotoxicity and permit us to develop the safest and most effective anesthetic strategies for the infants and children we care for.

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