Are We Moving Closer to Noninvasive Imaging and Monitoring of Neonatal Anesthesia–induced Neurotoxicity?

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It is now undisputed that anesthetic agents with effects on N-methyl-D-aspartate and/or γ-aminobutyric acid A receptors (e.g., isoflurane, sevoflurane, ketamine, or midazolam) can induce cellular damage in the rodent or nonhuman primate (NHP) brain when administered during a window of developmental vulnerability, coinciding with high rates of synaptogenesis, maturation of neurons, and neurogenesis.\(^1,2\) For rodents, this period spans from postnatal day 7 to 14,\(^3\) while in NHPs, postnatal day 6 has been highlighted.\(^4\) Anesthesia-induced neurotoxicity in the developing animal brain is associated with learning disabilities as well as abnormal behavior including anxiety.\(^1,5\)

Disturbingly, the question of whether or not anesthesia neurotoxicity occurs in the young human brain remains unanswered. Retrospective, observational human studies linking anesthesia exposures in young children to cognitive and behavioral deficits later on in life have yielded conflicting and somewhat inconclusive results.\(^6,7\)

This has in part been attributed to "lack of an obvious human phenotype for anesthesia neurotoxicity."\(^8\) Prospective clinical studies are currently underway (ClinicalTrials.gov, Bethesda, Maryland), with some very recent data raising doubts as to the clinical relevance of this effect.\(^9\) Given the uncertainty, there has also been a strong effort to search for new approaches to explore and validate the possibility of neonatal anesthesia neurotoxicity in the human brain.

Molecular imaging approaches can play an important role in shedding light on the mechanisms involved in neonatal anesthesia-induced neurotoxicity. Such methodology has been extremely informative in understanding neurochemical and metabolic changes in disease states such as depression,\(^10\) addiction to substances of abuse,\(^11,12\) multiple sclerosis,\(^13\) and Parkinson disease,\(^14\) leading to discovery of new diagnostic and therapeutic targets. In this issue of Anesthesiology, Zhang et al.\(^15\) take an innovative approach to this problem with a translational tool by using positron emission tomography (PET) in combination with a radiotracer, \(^{18}\)F-labeled fluoroethoxybenzyl-N-(4-phenoxypyridin-3-yl)acetamide (\(^{18}\)F-FEPPA), to explore the state of "inflammation" in the brain of neonatal NHP rhesus monkeys after exposure to 2.5% sevoflurane for 8 h. The authors report that \(^{18}\)F-FEPPA binding (measured quantitatively as "standard uptake values") is significantly increased in the frontal and temporal lobes 24 h after sevoflurane exposure compared to nonexposed neonatal controls and remains elevated up to 1 week after exposure. The authors conclude that increased \(^{18}\)F-FEPPA uptake in the neonatal NHP brain is representative of glial cell activation (inflammation) and associated sevoflurane-induced neurotoxicity; the latter was confirmed histologically in a separate series of animals. In addition, it is reported that acetyl-L-carnitine (ALC) effectively blocks the inflammation as defined by increased \(^{18}\)F-FEPPA standard uptake values, and it is suggested that ALC may be protective in the setting of neurotoxicity from sevoflurane exposure in the neonate.

What is the evidence for the presence of neuroinflammation in the setting of an increase in \(^{18}\)F-FEPPA binding in the brain? The \(^{18}\)F-FEPPA ligand binds with high affinity to the translocator protein (TSPO) site of 18 kDa,\(^16,17\) which in the central nervous system is expressed mainly in glial cells (microglia as well as astrocytes). Specifically, TSPO is located on the outer membrane of mitochondria in glial cells and is part of a larger complex comprising a voltage-dependent anion channel and the adenine nucleotide carrier.\(^16\) There

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Accepted for publication April 11, 2016. From the Department of Anesthesiology, Stony Brook Medicine, Health Sciences Center, Stony Brook, New York.

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Anesthesiology, V 125 • No 1 22 July 2016
are several other radiolabeled tracers available for the TSPO site, but 18F-FEPPA is advantageous due to its high affinity for the TSPO site, favorable kinetic and metabolic profile, and excellent penetration into the brain, and further that 18F-FEPPA binding can be quantified. One disadvantage pertaining to the second-generation TSPO PET tracers in the human brain, including 18F-FEPPA, is that a polymorphism (rs6971) in exon 4 of the TSPO gene influences binding, which necessitates genotyping in all study participants. This polymorphism is relatively high in humans of European ancestry (allelic frequency of about 30%), but does not appear to be present in NHP.

The observed increase in 18F-FEPPA binding in the neonatal NHP brain exposed to sevoflurane compared to non-exposed controls as shown by Zhang et al. is interpreted as a sign of glial cell activation—presumably microglial cell activation. Under normal conditions, microglia cells comprise 10% of the cellular population in the brain and are capable of changing from a surveilling, ramifying state to an amoebic, phagocytic reactive state in the setting of injury or infection. Activated microglia cells secrete a wide range of cytokines including interleukin-1, interleukin-6, and tumor necrosis factor α, and can also attract and interact with T cells. The microglial responses to various types of injury in the brain vary dependent on the challenge: for example, when removing apoptotic cells, microglia release antiinflammatory factors.

It is important to know that, although increased 18F-FEPPA binding in the brain is highly suggestive of reactive microglia cells (and/or astrocytes), it may also signify an increase in the mitochondrial population in each of the glia cells. The exact function of TSPO in activated microglia is still unclear. For example, in mice deficient of TSPO (C57BL/6-Tspotm1GuWu(W gouvernay War))–knockout mice), activation of microglia after neuronal injury appears to be unimpaired, suggesting lack of a specific “phenotype” of TSPO in the setting of inflammation. Nevertheless, post-mortem studies have documented the presence of activated microglia in several disease states, including Alzheimer disease, multiple sclerosis, or Parkinson disease, with parallelled findings using in vivo PET and 18F-FEPPA. For example, in a recent quantitative PET study, 18F-FEPPA was used to compare TSPO binding in patients with Alzheimer disease and in cognitively intact age-matched controls, and areas of increased binding were observed in the internal capsule and cingulum bundle. What is the potential clinical significance of increased 18F-FEPPA binding in the neonatal NHP brain? Based on in vivo and in vitro studies using the TSPO radioligands, it is clear that an increased binding signifies the presence of activated glial cells and neuroinflammation. However, it is well known that the microglial reactive response is very variable dependent on the “stimulus,” and it could be that the increased response in the setting of sevoflurane exposure is merely reflecting the necessary phagocytosis of apoptotic neurons. In this context, therefore, the effect of ALC documented by Zhang et al. would simply mean a reduction in neuroinflammation and microglial function. It is also intriguing that recent reports highlight the beneficial effect of an acute immune response in the brain to overcome injury. Further investigations are needed to clarify if ALC is neuroprotective after sevoflurane exposure, and whether or not it might reduce cognitive dysfunction later on in life. Nevertheless, it is clear that long-duration sevoflurane induces a glial cell response in the neonatal NHP brain, and this study has taken us one step closer toward a translational approach to exploring this problem. Furthermore, the study by Zhang et al. is one of the first studies to look at multiple points within the same animal group, introducing the possibility of studying recovery from anesthesia-induced neurotoxicity in the developing brain. Although radioactive exposure is not recommended in this age group, this response might in the near future be validated by this or other noninvasive methods in the young human brain.

Competing Interests
The authors are not supported by, nor maintain any financial interest in, any commercial activity that may be associated with the topic of this article.

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