Anesthetic Neurotoxicity: New Findings and Future Directions

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The development and refinement of practices for the safe administration of anesthesia to children is a major success story in modern medicine. During the past several decades, there have been significant improvements in safety standards, cardiopulmonary monitoring, delivery systems, and airway management specific to the pediatric patient undergoing anesthesia. Millions of children receive anesthesia each year for surgical, procedural, or diagnostic purposes, and the majority of these patients receive a general anesthetic.[1] Parents and care providers can be confident that the vast preponderance of these children will have a safe outcome with a low likelihood of major morbidity or mortality.[2]

The last several decades also have seen the discovery, and subsequent verification, that agents commonly used to induce and maintain general anesthesia in humans exhibit evidence of neurotoxicity in animal models.[3] This realization dates to the early 1980s, when exposure of pregnant rat dams to chronic, low-level halothane was found to result in abnormal synaptogenesis and behavior in their offspring.[4] Further concerns arose from the discovery in 1998 that the *N*-methyl-D-aspartate (NMDA) receptor antagonist nitrous oxide (also known as laughing gas) can be neurotoxic in rodents.[5] It was shown subsequently that when administered to neonatal rats during a period of critical synaptogenesis, a commonly used cocktail of anesthetics (including nitrous oxide, midazolam, and isoflurane) induces immediate widespread neuronal apoptosis and impairments in learning and memory that persist into adulthood.[6] Preclinical models from *Caenorhabditis elegans* to nonhuman primates now suggest that multiple anesthetic agents may be neurotoxic. These include positive allosteric modulators of the $GABA_A$ receptor (benzodiazepines, propofol, and the anesthetics isoflurane, sevoflurane, and desflurane), and the NMDA receptor antagonists ketamine and nitrous oxide.[3] The discovery of anesthetic neurotoxicity in animal models raises the disconcerting possibility that administration of what appears to be a safe general anesthetic may have long-lasting deleterious neurocognitive effects.

These discoveries and concerns, however, represent an inversion of the traditional use of preclinical models to study human diseases. In most circumstances, a human malady is recognized clinically and is sufficiently prevalent or severe that researchers develop animal models to study disease process and to refine diagnostic and therapeutic approaches. Anesthetic neurotoxicity was first discovered in animal models, with the possibility of detriment to human patients arising from that discovery. This atypical knowledge acquisition makes it unclear what neurocognitive or behavioral components comprise the clinical syndrome of anesthetic-induced developmental neurotoxicity. This uncertainty presents parents, clinicians, and researchers with a conundrum: given the millions of children that undergo general anesthesia for surgical, procedural, and diagnostic purposes each year, anesthetic neurotoxicity, although unproven in human patients, may represent a significant public health problem.

Two recently published human studies that suggest a lack of harm in otherwise-healthy children following a short duration anesthetic (approximately 1 hour) deserve early mention. The first of these trials is the General Anaesthesia compared to Spinal anaesthesia (GAS) Trial, which randomized infants undergoing inguinal hernia repair to either an awake-regional technique or a general anesthetic.[7] Secondary outcomes assessed at 2 years of age showed no increased risk of adverse neurodevelopment in children exposed to a general anesthetic. The Pediatric Anesthesia & Neurodevelopment Assessment (PANDA) study compared children who had undergone inguinal hernia repair with general anesthesia before 3 years of age with an unexposed sibling.[8] No difference in IQ was found between exposed and unexposed siblings. Further details regarding these studies are discussed herein. The results from these trials are encouraging and suggest that a short-duration anesthetic in otherwise-healthy children may have limited effects. Nevertheless, the concerns regarding anesthetic neurotoxicity are myriad and nuanced. This commentary is intended as a review for pediatricians, anesthesiologists, and surgeons of the animal studies that first raised these concerns, the historical context of these studies, and the human studies that are either completed or ongoing.

Neuronal Damage and Behavioral Changes in Animal Models

Exposure of rodents to anesthetic agents between postnatal day 7 and 14 is associated with decreased neuronal density, decreased neuronal numbers, and increased neuronal apoptosis.[3,6,9] Multiple anesthetics have been implicated, including ketamine, propofol, and halogenated anesthetic gases. A cocktail of nitrous oxide, midazolam, and sevoflurane sufficient to maintain a surgical plane of general anesthesia for 6 hours results in

NMDA *N*-methyl-D-aspartate

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apoptosis in multiple brain regions,[6] and even subanesthetic doses of propofol can be apoptogenic.[10] Nonhuman primates also are vulnerable to neuronal damage. When administered to postnatal day 5-6 macaques, a combination of inhaled nitrous oxide and isoflurane sufficient to maintain surgical anesthesia for 8 hours produces apoptotic and possibly necrotic neuronal damage in multiple brain regions.[11] Evidence for anesthetic neurotoxicity has been found throughout the nervous system, including the hippocampus, striatum, thalamus, amygdala, cerebellum, cerebral cortex, and spinal cord.[9] The damage is not limited to neurons; apoptosis also has been observed in oligodendrocytes and other glial cells.

In addition to neuronal damage, early-life anesthetic exposure in rodents has been associated with long-term behavioral deficits in spatial memory[3,6]; however, not all studies that have assessed behavior have found a difference between exposed and control animals.[9] Most studies that include behavioral outcomes have been performed in rodents; however, macaques exposed to a single 24-hour ketamine anesthetic also have long-lasting impairments in learning and motivation as compared with unexposed controls.[12]

Caveats of Animal Models

The aforementioned studies represent a mere fraction of the nearly 1000 articles related to anesthetic neurotoxicity that have been published (for an excellent review, see Disma et al[9]). The general consensus from these studies is that anesthetic agents may be neurotoxic; however, there is significant heterogeneity in the anesthetic(s) used, the duration of exposure, the age of the animals studied, the histologic methods by which neuronal damage was assessed, and the behavioral tasks performed.

Age of Exposure in Animals vs Humans

In mammals, NMDA- and GABA-mediated neuronal activity are important for synaptogenesis during a critical period of brain maturation.[13] The sequence of events involved in brain maturation including neurogenesis, synaptogenesis, myelination, and increases in brain weight, along with the development of behavioral milestones, proceed in an orderly and similar fashion in all mammalian species studied. These species include mice, rats, guinea pigs, cats, sheep, nonhuman primates, and humans.[14]

The postconceptual and postnatal age at which synaptogenesis begins and its duration, however, vary significantly between species. Even within the same species, different brain regions are maximally vulnerable to anesthetic neurotoxicity at different developmental time points.[3] This interspecies variation and differential vulnerability underscores an important challenge in conducting and interpreting experiments involving animal models of neurodevelopment, that of determining what postconceptual age in a human patient corresponds to a given postconceptual age in another species, and what brain region(s) may be most vulnerable at that time point.

For example, peak vulnerability to anesthetic neurotoxicity in rodents occurs at postnatal day 7 and remains present (albeit in different brain regions) up to postnatal day 21.[3] Synapse formation and elimination is thought to occur approximately between postconceptional days 10 and 60 in rats and days 25 and 1000 in humans.[14] There are myriad potential confounds in correlating interspecies brain maturation to exact postconceptual dates, however extending this argument to its logical conclusion suggests that the period of maximal vulnerability in humans actually may be in the second trimester and then last until nearly 3 years of age. Importantly, our goal here is not to use this interspecies comparison to define vulnerable dates in humans but rather to illustrate the complexity in applying animal data to human patients.

Duration of Anesthetic Exposure and Use of Multiple Agents

The likelihood of apoptosis is influenced by the duration of exposure and the use of multiple vs single anesthetic agents. For example, studies have found no evidence of apoptosis when either nitrous oxide or isoflurane was administered as the sole anesthetic, only when administered in combination.[6,11]

Additional studies that use isoflurane as the sole anesthetic agent, however, have shown evidence of apoptosis in neurons and oligodendrocytes.[6,15-17] The conflict regarding the apoptogenic nature of single-agent anesthetics is not unique to isoflurane. Single doses of ketamine also have been suggested either to cause or not to cause apoptosis.[18,19] Studies that show no evidence of neuronal injury are not necessarily reassuring, because the majority of studies performed thus far indicate that the likelihood of neuronal damage increases when repeated doses of a single agent are given, when multiple agents are administered together, and especially when anesthesia is maintained for durations longer than 2-3 hours.

Use of Apoptosis to Define Neuronal Toxicity

Another challenge in extrapolating data from animal studies to humans lies in the use of immunohistochemical markers of neuronal apoptosis to signify neurotoxicity. Apoptosis during brain maturation is a physiologic process that is necessary for the removal of excess neurons produced during normal development.[20] It can be argued that anesthetic exposure results in an exaggerated, pathologic increase in neuronal apoptosis of otherwise-healthy cells,[3,6,21] although the possibility exists that the increased apoptosis seen following anesthetic exposure in animals merely represents an acceleration of the death of neurons that would eventually have undergone physiologic pruning.[13] Regardless of whether it occurs under physiologic or pathologic conditions, the final steps of the apoptotic cascade include the activation of proteolytic effector caspases.[20]

Many animal studies of anesthetic neurotoxicity use staining for activated caspase-3 to mark neurons that have passed the point of no return and are committed to cell death; however, caspase-3 staining is only able to reveal neurons in the early stages of apoptosis (approximately 6-12 hours after cascade initiation), as eventual phagocytosis of the dead neurons leaves no substrate for immunoreactivity. Furthermore, neuronal apoptosis may actually be one of the least-sensitive ways to assess altered synaptogenesis. Short durations of anesthetic

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