General Anesthesia and Young Brain: What is New?

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Abstract: Considering that growing population of very young children is exposed to general anesthesia every year, it is of utmost importance to understand how and whether such practice may affect the development and growth of their very immature and vulnerable brains. Compelling evidence from animal studies suggests that an early exposure to general anesthesia is detrimental to normal brain development leading to structural and functional impairments of neurons and glia, and long-lasting impairments in normal emotional and cognitive development. Although the evidence from animal studies is overwhelming and confirmed across species examined from rodents to non–human primates, the evidence from human studies is inconsistent and not conclusive at present. In this review we focus on new developments in animal studies of anesthesia-induced developmental neurotoxicity and summarize recent clinical studies while focusing on outcome measures and exposure variables in terms of their utility for assessing cognitive and behavioral development in children.

Key Words: development, neurotoxicity, immature neurons, rodents, nonhuman primates, epigenetics

Mounting preclinical findings published over the past decade confirm the compelling evidence presented in the initial animal studies where the exposure to commonly used gaseous and intravenous general anesthetics (GAs) induces developing neurons to undergo widespread neuroapoptosis ultimately resulting in their demise.[1–4] Concerning was the demonstration of significant cognitive and behavioral impairments later in life not only in animals[1,5–10] but possibly in humans as well.[11–14] Although the causality between neuromorphologic damage and behavioral sequelae could not be established with certainty, concerns were raised regarding the safety of GAs, a class of agents that once were considered to be safe for the young brain.

In view of the fact that there is a large body of information available in this area of research, our review will focus on 2 important and rapidly growing bodies of work: very recent studies of anesthesia-induced epigenetic modulations during critical stages of mammalian brain development and mounting evidence of anesthesia-induced developmental neurotoxicity in non–human primates. As definitive proof of anesthesia-induced impairments of synaptogenesis and long-term behavioral sequelae may not be possible in humans considering the complexity of the phenomenon, non–human primate studies are quickly becoming invaluable in getting us a step closer to understanding potential relevance of currently available animal data to humans.

NEUROMODULATORY CHANGES FROM ANESTHETIC EXPOSURE IN THE DEVELOPING BRAIN

Neuronal Development and Activity

During early stages of brain development neurons undergo intense maturation and differentiation while actively migrating to their final destination to establish functional circuitries crucial for proper behavioral and cognitive development. Of particular interest for this review is the developmental period referred to as synaptogenesis which is marked by intense branching of dendritic processes and the formation of numerous synaptic contacts. Although the exact timing of developmental synaptogenesis in mammalian species is not easy to decipher and it varies from one species to another, it is believed that the critical stages in humans occur during the last trimester of in utero life and a first couple (perhaps a few) years of postnatal life. The evidence that neuronal firing and communication are essential for proper formation of neuronal circuitries, which form the foundation for cognitive and behavioral development,[15] suggests that perhaps nonphysiological interference with neuronal interactions during the GA state could contribute to enhanced neuronal demise observed in GA-induced developmental neurotoxicity.

GA-induced Structural Modulations During Brain Development

Over the last decade the scientific community has spent a great deal of effort identifying the features of injury and investigating the cellular pathways that are triggered by anesthetics in the developing brain so that neuroprotective strategies could be devised. Many anesthetic agents have been studied in preclinical laboratory experiments, both in vivo and in vitro, and found to have significant neurotoxic potential.

Although the intial focus was on neurons where extensive apoptotic damage was reported, it appears that GA-induced neurotoxicity could be detected in glial cells as well.[16–18] For example, oligodendrocytes, which are necessary for myelination in the central nervous system white matter, have been shown to undergo GA-induced apoptosis, both in fetal and neonatal non–human primates.[16,19–21] The observed neurotoxic effects occur at the point of maturation when the oligodendrocytes develop the ability to myelinate axons, suggesting that a deficit in myelination could partially explain GA-associated problems with neurobehavioral development.

When one considers GA-induced developmental neurotoxicity of GABAergic anesthetics it is important to recognize that unlike in adults, $GABA_A$ receptor activation during early stages of brain development is an excitatory phenomenon.[22,23] Thus, while GABAergic medications are commonly used to treat excitatory conditions such as status epilepticus in the mature brain, it is possible that sustained $GABA_A$ mediated excitation may contribute to GA-induced neurotoxicity in the developing brain. The switch from an excitatory to inhibitory $GABA_A$ effect is a gradual process that peaks at P7 in rodents and could contribute to the anesthetic toxicity seen experimentally in mouse and rat pup models. However, recent evidence suggests that this switch is likely to occur immediately before birth in humans, and even earlier in non–human primates.[16] Therefore, this mechanism may contribute to neurotoxicity following in utero, rather than postnatal exposure to anesthesia in humans and non–human primates.

Modulation of Neuronal Plasticity

As proper and timely formation of neuronal circuitries provides the fundamental neuronal network necessary for proper neurocognitive development, axonal and dendritic development are thus key features of early brain maturation. During axonal development, the establishment of axon-dendrite polarity, in which the axon becomes differentiated from the dendrites, is a crucial step in the development of a functional neuron.[24] Isoflurane, a volatile anesthetic and propofol, an intravenous anesthetic, both have been shown to delay this polarization process and to impair collapse of the axonal growth cone in response to a repulsive cue in an in vitro model of embryonic mouse neocortical neurons.[25,26] Exposure to isoflurane at higher concentrations or for longer durations was associated with a dose-dependent retardation of neuronal polarization.[26] Ketamine exposure has also been shown to impair normal formation of dendrites on GABAergic interneurons, which are crucial for the formation of neuronal networks.[27]

Impaired neurogenesis following early exposure to volatile anesthetics has also been shown to lead to memory deficits. When infant rats and mice were exposed to daily isoflurane anesthesia on 4 days for 35 minutes each day, they showed progressively severe memory impairments on subsequent testing over the next 4 weeks. On histologic examination, no increased cell death was seen 24 hours after the last isoflurane exposure; however, the hippocampal stem cell pool was significantly smaller 4 weeks after exposure, and the overall number of hippocampal neurons was lower in the young rodents who were exposed to isoflurane.[28] The underlying mechanism and significance of these findings have yet to be determined.

EPIGENETIC MECHANISMS OF ANESTHETIC NEUROTOXICITY

As early as the 1980s, there were reports of a variety of behavioral impairments was shown to be associated with GA exposure during fetal stages of brain development. Interestingly, in utero exposure of rodents to volatile anesthetics (eg, halothane and enflurane) caused cognitive impairments not only in the first generation offspring, but also in the second generation offspring never exposed to GA but born to dams exposed to GA in utero.[29] These findings suggest that a transient exposure to GA during a critical period of neuronal remodeling perhaps causes epigenetic changes that become embedded in the genetic information resulting in the impairment of proper and timely neuronal development. Could epigenetic modifications from exposure to GA agents be an explanation for long-lasting impairments in behavioral development observed in numerous animal and emerging human studies?

Behavioral development including cognitive development depends on gene expression which requires access to DNA. Because DNA is highly intertwined with chromatin[30,31] access to DNA depends not only on the epigenetic modulation of DNA itself (eg, methylation) but very much on chromatin remodeling and histone epigenetic modifications (eg, acetylation, methylation, phosphorylation, ubiquitination, and sumoylation) that could be impacted by environmental influences. As such, epigenetic mechanisms translate environmental influences into changes in the expression of target genes having significant roles in brain development. The importance of epigenetic mechanisms is gaining significant attention especially in view of the recent findings that some forms of chromatin remodeling were found to be implicated in intellectual disability disorders[32–34] and autism.[35,36] Furthermore, the human disease Rubinstein-Tayby syndrome, which is clinically manifested as significant mental retardation from an early age,[37] was found to be caused by dysfunctional and downregulated cAMP response element-binding protein-binding protein (CBP).[38] CBP plays an important role in epigenetic modulation of histones as

a histone acetyl transferase, which acetylates specific lysine residues in histones, thereby generating epigenetic changes that disrupt repressive chromatin structure and promotes DNA transcription. Interestingly, our recent findings suggest that an early exposure to GA causes significant downregulation of CBP leading to hypoacetylated state of histones (histone-3 in particular) which in turns results in downregulation of c-fos and brain-derived neurotrophic factor (BDNF), proteins considered to be responsible for memory storage and new memory formation.[39,40]

Along the same lines, the review of the literature reveals that administration of ethanol, the oldest anesthetic known to mankind, during critical stages of brain development causes significant chromatin remodeling[39,41] in the promoters of BDNF and c-Fos. This in turn downregulates their transcription and consequently leads to the impairment of long-term memory.[42,43] Epigenetic changes are critical for long-term memory storage. For instance, the downregulation of CBP protein together with significant reduction in histone acetylation were accompanied by impaired late phase hippocampal long-term potentiation and learning deficits[31,44,45] similar to those described post-GA exposure.[1] Furthermore, CBP mutations have been shown to result in reduced CBP protein content and in histone acetylation accompanied by impaired late phase hippocampal long-term potentiation and learning deficits.[44–46] Hence, it would appear that CBP modulation observed after an early exposure to GA or ethanol mimics the one observed with genetic disease diagnosed during early stages of human development such as Rubinstein-Tayby syndrome.[37]

As a critical contributor to long-term memory storage epigenetic modulations have been suggested for therapeutic purposes. For example, inhibition of histone deacetylase (HDAC), which removes acetyl groups from lysines on histone tails and increases histone acetylation has been used in some therapeutic strategies. Through histone hyperacetylation, HDAC inhibitors increase the expression of many genes, thus enhancing new memory formation[42,43] by increasing or "normalizing" histone acetylation which in turn relaxes chromatin structure and improves access to transcription factors. Collectively, these findings suggest that drugs or diseases that promote epigenetic modulations could induce long-term molecular signals leading to the impairment of neuronal development. Furthermore, a strategic use of global HDAC inhibitors can minimize the genetic influences of GA and reverse some aspects of GA-induced developmental neurotoxicity.[47] This notion is based on the fact that activity-dependent transcription enables neurons to convert brief cellular changes into stable alterations in brain function that constitute a form of "molecular memory"[48] hence suggesting that a short-term exposure to GAs during critical stages of neuronal activity could be culpable for long-lasting alterations in synaptogenesis and behavioral impairments. Over the past decade HDAC inhibitors have been used in clinical practice in cases where HDAC levels were found to be elevated or there was a need to modify histone acetylation status. Hence, in certain types

of cancer the HDAC inhibitors were reported to be an important epigenetic therapy of great benefit as adjuvants.[49] Many of them have been approved by the Food and Drug Administration[50] and have well-described pharmacological, pharmacokinetic and safety profiles with well-defined therapeutic index, dosing, and side-effect characteristics. Hence, the use of the HDAC inhibitors as adjuvants to commonly used GAs in cases where a child may need prolonged exposure to GA but has excessively elevated basal HDAC levels or perhaps low level of acetylated histones may not be an unreasonable option.

Interestingly newly available evidence suggests a link between epigenetic alteration and apoptotic cell dying process. Specifically, downregulation of CBP protein expression we and others have reported could be due to CBP degradation to fragments known to have impaired histone acetyl transferase activity.[51] Although the exact mechanism of CBP fragmentation remains to be determined, activation of apoptotic cascades, in particular, the activation of caspase-6, was shown to increase CBP cleavage, suggesting that CBP is very sensitive to apoptotic activation.[52] Considering that GA exposure during critical stages of synaptogenesis results in substantial apoptotic activation as shown previously[1] and increase in fragmentation of lamin,[47] a substrate of activated caspase-6, we have proposed that GA-induced epigenetic changes manifested as CBP fragmentation during critical stages of synaptogenesis might be an apoptosis-induced phenomenon. Interestingly, excessive CBP fragmentation has been implicated in Alzheimer disease and was reported to promote amyloid accumulation.[52] As stated earlier the issue of neuronal communication and activity during early stages of synaptogenesis is being recognized as an important element of timely and proper formation of neuronal circuitries. Along those lines, it is of no surprise that kinase pathways that drive c-fos and cAMP response element-binding protein phosphorylation, 2 transcription factors that control the promoters of many genes important for acquisition and storage of new memories[53,54] could be exquisitely sensitive to GA-induced changes in neuronal activity and communication.

The unique property of many epigenetic changes is that they can be detected long after the initial environmental or pharmacological influences are removed. In many instances, rapid and often transient epigenetic modifications of the immediate early gene family like c-fos and BDNF[55,56] have been shown to have long-lasting influence via a variety of activator proteins that bind to the promoters of numerous later response genes, the genes that participate in processes crucial for neuronal development and survival.[57] Along those lines, a recent work by Wu et al[58] has shown that an early exposure of young rats at the peak of their synaptogenesis to isoflurane, an inhaled anesthetic used in clinical practice, modulates histone acetylation and DNA methylation in the promoter region of BDNF (axon IV in particular) thus leading to downregulation of BDNF expression. Interestingly, the authors report that the epigenetic influences of enriched environment resulted in mitigation of the

isoflurane-induced changes in BDNF expression. This in turn lead to improved synthesis of synaptic proteins which then resulted in the improvement of the hippocampal synaptic activity and cognitive abilities when compared with isoflurane-treated animals living in the standard cage environment.

In addition to epigenetic modulations that could be observed in the enriched environment there are some inclinations that GA-induced long-term memory impairment could be pharmacologically ameliorated when the histone acetylation status is modulated with the use of HDAC inhibitors. For example, Zhong et al[59] have shown that HDAC inhibitor trichostatin A can reverse isoflurane-induced hypoacetylation of hippocampal histone 4 (H4K12) and increase c-fos expression in hippocampus while reversing isoflurane-induced memory impairments in mice exposed to isoflurane at the peak of their synaptogenesis.

There is also evidence that a relatively recently described class of molecules may play a significant role in anesthesia-induced epigenetic modulations and apoptotic damage to neuronal cells. These small RNA molecules, known as micro-RNA (miRNA), are noncoding RNA molecules that bind in complexes to messenger RNA, downregulating translation or increasing degradation of messenger RNA strands. When a group of 84 miRNAs were assayed in human embryonic stem cell-derived neurons after exposure to propofol, 20 of them were found to be downregulated.[60] Of these, several had already been established to have important roles in neuronal differentiation and the regulation of apoptosis[61,62]; however, 1 of them, miR-21 was of particular interest due to its well-established antiapoptotic activity. To further investigate the role of miR-21 in GA-induced apoptosis, stem cell-derived neurons were altered to either upregulate or knockdown the levels of miR-21. When these neurons were exposed to propofol, more apoptosis was seen in the knockdown neurons, whereas the neurons with upregulated miR-21 were protected from the apoptotic effect of propofol.[60] miRNAs may also play a role ketamine-induced neurotoxicity.[63] The effects of GA exposure on miRNA activity is being actively investigated.

All these finding collectively suggest that an early exposure to general anesthesia could be very powerful epigenetic modulator with far reaching consequences on neuronal maturation, proper circuitries formation and behavioral development. If indeed we could gain full understanding as to how an early exposure to general anesthesia becomes embedded in genetic information and how this could be prevented/modified, we could perhaps provide early interventions that would allow safe use on GAs while preventing devastating long-term sequelae we and others have been reporting for over a decade.

GA-INDUCED BEHAVIORAL MODULATIONS DURING BRAIN DEVELOPMENT

Although numerous rodent studies have reported the association between an early exposure to GA and long-lasting behavioral and cognitive impairments, more recent evidence with non–human primates suggests that GA-induced neuropathologic changes appear to be associated with persistent cognitive deficits and behavioral impairments later in life. For example, prolonged single exposure to ketamine in the first week of rhesus monkeys' life resulted in significant learning deficits as well as a deficit in accuracy, task performance and response speed notable even at 3 years of age.[64] More recent evidence from Dr. Baxter's laboratory have demonstrated that repeated exposure of infant rhesus monkeys to sevoflurane anesthesia (total of 3 exposures, 4 h each) resulted in significant increase in anxiety-related behaviors when examined at 6 months of age suggesting adverse long-term anesthesia effects.[65] Along the same lines, a very recent evidence where a single 5-hour exposure to isoflurane was compared with multiple exposures (total of 3 times) confirms that when compared with controls, multiple-exposed but not single-exposed monkeys exhibited motor reflex deficits at 1 month of age and responded to their new social environment with increased anxiety and affiliative/appeasement behavior at 12 months of age. The authors concluded that an early exposure to isoflurane results in long-lasting and detrimental effects on socioemotional development.[66] Although in odds with rapidly accumulating evidence regarding the detrimental effects of sevoflurane in non–human primates, one of the studies with Cynomolgus monkeys suggests that perhaps there is no correlation between an early exposure to sevoflurane and long-term learning deficits although the authors report a decreased envinromental behavior when compared with controls.[67] Although these data cannot be directly extrapolated to human children, they provide important information about the pathophysiology and potential functional consequences of anesthetic neurotoxicity.

CONCLUSIONS

In this review we have summarized some recently available advances in the field of anesthesia-induced disturbances in the development of the immature brain. As the body of evidence continues to mount, it is becoming increasing clear that the animal data, non–human primates ones in particular, are very suggestive of detrimental effects of GAs on the very young brain undergoing substantial growth and maturation. It is noteworthy though that existing human studies at present cannot completely address the potentially detrimental effects of general anesthesia due to the complexity of the experimental design and the complexity of human cognitive and behavioral development.

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