General anesthetics and neurotoxicity in the developing brain: a review of current literature

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Abstract

The question that general anesthetics (both inhaled and intravenous) might be neurotoxic for the developing brain has been asked for more than two decades, following an extensive number of animal studies. Nevertheless, the same effect on human developing brain was mostly ignored on the basis of difficulty with direct application of animal data to humans.

Recently, two epidemiological studies showed an association between learning disabilities and anesthesia during the first years of life, suggesting that general anesthetics could potentially be harmful. Human, prospective, randomized trials are underway to try and clarify this issue, but their results will be available only 5-6 years from now.

In this article, we are presenting a review of the rapidly growing scientific evidence for anesthesia-induced neurodegeneration and its implications for pediatric anesthesia.

Keywords: general anesthesia, pediatrics, neurodevelopment, neurotoxicity

Introduction

Since its introduction, millions of patients of all ages had general anesthesia with either inhalational or intravenous anesthetics. Although the exact molecular mechanisms by which these compounds render patients insensible to noxious stimulation are still not entirely understood, their interactions with a variety of ion channels, such as sodium, calcium, and potassium channels, as well as numerous neuronal receptors, such as γ-aminobutyrate (GABA), glycine, glutamate, nicotine, and serotonin present many potential targets [1]. Consequently, given the diversity of molecular interactions, it would not be surprising if anesthetics would also possess other, potentially harmful effects.

One of the most discussed topics in today’s neurobehavioral sciences and pediatric anesthesia is the concern of the anesthetics’ ability to cause widespread cellular death in the developing animal brain [2, 3] which has led some to seriously question the safety of pediatric anesthesia [4], while others have cautioned against the direct applicability of animal data to pediatric anesthesia practice [5, 6].

Recently, data from two epidemiological human studies have suggested an association between anesthesia and surgery in young children and subsequent behavioral abnormalities and learning disabilities [7, 8]. We are presenting a review of the rapidly growing scientific evidence for anesthesia-induced neurodegeneration and its implications for pediatric anesthesia.


Animal data

Apoptosis, or programmed cell death, is an inherent, energy-consuming process, which self-destructs and eliminates cells that are functionally redundant or potentially detrimental to the organism, utilizing a cascade of enzymes called caspases [9]. Anesthesia-induced neuroapoptosis can be triggered by both the intrinsic and extrinsic pathways of the apoptotic cascade [10] and is associated with a decrease in brain-derived neurotrophic factor (BDNF) [11], a protein integral to neuronal survival, growth, and differentiation.

It is important to understand, however, that widespread apoptotic cell death is common in developing mammalian brains, and is an integral part of normal brain development. Neurons are produced in excess during fetal and neonatal brain maturation and up to 70% are eliminated during normal brain development [12].

This process establishes the normal structure of the central nervous system and disruption of the physiological apoptotic cell death mechanism leads to embryonic brain malformation and intrauterine demise [13]. It remains unclear whether anesthesia accelerates apoptosis of cells destined to die by this physiological apoptosis, or whether it induces apoptosis of cells not otherwise destined to die (i.e. pathological apoptosis).

Data about delayed synaptogenesis and behavioral abnormalities in rat pups who were exposed in utero to subanesthetic doses of halothane [14] was already available since mild 1980s. Neuronal dendritic arborization, an essential process during synaptogenesis, is impaired in neuronal cell cultures treated with ketamine and propofol [15-17]. These effects, which include neuronal apoptotic lesions and neurodevelopmental impairments in learning, memory, attention, and motor function, have been seen in a variety of experimental models, including neonatal rats, mice, guinea pigs, and rhesus monkeys [18-21].

In-vivo and in-vitro subsequent animal studies have revealed neuronal cell death immediately following neonatal exposure to every currently used anesthetic or sedative that was investigated. Neurodegeneration or impaired neurological function has been noticed following an exposure to midazolam [22], diazepam [23], clonazepam [23], ketamine [19-20, 22, 24-26], propofol [16, 19], pentobarbital [27], nitrous oxide [28], halothane [14], enflurane [29], isoflurane [21, 28, 30, 31], sevoflurane [32], or xenon [33]. However, several studies have also documented a lack of harmful sequelae following an exposure to midazolam [16, 34], ketamine [15, 20], thiopental [19], propofol [35], nitrous oxide [28, 34], isoflurane [21], sevoflurane [28], or xenon [28]. One study even noted a decrease in neurodegeneration and improvement in juvenile memory retention following prenatal exposure to isoflurane in rats [36].

In general, when neuronal degeneration is observed, it is widespread and dependent on dose and exposure time [14-17, 25, 27, 37] and worsened by combinations of several anesthetics [19, 34].

Interestingly, the notable exception to this synergistic effect is the N-methyl-D-aspartate (NMDA)-antagonist xenon, which diminished isoflurane-induced cellular death [28, 33].

Neurobehavioral models of learning and memory have been used to investigate the long-term deficits after general anesthetic exposure of adult and aged rodents. Impaired learning was reported consistently within 2–3 weeks after exposure to anesthetic combinations in aged rats [38].

A 4-h exposure of aged rats to nitrous oxide alone has been reported to cause learning impairment for up to 2 weeks [39], which possibly correlate to the earlier finding of neuronal damage induced by nitrous oxide exposure [34].

Even though the neurotoxic potential of ketamine is well established [40], it was the demonstration that a commonly used drug combination produces long-lasting neurologic deficits in neonatal rats that shocked the medical community.

Although the adverse effects of ketamine appear to occur at doses considerably higher than those used clinically, adverse effects of isoflurane have been observed using dosing schedules similar to those used in human infants [41] and even at sub-anesthetic doses [37]. One recent study reported that propofol induces neuroapoptosis in mice at one-fourth the dose required to achieve a surgical plane of anesthesia [42].

The mechanism(s) of this anesthesia-induced enhancement of neuroapoptosis and neurodevelopmental impairment is not clear. General anesthetics suppress the expression of neurotrophins necessary for neuronal survival and normal dendritic spine development [e.g., brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF)].

One recent study in rats suggested that a triple agent cocktail frequently used in pediatric anesthesia (midazolam, isoflurane, and nitrous oxide) significantly enhanced BDNF-activated neuroapoptotic cascades in the cerebral cortex and thalamus [11]. Prolonged exposure to ketamine also increases BDNF levels in the developing rat brain [43].

Rat pups anesthetized on postnatal day 7 (P7) with combinations of midazolam, isoflurane, and nitrous oxide (triple cocktail) sufficient to maintain a surgical plane of anesthesia for 6 h showed excessive neuronal apoptosis in the short term, impaired electrophysiologic and behavioral measures of learning and memory as
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This publication has since achieved landmark status because of its persuasive combination of histopathology, electrophysiology, and behavioral experiments and the follow-up into adulthood of subjects exposed to the triple cocktail. Only isoflurane, when given alone, increased apoptosis in the neonate, but each additional drug (double and triple cocktails) further increased the number of apoptotic neurons (up to 15-fold). Exposure to the triple cocktail exactly at P7 caused impaired hippocampal long-term potentiation and mild deficits in spatial reference memory as adolescents (P28–32). Deficits in hippocampus-dependent learning and memory persisted into adulthood.

The “triple agent cocktail” has also been found to affect levels of synaptic proteins important in activity-induced synaptic plasticity (e.g., synaptophysin, synaptobrevin, amphiphysin, SNAP-25, CaM kinase H) [30]. This might explain the consistent finding that anesthetic agents appear to have the greatest neurodegenerative impact when exposure occurs during the period of rapid synaptogenesis. This critical period of synaptogenesis in the rat lasts from roughly day birth to day 10, peaking at day 7 [44]. In humans, this corresponds roughly to a period from the third trimester to 1 year of age. Thus, in theory, humans may be most susceptible to general anesthesia toxicity from the prepartum period through infancy.

A number of additional experimental studies have confirmed the immediate neurotoxic effect of anesthetics in immature rodents and have largely excluded the possibility of confounding alterations of homeostasis (e.g., hypoglycemia, hypothermia, hypoxia).

However, these studies generally did not address the important question of long-term cognitive consequences. Recent reports confirmed the early cellular-level toxicity of both isoflurane [31] and sevoflurane [45] when administered at P7-10, but differed significantly with respect to long-term cognitive outcomes. Behavioral deficits persisting into adulthood were found in the experiments using sevoflurane but not isoflurane.

Studies of injectable drugs suggest that anesthetic agents somewhat specific for NMDA or GABA receptors (ketamine, thiopental, and propofol, either alone or in combination) when administered to P10 mice can also cause behavioral deficits or alterations in adulthood [25].

Overall, this evidence, suggesting that anesthetics with different molecular mechanisms of action can potentially increase apoptosis in the rodent brain and lead to long-lasting neurobehavioral consequences.

The neonatal rodent model was the primary model used for these studies but similar results have been published on newborn rhesus monkeys.

**Human data**

The relevance of the animal findings to clinical anesthesia has been intensely debated in the pediatric anesthesia literature. Two principal arguments have been raised by skeptics of the clinical relevance of anesthetic neurotoxicity.

The first is that neuronal damage and its sequela (in rodent models without surgical intervention) are due to causes other than the anesthetic drugs. This argument was not confirmed. The second argument is based on the profound differences between mammalian species in neurodevelopmental time course and hence timing and relative duration of exposure in potentially anesthetic-sensitive critical periods. A study trying to align the anesthetic-sensitive critical periods in different mammalian species found that the vulnerable periods for rats (P7-10) and monkeys approximately correlate to the 20th and 26th week of human gestation, respectively [46].

The increasing successful management of premature babies and young infants in neonatal intensive care units (NICU), pediatric intensive care units, and tertiary care pediatric hospitals has allowed many of them to survive, some with different degrees of neurologic deficit. Many of these children require multiple surgical procedures in the first weeks of life, such as operative patent ductus arteriosus ligation, central line insertions, bowel resections, and repair of congenital anomalies. In addition, many of the children suffer from immature lung development, leading to a need for prolonged mechanical ventilation and sedative infusions.

Because of the complexity of the children’s illnesses and interventions that they undergo, the specific factors that contribute to poor neurological outcomes are difficult to identify. Among the possibilities are concomitant aspects of critical illness, sepsis, hypoglycemia, hypotension, hypertension, anemia and the stress of surgery.

Many factors complicate an attempt to generalize the results of animal studies to humans. First, the experimental conditions under which data are collected from rodents differ from the conditions experienced by neonates undergoing surgery and general anesthesia [47]. For example, because of difficulties measuring blood glucose, oxygen saturation, and vital signs, such as blood pressure in immature animals, the operative conditions for these animals may be significantly different from those experienced by human neonates. Second, in some (but not all) of the animal studies, the anesthetic dosage or the duration of anesthetic exposure was greater than what is generally experienced by human neonates undergoing general anesthesia (although not necessarily longer than the periods of sedation that some neonates experience in the NICU).
Third, there may be differences in brain plasticity between humans and rats that allow humans to accommodate apoptotic injury without clinically significant effects on neurodevelopment. Fourth, the neurodevelopmental impairments observed in animals, such as disruption of spontaneous behavior, and poorer performance on radial arm and elevated mazes [19], are of uncertain relevance to humans [48]. Lastly, since anesthetic drugs are given to blunt the stress response and perception of pain during surgery and painful procedures, the experimental models utilized in the preclinical studies disregard the effect of concurrent noxious stimulation during the administration of the anesthetic. In newborn rat pups, painful stimuli and maternal withdrawal cause abnormalities in long-term behavior and pain perception [49-51]. Fetuses and neonates subjected to pain and stresses associated with painful procedures are also at risk for long-term adverse outcomes.

There are clear benefits associated with the ablation of nociceptive stimuli during surgery; therefore in any clinical scenario the possible direct neuromodulating or toxic effects of the anesthetic agent must be weighed against the neuromodulating effect of the nociceptive stimulus [24].

Translation of these animal studies to humans is underway. There are several epidemiological studies that have positively linked exposure to general anesthesia before the age of 4 with an increased chance of developing learning deficits. In a data base review of 228,961 pediatric New York State Medicaid patients, Sun et al. found that that children that had surgery before the age of 3 had more utilization of Medicaid services for learning deficits than those enrollees that had no surgery [52].

Wilder et al. examined the extensive medical and school records of 5358 children in Rochester, Minnesota and determined that there was an association between children having more than 2 surgeries before age 4 and learning deficits in school [8]. A cross sectional study done by Kalkman et al. [53] in Utrecht, Netherlands surveyed a small number of parents of children who had surgery at a young age and determined that there was a trend toward greater prevalence of learning deficits in children who had early surgery.

All these studies demonstrate an apparent association between general anesthesia at a young age and neurodevelopmental disorders, but cannot support definitive conclusions regarding causation. In fact it is easy to generate competing explanations for worse neurologic outcomes in children who underwent surgeries at a young age, including premorbid conditions and the effects of surgery itself.

Finally, the first prospective randomized controlled trial to determine whether general anesthesia causes long-term neurotoxicity in children is underway [54]. Patients scheduled for inguinal herniorrhaphy are allocated to general or pure regional anesthesia in a randomized fashion. Neurodevelopmental assessments will occur at 2 and 5 years with standard neuropsychological tools.

The drawbacks to such a study are that it takes a long time to complete, it is expensive and it is difficult to quip the patients enrolled for such a long period of time.

If it is determined that there is no difference in the neurocognitive outcomes of children receiving regional and general anesthesia for inguinal herniorrhaphy, the findings would not bear directly on the question of whether prolonged use of a general anesthetics (greater than 2 h) or sedation of infants in the NICU for prolonged periods of time are similarly safe. It would also not answer the question of whether a general anesthetic performed with different agents or different doses is similarly safe.

Conclusions

The determination of whether general anesthetics are safe for infants is important to ascertain. Since the irrefutable laboratory evidence in non-human models demonstrates anesthetic-induced neurotoxicity in the developing brain, comparable experimental designs should be applied to human studies. It will take many different approaches with complementary study designs to accumulate the aggregate knowledge to say with confidence whether general anesthesia is safe or not for young children [52, 54]. If general anesthetics are determined to pose a risk to the neurodevelopmental outcome of young infants, it will be important to determine which general anesthetics or combinations of anesthetics pose the greatest risk. It is also important to remember that general anesthesia has allowed surgeons to perform life saving operations on very small children and that the risks of delaying surgery may be greater than any risk posed by general anesthesia.

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Rezumat

Întrebarea dacă anestezicele generale (atât inhalatorii cât și intravenoase) ar putea fi neurotoxice pentru dezvoltarea creierului s-a pus timp de peste două decenii, în urma unor studii extensive pe animale. Cu toate acestea, același efect asupra dezvoltării creierului uman a fost în mare măsură ignorat din cauza dificultății aplicării directe la om a datelor obținute de la animal.

Recent, două studii epidemiologice au semnalat o asocieri între dizabilitățile în procesul de învățare și anestezia din cursul primilor ani de viață, sugerând că anestezia ar putea fi potențial dăunătoare. Trialuri prospective, randomizate, pe subiecți umani sunt în curs, încercând să clarifice acest subiect, însă rezultatele vor fi disponibile numai peste 5-6 ani.

În acest referat, prezentăm o trecere în revistă a dovezilor științifice din ce în ce mai numeroase privind neurodegenerarea indusă de anestezie și implicațiile acesteia în anestezia pediatrică.

Cuvinte cheie: anestezie generală, pediatrie, dezvoltare neuronală, neurotoxicitate