Review Article

Effect of general anesthetics on the developing brain

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Abstract

Studies on rodents and subhuman primates suggest that prolonged exposure to general anesthetics may induce widespread neuronal cell death and neurological sequelae; seriously questioning the safety of pediatric anesthesia. This review presents recent developments in this rapidly emerging field. There is mounting and convincing preclinical evidence in rodents and nonhuman primates that anesthetics in common clinical use are neurotoxic to the developing brain *in vitro* and cause long-term neurobehavioral abnormalities *in vivo*. Prior to the publication of animal data and after the publication of animal data, there are several human cohort studies that demonstrate the association of poor neurodevelopmental outcome in neonates, who underwent major surgery during their neonatal period. This review summarizes our present understanding of some of the key components responsible for anesthesia-induced neuroapoptosis and offers some of neuroprotective strategies that could be beneficial as adjunct therapy in preventing anesthesia-induced death of developing neurons in the neonates. A randomized literature search was carried out using search words apoptosis, general anesthetics, and developing brain from 1979 to 2011 for effects of general anesthetics on developing brain in PUBMED and relevant published literature reviewed. General anesthetics may produce neurotoxicity and enduring cognitive impairment in young and aged animals, but the issue has not been adequately studied in humans. It is premature to recommend a change clinical practice based on the present data.

Key words: Apoptosis, delayed effects, general anesthetics, neurodegeneration

Introduction

Anesthesiology is a young and growing specialty. The delayed effects of anesthetics are not well known because potential interventions cannot be studied directly in humans. Anesthesia for obstetric and pediatric surgery is an unavoidable as pregnant mothers and newborn infants present with life-threatening conditions requiring surgery or prolonged stay in the intensive care unit. Although, brain development begins during the last trimester of intrauterine life, human brain is not fully developed at birth and continues to grow over the first couple of years of postnatal life.[1] A randomized literature search was carried out using search words apoptosis, general anesthetics, and developing brain from 1979 to 2011 for effects of general anesthetics on developing brain in PUBMED and relevant published literature reviewed.

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History

Young children exposed to a brief, single anesthetic did not show any evidence of adverse long-term effects on the brain, according to a new Danish study.[2] Studies in young animals and human primates have shown that several classes of general anesthetics, at concentrations within the range used for anesthesia, killed cells, and produced neurodegeneration, when the brain is developing. The applicability of animal data to humans undergoing anesthesia early in life remains uncertain, partly due to the difficulty in differentiating anesthetic exposure and pathology in animals to clinically meaningful effects in patients, but the data cannot be ignored.

Physiology of Synaptogenesis

It is well known that all key elements of neuronal development take place during the early stage of brain development, which is the time of great vulnerability. In this early stage, the blood-brain barrier is incomplete, allowing access to the brain of substances that would normally be prevented. Neurogenesis,

gliogenesis, and synaptogenesis occur at a high rate by migration, synapse formation, differentiation, and maturation of neuronal cells. The process of synaptogenesis depends upon constant neuronal signaling, communication, and feedback processing.[3] A very small percentages of neurons that do not make meaningful connections and feedback during synaptogenesis are considered redundant and are destined to die via the natural pruning process of apoptosis, or neuronal suicide, a process is referred to as programmed cell death.

The neurotrophins, a family of growth factors consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophic factor (NT-3, NT-4, and NT-5), are known to support neuronal survival, differentiation, and several forms of synaptic plasticity and therefore play an important role in the synaptogenesis of the mammalian brain. The signal transduction systems that mediate the diverse biological functions of neurotrophins are initiated via two different classes of plasma membrane receptors. They are tropomyocine receptor kinase (Trk) receptors and P75 neurotrophic receptors (P75ntr). Current data suggest that the main physiological functions of P75ntr are not only the regulation of Trk receptor activation and signaling but also activation of Trk-independent signal transduction cascade.[4] Both Trk-dependent and Trk-independent cascade modulate the activation or phosphorylation of the protein kinase-B (PKB) serin/threonine kinase, the pivotal factor in major survival pathway of neurons.[5] The neurotrophins are synthesized and released by neurons and both their biosynthesis and secretion depend on neuronal activity. Extensive depression of neuronal activity can impair survival-promoting signals that are regulated by neurotrophins and clinically used to promote apoptosis.

Mechanisms of Neuroapoptosis

Based on the work of Ikonomidou *et al.*[6] and the work of others over the last few years, it is widely accepted that the commonly used general anesthetics potentiate inhibitory transmission through gamma-amino-butyric-acid type A ($GABA_A$) receptors and the excitatory transmission is reduced through *N*-methyl-D-aspartic acid (NMDA) glutamate receptors at the peak of synaptogenesis causes widespread apoptotic neurodegeneration.[7] Furthermore, based on the studies by Jevtovic-Todorovic *et al.* it appeared that exposure to general anesthetics at the peak of synaptogenesis causes significant learning and memory deficiencies later on in life in comparison to control group, and progressively widening the gap in adulthood.[8]

In the adult, $GABA_A$ receptor activation leads to an influx of chloride ions (Cl^-) into the cell. This results in hyperpolarization and can lead to neuroprotection in many models of hypoxia and ischemia. However, in the developing brain, especially during synaptogenesis, intracellular concentration of Cl^- is high; activation of $GABA_A$ receptor results in Cl^- efflux and depolarization of the neuron. Consequently, depolarization-mediated rise in intracellular calcium concentration reaches levels that can be harmful to the cell, suggesting that this excitotoxic action of $GABA_A$ may contribute to neuronal injury. An imbalance between excitatory and inhibitory input in the central nervous system during synaptogenesis may trigger apoptosis and changes to the morphology of dendritic spines.

Pathways of General Anesthesia-induced Neuronal Apoptosis

Apoptosis takes place via different biochemical pathways resulting in activation of effector caspase as the final step. The pathways are:

- Intrinsic pathway or mitochondria-dependent pathway.
- Extrinsic pathway or receptor-dependent pathway.
- Neurotrophic factor dependent pathway.
- Neuronal cell dependent pathway or neuronal cell deletion.

Intrinsic pathway or mitochondria-dependent pathway

The intrinsic or mitochondria-dependent pathway involves the down-regulation of anti- apoptotic proteins from the (B-cell lymphoma-2) BCL-2 super family (ex: bcl-x_l), results in an increase in mitochondrial membrane permeability with an increase in release of cytochrome-c into the cytoplasm. This in turn activates caspas-9 and caspas-3 resulting in apoptotic neuronal cell death.[9] A study on the brains of rats, 7 days after postnatal age by Yon *et al.*[10] found that mitochondria-dependent cascade gets activated within 2 h of exposure to general anesthesia.

Extrinsic pathway or receptor-dependent pathway

Extrinsic pathway or receptor-dependent pathway is activated by the activation of death receptors that involves the formation of a death inducing signaling complex (DISC), this contains Fas (Fas: legend/receptor, a transmembrane protein, a member of the tumor necrosis factor family also known as CD95). DISC formation results by up regulation of Fas protein levels and significant activation of caspas-8 which activates caspas-3, executing the cell death.[10] Based on timing, it appeared that general anesthesia induced activation of the intrinsic pathway occurs before the activation of extrinsic pathway.

Neurotrophic factor dependent pathway

Lu *et al*. presented evidence that clinically used general anesthetics administered at peak of brain development usually 7 days of postnatal age in rats induce neuroapoptotic damage in the developing brain via brain-derived neurotrophic factor (BDNF) modulated apoptotic cascade.[11] Dual mechanisms play in anesthesia-induced neurotrophin-mediated apoptotic pathway, one via Trk-dependent and the second Trk-independent or P75ntr-dependent apoptotic cascade. The importance of either pathway seems to be brain region specific. In the thalamus, anesthesia causes a decrease in the BDNF protein level and the activated PKB levels, without any effect on P75ntr and ceramide levels resulting in activation of caspase-9 and caspase-3 activities, leading to apoptotic neurodegeneration. On the other hand in the cerebral cortex, anesthesia causes an increase in the BDNF levels while decreasing the levels of the activated PKB and increasing caspase-9 and caspase-3 activity, suggests activation of Trk-independent and P75ntr-dependent cascade.

Neuronal cell dependent pathway or neuronal cell deletion

An important question regarding general anesthesia induced neuronal deletion is when the neuroapoptosis of the developing brain is permanent or when it is only a transient and reversible phenomenon. Recent study on brains exposed to clinically relevant anesthesia at the peak of synaptogenesis (usually 7 days of postnatal life in rats and 35-40 days in guinea pigs) show a significant decrease in neuronal densities in all vulnerable cortical and subcortical regions of brain. Although physiological pruning of the redundant neurons is commonly observed in the developing mammalian brain, only a small percentage, usually less than 1% with some regional variation, are pruned. Of grave concern is the fact that clinically relevant general anesthesia severely jeopardizes the survival of many developing neurons, leading to an alarming increase in neuronal deletion. Despite maintenance of normal blood gas values including arterial oxygen saturation, blood pressure, and blood sugar throughout anesthesia administration, the immature neurons undergo significant apoptosis.[12]

Clinical Relevance

Prior to the animal data being published, several human cohort studies had demonstrated an association between major surgery in the neonatal period and poor neurodevelopmental outcome.[13-15] Premature infants who underwent laparotomy had poorer neurodevelopmental outcome compared with matched controls,[16] and children born with esophageal atresia have increased long-term learning emotional and behavioral problems compared with the general population.[17]

More recently, Wilder *et al*.[18] used a large established birth cohort maintained at the Mayo Clinic. Looking at children who had surgery or did not before the age of 4, they found the risk of learning disability increased with the number of anesthetics a child had received. Interestingly, there was no evidence for an increased risk of association after just one exposure. The association between disability and multiple exposures to anesthetics persisted when adjustment was made for chronic illness.

Di Maggio *et al*.[19] performed a cohort study using the New York State medical aid records comparing children who had hernia repair before the age of 3 matched with those who had no surgery. After adjusting for several potential confounding factors, they found children who had hernia repair had twice the risk of diagnosis of behavioral or developmental disorder.

Using the Mayo birth cohort, Sprung *et al*.[20] compared children who were born by cesarean section under general anesthesia, with those born by cesarean delivery under regional anesthesia and those born by vaginal delivery. They found that children born by cesarean delivery under regional anesthesia had less risk of a learning difficulty than those born by vaginal delivery and no difference between those born by cesarean under general anesthesia and vaginal delivery. However, the reason for this is unclear.

Limitations with clinical evidence

- It is very difficult to interpret the clinical data from these studies. This is partly because the animal data cannot precisely inform the age of exposure, which is important, the duration of anesthetic likely to cause injury and the outcome which is most likely to be relevant to anesthesia.
- Testing children at an early age will only detect major neurological problems and psychometric tests in young children are poor at predicting later outcome. Prospective studies take several years and may suffer from loss to follow-up. Retrospective studies may be quicker, but exposure cannot be controlled and/or data of exposure may be incomplete, and the anesthesia techniques may be outdated.
- The largest problem, however, is even more confounding. Anesthesia is usually associated with surgery or a diagnostic procedure. The surgery may result in inflammatory or humoral stress that may itself influence outcome. Surgery may also be associated with septic, metabolic, hemodynamic, respiratory events, and are very likely to have pathology, which will also influence neurobehavioral outcome. Infants who require surgery may be premature or have genetic or chromosomal abnormalities; all of which can be associated with developmental delay.
- The significance of any anesthesia related neurotoxicity

may be even more difficult to unravel when we consider the potential benefits of anesthesia. It is established that infants undergoing major surgery who have inadequate anesthesia or analgesia have a poorer outcome. It is presumed that surgery and pain result in harmful metabolic, immunologic, and humoral responses that could at least partly be reduced by adequate anesthesia and analgesia.[21]

Preclinical Advances in the Prevention of Apoptosis

Late third-trimester anesthesia should be minimized or avoided. The timing of surgery may be an important consideration. Particularly in very young children, anything that can be delayed until after the brain growth spurt should be postponed, if waiting does not entail additional risk to the patient. Anesthetic management should be kept simple and the dosage should be low as far as possible.

Olney *et al*. have proposed that anesthetic drug effects on fetal and neonatal γ-aminobutyric acid and *N*-methyl-D-aspartic acid receptors cause translocation of a Bcl-2-associated protein to mitochondrial membranes, leading to an apoptotic cascade.[22] If we can interfere with the apoptotic cascade in abnormally inhibited neurons, we might be able to prevent anesthetic-induced neuronal apoptosis. Some ways to do this, found in laboratory animals, are:

Melatonin

Melatonin, a hormone secreted by the pineal gland at night, was shown to modulate the mitochondria-dependent apoptotic cascade in vitro, *via* inhibition of the mitochondria-dependent apoptotic pathway by up regulating the protein level of bcl-xL and down regulating the protein levels of cytochrome c, and thus preventing anesthesia-induced apoptotic neuronal degeneration.[23]

Beta-estradiol

Beta-estradiol a steroid hormone was shown to play an important role in up regulation of phosphorylated PKB levels, thus down regulating the caspas-9 and caspas-3 activity ultimately protecting against anesthesia-induced apoptotic cell death offering another clinically attainable and potentially usefully preventive strategy.[24]

l-carnitine

l-carnitine is an l-lysine derivative and its main role lies in the transport of long chain fatty acids into mitochondria to enter the β-oxidation cycle,[25] and neutralization of toxic acylCoA production in the mitochondria,[26] which correlates with various pathological processes, including numerous diseases of the CNS such as neurodegenerative diseases. Bax is a proapoptotic protein, a pore-forming cytoplasmic protein that translocates to the outer mitochondrial membrane, influencing its permeability and inducing cytochrome-c release from the intermembrane space of the mitochondria into the cytosol, subsequently leading to cell death.[27] The anesthetic combination [nitrous oxide (75%) with isoflurane (0.55%)] resulted in a significant up-regulation of Bax protein compared with control, and this effect was blocked by the coadministration of l-carnitine (300 or 500 mg/kg) thereby protecting the neuronal cell.

Xenon

Pretreatment with xenon prevented nitrous oxide and isoflurane-induced neuroapoptosis (*in vivo* and *in vitro*) and cognitive deterioration (*in vivo*). Xenon pretreatment increased Bcl-2 expression and decreased both cytochrome-c release and protein 53 (P53) expressions thereby preventing neuronal degeneration.[28,]

Lithium

Lithium restored phosphorylated ERK1/2 levels (but not Akt) and prevented ketamine- and propofol-induced injury. It should, however, be noted that activation of ERK1/2 was demonstrated only with the higher dose of lithium (6 mg/kg) that was studied. Although 3 mg/kg did inhibit apoptosis, its effect *vis-à-vis* ERK1/2 activation remains to be defined.[29]

Dexmedetomidine

In vivo dexmedetomidine dose dependently prevented isoflurane-induced injury in the hippocampus, thalamus, and cortex. Isoflurane did induce long-term memory impairment. This neurocognitive deficit was prevented by administration of dexmedetomidine, which also inhibits isoflurane-induced caspase-3 expression in organotypic hippocampal slice cultures *in vitro*.[30]

Erythropoietin

Erythropoietin has also shown promise against *N*-methyl-D-aspartic acid receptor antagonist neurotoxicity in rat and mouse neonates and in hypoxic-ischemic injured neonatal rats.[31]

Conclusion

The potentially alarming issue of anesthesia-induced neuronal damage in the immature brain is gathering a lot of interest among practicing anesthesiologists. By improving our understanding of the mechanism by which anesthesia induces neuronal damage in the immature brain, we can devise the more effective preventive strategies to use the existing anesthetic drugs to their full advantage, without the risk of neurotoxic side effects.

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