PERSPECTIVE

Anesthesia-induced neurotoxicity in an animal model of the developing brain: mechanism and therapies

Children are being exposed to an increasingly greater variety of anesthetics with advances in pediatric and obstetric surgery. Recent animal and retrospective human data suggest that the general anesthetics commonly used in pediatric medicine could be damaging to the developing brain when used at clinical concentrations. *In vivo* primate and rodent models have shown that neonatal exposure to clinical concentrations of anesthetics causes neural apoptosis and long-term cognitive impairment. Many general anesthetics, such as isoflurane, sevoflurane, barbiturates, benzodiazepines, ketamine, propofol, and nitrous oxide, cause adverse changes in the neonatal rodent and primate brain. Animal and human data suggest an association between general anesthesia during the neonatal period and long-term cognitive impairment. Cohort studies involving humans have recently been started. The window of vulnerability to these neurotoxic effects of anesthetics is restricted to the period of synaptogenesis, also known as the "brain growth spurt" (BGS) period. To minimize the risk of neurodegeneration, it is necessary to study both the mechanism of neurotoxicity and preventative medicine. Neonatal anesthetic exposure affects many mechanisms of neurotoxicity. Mechanisms of anesthetic-induced neurotoxicity seem to involve altered expression of ligand-gated ion channels, disturbance of intracellular calcium homeostasis, and the mitochondria-mediated apoptotic pathway. Several agents reportedly help to prevent anesthesia-induced neurotoxicity, including hydrogen, melatonin, apocynin, and ketorolac, and should thus be co-administered with anesthetics. After anesthesia, only environmental enrichment can improve learning deficits due to anesthesia-induced neurotoxicity. Further studies of environmental enrichment (Wu et al., 2016) after anesthesia are necessary to develop preventative and therapeutic strategies for anesthesia-induced neurotoxicity.

Clinical findings: Children undergo general anesthesia for diagnostic imaging and surgical procedures more commonly than do adults. The safety of anesthesia in children remains a subject of debate; some retrospective studies have concluded that it causes learning deficits, and others have reported that it is not harmful. These inconsistent results are likely due to differences in the patients' clinical histories. It is reported that children who underwent surgery before 4 years of age exhibited a significantly lower listening comprehension and performance intelligence quotient (IQ) than did controls matched for age, sex, handedness, and socioeconomic status. Furthermore, long-term impairments in language ability and cognition were associated with lower gray matter density in the occipital cortex and cerebellum.

Cohort studies: Three cohort studies have recently reported their primary outcomes. The Pediatric Anesthesia Neurodevelopment Assessment (PANDA) project is a prospective neuropsychological assessment of 28 exposed–unexposed sibling pairs aged 6 to 11 years. No differences were found between the exposed and unexposed groups in verbal IQ, performance IQ, or full IQ. Another investigation was conducted by the General Anesthesia compared to Spinal anesthesia (GAS) consortium. This was a multicenter randomized controlled trial of infants receiving either awake-regional anesthesia or sevoflurane-based general anesthesia for inguinal hernia repair with 2-year follow-up. The results of this study showed that < 1 hour of sevoflurane anesthesia in infancy did not increase the risk of adverse neurodevelopmental outcomes at 2 years of age compared with awake-regional anesthesia.

Finally, Sun et al. (2016a) reported that children exposed to a single episode of anesthesia before 3 years of age showed no statistical difference in their global cognitive function score compared with healthy siblings with no anesthesia exposure.

Together, these data indicate that a short duration of, and single exposure to, anesthesia may be safe. Further follow-up is important in these studies. Long-term sedation in the intensive care unit may be harmful for children.

Factors influencing the degree of neurodegeneration in animal studies: The main factors that influence the toxicity of anesthetic agents are the duration, concentration, and frequency of anesthesia, and the sensitivity of the brain to the anesthetic agent. Studies have shown that longer durations, higher concentrations, and higher frequencies of anesthesia lead to more severe neurodegeneration. The period during which neurons are most vulnerable is the BGS, which is equivalent to synaptogenesis. In humans, the BGS occurs from the third trimester to 3 years of age, and in rodents during the first 2 weeks after birth. The peak of synaptogenesis occurs on postnatal day 7 in rodents. All investigators use clinical concentrations of anesthesia in investigations of anesthesia-induced neurotoxicity. Most studies have used arterial blood gas analysis to evaluate respiratory and circulatory depression. Of course, high doses of anesthetics are harmful. Therefore, all of these investigations limit the clinical concentrations of anesthetic agents.

To minimize the risk of neurodegeneration after anesthesia, it is necessary to study the mechanisms of neurotoxicity and to explore preventative options.

Neurotoxicity mechanisms (Figure 1): Coadministration of an N-methyl-D-aspartate (NMDA) receptor antagonist with a γ-aminobutyric acid type A ($GABA_A$) receptor agonist causes neonatal cell death in the brain and results in learning deficits in adult mice. Alcohol, an NMDA antagonist and $GABA_A$ agonist, causes fetal

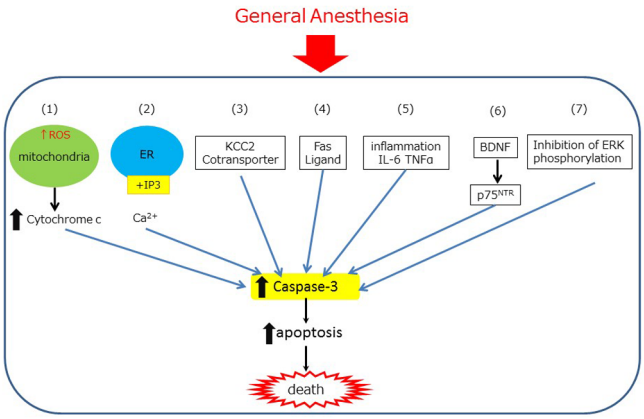

Figure 1 Anesthesia-induced pathways of neuronal apoptosis.
Anesthesia induces apoptosis by seven different pathways. (1) Mitochondrial pathway. Anesthesia induces excessive production of reactive oxygen species (ROS). This damages mitochondria, causing them to release cytochrome c and activate the apoptotic pathway. (2) Endoplasmic reticulum (ER) pathway. This involves anesthesia-induced activation of inositol 1,4,5-trisphosphate (IP3) receptors, leading to excessive Ca^{2+} release and subsequent downregulation of the mitochondrial anti-apoptotic protein BCL-X_L. (3) γ-Aminobutyric acid (GABA) pathway. The developmental transition from depolarizing to hyperpolarizing GABA-mediated neurotransmission is primarily mediated by an increase in the amount of the potassium-chloride cotransporter 2 (KCC2) during the brain growth spurt (BGS). (4) Death pathway. Anesthesia activates Fas protein, which induces apoptosis. (5) Inflammatory pathway. Inflammation involves anesthesia-induced neurotoxicity. (6) Pro-brain-derived neurotrophic factor (proBDNF) pathway. ProBDNF is involved in synaptogenesis and can induce neuronal apoptosis *via* p75 neurotrophic receptors. Neuronal activity stimulates tissue plasminogen activator (tPA) to convert plasminogen to the protease plasmin, which in turn cleaves proBDNF into mature BDNF (mBDNF). Anesthetics suppress tPA release from neurons, enhance p75 signaling, and reduce synapses, resulting in apoptosis. (7) Extracellular signal-regulated kinase (ERK) pathway. Anesthesia suppresses ERK phosphorylation. Apoptosis is caused by the inhibition of ERK phosphorylation during the BGS.

alcohol syndrome if the fetus is exposed during the BGS. In one study, stimulation of the $GABA_A$ receptor alone during the BGS induced neural apoptosis because of a developmental change in chloride gradients (Edwards et al., 2010). Other mechanisms include a decrease in brain-derived neurotrophic factor (Wu et al., 2016), activation of inositol 1,4,5-trisphosphate receptors (Wei et al., 2008), upregulation of Fas protein (Yon et al., 2005), activation of inflammatory markers (Shen et al., 2013), suppression of extracellular signal-regulated kinase phosphorylation (Yufune et al., 2016), and an increase in reactive oxygen species (ROS) (Boscolo et al., 2013). Excessive production of ROS induces mitochondrial damage, causing mitochondria to release proapoptotic proteins such as cytochrome c, thus initiating the apoptotic pathway.

Mechanistic understanding allows development of preventative therapies: If the mechanisms underlying neonatal anesthesia-induced damage are understood, preventative therapies can be developed. All drugs are used only in investigation, not in clinical practice. Protecting mitochondria from damage and preventing ROS accumulation has attracted much attention in the field of medical research. Melatonin (Yon et al., 2006), EUK-134, and hydrogen (Yonamine et al., 2013) have been found to alleviate anesthesia-induced neurotoxicity. Melatonin is a direct ROS scavenger, improves mitochondrial homeostasis, and stabilizes the inner mitochondrial membrane. EUK-134 is a synthetic ROS scavenger; its subcutaneous administration to rats on postnatal day 7 prevents anesthetic neurotoxicity. Molecular hydrogen is also an effective ROS scavenger and can be readily supplied as part of the carrier gas during anesthesia. Coadministration of hydrogen reduces oxidative stress induced by 6 hours of sevoflurane exposure. This nonselective antioxidant, used with intravenous or inhalational anesthetics, protects rodent neonates against neural apoptosis and long-term cognitive impairment.

Reducing excessive ROS is crucial for preventing the memory impairment caused by neonatal anesthetic exposure. In our own research, we have focused on nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, one of the most important sources of superoxide (Sun et al., 2016b). NADPH oxidase inhibitors, such as apocynin, show neuroprotective effects in traumatic brain injury and cerebral ischemia.

NADPH oxidase inhibitors are neuroprotective: We administered 3% sevoflurane to mice for 6 hours on postnatal day 6 after an intraperitoneal injection of apocynin (50 mg/kg) (Sun et al., 2016b). Sevoflurane exposure increased the concentrations of superoxide and the NADPH oxidase subunit p22phox in the brain, and this effect was decreased by the NADPH oxidase inhibitor apocynin. Neonatal sevoflurane exposure caused learning deficits in adult mice. However, apocynin suppressed apoptosis and mitochondrial damage and maintained long-term memory in the sevoflurane-exposed mice. Apocynin alone did not affect long-term memory. In a previous study, continuous administration of apocynin induced a significant change in cerebellar foliation and an alteration in motor behavior (Coyoy et al., 2013). These results indicate that NADPH oxidase is essential for normal brain development. Furthermore, the timing of apocynin administration is important for brain development.

Conclusions: Most general anesthetic agents at clinical concentrations are neurotoxic to the developing brain. Acute administration of general anesthetics causes neuronal apoptosis and persistent learning deficits. The mechanisms of anesthetic-induced neurotoxicity seem to involve alterations in the expression of ligand-gated ion channels and disturbances to intracellular calcium homeostasis and mitochondria-mediated apoptotic pathways. Interestingly, mice exposed to sevoflurane in the neonatal period have a normal brain structure as adults. These findings suggest that anesthetic exposure during the BGS may cause persistent functional, but not structural, changes and that these changes cannot be repaired by brain remodeling. Several agents reportedly prevent anesthesia-induced neurotoxicity, including hydrogen, melatonin, apocynin, and ketorolac, and they should therefore be coadministered with anesthetics. After anesthesia, only environmental enrichment can improve learning deficits due to anesthesia-induced neurotoxicity. Environmental enrichment increases the level of brain-derived neurotrophic factor and improves cognitive function. Further studies of environmental enrichment after anesthesia are necessary to develop preventative and therapeutic strategies for anesthesia-induced neurotoxicity. Environmental enrichment, such as an improved social life, may help to alleviate anesthesia-induced neurotoxicity in humans.

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