CLINICAL STUDY

Post-operative behavioural disorders in paediatric anaesthesia caused by anaesthetics and sedatives

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ABSTRACT
Neurotoxicity of anaesthetics have become one of the most discussed problems in paediatric anaesthesiology. The experimental studies on animal models have shown that the anaesthetics used in general anaesthesia should have an influence on neurodegenerative processes, neuroapoptosis and irregulated death of the neuronal cells.
Because of this fact, scientists are trying to discover the possibilities of how to minimize the adverse effects of anaesthesia and revise the other alternatives of prevention of anaesthesia-induced maladaptive behavioural disorders *(Tab. 1, Fig. 1, Ref. 21).* Text in PDF *www.elis.sk*
KEY WORDS: neurotoxicity of anaesthetics, maladaptive behavioural disorders, mechanism of neurotoxicity, post-anaesthetic behavioural changes in children, future of paediatric anaesthesiology.

Introduction

The results of recent studies indicate a neurotoxic effect of commonly used inhaled and intravenous anaesthetics on the developing brain of mammals, which has been long overlooked.

In retrospective and observational studies, the data in children who have undergone general anaesthesia appear to indicate behavioural and neurocognitive abnormalities. These disorders occur in up to 50% of children operated on at the youngest age and are resolved within 1 month after the operation. The frequency of this phenomenon is indirectly proportional to the child´s age and directly proportional to the painfulness of operation and restlessness of the introduction of anaesthesia.

In critically ill new-born children, especially those with low birth weight, a decrease in IQ, higher incidence of cerebral palsy and visual and hearing impairments have been reported at a later age. Especially at this age, it is very difficult to separate the consequences of stress, homeostasis disorders, surgery and several days of critical condition from the effect of anaesthetics.

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Influence of anaesthetics and sedatives on ontogenesis

The development and growth of the mammalian brain is a complex process starting with neurogenesis, continuing with the differentiation of neurons into different subpopulations, migration of nerve cells to their definitive localisation in the central nervous system (CNS), synaptogenesis, synapse formation and myelination of neuron-axon connections. These processes differ significantly depending on the gestational age and species of the mammal in direct relation to the expected life expectancy of the mammal.

Synaptogenesis begins in humans in the third trimester of pregnancy. Brain growth ends at the age of 2–3 years. During physiological development, CNS neurons are produced in considerable abundance. Their subsequent elimination is crucial for achieving normal brain size as well as its morphology. During ontogenesis, excess neurons are eliminated by 50–70 % by apoptosis (Moore, 2002).

Laboratory work on *in vitro* cultures demonstrates the dependence of the extent of neurodegenerative changes on the age of the individual, dose of anaesthetic and duration of exposure. Differences in regional distribution and cell-specific deleterious effects of anaesthetics on the developing brain as well as their persistence into adolescence in the dentate gyrus and *bulbus nervus olfactorius* areas were found. A similar effect is described after long-term administration of drugs (especially benzodiazepines) during treatment in intensive care units.

While the sensitivity of neurons to anaesthesia-indicated toxicity corresponds to a maximum in the development of synaptogenesis, the greatest susceptibility of oligodendrocytes during exposure to anaesthesia occurs at the time of myelination. Thus, both CNS components are highly sensitive to apoptotic neurodegeneration (Andropoulos, 2015).

Recent research points to a developmentally determined protective role of microglia during brain development and maturation because, under physiological conditions, the microglia alter the synaptic transmission and plasticity of the brain. Under certain conditions – hypoxia, infection, traumatic brain injury, autoimmune neurodegenerative processes – the microglial function is enhanced and can modify synaptic connections and plasticity of memory and learning (Kamat, 2019).

Many current research findings point to the neurotoxic effect of commonly used anaesthetics and sedatives on animal models after exposure to doses of anaesthetics used in paediatric anaesthesiology (O'Leary, 2017). The neurotoxicity of anaesthetics in animals, which persists into adulthood, depends on the dose and number of anaesthetics used, maturity of the developing brain at the time of exposure to the anaesthetic and presence of other factors, especially inflammatory processes in the body. The combination of all influences increases the sensitivity of the brain to the effect of the anaesthetic (Kamat, 2019). "Pharmaceuticals commonly used in intensive care units and operating theatres, such as isoflurane, benzodiazepines, barbiturates, etomidate, propofol and ketamine, are involved in the development of neurotoxicity in animals" (Kamat, 2019). Although these effects on the human body have not yet been clearly demonstrated, a link between anaesthetic exposure and acquired neurological development disorder in children is evident.

In particular, exposure of children to anaesthetics at an early age causes transient suppression of neurogenesis, ultrastructural abnormalities of synapses and alterations in the development of the signalling and neuroinflammatory neural networks, loss of neurons, production of free radicals and alterations of mitochondrial integrity. These side effects on the basis of developing neuronal connections can result in acute neuronal damage as well as long-lasting neurocognitive dysfunctions (Deshui, Yu, 2016, O´ Leary, 2016, Kamat 2019). Cognitive deficits are mainly related to the hippocampal region, where the extent of damage to their neurons is much greater compared to other brain regions.

This provides a possible explanation for the neuroapoptosis following anaesthesia described in this area by most studies, while long-term neurocognitive dysfunctions are described in only a few of them. An increase in the incidence of cell death after anaesthetic exposure does not necessarily lead to a significant reduction in neuronal density in old age. During development, 50–70 % of all CNS cells undergo natural cell death while maintaining the physiological structure of the CNS. It remains unclear whether anaesthesia accelerates the apoptosis of neurons that are primarily destined for death in physiological degeneration, or damages healthy neurons that are not primarily destined for death. Thus, the fact that cognitive deficit is caused by cell death with consequent loss of neurons, i.e, not only by cell death itself, remains an important finding. At the same time, it remains unclear whether neuroapoptosis is the only cause of cognitive dysfunction (Deshui Yu, 2016).

In children, associations have been found between long-term exposure to anaesthetics and sedatives, especially GABAergic, and subsequently lower levels of neurological development up to the age of 12–48 months.

A special group among these patients are children with congenital heart and vascular diseases, whose survival has increased by up to 90 % compared to the past due to neonatal surgery. In 30–50 % of these children, intelligence disorders, major or minor motor dysfunctions, and receptive and expressive speech disorders occur after undergoing cardiac surgery. Memory disorders, speech disorders, counting disorders and visual-motor coordination are observed at the age of school entry and integration of these children.

The mechanism of anaesthesia-induced neurotoxicity documented in studies

The effect of most anaesthetics results from their action as NMDA receptor antagonists and/or GABA A agonists. Anaesthesia-induced neurotoxicity is mediated through the mitochondrial apoptotic cascade (the internal part of the anaesthetic-induced apoptotic cascade), which activates the neurotropic cascade and subsequently the cascade leading to the destruction of these receptors. Thus, commonly used anaesthetics cause extensive apoptotic neurodegeneration in various parts of the brain during its development.

The main role of every mitochondrion in the cell is the production of energy through oxidative phosphorylation. In addition to the latter role however, mitochondria have many regulatory functions that are important for further survival as well as cell death, as exemplified by the intrinsic apoptotic cascade, which leads to organised and controlled cell death. The activation of this cascade is caused by the release of cytochrome c from the mitochondria into the cytosol. Cytochrome c forms apoptosomes followed by the activation of apoptotic protease factor (APAF-1) to form deoxyadenosine triphosphate (dATP) and adenosine triphosphate (ATP). The binding of the apoptosome complex activates procaspase -9. The activated caspase-9 activates caspase-3, resulting in deoxyribonucleic acid (DNA) fragmentation and cell death.

The physiological function of cytochrome c is the transfer of electrons between complexes III and IV of the respiratory chain during oxidative phosphorylation. However, the binding to cardiolipin can also have peroxidase effects, namely by inducing the oxidation of hydroperoxycardiolipin and thereby contributing to the development of pro-apoptotic stimuli. Oxidative stress contributes significantly to its production.

The following figure shows the internal and external pathways of the apoptotic cascade, as well as antiapoptotic effects of dexmedetomidine and erythropoietin (Fig. 1).

Many inhaled (isoflurane, sevoflurane) and intravenous (propofol) anaesthetics increase free radical production in the developing brain. The exposure to anaesthetics, even under conditions of normoxaemia, increases the production of free oxygen and nitrogen radicals in developing neurons, hippocampus, subiculum, and thalamus. Oxidative stress caused by anaesthetics leads to peroxidation of membrane lipids, damage to mitochondria and impairment of their integrity.

The binding of apoptosis-induced ligands to cell death receptors activates the external part of the anaesthetic-induced apoptotic cascade. The main pro-apoptotic ligands are tumour necrosis factor
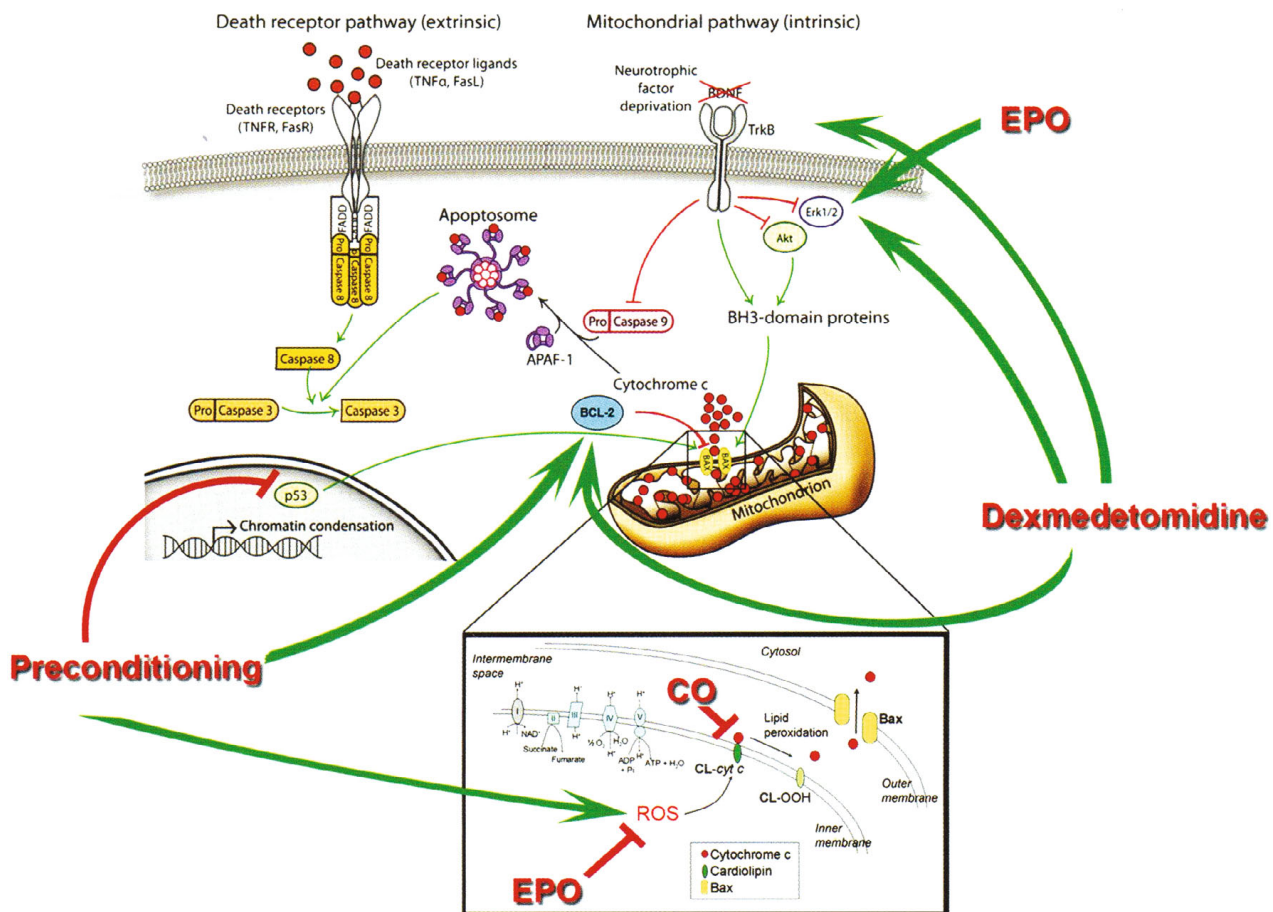
Fig. 1. Illustration of the internal and external pathways of the apoptotic cascade (taken from: Andropoulos DB, Anesthesia for Congenital Heart Disease, 3rd edition, Wiley, 2015, p. 188)

alpha (TNF), FasL and TNF-related apoptosis-including ligands (TRAILs). The activation of cellular death receptors by the adoption of the intracellular Fas-associated death domain (FADD) leads to the internalisation of procaspase-8, which results not only in its activation but also in the activation of caspase-3. Simultaneous exposure to isoflurane together with nitric oxide and midazolam upregulated Fas receptors and activated caspase-8 in the cortex of parietal and occipital lobes in an experiment in 7-day-old laboratory rats. While the internal pathway of the apoptotic part of the cascade was activated as early as 2 hours after the exposure to this combination of anaesthetics. The time difference was due to the dependence on Fas protein expression and upregulation.

NMDA antagonists and GABA receptor agonists cause neuronal cell death by activating the mitochondrial portion of apoptosis.

Neurotrophins belong to a group of growth factors that determine the survival and differentiation of neurons and the plasticity of synapses. They form a group of growth factors that determine neuronal survival, differentiation and plasticity of synapses. Already in 7-day-old laboratory rats exposed to a 6-hour mixture of isoflurane, nitric oxide and midazolam, a decrease in the activity of their main representative BDNF (brain-derived neurotrophic factor) in the developing thalamus was demonstrated.

There are suspicions that opioids may induce apoptosis of developing neurons. For example, infusion of continuous fentanyl results in an increase in caspase-3 levels in specific brain sections of 5-day-old pigs as compared to unexposed individuals. Other studies describe the determining effect of morphine on the developing cortex and amygdala. The area of the hippocampus remains surprisingly spared from these influences. The neurotoxic effects of propofol have also been extensively studied and its induced neuronal apoptosis has been described in both rodents and primates (Andropoulos, 2015).

The immature brain is very susceptible to anaesthesia-induced neuronal apoptosis. The fact remains that some neurons undergo cell death after exposure to anaesthetics, while other neurons survive intact. Hofacer et al. demonstrated that the neurotoxicity of anaesthetics depends more on the age of the neuron than on the age of the organism. They pointed out that postnatal gyrus cells undergoing isoflurane-induced neurodegeneration are young and relatively immature. They reach the maximum anaesthetic-induced

Tab. 1. Neurodegenerative and neuroprotective effects of anaesthetics (taken from: Andropoulos DB, Anesthesia for Congenital Heart Disease, 3rd edition, Wiley, 2015, p. 186.

Agent	Primary receptor binding site				Neurodegenerative effects		Neuroprotective effects	
	GABA	NMDA	μ- opioid	A2- adrenergic	Neuroapoptosis	Other neurodegenerative effects	Neuroprotection: anesthetic-induced neuroapoptosis	Neuroprotection: excitotoxic neuronal death hypoxia– ischemia
Halogenated anesthetics (sevoflurane, isoflurane, desflurane)	+++	–			+++	+	–	+++
Nitrous oxide		–			+/NE	NE	–	NE
Benzodiazepines	++				++	++	–	NE
Propofol	++				+	+	–	ND
Barbiturates	+				+	+	ND	ND
Etomidate	+				+	ND	ND	ND
Chloralhydrate	+				++	ND	ND	ND
Ketamine		–			+++	+	+	+++
Opioids			+++		+,NE	+	ND	NE
Dexmedetomidine				++	NE	NE	++	++
Xenon		–			NE	NE	++	+

vulnerability at the age of 2 weeks after birth. They have also focused their research on olfactory bulb cells which undergo neurogenesis into adulthood, where they have also demonstrated their susceptibility to anaesthesia-induced apoptosis.

The heterogeneity of susceptibility to neurotoxic effects may vary with age. This fact increases the possibility of confirming the assumption that anaesthesia-induced neurotoxicity depends on the age of the organism at the time of its exposure to the adverse effect. Furthermore, it indicates that the time of manifestation of the neurotoxic effect may exceed the time of early childhood. Young rhesus macaques which were repeatedly exposed to sevorane developed anxiety behaviour up to 6 months after exposure as compared to the unexposed sample. Thus, the study only confirms the effect of general anaesthesia on the development of behavioural disorders with a longer time interval from exposure to anaesthetics in primates (O´ Leary, 2016).

Table 1 summarises the neurodegenerative and neuroprotective effects of anaesthetics.

Consequences of neurotoxicity

The success of current intensive care therapy contributes significantly to the increased survival rate of critically ill patients. However, after overcoming this critical and life-threatening period, a significant proportion of paediatric patients have motor, cognitive, and psychological consequences. "The cognitive deficit after overcoming a critical illness leads to a decrease in IQ, poorer school performance and attention and memory disorders. Risk factors for cognitive impairment in patients after hospitalisation in intensive care units include artificial lung ventilation, extracorporeal vital signs, traumatic impairment, oncological and neurological diseases, and use of medication for sedation as well as opiates" (Kamat, 2019).

Postnatal neurogenesis is a critical period for the development of learning and memory which are located mainly in the hippocampus. The exposure to the anaesthetic during peak synaptogenesis significantly reduces the synaptic transmission and synapse density, thereby causing a lack of synaptic connections and inhibition of synaptic transmission.

The development of neurons also depends on the integrity of the CNS and proper function of the astroglia, Thus, there is a reasonable suspicion that the adverse effects of anaesthesia may also occur through its pathways. The adverse effect of reducing the levels of the excitatory neurotransmitters, namely of aspartate and glutamate in the cortex and hippocampus on the modulation of learning and memory is also expected (Deshui Yu, 2016).

The focus of some current laboratory research is on therapeutic and preventive strategies for neurotoxicity. Of these, the beneficial effects of dexmedetomidine and mechanism of upregulation of anti-apoptotic proteins, are evident (Andropoulos, 2016). Erythropoietin also crosses the blood-brain barrier, stimulates neurogenesis, induces neuronal differentiation, activates neurotropic signalling, and it has anti-apoptotic, antioxidant, and anti-inflammatory properties. Due to the anaesthetic-induced production of inflammatory mediators, and development of oxidative

stress described after the use of inhaled anaesthetics (isoflurane), the effects of vitamins (vitamins B3 and D3) and other nutrients are also studied (Andropoulos, 2016).

Conclusion

Anaesthesia-induced neuronal damage should have far-reaching consequences in the future of paediatric patients. Therefore, the research into the neurotoxicity of drugs used in patients in paediatric intensive care medicine and anaesthesiology should be aimed at elucidating the absolute and dose-anaesthetic effect of these drugs on the developing brain. Understanding the mechanism of toxicity of anaesthetics to the developing nervous system, in particular the mechanism by which anaesthesia impairs brain function for up to one month, would significantly contribute to the prevention and development of therapeutic strategies (Deshui Yu, 2016). Further research will be needed to clarify the stage of hippocampal neurogenesis with which anaesthetics interfere, thereby inducing impaired synapse development and their remodelling as a potential cause of cognitive dysfunction. Research will also focus on neurotoxicity therapy options, preventive methods and protection of the developing brain from neurotoxic effects. Some ongoing preclinical animal studies have already shown positive results. If the neurodegenerative effects of anaesthetics are explicitly demonstrated in the future, it will be important to apply therapy and procedures to maintain safety with minimal risk to the paediatric patient. It is also important to focus on the possibilities of adaptation of paediatric anaesthesiologists and paediatric intensivists to prevention and the possibilities of limiting the potential negative impact of anaesthesia on the neurological development of children.

Given the repeated observations of daily practice, other relevant and informative scoring systems will need to be developed in the near future. They should be specifically focused not only on the value for identifying behavioural changes but also on the speed and quality of recovery from original cognitive function as well as recovery time from potential adverse effects of anaesthesia.

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