Review Article

Perils of paediatric anaesthesia and novel molecular approaches: An evidence-based review

Address for correspondence:
Dr. Sukhminder Jit Singh Bajwa, House No-27-A, Ratan Nagar, Tripuri, Patiala, Punjab, India.
E-mail: sukhminder_bajwa2001@yahoo.com

Sukhminder Jit Singh Bajwa, Smriti Anand[1], Hemant Gupta[2]

Department of Anaesthesiology and Intensive Care, Gian Sagar Medical College and Hospital, Banur, Punjab, India, [1]Department of Anaesthesiology and Intensive Care, Maharishi Markendeshwar Medical College and Hospital, Kumarhatti, Solan, Himachal Pradesh, [2]Department of Paediatrics, Indira Gandhi Medical College, Shimla, Himachal Pradesh, India

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ABSTRACT

Evolution of anaesthesia has been largely helped by progress of evidence-based medicine. In spite of many advancements in anaesthesia techniques and availability of newer and safer drugs, much more needs to be explored scientifically for the development of anaesthesia. Over the last few years, the notion that the actions of the anaesthesiologist have only immediate or short-term consequences has largely been challenged. Evidences accumulated in the recent years have shown that anaesthesia exposure may have long-term consequences particularly in the extremes of ages. However, most of the studies conducted so far are *in vitro* or animal studies, the results of which have been extrapolated to humans. There have been confounding evidences linking anaesthesia exposure in the developing brain with poor neurocognitive outcome. The results of animal studies and human retrospective studies have raised concern over the potential detrimental effects of general anaesthetics on the developing brain. The purpose of this review is to highlight the long-term perils of anaesthesia in the very young and the potential of improving anaesthesia delivery with the novel molecular approaches.

Key words: Apoptosis, brain, general anaesthesia, neurodegeneration, paediatric anaesthesia, synaptogenesis

INTRODUCTION

Paediatric patients range from preterm infants to teenagers, and it would not be appropriate to call them miniature adults. There exist definite anatomical, physiological, pharmacological and psychological differences among the different paediatric age groups and between the paediatric and adult patients, which makes the safe anaesthetic delivery extremely challenging. Recently, there have been conflicting reports over the potential neurotoxicity of the general anaesthetics on the developing brain. Particularly intriguing are the long-term neurocognitive outcomes of anaesthesia in the very young.[1] This has generated a lot of concern because anaesthesia delivery among paediatric patients is no longer confined to the operation theatres and intensive care units but is also being increasingly used in the non-surgical settings such as long diagnostic procedures, radiological and interventional studies, to allay pain and anxiety and to maintain stable vitals: This has resulted not only in a tremendous increase in the number of anaesthetics being administered but also increased anaesthetic exposure in progressively younger age groups.[1]

Research articles, meta-analyses and systematic reviews and studies in various international and national bibliographic indices were extensively searched with emphasis on key words 'apoptosis, synaptogenesis, neurodegeneration, neurotoxicity, general anaesthesia (GA) in paediatric patients' published between the period of 1994 and 2014. The various search engines included Entrez (including PubMed), NIH.gov, Cochrane database for systematic reviews, Science direct, Scopus, WebMD.com, MedHelp.org, Searchmedica, MD consult and Google.com. The inclusion criteria was mainly focused on extraction of full text articles containing literary

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evidence of anaesthesia in paediatric patients from the previous two decades. Manual search was also carried out, and various text books of paediatric anaesthesia, anaesthesiology and paediatric surgery were looked into for evidences of possible effect of GA on the paediatric population.

APOPTOSIS AND SYNAPTOGENESIS

Apoptosis or programmed cell death is a normal physiological process occurring during the development of central nervous system to remove the redundant cells. It has particularly gained importance in relevance to paediatric anaesthesia. It has been found that stress responses (such as pain, hypoglycaemia, hypoxia and ischemia), environmental factors, genetic disturbances and drugs cause thwarting or exacerbation of apoptosis, a pathological phenomenon, resulting in suicide of vital brain cells manifesting as neurodevelopmental aberrations in foetuses and infants. Of particular significance is the stage of synaptogenesis, that is the period of 'brain growth spurt' which corresponds to 6th month of pregnancy and extends to 3rd year after birth in humans and the first 2 weeks of life in rodents and mice.[2] During this period, very few neurons are destined to die. However, interference of certain neurotransmitters during this period can cause widespread neuroapoptosis.

Receptor modulation and neurotoxicity

Excitatory N-methyl-D-aspartate (NMDA) and inhibitory gaba-amino-butyric acid (GABA) receptor stimulation are pivotal during neurogenesis. Excessive antagonism of NMDA receptors or agonism of GABA receptors triggers neuronal apoptosis. Most of the commonly used general anaesthetics have a strong affinity for these receptors thereby elucidating their mechanism of apoptosis. Several studies conducted on animal models have shown accelerated neurodegeneration through general anaesthetic-induced NMDA and GABA receptor modulation, which resulted in behavioural abnormalities in rats, mice, guinea pigs and non-human primates.[3] Inhalational anaesthetics like nitrous-oxide (N_2O),[4] isoflurane,[5] sevoflurane[6] and desflurane[7] as well as intravenous anaesthetics such as ketamine,[8] propofol,[9] thiopentone[10] and diazepam[11] used alone or in combination have been implicated as triggering neurotoxic agents. Ethanol a potent NMDA-antagonist and GABA-agonist causes widespread apoptotic neurodegeneration in the developing rat forebrain during the periods of synaptogenesis, which manifests in the humans in the form of neurobehavioral abnormalities collectively called as foetal alcohol syndrome.[11]

Pharmacological evidence of neurotoxicity

Ketamine, a potent NMDA receptor antagonist, is a widely used anaesthetic agent in paediatric patients. Its neurotoxicity in animal studies has been very well documented[12] [Table 1]. *In vitro* studies of ketamine on human embryonic stem cells (hESCs) – derived neural stem cells (NSCs) and neurons have shown that it increases NSC proliferation and causes neuronal apoptosis through reactive oxygen species (ROS) mediated mitochondrial pathway.[20]

Propofol, another potent intravenous anaesthetic agent, when continuously administered for 5 h, causes apoptosis of neurons and oligodendrocytes in both the foetal and neonatal non-human primate brain.[21] However, it induces neurotoxicity at one-fourth of the dose required for surgical anaesthesia.[9]

Barbiturates like pentobarbital and phenobarbital, as well as benzodiazepines like diazepam and clonazepam, have been found to trigger neurodegeneration in mouse and rat pups with consequent alterations in long-term neurobehavioral outcomes.[22]

Similar to GABA and NMDA receptors, opioid receptors are also involved in early brain development and synaptogenesis[23], which highlights their effects during synaptogenesis. Morphine,[24] buprenorphine and methadone[25] have all been incriminated as possible triggers. High dose fentanyl significantly accentuates white matter brain lesions-induced by glutaminergic overstimulation in mice.[26]

Inhalational anaesthetic agents are >100-fold less potent than intravenous anaesthetics and less target selective. Experimental studies with halothane, a commonly used inhalational anaesthetic, have shown that its chronic exposure during the developmental period in rats causes both neural and behavioural impairment.[16] Sevoflurane is the preferred inhalational agent in paediatric patients because of its rapid onset and offset, less irritation for airways, decreased pungency and stable haemodynamic profile.[27] It has both NMDA receptor antagonist and GABA receptor agonist properties. Animal studies link sevoflurane exposure in developing a brain with neuroapoptosis.[9] Feng *et al*. demonstrated that a single 6 h exposure of 2.3% sevoflurane in neonatal rats-induced significant apoptosis and decreased neuronal nitric acid synthase

Table 1: Evidence of anaesthetic drugs causing neurodegeneration

Name of the drug	Name of the study	Subject used	Possible toxicity/ hazard	Potential dose of toxicity	Mechanism of toxicity	Any other harmful effect
Ketamine	Slikker *et al*.[13] Young *et al*.[14] Wu X *et al*.[15]	Perinatal rhesus monkey Infant mouse brain	Neurotoxicity	Significant neuroapoptosis at 20-30 mg/kg and marginally non-significant toxicity at 10 mg/kg[15]	NMDA receptor antagonist. Interferes with ROS mediated mitochondrial pathway	Epileptogenic which further enhances general anaesthesia-induced apoptosis
Propofol	Cattano *et al*.[9]	Neonatal mice	Neurotoxicity	5 mg/kg (subcutaneous/ intraperitoneal) or repeated doses exceeding 20 mg/kg/h for 4-5 h	$GABA_A$ receptor agonist mediated apoptosis	
Barbiturates (phenobarbitol and pentobarbital)	Fredriksson *et al*.[10]	Neonatal mice	Neuroapoptosis	5 mg/kg of thiopentone, when co-administered with 25 mg/kg of ketamine causes significant apoptosis	$GABA_A$ receptor agonist mediated apoptosis	
Ethanol	Ikonomidou *et al*.[11]	Immature rat brain	Neurodegeneration		$GABA_A$ receptor agonist and NMDA receptor antagonist	Foetal alcohol syndrome
Halothane	Levin *et al*.[16]	Rats	Neurodegeneration		NMDA antagonist and GABA agonist	Behavioural syndrome including learning impairment decreased exploratory behaviour and decreased nociceptive reactivity
Sevoflurane	Feng *et al*.[17]	Neonatal rats	Neuroapoptosis	2.3% MAC	NMDA antagonist and GABA agonist. Causes decrease in nitric oxide synthase levels	
Isoflurane	Jevtovic-Todorovic *et al*.[4]	7 days old infant rats	Neurotoxicity	0.7-1.5% MAC	NMDA antagonist and GABA agonist	
Desflurane	Kodama *et al*.[18]	6 days old mice	Neurodegeneration	8% MAC	NMDA antagonist and $GABA_A$ agonist	
Nitrous-oxide	Jevtovic-Todorovic *et al*.[4]	7 days old infant rats	Neuroapoptosis		NMDA receptor antagonism	
Chloral hydrate	Cattano *et al*.[19]	Immature mouse pups	Neurodegeneration	100 mg/kg	GABA receptor agonist mediated neurotoxicity	

GABA – Gaba-amino-butyric acid; NMDA – N-methyl-D-aspartate; MAC – Minimum alveolar concentrations; ROS – Reactive oxygen species

levels in the hippocampus within 24 h exposure.[17] Furthermore, epileptiform activity with sevoflurane has been well documented in the literature.[28] Studies with isoflurane on rodents have shown its deleterious effects on neurogenesis and long-term behavioural outcomes.[5] When used in human neonates, hyperexcitation as evident by electroencephalography has been well documented.[29] Similarly, conflicting results have been reported with N_2O,[4] desflurane[7] and xenon.[30]

Chloral hydrate, a sedative agent, which is a chlorination product of ethanol, has largely been replaced by barbiturates and benzodiazepines in paediatric clinical practice. However, when used in doses of 100 mg/kg and above for sedation and radiological studies, it has been found to induce apoptosis in cerebral cortex and caudate-putamen complex in immature mouse pups.[19]

FACTORS AFFECTING APOPTOSIS BY GENERAL ANAESTHETICS

Dose

Low doses of the anaesthetic agent do not cause significant apoptosis while higher concentrations are consistently associated with neuro-pathological effects [Table 2]. Ketamine, for instance, when

Table 2: Risk factors enhancing apoptosis by general anaesthetics

Risk factors	Mechanism of injury	Possible consequences	Literary evidence
Dose and number of exposures of the anaesthetic agent	Higher doses and repeated administration of ketamine causes enhanced expression of NR1 subunit of NMDA receptor	Neuroapoptosis	Zou *et al*.[31]
Duration of exposure	Prolonged exposure, e.g., 6 h exposure to 0.25 MAC isoflurane causes increased hippocampal caspase-3 mRNA levels	Neuronal cytotoxicity	Zhao *et al*.[32]
Type of anaesthetic agent	Variability of effects of general anaesthetics on mitochondrial function	Enhanced neurotoxic profile of desflurane than equivalent doses of sevoflurane and isoflurane	Kodama *et al*.[18] Lei *et al*.[33]
Double and triple cocktail regimes	Increased anaesthetic depth, NMDA receptor antagonism, GABA receptor agonism, mitochondrial alterations	Augmented neurodegeneration	Young *et al*.[14]
Type of receptor	NMDA receptor antagonism and GABA receptor agonism	Apoptosis of neurons	Ikonomidou *et al*.[12]
Stage of brain development and age at the time of anaesthesia exposure	Developing brain is susceptible to general anaesthesia-induced mitochondrial injury, which results in increased ROS	Neurodegeneration with neurodevelopmental and neurobehavioural abnormalities later in life	Gutierrez *et al*.[34] Lei *et al*.[33] Kalkman *et al*.[35]
Surgical factors	IL-1β levels during surgery cause upregulation of $GABA_A$ receptors	Synergism with anaesthesia-induced neuroapoptosis	Wang *et al*.[36]
Presence of comorbities (low birth weight, hypoxia, hypoglycemia, congenital malformations etc.)	Cause developmental delay	Confounding factors	Taylor[37]

NMDA – N-methyl-D-aspartate; MAC – Minimum alveolar concentrations; GABA: Gaba-amino-butyric acid; ROS – Reactive oxygen species; IL-1β – Interleukin 1 beta

given in high doses induces seizures, which enhances neuroapoptosis. Using allometric scaling, animal drug doses with injectable anaesthetics comparable to human doses have been found to be 3-, 6- or 12-fold, respectively, for monkeys, rats or mice.[38] Using this scale it was found that plasma concentrations of ketamine were 3–10 times higher in rodents and small monkeys than those observed during clinical human practice thereby indicating that neurotoxic profile of large doses of injectable anaesthetics like ketamine cannot be extrapolated to humans. Doses of inhaled anaesthetics required to produce immobility in animals are, however, similar to clinically used doses.

Duration and number of exposure

Prolonged and repeated exposure to anaesthetic agents cause accelerated apoptosis. Isoflurane, for example, when administered at 5 minimum alveolar concentrations (MAC) is neuroprotective against ischaemia, but its repeated or prolonged exposure for 6 h in developing rat hippocampal neurons even at 25 MAC is significantly neuroapoptotic.[32] Similarly, ketamine exposure for 9 h causes significantly more apoptosis[13] than when exposed for 3 h.[31]

Type of anaesthetic agent

The anaesthetic agents differ in their neurotoxic profile. Kodama *et al*. demonstrated that administration of desflurane (8%) was more deleterious on neuronal cells in neonatal mice than exposure of equivalent doses of isoflurane (2%) and sevoflurane (3%).[18]

Combination of anaesthetic agents

Anaesthetics when given in combination are more deleterious on neurons than when given alone. A cocktail of isoflurane, midazolam and N_2O when administered to rat pups causes enhanced apoptosis.[14] Significant neurodegeneration was observed with triple than double cocktail regime.

Receptor type

N-methyl-D-aspartate and GABA receptor stimulation is pivotal for neuronal survival, and their excessive excitation or inhibition accelerates neuroapoptosis. This effect is exclusive to NMDA and GABA receptors as other excitatory or inhibitory systems like dopaminergic and muscarinic do not produce these effects.[12]

Stage of brain development

Experimental studies in rat brain have shown that anaesthesia-induced cell-death is age dependent peaking at postnatal day (PND) 7, declining by PND14 and reaching nil by PND21.[34]

Age at the time of anaesthesia exposure

The period of synaptogenesis during which the rodents are most vulnerable to anaesthetic insult approximates with the period of human development that occurs

from the third trimester in utero to the third year of life. Indeed, several retrospective cohort studies have linked early anaesthesia exposure at <3 years of age with unfavourable learning and behavioural outcomes.[39] In fact, human epidemiological studies have shown a higher incidence of neurocognitive derangements on delivery of anaesthesia to infants than to children above 1 year of age.[35]

Effects of surgery

During surgery, there is substantial inflammation, risk of infection, blood loss as well as fluid shifts, which can be tremendous. It has been found that there are adverse neurological outcomes with isoflurane when combined with surgical stimulation.[40] However, a study by Liu *et al*. has shown that noxious stimuli attenuate ketamine-induced neuroapoptosis.[41] Surgery is associated with increased levels of interleukin 1 beta (IL-1β), which causes up regulation of $GABA_A$ receptors on neurons thereby increasing the neurotoxic profile of the delivered anaesthetic agent.[36] Surgery therefore if not additive has a significant synergistic role in GA-induced neurodegeneration.

Presence of other comorbidities

Low birth weight, perinatal hypoxia, infection or haemorrhage as well as hypotension, hypoglycaemia and congenital malformations and abnormalities such as cyanotic congenital heart disease, congenital diaphragmatic hernia, and oesophageal atresia-all have confounding effects on anaesthesia-induced apoptosis.[37]

Pharmacogenetic factors

Pharmacogenetics deals with the hereditary basis for differences in inter-individual responsiveness to the therapeutic agents. Pharmacogenetics in paediatric anaesthesia is enigmatic because children in addition to exhibiting the same inter-individual genetic variability as seen in adults also present further differences arising from different stages of development.

Wilke *et al*. have identified a gene GABARE, which encodes for class epsilon of $GABA_A$ receptors (gene map locus Xq28).[42] Increased sensitivity to diazepam, barbiturates and propofol has been linked to the genetic variation in the gene coding for this subunit of GABA receptor. It has been found that volatile anaesthetics act on a different site on $GABA_A$ receptor molecule although nature and location of that site remains unclear. The genetic impact of the drug on its pharmacodynamic and pharmacokinetic profile determines its effects and side effects. An active drug exhibiting slow metabolism because of increased receptor sensitivity will have enhanced toxicity than an extensively metabolised drug due to reduced receptor sensitivity.

MOLECULAR MECHANISMS OF ANAESTHESIA-INDUCED NEUROTOXICITY

N-methyl-D-aspartate receptor antagonists and GABA receptor agonists trigger widespread cerebral apoptosis in the developing rodent brain manifesting in the long-term as neurobehavioral impairments. Zhou *et al*. demonstrated that 70% N_2O and 0.75% isoflurane exposure for 6 h caused enhanced apoptosis of glutaminergic, GABAergic and dopaminergic neurons in the developing brain but not of cholinergic neurons in basal forebrain[43] from which it can be deciphered that anaesthetics with NMDA receptor blocking or GABA receptor potentiating effects trigger neuronal damage in cell type specific manner.[33]

Anaesthesia exposure at the time of synaptogenesis has been found to interfere with mitochondrial morphology and functioning manifesting as anaesthesia triggered apoptosis acutely and long-term neurocognitive aberrations later in life.[33] It has been found that anaesthesia-inflicted-mitochondria generates ROS, which if inadequately scavenged causes excessive lipid peroxidation and consequent cell scathing.

Anaesthesia-induced increased levels of neuro-inflammatory mediators have also been implicated in neuronal insult. Experimental studies have shown that volatile anaesthetics increase pro-inflammatory cytokines like tumour necrosis factor alpha (TNF-α), IL-6, and IL-1β in brain tissues of mice[15] as also surgical trauma which causes augmented expression of TNF-α, IL-1β and microglia activation in hippocampus.[44] However, which of the two, that is, anaesthesia or surgery is bigger culprit is difficult to answer.

Alterations in the level of neurotrophins have also been associated with enhanced neuronal toxicity. One such factor is the brain-derived neurotrophic factor-BDNF, which normally binds with the Trk receptor, but its precursor form, that is, proBDNF binds with $P75^{NTR}$ and induces apoptosis.[45]

Similarly, other signal transduction pathways like up regulation of PKCα and p-JNK and down regulation of

p-ERK and Fos protein in hippocampus of neonatal rats are other possible mechanisms of inciting apoptosis as also the dysregulation of intracellular calcium haemostasis[33] and enhanced expression of tumour suppressor protein 53 (p53) expression by the general anaesthetics.[46]

EVIDENCE ON LONG-TERM PERILS OF GENERAL ANAESTHESIA

Several retrospective cohort studies have shown that paediatric patients exposed to general anaesthetics early in life have poor neurobehavioral outcomes than their unexposed peers. DiMaggio *et al*. in one such study compared 383 children who underwent inguinal hernia repair during first 3 years of life with 5050 children who did not and found that children who underwent surgery were twice as likely the controls to be diagnosed with behavioural or developmental disorders.[39] Wilder *et al*. investigated whether anaesthesia delivered at <4 years of age was associated with long-term learning disabilities and concluded that learning disabilities were more obvious in children who had >1 anaesthetic exposure and total duration of anaesthesia exposure of >2 h was associated with increased incidence.[47] Similarly, results from the Victorian Infant Collaborative Study Group of 1996 have concluded that premature or low birth weight infants requiring surgery and anaesthesia during primary hospitalization have poor sensorineural outcome.[48]

These neurobehavioral aberrations commonly manifest in the form of attention deficit hyperactivity disorder, autism and learning disabilities in humans.

At present several clinical trials are underway to assess long-term neurocognitive outcomes of anaesthesia exposure in children. These include:

General versus spinal anaesthesia study

It is a multicentric randomised controlled trial aimed at comparing the two common modes of anaesthesia, i.e., regional and GA for assessment of neurocognitive outcomes at 2 years and 5 years post-inguinal hernia repair[49] as this is the most common surgery done in infants and will enlighten on safety or toxicity of general anaesthetics in this age group.

Paediatric anaesthesia and the neurodevelopment assessment

This study is another multicentric trial aimed at studying two groups of children: Those exposed to single dose of GA for inguinal hernia repair before 3 years of age and those who are siblings of the first group who have not and assess their neurocognitive outcome between 8 and 15 years.

Mayo clinic research: Mayo safety in kids study

This study compares the performance of children with no anaesthetic exposure to those with single or multiple exposures prior to 3 years of age using a battery of neurocognitive tests including operant test battery. The results of all these trials are eagerly awaited as they will elucidate whether general anaesthetics have the same neurotoxic profile in human paediatric populations as in experimental animals.

NOVEL MOLECULAR APPROACHES IN IMPROVING GENERAL ANAESTHESIA IN PAEDIATRIC PATIENTS

Considering the neurotoxic profile of general anaesthetics, following novel neuroprotective molecular strategies have been explored to ameliorate this flaw [Table 3].

Dexmedetomidine

A centrally acting alpha-2 receptor agonist, dexmedetomidine produces sedation and analgesia without inhibiting respiration. It has a trophic role during neurogenesis apart from being neuroprotective. *In vivo* dexmedetomidine curtails isoflurane-induced injury in the hippocampus, thalamus and the cortex and also inhibits isoflurane-induced caspase-3 expression in hippocampal cultures *in vitro*.[50] Similarly, clonidine another alpha-2 receptor agonist abolishes ketamine-induced apoptosis in mice.[51]

Erythropoietin

Erythropoietin (EPO) has a neuroprotective role to play during periods of neuronal insults such as hypoxia, haemorrhage and ischaemia as elucidated in both *in vivo* and *in vitro* studies. Tsuchimoto *et al*. demonstrated that isoflurane-induced neuroapoptosis in PND 7 mice was significantly less when 50,000 IU/Kg of rEPO was administered subcutaneously prior to a 6 h exposure of isoflurane (1%) as compared to isoflurane alone.[52]

Lithium

Lithium, another promising agent, has been found to impart neuroprotection by pretreatment (100 mg/kg intraperitoneal) in rats exposed to sevoflurane by stimulating glycogen synthase kinase-3β and inhibiting apoptosis through the BDNF-Akt-Bcl-2 signalling pathway.[53]

Table 3: Neuro-protective evidence from novel approaches

Neuroprotective strategies	Mechanism of toxicity prevention	Literary evidence/ name of the study	Possible effects of strategies employed
Alpha-2-receptor agonists like dexmedetomidine and clonidine	Inhibits isoflurane-induced caspase-3 expression in hippocampal cultures *in vitro* as well as ketamine-induced apoptosis in mice. Neurotrophic	Sanders *et al*.[50] Pontén *et al*.[51]	Neuroprotection
EPO	Enhances neurotransmitter release and its pretreatment prior to isoflurane exposure prevents learning disability in mice. Also neurotrophic	Tsuchimoto *et al*.[52]	Attenuation of general anaesthesia-induced apoptosis.
Lithium	Stimulates glycogen synthase kinase 3-β and inhibits apoptosis by BDNF-Akt-Bcl-2 signalling pathway	Leyhe *et al*.[53]	Neuroprotection
Vitamins (thiamine, nicotinamide, Vitamin D_3 and Vitamin C)	Downregulation of Bax, inhibition of cytochrome c release, reduction in activated caspace-3 levels andinhibition of isoflurane-induced release of proinflammatory cytokines by nicotinamide and increased expression of calcium binding proteins by Vitamin D as well as antioxidant action of Vitamin C	Jaatinen and Rintala[54] Ullah *et al*.[55] Turner *et al*.[56] Naseer *et al*.[57]	Therapeutic as well as neuroprotective
Xenon	Increases Bcl-2 expression and inhibits cytochrome c release and p53 expression	Shu *et al*.[46] Reddy[58]	Neuroprotection
Brain preconditioning with anaesthetics	Low dose or short duration of exposure prevents damage from high dose or prolonged anaesthesia exposure in the immature brain	Wei *et al*.[59]	Neuroprotection
ADNP	Neuroprotection against NMDA receptor antagonists	Turner *et al*.[56]	Neuroprotection
Melatonin	Inhibits mitochondria-induced apoptotic pathway	Lei *et al*.[33]	Neuroprotection
Acetyl-L-carnitine	Inhibition of mitochondrial apoptotic pathway	Zou *et al*.[60] Scafidi *et al*.[61]	Prevents anaesthesia-induced cognitive impairment
B-estradiol	Upregulation of pro-survival proteins	Asimiadou *et al*.[62]	Neuroprotection
NSAIDS	Inhibit pro-inflammatory cytokines	Gong *et al*.[63]	Neuroprotection
L-type calcium channel blockers	Inhibit calcium influx	Wei[64]	Neuroprotection
Hypothermia	Inhibits cytochrome C release from mitochondria and caspase activation	Creeley and Olney[65]	Neuroprotection
Heat shock protein-72, EUK-134 and pramipexole	Restore mitochondrial integrity and prevent upregulation of ROS	Vizcaychipi *et al*.[66] Boscolo *et al*.[67]	Neuroprotection

EPO – Erythropoietin; ADNP – Activity dependant neuroprotective protein; NSAIDs – Non-steroidal anti-inflammatory drugs; NMDA – N-methyl-D-aspartate; ROS: Reactive oxygen species; Protein 53 – p_{53}

Vitamins

Vitamin B_1, also known as thiamine is pivotal for metabolic and cellular function, and its deficiency is associated with ethanol-induced cerebellar apoptosis.[54]

Nicotinamide a water soluble vitamin and coenzyme in numerous redox reactions, has a protective effect in a dose of 1 mg/kg against ketamine-induced apoptotic neurodegeneration in the developing brain of rats as documented by Ullah *et al*.[55] thereby emphasizing its therapeutic and neurodevelopmental potential in neurocognitive disorders.

Vitamin D_3 (1-α2, 5-dihydroxy-vitamin D_3) by causing an increase in calcium binding proteins is neuroprotective.[56] Similarly, Vitamin C can attenuate anaesthesia triggered neurotoxicity.[57]

Xenon

Pretreatment with xenon ameliorates N_2O and isoflurane-induced apoptosis (*in vivo* and *in vitro*) and cognitive deterioration *(in vivo)* by enhancing Bcl-2 expression, an anti-apoptotic protein, and diminishing both cytochrome–C release and p_{53} expressions.[46,58]

Brain preconditioning with anaesthetics

It has been found that anaesthetics can be used as weapons against anaesthesia-induced apoptosis. Pretreatment with low dose of anaesthetics or a shorter duration of anaesthetic exposure can prevent apoptosis due to high dose or prolonged anaesthetic exposure in the immature brain. Prevention of isoflurane-induced apoptosis by prior preconditioning with isoflurane has been well illustrated by Wei *et al*.[59]

Activity dependant neuroprotective protein

Activity dependant neuroprotective protein exerts its neuroprotective action on neurotoxicity mediated via NMDA receptor antagonists as shown by Turner *et al*.[56]

Melatonin

It is a hormone secreted by the pineal gland and exerts its neuroprotective effect by attenuating mitochondria-induced apoptotic pathway.[33]

Acetyl-L-carnitine

It confers protection against neurodegeneration mediated via combination of N_2O and isoflurane as well as that-induced by traumatic brain injury in developing rat brain.[60,61]

B-estradiol

It is a sex hormone that by up regulating prosurvival proteins exerts neuroprotection.[62]

Non-steroidal anti-inflammatory medications

Drugs such as ketorolac and parecoxib exhibit neuroprotection by inhibiting proinflammatory cytokines, which produce neurobehavioral deficits with sevoflurane.[63]

L-type calcium channel blockers

These confer neuroprotection by preventing excessive calcium influx.[64]

Hypothermia

It provides protection against natural, as well as anaesthesia triggered apoptosis.[65]

Other potential neuroprotectants are heat shock protein[66] and EUK-134 and pramipexole,[67] which maintain mitochondrial function and integrity and suppress anaesthesia mediated apoptosis. Evidence accumulated from the experimental studies justify the present concern regarding the credibility of anaesthesia exposure in the very young culminating in the long-term neurobehavioral consequences. This raises the vital question: Can these interpretations be extrapolated in humans? There are certain differences, which complicate the translation of animal studies to humans. These include:

- Uncertain doses and duration of exposure
- Differences in developmental timelines. E.g., the critical period for anaesthesia-induced apoptosis in rats corresponds to 20th week of gestation in humans while the rhesus monkey susceptible period approximates to 26th week of gestation in humans thereby highlighting peak neurotoxicity for premature human foetuses than-term neonates or infants.
- Presence of confounding factors in human studies, e.g., intraoperative core body temperature, hypocapnia and co-morbidities.

Furthermore, novel research avenues have been developed for detecting and deciphering mechanisms for anaesthesia-induced apoptosis like use of hESCs *(in vitro)* and radiotracers (PET), and preventive strategies are underway.[68]

EFFECTS OF LOCAL ANAESTHETIC AGENTS ON IMMATURE BRAIN

Local anaesthetic agents have a safe anaesthetic profile in neonates and children. It has been found that neuraxial analgesia administered to the delivering mother during labour and vaginal delivery had no deleterious effects on learning abilities. In fact, clinically regional anaesthesia has proven to be safe and effective in very young patients. However, lignocaine when given neuraxially in the dorsal root ganglia of neonatal rats causes cytotoxicity similar to that seen in adult rats.[69]

CONCLUSION

Epidemiological studies conducted so far regarding the safety of anaesthetic agents on the immature brain are intimidating, and various prospective clinical trials are underway, which will unfold the mystery. As for now, restricting anaesthesia exposure to unavoidable emergency procedures in the immature young brain and employing novel molecular approaches will reduce the incidence and severity of neuroapoptosis.

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Announcement

Dr. TN Jha and Dr. KP Chansoriya Travel Grants

For the year 2015 the Dr. TN Jha and Dr. KP Chansoriya travel grant will be awarded to the participants from 15 states. All the states can select their candidate during their annual conference and send them with the recommendation of the Secretary. Only one candidate is allowed from each state. In case if two states have a combined annual meet but separate as per the records, have to select one candidate from each state. If more than 15 states recommend the candidates for the award, selection will be made on first come first served basis.

Dr. Venkatagiri K M
Secretary - ISA
"ASHWATHI", Opp. Ayyappa Temple, Nullippady, Kasaragod - 671121, Kerala
Email: isanhq@gmail.com / secretaryisanhq@gmail.com Mobile: 093880 30395