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Minimally invasive biomarkers of general anesthetic-induced developmental neurotoxicity

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ABSTRACT

The association of general anesthesia with developmental neurotoxicity, while nearly impossible to study in pediatric populations, is clearly demonstrable in a variety of animal models from rodents to nonhuman primates. Nearly all general anesthetics tested have been shown to cause abnormal brain cell death in animals when administered during periods of rapid brain growth. The ability to repeatedly assess in the same subjects adverse effects induced by general anesthetics provides significant power to address the time course of important events associated with exposures. Minimally-invasive procedures provide the opportunity to bridge the preclinical/clinical gap by providing the means to more easily translate findings from the animal laboratory to the human clinic. Positron Emission Tomography or PET is a tool with great promise for realizing this goal. PET for small animals (microPET) is providing valuable data on the life cycle of general anesthetic induced neurotoxicity. PET radioligands (annexin V and DFNSH) targeting apoptotic processes have demonstrated that a single bout of general anesthesia effected during a vulnerable period of CNS development can result in prolonged apoptotic signals lasting for several weeks in the rat. A marker of cellular proliferation (FLT) has demonstrated in rodents that general anesthesia-induced inhibition of neural progenitor cell proliferation is evident when assessed a full 2 weeks after exposure. Activated glia express Translocator Protein (TSPO) which can be used as a marker of presumed neuroinflammatory processes and a PET ligand for the TSPO (FEPPA) has been used to track this process in both rat and nonhuman primate models. It has been shown that single bouts of general anesthesia can result in elevated TSPO expression lasting for over a week. These examples demonstrate the utility of specific PET tracers to inform, in a minimally-invasive fashion, processes associated with general anesthesia-induced developmental neurotoxicity. The fact that PET procedures are also used clinically suggests an opportunity to confirm in humans what has been repeatedly observed in animals.

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1. Introduction

In the USA more than one million children less than 5 years old undergo general anesthesia every year, including 1.5 million infants less than 12 months of age. Although general anesthesia is often required to alleviate pain and stress, stabilize vital signs and provide consistent conditions for surgery and diagnostic procedures, questions remain regarding the long-term neurotoxic and neurodegenerative effects of anesthetic exposure on the developing brain (Aker et al., 2015; Jevtovic-Todorovic, 2010; Mann and Kahana, 2015; Sun, 2010). Recent experimental evidence indicates that early exposure to general anesthetics can have adverse effects on the developing central nervous system (CNS). While anesthetic-induced neurotoxicity has been investigated primarily using neurophysiological, neuropathological and behavioral approaches, additional non-invasive biomarkers that allow for the dynamic detection and monitoring of adverse effects are highly desired (Pogge and Slikker, 2004; Zhang et al., 2013c). Molecular imaging technologies, such as magnetic resonance imaging (MRI), computed tomography (CT) and positron emission tomography (PET) allow for the noninvasive collection of imaging data providing anatomical and functional information regarding biochemical, physiological, pathological and pharmacological processes in vivo.

Amongst a variety of molecular imaging systems, PET is a unique modality with both high spatial resolution (typically ~2 mm for microPET scanners appropriate for use with small animals) and high sensitivity that offers relative and absolute quantitation (Chatziioannou, 2002; Luker et al., 2003; Myers, 2001; Phelps, 2000; Walker et al., 2004; Zhang et al., 2013c). With the high sensitivity of PET (nanomolar to picomolar concentrations can be detected), biological processes of interest can be studied by measuring the uptake and retention of radiotracers that target those processes (Jacobs et al., 2003; Zimmer et al., 2014c). PET imaging can, thus, provide valuable insights into brain-related biological processes, including those associated with neuronal plasticity, neuronal apoptosis, degeneration, regeneration, and neurotoxicity (Hammoud, 2016; Ory et al., 2015; Ory et al., 2016;

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Pagano et al., 2016; Roy et al., 2016; Wang et al., 2007; Zimmer et al., 2014a; Zimmer et al., 2014b; Zimmer et al., 2014c). MicroPET imaging using animal models of human diseases allows for repeated kinetic analyses of special molecular events in the same experimental subject, providing minimally-invasive assessments of therapeutic effects over time (Jacobs et al., 2003; Zhang et al., 2013c).

2. General anesthetic-induced neurotoxicity in the developing brain

General anesthesia is widely utilized in pediatric patients for surgery, diagnostic and therapeutic procedures. Although the exact neural mechanisms that result in general anesthetic-induced loss of consciousness, analgesia, immobility and, importantly, amnesia remain unknown, both intravenous and volatile general anesthetics are thought to exert their primary effects by interfering with neurotransmission (Aker et al., 2015; Culley et al., 2007; Mann and Kahana, 2015; Soriano and Anand, 2005). Most of the currently used general anesthetics have either NMDA receptor blocking properties, such as ketamine and nitrous oxide, or GABA receptor enhancing effects, such as isoflurane and propofol (Bianchi et al., 2008; Xie et al., 2007). The action of these receptors involves ligand-gated ion channels or G proteins and is crucial for early neurodevelopmental events including synapse formation, neuroplasticity, and neuronal survival (Wang and Slikker, 2008; Zhang et al., 2009; Zhang et al., 2013c).

Neurotoxicity, as discussed here, will be defined as the occurrence of adverse effects on the structure or function of the nervous system induced by exposure to biological, chemical or physical agents (Costa, 1996; Culley et al., 2007; Harris and Blain, 2004; Pogge and Slikker, 2004; Slikker et al., 2004). From the third trimester of gestation to the 3rd years of life, the human brain undergoes a rapid growth spurt during which the brain grows at an accelerated rate characterized by neuronal proliferation and differentiation, and dendritic arborization and synaptogenesis (Aker et al., 2015; Culley et al., 2007; Soriano and Anand, 2005). This critical period of rapid growth occurs at different times relative to birth in different species occurring during postnatal days 1–14 in rats and mice (du Bois and Huang, 2007). During this eruption of neurogenesis, gliogenesis and synaptogenesis the brain is exquisitely vulnerable. Environmental insults during this period can adversely impact brain development and cause long-lasting functional deficits (Aker et al., 2015; Culley et al., 2007; Dobbing and Sands, 1979; Soriano and Anand, 2005; Wang and Slikker, 2008). For example, continuous blockade of NMDA receptors or activation of GABA receptors by general anesthetics during a critical period of CNS development may reduce the establishment of important synapses (du Bois and Huang, 2007; Forcelli et al., 2011; Ikonomidou et al., 1999; Olney et al., 2002; Scallet et al., 2004; Slikker et al., 2005; Slikker et al., 2007a; Slikker et al., 2007b; Stefovska et al., 2008; Zhou et al., 2011).

2.1. Ketamine

As a non-competitive antagonist of NMDA receptors, ketamine is widely used to induce and maintain general anesthesia in pediatric patients (Kurdi et al., 2014). NMDA receptor blockade by ketamine leads to inhibition of neuronal activity and, in the immature CNS, can trigger abnormal neuronal apoptosis (Brambrink et al., 2012; Ikonomidou et al., 1999; Olney et al., 2002; Scallet et al., 2004; Slikker et al., 2007a; Wang et al., 2005; Wang et al., 2006). Previous studies indicate that multiple doses of ketamine (20 mg/kg every 2 h for 6 times) given to PND 7 rats triggers a massive wave of apoptotic neurodegeneration affecting many neurons in several major regions of the developing brain, especially the frontal cortex (Zou et al., 2009b). Newborn rhesus monkeys (postnatal day 5 or 6) anesthetized via intravenous ketamine infusions for either 9 or 24 h exhibited significantly increased neuronal cell death in layers II and III of the frontal cortex (Zou et al., 2009a). Five hours of ketamine-induced general anesthesia in postnatal day 6 rhesus neonates or gestation day 120 fetuses (pregnant rhesus females anesthetized) was also shown to cause significant increases in neuronal apoptosis in both the fetal and neonatal brains (Brambrink et al., 2012). Ketamine-induced neuronal apoptosis has also been shown to occur in primary cultured rat neurons (Li et al., 2014; Liu et al., 2013a,b). Neurodegeneration induced by early exposure to ketamine is associated with long-term cognitive deficits, including impaired learning and memory in animals when tested as adults (Huang et al., 2012; Paule et al., 2011).

2.2. Isoflurane plus N_2O

The combination of nitrous oxide (N_2O), an NMDA receptor antagonist, with an inhalational general anesthetic can significantly decrease the amount of anesthetic agent necessary to produce loss of consciousness and analgesia. Therefore, the combination of N_2O gas and isoflurane (ISO) vapor is widely used in pediatric surgical procedures (Zhang et al., 2013b; Zou et al., 2008, 2011). In PND7 rat pups exposed to N_2O (75%) plus ISO (0.55%) for 6 or 8 h, massive neuronal apoptosis was observed, especially in layers II and III of the frontal cortex (Zou et al., 2008). The neurotoxic effects of ISO + N_2O have also been investigated in a nonhuman primate (NHP) rhesus monkey model. Exposure of PND 5 or 6 animals to N_2O (70%) plus ISO (1.0%) for 8 h lead to a significant increase in the number of caspase-3-, silver stain- and Fluoro-Jade C-positive cells in the frontal cortex, temporal gyrus and hippocampus (Zou et al., 2011).

2.3. Sevoflurane

Sevoflurane is a one of the most widely used inhaled anesthetics for the induction and maintenance of general anesthesia in both adults and children (Delgado-Herrera et al., 2001; Li et al., 2013). Sevoflurane is a volatile, non-flammable and nonexplosive liquid administered after vaporization. Anesthesia induced by inhalation of sevoflurane causes little airway irritation, is associated with minor cardiovascular and respiratory side effects, and has minimal interactions with other drugs (Delgado-Herrera et al., 2001; Li et al., 2013). Due to the rapid induction and recovery from sevoflurane-induced anesthesia, it has been widely used in infants and children for pediatric inpatient and outpatient surgery (Lerman et al., 1994; Li et al., 2013; Zhou et al., 2012). Sevoflurane is believed to activate glycine and $GABA_A$ receptors and inhibit NMDA receptors (Brosnan and Thiesen, 2012; Hollmann et al., 2001; Nishikawa and Harrison, 2003; Tagawa et al., 2014). In a study employing neonatal Cynomolgus monkeys, no significant learning or memory deficits or behavioral abnormalities were observed early in life after receiving sevoflurane anesthesia at surgical plane (2–2.6%) for 5 h on PND 6 (Zhou et al., 2015). In contrast, single episodes of sevoflurane induced general anesthesia (2–2.5% for 4–6 h) on PND 7 were reported to lead to widespread neuronal apoptosis in several brain regions and cause long-term behavioral impairments and memory dysfunction in rats (Fang et al., 2012; Zheng et al., 2013; Zhou et al., 2012). After exposure to 2.0% sevoflurane for 5 h, early cell death was found in organotypic hippocampal slices (OHS) from rat pups on PND14. At 72 h, cell death was significantly detected in the OHS prepared from the PND7 and 4 rat pups (Piehl et al., 2010). Repeated exposures to sevoflurane during gestation (in utero) in the rat also resulted in abnormal levels of neuronal apoptosis in the brains of offspring (Wang et al., 2012b). Prolonged sevoflurane treatment (6 h) also reduced the regeneration of hippocampal neural stem cells isolated from Sprague-Dawley rat embryos (Nie et al., 2013). In the mouse, exposure to sevoflurane on PND 7 resulted in increased neuroapoptosis in the hippocampal region and was associated with subsequent abnormal social behaviors and deficits in fear conditioning in adulthood (Liang et al., 2010; Lu et al., 2010; Satomoto et al., 2009; Tagawa et al., 2014; Takaenoki et al., 2014; Yonamine et al., 2013; Zhang et al., 2008). It has been postulated that sevoflurane causes neuronal apoptosis via a MEK/ERK1/2 MAPK signaling pathway (Nie et al., 2013; Wang et al., 2012a; Wang et al., 2013). DNA microarray analysis of frontal

cortical tissues from the brains of rhesus monkeys exposed on PND 5 or 6 to 9 h of 2.5% sevoflurane also demonstrated that essential lipid components were significantly down regulated. Abnormal levels of several cytokines and increased Fluoro-Jade C staining were also observed in the brains of these animals (Liu et al., 2015).

3. MicroPET imaging of general anesthetic-induced neurotoxicity

The application of microPET imaging technologies to the study of living animals has provided the ability to repeatedly collect sensitive and quantitative three-dimensional molecular information with increased resolution from the brains of experimental animals such as mice, rats and nonhuman primates (Chen et al., 2004; Chen et al., 2009; Jang, 2013; Kornblum et al., 2000; Lang, 2000; Ohashi et al., 2008). In order to repeatedly assess the adverse neuronal effects associated with early exposure to general anesthetics, multiple microPET protocols have been developed under which it is possible to measure the brain's uptake of radiotracers as quantitative markers of metabolic activity, neuronal apoptosis and damage in the living rat and monkey (Liu et al., 2014; Liu et al., 2013a,b; Zhang et al., 2009; Zhang et al., 2011; Zhang et al., 2013a; Zhang et al., 2013b; Zhang et al., 2016; Zhang et al., 2012). After injection of a radiotracer into animal subjects, the labeled molecule often binds to specific targets and, thus, via visualization with the microPET, it is possible to characterize the bio-distribution of that specific tracer. The radiation emitted by the labeled tracer is detected by the PET scanner allowing for the detection, localization and quantification of signal intensity. By utilizing multiple radioactive tracers, several biological or pathological processes such as tissue perfusion, metabolism, receptor binding and protein expression can be qualitatively and quantitatively assessed (Lancelot and Zimmer, 2010; Myers and Hume, 2002; Schnockel et al., 2010; Wagner and Langer, 2011; Zhang et al., 2013c).

3.1. MicroPET imaging of neurotoxicity utilizing a marker of apoptosis

Apoptosis, also called as programmed cell death, is a regulated, energy-dependent process that results in the generation of cellular debris that is subsequently phagocytized. In contrast to necrosis, apoptosis is characterized by membrane blebbing, cell shrinkage, loss of membrane asymmetry and attachment, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation (Tait, 2008; Wolters et al., 2007). Always initiated by either the presence of a stimulus or the removal of a suppressing agent, apoptosis is known as a programmed process for eliminating superfluous or unnecessary cells or cells with DNA damage. Being a fundamental physiological process, apoptosis likely plays a critical role in many physiological disorders (Aloya et al., 2006). As demonstrated by studies in animal models, exposure to general anesthetics during the brain growth spurt can induce widespread nerve cell loss, typically by increasing apoptotic processes (Hayashi et al., 2002; Jevtovic-Todorovic et al., 2003; Olney et al., 2002; Scallet et al., 2004; Slikker et al., 2007b; Wang et al., 2005; Zou et al., 2009b). To obtain information about the life-cycle of the apoptotic events associated with early exposure to general anesthetics, apoptotic processes were investigated using microPET ligands that targeted those processes.

Apoptosis involves several specific biochemical pathways and most apoptotic imaging probes are designed to target specific molecules that are involved in the apoptotic cascade. Based on the cellular processes that apoptotic tracers are designed to target, they can be characterized into five main categories (Neves and Brindle, 2014; Tait, 2008): 1) molecules that target caspase-3/7 activation; 2) radiolabeled probes based on phosphonium cations that can be used to assess the mitochondrial membrane potential; 3) agents that detect plasma membrane phospholipid asymmetry and phosphatidylserine (PS) exposure; 4) molecules that target depolarization of plasma membrane; and 5) compounds that accumulate in apoptotic cells (Berridge et al., 2009; Neves and Brindle, 2014; Tait, 2008).

3.1.1. MicroPET imaging of apoptosis using [^{18}F]-annexin V

When a cell is entering apoptosis, phosphatidylserine (PS), one of the four major phospholipids that make up the cell membrane, will externalize from the intracellular to the extracellular side of the plasma membrane (Allen et al., 1997; Fadok et al., 1992; Lahorte et al., 2004; Saraste and Pulkki, 2000; Zijlstra et al., 2003). This redistribution of PS to the exterior of the cell membrane is one of the early characteristics of apoptotic cells and serves as a signal to macrophages and adjacent normal cells to phagocytize and digest the components of the cell undergoing apoptosis (Schlegel and Williamson, 2001; Strauss et al., 2008; Yagle et al., 2005). Therefore, externalized PS proved to be a very promising biomarker for the early detection of apoptotic cells in vivo. Annexin V, a member of the superfamily of annexin proteins, is a 36-kDa human protein that exhibits Ca^{2+}-dependent binding to PS with high affinity. Annexin V, thus, has been widely utilized as an apoptotic radiotracer when labeled with various isotopes (Grierson et al., 2004; Kwak et al., 2015; Lahorte et al., 2004; Lu et al., 2015; Neves and Brindle, 2014; Strauss et al., 2008; Toretsky et al., 2004; Zijlstra et al., 2003).

Relevant to the present topic, neuronal apoptosis induced by ketamine exposure was analyzed in rodent model using PET imaging with radiolabeled Annexin V (Zhang et al., 2009; Zhang et al., 2013c). On PND 7, rat pups were randomly assigned to control or treated groups to receive either ketamine or saline subcutaneously every 2 h. Ketamine (20 mg/kg/injection) was administered in six injections (Zou et al., 2009b) and control animals received six injections of saline under the same schedule MicroPET scanning was performed on PND 35 when each rat was scanned for 2 h after the i.v. injection of [^{18}F]-annexin V. While the uptake of [^{18}F]-annexin V was evident in the brains of both control and ketamine treated rats with maximal levels being attained 5 min after the injection, the high radioactive signal in the regions of interest in controls decreased rapidly, whereas the radioactivity in the ketamine-treated brains remained at relatively high levels for the duration of the 40 min PET scan. This persisting signal in the ketamine-treated brain is thought to represent radiolabeled annexin V bound to the outer membranes of ketamine-induced apoptotic neurons, the sites expressing PS. These results demonstrated that cellular degeneration caused by a PND 7 exposure to ketamine persisted for at least 4 weeks.

As an imaging agent that binds to externalized PS, annexin V can also accumulate on necrosing neurons with ruptured plasma membranes and, thus, it is difficult to clearly differentiate apoptotic from necrotic cells (Lahorte et al., 2004) using this compound. Additionally, annexin V also binds to neurons that are under other forms of pathophysiological stress (Kim et al., 2006; Lorberboym et al., 2006; Strauss et al., 2008; Zeng et al., 2008). According to its bio-distribution and pharmacokinetic profile, annexin V is cleared slowly from the blood, has a poor signal-to-noise ratio, limited brain access, and is characterized by high background activity, especially in the liver, kidneys, and gut (Kwak et al., 2015; Neves and Brindle, 2014; Niu and Chen, 2010; Reshef et al., 2010).

3.1.2. MicroPET imaging of apoptosis using [^{18}F]-DFNSH

[^{18}F]-5-(dimethylamino)-*N*′-(4-fluorobenzylidene) naphthalene-1-sulfonohydrazide ([^{18}F]-DFNSH) is included in the group of compounds that accumulate in the cytoplasm of cells undergoing apoptosis. All of the compounds belonging to this group are small molecules with a fluorescent dansyl core and they can be synthesized with high yield and efficiency (Reshef et al., 2010; Zeng et al., 2008). Compared with [^{18}F]-annexin V, which binds to PS localized on the external leaflet of plasma membrane, DFNSH is taken up into the cytoplasm of different types of apoptotic cells. The mechanism(s) that underlies the intracellular uptake of DFNSH is not clearly known, but it has been postulated to involve the loss of plasma membrane potential, activation of the membrane phospholipid scramblase system, and the acidification of the external plasma membrane leaflet and cytosol of apoptotic cells. The intracellular uptake of [^{18}F]-DFNSH differentiates apoptotic cells from viable and necrotic cells and it has an improved signal-to-noise ratio, making [^{18}F]-

DFNSH a more specific apoptotic tracer than annexin (Cohen et al., 2009; Grimberg et al., 2009; Reshef et al., 2010). Having a low molecular weight, this small probe is cleared predominantly by the kidney and has a fast elimination rate (Neves and Brindle, 2014; Niu and Chen, 2010).

Rats exposed to ketamine on PND 7 were scanned on PND 35 with ^{18}F labeled DFNSH to investigate the anesthetic-induced neuronal apoptosis (Zhang et al., 2011; Zhang et al., 2013b). The microPET imaging data demonstrated that an initial distribution of [^{18}F]-DFNSH into brain was evident in both control and ketamine treated rats. However, the radioactivity in the frontal cortex of ketamine-treated brains remained at relatively high levels for at least 40 min compared to controls. The persistent signal in ketamine treated brains is consistent with our earlier study using [^{18}F]-annexin V (Zhang et al., 2009) and supports the interpretation that the cellular degeneration caused by a single PND 7 exposure to ketamine is sustained for a much longer time than previously known. Since the half-life of ketamine in the rat is relatively short (Scallet et al., 2004; Slikker et al., 2007b), the noted persistent apoptosis must be a result of processes triggered by the initial exposure but not dependent upon the continued presence of ketamine.

Neuronal apoptosis induced by the combined administration of ISO (1%) and N_2O (70%) was also evaluated using [^{18}F]-DFNSH and microPET imaging (Zhang et al., 2013b). On PND 7, rats in the experimental group were exposed to ISO/N_2O for 8 h with or without acetyl-L-carnitine (ALC), and control rats were exposed to room air only, with or without ALC. ALC, an esterified compound of L-carnitine (LC), may provide neuroprotective benefits in neurodegenerative and aging situations (Abdul et al., 2006; Abdul and Butterfield, 2007; Calabrese et al., 2005; Calabrese et al., 2006; Ishii et al., 2000; Virmani et al., 2001; Zaitone et al., 2012; Zanelli et al., 2005). Although the mechanism(s) underlying the neuroprotective effect(s) of ALC are not known, they may involve improvement of mitochondrial function, antioxidant activity, stabilization of membranes, and/or modulation of protein and gene expression (Barhwal et al., 2008; Jones et al., 2010; Scafidi et al., 2010).

On PNDs 14, 21, and 28, microPET/CT images were obtained using [^{18}F]-DFNSH for 90 min. In PND 14 treated rats, the uptake of [^{18}F]-DFNSH in frontal cortex was increased and the duration over which the tracer was washed out was prolonged. The noted increase radiotracer uptake remained significant for at least one week after exposure. ALC greatly attenuated the DFNSH uptake suggesting that it can effectively block the anesthetic-induced neuronal apoptosis.

Sevoflurane-induced neuronal apoptosis was also assessed in the rat using PET imaging with [^{18}F]-DFNSH. In this experiment, neonatal rats were exposed for 9 h to sevoflurane at 2.5% in oxygen on PND 7. MicroPET scans were performed following the injection of [^{18}F]-DFNSH on PNDs 14, 28 and 63. At PND 14, the radioactivity in frontal cortical areas in exposed rats was significantly higher than that seen in controls. In contrast, radioactivity in the same regions of interest in animals exposed to sevoflurane for either 3 or 6 h were not significantly different from those of control animals at PNDs 14, 28, and 63. There was an exposure duration increase in radiotracer signal but the effects were not statistically significant. These data indicate the results from imaging studies can provide dose-response data (Liu et al., 2013a,b).

3.2. MicroPET imaging of neurotoxicity using other tracers

3.2.1. MicroPET imaging using [^{18}F]-FLT

3′-Deoxy-3′-^{18}F-fluorothymidine ([^{18}F]-FLT) is a radioactive thymidine analog that is used in vivo as a marker of cell proliferation (Liu et al., 2014; Rasey et al., 2002; Tseng et al., 2005; Viertl et al., 2011). In cells, FLT can be phosphorylated by cytosolic thymidine kinase-1 (TK1) which is resistant to dephosphorylation by thymidine phosphorylase. [^{18}F]-FLT, thus, gets trapped within the cytosol after being monophosphorylated by TK1 (Lee et al., 2011; Rasey et al., 2002; Tseng et al., 2005). Since TK_1 is a key enzyme in the DNA-salvage pathway, it is strongly regulated by the cell cycle and selectively upregulated before and during the S phase. The uptake and retention of [^{18}F]-FLT provides a metric of the activity of TK1 and can be used as a noninvasive biomarker of cell proliferation (Lee et al., 2011; Liu et al., 2014; Rasey et al., 2002). It has been recently reported that PET imaging of [^{18}F]-FLT has been used for the non-invasive monitoring of endogenous neural stem cell proliferation in the normal and ischemic adult rat brain in vivo (Jacobs et al., 2007; Rueger et al., 2010).

In one of our previous studies, aspects of sevoflurane-induced neuronal toxicity were investigated using [^{18}F]-FLT microPET. Neonatal rats were exposed to 2.5% sevoflurane or room air for 9 h on PND 7. On PND 21, 2 weeks following the exposure, standard uptake values (SUVs) for [^{18}F]-FLT in the hippocampal formation were significantly attenuated in the sevoflurane-exposed rats, suggesting decreased cell proliferation in this region. Four weeks following exposure, the effects of sevoflurane exposure were no longer evident. These investigations indicated that a single prolonged exposure to sevoflurane during rapid brain development causes signification inhibition of neural progenitor cell proliferation that lasts for at least 2 weeks (Liu et al., 2014).

3.2.2. MicroPET imaging with [^{18}F]-FEPPA

In the CNS, translocator protein (TSPO), previously referred to as the peripheral benzodiazepine receptor (PBR), is mainly located in glial cells, particularly in microglia and astrocytes, with the highest densities in the olfactory bulb, choroid plexus, and the ependymal lining of the ventricles(Imaizumi et al., 2008; Lang, 2002). In the CNS, TSPOs participate in multiple physiological functions including neurosteroid synthesis, nutritional support of neurons and modulation of CNS immune reactions. The expression of the TSPO in brain is significantly increased in response to a wide variety of insults. Experimental results show that such increases are mainly due to activated glial cells. In response to intercellular signaling induced by neurotoxicants, microglial activation usually begins several hours after exposure and lasts for several days after injury onset (Banati, 2003; Ito et al., 2010; Takeuchi et al., 1998). In their activated state, microglia undergo morphological changes, accumulate and proliferate at the site of neuronal damage, synthesize proinflammatory cytokines, and release toxic molecules and metabolites to eliminate damaged cells (Briard et al., 2008; Ito et al., 2010; Papadopoulos et al., 2006). Various studies have demonstrated that TSPOs are involved in numerous nervous system disorders such as multiple sclerosis, cerebral ischemia and stroke, epilepsy, brain injury, neurotoxic brain damage, and neurodegenerative diseases (Benavides et al., 1987; Briard et al., 2008; Lang, 2002; Oku et al., 2010; Papadopoulos et al., 2006). Following exposure to neurotoxicants such as general anesthetics and other insults, the levels of TSPO increase significantly in both astrocytes and microglia in damaged brain areas in a time-dependent and region-specific manner (Kuhlmann and Guilarte, 1999; Kuhlmann and Guilarte, 2000; Lang, 2002).

^{18}F-labeled fluoroethoxybenzyl-*N*-(4-phenoxypyridin-3-yl) acetamide ([^{18}F]-FEPPA) is a specific TSPO ligand that can be efficiently synthesized. With high radiochemical yields and high specific activity, [^{18}F]-FEPPA has proved useful in nonhuman primate and human studies (Bennacef et al., 2008; Rusjan et al., 2011; Schweitzer et al., 2010; Wilson et al., 2008; Zhang et al., 2012; Zhang et al., 2013b; Zhang et al., 2013c). Due to the increased expression of TSPOs in areas of neuronal injury, [^{18}F]-FEPPA is used as a marker of microglial activation and, thus, as a surrogate marker of neuronal damage. (Zhang et al., 2012; Zhang et al., 2013a; Zhang et al., 2013b; Zhang et al., 2013c; Zhang et al., 2016).

To monitor the neurotoxicity induced by ISO + N_20 in a nonhuman primate model, PND 5 or 6 rhesus monkey neonates were exposed to a mixture of 70% N_2O/30% oxygen and 1% ISO for 8 h and control monkeys were exposed to room air only. One day later, [^{18}F]-FEPPA (56 MBq) was injected into the lateral saphenous vein and microPET/CT images were obtained over the next 2 h. MicroPET/CT scans were repeated for each monkey one week, three weeks and 6 months after the anesthetic

exposure (Zhang et al., 2013a; Zhang et al., 2012). During each microPET imaging session, [^{18}F]-FEPPA quickly distributed into the brains of both treated and control monkeys. One day after anesthetic exposure the uptake of [^{18}F]-FEPPA was significantly increased in the temporal lobe of treated animals. One week after exposure the uptake of [^{18}F]-FEPPA in the frontal lobe of treated animals was significantly greater than that in controls. These effects were gone three weeks after exposure. Significant brain injury in both the temporal cortex at one day, and frontal cortex at one week after anesthetic exposure was suggested by the time course of the FEPPA retention (Zhang et al., 2013a; Zhang et al., 2012). Although [^{18}F]-FEPPA does not directly target dying neurons, increases in the expression of TSPOs are thought to co-occur with insults such as neuronal damage and death. Repeatedly assessing the uptake of [^{18}F]-FEPPA in vulnerable brain areas provides the opportunity to evaluate the severity and time course of anesthetic-induced glial activation, that presumably occurs in concert with, or in reaction to, neural damage. The reasons underlying the different time courses in different brain areas are not known but would seem to indicate differential sensitivities of different brain areas to anesthetic-induced neurotoxicity, which is also seen using other metrics of damage such as fluoro jade and caspase-3 stains.

Sevoflurane induced neurotoxicity in a nonhuman primate model was also assessed using microPET imaging with [^{18}F]-FEPPA (Zhang et al., 2016). Neonatal rhesus monkeys (postnatal day 5 or 6, 3–6/group) were exposed for 8 h to 2.5% sevoflurane with or without ALC. Control monkeys were exposed to room air with or without ALC. Following the exposure, microPET/CT scans using [^{18}F]-FEPPA were performed repeatedly on day one, one and three weeks, and two and six months after exposure. The uptake of [^{18}F]-FEPPA in the frontal and temporal lobes was increased significantly 1 d and one week after exposure, respectively. Co-administration of ALC anesthetic exposure, the uptake of [^{18}F]-FEPPA in the anesthetic-exposed monkeys remained higher than that seen in the controls at most time points, but this effect was not statistically significant at this point, likely due to the small number of subjects utilized. At 2 and 6 months of age, the uptake of [^{18}F]-FEPPA in the anesthetic-exposed monkeys returned to levels similar to those observed in controls (Zhang et al., 2016). According to microPET data, exposing the developing monkey brain to sevoflurane during a period of rapid brain development can induce adverse effects in several brain regions including the frontal cortex and temporal lobe, as evidenced by increases [^{18}F]-FEPPA uptake indicating microglial activation. Co-administration of ALC, while not affecting depth of anesthesia, blocked, at least partially, this anesthetic–induced effect (Zhang et al., 2016).

4. Limitations and safety issues of clinical applications

The development of molecular imaging approaches constitutes a step towards meeting the clinical needs for biomarkers of neurotoxicity. MicroPET/CT imaging using made-for-purpose radiotracers has the potential to provide great assistance in clinical practice with respect to early and accurate diagnoses as well as in monitoring the progression of neurodegeneration induced by anesthetic agents. Although research results in preclinical studies have proved to be very promising, additional efforts are required to make an effective translation into the clinic (Heneweer and Grimm, 2011; Preuss et al., 2014; Shulkin, 2004). With high sensitivity and specificity, PET imaging can facilitate detailed mapping of radiotracer distribution using only picomolar concentrations which greatly reduces the likelihood of toxicity (Heneweer and Grimm, 2011; Massoud and Gambhir, 2003). Dosimetry of radiotracers, however, needs to be carefully considered in pediatric patients since radiation is a well known carcinogen. Compared with adults, the lifetime risk of acquiring malignant solid tumors per unit of radiation is higher in all pediatric age groups, especially in infants and small children (Fahey et al., 2016a,b; Gelfand and Lemen, 2007). Recently, hybrid multi-modality imaging systems, such as PET/CT, have been widely introduced and frequently used. PET/CT systems provide additional information and enhanced anatomical localization, improve image resolution and increase the accuracy of diagnosis. CT scan based attenuation corrections can reduce total imaging time by avoiding transmission scans and consequently reducing the time of anesthesia. This improves safety by reducing the length of sedation and anesthesia, however, radiation exposures in small children are not without risk (Preuss et al., 2014; Roberts and Shulkin, 2004; Shulkin, 2004).

5. Summary

While general anesthetics are widely applied and required in pediatric patients for surgery and diagnostic procedures, the safety of general anesthesia in infants and children is still under review. Recent studies using animal models indicate that early exposure to commonly used general anesthetics can adversely affect developing nervous tissue and even result in significant abnormal neuronal apoptosis in vulnerable brain areas. Preclinical and clinical findings suggest an association between developmental exposures to general anesthetics and subsequent deficits in cognitive function. Molecular imaging modalities, such as microPET, provide the opportunity to repeatedly, and in a minimally-invasive fashion, monitor anesthetic-induced neurotoxicity by targeting specific molecular and cellular events in vivo.

Transparency document

The Transparency document associated to this article can be found, in the online version.

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This document has been reviewed in accordance with United States Food and Drug Administration (FDA) policy and approved for publication. Approval does not signify that the contents necessarily reflect the position or opinions of the FDA nor does mention of trade names or commercial products constitute endorsement or recommendation for use. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the FDA.

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