NeuroToxicology 39 (2013) 45–56

Contents lists available at ScienceDirect

NeuroToxicology

Early life exposure to sevoflurane impairs adulthood spatial memory in the rat

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ARTICLE INFO

Article history:
Received 9 May 2013
Accepted 17 August 2013
Available online 27 August 2013

Keywords:
Sevoflurane
Memory
Neonate
Pediatrics
Inhalation anesthesia
Neuronal toxicity

ABSTRACT

Sevoflurane is a general anesthetic commonly used in the pediatric setting because it is sweet-smelling, nonflammable, fast acting and has a very short recovery time. Although recent clinical data suggest that early anesthesia exposure is associated with subsequent learning and memory problems, it is difficult to determine the exact scope of developmental neurotoxicity associated with exposure to specific anesthetics such as sevoflurane. This is largely due to inconsistencies in the literature. Thus, in the present studies we evaluated the effect of early life exposure to sevoflurane (1%, 2%, 3% or 4%) on adulthood memory impairment in Sprague-Dawley rats. Animals were exposed to different regimens of sevoflurane anesthesia on postnatal days (PNDs) 3, 7, or 14 or at 7 weeks (P7W) of age and spatial memory performance was assessed in adulthood using the Morris Water Maze (MWM). Rats exposed to sevoflurane exhibited significant memory impairment which was concentration and exposure duration dependent. Disruption of MWM performance was more severe in animals exposed on both PNDs 3 and 7 than in animals exposed on both PNDs 3 and 14. The younger the animal's age at the time of exposure, the more significant the effect on later MWM performance. Compared to the neonates, animals exposed at P7W were relatively insensitive to sevoflurane: memory was impaired in this group only after repeated exposures to low doses or single exposures to high doses. Early life exposure to sevoflurane can result in spatial memory impairments in adulthood and the shorter the interval between exposures, the greater the deficit.

1. Introduction

Due to rapid advances in anesthesiology over the past decades, surgical, dental and diagnostic imaging procedures in pediatric patients now have become routine and safe (Somri et al., 2012). However, serious safety concerns were raised to the potential long-term effects of anesthesia on the developing brain after the publication by Jevtovic-Todorovic et al. (2003) who found that laboratory exposure to anesthetics like midazolam, nitrous oxide, and isoflurane in 7-day old rodents caused persistent learning deficits. Subsequently, a body of studies compared the neurotoxic effects of different fluorinated gaseous anesthetics including desflurane, isoflurane, and sevoflurane, and reported that anesthetic-associated neurotoxicity on the developing brain is a common feature (Istaphanous et al., 2011; Lin and Zuo, 2011; Liu et al., 2012; Satomoto et al., 2009; Zhu et al., 2010). In clinical settings, the results were inconsistent. Some found significant deficiency in cognition in children after anesthesia exposure (DiMaggio et al., 2011; Wilder et al., 2009), but others did not (Bartels et al., 2009; Hansen et al., 2011). A more recent study by Ing et al. (2012) analyzed the long-term differences in language and cognitive function in 2868 Australian children exposed or unexposed to anesthesia before age 3 and found that those exposed to anesthesia before age 3 had a higher relative risk of language and abstract reasoning deficits at age 10 than peers without exposure. Therefore, further studies are needed to clarify the potential adverse effect of general anesthetics on the developing brain.

General anesthesia-induced neurocognitive dysfunction includes changes in memory, attention, learning and motor activity (Bercker et al., 2009; Istaphanous et al., 2011; Jevtovic-Todorovic et al., 2003; Lin and Zuo, 2011; Paule et al., 2011; Satomoto et al., 2009; Zhu et al., 2010). In animal studies, the exposure time was

Abbreviations: AUC, area under curve; BID, twice a day; MAC, minimum alveolar concentration; MWM, Morris Water Maze; P3D, postnatal 3 days; P7D, postnatal 7 days; P14D, postnatal 14 days; P7W, postnatal 7 weeks; SEM, standard deviation; SID, once a day; TID, three times a day.

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http://dx.doi.org/10.1016/j.neuro.2013.08.007

longer (4–6 h) and the concentration was higher (3–12% of inhalational anesthetics) than those used in the pediatric clinics where the anesthesia time in general is less than 2 h, and the concentration is much lower (McCann and Soriano, 2012; Somri et al., 2012). Even so, the latest evidence has suggested that early anesthesia exposure is an independent risk factor for retarded learning and memory during early learning stages (Flick et al., 2011; Ing et al., 2012), and this negative effect was more significant in preterm babies (Filan et al., 2012). No matter the effect of anesthesia on the developing brain, either negative (Bartels et al., 2009; Hansen et al., 2011; Pham et al., 2010) or positive (DiMaggio et al., 2011; Filan et al., 2012; Flick et al., 2011; Ing et al., 2012; Wilder et al., 2009), pediatric clinicians and anesthesiologists should still be cautious and alert when anesthesia is ready to be given.

Sevoflurane, a volatile anesthetic and a highly fluorinated methyl isopropyl ether, is now the most common agent for pediatric patients due to its sweet-smelling property as well as fast onset and recovery (Lerman and Jöhr, 2009; Singh et al., 2009). It is also recommended because of its lesser irritation to mucous membranes (Klock et al., 2001; Lee et al., 2007), little toxicity, and relatively stable hemodynamics (Michel and Constantin, 2009; Patel and Goa, 1996). For sevoflurane, Callaway et al. (2012) did not find significant impairment of acquisition learning and retention memory in young adult (8–10 weeks) and aged rats (>20 months) when 1 minimum alveolar concentration (MAC) of sevoflurane (~3%) was given, which was almost the same in patients who underwent sevoflurane anesthesia (Bartels et al., 2009; Kadoi and Goto, 2007). However, other studies found a causal relationship between sevoflurane and cognitive disability in 6–8 days old mice (Istaphanous et al., 2011; Satomoto et al., 2009), in 12-month-old rats (Peng et al., 2011), children (Millar et al., 2006), and adult and aged patients (Kanbak et al., 2007; Rohan et al., 2005). The more interesting thing is that in the context of cerebral ischemia, sevoflurane anesthesia can prevent deficits in cognitive function (Eberspächer et al., 2009). Because the effects of sevoflurane on cognition and memory are unclear, it is hard to draw a conclusion as to whether or not sevoflurane is a causative agent of post-anesthesia cognitive decline in different aged patients.

Therefore, we hypothesized that early but not adult exposure to sevoflurane would cause a late-life decline in cognition and memory. In the present study, we investigated the potential causal effect of different concentrations of sevoflurane on adult spatial memory under different anesthetic regimens. The results demonstrated that when sevoflurane was given in multiple exposures in a short time period, high concentrations with a short duration or in a single exposure with a long duration, cognitive retardation and memory deficiency were present in adulthood.

2. Materials and methods

2.1. Animal care and ethics

After approval by the Institutional Committee of Animal Care and Use, male Sprague-Dawley rats aged from 3 days (postnatal 3 days, P3D) to 25 weeks were used for sevoflurane intervention and behavioral tests. The pups (P3D–P14D) were housed with maternal rats to a same plastic cage with soft bedding on a reverse 12:12 h light/dark cycle with lights on at 8:00 AM and maintained in climate with 23 ± 1 °C housing temperature and free access to food and water. The pups were returned to their mother cage after sevoflurane anesthesia. Three weeks after birth, the offspring were separated from maternal rats and housed one animal to one cage until the completion of behavior tests. The littermates were randomly assigned to each testing group. The random numbers were generated by means of the QuickCalcs (GraphPad Software Inc, La Jolla, San Diego, CA; Online Calculators for Scientists, available at http://www.graphpad.com/quickcalcs/RandMenu.cfm. Last accessed April 03, 2013.). Anesthesia was conducted during the light phase between 08:00 AM and 05:00 PM in a quiet room maintained at 22–24 °C. Each animal was used for the same anesthesia regimen and was euthanized after completion of the water maze behavioral experiment by administering a lethal dose of pentobarbital.

2.2. Sevoflurane exposure procedures

Animals were exposed to sevoflurane (Abbott Laboratories, Animal Health Division, IL, USA) through a specific vaporizer (Penlon Sigma Delta Sevoflurane Vaporizer; Kent Scientific Co., Torrington, CT, USA). A total of 400 rats aged from P3D to P14D were used in different exposure sessions. Sevoflurane was given through conical tubes with different sizes to different aged animals under 100% oxygen.

2.2.1. MAC determination and survival rate

Given that age affects the MAC of inhalational anesthetics (Eger, 2001; Matsuura et al., 2009), we calculated the MAC of sevoflurane in our animals at 3, 7, and 14 days old at varying durations of anesthesia maintenance. Effective anesthesia was defined by a lack of motor responses to painful stimuli when clamping the tail with toothed forceps. After 3-min equilibration at 3% sevoflurane (gross estimation), the clamp was left in place for 30 s. or when substantial movement of the head or extremities was evident. When motor responses were exhibited, the concentration of sevoflurane was increased by 1% and retested 5 min later after re-equilibration; but if the rat displayed no responses, the concentration of sevoflurane was decreased by 1% and retested 5 min later after re-equilibration. The MAC was determined as the mean value of the concentration at which the animal did not show motor responses to tail clamping, i.e. the highest concentration. Each rat was determined in triplicate and the data were presented as mean and standard error. The survival rates of the animals were calculated when exposed to sevoflurane for different duration of anesthesia at 1 MAC ($n = 20$). The body temperature of the anesthetized animals was maintained using a heating pad (Model: HP15RB; Conair®, East Windsor, NJ, USA) on which the surface temperature was kept at 37 ± 1 °C.

2.2.2. Session 1: Lower concentration but repeated exposure on the same day

A total of 40 P3D rats were randomly scheduled for four groups for 1% sevoflurane exposure: only air, once a day (SID), twice a day (BID) and three times a day (TID) with 10 rats each, and the exposure lasted 2 h each time. After sevoflurane anesthesia, the animals were returned to maternal cages for three weeks and then separated into individual cages until the water maze test was performed at the 7th week after birth. The Morris Water Maze (MWM) training was given once a day for seven consecutive days and followed by measurement at 1, 2, 3, 4, 5, 6, 7, 10, and 15 weeks after training (Fig. 1).

2.2.3. Session 2: Single exposure with changing concentrations on the same day

To investigate the effect of the changing concentrations of single sevoflurane anesthesia on the adult spatial memory, we randomly assigned 40 P3D rats into another four groups with 10 animals each: air, 1%, 2%, and 4% of sevoflurane for 2 h exposure. The MWM test procedure and time points were the same throughout testing sessions.

2.2.4. Session 3: Multiple exposures with a lower concentration on different days

In this session, we assess the potential effect of multiple exposures to a lower concentration of sevoflurane (1%) at different days. In total, 50 P3D rats were randomized into 5 groups ($n = 10$):