Neurotoxicology and Teratology 61 (2017) 92–103

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Carbon monoxide incompletely prevents isoflurane-induced defects in murine neurodevelopment☆

Li Wang [a], Aili Wang [b], William W. Supplee [c], Kayla Koffler [b], Ying Cheng [d], Zenaide M.N. Quezado [a], Richard J. Levy [b,*]

[a] The Sheikh Zayed Institute for Pediatric Surgical Innovation, Division of Pain Medicine, Children's National Health System, Children's Research Institute, The George Washington University School of Medicine and Health Sciences, United States
[b] Department of Anesthesiology, Columbia University Medical Center, United States
[c] Rutgers New Jersey Medical School, United States
[d] Center for Genetic Medicine Research, Children's National Health System, Children's Research Institute, The George Washington University School of Medicine and Health Sciences, United States

ARTICLE INFO

Article history:
Received 20 October 2016
Received in revised form 17 January 2017
Accepted 24 January 2017
Available online 26 January 2017

Keywords:
Anesthesia
Isoflurane
Neurotoxicity
Carbon monoxide
Spatial reference memory
Social behavior
Brain
Development
Apoptosis

ABSTRACT

Background: Commonly used anesthetics have been shown to disrupt neurodevelopment in preclinical models. It has been proposed that such anesthesia-induced neurotoxicity is mediated by apoptotic neurodegeneration in the immature brain. Low dose carbon monoxide (CO) exerts cytoprotective properties and we have previously demonstrated that CO inhibits isoflurane-induced apoptosis in the developing murine brain. Here we utilized anti-apoptotic concentrations of CO to delineate the role of apoptotic neurodegeneration in anesthesia-induced neurotoxicity by assessing the effect of CO on isoflurane-induced defects in neurodevelopment.

Methods: C57Bl/6 mouse pups underwent 1-hour exposure to 0 ppm (air), 5 ppm, or 100 ppm CO in air with or without isoflurane on postnatal day 7. Cohorts were evaluated 5–7 weeks post exposure with T-maze cognitive testing followed by social behavior assessment. Brain size, whole brain cellular content, and neuronal density in primary somatosensory cortex and hippocampal CA3 region were measured as secondary outcomes 1-week or 5–7 weeks post exposure along with 7-day old, unexposed controls.

Results: Isoflurane impaired memory acquisition and resulted in abnormal social behavior. Low concentration CO abrogated anesthetic-induced defects in memory acquisition, however, it also resulted in impaired spatial reference memory and social behavior abnormalities. Changes in brain size, cellular content, and neuronal density over time related to the age of the animal and were unaffected by either isoflurane or CO.

Conclusions: Anti-apoptotic concentrations of CO incompletely prevented isoflurane-induced defects in neurodevelopment, lacked concentration-dependent effects, and only provided protection in certain domains suggesting that anesthesia-related neurotoxicity is not solely mediated by activation of the mitochondrial apoptosis pathway.

1. Introduction

Postnatal exposure to the most commonly used anesthetic agents has been shown to disrupt neurodevelopment in preclinical models, resulting in cognitive impairments and behavioral abnormalities later in life (Jevtovic-Todorovic et al., 2003; Stefovska et al., 2008; Istaphanous and Loepke, 2009; Brambrink et al., 2010; Istaphanous et al., 2011; Rizzi et al., 2010). Such toxicity has been demonstrated in many different animal species, including nonhuman primates, using a variety of exposure paradigms (Jevtovic-Todorovic et al., 2003; Stefovska et al., 2008; Istaphanous and Loepke, 2009; Brambrink et al., 2010; Istaphanous et al., 2011; Rizzi et al., 2010; Schenning et al., 2016; Raper et al., 2015; Liu et al., 2015). Although a causal relationship has yet to be established in children, several retrospective studies have suggested that anesthesia exposure at a young age is associated with subsequent defects in learning and scholastic performance (Wilder et al., 2009; DiMaggio et al., 2009; Flick et al., 2011; Psaty et al., 2015). Despite over a decade of rigorous experimentation, however, the exact mechanisms of anesthesia-induced neurotoxicity remain undefined.

Seminal work demonstrated widespread apoptotic neurodegeneration in the immature rodent brain following exposure to MK801 and

☆ Supported by NIH/NIGMS R01GM103842-01 (RJL), NIH/NIEHS P30 ES009089 (RJL), FAER MSARF (WWS)

* Corresponding author at: Department of Anesthesiology, College of Physicians and Surgeons of Columbia University, 622 W. 168th Street, New York, NY 10032, United States.
E-mail address: rl2740@cumc.columbia.edu (R.J. Levy).

http://dx.doi.org/10.1016/j.ntt.2017.01.004
0892-0362/

ethanol, raising concern for the neurodevelopmental consequences of perinatal drug abuse and providing a potential explanation for the reduction in brain mass and behavioral abnormalities seen with fetal alcohol syndrome (Ikonomidou et al., 1999; Ikonomidou et al., 2000). As an extension of these findings, it was found that exposure to anesthetics also triggered widespread apoptotic neuronal cell death at a critical period during neurodevelopment (Jevtovic-Todorovic et al., 2003). Although the upstream mechanisms are unknown, it has been proposed that such anesthesia-induced neurotoxicity may be mediated by the oxidative stress-associated mitochondrial apoptosis pathway (Olney et al., 2004; Yon et al., 2005; Bai et al., 2013; Boscolo et al., 2013; Zhang et al., 2010).

Programmed neuronal cell death is a natural developmental process, necessary for selective elimination of excess neurons and aberrant connections (Vanderhaeghen and Cheng, 2010; Sanno et al., 2010; Chan et al., 2002). During development, inhibition of the mitochondrial pathway of apoptosis or innate defects in physiologic programmed neuronal cell death result in excess number of neurons and megalencephaly while pathologic apoptotic neurodegeneration can decrease the pool of neurons and diminish the size of the forebrain (Vanderhaeghen and Cheng, 2010; Cheng et al., 2012; Cheng et al., 2013). As an example of the latter, ethanol exposure on postnatal day 7 (P7) has been shown to reduce total murine brain volume by 10–13%, reduces neocortical volume and surface area, and leads to a significant loss of GABAergic neurons (Smiley et al., 2015). Although early work suggested anesthesia-induced neuronal deletion in the developing rat brain, more recent studies indicate that postnatal anesthetic exposure does not result in a decrease in neuron numbers or neuron density despite widespread induction of apoptosis (Nikizad et al., 2007; Loepke et al., 2009; Osterop et al., 2015). Unlike ethanol toxicity, lack of a measureable effect of anesthetics on the net number of neurons and brain morphology raises questions about the mechanistic role of apoptotic neurodegeneration in anesthesia-induced neurotoxicity.

Low concentrations of carbon monoxide (CO) have been shown to exert cytoprotective and anti-apoptotic properties in a variety of tissues including the brain (Kim et al., 2005; Otterbein et al., 1999; Hoetzel et al., 2008; Lavitrano et al., 2004; Vieira et al., 2008; Mahan et al., 2012; Queiroga et al., 2012; Wang et al., 2011a). We have previously demonstrated that 3-hour exposure to low concentration CO inhibits natural apoptosis in the developing murine brain on P10, leading to measurable increases in neuronal content and neuron numbers, detectable increases in relative brain size, and quantifiable defects in memory, learning, and social behavior (Cheng et al., 2012). One-hour exposure to the same CO concentrations on P7 had variable effects on programmed cell death, however, modulated oxidative stress in the developing murine brain during isoflurane exposure and inhibited isoflurane-induced apoptosis in a dose-dependent manner (Cheng and Levy, 2014; Cheng et al., 2015). Based on these findings, we aimed to test the hypothesis that apoptotic neurodegeneration is causally linked to anesthesia-induced impairments in neurodevelopment. We hypothesized that low concentration CO would prevent isoflurane-induced defects in memory, learning, and social behavior following postnatal exposure. As secondary outcome measures, we assessed brain size, relative cellular content, and estimated neuronal density following neonatal isoflurane exposure. Postnatal isoflurane exposure induced subtle, but detectable impairments in neurocognition and socialization, but had no effect on murine brain weight or volume, cellular content, or neuron density. Low concentration CO abrogated anesthetic-induced defects in memory acquisition, however, resulted in impaired spatial reference memory and social behavior abnormalities following postnatal exposure with or without isoflurane. The data suggest anesthesia-mediated defects in neurodevelopment are not completely prevented by cytoprotective doses of CO despite prevention of isoflurane-induced apoptosis. Therefore, anesthesia-induced neurotoxicity may not be mediated solely by activation of the mitochondrial apoptosis pathway.

2. Methods

2.1. Animal exposures

The care of the animals in this study was in accordance with NIH and Institutional Animal Care and Use Committee guidelines. Study approval was granted by the Children's National Medical Center and Columbia University Medical Center. Six to eight week old breeding pairs of C57Bl/6 mice (20–30 g) were acquired (Charles River, Wilmington MA) to yield newborn pups. A total of 24 dams were used to complete the study. On P7, we exposed male and female C57Bl/6 mouse pups to air (0 ppm CO), 5 ppm CO in air, or 100 ppm CO in air with and without isoflurane (2 vol%) for 1 h in a 7-liter Plexiglas chamber (25 cm × 20 cm × 14 cm). The chamber had a port for fresh gas inlet and a port for gas outlet which was directed to a fume hood exhaust using standard suction tubing. Specific concentrations of CO in air (premixed gas H-cylinders, Air Products, Camden, NJ) were verified using an electrochemical sensing CO detector (Monoxor III, Bacharach, Anderson, CA). Designated CO mixtures were delivered through a variable bypass isoflurane vaporizer and exposure chamber at a flow rate of 4 l per minute. Inhaled concentration of anesthetic was determined within the chamber (RGM 5250; Datex-Ohmeda Inc., Louisville, CO) and was maintained at 2% isoflurane for the duration of exposure. Mouse body temperature was maintained between 36 and 37 °C with an infrared heating lamp (Cole-Parmer, Court Vernon Hills, IL). An equal number of male and female mice were evaluated and were randomly exposed to a designated CO concentration with or without isoflurane. Littermates were assigned to different treatment groups. Following exposure, pups were returned to their respective dams. P7 was chosen because synaptogenesis peaks at day 7 in rodents and is completed by the second or third week of life (Sanno et al., 2010; Rice and Barone, 2000). One-hour exposure to 2% isoflurane has previously been shown to induce neuronal degeneration in 7 day old mice and represents a brief anesthetic exposure (Cheng and Levy, 2014; Johnson et al., 2008). Furthermore, 1-hour exposure to such concentrations of CO inhibited isoflurane-induced apoptosis in various regions of mouse forebrain in a dose-dependent manner (Cheng and Levy, 2014).

2.2. Brain weight and volume

Separate cohorts of mice were evaluated 1-week post exposure or 5–7 weeks post exposure. An unexposed, naïve cohort was also evaluated on P7. Following euthanasia with pentobarbital (150 mg/kg, ip), body weight was measured with a calibrated electronic scale. Fresh whole brain was dissected, dura removed, and medulla severed just distal to the cerebellum. Brain weight was measured with an analytical balance. Brain volume was determined from the amount of saline displaced by fresh whole brain based on Archimede's principle (Karlen and Krubitzer, 2009). Brain weight-to-body weight ratios and brain volume-to-body weight ratios were calculated.

2.3. Immunoblot analysis

10 μg samples of homogenized whole brain protein were subjected to SDS-acrylamide gel electrophoresis and immunoblotting. Blots were labeled with a primary polyclonal antibody to bovine NeuN (Molecular Probes, Eugene, Oregon, USA), a primary monoclonal antibody to rabbit S100β (Cell Signaling, Danvers, MA, USA), a primary polyclonal antibody to rabbit adenomatous polyposis coli (APC) (Abcam, Cambridge, MA, USA), a primary monoclonal antibody to rabbit ionized calcium binding adapter molecule 1 (Iba1) (Abcam, Cambridge, MA, USA), and a primary monoclonal antibody to mouse β-Actin (Thermo Fisher Scientific, Waltham, MA, USA) and secondarily exposed to goat anti-bovine IgG or donkey anti-rabbit IgG (Santa Cruz Biotechnology Inc., Santa Cruz, California) or horse anti-mouse IgG (Cell Signaling,