NeuroToxicology 46 (2015) 155–164

Contents lists available at ScienceDirect

NeuroToxicology

Pre-administration of curcumin prevents neonatal sevoflurane exposure-induced neurobehavioral abnormalities in mice

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ARTICLE INFO

Article history:
Received 5 September 2014
Accepted 9 November 2014
Available online 15 November 2014

Keywords:
Cognition
Sevoflurane
Neurotoxicity
Curcumin

ABSTRACT

Sevoflurane, a commonly used inhaled anesthetic, can induce neuronal apoptosis in the developing rodent brain and correlate with functional neurological impairment later in life. However, the mechanisms underlying these deleterious effects of sevoflurane remain unclear and no effective treatment is currently available. Herein, the authors investigated whether curcumin can prevent the sevoflurane anesthesia-induced cognitive impairment in mice. Six-day-old C57BL/6 mice were exposed to 3% sevoflurane 2 h daily for 3 consecutive days and were treated with curcumin at the dose of 20 mg/kg or vehicle 30 min before the sevoflurane anesthesia from postnatal days 6 (P6) to P8. Cognitive functions were evaluated by open field, Morris water maze, and fear conditioning tests on P61, P63–69, and P77–78, respectively. In another separate experiment, mice were killed on day P8 or P78, and the brain tissues were harvested and then subjected to biochemistry studies. Our results showed that repeated neonatal sevoflurane exposure led to significant cognitive impairment later in life, which was associated with increased neuronal apoptosis, neuroinflammation, oxidative nitrosative stress, and decreased memory related proteins. By contrast, pre-administration of curcumin ameliorated early neuronal apoptosis, neuroinflammation, oxidative nitrosative stress, memory related proteins, and later cognitive dysfunction. In conclusion, our data suggested that curcumin pre-administration can prevent the sevoflurane exposure-induced cognitive impairment later in life, which may be partly attributed to its ability to attenuate the neural apoptosis, inflammation, and oxidative nitrosative stress in mouse brain.

1. Introduction

Inhalational anesthetics including sevoflurane are currently the most common agents used for general anesthesia induction and maintenance, especially for the pediatric patients (Boscolo et al., 2012; Pellegrini et al., 2014; Sanchez et al., 2011; Shen et al., 2013a,b; Vlisides and Xie, 2012). In humans, anesthetics are often administered during the brain growth spurt period that occurs from the third trimester to the age of approximately 2 years, and this time period is equivalent to the first week after birth in mice and rats (Jevtovic-Todorovic et al., 2003; Yuede et al., 2010). It has been suggested that general anesthetics can induce neuronal apoptosis (Yonamine et al., 2013), disturb neurogenesis (Zhu et al., 2010), and impair long-term neurocognitive function in the infant rodent brain (Flick et al., 2011; Boscolo et al., 2012). Notably, children who have multiple exposures (e.g., three times) to anesthetics and surgery at an early age also are at an increased risk of developing learning disabilities (Flick et al., 2011; Shen et al., 2013a,b; Wilder et al., 2009). Like other general anesthetic agents, sevoflurane is a potential neurotoxic to the developing brain (Boscolo et al., 2012; Pellegrini et al., 2014; Sanchez et al., 2011; Vlisides and Xie, 2012), which needs further studies to explore the exact underlying mechanisms and develop the therapeutic strategies to prevent this kind of neurocognitive dysfunction.

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; Cur, curcumin; DMSO, dimethyl sulfoxide; IL, interleukin; ELISA, enzyme-linked immunosorbent assay; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor-κB; Nox2, nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase; P, postnatal; $PaCO_2$, partial pressure of arterial carbon dioxide; PaO_2, partial pressure of arterial oxygen; PSD95, post synaptic density protein 95; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha.

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http://dx.doi.org/10.1016/j.neuro.2014.11.003
0161-813X/

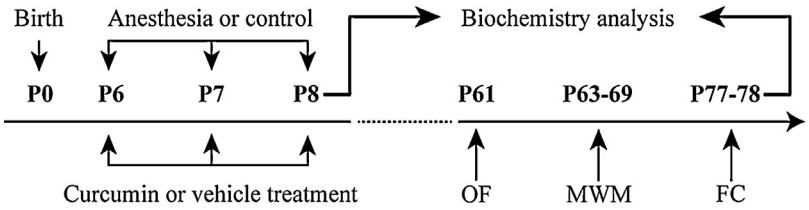

Fig. 1. Schematic timeline of the experimental procedure. Six-day-old mice were exposed to 3% sevoflurane 2 h daily for 3 days and were treated with curcumin (20 mg/kg) or vehicle 30 min before the sevoflurane anesthesia from postnatal days 6 (P6) to P8. The behavioral tests including open field (OF), Morris water maze (MWM), and fear conditioning (FC) tests were assessed on P61, P63–69, and P77–78, respectively.

Although the precise mechanisms underlying these deleterious effects of general anesthetics remain unclear, accumulating evidence has implicated neuroapotosis, neuroinflammation, reactive oxygen species accumulation, neurotransmitter disturbances, and mitochondrial dysfunction as contributing factors (Boscolo et al., 2012; Pellegrini et al., 2014; Sanchez et al., 2011; Shen et al., 2013a,b; Vlisides and Xie, 2012). In support of this notion, strategies focusing on these targets have presented an improvement in general anesthetics-induced cognitive dysfunction (Boscolo et al., 2012; Pellegrini et al., 2014; Sanchez et al., 2011; Shen et al., 2013a,b).

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] (diferuloyl methane), a phytochemical derived from the rhizome of curcuma longa, which can enter the central nervous system because of its lipophilic nature, exhibits a wide range of biological actions including potent antiapoptosis (Tiwari and Chopra, 2012), antiinflammation (Zhu et al., 2014), antioxidant activities (Kuo et al., 2011; Tiwari and Chopra, 2012), and preservation of mitochondrial integration (Liu et al., 2014a,b). Moreover, curcumin has been reported to take a positive effect on the neurogenesis in the hippocampus and brain-derived neurotrophic factor (BDNF) expression, reduction of which is associated with the detrimental effects of general anesthetics (Liu et al., 2014a,b; Yu et al., 2013a,b). These previous studies strongly suggest that curcumin can exert preventive or therapeutic properties for the sevoflurane anesthesia-induced cognitive impairment, which remains unclear currently. The present study was therefore designed to investigate whether pre-administration of curcumin could prevent sevoflurane exposure-induced cognitive impairment in neonatal mice and the underlying mechanisms.

2. Materials and methods

2.1. Animals and housing

The study protocol was approved by the Ethics Committee of Jinling Hospital, Nanjing University and the experiment was performed in accordance with the Guideline for the Care and Use of Laboratory Animals from the National Institutes of Health, USA. Six-day-old male C57BL/6 mice were purchased from the Animal Center of Jinling Hospital, Nanjing, China. The pups from postnatal day 6 (P6) to P20 were housed with maternal mice in a standard condition under a 12-h light/dark cycle (light from 07:00 to 19:00), with room temperature of 24 ± 1 °C and free access to food and water. At P21, the pups were weaned and housed 4–6 per cage in a standard environment. To minimize the influence of litter variability, no more than 2 pups from each litter were used for each experimental subgroup. Furthermore, we used only male offspring (n = 11–12 per group) in the behavioral tests and biochemical analysis to exclude the influence of estrogen (Kramár et al., 2013). The animals for the behavioral tests and biochemistry studies were all randomly selected. For biochemistry studies, no more than 2 pups from the same litter were used for each experimental subgroup. The flow chart for the study protocol is summarized in Fig. 1.

2.2. Animal grouping

The P6 mice were randomly assigned to the following four groups: control + vehicle (10 ml/kg) group; control + curcumin (20 mg/kg) group; sevoflurane + vehicle (10 ml/kg) group; and sevoflurane + curcumin (20 mg/kg) group. Animals in the sevoflurane groups received 3% sevoflurane in 40% oxygen (O_2)/air for 2 h daily for 3 consecutive days in the anesthetizing chamber where the mice were kept warm on a plate heated to 37 ± 1 °C. The mice breathed spontaneously and the concentrations of sevoflurane and O_2 were continuously monitored (GE Datex-ohmeda, Tewksbury, MA, USA). Pups exposed to sevoflurane were returned to their mothers in the cage after return of the righting reflex. The mice in the two control groups were treated in an identical chamber for the same time expect that they were not exposed to sevoflurane.

2.3. Drug administration

Curcumin (Sigma, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO, Sigma, St. Louis, MO, USA) and the concentration of DMSO was 0.1% of the total volume. For the dose–response study, curcumin at 10, 20, or 40 mg/kg was intraperitoneally administered 30 min before the start of the 3-day sevoflurane anesthesia sessions to determine the optimal dose for the prevention of sevoflurane anesthesia induced neurotoxicity. Our data suggested that 20 mg/kg was the optimal dose effective in increasing the freezing behavior. Next, 20 mg/kg curcumin was used in the control + curcumin and sevoflurane + curcumin groups. The equal volume (10 ml/kg) of DMSO solution (0.1%) was administered in the control + vehicle and sevoflurane + vehicle groups. All the P6–8 mice weighed about 2–4.5 g in this study and thus 20–45 microliters were intraperitoneally injected for each mouse.

2.4. Arterial blood gas analysis

Twenty-four mice not involved in other experiments were used to analyze the effect of sevoflurane on arterial blood gas immediately after the last sevoflurane anesthesia or 40% O_2 exposure (P8) by thoracotomy and orthoptic puncture of the left ventricle with an arterial blood gas analyzer (GEM Premier 3000, Instrumentation laboratory, USA). The experiments were terminated by decapitation.

2.5. Behavioral and cognitive tests

All behavioral tests were conducted at 14:00–16:00 p.m. in a sound-isolated room with the instruments provided by the Xinruan Corporation (Shanghai, China). All behavioral data were recorded by the same person who was blinded to the animal grouping as previous described in our previous studies (Ji et al.,