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Lipoic acid inhibited desflurane-induced hippocampal neuronal apoptosis through Caspase3 and NF-KappaB dependent pathway

Hui Zhao[a], Meimei Bu[b], Binglu Li[c], Yong Zhang[a,*]

[a] Galactophore Department, The Maternal and Child Health Hospital of Jinan City, Jinan, Shandong, China
[b] Anesthesia Department, The Maternal and Child Health Hospital of Jinan City, Jinan, Shandong, China
[c] Pharmacy Department, The Infectious Diseases Hospital of Jinan City, Jinan, Shandong, China

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ABSTRACT

Desfluraneis a widely-used general anesthetics. However, recent reports showed its significant side effect in the nervous system. Desflurane could lead to the neuronal death and affect the working memory. Unfortunately, the mechanism underlying the action of desflurane is still not clear and there is still no potent medicine to prevent the lesion in the central nervous system caused by general anesthetics. In this study, we found α-lipoic acid, an antioxidant exerting protective effect on multiple cells tissues, could resist the neurotoxicity caused by desflurane exposure. Lipoic acid possessed strong anti-apoptotic effect on the desflurane-treated hippocampal neurons, which was mediated by the Caspase-3 dependent pathway and NF-kappaB signaling. Collectively, we found a promising candidate to be clinically applied in intervention against the damage in nervous system by the desflurane.

1. Introduction

General anesthetics including desflurane and sevoflurane are required for surgical procedures. Recently, desflurane is gradually replacing isoflurane in the human use. Mechanistically, as a halogenated compound, desflurane interact with GABA-A receptor (Nishikawa and Harrison, 2003). Importantly, the binding interaction between desflurane and ligand-gated ion channel has been disclosed (Nury et al., 2011). Although the advantage of desflurane as general anesthetic is apparent, there are still some concerns in its clinical application, one of which is cytotoxicity. Previous studies showed that desflurane increase the cytotoxicity of intracellular and extracellular amyloid β (Yu and Zhang, 2013). Using animal models, it was demonstrated that desflurane exposure in neonatals induced severe neuroapoptosis and impaired working memory (Kodama et al., 2011). It was also reported that desflurane impairs outcome of organotypic hippocampal slices (Krings et al., 2016). To reduce the potential lesion during the desflurane application, it is significance to find out the substance counteracting the cytotoxic effect of desflurane.

During the process of general anesthetics, neurotoxicity is one of the major concerns, depending on the dose and duration (Perez-Castro et al., 2009). It was also shown that isoflurane induced neuronal degeneration in hippocampal slice cultures (Wise-Faberowski et al., 2005). All three anesthetic reagents including desflurane, isoflurane, and sevoflurane showed similar neurotoxic profiles (Istaphanous et al., 2011). By comparison, desflurane is a safer anesthetic than isoflurane in AD patients (Zhang et al., 2012). In preinjured neurons, isoflurane postexposure aggravates more severe neurotoxicity than sevoflurane or desflurane (Schallner et al., 2014). However, it is still not clear how to reduce or eliminate the neurotoxic potency of anesthetics. Recently, it was reported that Lipoic acid (LA) may hold potentials in preventing neuronal damage. Lipoic acid is also known as α-lipoic acid is an antioxidant, exerting multiple roles in regulating biological function in various organs and tissues (Zhang et al., 2007). In the nervous system, lipoic acid shows a critical role in preventing the neuronal injury induced by bupivacaine application (Wang et al., 2010). Later, similar function of lipoic acid was elucidated in counteracting the harmful effect of sevoflurane (Ma et al., 2016). This effect of lipoic acid is mediated by PI3K/PKG/ERK1/2 (Antonio and Druse, 2008) as well as PI3K/Akt signaling pathways(Zhang et al., 2001). Collectively, these data indicated a direct correlation between lipoic acid and protective effect against the neuronal damage induced by general anesthetics.

In the current study, we firstly reported lipoic acid could protect hippocampal neurons from the damage from desflurane exposure. This effect was correlated with the inhibition in caspase-3 dependent apoptotic pathway and activation of NF-kappaB pathway leading the enhancement in cell survival. Therefore, this study put forward a potent candidate to resist the apoptotic effect of desflurane on the central

* Corresponding author at: Anesthesia Department, the Maternal and Child Health Hospital of Jinan City, Jinan, Shandong, 250001, China.
E-mail address: yongzhangmchh@sina.com (Y. Zhang).

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nervous system.

2. Materials and methods

2.1. Chemicals

All the reagents were purchased from Sigma-Aldrich except for specified. Caspase-3 inhibitor, zDEVD-FMK was purchased from SM Biochemicals (Anaheim, CA). Inhibitors to NF-kappaB, PDTC or SN50, were purchased from Cayman Chemical (Ann Arbor, MI, USA).

2.2. Hippocampal neuronal culture

All the animal procedures were approved by the animal welfare commission of the Maternal and Child Health Hospital of Jinan City. Mouse hippocampal neurons were obtained from hippocampus of neonatal mouse. 7–9 neonatal mice were used in each neuronal culture preparation. Hippocampus was dissected and digested with 0.25% Trypsin-EDTA to make single cell suspension. The cells were seeded in Poly-D-lysine/laminin-coated plate and cultured in Neurobasal A medium, supplemented by 1%penicillin/streptomycin, 1% N2,10 ng/ml bFGF, 20 ng/ml NGFand 1% B27 for 7days. All chemicals were purchased from Thermofisher. A half-volume of medium was replaced with fresh medium once every 3 days.

2.3. Desflurane exposure

The exposure to desflurane was conducted as previously described (Xu et al., 2016). Briefly, culture dishes were placed in an airtight modular incubator chamber (Billups-Rothenberg). Desflurane mixed with medical air gas was vaporized using a Datex-OhmedaAestiva/5 vaporizer and concentrations were monitored with a GE Healthcare Gas Analyzer. We generally used clinically relevant concentrations (roughly 0.5 minimum alveolar concentration equivalent) of desflurane (~4%). Cells exposed to medical air onlywere set as controls. After various durations of exposure, the neurons were placed back and maintained in an incubator (37 °C, 5% CO2) until use.

2.4. Lipoic acid treatment

To elucidate the protective effect of lipoic acid, the neurons were pretreated with 5 μM lipoic acid before exposed to desflurane.

2.5. MTT assay

MTT was used to detect effect of desfluraneand lipoic acid on the viability of hippocampal neurons. Briefly, MTT was incubated with cells for at least 4 h to produce formazan. When formazan was completely dissolved by SDS-HCl, the absorbance at 570 nm was measured with a Universal Microplate Reader (Bio-Tek instruments), and OD (treated group)/OD (blank control group) was calculated.

2.6. TUNEL assay

Terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) assay was performed by using an in situ cell death detection kit (Santa Cruz Biotechnology). Briefly, hippocampal neurons were fixed with 4% paraformaldehyde and permeabilized with 0.2% Triton X-100. Fragmented DNA was labeled with fluorescein-12-dUTP at 37 °C for 1 h. After rinsing twice in PBS, TUNEL-positive nuclei were detected with a fluorescent microscope (Nikon).

2.7. Western blotting

2 μg cell lysates were loaded on each lane of 10% polyacrylamidegel, and then blotted onto a polyvinylidenedifluoride (PVDF) membrane. After blocking with a PBST containing 5% nonfat dry milk, the membrane was incubated with antibodies against cleaved caspase-3, BCL2, BAX, IKKβ, P65, P-AKT, CyclinD1, C-MYC or GAPDH. All the primary antibodies were purchased from Cell Signaling Technologies (Massachusetts, USA). Peroxidase-linked anti rabbit, goat or mouse IgG (purchased from Thermofisher) were used as secondary antibodies. These proteins were visualized by using an ECL western blotting detection kit (Amersham Biosciences).

2.8. Cell transfection

To visualize the alteration in neuronal morphology, the hippocampal neurons were transfected with EGFP-N2 plasmid by Lipofectamine 3000 (Thermofisher). The procedure was strictly followed by the manual of transfection reagent. Briefly, neurons in 24 well plate was transfected with 0.8 μg plasmid plus 1.5 μl transfection reagent. To minimize cytotoxicity, transfection reagent was removed at 6 h after incubation. The morphological alteration was analyzed at 24 h after transfection.

2.9. Quantitative PCR

Total RNA was isolated using TRIZOL reagent (Life Technologies). Synthesis of cDNA was performed by using one-step RT-PCR kit from Takara. SYBR Green (Toyobo) RT-PCR amplification and real time fluorescence detection were performed using the PRISM 7300 sequence detection system (Applied Biosystems). Relative gene expression was calculated by the ΔΔCt method.The primer sequences were as follows, MAP2, 5′-TGCCACCTGTTTCTCTCCAC-3′ and 5′-TCTTTTGCTTGCTCG GGATT-3′; GAPDH, 5′-GGTATCGTGGAAGGACTCATGAC-3′ and 5′-ATGCCAGTGAGCTTCCCGT TCAGC-3′.

2.10. Analysis of cell cycle phase by flow cytometry

Following the treatment of desflurane with or without lipoic acid, the hippocampal neurons were resuspended in PBS twice before fixation by adding dropwise into to 95% precooled ethanol. Prior to analysis, the cells were warmed, centrifuged at 450*g* for 5 min and resuspended twice in PBS, then stained with PI (containing RNase A at 50 μg/ml) at room temperature in the dark for 30 min. The DNA content was analyzed by flow cytometry using the CellQuest program (Becton-Dickinson and Co., USA).

2.11. Statistical analysis

All the data were deprived from three independent preparations. Data were presented as mean ± SEM. Relative density of protein expression level was analyzed by Image J software. Length of neurite was calculated by SPOT software. Unpaired Student's *t*-test was used to determine significant difference. A p value less than 0.05 is considered as significantly different.

3. Results

3.1. Desfluraneinhibited neuronal maturation and promoted apoptosis in hippocampal neurons

Firstly, we determined whether desflurane could affectthe hippocampal neuronal growth. The general viability of hippocampal neurons was impaired in the presence of desflurane, certified by the MTT assay (Fig. 1A). This cytotoxic effect showed dose dependent tendency based on the exposure time (Fig. 1A). Then, we asked whether desflurane cause the neuronal death. The exposure to desfluraneincreased the ratio of TUNEL+ cells in treated groups (Fig. 1B). Besides, the expression level of cleaved Caspase-3 was increased in desflurane-treated hippocampal neurons (Fig. 1C). These data indicated thatdesflurane