

Original Article

Nicotinamide improves sevoflurane-induced cognitive impairment through suppression of inflammation and anti-apoptosis in rat

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Received August 21, 2015; Accepted October 13, 2015; Epub November 15, 2015; Published November 30, 2015

Abstract: Nicotinamide is amide form of vitamin B3, participate in oxidation-reduction reaction, and it plays an important role in the maintenance of normal life activities in cells; it has broad application prospects in the treatment of heart blood-vessel disease, respiratory disease, type 1 diabetes and inflammatory autoimmune diseases. Thus the present study aimed to identify whether the nicotinamide improves sevoflurane-induced cognitive impairment and its potential mechanisms in rat. Firstly, Male Sprague-Dawley rats were induced by 2.1% sevoflurane for 6 h. Protective function of nicotinamide on cognitive impairment was evaluated using Morris water maze test in the rats. Next, NF- κ B and caspase-3 activities, and p53, Bax and Bcl-2 protein expression was executed using commercial kits and Western blot analysis, respectively. Preconditioning with nicotinamide could improve cognitive impairment in the rats. Administrate of nicotinamide suppressed the activation of NF- κ B and caspase-3, reduced the protein expression of Bax, and promoted Bcl-2 protein expression in rats. The present results suggested nicotinamide improves sevoflurane-induced cognitive impairment and has an anti-inflammatory and anti-apoptotic effect against sevoflurane-induced damages.

Keywords: Nicotinamide, sevoflurane, cognitive impairment, inflammation, apoptosis

Introduction

Today's age structure more and more tends to an aging society, and the proportion subsequently increases in elderly patients undergoing surgery in the clinical treatment [1]. Postoperative elderly patients are more prone to the characteristic clinical manifestations of mood and personality changes, decreased memory, mental disorders, etc. After the surgery, the changes in Cognitive abilities, personality, social skills and the ability of memory loss is called Postoperative Cognitive Dysfunction (POCD) [2, 3].

Sevoflurane is a kind of volatile inhalation anesthetics, colorless, no excitant with fruit aroma, and induction and wake-up are smooth and with quickly, good anaesthesia effect, but it affects the liver and kidney function, as one of the widely used clinically inhaled anesthetics [4]. In recent years, the relationship between

POCD and inhaled anesthetics get more and more attention. The studies suggested that sevoflurane may be caused by the central nervous system inflammation, oxidative stress reaction and intracellular calcium ion balance to influence the cognitive function of rats, studies adopt different concentrations of sevoflurane pretreatment, also find the neural function plays a protective role in rats. Some scholars currently think that POCD is basis on the patient's own degradation of the central nervous system, a variety of external factors can further damage the metabolism of the central nervous system and neurotransmitters caused by transfer function [5, 6].

Nicotinamide is the amide form of niacin, it is the synthesis coenzyme I dihydrouracil dehydrogenase (NAD⁺) and coenzyme II nicotinamide nicotinamide-adenine dinucleotide phosphate (NADP) precursor, by participating in the cell energy metabolism and play a role of protection

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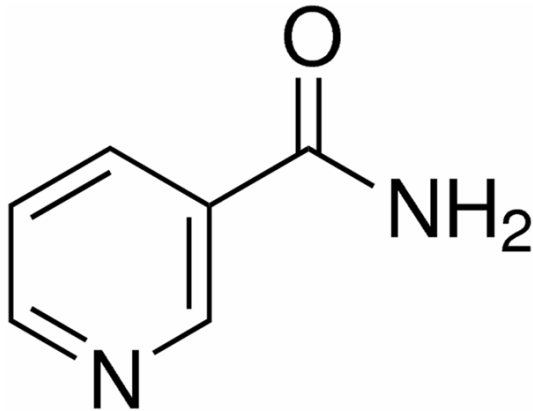


Figure 1. The chemical structure of nicotinamide.

in oxidative stress injury or inflammation, it can effectively prevent cell and cell membrane from damage by free radicals [7]. Nicotinamide also including the immune system dysfunction, diabetes and neural degenerative diseases affect the regulation of cell survival and death during the period of multiple oxidative stress pathways [8]. Nicotinamide can activate the inflammatory cells, early apoptosis cells and advanced nuclear DNA break down [9]. But the neuroprotective effect and underlying molecular mechanisms of nicotinamide on sevoflurane-induced cognitive impairment are still unknown. Therefore, we hypothesized whether the neuroprotective effect of nicotinamide improves sevoflurane-induced cognitive impairment through suppression of inflammation and anti-apoptosis in rat. It might play a role in the sevoflurane-induced memory impairment in rats.

Materials and methods

Animals, surgery and anesthesia treatment

Male Sprague-Dawley rats (200-250 g) were obtained from Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China), and were maintained under a 12:12 h light-dark cycle with a room temperature of $22 \pm 1^\circ\text{C}$, and given continuous access to food and water. All procedures were approved by the Utilization Committee of Dalian Medical University and confirmed by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

SD rats were placed into a chamber and exposed with 2.1% sevoflurane for 6 h. The

total gas flow was 1.5 l/min, using 70% O₂ as a carrier. The oxygen and anesthetic agent fractions were measured with gas analysis system. Then, the chamber was maintained at $37 \pm 1^\circ\text{C}$ with an infrared heat lamp. SD rats were sacrificed after 6 h of anesthesia.

Experimental design

All rats were randomly divided into three groups: the sham anesthesia group (Control), the sevoflurane anesthesia group (Sevoflurane) and nicotinamide-treated group (Treated). In sham anesthesia group, normal rats were administered saline. In sevoflurane anesthesia group, sevoflurane-induced rats were administered saline. In nicotinamide-treated group, sevoflurane-induced rats were 500 mg/kg nicotinamide for 7 continuous days.

Morris water maze (MWM) test

After the treatment with nicotinamide for 7 days, the spatial memory abilities were tested by using the (MWM) test [10, 11]. All rats were received the addition of black non-toxic ink twice per day (every morning and afternoon) for the first 5 days, a probe trial on the 6th day, and a visible platform trial on the 7th day. The water temperature was maintained at $25 \pm 1^\circ\text{C}$. The apparatus consisted of a circular water tank (180 cm diameter, 50 cm deep) was filled with water to a depth of 30 cm. Each time, rat was put into the pool from different quadrants for training for 120 sec. After arriving at the platform, the rat was allowed to stay on it for 30 sec, and the next training was performed after 120 sec of rest. On the 5th day, the hidden platform was removed, and each rat was allowed to swim freely for 120 sec.

Measurement of nuclear transcription factor-kappa (NF- κ B) and caspase-3 activities

After the treatment with nicotinamide for 7 days, the brain tissue was homogenized in physiological saline. Miscible liquids were centrifuged at 12,000 g for 15 minutes at 4°C . The clear upper supernatants were collected for analysis of NF- κ B and caspase-3 activities using the manufacturer's instructions (KeyGen Biotech, Nanjing, China).

Western blot analysis

After the treatment with nicotinamide for 7 days, the brain tissue was homogenized in

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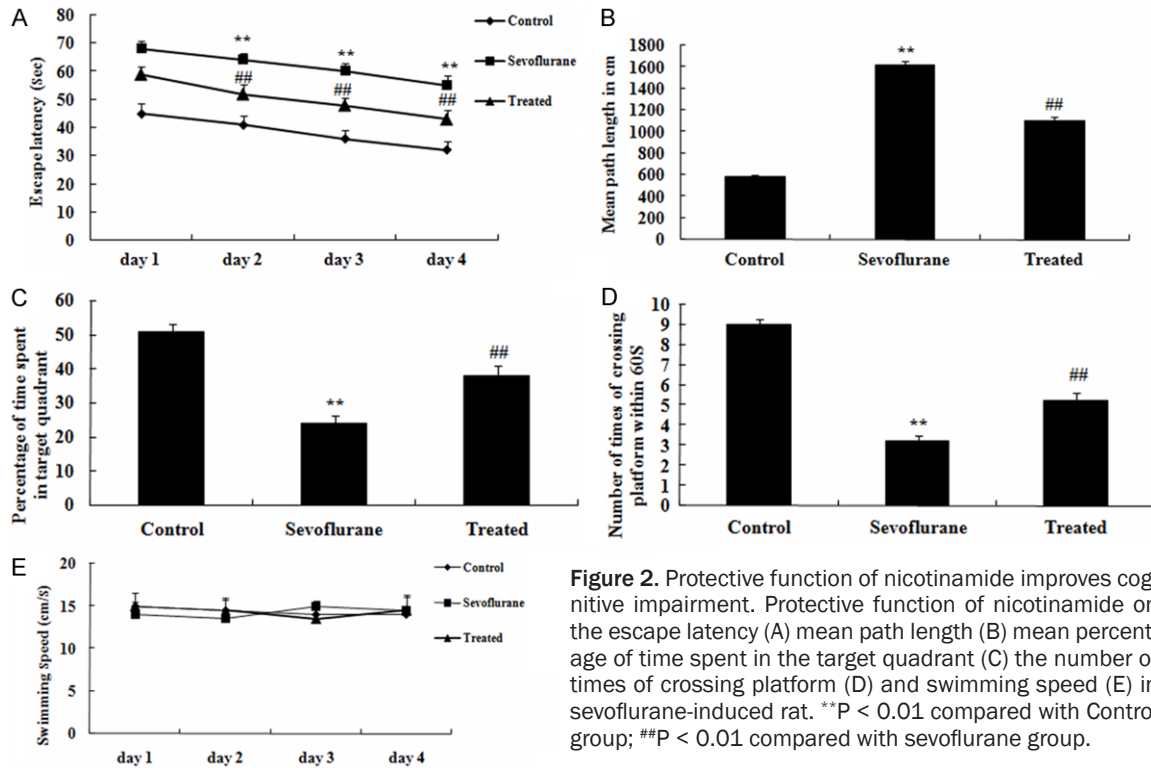


Figure 2. Protective function of nicotinamide improves cognitive impairment. Protective function of nicotinamide on the escape latency (A) mean path length (B) mean percentage of time spent in the target quadrant (C) the number of times of crossing platform (D) and swimming speed (E) in sevoflurane-induced rat. ** $P < 0.01$ compared with Control group; ## $P < 0.01$ compared with sevoflurane group.

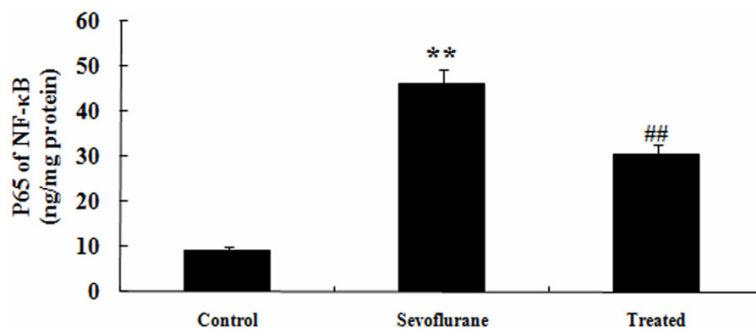


Figure 3. Protective function of nicotinamide against NF- κ B activity. ** $P < 0.01$ compared with Control group; ## $P < 0.01$ compared with sevoflurane group.

radioimmunoprecipitation assay lysis buffer, pH 7.4. Miscible liquids were centrifuged at 12,000 g for 15 minutes at 4°C. The clear upper supernatants were collected for analysis of the total protein contents using bicinchoninic acid (BCA) protein assay (Beyotime Institute of Biotechnology, Haimen, China). Equal quantities were loaded onto 10% SDS-polyacrylamide gel electrophoresis gel and transferred into nitrocellulose membranes (EMD Millipore, Billerica, MA, USA) for 2-3 hours. The membrane was blocked with 5% non-fat milk and incubated with anti-Bax (1:500, Santa Cruz Biotechnology, USA), anti-Bcl-2 (1:1000, Santa

Cruz Biotechnology, USA), anti-p53 (1:1000, Santa Cruz Biotechnology, USA) β -actin (1:500, Santa Cruz Biotechnology, USA) overnight at 4°C with shaking. Immunocomplex were visualized using an enhanced chemiluminescence-detection reagent (EMD Millipore).

Statistical analysis

All statistical analysis was done with SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA) and analyzed using analysis of variance (ANOVA). Values were expressed as mean standard deviation (SD) and a P value < 0.05 was considered statistically significant.

Results

Protective function of nicotinamide improves cognitive impairment

The chemical structure of nicotinamide (purity ≥ 99.5 , HPLC, Sigma, St. Louis, MO, USA) was represented in **Figure 1**. During the sevoflurane anesthesia, the escape latency and the mean

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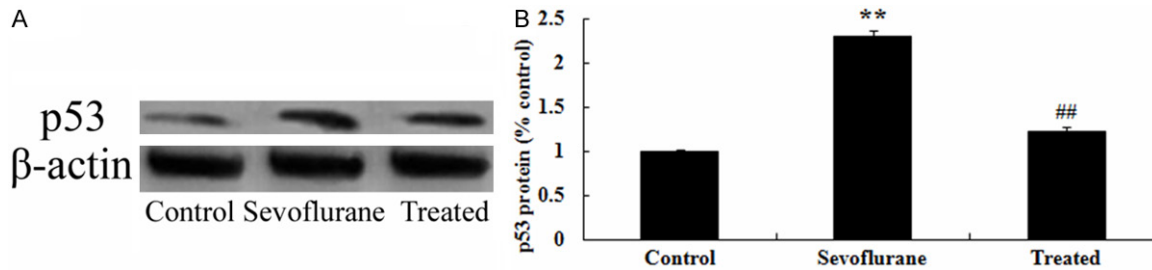


Figure 4. Protective function of nicotinamide against p53. Protective function of nicotinamide against p53 protein expression using western blotting analysis (A) and statistical analysis of p53 protein expression level (B) in sevoflurane-induced rat. **P < 0.01 compared with Control group; ##P < 0.01 compared with sevoflurane group.

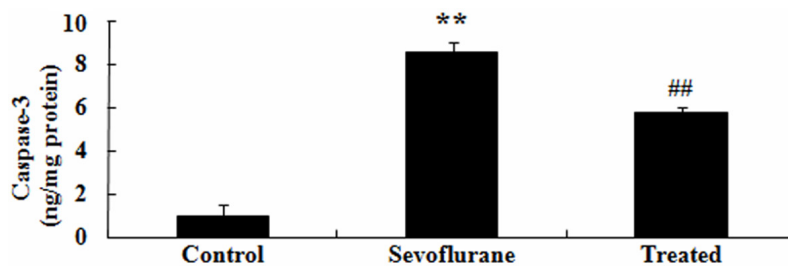


Figure 5. Protective function of nicotinamide against caspase-3 activity. **P < 0.01 compared with Control group; ##P < 0.01 compared with sevoflurane group.

Protective function of nicotinamide against NF- κ B activity

We examined whether the protective function of nicotinamide attenuates NF- κ B activity of rat with sevoflurane-induced. This result indicates that sevoflurane activated the NF- κ B activity in rats, compared to that of control group (Figure 3).

Increased activation of NF- κ B was suppressed by pre-treatment with nicotinamide, compared to that of sevoflurane group (Figure 3).

Protective function of nicotinamide against p53

To explore the protective function of nicotinamide affected on the protein expression of p53, we searched by western blot analyses in the rat brain. The proapoptotic protein, the p53 protein expression level of sevoflurane treatment, compared to that of control group (Figure 4A, 4B). However, pre-treatment nicotinamide had significant suppressed the sevoflurane-induced up-regulation of the p53 protein (Figure 4A, 4B).

Protective function of nicotinamide against caspase-3 activity

We inspected whether the protective function of nicotinamide affected on caspase-3 activity of rat with sevoflurane-induced. Compared with that of control group, sevoflurane caused a significant increased caspase-3 activity in rats (Figure 5). Nicotinamide treatments against

path length were enhanced by sevoflurane-induced in rats, when compared to that of control group (Figure 2A, 2B). The protective function of nicotinamide weakened the escape latency and the mean path length in sevoflurane-induced rat, compared to those of sevoflurane group (Figure 2A, 2B).

In the probe trials, sevoflurane spent less time in the target quadrant, compared to that of control group, and these differences were statistically significant (Figure 2C). The protective effect of nicotinamide augmented the time in the target quadrant of rat with sevoflurane-induced, compared to that of sevoflurane group (Figure 2C). Meanwhile, sevoflurane decreased the number of times the animals crossed the former platform location, when compared to that of control group (Figure 2D). The protective effect of nicotinamide recovered the number of times the animals crossed the former platform location, compared to that of sevoflurane group (Figure 2D). Interesting, there is no significant difference in the swimming speed between control, sevoflurane and nicotinamide group (Figure 2E).

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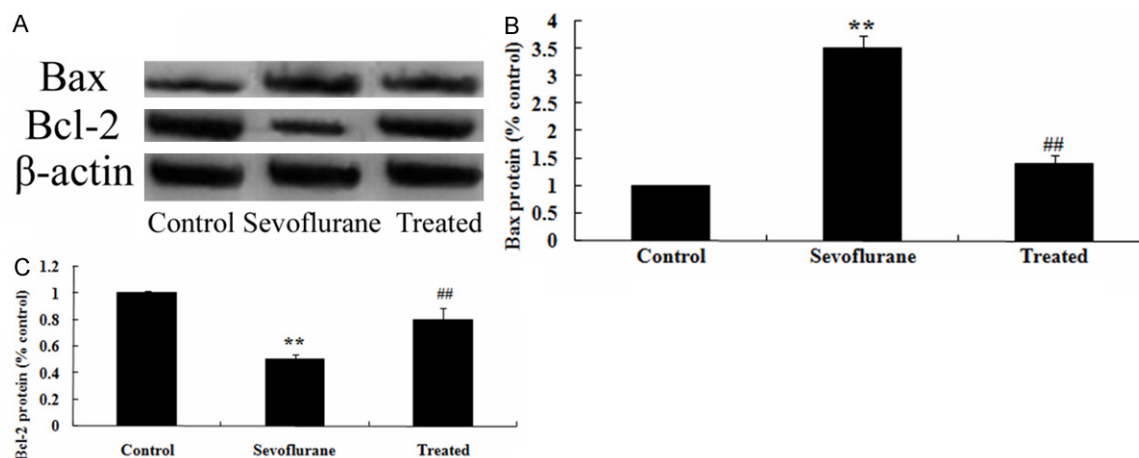


Figure 6. Protective function of nicotinamide against Bax and Bcl-2. Protective function of nicotinamide against Bax and Bcl-2 protein expressions using western blotting analysis (A) and statistical analysis of Bax (B) and Bcl-2 (C) protein expression levels in sevoflurane-induced rat. ** $P < 0.01$ compared with Control group; ## $P < 0.01$ compared with sevoflurane group.

sevoflurane resulted concurrent decrease caspase-3 activity in sevoflurane-induced rat, compared to that of sevoflurane group (Figure 5).

Protective function of nicotinamide against Bax and Bcl-2

To investigate the protective function of nicotinamide affected on protein levels of Bax and Bcl-2, we performed western blot analyses in the rat brain. Western blot analyses confirmed increased levels of Bax and reduced Bcl-2 in sevoflurane group compared to that of control group (Figure 6A-C). These results revealed that the protective function of nicotinamide reversed the protein expression of Bax and Bcl-2 in rats with sevoflurane-induced, compared to that of sevoflurane group (Figure 6A-C).

Discussion

In recent years, studies have shown that 1.3% of sevoflurane inhalation can improve learning and memory function of rats, it also has experiments show that sevoflurane can lead to cognitive impairment in rats [12]. Inhaled sevoflurane has certain influence on cognitive function in aged rats and the effect with inhaled concentration have a significant relationship; the greater the concentration the more significant. The mechanism may be caused by sevoflurane treatment after the increase of Amyloid we pro-

tein (APP), the Human Beta-Site APP-Cleaving Enzyme-1 (BACE-1), eventually lead to the increase of A beta [13]. This process may be related to Caspase activation and apoptosis, etc. The results showed that the protective function of nicotinamide improved cognitive impairment in the sevoflurane-induced rats. Shetty et al. reported that pre-treatment nicotinamide improves neuronal function after severe hypoxia [14]. Liu et al. suggested that nicotinamide forestalls pathology and cognitive decline in Alzheimer mice [15].

Nuclear transcription factor the NF-kappa B, in the resting state in dimer form, plasma combined with I kappa B predominate within the cell, activated and dissociation with I kappa B, the NF-kB nuclear factor positioning sequence, NF-kB entering the nuclei, and plays a role of transcriptional regulation in the form of a dimer, thus induced interleukin 1, intercellular adhesion molecules, tumor necrosis factor- α (TNF- α) and so on, in a variety of inflammatory mediators mediated by white blood cells, move, adhesion and ooze to vascular endothelial cell, involving in the inflammatory cascade reaction, eventually leading to ischemic neuronal damage [16, 17]. Along with the activation of p53 pathway brain aging process, aging brain inflammation factors (such as AA metabolic pathways of PGE2, cytokines) can improve the level of p53 neurons [18]. The above studies suggest the NF-kB/p53 pathway plays an

important role in the pathological process of inflammatory brain aging. The results from the present study supported this finding and showed that administration of nicotinamide weakened the activation of NF- κ B and the p53 protein expression in rats with sevoflurane-induced. A previous study showed that nicotinamide treatment reduces the NF- κ B, p53, and Bax levels in A β (1-42)-induced rat model of Alzheimer's disease [19]. Moreno-Vinasco et al. reported that nicotinamide inhibits inflammatory lung injury and NF- κ B levels in murine models [20].

Experiments found that cause nerve cell apoptosis, and longer the duration of ischemia, rats persistent cognitive dysfunction, that means the cells apoptosis participate in the process of cognitive dysfunction due to chronic ischemia VD [21]. Cell apoptosis with some apoptosis promote or inhibit the expression of genes, these genes of apoptosis occurred in the process of intervention and regulation of apoptosis. The Bcl-2 is an apoptosis inhibitor, it probably overexpresses through inhibiting cytochrome C from mitochondria spillover, which blocks apoptosis. Otherwise, the Bcl-2 may through reducing the formation of oxidation reaction material in neurons, inhibiting calcium overload, regulating the cell signal transduction pathway to play a role of antiapoptotic level is higher, and the degree of cell apoptosis lighter [22, 23]. Our results confirmed that administration of nicotinamide reduced caspase-3 activity and the protein expression of Bax, and decreased Bcl-2 protein expression in rats with sevoflurane-induced. Ullah et al. showed that nicotinamide inhibits activation of caspase-3 and Bax the protein expression, and suppressed alkylating agent-induced apoptotic neurodegeneration in the developing rat brain [24]. Ullah et al. suggested that the protective function of nicotinamide inhibits expression of activated caspase-3 and Bax, receded ketamine-induced apoptotic neurodegeneration in the infant rat brain [25].

In conclusion, the present study demonstrated that the nicotinamide improves sevoflurane-induced cognitive impairment through down-regulating inflammation and anti-apoptosis in rat. Finally, nicotinamide may have a potential neuroprotective activity and promises a potential therapeutic value in neuropathological conditions.

Disclosure of conflict of interest

None.

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