

Original Research Article

Sevoflurane and fentanyl exert protective effect on cognitive function in aged rats via regulation of inflammatory response in the brain

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Abstract

Purpose: To investigate the effects of sevoflurane and fentanyl on cognitive function in aged rats, and to determine the mechanism of action.

Methods: A total of 160 adult male Wistar rats were randomly assigned to four groups of 40 rats each. With the exception of control, the rats were surgically operated on. Sevoflurane group received sevoflurane (2 %) via inhalation for 2 h/day for 7 days, while the fentanyl group received fentanyl (50 µg/kg body weight) for 1 h via their tail veins for 7 days. The cognitive function of the rats was evaluated by shuttle box and Morris water maze (MWM) tests, while interleukin-6 (IL-6), vascular endothelial growth factor (VEGF) and tumor necrosis factor α (TNF-α) were evaluated using ELISA kits.

Results: The learning and memory latencies of the rats were significantly prolonged in surgery, with prolongation greater in sevoflurane and fentanyl groups than in control group; however, the latencies were significantly shorter in sevoflurane and fentanyl groups than in surgery group ($p < 0.05$). The levels of VEGF, IL-6 and TNF-α were significantly higher in the surgery, sevoflurane and fentanyl groups than in control group ($p < 0.05$).

Conclusion: Sevoflurane and fentanyl improve cognitive function in aged rats via a mechanism involving the regulation of inflammatory response in the brain.

Keywords: Sevoflurane, Fentanyl, Aged rats, Cognitive function, Inflammatory factors

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INTRODUCTION

Cognitive function is an essential neurological activity which enables animals to adapt to their environment, receive and process information, and acquire knowledge and experience. According to statistics, about 25 % of elderly patients develop postoperative cognitive dysfunctions which are characterized by changes

in personality, social abilities and cognitive abilities [1]. The specific causes, and processes involved in patient postoperative cognition decline, especially in the elderly, have not been fully elucidated. However, it has been hypothesized that brain damage and suppression of central neurotransmitter function may be involved [2,3]. Anesthesia is an indispensable medical step in surgical procedures. However,

the effect of anesthesia on cognitive ability, and the organs and molecules involved remain unclear. The aim of the present study was to investigate the effects of sevoflurane and fentanyl on cognitive function in aged rats, and to assess the underlying mechanism.

EXPERIMENTAL

Materials and equipment

Adult male Wistar rats were obtained from Nanjing Qinglongshan animal farm. Sevoflurane and fentanyl were products of Jiangsu Hengrui Medicine Co. Ltd, while VEGF, IL-6 and TNF- α kits were purchased from Beijing Lidman Biochemical Co. Ltd. Shuttle box and Morris-water maze were products of Anhui Zhenghua Biological Instrument Equipment Co. Ltd. Video monitoring and analysis system was purchased from Shanghai Xin Soft Information Technology Co. Ltd.

This research received approval from the Animal Ethical Committee of Anesthesia Department, Linzi District People's Hospital, Huangonglu 139, Linzi, Zibo, Shandong, China (approval no. 20186640), and was conducted in line with "Principles of Laboratory Animal Care" [4].

A total of 160 adult male Wistar rats (300 - 500 g) aged 20 months were randomly assigned to four groups of 40 rats each: control group, surgery group, sevoflurane group and fentanyl group. They were kept under standard conditions thus: 24 h light, stable temperature and humidity, and free access to feed and water. The rats were subjected to water maze test prior to enrollment, and those that had dyskinesia or cognitive impairment were excluded.

Surgical procedure

After anesthesia, the chests of rats in surgery, sevoflurane and fentanyl groups were cut open under auxiliary breathing, and their left coronary were arteries ligated within 30 min, and then re-passed for 2 h. Changes in the appearances of their myocardia or occurrence of arrhythmia were carefully observed and noted, after which the chests were sutured. In the control group, rat chests were opened and their hearts exposed for 2 h, and were sutured without coronary artery ligation.

Administration of anesthesia

Control and surgery groups: They received physiological saline for 7 days.

Sevoflurane group: Sevoflurane (2 %) was inhaled for 2 h/day for 7 days.

Fentanyl group: The tail vein was injected with fentanyl at a dose of 50 μ g/kg bwt, and venous pump was used to continuously pump the vein for 1 h for 7 days.

Evaluation of cognitive function

Active avoidance learning ability test

The rats were put in a shuttle box and permitted free movement for 5 min to eliminate exploration and reflection, after which they were set to start the buzzer. At the end of 20 sec of activity, electric shock stimulation was given for 10 sec, and the rats either fled to the opposite shore, or turned off the buzzer and electric shock. Rats fleeing to safe areas within 10 sec of buzzer activation were taken as having performed active avoidance. On the 30th and 60th days, the number of shocks and active avoidances were recorded and analyzed.

Morris water maze (MWM) test

The praxiological information of rats in the water maze was collected using ANY-maze video acquisition system. The rats were introduced into the pool at one of the four entries and different entries were used in a day. They were given 60 sec to find the site of the platform, and once they did, they remained there for another 10 sec. Rats unable to locate the platform within 60 sec were put on it for 10 sec, and thereafter taken out of the pool. The escape latency of rats, from water to searching and climbing on the platform (index reflecting memory ability) was observed and recorded.

Determination of levels of inflammatory factors

After the evaluation of cognitive function on the 30th and 60th days, the rats were sacrificed in batches, and their brain tissues excised and stored at -80 °C. The brain tissues were homogenized in ice-cold saline, and subjected to centrifugation at 3000 rpm for 10 min. The resultant supernatants were used for determination of levels of inflammatory factors using ELISA kits.

Statistical analysis

Data are expressed as mean \pm SEM. Statistical analysis of data was carried out with SPSS (19.0). Group comparison was done with *t*-test. Statistical significance was fixed at $p < 0.05$.

RESULTS

Cognitive function

Table 1 shows that on the 30th and 60th days, the number of electric shocks were significantly higher in surgery, sevoflurane and fentanyl groups than in the control group. However, the number of active avoidances of electric shocks were significantly lower in surgery, sevoflurane and fentanyl groups, relative to control group ($p < 0.05$). Active avoidances of electric shocks were significantly higher in sevoflurane and fentanyl groups than in the surgery group, but the sevoflurane group had significantly less avoidance than the fentanyl group ($p < 0.05$).

Table 1: Cognitive function in the various groups

Group	Electric shocks (time/min)		Active avoidance of electric shocks (time/min)	
	Day 30	Day 60	Day 30	Day 60
Control	53.41 ± 9.72	53.63 ± 6.55	86.49 ± 7.74	85.66 ± 6.29
Surgery	91.33 ± 5.75*	97.65 ± 6.52*	51.07 ± 10.24*	50.86 ± 7.05*
Sevoflurane	73.27 ± 6.83 [#]	74.49 ± 5.94 [#]	78.86 ± 9.49 [#]	78.04 ± 7.94 [#]
Fentanyl	85.62 ± 7.68 [#]	86.02 ± 6.11 [#]	66.08 ± 9.53 [#]	67.34 ± 5.82 [#]
<i>f</i>	235.39	427.07	119.49	117.06
<i>p</i>	0.000	0.000	0.000	0.000

* $P < 0.05$, relative to control group; [#] $p < 0.05$, relative to surgery group

Outcomes of MWM test

On the 30th and 60th days of the MWM test, learning and memory latencies of rats were significantly prolonged in surgery, sevoflurane and fentanyl groups, relative to sham-operated group ($p < 0.05$). In addition, learning and memory latencies of rats were significantly shorter in sevoflurane and fentanyl groups than

in surgery group, and were shorter in sevoflurane group than in fentanyl group ($p < 0.05$; Table 2).

Table 2: Morris-water maze test results

Group	Learning latency (s)		Memory latency (s)	
	30 ^m day	60 ^m day	30 ^m day	60 ^m day
Control	67.98 ± 8.85	68.77 ± 7.32	60.94 ± 5.88	61.07 ± 5.83
Surgery	177.68 ± 9.02*	178.09 ± 8.07*	153.94 ± 6.02*	152.60 ± 6.14*
Sevoflurane	145.92 ± 10.07 [#]	146.83 ± 7.99 [#]	120.56 ± 5.95 [#]	121.73 ± 6.09 [#]
Fentanyl	158.86 ± 8.27 [#]	160.07 ± 8.86 [#]	138.68 ± 5.98 [#]	139.08 ± 5.97 [#]
<i>f</i>	604.51	831.62	989.01	1061.15
<i>p</i>	0.000	0.000	0.000	0.000

* $P < 0.05$, when compared to control group; [#] $p < 0.05$ when compared to surgery group

Inflammatory factors

As shown in Table 3, TNF- α and VEGF were markedly higher in the surgery, sevoflurane and fentanyl groups, relative to the sham-operated group, and VEGF level was significantly higher in sevoflurane and fentanyl groups than in the surgery group ($p < 0.05$). Moreover, IL-6 was significantly upregulated in surgery, when compared to the fentanyl and sevoflurane groups.

DISCUSSION

Postoperative cognitive dysfunction which occurs in the elderly can delay their recovery from unconsciousness, significantly affect their quality of life, and may increase their mortality. Such cognitive decline can weaken the ability of organs to respond to surgical and traumatic stress. Sevoflurane and fentanyl are often used as anesthetics. However, there is limited evidence as to their involvement in the pathogenesis of postoperative cognitive dysfunction in elderly patients [5,6].

Table 3: Levels of inflammatory factors in the various groups

Group	VEGF (ng/L)		IL-6 (ng/L)		TNF- α (ng/L)	
	30 ^m day	60 ^m day	30 ^m day	60 ^m day	30 ^m day	60 ^m day
Control	53.09 ± 3.21	54.49 ± 6.01	14.67 ± 2.98	15.07 ± 2.38	17.15 ± 3.28	16.89 ± 2.45
Surgery	65.27 ± 4.02*	69.85 ± 5.93*	29.11 ± 3.12*	30.67 ± 2.15*	34.82 ± 4.06*	35.17 ± 3.23*
Sevoflurane	85.56 ± 4.77 [#]	86.29 ± 6.35 [#]	20.37 ± 2.87 [#]	21.05 ± 2.97 [#]	23.68 ± 3.97 [#]	24.78 ± 3.16 [#]
Fentanyl	78.32 ± 3.96 [#]	78.85 ± 5.76 [#]	22.26 ± 3.20 [#]	22.98 ± 3.21 [#]	27.52 ± 3.45 [#]	28.55 ± 3.76 [#]
<i>f</i>	321.47	123.44	117.69	144.76	94.73	162.37
<i>p</i>	0.000	0.000	0.000	0.000	0.000	0.000

* $P < 0.05$, relative to control group; [#] $p < 0.05$, relative to surgery group

In this investigation, MWM test revealed that the number of electric shocks were significantly higher in the surgery, sevoflurane and fentanyl groups than in control group. However, the number of active avoidances of electric shocks were significantly reduced in these groups than in control. The number of active avoidances of electric shocks were significantly higher in sevoflurane and fentanyl groups than in surgery group. The number of electric shocks was significantly lower in sevoflurane group than in fentanyl group. These results suggest that sevoflurane and fentanyl may exert some degree of protection on cognitive function in aged rats, and are in agreement with those previously reported [7-9]. Studies have shown that sevoflurane reduces myocardial infarct size via inhibition of the inflammatory response. The protective effect of sevoflurane on cognitive function in aged rats has been speculated to involve inhibition of the inflammatory response [10]. Increased levels of VEGF promote formation of blood vessels and nerves [11,12]. Interleukin-6 (IL-6), and TNF- α can directly or indirectly damage neurons of the hippocampus [13-15]. In this study, the levels of IL-6, VEGF and TNF- α were markedly higher in surgery, sevoflurane and fentanyl groups than in control group, and were significantly higher in sevoflurane and fentanyl groups than in surgery group. The level of IL-6 was significantly higher in surgery group than in fentanyl and sevoflurane groups. However, there were significant reductions in IL-6 and TNF- α levels in the sevoflurane and fentanyl groups, when compared with the surgery group. These results appear to suggest that sevoflurane and fentanyl may improve brain function in aged rats by reducing the levels of damaging inflammatory factors involved in the apoptosis of brain cells. These findings are consistent with those obtained in previous studies [16-18].

CONCLUSION

Sevoflurane and fentanyl improve cognitive function in aged rats through a mechanism involving the regulation of inflammatory response in the brain. This finding may also provide useful guides for the discovery of other therapeutic agents for improving cognitive function in the elderly through regulation of brain inflammation.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication. Peixiang Li conceived and designed the study, while Xin Zhao, Peixiang Li collected and analyzed the data. Xin Zhao wrote the manuscript.

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