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Original Article

The inflammatory response of two different kinds of anesthetics on vascular cognitive impairment rats and the effect on long term cognitive function

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Abstract: Vascular cognitive impairment, caused by vascular injury and inflammation, affects brain function. Present treatment for vascular injury primarily relies on combination therapy of surgery with anesthesia. In this study, we sought to determine the effects of anesthetics, sevoflurane and fentanyl, on long-term cognitive function in brain tissue of rats, and potential correlations with inflammatory factors such as VEGF, IL-1β, TNF-α. We used shuttle box and water maze tests to study the cognitive function of Wistar rats. The results demonstrated that rats treated with sevoflurane or fentanyl performed less shock times and more active escape times compared with rats model undergoing vascular cognitive impairment. Treatment of anesthetics also shortened the periods of learning and memory incubation, suggesting a protective role in cognitive function. In addition, our results unraveled a reducing expression of TNF-α and IL-1β but an increasing level of VEGF in head tissues of rats implemented with anesthetics. These findings underscore the improving role of sevoflurane and fentanyl in the recovery of vascular cognitive impairment rats as well as the cognitive function in rats, by regulating the expression of inflammatory factors.

Keywords: Sevoflurane, fentanyl, inflammatory factors, vascular cognitie impairment, cognitive function

Introduction

Previous studies have confirmed that vascular cognitive impairment is directly related to vascular injury and inflammation, while the damage degree is positively correlated with inflammation and vascular injury [1]. Study on physiological functions showed that brain plays its own special role in cognitive functions [2]. Therefore, conprehensive brain protection may contribute reduction of the vascular cognitive impairment in case of acute myocardial ischemia-reperfusion injury. Sevoflurane played a positive role against myocardial and cerebral injury in this scenario, as it facilited to decrease myocardial oxygen consumption, inhibit movements of myocardial calcium ions, activate mitochondrial signaling pathways and prevent the generation of inflammatory factor [3]. However, there is no direct and systemic verification in regard to its effect on the treatment of vascular cognitive impairment. Thus, we intend to investigate the cerebral protective effect of sevoflurane by intervening vascular cognitie impairment rats.

Materials and methods

Experimental animals

Eighty female Wistar rats weighted 300~350 g were provided from the animal center of Anhui Medical University. The rats were randomly divided into four groups. Food, but not water, was withdrawn 12 hours before the study started. The protocol was approved by the animal experiment ethical committee of Anhui Medical University.

Reagents and instruments

VEGF, IL-1β, and TNF-α detection kits were purchased from CapitalBio co., LTD (Beijing, China). Pentobarbital sodium and Evans blue were supplied by the Shanghai chemical reagent purchasing and supply station. Sevoflurane and fentanyl were manufactured by Nhwa pharma

Table 1. Shuttle box test ($\bar{x} \pm s$)

Group (n)	Shock times (/min)		Active escape times (/min)	
	Week 4	Week 8	Week 4	Week 8
Group A (n)	55.4 ± 8.1	52.0 ± 9.0	82.4 ± 7.2	85.6 ± 8.1
Group B (n)	92.2 ± 8.3[a]	98.1 ± 7.2[a]	55.2 ± 4.8[a]	51.7 ± 6.8[a]
Group C (n)	72.2 ± 7.1[a,b,c]	73.8 ± 6.2[a,b,c]	72.5 ± 6.3[a,b,c]	72.0 ± 5.1[a,b,c]
Group D (n)	83.2 ± 6.8[a,c]	85.5 ± 6.9[a,c]	67.2 ± 6.1[a,c]	67.3 ± 5.8[a,c]

[a], compared with group A, P < 0.05; [b], compared withgroup D, P < 0.05; [c], compared with group B, P < 0.05.

Table 2. Water maze test ($\bar{x} \pm s$)

Group (n)	Learning incubation period (s)		Memory incubation period (s)	
	Week 4	Week 8	Week 4	Week 8
Group A (n)	66.7 ± 5.8	64.1 ± 5.1	60.2 ± 4.3	58.3 ± 6.0
Group B (n)	172.4 ± 6.3[a]	177.6 ± 3.5[a]	153.4 ± 11.7[a]	148.7 ± 7.8[a]
Group C (n)	142.5 ± 8.3[a,b,c]	141.3 ± 7.1[a,b,c]	122.3 ± 6.8[a,b,c]	117.3 ± 8.1[a,b,c]
Group D (n)	157.3 ± 7.2[a,c]	155.7 ± 8.3[a,c]	135.5 ± 6.7[a,c]	129.3 ± 8.3[a,c]

[a], compared with group A, P < 0.05; [b], compared withgroup D, P < 0.05; [c], compared with group B, P < 0.05.

co., LTD (Jiangsu, China). The diving platform and shuttle box were provided from the Chongqing key laboratory of neurology. Small animal breathing machine was from the United States (Harvard type 638).

Animal model preparation

After pentobarbital anesthesia, the rats were intubated endotracheally and connected to breathing machine, with vital signs and electrocardiogram (ECG) being monitored. Femoral artery was punctured and inserted with tube. The chest and heart were initially exposed for the ligation of left coronary artery. ECG was observed after tightening the ligature. If the ST-T segment decreased for more than half, it revealed the appearance of myocardial ischemia. Myocardial ischemia occurred after the ligature being clipped for 30 min. Myocardial perfusion was evaluated by the observation of myocardial color or reperfusion arrhythmia. After 120 min reperfusion, the rats were sutured and put back into the box.

Rats in group A received sham operation by seton instead of ligation of the left coronary artery. Rats in group B were set as a model of vascular cognitive impairment. Rats in group C inhaled 2% sevoflurane and sustained 2 h for 5 consecutive days on base of group B, and the sevoflurane concentration was monitored. Rats in group D were intravenous injected with 50 μg/kg fentanyl, then maintained with 2 μg/kg·min intravenous infusion and sustained 1 h for 5 consecutive days based on group B.

Shuttle box and water maze tests were applied to train the cognitive function in rats. The cognitive function was evaluated after treatment for 4 weeks and 8 weeks, respectively. The hippocampus and cortex were separated for brain tissue specimen. ELISA was used to determine the levels of inflammatory cytokines such as VEGF, IL-1β, and TNF-α, in the brain. The shuttle box specification was 60 cm × 16 cm × 25 cm. A buzzer was carried out 20 s after the rats were placed, then the electric shock at 30 V, 50 Hz was given after 5 s. The buzzer and electric shock disappeared until the rats fled to the other side. The number of shock times and active escape times were recorded. The depth and temperature of the water maze were 62 cm, 20°C, respectively. It was mainly used to gather statistics of rats learning incubation period and memory incubation period.

Statistical analysis

SPSS 19.0 statistical software (SPSS, Chicago, IL, USA) was used for data analyses. Measurement data were presented as mean ± S.D. F test was applied for multiple sets comparison, while N-K test was applied between two groups with statistically significant differences defined as $P < 0.05$. Spearman correlation analysis between the expression of inflammatory factor and cognitive function was applied.

Results

Shuttle box test

The shock times in group B, C, D were significantly higher than that of group A, while the active escape times were significantly lower (*P*

Table 3. Inflammatory factors expression ($\bar{x} \pm s$)

Group (n)	VEGF (ng/L)		IL-1β (ng/L)		TNF-α (ng/L)	
	Week 4	Week 8	Week 4	Week 8	Week 4	Week 8
Group A (n)	59.0 ± 5.1	59.5 ± 6.3	16.3 ± 4.2	16.8 ± 5.1	16.6 ± 5.3	18.2 ± 6.0
Group B (n)	81.3 ± 6.0[a]	84.5 ± 7.1[a]	31.2 ± 5.1[a]	31.5 ± 4.2[a]	38.7 ± 6.0[a]	38.4 ± 5.1[a]
Group C (n)	98.3 ± 7.1[a,b,c]	104.3 ± 5.8[a,b,c]	23.3 ± 4.2[a,b,c]	23.8 ± 3.8[a,b,c]	24.3 ± 4.8[a,b,c]	24.5 ± 6.7[a,b,c]
Group D (n)	90.1 ± 5.1[a,c]	92.3 ± 6.1[a,c]	27.1 ± 4.1[a,c]	27.7 ± 4.4[a,c]	31.1 ± 5.8[a,c]	32.8 ± 6.0[a,c]

[a], compared with group A, $P < 0.05$; [b], compared with group D, $P < 0.05$; [c], compared with group B, $P < 0.05$.

< 0.001) both in week 4 and week 8. Rats in group C and D received less shock times and appeared more active escape times compared with group B ($P < 0.001$). Of note, similar phenomena were also observed when comparing group C and group D (**Table 1**).

Water maze test

The results of water maze experiment presented a trend similar to that in shuttle box test: in each time point, data in group A exhibited the optimal effect, followed by group C, group D and group B. In comparison, there are statistically significant differences between two independent groups ($P < 0.05$) (**Table 2**).

Inflammatory factors expression

The VEGF changes trend among each group is: group C exhibited highest level in each time point, followed by group D, group B and group A, the difference had statistical significance ($P < 0.01$). However, the expression of IL-1β and TNF-α in group B showed highest level in each time point, followed by group D, group C and group A ($P < 0.05$) (**Table 3**).

Correlation analysis between inflammatory factors and cognitive function

Correlation analysis was only performed among rats in group B, C, D that showed vascular cognitive impairment. VEGF presented being negative correlated with number of shock times, learning and memory incubation period (r = -0.314, -0.381, -0.31), while positive correlated with the number of active escape times (r = 0.411). Both IL-1β and TNF-α showed positive correlation with the number of shock times, learning and memory incubation period (r = 0.417, 0.455, 0.417; 0.381, 0.355, 0.321), but a negative correlation with the number of active escape times (r = -0.505; -0.417). The differences were likewise statistically significant ($P < 0.05$).

Discussion

Reperfusion of ischemic tissues associated with microvascular injury is caused by direct consequence of activation of the inflammatory response. The present clinical remedy for vascular injury is mainly surgery, with the combination of anesthesia. However, conventional anaesthetics such as propofol may cause independent cognitive dysfunction, which is not conducive to the recovery of vascular cognitive impairment [4, 5]. Based on this, we attempted to find out whether the anesthesia scheme favors the cognitive function recovery by using shuttle box test and water maze experiment. Our data illuminated that sevoflurane and fentanyl may both have a certain effect, while the former shows preferable profiles for treatment. Shuttle box test results confirmed that firstly, cognitive function in group B, C, D was severely damaged compared to that in group A; secondly, cognitive impairment was partly eased in group C and D after using anesthetics; lastly, cognitive impairment in group C was lighter than in group D. Water maze experiment results also showed in a similar fashion, which suggested that sevoflurane and fentanyl reduce the damage of cognitive function in rats caused by myocardial ischemia reperfusion. The comparison among groups in each time point further illustrated that there was a dramatic decline in rats' cognition as time went by. Eberspacher E also suggested that sevoflurane might have a positive impact on motor function and histopathology index of the rats after cerebral ischemia [6, 7].

A wide range of studies on protective and preprocessing protective roles demonstrated sevoflurane in vitro suppresses the production of inflammatory cytokines and reduces the area of myocardial infarction caused by ischemic reperfusion injury [8, 9]. However, the association between inflammatory mediators

and damaged cognitive function still need to be further investigated. In our cognitive function study, we found that myocardial ischemia reperfusion led to cognitive function impairment in rats; meanwhile, both sevoflurane and fentanyl alleviated the relevant damage. To explore its mechanism, we focused on the traditional inflammatory factor TNF-α and IL-1β and found the expressions of these cytokines in brain tissue were inhibited by sevoflurane [10, 11]. Previous research indicated TNF-α and IL-1 as the first identified inflammatory cytokines related to the disease [12, 13]. Some studies have pointed out that intraventricular injection IL-1β ameliorated the impairment of rat spatial learning ability [14], while the increasing level of TNF-α is associated with progressive cognitive deficits in chronic stress reaction [15]. In accordance with the above conclusion, our results also revealed that the inhibitory expression of TNF-α and IL-1β performs a reduction of the cognitive function damage in rats with ischemic injury, indicating a certain correlation between inflammatory cytokines and cognitive function, which is also in line with previous studies [16, 17]. There is no evidence showing that impeding the expression of inflammatory cytokines decreases cognitive impairment and promotes its natural recovery at the same time. To further investigate the lightening role of sevoflurane on rats' cognitive impairment, we focused the study on the VEGF, a growth factor, to promote the growth of blood vessels and the establishment of collateral circulation, and prevent vascular injuries caused by myocardial infarction [18]. Its elevated level contributed to the recovery of brain damage [19]. An increase of VEGF expression is elicited in the body's response to stress after the formation of cognitive impairment, while in our findings, higher level of sevoflurane in group C increments VEGF level for the recovery of brain tissue damage. Chen et al. also had shown that VEGF have a certain brain protection function [18, 20]. Thus, we speculated that sevoflurane might play protective function by promoting the expression of VEGF. Taken together, we conclude that sevoflurane and fentanyl may contribute to the recovery of cognitive function caused by vascular cognitive impairment in rats, in achieving a higher level of VEGF but lower levels of TNF-α and IL-1β expression.

Disclosure of conflict of interest

None.

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References

[1] Kitamura T, Sato K, Kawamura G, Yamada Y. The involvement of adenosine triphosphate-sensitive potassium channels in the different effects of sevoflurane and propofol on glucose metabolism in fed rats. Anesth Analg 2012; 114: 110-116.

[2] Zhang FJ, Ma LL, Wang WN, Qian LB, Yang MJ, Yu J, Chen G, Yu LN, Yan M. Hypercholesterolemia abrogates sevoflurane-induced delayed preconditioning against myocardial infarct in rats by alteration of nitric oxide synthase signaling. Shock 2012; 37: 485-491.

[3] Li Y, Liu C, Zhao Y, Hu K, Zhang J, Zeng M, Luo T, Jiang W, Wang H. Sevoflurane induces short-term changes in proteins in the cerebral cortices of developing rats. Acta Anaesthesiol Scand 2013; 57: 380-390.

[4] De Oliveira GS Jr, Fitzgerald PC, Ahmad S, Marcus RJ, McCarthy RJ. Desflurane/fentanyl compared with sevoflurane/fentanyl on awakening and quality of recovery in outpatient surgery using a laryngeal mask airway: a randomized, double-blinded controlled trial. J Clin Anesth 2013; 25: 651-658.

[5] Chen ZY, Tu WF, He H, Huang JX, Shi C. [Measurement of the minimum alveolar concentration of sevoflurane during combined anesthesia with sevoflurane, small-dose dexmedetomidine and fentanyl]. Nan Fang Yi Ke Da Xue Xue Bao 2011; 31: 718-720.

[6] Eberspacher E, Eckel B, Engelhard K, Muller K, Hoffman WE, Blobner M, Werner C. Effects of sevoflurane on cognitive deficit, motor function, and histopathology after cerebral ischemia in rats. Acta Anaesthesiol Scand 2009; 53: 774-782.

[7] Saunders AB, Hanzlicek AS, Martinez EA, Stickney MJ, Steiner JM, Suchodolski JS, Fosgate GT. Assessment of cardiac troponin I and C-reactive protein concentrations associated with anesthetic protocols using sevoflurane or a combination of fentanyl, midazolam, and sevoflurane in dogs. Vet Anaesth Analg 2009; 36: 449-456.

[8] Kim YJ, Lee H, Kim CH, Lee GY, Baik HJ, Han JI. Effect of flumazenil on recovery from anesthe-

sia and the bispectral index after sevoflurane/fentanyl general anesthesia in unpremedicated patients. Korean J Anesthesiol 2012; 62: 19-23.
[9] Moritz S, Schmidt C, Bucher M, Wiesenack C, Zimmermann M, Schebesch KM, Kasprzak P, Metz C. Neuromonitoring in carotid surgery: are the results obtained in awake patients transferable to patients under sevoflurane/fentanyl anesthesia? J Neurosurg Anesthesiol 2010; 22: 288-295.
[10] Lima-Rodriguez JR, Garcia-Gil FA, Garcia-Garcia JJ, Rocha-Camarero G, Martin-Cancho MF, Luis-Fernandez L, Crisostomo V, Uson-Gargallo J, Carrasco-Jimenez MS. Effects of premedication with tiletamine/zolazepam/medetomidine during general anesthesia using sevoflurane/fentanyl in swine undergoing pancreas transplantation. Transplant Proc 2008; 40: 3001-3006.
[11] Osama M, Asaad MH, Mohamed Y. Mohamed Sherif S El-mahgoup. Comparative study between prophylactic single dose of fentanyl and dexmedetomidine in the management of agitation after sevoflurane anesthesia in children. Egyptian Journal of Anaesthesia 2011; 27: 31-37.
[12] Peng S, Zhang Y, Li GJ, Zhang DX, Sun DP, Fang Q. The effect of sevoflurane on the expression of M1 acetylcholine receptor in the hippocampus and cognitive function of aged rats. Mol Cell Biochem 2012; 361: 229-233.
[13] Lovstad RZ, Stoen R. Postoperative epidural analgesia in children after major orthopaedic surgery. A randomised study of the effect on PONV of two anaesthetic techniques: low and high dose i.v. fentanyl and epidural infusions with and without fentanyl. Acta Anaesthesiol Scand 2001; 45: 482-488.
[14] Searle NR, Martineau RJ, Conzen P, al-Hasani A, Mark L, Ebert T, Muzi M, Hodgins LR. Comparison of sevoflurane/fentanyl and isoflurane/fentanyl during elective coronary artery bypass surgery. Sevoflurane Venture Group. Can J Anaesth 1996; 43: 890-899.
[15] Reilly S, Seddighi R, Egger CM, Rohrbach BW, Doherty TJ, Qu W, Johnson JR. The effect of fentanyl on the end-tidal sevoflurane concentration needed to prevent motor movement in dogs. Vet Anaesth Analg 2013; 40: 290-296.
[16] Milic M, Goranovic T, Knezevic P. Complications of sevoflurane-fentanyl versus midazolam-fentanyl anesthesia in pediatric cleft lip and palate surgery: a randomized comparison study. Int J Oral Maxillofac Surg 2010; 39: 5-9.
[17] Katoh T, Kobayashi S, Suzuki A, Iwamoto T, Bito H, Ikeda K. The effect of fentanyl on sevoflurane requirements for somatic and sympathetic responses to surgical incision. Anesthesiology 1999; 90: 398-405.
[18] Ledowski T, Manopas A, Lauer S. Bronchial mucus transport velocity in patients receiving desflurane and fentanyl vs. sevoflurane and fentanyl. Eur J Anaesthesiol 2008; 25: 752-755.
[19] Citerio G, Franzosi MG, Latini R, Masson S, Barlera S, Guzzetti S, Pesenti A. Anaesthesiological strategies in elective craniotomy: randomized, equivalence, open trial--the NeuroMorfeo trial. Trials 2009; 10: 19.
[20] Juckenhofel S, Feisel C, Schmitt HJ, Biedler A. [TIVA with propofol-remifentanil or balanced anesthesia with sevoflurane-fentanyl in laparoscopic operations. Hemodynamics, awakening and adverse effects]. Anaesthesist 1999; 48: 807-812.