ORIGINAL ARTICLE

Effect of sevoflurane and halothane anesthesia on cognitive function and immune function in young rats

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Abstract In the current study, we scrutinized the effect of sevoflurane and halothane on cognitive and immune function in young rats. The rats were divided into following groups: sevoflurane, halothane and sevoflurane + halothane groups, respectively. The rats were regularly treated with the pre-determined treatment. We also scrutinized the serum proinflammatory cytokines including IL-10, IL-4 and IL-2; brain level IL-1β; hippocampal neuronal apoptosis concentration were estimated. The water maze test was performed in rats for the estimation of cognitive ability. During the water maze test, on the 1st day the sevoflurane group showed the latency; sevoflurane and sevoflurane + halothane group demonstrated the declined latency gradually as compared to the control group rats after the 3 days. The latency of the control, halothane, sevoflurane + halothane group rats showed the reduced latency and also showed the reduced crossing circle times. The hippocampal neuron apoptosis was significantly increased in halothane and sevoflurane + halothane group as compared to control group rats, respectively. Control group rats demonstrated the increased neuron apoptosis. The proinflammatory cytokines including IL-10 and IL-4 was significantly higher in sevoflurane, halothane and sevoflurane + halothane group rats after anesthesia and the whole brain IL-1β was significantly decrease in the sevoflurane, halothane and sevoflurane + halothane as compared to control group. Sevoflurane can inhibit the anesthesia effect of halothane on the immune and cognitive function of rats.

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Peer review under responsibility of King Saud University.

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1. Introduction

Few of the studies showed the intravenous anesthetics does not show effects as analgesic but as having side effects on reducing respiration, which is commonly used in the clinical surgical treatmentv. Several studies revealed that the use of anesthesia can induce mental illness and cognitive destruction and other

adverse effects (Gan et al., 2011; Chen, 2012). Infant's and neonatal central nervous system and immune response are still in the expansion stage and predominantly responsive to the external environment. The mechanism of the action of the anesthesia effect on the immune function and central nervous system is also still unclear (Sun et al., 2010). Moreover, the current experimental study aim was to scrutinize the possible effect of the sevoflurane and halothane on the immune response and cognitive function in young rats.

2. Material and methods

2.1. Experimental study

Swiss Albino (Wistar strain) rats were used for the current study. All the animals were procured from the department animal house. All the animals were kept in the single cage and fed with the normal diet and *ad libitum*. The rats were stored in the standard environmental condition; the rats were stored in the normal temperature 25 ± 2, relative humidity.

2.2. Experimental model

The Wistar rats were divided into the following groups and each group contains 20 rats. Before the experimentation study the rats adopted breeding for one week in animal house. The rats were divided into the following groups: group A, B and C. The rats from the normal control received the intraperitoneal injection of saline (0.9%) 1 mL every 2 h, followed for 3 days. Group A rats received the 1 mL intraperitoneal injection of sevoflurane (80 mg/kg) every 2 h, followed for 3 days. Group B rats received the 1 mL intraperitoneal injection of halothane (80 mg/kg) every 2 h, followed for 3 days. Group C rats received the 1 mL intraperitoneal injection of sevoflurane + halothane (80 mg/kg) every 2 h, followed for 3 days. In the current study, we used the intravenous injection dose 1 mL, if the dose is less than the 1 mL the drug dissolved in the saline. After 15 min of the anesthesia, half of the rats were sacrificed, and rest of the animal was using for the Morris water maze test after 3 weeks. All abandoned or died rats in midway were supplemented via modeling again.

2.3. Estimation of immune parameters

For the estimation of the immune parameters, all group rats' blood samples were collected from puncturing the percutaneous at the point of maximal impulse and collecting in the sterile EP tube. After collecting the blood samples, the blood samples were centrifuged at 3000 rpm for 30 min at 4 °C. The serum samples were separated after the centrifuged; the separated serum samples were stored in the −80 °C. The immune parameters such as IL-10, IL-4 and IL-2 concentration were estimated via using the ELISA.

2.4. Collection, preparation and indicator test of Brain tissue

After collection of blood samples, half of the rats were immediately selected. The rats heart was exposed through thoracotomy and the perfusion needle was inserted into the ascending arota from the left ventricle and fixed. Then the auricle (right) was cut into small pieces and washed using the saline at 4 °C until the right atrium was clean and clear. After that it was fixed into the paraformaldehyde (4%) phosphate buffer. Brain tissue was used for the separation of the hippocampal when the body and the body tissue were hard. The tissue was cut and paraffin embedded. The terminal deoxynucleotidyl transferase mediated nick end labeling (TUNEL) technique was used for the estimation of neuronal apoptosis. The 6 horizons were randomly selected and their average optical density was estimated. The apoptosis index and positive intensity was estimated using the following formula

$$Apoptotic\ index\ (AI) = MOD \times Area\% \times 100$$

where MOD represents the average gray level; the area% denotes the percentage of total positive nucleus area in the total nucleus area. The rest of the rats cerebral were obtained immediately via opening the cranium and tissue of brain was mixed in saline using the homogenizer. The brain tissues (10%) were homogenized and centrifuged at 3000 rpm for 20 min at 4°C. The supernatant samples were stored in the −80 °C for further use. The whole brain IL-1β was estimated via using the ELISA.

2.5. Morris water maze test

Morris water maze test used for scrutinized the behavior of the rats. Morris water maze test contains the four quadrants on the round tanks and at the fourth quadrant was fixed, which was fixed in the underwater 1 cm (Marsden et al., 2010). The rats were randomly selected and put into the water of the selected quadrants, and the camera was used for counting the swim tracks of the rats. The rats were finding the latency, we also calculated. After performing the current test, the platform was successfully removed and rats were dipped into the water till the same point, the time crossing of the rats was also calculated.

2.6. Statistical analysis

The result of the current study was presented as mean ± SD value and graph pad prism software was used for analysis of the data. $P < 0.05$ was considered as the statically significant.

3. Result

3.1. Effect of sevoflurane and halothane on hippocampal neuron apoptosis

The apoptosis assay of the hippocampal neurons was performed on the current study. The experimental group sevoflurane confirms the significantly enhanced hippocampal neuron apoptosis (15.4 ± 7.4%), as compared to the hippocampal neuron apoptosis (2.9 ± 1.3%) of the control group rats. But the halothane group rats showed the hippocampal neuron apoptosis (5.1 ± 2.1%), which was not significant as compared to the control group rats. The sevoflurane + halothane group rats confirm the increased hippocampal neuron apoptosis (11.3 ± 5.7%) as compared to the control group rats and significantly decreased as compared to the sevoflurane group rats.

3.2. Estimation of immune response and whole brain level

All group rats showed that the IL-2 level in the serum of the all group rats was no significant (Fig. 1). The sevoflurane, halothane and sevoflurane + halothane group rats showed the increased level of IL-10 and IL-4 as compared to the control group rats (Figs. 2 and 3). The level of IL-10 and IL-4 in the sevoflurane + halothane was lower as compared to the sevoflurane group rats. The whole brain level IL-1β was lower observed in the sevoflurane, halothane and sevoflurane + halothane group rats as compared to the control group rats. The sevoflurane + halothane group rats showed the increased whole brain IL-1β level as compared to the sevoflurane control group rats (Fig. 4).

3.3. Observation of rat behavior

The latency of the sevoflurane group rats was unchanged at end of the study. While control, halothane and sevoflurane + halothane group rats showed the gradually reduced latency on the 1st and 3rd days, which was consider as significant ($P < 0.05$). The latency of sevoflurane, halothane and sevoflurane + halothane groups was gradually reduced after the 3 days of the experimental study. Halothane and sevoflurane + halothane group rats confirmed the different latency after the 3 days as compared to the sevoflurane group rats. Halothane and sevoflurane + halothane group rats showed the different crossing circle time as compared to the sevoflurane group rats ($P < 0.05$) (Fig. 5).

4. Discussion

Several studies showed that the use of the narcotic drug is strongly associated with the understanding the dysfunction, because postoperative cognitive dysfunction gain the attention. Several researchers confirm that the hippocampal neuron is directly connected to the long term memory (Wu, 2013). The injury of the neurons synapse can considerably influence the capacity to understand the memory and learning in animal. Postsynaptic membrane receptor mixed up with the rat's cognitive function, and also involved in the Alzheimer's patient pathogenesis which reduced the expression of the receptors. During the anesthesia condition, reduced the immune changes, attract to the researchers (Meyer et al., 2010). Sevoflurane and halothane, both anesthesia used as the first line anesthesia.
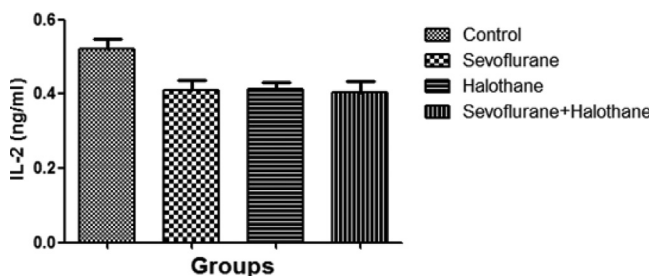

Figure 1 Effect of the IL-2 on the control, sevoflurane, halothane and sevofluarnae + Halothane group rats. Differences were considered significant at $^*P < 0.05$ when compared Test groups and # compared with the experimental group.
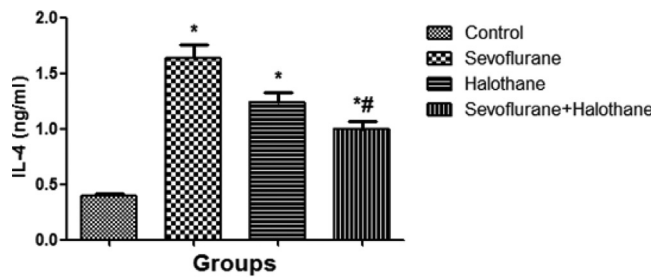

Figure 2 Effect of the IL-4 on the control, sevoflurane, halothane and sevofluarnae + Halothane group rats. Differences were considered significant at $^*P < 0.05$ when compared Test groups and # compared with the experimental group.

Figure 3 Effect of the IL-10 on the control, sevoflurane, halothane and sevofluarnae + Halothane group rats. Differences were considered significant at $^*P < 0.05$ when compared Test groups and # compared with the experimental group.
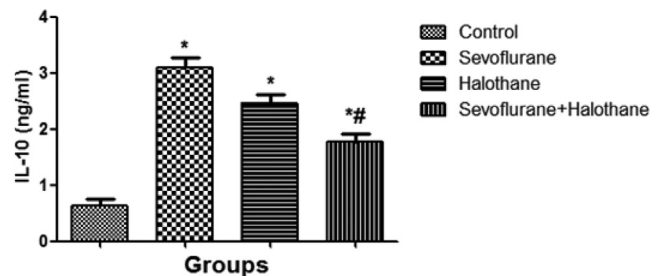

Figure 4 Effect of the IL-1β on the control, sevoflurane, halothane and sevofluarnae + Halothane group rats. Differences were considered significant at $^*P < 0.05$ when compared Test groups and # compared with the experimental group.
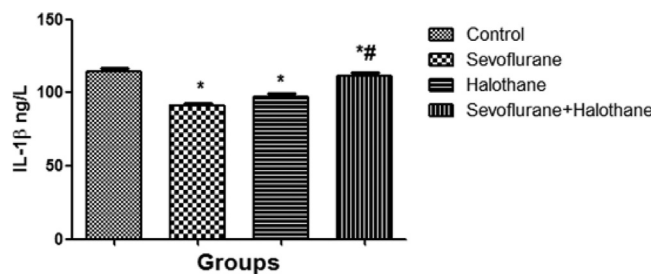
Both the anesthesia regulated the central nervous system via reducing the receptor of postsynaptic membrane, but the changes of the immune and cognitive function of the both anesthesia still are unclear till date. In the current study, the authors make effort to investigate the possible effect of the sevoflurane and halothane on the immune system and cerebral expansion.

For the estimation of the memory mechanism of the rats, a classical method Morris water maze test was used (Chen, 2007; Jiang et al., 2009). In the current study, we choose the classic method for the estimation the anesthesia effect of sevoflurane and halothane on the cognitive effect on the rats. The result of the current study showed that the use of the sevoflurane anesthesia latency at the 3 days is not significantly shorter as
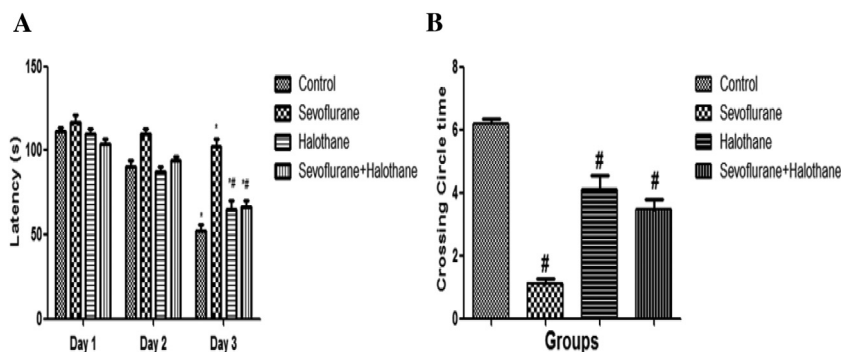
Figure 5 Effect of the control, sevoflurane, halothane and sevofluarnae + Halothane group rats on the Morris water maze test. A: shows the latency of the different groups and B: shows the crossing time of the different groups. Differences were considered significant at $^*P < 0.05$ when compared Test groups and # compared with the experimental group.

compared to the day 1 of rats after the use of the halothane anesthesia. But the sevoflurane anesthesia group rats revealed that the longer exploration time as compared to the normal control rats. The Morris test showed that the sevoflurane anesthesia treated rats demonstrated the increased cross times as compared to the normal control, which confirm that the sevoflurane can inhibit the memory and spatial learning capacity in the rats, but the sevoflurane group demonstrated the significant less degree.

Several studies demonstrated that the abusers of sevoflurane have a long memory injury, and its mechanism of action to damage the hippocampal neurons, which induce the rapid neuronal apoptosis in the rats (Erasso et al., 2012). Another drug halothane does not have effect on the cognitive function of rats, but few studies confirm that the halothane causes the affect on the brain expansion and degradation of nerve. Sevoflurane + halothane group rats showed the memory ability and spatial learning similar to the halothane group rats, and the latency of the sevoflurane + halothane shorter than the halothane group rats. Sevoflurane + halothane group rats confirm the flat time of cross also significant enhanced, the result of the current group confirm that the halothane reduced the effect of the sevoflurane on the cognitive functions. Sevoflurane + halothane treated group rats confirmed the decreased apoptosis rate as compared to the halothane via performing the study on the apoptosis of hippocampal neuronal, which is in accordance with change of the memory ability and spatial learning capacity in the rats and confirmed that halothane significantly inhibits the hippocampal neuron apoptosis in the rats. The current data revealed that the mechanism that halothane can inhibit the sevoflurane effect on rats cognitive lies in the apoptosis in hippocampal neuronal. With enhance the apoptosis in hippocampal neuronal, the memory dysfunction and long term memory learning are more prominent. Numerous investigation also confirm that the use of the halothane in the animals and humans have potential effect on the cerebral. The protective effect of halothane on the memory enhancing due to the inhibit the intracranial pressure and cerebral metabolic rate and also inhibiting the secretion of the excitatory amino acid glutamate neurotransmitter, completely bock the glutamate pathways, inhibit the neurotoxicity caused pathological injury, reduced the lipid peroxidation, avoiding the denaturation of protein, secretion of the inflammatory mediators and inhibiting the secretion of the free radicals.

Immunity is considered as the foremost effects of the anxiety response. The current study showed that the immune response in the rats still under expansion stage, and also sensitive to the encouragement of the external features. Usually it is supposed that the impact of the stress on the mainly due to the regulation and suppression effect on the immune functions. In the immune system, T lymphocytes are considered as the important component (Schoen et al.,2011). To maintain the effective body immune function needs the moderate concentration of homeostasis and response. The Th1 and Th2 cells play an important role in the balancing of the immune function. If the body showed the imbalance of the Th cells, it also confirmed the imbalance of the Th1/Th2 ratio and immune functions (Lewis et al., 2012; Ye et al., 2013). This imbalance also takes part in the secretion or activation of the inflammatory cells. The result of the current study confirms that the all group rats showing the effect on the secretion of the IL-2, but the serum tic LI-10 and IL-4 were considerably enhanced, while the IL-1β level was significantly reduced. Few investigations claim that the stress response of the young rats is different from that of adult rats. The adult rats showed the powerful adaptability, while young rats confirm the long lasting effects to stress response. Proinflammatory cytokines such as IL-1β secreted during the infection of the cells and the inflammatory states, which confirm the various physiological effects. Due to the effect on the physiological factor, it is also called the central stress arbitrated response (Erdogan et al., 2012; Rezaei et al., 2012).

5. Conclusion

In conclusion, sevoflurane can reduce the cognitive effect in young rats and confirm the less toxic effect on the hippocampal neurons. Sevoflurane used in combination with halothane, can inhibit the neurotoxicity and produce the protection to the brain tissue. Sevoflurane can reduce immune response in young rats, while halothane can inhibit sevoflurane's reduction to the immune response.

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