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Research Article
Induction of Beta-amyloid Protein by Sevoflurane Is Associated with Cognitive Impairment During Anesthesia in Aged Rats

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Abstract

Background and Objective: Sevoflurane is known to be associated with cognitive impairment during anesthesia in Alzheimer's Disease (AD) patients. However, the molecular mechanism underlying the pathogenesis caused by sevoflurane-induced anesthesia is not properly understood. The present investigation was an attempt to understand the molecular mechanism of sevoflurane anesthesia in causing cognitive decline in AD patients using aged rats as the model organism. **Materials and Methods:** In this study, aged rats (n = 60) were categorized into six different groups (CON, SLF-0, SLF-2, SLF-4, SLF-6 and SLF-8) having a population size of 10 rats in each group. The Control (CON groups were given 40% O_2 for 2 h) and the SLF groups were placed under anesthesia with 2.2% sevoflurane and 30% O_2 for 60 min. The rats in each of the SLF groups were analyzed for the exposure. The MWM (Morris water maze) test was assessed for assessing the cognitive function of the aged rats and the expression level of APP (Amyloid Precursor Protein), BACE-1 (β-site APP Cleavage Enzyme-1) and Aβ42 (Beta-amyloid-42) oligomers were analyzed compared to the CON group. **Results:** The study observed that the protein expression levels of APP mRNA were increased because of sevoflurane-induced anesthesia thereby promoting the overproduction of Aβ42 oligomers and depletion of APP protein. Interestingly, the expression of BACE-1 was not affected. Moreover, the SLF groups showed an increase in the escape latency and impaired memory. **Conclusion:** The study suggested that sevoflurane-induced anesthesia contributed to the cognitive decline in aged rats due to had increased expression of APP mRNA and oligomerization of Aβ42 peptide.

Key words: Alzheimer's disease, sevoflurane anesthesia, cognitive impairment, beta-amyloid

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

AD is a neurodegenerative disease which is characterized by changes in orientation, personality and cognitive abilities. AD is the major cause of dementia and cognitive decline[1]. It is usually prevalent in the elderly person with 65 years and above. The cause of Alzheimer's is not properly known, however, a majority of the risk is believed to be because of the genetic factor. AD is also caused by several other risk factors which include hypertension, injuries in brain and depression and there are no medicines to reverse its progression[2]. The beta-amyloid (Aβ) is of 36-43 amino acids long peptides which is present in the brains of Alzheimer's Disease (AD) patients[3]. Aβ is the major component of the amyloid plaques and the cleavage products of the transmembrane amyloid precursor protein[4]. The Aβ molecules sometimes aggregated and formed oligomers in different forms such as Aβ40 and Aβ42 which are toxic to nerve cells. In fact, the oligomers of Aβ are the major contributor to the loss of memory and cognitive decline in AD patients[5]. Additionally, there are various reports on the animal study and biochemical study that support the role of Aβ in the development of cognitive decline and Alzheimer's disease pathology[6,7]. There are also theories which suggest that the cytotoxic aggregated ubiquity of Aβ oligomers in AD patients due to Aβ peptide misfolding which is based on the amyloid cascade hypothesis[8]. The amyloid cascade hypothesis is based on the aggregate association between the Aβ oligomers and to death of the neuronal cells which is mainly because of oxidative stress and calcium dysregulation[9]. In fact, the etiology of AD is mainly due to the weakness of the neurons and synapses. It's primarily cellular etiology is related to the death of the neuronal cells and weakness of the synaptic function in limbic and cortical structures, thereby distressing the amygdala and hippocampus. Thus, forming the accumulation and overproduction of Aβ oligomers and peptides[10]. The β-amyloid is formed by the proteolysis of the Amyloid Precursor Protein (APP) by aspartyl protease β-site APP cleavage enzyme-1 (BACE-1)[11].

The beta-amyloid which derived from the APP is a type I transmembrane protein comprising of a large extracellular domain, short intracellular domain and a single transmembrane[12]. However, the APP is processed in two different pathways, viz. the amyloidogenic pathway and the non-amyloidogenic pathway[9]. In the non-amyloidogenic pathway metabolic pathway, the APP is cleaved by α-secretase and release the N-terminal soluble ectodomain (sAβPP α) which generates a 3 amino acid-long membrane. While the second cleavage of the APP by γ-secretase release the two major AB isoforms: Aβ42 (42 residues long) and Aβ40 (40 residues long)[13]. On the other hand, the expression of BACE-1 in AD patients with the deposited of Aβ was found to be higher in AD patients[14]. BACE-1 is also a major cause of the overproduction of Aβ and accumulation in the brain of AD patients. In fact, the accumulation and overproduction of Aβ are considered as the primary pathogenesis of AD[15]. There are also studies which suggest the administration of general anesthesia observe a higher incidence of Alzheimer's disease in aged patients. Thus indicating a high-risk factor for developing Alzheimer's disease in administering general anesthesia[10,15]. The present investigation was an attempt to study the risk factors arising from the administration of general anesthesia in the development of AD patients. Therefore, it is important to study the underlying mechanism between general anesthesia and the cause of cognitive decline in aged patients that contributes to reduce the important risk factors in AD patients. The present investigation deal with the effects of sevoflurane based on APP, β-amyloid and BACE-1 expression in the hippocampus of aged rats.

MATERIALS AND METHODS

Ethical statement: The protocol was approved by the Jilin University Ethical Committee. All procedures were performed in accordance with the 1964 Helsinki declaration and its later amendments or similar ethical standards.

Animals: A total of sixty aged Wistar rats (around 2 years old) were used as the animal model in the present investigation. The rats were fed with food and water and kept under optimum temperature in standard animal laboratory cages (23±2°C, 12 h light cycle). All animal work and protocol was approved by the Animal Care and Ethical Committee, China.

Experimental design: The Wistar rats were categorized into 6 different groups (Table 1) having a population of 10 rats in each group with a total population of 60 rats (n = 60). The first group was kept as Control Group (CON) while the rest were further categorized based on their day of sacrifice as sevoflurane induced anesthesia groups (SLF-0D, SFL-2D, SFL-4D, SFL-6D and SFL-8D).

Ten rats of each group were taken randomly for the Morris Water Maze (MWM) test and the rats in the CON (control) group were given 40% O_2 for 2h at 37°C. While the rats in SLF (sevoflurane) groups were placed under anesthesia with 2.2% sevoflurane and 30% O_2 for 60 min. After the sevoflurane anesthesia, the ISO groups were kept in 40% O_2 for another

Table 1: Categorization of the rats groups

SN	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Sacrifice Day		0	2	4	6	8
Group	CON	SLF-0D	SLF-2D	SLF-4D	SLF-6D	SLF-8D
Popn (n)	10	10	10	10	10	10

60 min at 37°C for fast recovery. The rats in the CON were then sacrificed for cognitive examination after the exposure. The rats in the SLF-0 group were also sacrificed on the same day (Day 0) right after the anesthetic recovery and cognitive examination. While the rats in the other SLF groups were sacrificed on Day 2, 4, 6 and 8, respectively right after the cognitive examination. The hippocampi of the sacrificed CON groups and SLF group rats were collected and their hippocampus and right cerebral cortex were kept in liquid nitrogen at -80°C for further analysis. Quantitative real-time PCR (RT-qPCR) was technique was used for analyzing the amyloid precursor protein and β-secretase-1 mRNA from the hippocampal samples.

Sevoflurane anesthesia: The rats were placed under anesthesia with 2.2% sevoflurane and 30% O_2 in the glass box for 60 min. While the control rats (CON) were given 40% O_2 for 2 h at 37°C. The rectal body temperature of the rats was maintained using an electric blanket. The rats were then monitored for their Respiratory Rate (RR), oxygen saturation meter for detecting the pulse oximeter oxygen saturation (SpO2), anesthetic inhalation Minimum Alveolar Concentration (MAC) values and Heart Rate (HR) using a multi-function monitor.

Morris Water Maze (MWM) experiments: The MWT starts with the first 3 days of adaptive training in a circular pool comprising of lukewarm water (25°C). A hidden platform was submerged beneath the water in one of the quadrants for analysis purpose. Seven rats from each group were chosen randomly for the MWM training. The rats were placed for navigation test for the consecutive 6 days prior to anesthesia or control exposure by placing four times randomly from the quadrants in the water maze and the rats were allowed to discover the hidden platform freely. The hidden platform was removed for conducting the probe trial on day 0, 2, 4, 6 and 8th day after sevoflurane anesthesia and the rats were sacrificed. The trajectories of the swimming path, latency and its speed were recorded and analyzed by the automatic tracking camera system and software.

Quantitative real-time PCR: Total mRNA was isolated from the hippocampus of the aged rat subjects using mRNA isolation kit as per the manufacturer's protocol (TransGen Biotech, Beijing, China). Further, the kit extracted mRNA was treated with DNA I-ase to remove any further contamination from genomic DNA. The purity of the mRNA was assessed using a spectrophotometer. Enzyme-specific PCR primers were designed for the RT-qPCR experiment. The transcripts were quantified for analyzing the relative mRNA expression and the target gene threshold values were normalized corresponding to reference gene.

Western blotting: The hippocampal samples were extracted using iced cold RIPA lysate buffer supplemented with 1 mM PSMF and the total protein was extracted using a standard Protein Extraction Tool Kit and the concentration of the protein content was quantified based on BCA assay kit (Beyotime, Jiangsu, China). The protein was then loaded into a separate lane and separated by 10% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel). The bands were then transferred into a polyvinylidene difluoride (PVDF) membrane. The membrane was incubated and blocked with 7% skimmed milk for 3 h and then probe with the corresponding antibody anti-APP, anti-BACE-1 and anti-Aβ42.

Statistical analysis: Statistical analysis was carried out using SPSS Statistical Software (Chicago, IL, USA). The data were represented based on the mean value of ±SD. The MWM test statistical analysis was carried out based on one-way ANOVA. The $p<0.05$ (two-tailed) was considered as statistically significant.

RESULTS

Influence of sevoflurane anesthesia on MWM experiment: The study observed that sevoflurane was able to induce the spatial memory of the aged rats based on the MWM test. In fact, the MWM test was first established to test the hippocampal-dependent learning which included the acquisition of spatial memory and long-term spatial memory in rodents[16]. In the present investigation, it was observed that prior to anesthesia; the aged rats were able to discover the hidden platform which was submerged in the water after 7 days of MSM training. The rats in all the groups were able to discover the submerged platform within 35-40 sec on the 6th and the 7th day. After the administration of sevoflurane anesthesia in the SLF groups, these rats tend to escape at a higher latency value compared to the CON groups (Fig. 1a). In Fig. 1b, it was observed that there was a decrease in the

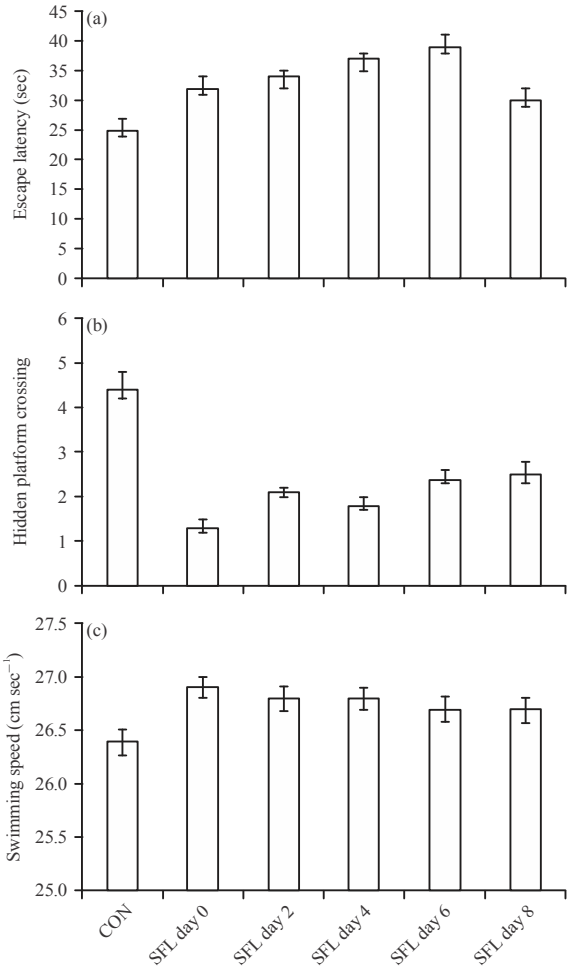

Fig. 1(a-c): Sevoflurane induced anesthesia of the aged rats on of both the CON and SFL groups on (a) Morris water maze (MWM) test analysis on escape latency and impaired memory in discovering the hidden platform, (b) Hidden platform crossing in the test and (C) Swimming speed

number of platform crossings in the SLF aged rat groups compared to CON group. However, no significant difference was observed in the speed of the swimming between the SLF aged rat groups (SLF-0D, SLF-2D, SLF-4D, SLF-6D and SLF-8D) and the CON rat groups (Fig. 1c).

Influence of sevoflurane anesthesia on APP expression: In Fig. 2, it was observed that the expression of APP was promoted by sevoflurane anesthesia in the hippocampus of aged rats. However, it did not promote the expression of BACE-1 mRNA based on the measurement by RT-qPCR in the hippocampus of these aged rats. It was also observed that the expression level of APP mRNA in the SLF-0D and SLF-2D aged

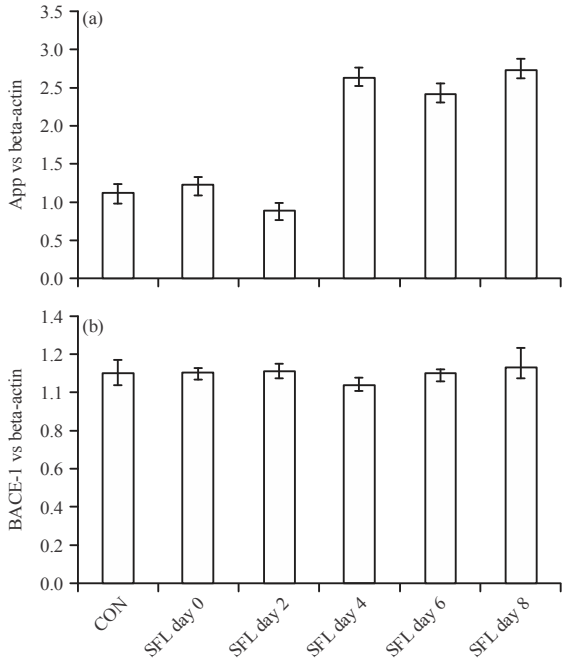

Fig. 2(a-b): mRNA expression level of (a) APP and (b) BACE-1 over Beta-actin after sevoflurane anesthesia in the hippocampus of aged rats measured by RT-qPCR

rat group were not changed compared to the CON group. It was observed that there was a significant increase in APP expression in SLF-4D, SLF-6D and SLF-8D aged rat groups (Fig. 2a). However, there was no significant difference in the mRNA expression level of the APP among SLF-4D, SLF-6D and SLF-8D aged rat groups. On the other hand, the expression level of BACE-1 mRNA between the control (CON) aged rat groups and the sevoflurane anesthesia rat groups (SLF-0D, SLF-2D, SLF-4D, SLF-6D and SLF-8D) was more or less similar (Fig. 2b).

Influence of sevoflurane anesthesia on production of $A\beta_{42}$ peptide: In this study, it was observed from Fig. 3 that the sevoflurane anesthesia up-regulate $A\beta_{42}$ peptide production while it depleted APP protein. However, sevoflurane anesthesia did not affect the over expression of BACE-1 based on the Western blot measurements. In Fig. 3a, the levels of APP protein tends to decrease towards sevoflurane anesthesia (SLF) age rat groups with the minimum in the SLF-8 group ($p<0.05$). Whereas in Fig. 3b, the expression level of BACE-1 of the sevoflurane anesthesia (SLF) rat groups were similar to that of the control rat groups (CON) and no significant variation was among these groups. Figure 3c revealed that the $A\beta_{42}$ oligomers were observed in different molecular weight (22 kDa-31 kDa). It was evident from the figure that the

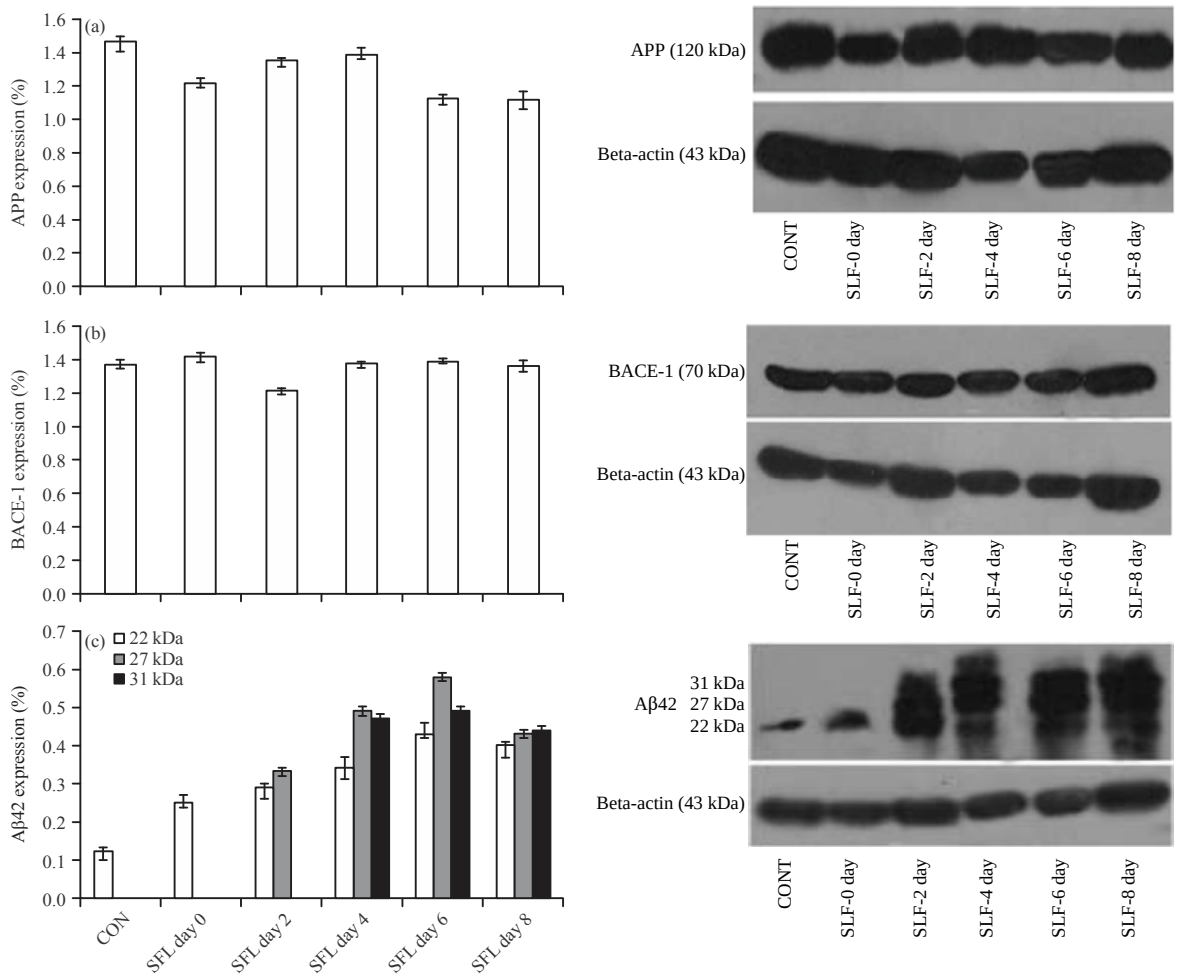

Fig. 3(a-c): Effects of sevoflurane induced anesthesia on the expression level of (a) APP, (b) BACE-1 and (c) Aβ42 oligomers in the hippocampal measured by the Western blot

expressions of $A\beta_{42}$ oligomers were considerably higher in the sevoflurane anesthesia SLF-0D towards SLF-8D rat groups compared to the CON groups. Thus, confirming that $A\beta_{42}$ was unregulated from SLF-0D towards SLF-8D.

DISCUSSION

In this study, it was observed that sevoflurane-induced long-term spatial memory in aged rats. This observation was based on the evaluations of the certain protein associated with Alzheimer's disease such as beta-amyloid protein in the aged rat's hippocampus. It was also observed that the sevoflurane anesthesia was not responsible for the spatial memory impairment because the speed of the rats swimming in the water was not affected even by the test exposures. Thus indicating that the motor deficits were not the primary factor for cognitive impairment.

In fact, sevoflurane is a commonly used clinical anesthetic compound. But the safety and accuracy of sevoflurane dosage vary from experiment to experiment[17]. Moreover, there are no concrete reports or evidence of sevoflurane inhalation anesthesia which have a negative influence on the Central Nervous System (CNS)[18]. Therefore, it is necessary for us to investigate the mechanism of sevoflurane inhalational anesthesia by detecting its biological target. In this investigation, the effects of sevoflurane associated with cognitive decline during anesthesia in aged rats were studied. The study also observed that the aged rats exposed to 2.2% sevoflurane anesthesia showed proper memory impairments in the MWM training. Thus, revealing that the appropriate dosage of sevoflurane anesthesia was able to induce the cognitive impairment in aged rats. Based on the reports by Zhang *et al.*[19] sevoflurane is one of the most commonly used inhalation anesthetics in patients and Inhaled sevoflurane may

promote neuropathogenesis in AD patients. But one previous study by Liu *et al.*[20] observed that sevoflurane anesthesia does not impair acquisition learning in mice and the long-term cytotoxic effects of sevoflurane, caused damage in the hippocampal neurons in neonatal mice. However, there are other several studies which report that low dose of sevoflurane exposure manifest improvement in the behavioral learning and retention in rats[21]. However, the difference may be because of the experimental conditions and the ages of the laboratory animal and the concentration of the chemicals used for anesthesia. Moreover, the escape latency of the SLF-8 and pattern of the hidden platform for SLF-4D, SLF-6D and SLF-8D did not come back to its normal level. Hence, the study cogitates that 2.2% sevoflurane anesthesia for 60 min induces cognitive impairment in the studied animal model. In this study, 2.2% of sevoflurane for 60 min was able to increase the APP mRNA but it decreases the protein expression of APP in the aged rats' hippocampi. In fact, there reports of sevoflurane which indicates the abnormal promotion of APP thereby progressing the pathogenesis of AD[22]. In this study, it was observed that the expression of APP protein in the sevoflurane-treated rat groups tends a sliding tendency after sevoflurane anesthesia. However, the expression of Aβ42 oligomers tends to upregulate after sevoflurane anesthesia. Earlier, it was reported by Lu *et al.*[23] that exposure of 2.5% sevoflurane for several hours initiate the promotion of APP and induced the production of Aβ oligomers. And the same finding is in agreement with this study from 2.2% sevoflurane anesthesia for 60 min which have an impact on APP processing in the hippocampi of aged rats. Surprisingly, it was observed that sevoflurane does not affect the expression of BACE-1 which usually elevates in the brain of AD patients. On the other hand, it might be also possible that the secretion level of γ-secretase is altered by sevoflurane thereby promoting the progression of APP in the hippocampus of the aged rats. In fact, BACE-1 aids in the proteolysis of APP thereby producing Aβ. After sevoflurane anesthesia the primary oligomers of Aβ42 were detected which belongs to the ADDLs category. In fact, ADDL is responsible for the damage of mature neuronal cells and synaptic dysfunction in AD patients[24]. In this study, it was put forward the concept that the $A\beta_{42}$ oligomers were overproduced because of the peptide oligomerization promotion due to sevoflurane anesthesia. It is also observed that, with the increasing $A\beta_{42}$ level in the aged rat's hippocampus, the rats tend to show a decline in the cognitive behavior after sevoflurane anesthesia. The same result has also been observed by Yue *et al.*[25] where the workers also observed a relation between increase level of $A\beta_{42}$ oligomers and cognitive impairment. The present study implies that the Aβ oligomers were responsible for the sevoflurane induced cognitive decline in the studied animal model. The study also observed that the decline in the cognitive recognition may be because of the AD and its associated diseases. The study also observed that sevoflurane anesthesia up-regulate the APP mRNA expression and over expressed $A\beta_{42}$ peptide and its oligomerization. Because of these reasons, the aged rats after sevoflurane anesthesia were observed with cognitive decline. Lastly, the study also provide an impetus on the biological mechanism of sevoflurane anesthesia in the induction of cognitive impairment which should be further noted by future researchers and scientists to explain the pathogenesis of AD.

CONCLUSION

To conclude, the study observed that sevoflurane anesthesia was associated with cognitive decline in aged rats. This is because; the APP mRNA expression was significantly higher in the hippocampus of the aged rats thereby depleting the APP protein. Thus, promoting the oligomerization of $A\beta_{42}$ peptide which is toxic to the neuronal cells thereby contributing to cognitive impairments. The study also recommends that the present findings on sevoflurane anesthesia should be further noted by future researchers and scientists to elucidate the pathogenesis of Alzheimer's disease in aged patients.

SIGNIFICANCE STATEMENT

The significance of the study is that sevoflurane anesthesia is associated with cognitive decline because of the APP mRNA expression. The study is also first such report which observed the induction of beta-amyloid protein by sevoflurane. Hence, the study recommend the limited use of sevoflurane in aged patients.

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