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Isoflurane and sevoflurane affects Wnt/β-catenin signaling pathways in hippocampal formation of neonatal rats

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Abstract. – OBJECTIVE: General anesthesia impairs spatial learning and memory in neonatal rats. The aim of this study was to investigate whether the Wnt pathway was involved in neonatal isoflurane and sevoflurane exposure-induced neurocognitive impairment.

MATERIALS AND METHODS: Sprague-Dawley rats were randomly assigned to administration isoflurane or sevoflurane for 6 hours at postnatal 7 days. Wnt inhibitor XAV 939 was administrated 30 min before anesthesia. Morris water maze was used to test the learning and memory at 5-week and 10-week. Hematoxylin and Eosin (H&E) stain was performed to evaluation the neuronal death in the hippocampus. Quantitative Real-time PCR (q-PCR) and Western blot assays were used to measure mRNA and proteins expression levels of the Wnt3a, GSK 3β and β-catenin, respectively.

RESULTS: The results showed that isoflurane or sevoflurane could significantly increase neonatal death and cell lost in the developing brain and the Wnt inhibitor could improve the cell degeneration. It demonstrated that isoflurane or sevoflurane could impair the P7 rats learning and memory capability, while these effects were reduced over time. When rats treated Wnt inhibitor at 30 min before anesthesia, the impairment of brain could relieve. q-PCR and Western blot demonstrated that isoflurane or sevoflurane affects expression levels of Wnt3a, GSK 3β and β-catenin. These results suggested that impairment of learning and memory in P7 rats may be related to the Wnt signaling pathway.

CONCLUSIONS: The results suggested general anesthesia treatment led to increased brain cell degeneration and impaired learning and memory in P7 rats via Wnt signaling pathway.

Key Words:
Isoflurane, Sevoflurane, Wnt signaling pathway, P7 rats, Morris water maze.

Introduction

Millions of pregnant women and neonates undergo anesthesia to prevent pain during childbirth or for surgical procefures[1]. Jevtovic-Todorovic et al[2] first reported that an anesthetic combination of isoflurane, nitrous oxide and midazolam could kill brain cells and impaired learning and long-term neurocognitive functions. General anesthesia is inhaled or injected anesthetic agents to cause a state of unconsciousness during the surgery. However, the mechanism of anesthesia is still not understood very well. Jevtovic-Todorovic's findings are growing concern relevant to human health within the public and anesthesia community[3].

Volatile anesthetics, such as isoflurane and sevoflurane, could inhale and induce rapid induction and faster recovery times. Due to those advantages, volatile anesthetics are widely used in pediatric patients[4,5]. The brain of neonate is developing brain, which is in a dynamic state of establishing and strengthening neural connections. Many studies have reported that children before 4-year old exposure to general anesthesia could increase the risk of developing learning disabilities[6,7]. The postnatal day 7 rats are common non-primate model to study the effects of anesthetics on neonates[8]. The administration of general anesthetics, such as isoflurane and sevoflurane could kill brain cells and lead long-term neurocognitive dysfunction[9-12]. Although the preclinical results exhibited anesthetics caused brain cell death, the relationship between the brain injury and behavior and cognitive deficit are not clear[13-15]. Several clinical studies also determined that general anesthesia could decrease cognitive function. The first study reported there was no difference in neurodevelopment between 2-year old infants

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who has inguinal hernia repairs under either the general or neuraxial anesthesia[16]. Another study also found no significant differences in cognitive function or behavior between infants who exposed general anesthesia during inguinal hernia repair surgery and their sisters or brothers[17]. Most recently, a study reported that children had a deficit that received anesthesia from ages 2-4, compared to age-matched control[18]. The mechanism still needs to be elucidated. The Wingless/Int (Wnt) pathway plays an important role in neural development and neurodegenerative diseases[19,20]. The Wnt is a secreted glycolipoprotein, which could be activated by its ligands, one of the most vital endogenous activators of Wnt, in the central nervous system. Ligand binds to Wnt receptor frizzled and then initiates the cascade that results in inactivates the β-catenin destruction complex. The step prevents β-catenin degradation through its phosphorylation and β-catenin accumulates in the cytoplasm and translocates to the nucleus and then binds to lymphoid enhancer-binding factor/T-cell specific transcription factor (Lef/Tcf). This leads to the transcription of Wnt-related genes involved in cell proliferation, survival, differentiation, neurogenesis and inflammation[21,22]. However, in the absence of Wnt ligands, the β-catenin could be phosphorylated by CK-1 and glycogen synthase kinase 3β (GSK-3β), which could increase β-catenin ubiquitination and degradation through the proteasomal pathway. In this study, postnatal day 7 rats were used to evaluate the effects of isoflurane and sevoflurane and Wnt inhibitor on learning and memory through Wnt/β-catenin pathway. The mRNA and protein expression levels of Wnt, β-catenin, and GSK-3β were measured with quantitative Real-time polymerase chain reaction (qPCR) and Western blot, respectively

Materials and Methods

Animal's Anesthesia

One hundred postnatal day 7 (P7) Sprague-Dawley rats were housed in a temperature and humidity controlled room with a 12-h light-dark cycle. The rats were randomly assigned into five groups (n=20): Control group, ISA group, SEA group, ISAI group and SEAI group. The P7 rats were anesthetized in an acrylic box with 0.75% isoflurane (ISA group) or 0.85% sevoflurane for 6 h (SEA group). Isoflurane and sevoflurane were obtained from Shanghai Yuyan Instrument Co., Ltd (Shanghai, China). The isoflurane with Wnt inhibitor (ISAI group) and sevoflurane with Wnt inhibitor (SEAI group) were injected with 100 μl 2 mg/ml Wnt inhibitor XAV 939 (Abmole China, Shanghai, China) at 30 min before anesthesia. The control group rats were under the same treatment and environment, except that the control group rats were exposed to air for 6 h. Hemoglobin oxygen saturation and heart rate were detected with a rodent trans-reflectance sensor. Animals were kept for another 5 or 10 weeks with free access to water and food. The present work was conducted with approval from the Animal Ethics Committee of the University.

Morris Water Maze Test

The Morris water maze (NatureGene Corp., Beijing, China) was a circular pool (120 cm in diameter and 50 cm deep) filled with warm water (25 ± 1°C) with four quadrants. The white, 15 cm diameter hidden platform was submerged 1.5 cm below the water surface. The hidden platform was placed at the same position during the training experiments. A digital camera was set up to record the tracking of the path. Rats were received four training trials per day for five consecutive days. Each rat was released facing wall of water maze at different starting positions. The investigator recorded the time of the rats taken to reach the hidden platform (latency); the cut-off time was 60 s. If the rat did not reach the platform within 60 s, the investigator would guide it to the hidden platform. It was allowed to stay on the platform for 15 s and the latency was recorded as 60 s. At the end of the test, the probe trial was administered with the platform removed from the circular pool. The time spent of the first time to reach the platform (T1), the time spent in the target quadrant (T2) and the times of crossing the platform were recorded and analyzed.

Histopathological Assay

After animals behavioral studies, the rats were killed by decapitation, the hippocampus was removed and further fixed in 10% phosphate-buffered formalin for overnight, dehydrated and embedded in paraffin. Then it was cut on a microtome into 5 μm thick slices according to previously described[23]. Sections were dried, deparaffinized in xylene and rehydrated in ethanol, stained with hematoxylin and eosin. Briefly, they were placed in hematoxylin for 5 min, washed with tap water and left for 5 min, followed by rinsing in 1% acid alcohol and washing in tap

water. After that, sections were added in eosin solution (1%) for 5 min, followed by washing in tap water, dehydrated. All stained sections assessed under light microscope. All the above chemical reagents were obtained from Shanghai R&S Biotechnology Co., Ltd (Shanghai, China).

qRT-PCR Assay

Quantitative Real-time PCR was performed to evaluate the expression level of Wnt3a, GSK 3β and β-catenin. For RNA isolation, total RNA was extracted with Trizol reagent (Gibco, Grand Island, NY, USA), the concentration and purity of RNA were determined at 260/280 nm using a nanodrop spectrophotometer (Berthold Detection Systems GmbH, Pforzheim, Germany). 1 μg of each isolated RNA was subjected to cDNA synthesis. RTcDNA synthesis was conducted in a 14 μl reaction buffer, containing 1 μl reverse transcriptase (50 U) and 1 μl ologo (dT) primer, according to manufacturer's instructions (TaKaRa, Otsu, Shiga, Japan). With the obtained for cDNA as a template, the relative expression levels of Wnt3a, GSK 3β and β-catenin from rats receiving experimental treatment were determined by PCR. The sequences of the primers for RT-PCR are as follows,

Wnt 3a: Forward, 5'-ATGGGCGGGAGGGGACA-3'; and Reverse, 5'-CGCCCATTGGATCCTTAAG-3';
GSK 3β: Forward, 5'-AACACCAAGGGAGCAAA-3', and Reverse, 5'-GAGCGTGAGGAGGGATAAGG-3';
β-catenin: Forward, 5'-CGTTTCGCCTTCATGGACTA-3', and Reverse, 5'-GCCGCTGGGTGTCCTGATGT-3';
β-actin: Forward, 5'-GTGGGGATAATGAACTTGCAG-3', and Reverse, 5'-GGAACCCCTGGTAGAACAGT-3'.

Each 20 μl reaction system comprised 2 μl of cDNA, 10 μl SYBR Premix Ex Taq II, 10 μmol/l of both sense and antisense primers. All data for each sample were measured in triplicate and using $2^{-\Delta\Delta Ct}$ method.

Western Blot Assay

The hippocampal tissues were homogenized in a buffer composed of 10 mmol/L Tris-HCl, 0.5 mmol/L EDTA, 250 mmol/L sucrose, 1 mmol/L phenyl methyl sulfonyl fluoride, 1 mmol/L Na_4VO_3 and protease inhibitor cocktail. The protein samples from each group were resolved by SDS-PAGE. The protein concentrations were determined using a BCA Protein Assay reagent kit (Thermo Fisher Scientific, Beijing, China). Equal amounts of protein (50 μg) were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS/PAGE), transferred to polyvinylidene fluoride (PVDF) membranes, the nonspecific binding of antibodies were blocked with 5% non-fat dried milk in phosphate buffered saline (PBS) and then incubated with the primary antibodies Wnt3a, GSK-3β, p-GSK-3β, β-catenin, p-β-catenin (Abcam, Cambridge, MA, USA), and β-actin (Abcam, Cambridge, MA, USA) were used followed by the application of the secondary antibodies consisting of, HRP-conjugated goat anti-rabbit IgG (Abcam, Cambridge, MA, USA). The protein bands were detected by enhanced chemiluminescence reagents ECL. The images were analyzed with Quantity One molecular image System. All the chemical reagents were obtained from Shanghai R&S Biotechnology Co., Ltd (Shanghai, China).

Statistical Analysis

Data are expressed as mean ± SD. Statistical differences were evaluated by software SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Statistical analysis was performed using one-way analysis of variance, and LSD test was used to post-hoc test ANOVA. *p*-values of less than 0.05 were considered as statistically significant.

Results

Isoflurane and Sevoflurane Exposure Induces Spatial Learning and Memory Impairment

Morris water maze test was used to investigate whether isoflurane and sevoflurane affect spatial learning and memory. These experiments were performed at 5 weeks and 10 weeks after anesthesia. As shown in Table I, the latency times of ISA, SEA group were 26.08±11.68 s, 25.12 ± 11.23 s, respectively, while the latency time of control group was 15.72 ± 7.06 s at 5-week. The difference was significant ($p < 0.05$). When rats treated with Wnt inhibitor XAV 939 at 30 min before anesthesia, the latency times were markedly reduced than that of ISA and SEA group, respectively. This experiment was tested again at 10-week post anesthesia. The latency time of ISA and SEA groups were significantly diminished than those at 5-week ($p < 0.05$). The probe trial

Table I. Escape latency of rats after treatment in Morris water maze (s).

Group		1 d	2 d	3 d	4 d	5 d
5 weeks	Control	63.02 ± 19.62	34.15 ± 15.35	28.42 ± 13.15	19.52 ± 11.26	15.72 ± 7.06
	ISA	68.75 ± 20.25	40.13 ± 16.03	35.62 ± 15.63	29.46 ± 12.08	26.08 ± 11.68*
	SEA	68.93 ± 20.16	40.02 ± 15.97	34.96 ± 15.42	28.86 ± 11.98	25.12 ± 11.23*
	ISAI	66.75 ± 19.86	38.69 ± 15.39	30.46 ± 14.85	22.62 ± 11.54	16.56 ± 8.02#
	SEAI	66.43 ± 19.76	37.26 ± 14.87	32.74 ± 15.13	26.97 ± 11.36	15.87 ± 7.79▲
10 weeks	Control	51.46 ± 17.32	30.92 ± 14.25	23.36 ± 12.76	16.43 ± 9.26	10.23 ± 5.14
	ISA	57.62 ± 18.43	36.52 ± 15.24	30.01 ± 12.97	24.36 ± 11.07	15.76 ± 6.98#
	SEA	57.84 ± 18.52	36.13 ± 15.16	29.53 ± 12.14	23.86 ± 10.95	15.23 ± 6.72▲
	ISAI	54.65 ± 17.96	33.18 ± 14.09	26.32 ± 11.94	20.32 ± 9.76	12.46 ± 5.87
	SEAI	53.76 ± 17.41	32.59 ± 13.74	25.96 ± 11.25	19.82 ± 9.54	12.05 ± 5.65

Note: vs. 5 weeks control group *$p < 0.05$; *vs.* 5 weeks ISA group #$p < 0.05$; *vs*. 5 weeks SEA group ▲$p < 0.05$.

was conducted to measure reference memory at the end of learning. The hidden platform was removed from the circular pool. Isoflurane and sevoflurane tended to increase the time needed to reach the platform for the first time (T1, Figure 1, $p < 0.05$), and significantly reduced the time spent in the target quadrant (T2) and also times crossing the platform (N) were markedly reduced at 5 weeks after anesthesia. When rats were administrated with Wnt inhibitor XAV 939 at 30 min before anesthesia, T1 was significantly reduced while T2 and N were markedly increased when compared with ISA and SEA group, respectively. There was no statistical difference of T1, T2 and N among 5 different groups at 10 weeks post anesthesia (Figure 1, $p > 0.05$). However, T1, T2 and N were significantly different at 10-week compared to the same group at 5-week (Figure 1, $p < 0.05$). These results demonstrated that isoflurane and sevoflurane could impair P7 rats' learning and memory capability, while these effects were reduced over time. When rats were treated with Wnt inhibitor XAV 939 at 30 min before anesthesia, the impairment of brain could relieve. These findings suggested that impairment of learning and memory in P7 rats may through the Wnt signaling pathway.

Histopathological Assay

Histological features of the hippocampus of P7 rats, which were treated with anesthetic isoflurane and sevoflurane, were shown in Figure 2. In the control group, there was no marked pathology change in the hippocampus, and the neurons were clear and of moderate size, with normal ultrastructure. Whereas isoflurane and sevoflurane treated group, the neurons were significant shrinkage, loss of neurons and widespread damage in ISA and SEA group at 5-week after anesthesia (Figure 2). However, P7 rats treated with Wnt inhibitor XAV 939 could markedly relief that damage. Those morphological changes were significantly moderated and reduced neuronal loss at 10-week (Figure 2).

Isoflurane and Sevoflurane Exposure Affects Wnt3a, GSK-3β and β-catenin mRNA Expression

Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) results revealed that the mRNA expression levels of Wnt3a and β-catenin were significantly higher in the ISA and SEA groups, compared to control group at 5-week (Figure 3A, C, $p < 0.05$), while GSK-3β expression level was significantly lower than that of control group (Figure 3B, $p < 0.05$). As shown in Figure 3, the expression levels of Wnt3a and β-catenin (Figure 3A, C, $p < 0.05$) were significantly reduced after Wnt inhibitor XAV 939 treatment, while GSK-3β expression level was dramatically increased in ISAI and SEAI groups (Figure 3B, $p < 0.05$). However, there was no marked difference of mRNA expression levels of Wnt3a, β-catenin and GSK-3β in ISA and SEA groups compared to control group (Figure 3 D-F, $p > 0.05$) at 10-week.

Isoflurane and Sevoflurane Exposure Affects Wnt3a, GSK-3β and β-catenin Proteins Expression

Wnt/β-catenin pathway related proteins were determined in this experiment. As is clearly shown in Figure 4, the proteins expression levels of Wnt and phosphorylated-β-catenin were significantly up-regulated in ISA and SEA group at 5 weeks post-anesthesia compared

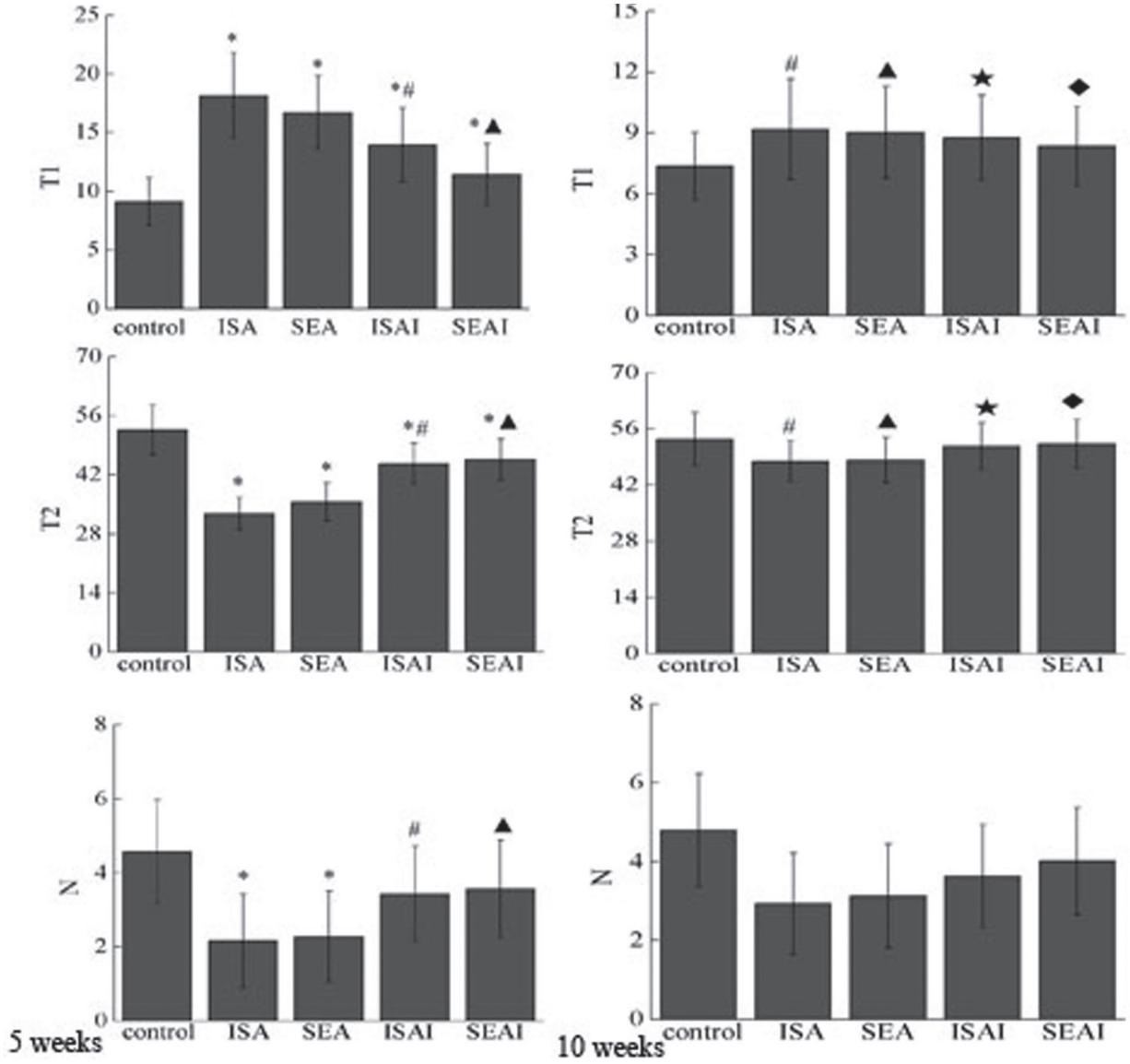

Figure 1. Effect of isoflurane and sevoflurane on the memory rentention in a probe trial. *$p < 0.05$, compared with 5 weeks control group; #$p < 0.05$, compared with 5 weeks ISA group; ▲$p < 0.05$, compared with 5 weeks SEA group; ★$p < 0.05$, compared with 5 weeks ISAI group; ◆$p < 0.05$, compared with 5 weeks SEAI group.

to control group (Figure 4 A, C, $p < 0.05$), while phosphorylated- GSK-3β was markedly decreased in ISA and SEA groups (Figure 4 B, $p < 0.05$). Wnt inhibitor XAV 939 was significantly inhibited the up-regulation of Wnt and phosphorylated-β-catenin and increased phosphorylated-GSK-3β expression in the hippocampus in ISA and SEA groups, as compared with the control group at 5 weeks (Figure 4 A, B, C, $p < 0.05$). After 10 weeks, Wnt and phosphorylated-β-catenin were also markedly up-regulated, and phosphorylated- GSK-3β was dramatically decreased in ISA and SEA group, compared with control group (Figure 5 A, B, C, $p < 0.05$), but the degree of regulation was much lower than the same group at 5-week ($p < 0.05$). These data indicated that isoflurane and sevoflurane could affect Wnt/β-catenin pathway related proteins, and these results consist of the mRNA expression pattern.

Discussion

We showed that 4-h isoflurane and sevoflurane treatment caused a significant increase of cell

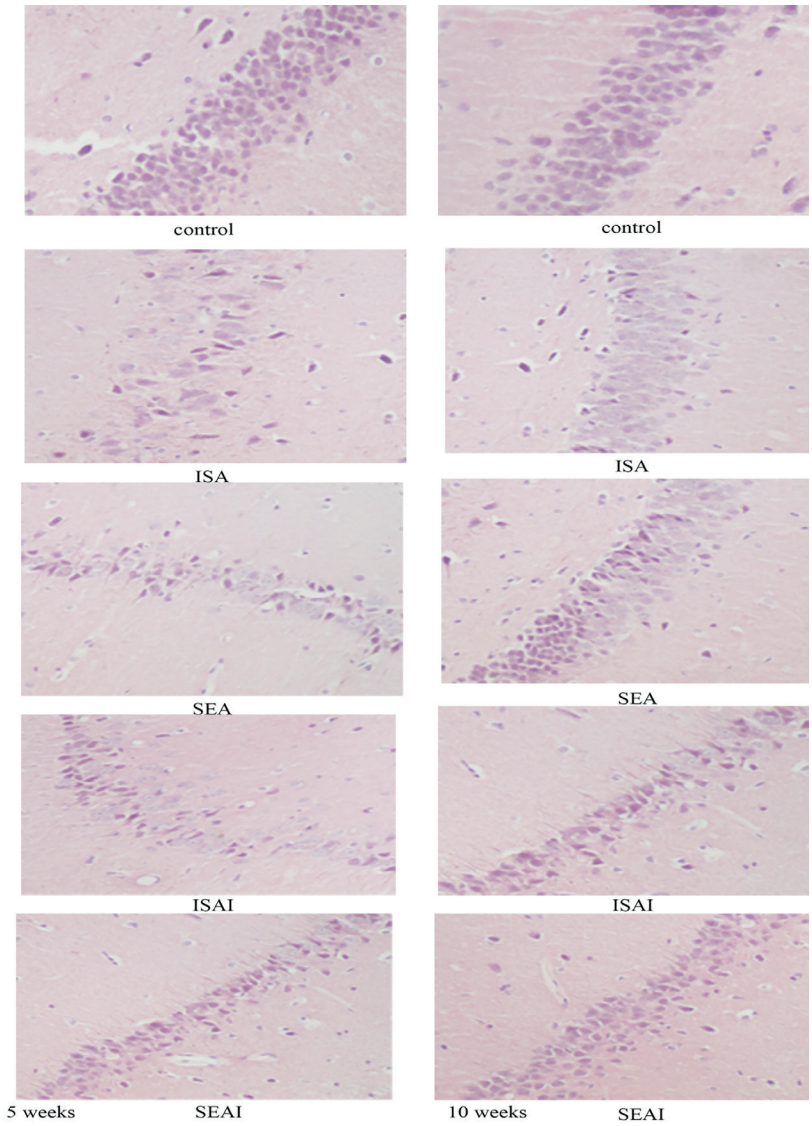

Figure 2. Effect of isoflurane and sevofluraneon hippocampal neuronal damage at 5-week and 10-week post-anesthesia (×200).

death in the hippocampus of the post-natal day 7 rats compared with control exposed only to air. The cell death caused a long-term neurocognitive deficit, which was evident in learning and memory study. The finding for isoflurane and sevoflurane is consistent with the previous report by Satomoto et al[9], who reported that neonatal P6 mice administrated with sevoflurane for 6 hours could markedly increase neuroapoptosis in the hippocampus. While Johnson et al[23] also observed that neonatal exposure to isoflurane induces a marked deficit on learning and memory capability. Jevtovic-Todorovic et al[2] found that isoflurane could significantly increase the neuronal degeneration in a dose-dependent model in P7 rats. One possible mechanism would be the isoflurane and sevoflurane are toxic to neural precursors and cause neurons decrease in proliferation at P7 rats. However, it is not clear that the effects of isoflurane and sevoflurane in proliferation occur at cell cycle exit or cell cycle arrest.

One key finding of this work is a decrease in neural proliferation caused by isoflurane and sevoflurane at 5-week after anesthesia and relieve at 10-week. The time course of anesthetic effect on proliferation in neonatal rats would be important for understanding if and how anesthetic effect on neurogenesis affects long-term cognitive outcome. The Morris water maze was used to assess hippocampus dependent learning and memory capability in our study. The memory deficits of P7 rats at 5 weeks after inhaled anesthetics and the fewer deficits were found at 10 weeks compared with the same

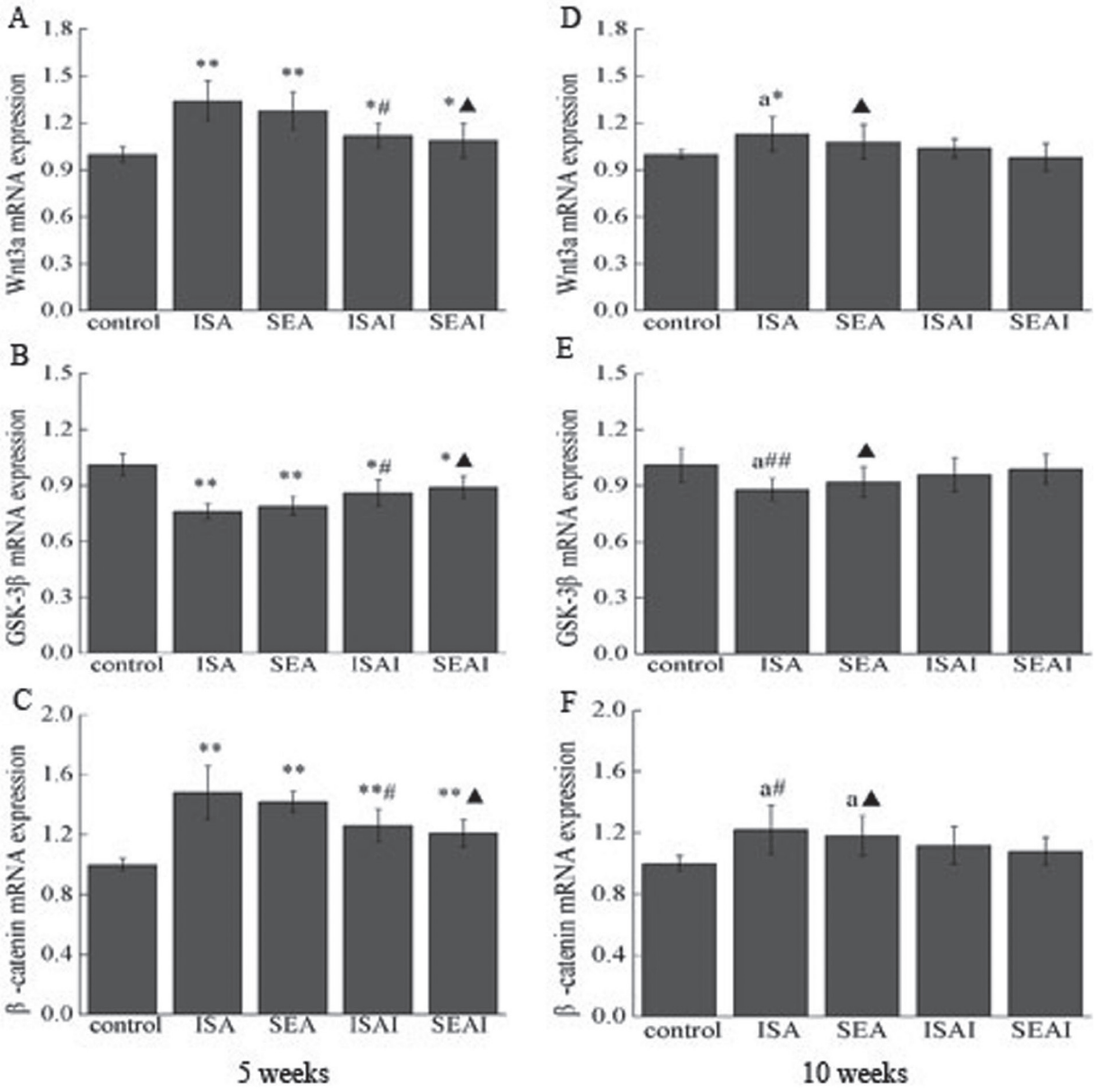

Figure 3. The effects of isoflurane and sevoflurane on related mRNA expression with Wnt/β-catenin pathway in the hippocampus of P7 rats. ***A-C,*** qPCR anlysis of Wnt, β-catenin and GSK-3β at 5-week post-anesthesia. ***D-F,*** qPCR anlysis of Wnt, β-catenin and GSK-3β at 10-week post-anesthesia. *$p < 0.05$, compared with 5 weeks control group; **$p < 0.01$, compared with5 weeks control group; $^{\#}p < 0.05$, compared with 5 weeks ISA group; $^{\#\#}p < 0.01$, compared with 5 weeks ISA group; $^{\blacktriangle}p < 0.05$, compared with 5 weeks SEA group; $^{a}p < 0.05$, compared with 10 weeks control group.

group at 5 weeks. These results suggested that the deficits were transient, which is consistent with Culley's results[24]. Culley et al[24] found that the neurocognitive deficits at 5 weeks after 2 h exposure isoflurane while no deficits were found at 8 weeks.

Previous studies showed that the neuron cell death occurs immediately after isoflurane and sevoflurane exposure, cognitive deficits occur at 4-6 weeks after the surgery and would gradually improve over time. Morris water maze is usually considered as a hippocampal-dependent spatial reference memory assay. The hippocampal lesions profoundly affect spatial reference memory in rodents. To understand the possible mechanism of anesthetic induced neurocognitive decline in P7 rats, Wnt signaling pathway related mRNA and proteins were

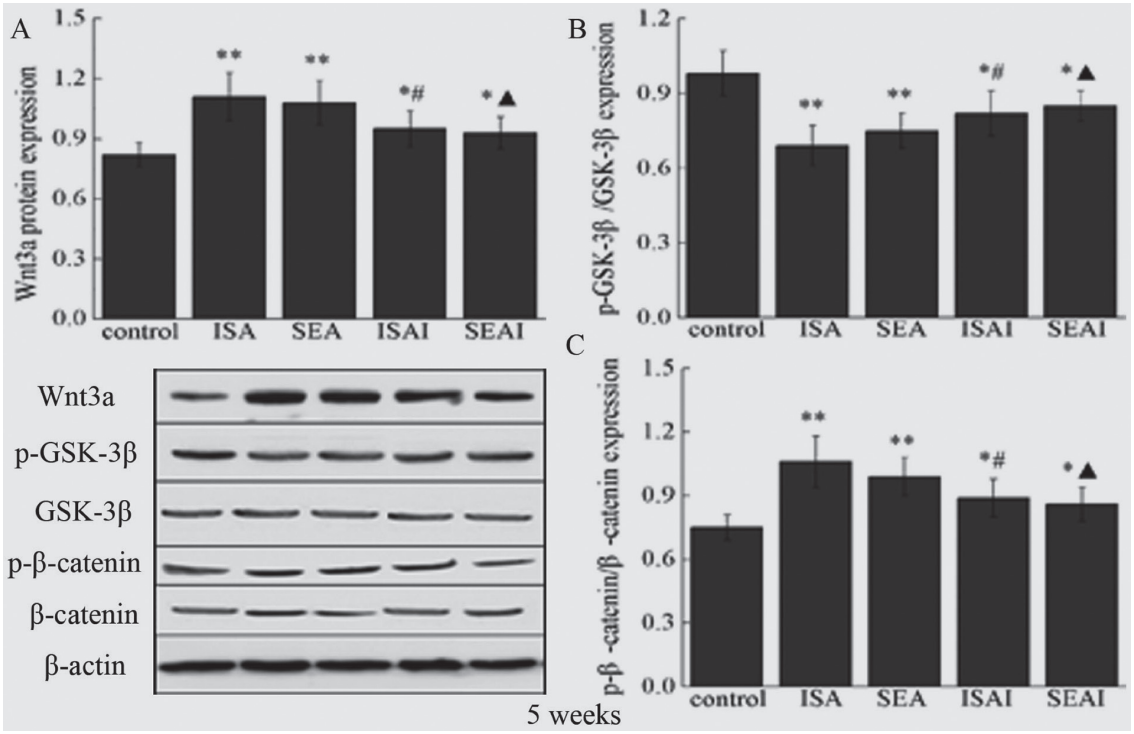

Figure 4. Effects of isoflurane and sevoflurane on related proteins expression with Wnt/β-catenin pathway in the hippocampus of P7 rats at 5-week post-anesthesia. ***A,*** Wnt3a; ***B,*** GSK-3β; ***C,*** β-catenin. *$p < 0.05$, compared with 5 weeks control group; **$p < 0.01$, compared with 5 weeks control group; $^{\#}p < 0.05$, compared with 5 weeks ISA group; $^{\blacktriangle}p < 0.05$, compared with 5 weeks SEA group.

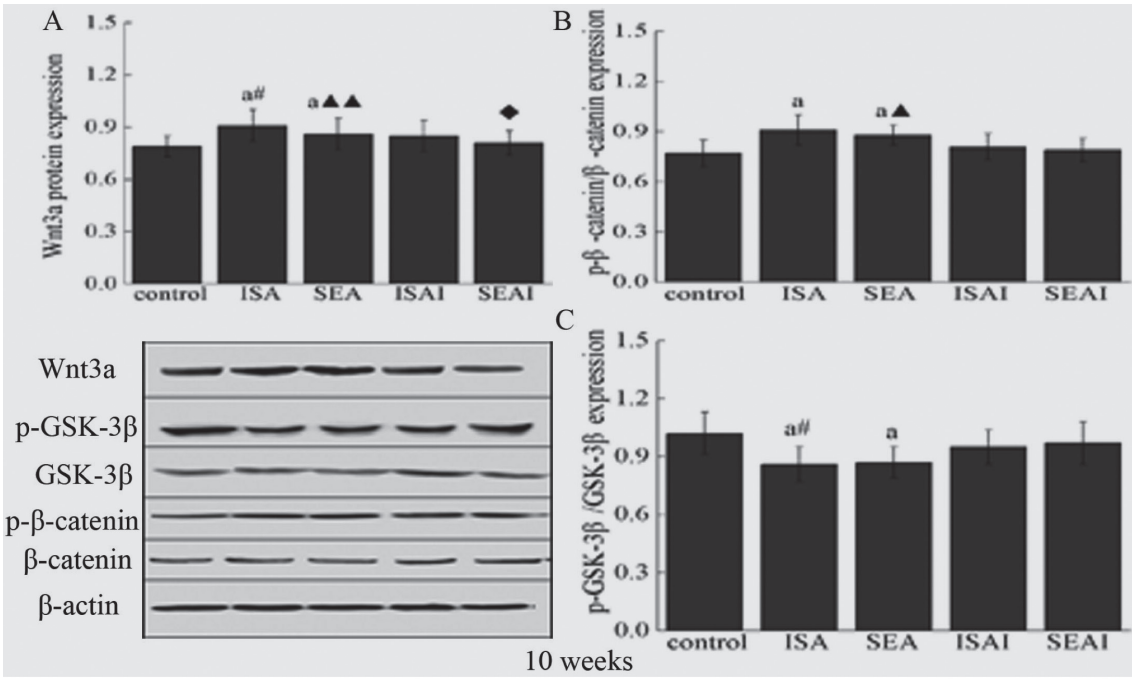

Figure 5. The effects of isoflurane and sevoflurane on related proteins expression with Wnt/β-catenin pathway in the hippocampus of P7 rats at 10-week post-anesthesia. ***A,*** Wnt3a; ***B,*** GSK-3β; ***C,*** β-catenin. *$p < 0.05$, compared with 5 weeks ISA group; $^{\blacktriangle}p < 0.05$, compared with 5 weeks SEA group; $^{\blacktriangle\blacktriangle}p < 0.01$, compared with 5 weeks SEA group; $^{\blacklozenge}p < 0.05$, compared with 5 weeks SEAI group; $^{a}p < 0.05$, compared with 10 weeks control group.

assessed in this study. The Wnt pathway plays a vital role in neural development during embryogenesis[25-27]. GSK-3 family has two members, GSK-3α and GSK-3β that show 98% sequence identity within their domains and share 85% amino acid sequence identity. The two GSK-3 isoforms were mainly expressed in the nervous system including brain and spinal cord, while the GSK-3β is highly expressed in the developing brain. GSK-3β plays a vital role in the neuronal differentiation and maintenance of neurons during nervous system development. GSK-3β is a key regulator of Wnt pathway, which is a neuron-specific intracellular serine-threonine kinase. GSK-3β could increase β-catenin degeneration in the cytoplasm and prevent β-catenin translocate to nuclear and inhibit the activation of the Wnt/β-catenin pathway. In the presence of Wnt ligand, the Wnt pathway is activated, β-catenin is not phosphorylated by the destruction complex including GSK-3β, and then β-catenin accumulates in the cytoplasm and migrates in the nucleus where transcripts Wnt target genes[28,29]. However, in the absence of the Wnt ligand, β-catenin is degraded by GSK-3β and Wnt target genes are not transcripted. The Wnt/β-catenin signaling pathway plays an important role in regulation of several functions in neurogenesis. The mRNA and proteins expression of Wnt, β-catenin were significantly up-regulated and GSK-3β markedly reduced after anesthetics in immature rats, when P7 rats treated with Wnt inhibitor XAV 939 could prevent neuronal cell death and reverse the mRNA and proteins expression trend in ISA and SEA group. As the results shown in this study, the inhibitor of Wnt can reverse the impairment of neuron death caused by isoflurane and sevoflurane. Yi et al[30] found Wnt signaling pathway played an important role in some cancers, while our results also suggested that Wnt signaling pathway was involved in anesthetics induced neurocognitive deficits in developing brain.

Conclusions

We demonstrated that memory and learning impairment induced by neonatal exposures to isoflurane and sevoflurane are associated with Wnt signaling pathway in the hippocampus, which probably affects the downstream expression of learning and memory-related genes.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Stratmann G, Sall J, May L, Loepke A, Lee M. Beyond anesthetic properties: the effects of isoflurane on brain cell death, neurogenesis, and long-term neurocognitive function. Anesth Analg 2010; 110: 431-437.
2) Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, Olney JW, Wozniak DF. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. J Neurosci 2003; 23: 876-882.
3) Soriano SG, Anand KJ, Rovnaghi CR, Hickey PR. Of mice and men: should we extrapolate rodent experimental data to the care of human neonates? Anesthesiology 2005; 102: 866-868.
4) Olsen RW, Li GD. GABA (A) receptors as molecular targets of general anesthetics: identification of binding sites provides clues to allosteric modulation. Can J Anaesth 2011; 58: 206-215.
5) Palmer L. Anesthesia: everything you need to know. Plast Surgical Nurs 2013; 33: 164-171.
6) Flick RP, Katusic SK, Colligan RC, Wilder RT, Voigt RG, Olson MD, Sprung J, Weaver AL, Schroeder DR, Warner DO. Cognitive and behavioral outcomes after early exposure to anesthesia and surgery. Pediatrics 2011; 128: e1053-1061.
7) Wilder RT, Flick RP, Sprung J, Katusic SK, Barbaresi WJ, Mickelson C, Gleich SJ, Schroeder DR, Weaver AL, Warner DO. Early exposure to anesthesia and learning disabilities in a population-based birth cohort. Anesthesiology 2009; 110: 796-804.
8) Lee JH, Zhang J, Wei L, Yu S. Neurodevelopmental implications of the general anesthesia in neonate and infants. Exp Neurol 2016; 272: 50-60.
9) Satomoto M, Satoh Y, Terui K, Miyao H, Takishima K, Ito M, Imaki J. Neonatal exposure to sevoflurane induces abnormal social behaviors and deficits in fear conditioning in mice. Anesthesiology 2009; 110: 628-637.
10) Zhang L, Zhang J, Yang L, Dong Y, Zhang Y, Xie Z. Isoflurane and sevoflurane increase interleukin-6 levels through the nuclear factor-kappa B pathway in neuroglioma cells. Br J Anaesth 2013; 110 (Suppl 1): i82-91.
11) Tao G, Zhang J, Zhang L, Dong Y, Yu B, Crosby G, Culley DJ, Zhang Y, Xie Z. Sevoflurane induces tau

phosphorylation and glycogen synthase kinase 3beta activation in young mice. Anesthesiology 2014; 121: 510-527.

12) Shen X, Dong Y, Xu Z, Wang H, Miao C, Soriano SG, Sun D, Baxter MG, Zhang Y, Xie Z. Selective anesthesia-induced neuroinflammation in developing mouse brain and cognitive impairment. Anesthesiology 2013; 118: 502-515.

13) Brambrink AM, Evers AS, Avidan MS, Farber NB, Smith DJ, Zhang X, Dissen GA, Creeley CE, Olney JW. Isoflurane-induced neuroapoptosis in the neonatal rhesus macaque brain. Anesthesiology 2010; 112: 834-841.

14) Loepke AW, Istaphanous GK, McAuliffe JJ, Miles L, Hughes EA, McCann JC, Harlow KE, Kurth CD, Williams MT, Vorhees CV, Danzer SC. The effects of neonatal isoflurane exposure in mice on brain cell viability, adult behavior, learning, and memory. Anesth Analg 2009; 108: 90-104.

15) Stratmann G, Sall JW, May LD, Bell JS, Magnusson KR, Rau V, Visrodia KH, Alvi RS, Ku B, Lee MT, Dai R. Isoflurane differentially affects neurogenesis and long-term neurocognitive function in 60-day-old and 7-day-old rats. Anesthesiology 2009; 110: 834-848.

16) Davidson AJ, Disma N, de Graaff JC, Withington DE, Dorris L, Bell G, Stargatt R, Bellinger DC, Schuster T, Arnup SJ, Hardy P, Hunt RW, Takagi MJ, Giribaldi G, Hartmann PL, Salvo I, Morton NS, von Ungern Sternberg BS, Locatelli BG, Wilton N, Lynn A, Thomas JJ, Polaner D, Bagshaw O, Szmuk P, Absalom AR, Frawley G, Berde C, Ormond GD, Marmor J, McCann ME; GAS consortium. Neurodevelopmental outcome at 2 years of age after general anaesthesia and awake-regional anaesthesia in infancy (GAS): an international multicentre, randomised controlled trial. Lancet 2016; 387: 239-250.

17) Sun LS, Li G, Miller TL, Byrne MW, Bellinger DC, Ing C, Park R, Radcliffe J, Hays SR, DiMaggio CJ, Cooper TJ, Rauh V, Maxwell LG, Youn A, McGowan FX. Association between a single general anesthesia exposure before age 36 months and neurocognitive outcomes in later childhood. JAMA 2016; 315: 2312-2320.

18) Stratmann G, Lee J, Sall JW, Lee BH, Alvi RS, Shih J, Rowe AM, Ramage TM, Chang FL, Alexander TG, Lempert DK, Lin N, Siu KH, Elphick SA, Wong A, Schnair CI, Vu AF, Chan JT, Zai H, Wong MK, Anthony AM, Barbour KC, Ben-Tzur D, Kazarian NE, Lee JY, Shen JR, Liu E, Behniwal GS, Lammers CR, Quinones Z, Aggarwal A, Cedars E, Yonelinas AP, Ghetti S. Effect of general anesthesia in infancy on long-term recognition memory in humans and rats. Neuropsychopharmacology 2014; 39: 2275-2287.

19) Libro R, Bramanti P, Mazzon E. The role of the wnt canonical signaling in neurodegenerative diseases. Life Sci 2016; 158: 78-88.

20) Guo QH, Yang HJ, Wang SD. Olanzapine inhibits the proliferation and induces the differentiation of glioma stem-like cells through modulating the Wnt signaling pathway in vitro. Eur Rev Med Pharmacol Sci 2015; 19: 2406-2415.

21) Angers S, Moon RT. Proximal events in Wnt signal transduction. Nat Rev Mol Cell Biol 2009; 10: 468-477.

22) Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. J Neurochem 2008; 104: 1433-1439.

23) Johnson SA, Young C, Olney JW. Isoflurane-induced neuroapoptosis in the developing brain of nonhypoglycemic mice. J Neurosurg Anesthesiol 2008; 20: 21-28.

24) Culley DJ, Raghavan SV, Waly M, Baxter MG, Yukhananov R, Deth RC, Crosby G. Nitrous oxide decreases cortical methionine synthase transiently but produces lasting memory impairment in aged rats. Anesth Analg 2007; 105: 83-88.

25) Harrison-Uy SJ, Pleasure SJ. Wnt signaling and forebrain development. Cold Spring Harb Perspect Biol 2012; 4: a008094.

26) Wang J, Wynshaw-Boris A. The canonical wnt pathway in early mammalian embryogenesis and stem cell maintenance/differentiation. Curr Opin Genet Dev 2004; 14: 533-539.

27) Ille F, Sommer L. Wnt signaling: multiple functions in neural development. Cell Mol Life Sci 2005; 62: 1100-1108.

28) Wu D, Pan W. GSK3: a multifaceted kinase in Wnt signaling. Trends Biochem Sci 2010; 35: 161-168.

29) Forlenza OV, De-Paula VJ, Diniz BS. Neuroprotective effects of lithium: implications for the treatment of Alzheimer's disease and related neurodegenerative disorders. ACS Chem Neurosci 2014; 5: 443-450.

30) Yi SJ, Li LL, Tu WB. MiR-214 negatively regulates proliferation and WNT/β-catenin signaling in breast cancer. Eur Rev Med Pharmacol Sci 2016; 20: 5148-5154.