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Full Paper

Dexamethasone preconditioning may improve isoflurane-induced memory impairment by stabilising blood-brain barrier

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Abstract: Perioperative neurocognitive disorder (PND) is a serious neurological complication that often follows surgery and anesthesia. It commonly impairs memory, attention, consciousness, and information-processing ability, yet its pathogenesis remains elusive. Studies suggest that anesthesia may be a risk factor for PND, and glucocorticoids are known to influence cognitive functions. Both abnormal and appropriate glucocorticoid levels can affect cognitive functions differently. This study explores the potential mechanism of dexamethasone preconditioning on postoperative cognitive function in a PND model involving long-term isoflurane anesthesia. Male C57BL/6 mice received a single dose of either dexamethasone or saline on the day of long-term isoflurane anesthesia. Spatial memory, contextual fear memory, permeability of the blood-brain barrier (BBB), and the expression of tight junction proteins were measured on the corresponding days following anesthesia. Results show that long-term isoflurane anesthesia causes spatial and episodic memory impairments in mice, while 5 mg/kg dexamethasone preconditioning ameliorated these impairments. Additionally, long-term isoflurane anesthesia increases BBB permeability and decreases the expression of tight junction proteins. In contrast, 5 mg/kg dexamethasone preconditioning mitigates these effects. Thus, dexamethasone preconditioning has a protective effect on memory function in mice subjected to long-term isoflurane anesthesia.

Keywords: dexamethasone preconditioning, isoflurane anesthesia, blood-brain barrier stability, perioperative neurocognitive disorder

Maejo Int. J. Sci. Technol. **2024**, *18*(01), 27-36

INTRODUCTION

Perioperative neurocognitive disorder (PND) is a significant neurological complication that can occur following anesthesia and surgery, often leading to impairments in memory, attention, consciousness, and information-processing abilities [1]. Patients who experience PND are at risk of facing severe complications including the development of dementia and, in some cases, even death . The concept of anesthesia negatively impacting the elderly was first introduced in 1995 [2]. Since then, numerous risk factors for PND have been identified, encompassing surgical procedures, types of anesthesia used, and patient age [3, 4]. Studies have indicated that neurocognitive disorders affect a substantial percentage of elderly individuals, with prevalence rates ranging from 14% to 48% in those over the age of 70 who have mild cognitive impairment, and 10% among those with dementia [5]. The global increase in the aging population presents a significant challenge to healthcare systems worldwide. Investigating the pathogenesis of PND and developing strategies for prevention and treatment can facilitate postoperative recovery and enhance the overall quality of life for the elderly.

Glucocorticoids play a crucial role in the development and functioning of cognitive processes in individuals. Maintaining stable glucocorticoid levels is essential for the proper development of cognitive functions during growth and maturation. Elevated glucocorticoid levels, particularly during embryonic or neonatal stages, can severely impair cognitive function [6]. In cases where individuals receive glucocorticoid treatment, such as for preterm birth, there is a risk of embryonic brain developmental disorders leading to cognitive dysfunction later in life. Conversely, insufficient glucocorticoid levels can also result in cognitive impairment. Studies involving rats that undergo adrenal removal show significant cognitive function impairment. Therefore, maintaining appropriate glucocorticoid levels is essential for preserving cognitive function. Some individuals have been found to exhibit better memory and cognitive abilities when glucocorticoid levels are within the optimal range. Notably, the relationship between glucocorticoid dosage and cognitive function in the hippocampal CA1 area follows a U-shaped curve, indicating the importance of maintaining the right balance [7]. Dexamethasone, a widely used synthetic glucocorticoid, has a significant impact on neurocognitive function [8]. Several animal experimental models have been developed for the study of PND, each mimicking different aspects of the disorder. These models include the open tibial fracture model, long-term isoflurane anesthesia model, and abdominal surgery model [9, 10]. Isoflurane is a commonly used inhaled anesthetic in clinical practice. Researchers have observed significant impairments in spatial and episodic memory in mice following prolonged exposure to isoflurane anesthesia [11, 12]. This study aims to investigate the potential role of dexamethasone preconditioning in mitigating postoperative cognitive dysfunction in a PND model induced by extended isoflurane anesthesia and to elucidate the underlying mechanisms.

This research seeks to address a critical gap in our understanding and management of PND, a condition that has a profound impact on cognitive functions, particularly memory and attention, following surgery and anesthesia. Although the potential adverse effects of anesthesia on cognitive function are well-documented, the precise mechanisms underlying PND remain largely elusive. Given the escalating challenges faced by healthcare providers due to increasing prevalence of PND, particularly among the elderly population, addressing this knowledge gap is of paramount importance [13, 14]. The primary objective of this study is to explore whether dexamethasone, a synthetic glucocorticoid, can mitigate cognitive decline associated with prolonged isoflurane anesthesia, a commonly employed anesthetic agent in medical practice. By examining the potential

Maejo Int. J. Sci. Technol. **2024**, *18*(01), 27-36

of dexamethasone preconditioning to maintain optimal glucocorticoid levels and stabilise the blood-brain barrier, this research aims to provide novel insights into the prevention and treatment of PND while safeguarding cognitive function. This has the potential to significantly benefit clinical practice by offering a viable strategy to enhance postoperative cognitive recovery and improve the overall quality of life for surgical patients, especially the geriatric population, which is more susceptible to postoperative cognitive impairment and related psychological side effects.

MATERIALS AND METHODS

All animal experiments were conducted by a protocol approved by the ethics committee of animals experiments of Nanjing Drum Tower Hospital (Approval No.20160508). The mice used in this study were all male C57BL/6 mice aged eight weeks, weighing 20-25 g, and the animal license number was SCXK (su) 2015-0001. The temperature and humidity of the feeding environment of the mice were maintained at a level suitable for them. The light mode was strictly 12 hr bright and 12 hr dark. The light was turned on at 8:00 hr and turned off at 20:00 hr.

Group

The mice were randomly divided into eight groups: the control group (group C), anesthesia group (group A), dexamethasone group (group D1.25, group D2.5 and group D5), and dexamethasone + anesthesia group (group D1.25 + A, group D2.5 + A and group D5 + A). In the experiment the sample size for each group was 7. Mice in groups D and D + A were intraperitoneally injected with normal saline solution once a day before anesthesia. The doses for the mice in group D (group D1.25, group D2.5, and group D5) and group D + A (group D1.25 + A, group D2.5 + A, and group D5 + A) were 1.25 mg/kg, 2.5 mg/kg, and 5 mg/kg respectively, and the volume was 0.25 ml. Groups C and A were given equal volumes of saline similarly.

Anesthesia

In this experiment the mice in groups A and D + A received long-term isoflurane inhalation anesthesia for 6 hr. The anesthesia was administered in a transparent box (28 cm × 20 cm × 20 cm). The isoflurane anesthesia induction concentration was 4% combined with 100% oxygen, and the duration was approximately 1 min. The concentration of isoflurane was maintained at 1–1.5% during the maintenance period, and the mice were able to breathe autonomously as their vital signs were maintained within normal levels. Groups C and D were administered oxygen in the same anesthesia box.

Water Maze

The spatial memory of the mice was assessed using a water maze. Mice were randomly divided into eight groups, with seven mice in each group. The first day after anesthesia and the following six consecutive days were divided into the learning and recall periods. The first five days were the learning period. The water maze was divided into four quadrants, each with a sign of a different shape and colour on the wall to provide visual clues to the mice. The fourth quadrant had a transparent circular platform, approximately 10 cm in diameter and 1 cm below the water surface. Before the experiment, a certain amount of titanium dioxide (harmless to the mice) was injected into the water to make it cloudy and prevent the mice from seeing where the platform was. The water temperature in the water maze was kept at around 25^{o}C. During the experiment, the mice were placed in the water from the side of the sign. Mice were allowed to observe the sign for 3

seconds, and then the time when the mice found the platform was recorded. If a mouse failed to find the platform within 1 min., it was guided to the platform and allowed to remain on the platform for 30 sec. to observe its spatial position. Day 6 was the recall period. After the first five days of learning, the mice got some memory of the spatial position of the platform, so the percentage of the time the mice spent exploring the platform quadrant and the time of crossing the platform position represented the strength of the mouse memory. A near-quadrant and a far-quadrant of opposite angles were randomly selected for the experiment, and the time percentage of platform exploration by the mouse and the time of crossing the platform position were recorded for 1 min.

Fear Conditioning

The contextual fear memory of the mice was assessed using a fear conditioning system. Mice were randomly divided into eight groups, with seven mice in each group. The conditioned fear memory experiment was divided into two stages: stimulus and recall. The first day of the experiment was the stimulus period. The mice were placed in a test box containing conditioned fear memory. After 5 min. of adaptation, the mice were given 30-sec. sound stimulation. When the sound was over, a 2-sec. electric stimulation was immediately administered. After placing the mice in a test box, the whole system was run by a preset computer program that recorded the time percentage of freezing behaviour in different stages. After the test, the faeces and urine at the bottom of the test box were wiped with alcohol to remove the influence of the former mouse on the next mouse. To prevent the irritating smell of alcohol from affecting the mice, the next mouse was tested after the alcohol had evaporated. The test process was quiet to reduce the impact of the external environment on the activity of the mice. The recall was assessed 24 hr after stimulation. The mice were placed back into the test box for electric stimulation. No sound or electric stimulation was detected during the recall period for 5 min., and the percentage of the time of trembling was recorded to reflect the episodic memory of the mice.

Blood-Brain Barrier (BBB) Permeability

After anesthesia, the mice in each group were intraperitoneally injected with 100 Evans Blue (2% in PBS, Sigma) on day three. Two hours later, the mice were decapitated and sacrificed by transcardial perfusion with PBS until colourless. The brains were removed, homogenised in formamide and incubated at 60°C for 72 hr. The homogenate was centrifuged at 14000 rpm for 30 min., and the concentration of Evans Blue in the supernatant was measured using a spectrophotometer at 620 nm.

Western Blot

Three days after anesthesia induction, the mice were sacrificed. Total protein was extracted from both hemispheres using an ice-cold radio immunoprecipitation assay buffer (Beyotime, China). The bicinchoninic acid protein assay kit (Sigma, USA) measured the protein concentration at equal concentrations in each sample. Protein samples were loaded onto gels and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The gel was then transferred onto polyvinylidene difluoride membranes (ThermoFisher Scientific, USA) and blocked with 5% bovine albumin (Beyotime, China) dissolved in Tris-buffered saline (Beyotime, China) with Tween-20, 10X (Beyotime, China) buffer for 1 hr at 37°C. The membranes were incubated overnight in a 4-°C refrigerator with primary antibodies: anti-claudin-5 (1:1000, Cell Signaling Technology, USA) and anti-ZO-1 (1:1000, Abcam, UK). After washing with Tris-buffered saline with Tween-20, 10X, the

membranes were incubated with a secondary antibody (1:5000, Abcam, UK) and visualised with enhanced chemiluminescence system (Beyotime, China). All experiments were repeated three times. Glyceraldehyde-3-phosphate dehydrogenase (1:1000, Abcam, UK) was used as an internal control.

Statistics

SPSS19.0 statistical software was used to analyse the water maze results by using ANOVA with repeated measures followed by Tukey's test. Fear conditioning results were processed by one-way analysis of variance (one-way ANOVA with repeated measures followed by Tukey's test). All experimental results were expressed as mean ± SEM, with $P < 0.05$ indicating a statistically significant difference.

RESULTS AND DISCUSSION

Single Dexamethasone Preconditioning and Long-Term Isoflurane-Induced Spatial Memory Impairment

Spatial memory in each group of mice was assessed using a water maze, with a sample size of 7 mice per group. The results from the learning period are presented in Table 1. Initially, there were no significant differences in the time taken for the mice to locate the platform within the first two days after anesthesia across all groups. However, between days 3 and 5 after anesthesia, there was a noteworthy increase in the time taken by group A to find the platform compared to group C (day 3, $P < 0.001$; day 4, $P = 0.004$; day 5, $P = 0.002$). Conversely, group D5 + A displayed a significant reduction in the time taken to find the platform compared to group A (day 3, $P = 0.016$; day 4, $P = 0.027$; day 5, $P = 0.013$). Additionally, the platform exploration time for group C was

Table 1. Time (sec.) to find platform in mice of each group 1-5 days after anesthesia in water maze

Group	1 day	2 days	3 days	4 days	5 days
Group C	54.4±1.17	39.9±1.33	20.8±0.54	19.8±0.92	17.6±0.64
Group A	50.7±0.87	48.1±0.34	39.7±1.11[a]	34.7±0.78[a]	32.5±1.02[a]
Group D1.25	55.3±0.85	42.8±1.24	36.2±1.13[a]	34.2±0.81[a]	28.4±0.65
Group D1.25+ A	54.7±0.79	47.0±1.06	35.4±0.71[a]	33.7±0.85[a]	27.9±0.92
Group D2.5	50.3±1.13	47.6±1.54	36.8±0.70[a]	34.0±0.56[a]	25.9±0.83
Group D2.5 + A	47.4±0.83	34.3±1.05	33.9±1.57	27.8±1.56	23.6±1.09
Group D5	51.0±1.24	43.0±1.98	36.0±1.26[a]	33.9±1.28[a]	26.3±1.38
Group D5 + A	48.6±1.24	36.3±1.67	25.5±1.05[b]	22.4±0.50[b]	19.8±0.51[b]

[a]$P < 0.05$, difference with group C; [b]$P < 0.05$, difference with group A. Sample size for each group is 7.

significantly shorter than that of both group D5 (day 3, $P = 0.008$; day 4, $P = 0.007$) and group D2.5 (day 3, $P = 0.004$; day 4, $P = 0.006$) on days 3 and 4. These findings collectively suggest that long-term isoflurane anesthesia can induce spatial memory impairment in mice, and pretreatment with 5 mg/kg dexamethasone can effectively alleviate this spatial memory impairment induced by long-term isoflurane anesthesia. Notably, it is worth mentioning that all three dexamethasone

Maejo Int. J. Sci. Technol. **2024**, *18*(01), 27-36

pretreatment groups exhibited transient spatial memory impairment even without long-term isoflurane anesthesia.

The test results for the recall period are shown in Figure 1. The platform was removed on the sixth day after anesthesia. The frequency of the mice in group A through the platform was significantly lower than in group C (P = 0.001) and group D5 + A (P = 0.02). At the same time, the time the mice in group A took probing in the platform quadrant was significantly lower than that of the mice in group C (P = 0.004) and group D5 + A (P = 0.031). These results suggest that long-term isoflurane anesthesia can damage spatial memory in mice, which can be sustained for at least six days after anesthesia. A single dose of dexamethasone (5 mg/kg) before anesthesia can alleviate long-term isoflurane anesthesia-induced spatial memory impairment.

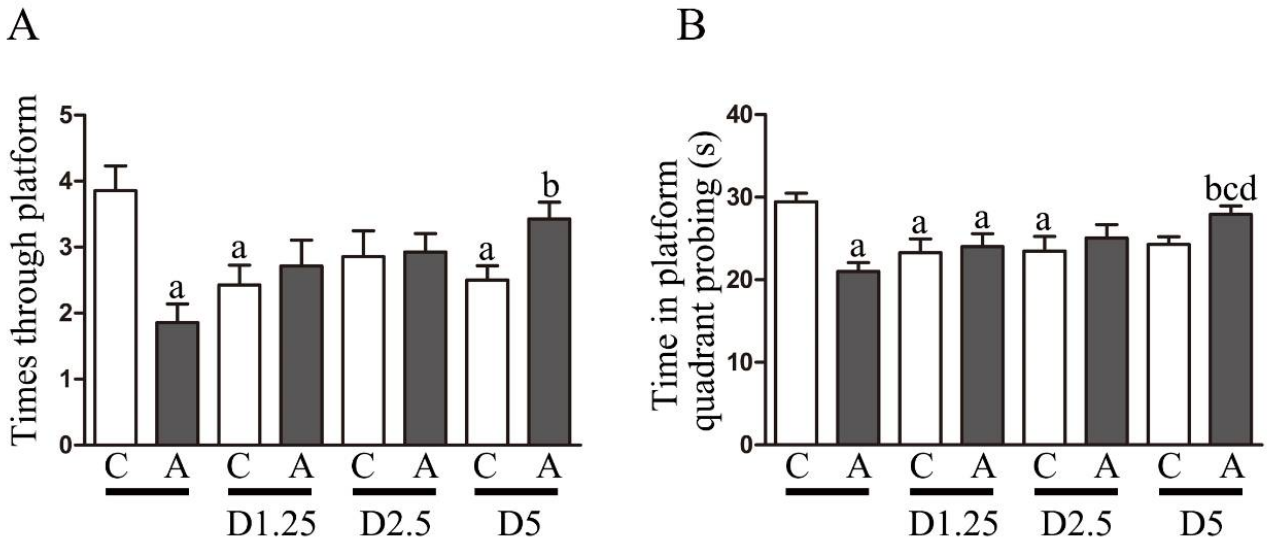

Figure 1. Graph A shows the number of times the mice in each group crossed platform on 6th day after anesthesia; Graph B shows exploration time (sec.) of the mice in each group in the quadrant of platform on 6th day after anesthesia. $^{a}P < 0.05$ indicates difference with group C; $^{b}P < 0.05$ indicates difference with group A; $^{c}P < 0.05$ indicates difference with group D1.25; $^{d}P < 0.05$ indicates difference with group D2.5.

Single Dexamethasone Preconditioning and Long-Term Isoflurane-Induced Contextual Fear Memory Impairment

Contextual fear memory in the mice was evaluated through a fear conditioning experiment, with each group consisting of 7 mice. The experimental outcomes are depicted in Figure 2. On the first day following anesthesia, there were no notable differences in the percentage of time spent trembling among the mice in each group. However, three days after anesthesia, the freezing time percentage in group A was significantly lower than that in group C (P = 0.004). Moreover, the freezing time of mice in group D5 decreased compared to those in group C (P < 0.018). The percentage of freezing time in groups DA5, D2.5, D2.5 + A, D1.25 and D1.25 + A did not significantly differ from that of mice in group C (P > 0.05). Interestingly, the freezing time in group A was reduced compared to that in groups D5 + A (P = 0.012), D2.5 (P = 0.006), D2.5 + A (P = 0.005), D1.25 (P = 0.024) and D1.25 + A (P = 0.011). Moving on to the 7th day following anesthesia, there were no significant disparities in the percentage of freezing time among all groups, except for group A, where the percentage of freezing time remained significantly lower than that in

Maejo Int. J. Sci. Technol. **2024**, *18*(01), 27-36

group C (P = 0.002). These findings indicate that long-term isoflurane anesthesia can induce contextual fear memory impairment in mice, and pretreatment with 5mg/kg dexamethasone can ameliorate the contextual fear memory impairment caused by long-term isoflurane anesthesia in mice. Additionally, it is worth noting that 5mg/kg dexamethasone was found to induce transient contextual fear memory impairment.

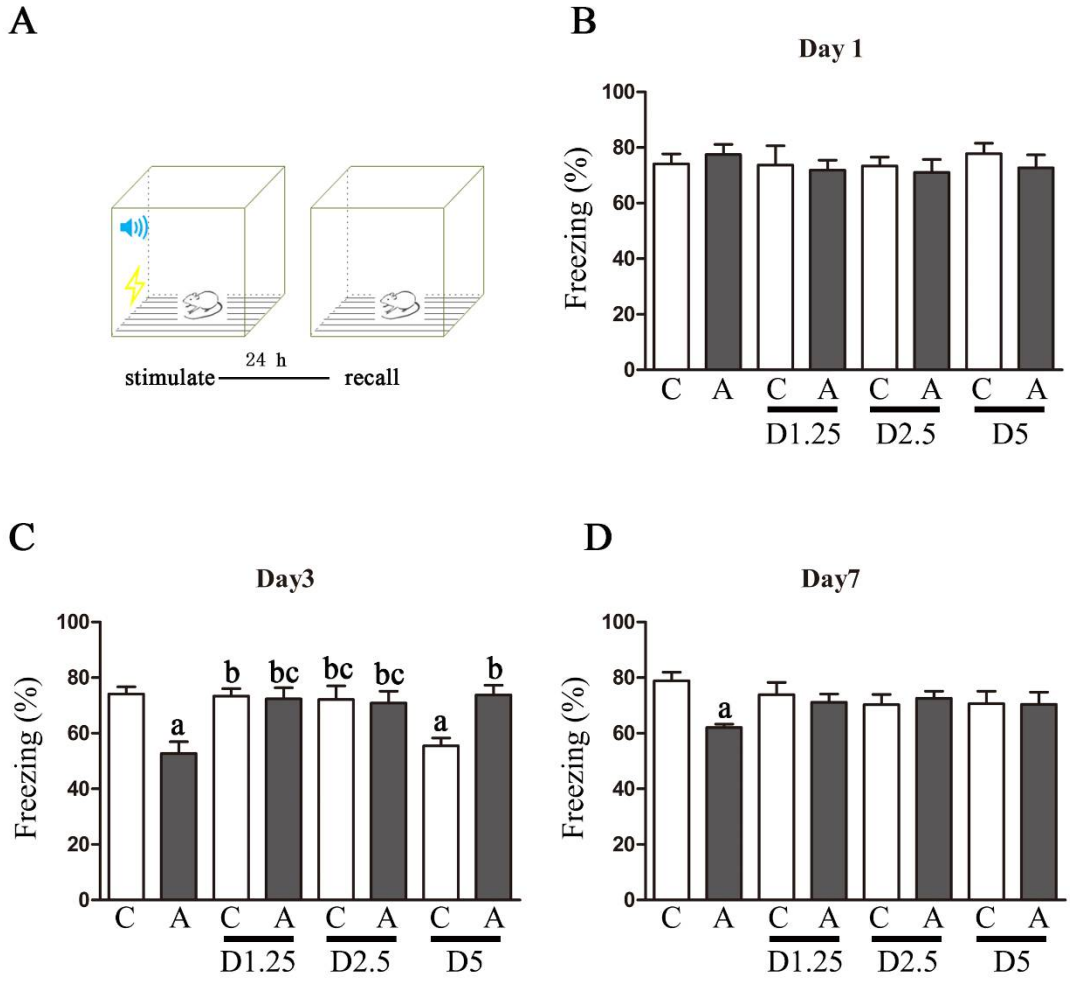

Figure 2. Graph A is schematic diagram of conditioned fear experiment. Graphs B, C and D show percentages (%) of freezing time of mice in each group on 1st, 3rd, and 7th days after anesthesia. $^{a}P < 0.05$ indicates difference with group C; $^{b}P < 0.05$ indicates difference with group A; $^{c}P < 0.05$ indicates difference with group D5.

Single Dexamethasone Preconditioning and BBB Impairment Induced by Long-Term Isoflurane Anesthesia in Mice

The permeability of the BBB in mice was assessed using Evans Blue intraperitoneal injection, with a sample size of 4 in each group. The experimental findings are illustrated in Figure 3. On the third day following long-term isoflurane anesthesia, the BBB permeability in group A mice was significantly higher than that in group C mice (P < 0.001). Moreover, it was also significantly higher than the BBB permeability observed in mice from groups D5 + A (P = 0.004), D2.5 + A (P = 0.004) and D1.25 + A (P < 0.001). These outcomes indicate that long-term isoflurane anesthesia has the capacity to elevate BBB permeability, while pretreatment with dexamethasone can effectively counteract this effect.

Maejo Int. J. Sci. Technol. **2024**, *18*(01), 27-36

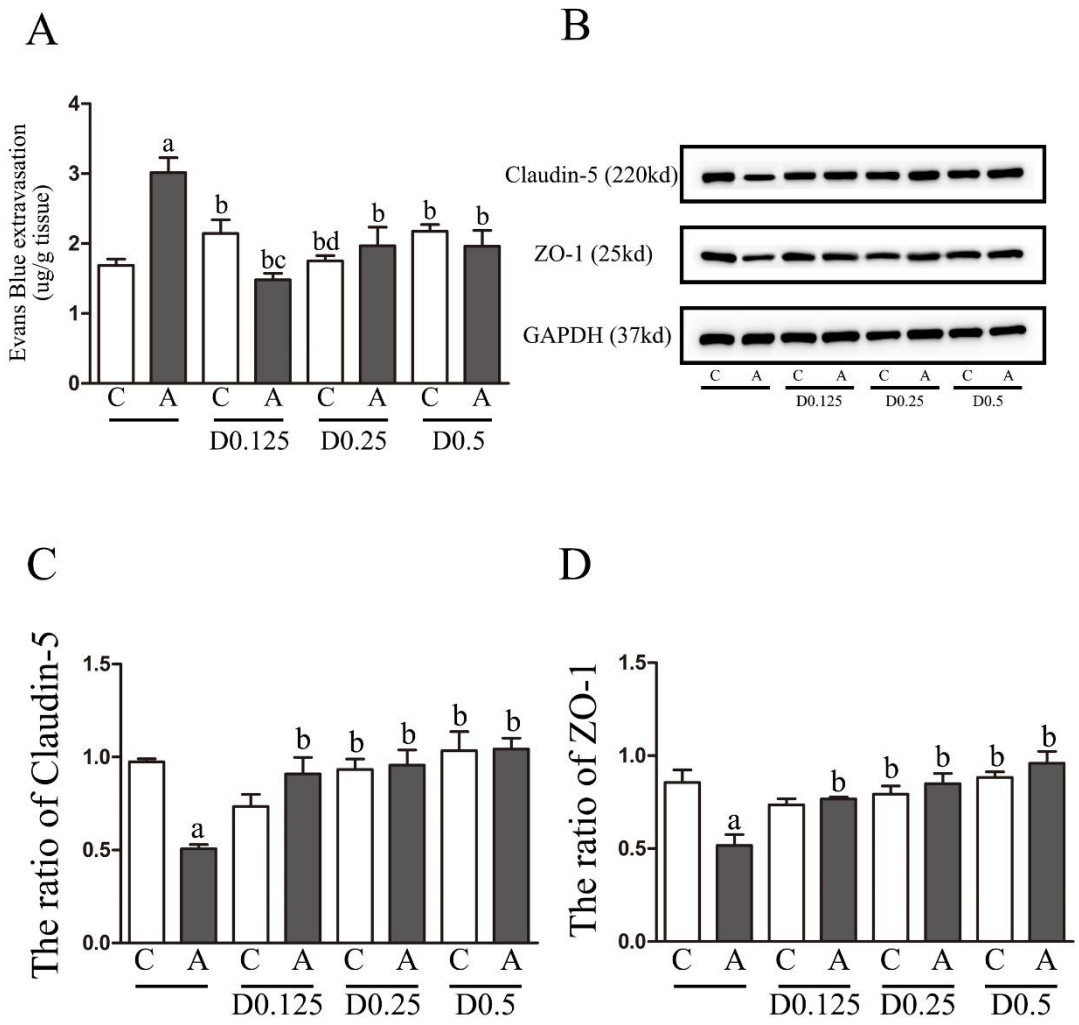

Figure 3. Graph A describes Evans Blue extravasation in the brain tissue of mice in each group. $^{a}P < 0.05$ indicates difference with group C; $^{b}P < 0.05$ indicates difference with group A; $^{c}P < 0.05$ indicates difference with group D1.25; $^{d}P < 0.05$ indicates difference with group D1.25 + A. Graph B describes changes of brain tissue Claudin-5 and ZO-1 protein expression on day 3 after anesthesia in all groups. Graph C and Graph D describe brain tissue Claudin-5 and ZO-1 relative protein levels on day 3 after anesthesia in all groups. $^{a}P < 0.05$ indicates difference with group C; $^{b}P < 0.05$ indicates difference with group A.

Single Dexamethasone Preconditioning and Claudin-5 and ZO-1 Expression Induced by Long-Term Isoflurane Anesthesia in Mice

Figures 3C and 3D show that the expression of claudin-5 (P = 0.003) and ZO-1 (P = 0.003) in group A was significantly lower than in group C on day 3 after anesthesia. At the same time, the claudin-5 level in group A also significantly decreased compared to that in groups D1.25 + A (P = 0.012), D2.5 + A (P = 0.005) and D5 + A (P < 0.001). The expression of ZO-1 in group A was also significantly lower than that in groups D1.25 + A (P = 0.036), D2.5 + A (P = 0.003) and D5 + A (P < 0.001). These results suggest that long-term isoflurane anesthesia can decrease claudin-5 and ZO-1 expression on the BBB, while dexamethasone preconditioning can prevent this effect.

The findings of this study reveal the protective role of single dexamethasone preconditioning against long-term isoflurane-induced cognitive impairment in mice. Long-term isoflurane anesthesia was shown to induce spatial and contextual fear memory impairment, elevate

Maejo Int. J. Sci. Technol. **2024**, *18*(01), 27-36

BBB permeability, and reduce claudin-5 and ZO-1 expression, all of which were effectively mitigated by dexamethasone preconditioning. Additionally, it should be noted that transient cognitive impairments were observed following dexamethasone administration alone. These results provide valuable insights into the potential use of dexamethasone as a protective measure against anesthesia-induced cognitive deficits, offering promise for improving the postoperative cognitive recovery and overall quality of life for surgical patients.

CONCLUSIONS

The findings of this study demonstrate that long-term isoflurane anesthesia leads to spatial and contextual fear memory impairments in mice. This memory deficit is consistent with the clinical manifestation of PND observed in humans, which often includes memory impairment, attention deficit, and altered cognitive function. One of the key observations in this study is that dexamethasone preconditioning, administered at a dose of 5 mg/kg, exerts a protective effect on memory function in mice subjected to long-term isoflurane anesthesia. This protective effect is particularly significant in the context of spatial and episodic memory, as evidenced by improved performance in water maze and fear conditioning tests. These findings suggest that dexamethasone, a glucocorticoid, may play a crucial role in preserving cognitive function in the perioperative period.

Long-term isoflurane anesthesia increases BBB permeability, which can have profound implications for the entry of potentially harmful substances into the brain. Furthermore, the expression of tight junction proteins, which are critical for maintaining the integrity of the BBB, is reduced following anesthesia. These changes in BBB integrity likely contribute to the cognitive impairment observed in the mouse model. Dexamethasone preconditioning emerges as a key factor in mitigating these BBB disruptions. Dexamethasone exerts its protective effects, at least in part, by stabilising the BBB. This is a novel and significant finding, as it provides a potential mechanistic explanation for the cognitive benefits of dexamethasone preconditioning in the context of PND. It is plausible that the anti-inflammatory action of dexamethasone contributes to maintaining BBB integrity in the face of long-term isoflurane exposure. Further research is needed to elucidate the precise mechanisms involved and to determine the optimal dosing and timing of dexamethasone administration. Nonetheless, this study represents a significant step toward addressing the challenging problem of PND and its impact on the cognitive function in surgical patients.

AUTHORS' CONTRIBUTION

X. S. and X. L. contributed equally to this work.

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