

Mammalian target of rapamycin/p70 ribosomal S6 protein kinase signaling is altered by sevoflurane and/or surgery in aged rats

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Received October 25, 2014; Accepted September 28, 2015

DOI: 10.3892/mmr.2015.4444

Abstract. The mammalian target of rapamycin (mTOR)/p70 ribosomal S6 protein kinase (p70S6k) pathway exerts anti-apoptotic effects that may contribute to disease pathogenesis. The memory impairment in patients with Alzheimer's disease (AD) has been suggested to be contributed to by abnormal mTOR signaling. The aim of the current study was to investigate the association between sevoflurane and/or surgery and AD through the mTOR/p70S6K signaling pathway. Sprague-Dawley rats were randomly assigned to the sevoflurane, surgery or control groups. The animals in the surgery group received a partial hepatectomy under sevoflurane anesthesia. The hippocampal levels of phosphorylated (p)-mTOR, p-p70S6K, caspase-3 and p-tau/total (t)-tau were analyzed. The Morris water maze (MWM) was used to evaluate cognitive function following treatment. The levels of p-mTOR and p-p70S6K were reduced, whereas caspase-3 levels were increased in the surgery group compared with the sevoflurane group. The p-tau/t-tau levels were increased, however, tau mRNA was unaffected by sevoflurane and/or surgery. The rats in the surgery group required a significantly longer time to locate the platform in the MWM test compared with the control and sevoflurane groups. Sevoflurane treatment and/or surgery reduced anti-apoptotic activity, and the postoperative cognitive dysfunction following surgery may be due to mTOR signaling pathway inhibition in aged rats. Increased neuronal apoptosis and tau phosphorylation are suggested to be involved in the association between anesthesia and AD occurrence.

Introduction

Mammalian target of rapamycin (mTOR) is a member of the phosphoinositol kinase-related kinase family (1). mTOR is a modulator of cell growth and proliferation and regulates protein translation efficiency within cells via its downstream targets (2). Furthermore, mTOR is suggested to act primarily via the phosphorylation of eukaryotic initiation factor 4E binding protein (4E-BP1) and p70 ribosomal S6 protein kinase (p70S6K), which are key regulators of protein translation (3).

The mTOR pathway is suggested to contribute to long-term potentiation, long-term depression and synaptic plasticity via protein synthesis regulation (4). Rapamycin disrupts protein synthesis, which is required to stabilize short-term memory into long-term memory (5), and impairs the generation of long-term fear memories when injected into the amygdala (6). This suggests that the enhancement of neuronal mTOR activity may increase memory function. Alternatively, an increase in mTOR activity may disrupt memory processing, as demonstrated by tuberous sclerosis, which is associated with overactive mTOR. Rapamycin treatment rescued memory deficits in a transgenic mouse model of tuberous sclerosis (7), which suggests memory impairments are contributed to by abnormal mTOR signaling.

Alzheimer's disease (AD) is the most common neurodegenerative condition worldwide, in addition to being the predominant cause of dementia (8). Although no conclusive studies have identified an association between anesthesia and AD occurrence, the age of AD onset has been inversely correlated with the cumulative exposure to general anesthesia prior to the age of 50 (9). A previous study suggested that the inhaled anesthetic, sevoflurane, may increase caspase activation, in addition to inducing modest increases in β -site amyloid precursor protein-cleaving enzyme levels, which may lead to increased amyloid- β (A β) levels in the mouse brain (10). Furthermore, a previous study suggested that sevoflurane accelerated the progression of amnesic mild cognitive impairment (MCI) to progressive MCI in a Chinese population (11).

Studies that have assessed human tissue have identified increased mTOR pathway activation in the affected areas of AD brains compared with non-diseased control tissue (12). mTOR activation was accompanied by reduced translational activity (13) and increased oxidative-damage to ribosomal RNA (14). However, alternative studies have demonstrated

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Key words: mTOR signaling, tau protein, Alzheimer's disease, anesthesia, surgery

that A β reduced mTOR signaling (15). In addition, studies that have assessed tau are contradictory. For example, mTOR activity has been demonstrated to increase tau protein production, which suggests mTOR activation may occur upstream of tau pathology (16). Conversely, in a *Drosophila melanogaster* model of tauopathy, wild-type or mutant tau overexpression induced mTOR activity, neural cell cycle activation and neurodegeneration (17). A β_{1-42} has been reported to rapidly and persistently downregulate mTOR/p70S6k phosphorylation in cells, and was associated with caspase-3 activation in a mouse model of AD (18). In addition, phosphorylated (p)-p70S6k expression was significantly reduced in the lymphocytes of patients with AD, and p-p70S6k levels were significantly correlated with Mini Mental Status Examination (MMSE) scores (18). These studies suggest that the mTOR/p70S6k pathway, which regulates cell proliferation, protein translation, apoptosis and autophagy, is altered in cellular and transgenic models of AD, in addition to in the peripheral cells of patients, thus, these alterations may contribute to disease pathogenesis.

The current study hypothesized that mTOR/p70s6k signaling is downregulated and caspase-3 is activated following exposure to sevoflurane and/or surgery, and that these alterations are associated with cognitive dysfunction following surgery. In the present study, the association between sevoflurane and/or surgery and AD and the role of the mTOR/p70S6K signaling pathway was investigated in the hippocampi of aged rats.

Materials and methods

Ethics statement and animals. All experiments were approved by the Institutional Animal Ethics Committee at Beijing Military Hospital (Beijing, China) with the following approval number: 2014-01-11A. A total of 100 female Sprague-Dawley rats aged 20 months were obtained from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China) and used in the experiments. The animals were group housed in a controlled environment (21 \pm 2°C, 50% humidity) and were maintained in a 12/12 h light/dark cycle with the lights on at 7 am. Food and water were available *ad libitum* throughout the experiment, with the exception of during sevoflurane and/or surgery exposure and behavioral tests.

Experimental protocol. All rats underwent baseline Morris water maze (MWM) tests, and the rats that swam slowly or remained stationary were excluded from the study. Rats (n=100) were randomly assigned to the sevoflurane (n=40), surgery (n=40) or control (n=20) groups (Fig. 1). The animals in the sevoflurane group received continuous exposure to 2% sevoflurane for 2 h. The animals in the surgery group received a partial hepatectomy under sevoflurane anesthesia and were subsequently exposed to sevoflurane anesthesia for 2 h. A total of 15 rats in the sevoflurane and surgery groups were used for western blotting, and 15 rats were used for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis at 1, 3 and 7 days post-treatment, with 5 rats sacrificed by decapitation at each time point. In the control group, 5 rats were used for western blotting and 5 rats were used for RT-qPCR. The remaining 10 rats in each group underwent MWM testing at 8-12 days post-treatment.

Sevoflurane anesthesia. An anesthesia chamber (30x30x15 cm) was used to administer sevoflurane/oxygen to 5 rats at a time. Oxygen was delivered using a calibrated flow meter (LZM; Jintai Instrument Co., Ltd., Zhejiang, China) with a flow rate of 3 l/min. Sevoflurane was delivered using an agent-specific vaporizer (Sevoflurane Dräger Vapor 2000; Drägerwerk AG & Co. KGaA, Lübeck, Germany) which delivered 2% anesthetic into the chamber. The animals were maintained under the following conditions: Normothermic (37-39°C), systolic blood pressure (120-135 mmHg), diastolic blood pressure (80-100 mmHg; SurgiVet V6004; Smiths Medical PM, Inc., Norwell, MA, USA), arterial partial pressure of carbon dioxide (36-38 mmHg), arterial partial pressure of oxygen (85-88 mmHg) and hemoglobin concentration (140-120 g/l; GEM Premier 3000; Instrumentation Laboratory Co., Lexington, MA, USA) throughout the experiment. Following the initial equilibration of sevoflurane/oxygen inside the chamber, the concentrations of chamber gas composition, including sevoflurane, carbon dioxide and oxygen, were analyzed using spectrometry (FTIR-650; Guangdong Technology Co., Ltd., Tianjin, China).

Surgery. Partial hepatectomy was performed as previously described by Tanaka *et al* (19). The left main branch of the portal vein was dissected and ligated to the left hepatic duct and artery using a 6-0 silk (Mersilk; Johnson-Johnson Co., Ltd., Shanghai, China) ligature. When an ischemic change in the medial and left lateral lobes was identified, the left lateral lobe was ligated at the pedicle using a 3-0 silk ligature, and was subsequently removed. The median lobe at the pedicle of its left portion was ligated using a 3-0 atraumatic needle and thread, and transfixing suturing of the right portion of the pedicle was performed, following which the lobe was removed. It was necessary to make these two ligatures 5 mm from the inferior vena cava to avoid vessel constriction. The abdominal incision was closed using continuous sutures. The animals were subsequently exposed to sevoflurane anesthesia for 2 h as previously described.

Behavioral tests. Hidden platform tests were conducted in a MWM. The tests were conducted in a circular tank (DB001, Zhishu Technology Co., Ltd., Beijing, China), which was 180 cm in diameter and 76 cm deep, in a room with extra maze cues (e.g., references hung on the wall outside the pool at a height above the eye level of the rat). A 10 cm diameter, black round platform was placed in the center of the north-east quadrant 1.5 cm below the surface of the water, which was maintained at 22°C throughout the test duration. Animal training was conducted over a 5-day acquisition phase, during which all four quadrants were used once per day. When a rat failed to find the platform within 120 sec, it was guided to the platform by the researcher and held there for 30 sec. The training was recorded by a video camera mounted on the ceiling in conjunction with a computerized animal tracking system (Ethovision 8.0; Noldus Information Technologies, Wageningen, The Netherlands).

Western blotting. The hippocampus, which was stored in liquid nitrogen, was homogenized (F6/10-10G; FLUKO Equipment Shanghai Co., Ltd., Shanghai, China), and proteins

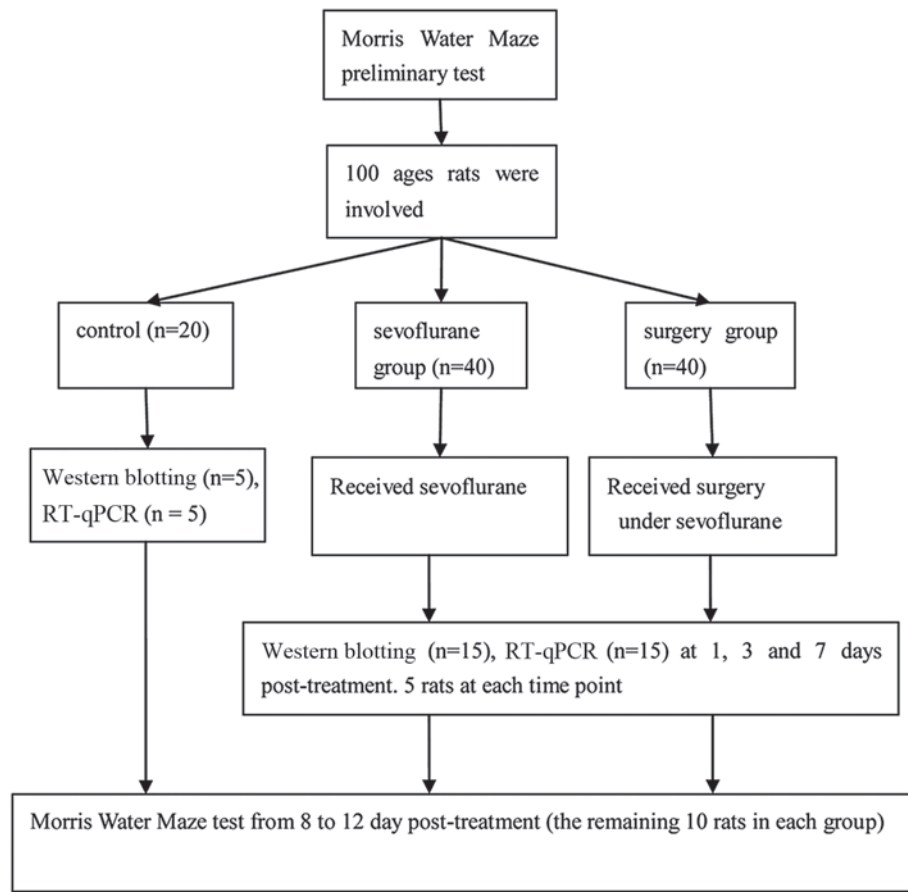


Figure 1. Schematic image of the experimental design. RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

were extracted using a total protein extraction reagent kit (ProMab Biotechnologies, Inc., Richmond, CA, USA). The protein concentrations were measured using a bicinchoninic acid protein assay kit (Biyuntian Biotechnology Co., Jiangsu, China). Following denaturation at 95°C, the proteins were separated via electrophoresis (12% gel; Pierce Companies, Billings, MT, USA) and transferred onto nitrocellulose membranes (Pierce). The membranes were then incubated in 5% skimmed milk (diluted in phosphate-buffered saline; Shanghai Mingrui Biotech Co., Ltd., Shanghai, China) at 37°C for 2 h. The membranes were incubated with monoclonal rabbit anti-p-mTOR (ab32028; 1:1,000; Abcam, Cambridge, MA, USA), polyclonal rabbit anti-p-p70S6K (#9205; Cell Signaling Technology, Inc., Danvers, MA, USA), monoclonal goat anti-p-tau (ab151559; Thr 231; 1:500; Abcam), polyclonal goat anti-tau (ab64193; 1:500; Abcam), and polyclonal rabbit anti-caspase-3 (1:1,000; ab2302; Abcam) antibodies at 4°C overnight, followed by secondary antibodies (1:40,000; ab7010; goat anti-rat IgG; Abcam) at 37°C for 1.5 h. Immunoreactive bands were detected using an enhanced chemiluminescence detection system (Cell Signaling Technology, Inc., Danvers, MA, USA). The protein levels were expressed as a band intensity ratio of the protein of interest and mouse monoclonal β -actin (1:800; SC-365062; Santa Cruz Biotechnology, Inc., Dallas, TX, USA).

RT-qPCR analysis. As previously described (20), total RNA was extracted from frozen hippocampal tissues using TRIzol

reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). The concentration and quantity of total RNA was measured by spectroscopy (SMA1000; Merinton Technology, Beijing, China), with an expected A260/A280 ratio of all RNA samples 1.8-2.0. The integrity of the total RNA was assessed by denaturing agarose gel electrophoresis, with the results of the 18S and 28S ribosomal RNA bands being clearly visible when stained with ethidium bromide. Total RNA (2 μ g) from each sample was reverse-transcribed in a volume of 20 μ l to produce cDNA using the first-strand cDNA synthesis kit (Invitrogen Life Technologies). The cDNA was subsequently stored at -20°C. RT-qPCR was performed in a volume of 25 μ l with 12.5 μ l SYBR Green PCR Master mix (Applied Biosystems, Inc., Carlsbad, CA, USA), 1.5 μ l primer mix, and 1 μ l cDNA. All PCR reactions used the following conditions: 94°C for 3 min followed by 40 cycles at 94°C for 30 sec and 72°C for 45 sec. The data were analyzed using the $\Delta\Delta$ Cq method (21), with β -actin as the internal control. The primer sequences, the expected PCR product sizes (base pairs; bp), and the annealing temperatures were as follows: mTOR, sense 5'-TGTCGAGAG GCAACAACCTC-3' and antisense 5'-TTGTTGGCTGCA TTGTGACG-3' (298 bp, 60°C); p70S6K, sense 5'-GGATTT CTGGGACGAGGTG-3' and antisense 5'-AGAGTTGGG CTGTCGGATTG-3' (148 bp, 58°C); tau, sense 5'-CTGAAG AAGCAGGCATCGGA-3' and antisense 5'-GGTGGTTCA CCTGATCCTGG-3' (265 bp, 59°C); caspase-3, sense 5'-CGG ACCTGTGGACCTGAAA-3' and antisense 5'-TAA CCGGGTGC GG TAGAGTA-3' (216 bp, 60°C); β -actin,

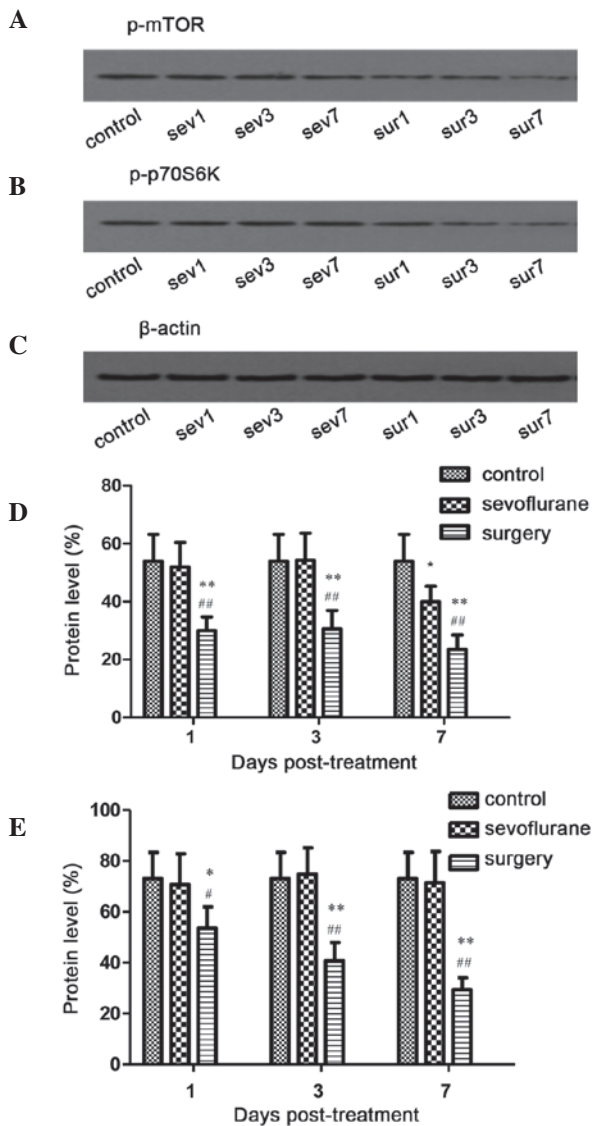


Figure 2. Western blots of p-mTOR and p-p70S6K in the hippocampus of the aged rats. Representative western blots that indicate the expression of (A) phosphorylated mTOR, (B) p70S6K, and (C) β -actin, respectively. Densitometry of the (D) p-mTOR and (E) p-p70S6K levels compared with β -actin in the hippocampus at 1, 3 and 7 days after treatment. The data are presented as the mean \pm standard error of the mean, $n=5$. * $P<0.05$ and ** $P<0.01$ vs. the control group. # $P<0.05$ and ## $P<0.01$ vs. the sevoflurane group. p-mTOR, phosphorylated mammalian target of rapamycin; p-p70S6K, p70 ribosomal S6 protein kinase; sev, sevoflurane group; sur, surgery group.

sense 5'-CTCATGACCACAGTCCATGC-3' and antisense 5'-CTCATGACCACAGTCCATGC-3' (155 bp, 59°C).

Statistical analysis. Statistical analyses were performed using the SPSS 13.0 software package (SPSS Inc., Chicago, IL, USA). Two-way analysis of variance and Bonferroni post hoc test or one-way analysis of variance followed by the post hoc least significant difference test were used. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Sevoflurane and surgery impairs mTOR/p70S6K signaling in the hippocampus. The p-mTOR and p-p70S6K levels were

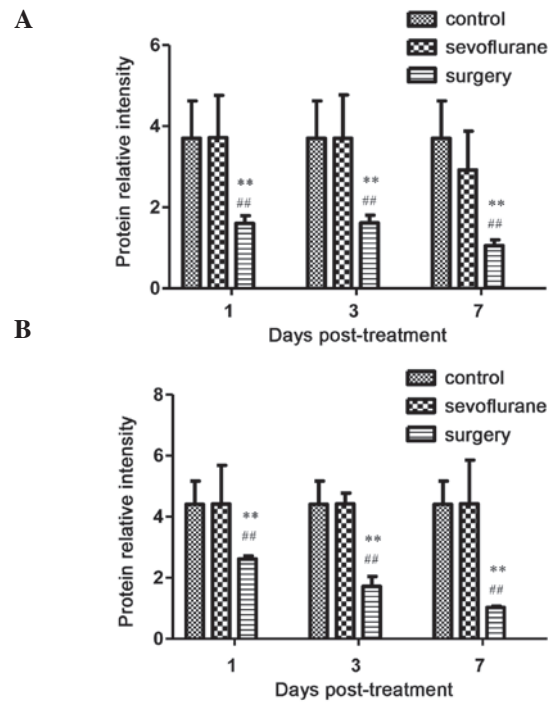


Figure 3. (A) Mammalian target of rapamycin and (B) p70 ribosomal S6 protein kinase mRNA levels determined using reverse transcription-quantitative polymerase chain reaction in aged rats. ** $P<0.01$ vs. the control group. ## $P<0.01$ vs. the sevoflurane group.

significantly reduced in the surgery group compared with the sevoflurane group on days 1, 3 and 7 (Fig. 2A-E). The p-mTOR levels were significantly reduced on day 7 in the sevoflurane group and days 1, 3 and 7 in the surgery group compared with the control group ($P<0.01$; Fig. 2D). The p-p70S6K levels were unaltered in the sevoflurane group, however, on days 1, 3 and 7, the p-p70S6K levels were significantly reduced in the surgery group compared with the control group (Fig. 2E).

The mTOR and p70S6K mRNA levels were significantly reduced in the surgery group compared with the sevoflurane group on days 1, 3 and 7. The mTOR and p70S6K mRNA levels were not significantly different in the sevoflurane group however were reduced on days 1, 3 and 7 in the surgery group compared with the control group ($P<0.01$; Fig. 3A and B).

Sevoflurane and/or surgery increases caspase-3 expression in the hippocampus. The caspase-3 levels were significantly increased in the surgery group compared with the sevoflurane group on days 1, 3 and 7 (Fig. 4A-C). The caspase-3 levels were significantly increased on day 7 in the sevoflurane group, whereas the surgery group exhibited an increase at days 1, 3 and 7 compared with the control group (Fig. 4C).

The caspase-3 mRNA levels were significantly increased in the surgery group compared with the sevoflurane group on days 1, 3 and 7, with a significant increase on day 7 in the sevoflurane group compared with the control group (Fig. 4D).

Sevoflurane and surgery, however not sevoflurane alone, increases the p-tau/t-tau ratio in the hippocampus. The p-tau/t-tau ratio was significantly increased in the surgery group compared with the sevoflurane group on days 1, 3 and 7. However, in the sevoflurane group, no significant

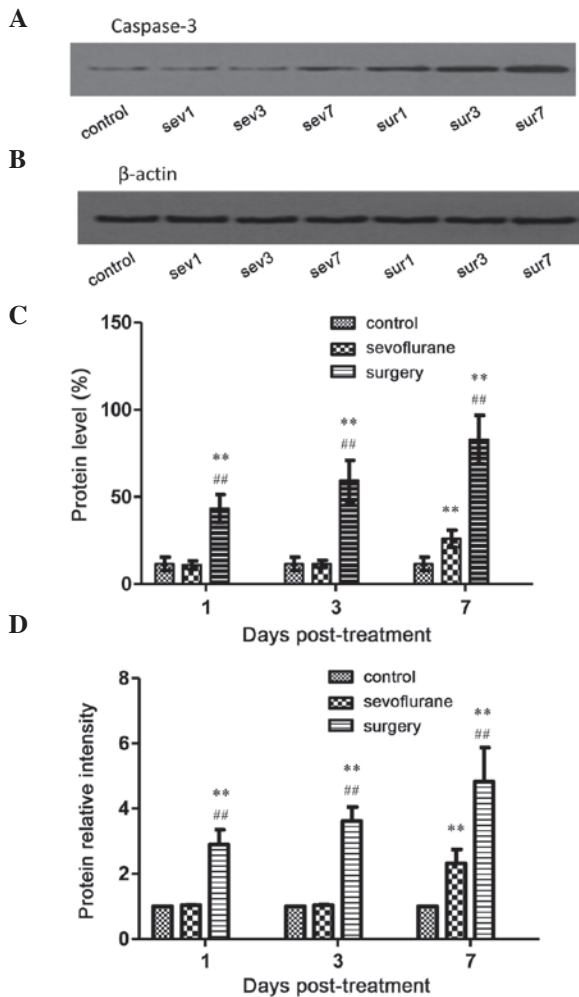


Figure 4. Representative western blots of proteins extracted from the hippocampus of aged rats and incubated with (A) anti-caspase-3 and (B) β-actin antibodies. (C) Densitometric analysis of the levels of caspase-3 compared with β-actin in the hippocampus on days 1, 3 and 7 in aged rats. (D) Levels of caspase-3 mRNA measured using reverse transcription-quantitative polymerase chain reaction in aged rats. Values are presented as the mean ± standard error, n=5. **P<0.01 vs. the control group. ##P<0.01 vs. the sevoflurane group. sev, sevoflurane group; sur, surgery group.

differences were observed compared with the control group (Fig. 5A-D).

RT-qPCR demonstrated that there were no significant effects of treatment or time on the caspase-3 mRNA expression (Fig. 5E). Taken together, these data suggest that the effects of sevoflurane and/or surgery on p-mTOR/p-p70S6K signaling, caspase-3 and p-tau/t-tau were significantly different. Of note, the effects of sevoflurane and surgery were greater than the effects of sevoflurane alone. Sevoflurane and/or surgery did not increase tau mRNA expression, which indicates the t-tau levels were not significantly different in the three groups, whereas the p-tau levels were significantly increased in the surgery group.

Sevoflurane and surgery, however not sevoflurane alone, impair cognitive function. The rats in the surgery group required a significantly longer time to locate the platform compared with the control group at all time points, and compared with the sevoflurane group on days 1, 2, 3 and 5. By

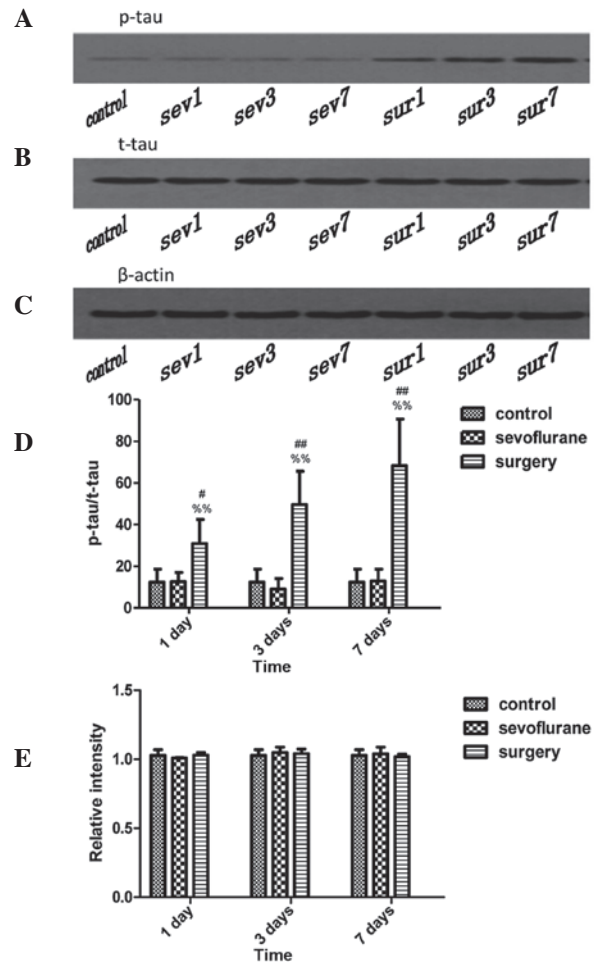


Figure 5. Representative western blotting of proteins extracted from the hippocampus of aged rats and incubated with (A) p-tau, (B) t-tau and (C) β-actin antibodies. (D) Densitometric analysis of the p-tau/t-tau ratio from blots compared with β-actin in the hippocampus on days 1, 3 and 7 days in aged rats. (E) Levels of tau mRNA measured using reverse transcription-quantitative polymerase chain reaction in aged rats. Values are presented as the mean ± standard error, n=5. *P<0.05 and **P<0.01 vs. the control group. ##P<0.01 vs. the sevoflurane group. p-tau; phosphorylated tau; t-tau, total tau; sev, sevoflurane group; sur, surgery group.

contrast, there was no significant difference between the sevoflurane and control groups at any time point (Fig. 6A). The rats in the surgery group swam a longer distance compared with the control and sevoflurane groups at all time points, however, there was no significant difference between the sevoflurane and control groups (Fig. 6B).

These data suggest that the aged rats in the surgery group exhibited more substantial memory impairment compared with the sevoflurane group, and that sevoflurane alone did not lead to postoperative cognitive dysfunction.

Discussion

The current study investigated the effects of sevoflurane and/or surgery on mTOR/p70S6K signaling, caspase-3 expression and p-tau/t-tau in the hippocampus of aged rats, in addition to measuring cognitive function following treatment. These data

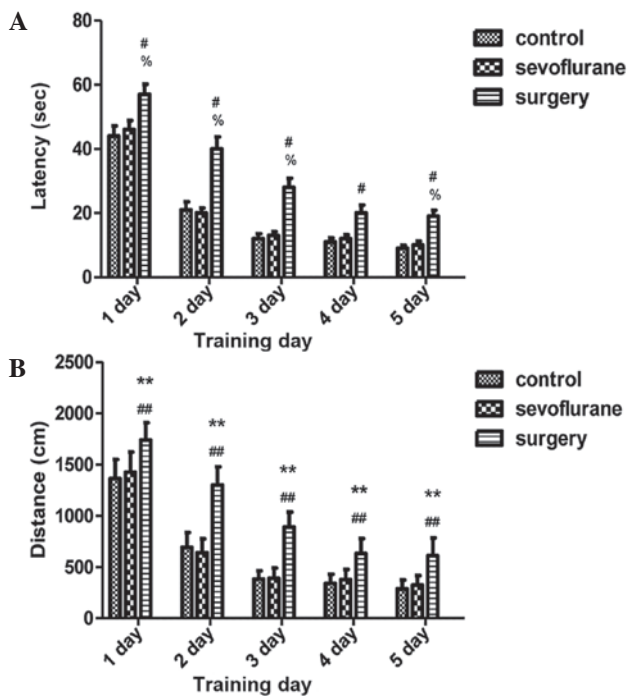


Figure 6. Rats in the surgery group exhibited greater memory decline from day 8-12 following treatment compared with those in the sevoflurane group. Following treatment (days 8-12 post sevoflurane and/or surgery), the rats were trained for 5 days to find the hidden platform. (A) Latency to find the platform during the test. (B) Distance swam to the platform during the test. Values are presented as the mean \pm standard error. ** $P < 0.01$ vs. the control group. # $P < 0.05$ and ## $P < 0.01$ vs. the sevoflurane group. % $P < 0.01$ vs. the surgery group.

suggest that sevoflurane and/or surgery impairs mTOR/p70S6K signaling and increases caspase-3 expression. Sevoflurane and surgery, however not sevoflurane alone, increased p-tau/t-tau and led to postoperative cognitive dysfunction.

The aged rats in the sevoflurane group did not exhibit memory impairment in the hidden platform tests, whereas the rats in the surgery group required a significantly longer time to locate the platform at 8-12 days post-treatment. These results were consistent with those of a previous study (22). In addition to the inflammatory reaction caused by sevoflurane and the surgical procedure, the inhibition of mTOR signaling may have contributed to the cognitive decline. Previous studies have demonstrated that protein synthesis is essential for long-term synaptic plasticity (23), and synaptic protein synthesis occurs locally in dendrites and at synaptic regions (24). The inhibition of TOR signaling and its upstream activators, phosphatidylinositol 3-kinase and Akt, was demonstrated to block N-methyl-D-aspartate receptor-dependent dendritic green fluorescent protein synthesis, in addition to the dendritic synthesis of endogenous proteins involved in synaptic plasticity, including tetanic stimulation-induced calmodulin-dependent protein kinase II α (α CaMKII) and microtubule-associated protein 2, in hippocampal slices (25). These studies provide evidence for the critical role of the mTOR signaling pathway in the regulation of synaptic activity-induced dendritic protein synthesis in the hippocampus. Protein synthesis is a well-established requirement for memory formation. The formation of long-term fear memory has been reported to be impaired when rapamycin is bilaterally injected into the amygdala

in rats during training. Rapamycin specifically impaired memory consolidation to long-term storage, which depends on additional brain regions, including hippocampus-dependent spatial memory (26) and auditory cortex-dependent memory. The data of the current study suggest that although sevoflurane and/or surgery impaired mTOR signaling in the hippocampus, cognitive function declined only in aged rats in the sevoflurane and surgery group. It is possible that sevoflurane alone has a weaker effect on mTOR signaling and cognitive function compared with the combination of sevoflurane and surgery.

mTOR phosphorylates p70S6k at Thr 389, and a previous study demonstrated that A β_{1-42} produced a rapid and persistent downregulation of mTOR/p70S6k phosphorylation in murine neuroblastoma cells, which was associated with caspase-3 activation (27). p-p70S6k expression has been observed to be significantly reduced in the lymphocytes of patients with AD, with p-p70S6k levels significantly correlated with the MMSE scores (18). mTOR inhibition via rapamycin led to an increase in A β -induced cell death in cultured cells (27). This observation suggests that mTOR/p70S6K activity is beneficial for cell survival, and that mTOR/p70S6k pathway inhibition may contribute to the degenerative process in the brains of patients with AD. The data of the current study are consistent with a previous study (18). In the current study, sevoflurane and/or surgery inhibited mTOR/p70S6K signaling and reduced caspase-3 levels in the hippocampus. However, previous studies have suggested that neurofibrillary tangle formation and tau phosphorylation are significantly associated with increased mTOR phosphorylation, in addition to with its targets, p70S6K and 4E-BP1 (12,28). Previously, the levels of p-p70S6K at the Thr 421/Ser 424 and Thr 389 sites were observed to correspond with increased tau phosphorylation at Ser 262 (29). Regression analyses have demonstrated that t-tau and p-tau levels were dependent on p-p70S6K, however not at the site typically phosphorylated by mTOR (28), which suggests mTOR may have both positive and negative effects. In the current study, the mTOR-mediated p-p70S6K levels were measured, which demonstrated that sevoflurane and surgery increased p-tau/t-tau in the hippocampus, whereas sevoflurane alone did not increase the ratio. Further studies are required to investigate additional pathways that may induce tau phosphorylation.

A previous study demonstrated that exposure to clinically relevant doses of isoflurane increased tau phosphorylation and insoluble and aggregated forms of tau, and induced the detachment of tau from microtubules (30). The insoluble tau levels were increased one week following anesthesia, which suggests that anesthesia precipitates brain alterations which may promote the subsequent development of tauopathy (30). Among the kinases that are able to phosphorylate tau, glycogen synthase kinase-3 β , cyclin-dependent kinase-5, mitogen-activated protein kinase/extracellular signal-regulated kinase, p38 and c-Jun N-terminal kinase are considered the key physiological and pathological tau kinases (31-33). Furthermore, CaMKII has been suggested to serve a major role in the regulation of tau phosphorylation at the epitopes that modulate tau binding to microtubules (34,35).

Anesthesia-induced hypothermia leads to overt tau hyperphosphorylation in normal mouse brains, however, the duration of anesthesia or the types of anesthetics were not observed to

alter the pattern of hypothermia-induced kinase activity. One week following a 4 h exposure to isoflurane, all kinase profiles were identical compared with the control-treated animals (30). Alternatively, studies have suggested that tau hyperphosphorylation is associated with abnormal glucose metabolism due to hypothermia (36). However, in the current study, the body temperature of the rats was maintained at $\sim 37^{\circ}\text{C}$, which thus eliminates the potential effect of body temperature on tau phosphorylation.

In the current study, the effects of sevoflurane and surgery on postoperative cognitive dysfunction, mTOR signaling and caspase-3 were observed to be greater than that of sevoflurane anesthesia alone. A previous study has additionally demonstrated that 18 month-old mice that underwent abdominal surgery with local anesthesia alone exhibited cognitive impairment (37). The surgery alone induced neuroinflammation which may result in cognitive dysfunction through the inhibition of synaptic function and neurogenesis, and may contribute to post-operative cognitive dysfunction (38,39). In addition, the stress and pain associated with surgery may contribute to cognitive dysfunction (40,41). Therefore, the alterations in mTOR signaling and caspase-3 resulting from anesthesia and surgery in the current study may be associated with this.

The current study has several limitations. Due to the use of multiple techniques, the sample size of each group was small, therefore, future studies are required to verify these data using a larger sample of animals. The present study did not investigate the effect of restoring mTOR/p70S6K signaling on the alterations in caspase-3 or post-operative cognitive function. Therefore future studies may undertake this approach to further reinforce the current evidence. Furthermore, future studies are required to investigate the association between the mTOR signaling pathway and post-operative cognitive dysfunction.

In conclusion, compared with the sevoflurane group, the levels of hippocampal p-mTOR and p-p70S6K were reduced in the surgery group, whereas the caspase-3 levels were increased. Surgery increased the ratio of p-tau/t-tau in the hippocampus of aged rats. The postoperative cognitive dysfunction following surgery is, in part, a result of mTOR signaling pathway inhibition in aged rats. Overall, the present study suggests that increased neuronal apoptosis and tau phosphorylation may be involved in the association between anesthesia and the occurrence of AD. It is thus suggested that sevoflurane anesthesia should not be the first choice in patients with AD or high-risk groups, however further investigation is required to verify this.

Acknowledgements

The current study was supported by the Department of Anesthesiology, Beijing Military General Hospital (Beijing, China).

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