

Changes in cognitive function due to combined propofol and remifentanil treatment are associated with phosphorylation of Tau in the hippocampus, abnormal total water and calcium contents of the brain, and elevated serum S100 β levels

X.-L. ZHI^{1,2}, C.-Y. LI^{1,2}, M. XUE³, Y. HU⁴, Y. JI⁵

¹Pharmacy College, Nanjing University of Chinese Medicine, Nanjing City, China

²Jiangsu Collaborative Innovation Center of Chinese Medicinal Resources Industrialization, Nanjing City, China

³Suzhou Institute for Food and Drug Control, Suzhou City, China

⁴Department of Anesthesiology and Medical Equipment, The 452nd Hospital of PLA, Chengdu, Sichuan, China

⁵Funing County Hospital, Yancheng City, China

Abstract. – OBJECTIVE: Propofol and remifentanil are commonly used combined for anaesthesia and can cause cognitive dysfunction. We hypothesized that combined treatment with these drugs would exert its effect via increased phosphorylation of Tau protein in the brain hippocampus. To address this, we assessed cognitive function and extent phosphorylation of Tau in experimental animals treated with either drug or their combination. In addition, we documented other biochemical abnormalities, such as brain total calcium and water contents, and serum levels of S100 β protein.

MATERIALS AND METHODS: 60 Sprague-Dawley (SD) rats were divided into 5 groups: control, model, propofol-treated, remifentanil-treated, and combined treatment groups (12 animals per group). The Morris water maze test assessed latent periods as a measure of cognitive function in experimental animals. Tau phosphorylation was quantified by immunohistochemistry and expressed as a number of cells with positive Tau expression and as average staining area. Brain water content was assessed by measuring wet and dry brain weights, and calcium content was evaluated by the flame atomic absorption spectroscopy method. Serum S100 β levels were assessed by ELISA.

RESULTS: Treatment with propofol and remifentanil markedly increased the latent period in the Morris water maze test, increased number the extent of Tau phosphorylation in the hippocampus, adversely modulated total water and calcium content in the brain, and elevated serum

S100 β levels. Under all conditions, combined treatment caused more pronounced effects on the studied outcomes.

CONCLUSIONS: Propofol combined with remifentanil induces a cognitive decline which is associated with Tau phosphorylation and modulation of local and systemic biochemical parameters.

Key Words:

Cognitive function, Propofol, Remifentanil, Tau, Phosphorylation, Hippocampus, Water content, Calcium content, Brain, S100 β .

Introduction

Postoperative cognitive dysfunction occurs most commonly in elderly patients after anaesthesia¹⁻³, and the mechanism of this disorder is unclear^{4,5}. Anaesthetics are suspected causative factors of postoperative cognitive dysfunction⁶. Propofol is commonly employed as an intravenous anaesthetic drug and may exert protective effects against brain injury⁷⁻⁹. However, propofol has an excessive sedative effect, which is not beneficial for patient recovery, and cannot be used alone^{10,11}. Remifentanil is an agonist of the μ -opioid-receptor, characterized by a strong analgesic effect and fast onset of action¹²⁻¹⁴. Both drugs are often used in combination.

Phosphorylation of Tau protein in the hippocampus has been reported to be associated with a decline in cognitive function¹⁵. We hypothesized that combined treatment with propofol and remifentanil would exert cognitive dysfunction via increased phosphorylation of Tau protein. This hypothesis was tested in the present study using experimental animals. Since total brain water, calcium contents, and serum levels of S100 β protein are associated with the extent cerebral injury^{16,17}, we assessed these parameters before and after combined treatment with propofol and remifentanil.

Materials and Methods

Animals and Drugs

Sixty healthy adult male SD rats (body weight 250 – 300 g) were purchased from the Kunming Institute of Zoology. Propofol was purchased from Guorui Pharmaceutical, (Sichuan, China), whereas remifentanil was provided by Langfang Branch Industry (Langfang, Hebei, China).

The rats were randomly divided into 5 groups: control group, model group, remifentanil-treated group, propofol-treated group, and combined treatment group. Each group comprised 12 rats. The rats were allowed to adjust to the new environment for 7 days at 60% humidity and 23° C. Animals from control group received an injection of normal saline into the caudal vein. Animals in the model group did not receive any treatment. Animals in the remifentanil-treated group received a slow injection of 25 mg/kg remifentanil via the caudal vein. When righting reflex disappeared, additional 50 mg/kg-hour of remifentanil were injected into the caudal vein for 0.5 hours. Animals in the propofol-treated group were slowly administered 15 mg/kg of propofol into the caudal vein. After the disappearance of the righting reflex, 30 mg/kg-hour propofol were injected into the caudal vein for 0.5 hour. Animals in the combined treatment group received both drugs administered as above.

Morris Water Maze test to Assess Cognitive Function

Experimental animals started the water maze training 7 days before drug administration. In the first 6 days, navigation training was done 5 times per day. The latent period was considered as the time that a rat required to find the platform. If the rat did not find the platform within 60 sec, the la-

tent period was counted as 60 sec. The 20th latent period was considered as the basal value before drug administration. Twenty-four hours after drug administration, navigation training was repeated, and latent periods after drug administration were recorded.

Assessment of Tau Phosphorylation

Tau phosphorylation has been assessed by immunochemistry. After the last water maze test, animals were sacrificed by cervical dislocation, and their brain tissues were collected and fixed in 4% paraformaldehyde for 24 hours. The tissues were sliced and processed by conventional dehydration, transparency, dipping wax, and embedding. Continuous coronal sections were made using the tissue 4 mm dorsal of the optic chiasma. Three adjacent slices were selected from the brain tissue of each rat, each at the 4- μ m thickness, and were immunostained. Briefly, the slices were dewaxed and dehydrated. Then, 3% H₂O₂ was used for 10 min to block endogenous peroxidase. Afterwards, the rabbit polyclonal antibody against phosphorylated Tau was added at a 1:200 dilution and incubated overnight at 4° C. Next, the HRP-labelled goat anti-rabbit IgG (1:1000) was added to the slide and incubate for 15-20 min at room temperature (all antibodies were purchased from CUSABIO Company, Wuhan, China). This was followed by DAB staining, counterstaining with hematoxylin, decolorization, dehydration, transparency, and mounting with neutral gum. The Image-Pro Plus 6.0 Image Analysis Software was utilized to determine the number of positive cells and average grey value, and both were used to assess the extent of Tau phosphorylation.

Brain Water and Calcium Contents

Brain tissue was taken out by craniotomy, and wet weight was determined using a weighing cup. Then, the tissue was placed into a beaker. The tissue was dried in a drying oven to obtain dry weight. The water content was calculated according to Elliot Formula (Elliott 1949): water content = [(wet weight – dry weight) / wet weight] \times 100%¹⁸.

Dry brain tissue, obtained as above, was digested into a colorless transparent liquid using a mixture of nitric and perchloric acids at the 3:1 ratio. Then, nearly boiling water was used for metered volume. The flame atomic absorption spectroscopy method was utilized to quantify the calcium content.

Table I. Length of latent periods.

Groups	Before drug administration, sec	After drug administration, sec
Control	8.14 ± 3.48	7.58 ± 3.25
Model	8.31 ± 3.61	7.83 ± 3.10
Remifentanil-treated	8.47 ± 3.74	17.59 ± 6.37 ^{a,b}
Propofol-treated	8.20 ± 2.51	18.52 ± 5.94 ^{a,b}
Combined treatment	8.29 ± 3.17	27.91 ± 6.41 ^{a,b,c}

Data are mean ± SD. ^a $p < 0.05$ vs. control group; ^b $p < 0.05$ vs. model group; ^c $p < 0.05$ vs. remifentanil- and propofol-treated groups.

Serum S100 β levels

Arterial blood (1.5 mL) was drawn from the right common carotid artery and allowed to stand for 20 min. Then, blood was centrifuged for 10 min at 1000 rpm. The serum was collected and used to quantify the level of S100 β by the ELISA (BioVendor – Laboratorní Medicína a.s., Karásek, Brno, Czech Republic).

Statistical Analysis

The SPSS 22.0 (IBM Corporation, Armonk, New York, USA) statistical package was used for statistical analyses. Quantitative and qualitative data were analyzed using, respectively, the t and chi-square tests. The $p < 0.05$ represented statistical significance.

Results

Latent Periods

As shown in Table I, latent periods in remifentanil-treated, propofol-treated, and the combined treatment group were significantly longer than in both control and model groups ($p < 0.05$; Table I). Furthermore, the latent period in the combined treatment group was significantly longer than those in remifentanil- and propofol-treated groups ($p < 0.05$; Table I).

Phosphorylation of Tau in the Hippocampus

The number of cells expressing phosphorylated Tau was comparably low in the brain slices from the animals from control and model groups (Figure 1). Both tested drugs markedly increased the number of cells positive for Tau phosphorylation ($p < 0.05$; Figure 1), and combined treatment further elevated the number of positive cells (Figure 1).

We further determined average gray in the slides as an alternative measure of expression

of phosphorylated Tau in the hippocampus. As shown in Table II, the average gray value of phosphorylated Tau was significantly higher in remifentanil- and propofol-treated animals, and in animals treated with both drugs ($p < 0.05$ vs. control and model groups; Table II). Furthermore, Tau phosphorylation was significantly more extensive in the animals treated with both drugs, compared with their counterparts treated with one drug only ($p < 0.05$; Table II).

Total Water and Calcium Content in the Brain

As shown in Table III, the brain water content was high in the control group, but significantly decreased in all other experimental groups. Furthermore, water was more depleted from the brains of the animals treated with remifentanil, propofol or both drugs ($p < 0.05$ vs. model group; Table III). While there was no significant difference in the

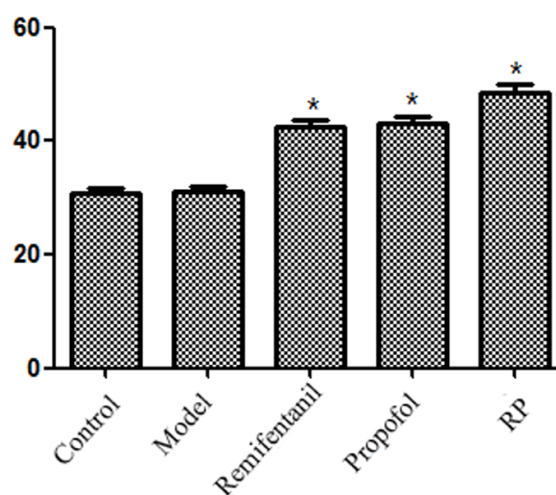


Figure 1. Number of hippocampal cells stained positive for phosphorylated Tau. Data are expressed as mean ± SD. “PR”: combined treatment. * $p < 0.05$ compared with control group.

Table II. Tau phosphorylation in the hippocampus.

Groups	Number of experimental animals	Average gray area
Control	12	0.234 ± 0.004
Model	12	0.233 ± 0.003
Remifentanyl-treated	12	0.256 ± 0.005 ^{a,b}
Propofol-treated	12	0.258 ± 0.007 ^{a,b}
Combined treatment	12	0.271 ± 0.006 ^{a,b,c}

Data are mean ± SD. ^a*p* < 0.05 vs. control group; ^b*p* < 0.05 vs. model group; ^c*p* < 0.05 vs. remifentanyl- and propofol-treated groups.

brain water content between either tested drug, treatment with both drugs lowered the brain water content even further (Table III).

Total calcium content in the brains of experimental animals is presented in Figure 2. The model group showed an increase in total calcium content, whereas the three treated groups showed a reverted trend (Figure 2).

Serum S100β levels

Serum S100β levels were significantly higher in remifentanyl-treated, propofol-treated, and combined treatment groups than in control and model groups (*p* < 0.05; Figure 3). In addition, animals in the combined treatment group exhibited serum levels of this marker significantly higher than animals treated with one drug only (*p* < 0.05; Figure 3).

Discussion

Our study presents experimental evidence that combined treatment with propofol and remifentanyl causes cognitive dysfunction and various biochemical abnormalities in the brain and on the systemic level.

Propofol is a new, short-acting intravenous general anaesthetic drug, practically insoluble in

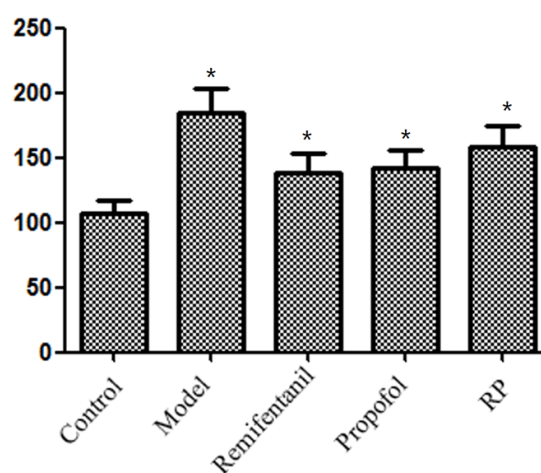


Figure 2. Total calcium content in the brain tissue. Data are expressed as mean ± SD, µg/dg. “PR”: combined treatment. **p* < 0.05 compared with control group.

water, but with the capability of quickly passing through the blood-brain barrier. Its advantages are the fast onset of anaesthesia and quick patient awakening¹⁹. Furthermore, propofol causes dilation of peripheral vessels, block sympathetic nerve system, and inhibit the vasomotor centre, thus inhibiting the effects norepinephrine and stress response mediators. However, propofol do-

Table III. Total water content in the brain.

Groups	Number of animals	Total water content (%)
Control	12	76.14 ± 0.05
Model	12	80.96 ± 0.11 ^a
Remifentanyl-treated	12	78.81 ± 0.09 ^{a,b}
Propofol-treated	12	78.74 ± 0.07 ^{a,b}
Combined-treatment	12	77.63 ± 0.08 ^{a,b,c}

Data are mean ± SD. ^a*p* < 0.05 vs. control group; ^b*p* < 0.05 vs. model group; ^c*p* < 0.05 vs. remifentanyl- and propofol-treated groups.

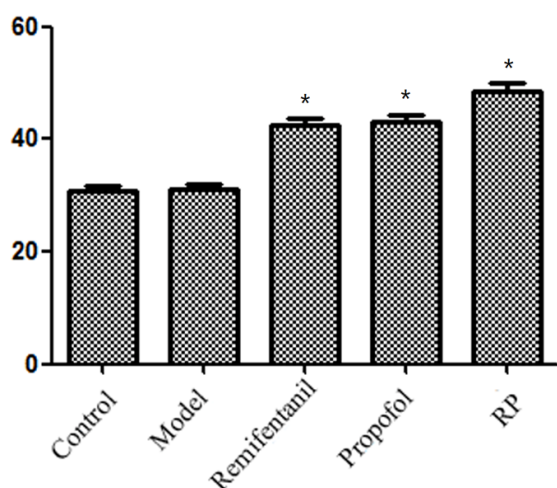


Figure 3. Serum levels of S100 β . Data are expressed as mean \pm SD, pg/mL. "PR": combined treatment. * $p < 0.05$ compared with control group.

se-dependently affects memory function and may cause anterograde and retrograde amnesias^{20,21}. Furthermore, propofol exerts an effect on blood glucose levels and antagonizes excitability, as well as modulates the supply and demand balance of cerebral oxygen. These effects are beneficial for cerebral protection, as they decrease the generation of oxygen radicals and facilitate patient recovery from brain injury²². Remifentanyl is a μ -opioid receptor agonist with ultrashort effect, characterized by a fast onset of action and quick clearance. Its metabolism is not affected by hepatic and renal function. Non-specific esterases in plasma and tissues quickly hydrolyze the ester bond in remifentanyl, with no cumulative effect^{23,24}. The recommendatory dose of remifentanyl for general anaesthesia is 0.1-0.5g / kg·min²⁵.

Calcium is one of the important ions of the body that can transfer extracellular information to intracellular compartments. Intracellular calcium overload causes cytotoxicity via the following mechanisms²⁶: (1) uncoupling of mitochondrial electron transport blocks oxidative phosphorylation, reduces generation of ATP, promotes anaerobic metabolism, thus aggravating acidosis and reducing ATP; (2) damage to the Na⁺-K⁺-ATP enzyme activity, enhancement of membrane permeability to other ions, which causes increase of osmotic pressure and intracellular water content, with the subsequent cytotoxic cerebral edema; (3) activation of neutral protease which causes neural cytoskeletal disruptor and neural microtubule de-

polymerization, with a strong impact on normal axonal transport or even leading to the neuronal cell death. Studies indicated that decreasing the extent of calcium overload modulates cerebral ischemia²⁷.

Tau is a low molecular weight glycoprotein mainly presented in the central nervous system cells²⁸. At present, six Tau isoforms have been detected in the human brain. The gene encoding Tau includes 16 exons and is located on the long arm of the chromosome 17. Eleven of these 16 exons encodes the protein, with 3-4 repeating domains near the C-terminus in each Tau. There are 2-3 phosphorylation sites in Tau. In addition to phosphorylation, Tau can also undergo other post-translational modification, such as saccharification, ubiquitination and abnormal glycosylation. Once it undergoes a post-translational modification, Tau loses the stabilizing effect on microtubules, thus damaging the biological function of nerve fibers²⁹. Studies demonstrate that significant phosphorylation occurs at different sites of the hippocampal Tau when experimental animals have been exposed to propofol for anaesthesia³⁰.

Conclusions

We demonstrate that propofol combined with remifentanyl induces a cognitive decline after anaesthesia which is associated with Tau phosphorylation, and modulation of biochemical parameters in the brain and on the systemic level.

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Conflicts of interest

The authors declare no conflicts of interest.

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