Insulin-like growth factor-1 improves postoperative cognitive dysfunction following splenectomy in aged rats

BIN WANG¹, XU LIN¹, JIAHUI ZHOU¹, CHUNHUI XIE², CHUAN LI², RUI DONG¹, GAOFENG ZHANG¹, XIAOPENG SUN¹, MINGSHAN WANG¹* and YANLIN BI*¹

¹Department of Anesthesiology, Qingdao Municipal Hospital Affiliated to Qingdao University, Qingdao, Shandong 266071; ²Department of Anesthesiology, Weifang Medical University, Weifang, Shandong 261042, P.R. China

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Abstract. Postoperative cognitive dysfunction (POCD) is a serious complication following anesthesia and operations in aged patients undergoing surgical intervention. It is characterized by temporary or permanent cognitive decline, memory impairment and deterioration in language comprehension and social adaption ability. Therefore, the development of POCD prevention and treatment tools has become an area of interest. The current study assessed the therapeutic effects of insulin-like growth factor-1 (IGF-1) on POCD in aged rats and explored the underlying mechanisms. Model rats underwent splenectomy under 1.5-2% isoflurane and mechanical ventilation. IGF-1 (50 µg/kg) was diluted in normal saline and administered by abdominal hypodermic injection daily from the operation to day 7 post-operation. Following splenectomy, the animals showed marked cognitive impairment as determined by the Morris water maze test. Hippocampal protein levels of amyloid precursor protein (APP), β-site APP-cleaving enzyme-1 (BACE-1), amyloid-β (Aβ), caspase3, Bax and Bcl-2 were assessed by immunoblotting. Neuronal apoptosis in the hippocampus was analyzed using a TUNEL assay. The results indicated that IGF-1 decreased Aβ-protein production and inhibited neuronal apoptosis in the hippocampus following splenectomy, subsequently alleviating POCD.

Introduction

Postoperative cognitive dysfunction (POCD) represents a serious complication following anesthesia and surgical procedures for patients undergoing surgical intervention (1). POCD is characterized by temporary or permanent cognitive decline, memory impairment, deterioration in language comprehension and social adaption ability, and particularly affects elderly people (>65 years) (2). POCD can lead to increased mortality, prolonged hospitalization, other complications such as Alzheimer's disease and higher treatment costs (3). Although the pathogenic mechanisms for POCD remain unknown, its risk factors comprise trauma surgery, postoperative pain and neuronal apoptosis (4). Therefore, the development of POCD prevention and treatment tools has become a focus of interest for research.

Amyloid precursor protein (APP) is hydrolyzed in two ways: i) Degradation by α-secretase during normal physiological conditions; or ii) generation of soluble β-APP8 and C99 by β-site APP-cleaving enzyme-1 (BACE-1), followed by C99 hydrolyzation by γ-secretase to generate insoluble amyloid-β (Aβ) (5). APP is distributed in neuronal synapses (6). Aβ, a 36-43-amino acid peptide, is the main constituent of amyloid plaques in Alzheimer's disease (AD) (7). It is widely accepted that Aβ oligomers are causally associated with neurodegenerative processes accompanying AD (8). The most common isoforms of Aβ are Aβ42 and Aβ40 (9), which serve important roles in POCD (10). A previous study revealed that POCD was associated with apoptosis of hippocampal neurons in rats (11). Therefore, effective inhibition of Aβ and apoptosis of hippocampal neurons demonstrated potential for the prevention and treatment of POCD.

Insulin-like growth factor I (IGF-I) serves critical roles in regulating body growth and metabolism and affects multiple cerebral functions (12). IGF-I promotes brain development, neuronal excitability, myelin sheath production, angiogenesis, synaptogenesis and neuronal survival, growth and differentiation (13). Additionally, IGF-I stimulates cell proliferation and survival in multiple cell types (14-16) and is considered a

Correspondence to: Professor Yanlin Bi, Department of Anesthesiology, Qingdao Municipal Hospital Affiliated to Qingdao University, 5 Donghai Middle Road, Qingdao, Shandong 266071, P.R. China
E-mail: yanlinbi68@sina.cn

*Contributed equally

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universal cytoprotective molecule, protecting cells from free radicals and apoptosis (17). Notably, a reduction in the amount of IGF-1 markedly contributed to age-associated cognitive impairment (18). A previous study revealed that IGF-1 expression was negatively associated with the progression of cognitive impairment (19).

Therefore, the current study aimed to assess whether IGF-1 improved POCD by mediating apoptosis and Aβ production. The present study studied cognitive function in aged rats following surgery with or without IGF-1 administration to investigate the protective effects of IGF-1 on splenectomy-induced POCD.

Materials and methods

Animals and groups. A total of 150 male Wistar rats (age, 16-18 months; weight, 350-550 g) were purchased from the SPF Beijing Biotechnology Co., Ltd. (license no. SCXK-2016-0002) and examined. The animals were housed under a 12-hour light/dark cycle with free access to water and rodent chow. All animal experiments followed the Guidance Suggestions for the Care and Use of Laboratory Animals by the Ministry of Science and Technology of the People's Republic of China (20). Approval was obtained from the Animal Ethics Committee of Qingdao Municipal Hospital (Qingdao, China).

Rats were housed under standard conditions with food and water available ad libitum and were allowed to acclimatize at 24-26°C for 1 week prior to experiments. The living environment of the rats was clean and tidy and suitable for survival. The rats were randomized into five groups (n=30/group), as follows: i) Control (C); ii) isoflurane (I); iii) splenectomy (S); iv) S + normal saline (S + NS) and v) S + IGF-1 (S + IGF-1).

Surgery and injection. The control group underwent no treatment. Rats in the I group were given continuous inhalation of 1.5-2% isoflurane for intubation and given 1.5% isoflurane and mechanical ventilation with 100% oxygen for anesthesia maintenance. This anesthetic procedure was selected due to its clinical relevance; additionally, anesthetics are considered to play a role in cognitive impairment (21). Rats in Group S underwent splenectomy following the same anesthesia as those in Group I. Briefly, the animals were placed in a supine position followed by skin shaving. After disinfection, an incision was made 1.5-2.0 cm below the costal margin for spleen removal. The splenic artery and vein were ligatured with silk threads. The abdominal cavity was closed after hemostasis was achieved. The splenic artery and vein were ligatured with silk threads. The abdominal cavity was closed after hemostasis was achieved. The splenic artery and vein were ligatured with silk threads. The abdominal cavity was closed after hemostasis was achieved.

The rats in Group S + NS were given NS (0.9% sodium chloride) via abdominal hypodermic injection every day from 1.5-2% isoflurane for 1 h at room temperature. This was followed by overnight incubation at 4°C with the following primary antibodies: Anti-β-actin (1:2,000; cat. no. ab8226; Abcam), anti-APP (1:2,000; cat. no. ab32136; Abcam), anti-BACE1 (1:1,000; cat. no. ab183612; Abcam), anti-caspase-3 (1:200; cat. no. ab13847; Abcam), anti-Bax (1:200; cat. no. ab32503; Abcam), anti-Bcl2 (1:200; cat. no. ab32124; Abcam) and anti-Aβ (1:2,000; cat. no. ab126649; Abcam).

Following overnight incubation, the membranes were washed with TBS-T three times (10 min each) and were further incubated with goat anti-mouse secondary antibody (1:5,000; cat. no. SAA544Mu19; Cloud-Clone Corp.) and goat anti-rabbit secondary antibody (1:5,000; cat. no. bs-0295G-HRP; Bioss) for 1 h at room temperature, membranes were then washed with TBS-T three times (10 min each). The generated immune complexes were detected with an enhanced chemiluminescence detection system (Amersham; Cytiva), and visualization was performed as previously reported (30) to detect proteins. The hippocampus was lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology) and protease inhibitors (PMSF; Beyotime Institute of Biotechnology), homogenized and placed in ice for full lysis for 40 min, followed by 20 min of centrifugation (1,600 x g; 4°C). Total protein was quantified using a bicinchoninic acid kit. Proteins (30 µg/lane) were resolved by 10% SDS-PAGE and electrotransferred onto polyvinylidene fluoride membranes (EMD Millipore), which were blocked with 10% skimmed milk containing TBS-Tween-20 (TBS-T; 0.1%) in ambient conditions for 1 h at room temperature. This was followed by overnight incubation at 4°C with the following primary antibodies: Anti-β-actin (1:2,000; cat. no. ab8226; Abcam), anti-APP (1:2,000; cat. no. ab32136; Abcam), anti-BACE1 (1:1,000; cat. no. ab183612; Abcam), anti-caspase-3 (1:200; cat. no. ab13847; Abcam), anti-Bax (1:200; cat. no. ab32503; Abcam), anti-Bcl2 (1:200; cat. no. ab32124; Abcam) and anti-Aβ (1:2,000; cat. no. ab126649; Abcam).

Western blotting. Western blotting was performed as previously reported (30) to detect proteins. The hippocampus was lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology) and protease inhibitors (PMSF; Beyotime Institute of Biotechnology), homogenized and placed in ice for full lysis for 40 min, followed by 20 min of centrifugation (1,600 x g; 4°C). Total protein was quantified using a bicinchoninic acid kit. Proteins (30 µg/lane) were resolved by 10% SDS-PAGE and electrotransferred onto polyvinylidene fluoride membranes (EMD Millipore), which were blocked with 10% skimmed milk containing TBS-Tween-20 (TBS-T; 0.1%) in ambient conditions for 1 h at room temperature. This was followed by overnight incubation at 4°C with the following primary antibodies: Anti-β-actin (1:2,000; cat. no. ab8226; Abcam), anti-APP (1:2,000; cat. no. ab32136; Abcam), anti-BACE1 (1:1,000; cat. no. ab183612; Abcam), anti-caspase-3 (1:200; cat. no. ab13847; Abcam), anti-Bax (1:200; cat. no. ab32503; Abcam), anti-Bcl2 (1:200; cat. no. ab32124; Abcam) and anti-Aβ (1:2,000; cat. no. ab126649; Abcam). Following overnight incubation, the membranes were washed with TBS-T three times (10 min each) and were further incubated with goat anti-mouse secondary antibody (1:5,000; cat. no. SAA544Mu19; Cloud-Clone Corp.) and goat anti-rabbit secondary antibody (1:5,000; cat. no. bs-0295G-HRP; Bioss) for 1 h at room temperature, membranes were then washed with TBS-T three times (10 min each). The generated immune complexes were detected with an enhanced chemiluminescence detection system (Amersham; Cytiva), and visualization...
was performed with the Sygene Bio Image system (Vilber). Gray values for APP, BACE-1, Aβ, caspase3, Bax and Bcl-2 were detected by ImageJ 1.8.0 (National Institutes of Health). The ratio of the target protein to the internal control, β-actin, was used in the final analysis.

**TUNEL assay.** TUNEL staining was performed according to a previous study (31). Neuronal apoptosis in hippocampal samples was analyzed by TUNEL assays. The samples were fixed with 4% paraformaldehyde at 4°C for 4 h. Following this, samples were treated with 3% hydrogen peroxidase...
and incubated in a labeling reaction mixture comprised of terminal deoxynucleotidyl transferase and deoxynucleotides overnight at 4°C. Sections were then subjected to further incubation with horseradish peroxidase (1:500; Shanghai Macklin Biochemical Co., Ltd.) for 30 min and treatment with 3,3'-diaminobenzidine for 15 min at 37°C in the dark. Reactions were stopped with running water and counterstaining was performed with hematoxylin at 37°C for 10 min. Following dehydration with a graded ethyl alcohol series and xylene treatment, tissue samples were mounted on coverslips with neutral gum. Apoptotic nuclei appeared as dark brown dots. Apoptotic cells in the CA1 region were assessed by light microscopy (magnification, x400) in a blinded manner in five random high-power fields.

Statistical analysis. Data are presented as the mean ± SD. All parameters were assessed by one-way ANOVA followed by Tukey’s post hoc test. SPSS (version no. 20.0; IBM Corp.) was used for data analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Cognitive function declines in aged rats following splenectomy. The MWM was performed to assess spatial learning and memory abilities. In Group S, the swimming distance (Fig. 1A) and escape latency (Fig. 1B) were significantly longer at days 1, 3 and 7 post-surgery compared with the C and I groups. The C and I groups presented similar values for swimming distance and escape latency throughout the experiment. These results indicated that surgery aggravated cognitive impairment. Swimming distance and escape latency in the S + IGF-1 group were significantly shorter at days 1, 3 and 7 post-surgery compared with the S and S + NS groups, indicating that IGF-1 improved cognitive function following splenectomy.

IGF-1 decreases Aβ protein production in the hippocampus of aged rats following splenectomy. Protein levels of APP, BACE-1 and Aβ were assessed in hippocampal specimens following surgery by immunoblotting. Splenectomy significantly upregulated APP, BACE-1 and Aβ expression at the protein level in hippocampal samples from aged rats at days 1, 3 and 7 compared with the C and I groups (Fig. 2). IGF-1 administration following splenectomy significantly decreased the protein amounts of APP, BACE-1 and Aβ in the hippocampus of aged rats at days 1, 3 and 7 compared with the S + NS group.

IGF-1 inhibits the apoptosis of neurons in the hippocampal CA1 region in aged rats following splenectomy. Immunoblotting was performed to assess the protein levels of caspase-3, Bax and Bcl2 in rat hippocampi following surgery.
The results demonstrated that splenectomy significantly upregulated caspase-3 and Bax expression, and significantly downregulated Bcl2 expression in the hippocampal CA1 region of aged rats at days 1, 3 and 7 post-surgery compared with the C and I groups (Fig. 3). Furthermore, IGF-1 administration following splenectomy significantly reduced caspase-3 and Bax protein levels, and significantly increased Bcl2 levels in hippocampal samples in aged rats at days 1, 3 and 7 postsurgery compared with the S and S + NS groups.

The TUNEL assay revealed that IGF-1 administration markedly reduced neuronal apoptosis associated with surgery in the hippocampal CA1 region of animals in the S + IGF-1 group compared with the S and S + NS groups (Fig. 4).

Discussion

The current study demonstrated that swimming distance and escape latency increased post-operatively, indicating splenectomy induced POCD in aged rats. This was consistent with previous studies which indicated markedly aggravated cognitive dysfunction in rats that underwent splenectomy (27,32). Furthermore, the results of the current study revealed that splenectomy induced the overexpression of APP, BACE-1 and Aβ in the hippocampus. This indicated that changes in Aβ-protein may be associated with early POCD, which is consistent with findings published by Canet et al (33). Furthermore, IGF-1, a multifunctional polypeptide essential for normal growth and development (12), inhibited the production of upstream proteins APP and BACE-1, attenuated Aβ production and improved surgery-induced POCD. These results indicated IGF-1 had a protective role in POCD by attenuating Aβ production in aged rats. Cognitive dysfunction persists transiently due to the acute production of APP and Aβ (34). Neuroinflammation associated with Aβ aggregation is an essential factor in cognitive dysfunction (35). Cleavage of APP by BACE-1 produced soluble β-APP8 and C99, and C99 is hydrolyzed by γ-secretase to produce insoluble Aβ (36). Research has demonstrated that Aβ is located in the brain as metastable monomeric Aβ is constantly produced by APP under conditions of catalysis by secretases (37). AD and POCD have similar neuropathogenesis (38). Given that cognitive function is unavoidably impaired by major surgeries in aged patients, developing efficient therapeutic tools is of high significance (39). The present work recommended IGF-1 as a novel potent drug as it improved POCD by reducing the generation of Aβ. A previous study reported that IGF-1 inhibited JNK activity (40) and enhanced APP phosphorylation at Thr668 in rat hippocampal tissue (41). Furthermore, IGF-1 increased α-secretase expression in the hippocampus and lowered the levels of BACE1 and γ-secretase, thereby reducing the levels and deposition of Aβ in hippocampal tissue (42).

Additionally, the results of the current study revealed that IGF-1 downregulated caspase-3 and Bax, and upregulated Bcl2 following splenectomy in hippocampal samples at days 1, 3 and 7 post-surgery. IGF-1 administration markedly reduced neuronal apoptosis associated with surgery in the hippocampal CA1 region of rats. Apoptosis is another important mechanism for POCD development (43). The present study demonstrated that in the hippocampus of aged rats following splenectomy, caspase-3 and Bax were significantly increased,
while Bcl-2 was significantly decreased. The Bcl-2 family protein is located upstream of the mitochondria and is an important regulator of mitochondrial membrane permeability, which controls the release of cytochrome c and activates downstream caspase-3 proteases, mediating cell survival or death (44,45). Under the condition of apoptosis inducer signals, caspases are activated by the combination of specific cofactors (46). Once caspases are activated, degradation of cellular proteins occurs, eventually causing irreversible cell death (47). Bcl-2 localization in the outer membrane of mitochondria is mediated by the indirect action of the caspases (48). Apoptotic protease activating factor 1 is targeted to the mitochondrial membrane by Bcl-2, which blocks the activation of the apoptotic protease by regulating its structure and regulates the action of cry-e (49). IGF-1 prevents apoptosis by inducing the signaling pathway mediated by PI3K and its downstream target Akt (50). The interaction between IGF-1 and the IGF-1 receptor phosphorylates tyrosine kinase or activates PI3K by activating the insulin receptor substrate (51). When activated, PI3K subunits phosphorylate phosphoinositide-dependent protein kinases and activate gene expression and protein translation of downstream target Akt. When the upstream signal activates the main target enzyme Akt, anti-apoptotic genes are upregulated, and Akt regulates Bcl-2 protein expression and enhances Bcl-2 activity (52). Peruzzi et al (53) demonstrated that PI3K inhibitors prevented IGF-1 from upregulating Bax and downregulating Bcl-2. Therefore, IGF-1 inhibited the apoptotic pathway (54).

Certain limitations of the current study must be discussed. Firstly, the number of animal experiments was limited. Sample sizes will be increased in future experiments. Secondly, the behavioral memory of rats can be measured comprehensively using behavior tests, including contextual fear conditioning test and the elevated plus maze test. Thirdly, since animal models cannot completely reproduce complex clinical situations, it remains essential to confirm whether similar changes occur in patients following surgery.

Overall, the current study reported a neuroprotective role for IGF-1 for POCD in aged rats. The mechanism involved decreased Aβ-protein production and inhibited neuronal apoptosis in the hippocampus. The present results indicated the use of IGF-1 for preventing POCD in aged patients.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

YB and MW conceived and designed the current study and drafted the manuscript. CX, CL and JZ performed the experiments at the physical laboratory of Qingdao University, China. RD, XL and XS analyzed data. GZ and BW performed the experiments and wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics and approval and consent to participate

The present study followed the recommendations of the National Institute of Health guidelines for the care and use of laboratory animals and obtained approval from the Clinical Trial Ethics Committee of Qingdao Municipal Hospital, Qingdao, China.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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