Neuropharmacology and analgesia

Dexmedetomidine improves early postoperative cognitive dysfunction in aged mice

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A B S T R A C T

Postoperative cognitive dysfunction (POCD) is a frequent complication following major surgery in the elderly. However, the exact pathogenic mechanisms are still unknown. Dexmedetomidine, a selective alpha 2 adrenal receptor agonist, was revealed anesthesia and brain protective role. The present study aimed to examine whether dexmedetomidine protects against POCD induced by major surgical trauma under general anesthesia in aged mice. In the present study, cognitive function was assessed by Y-maze. Pro-inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor (TNF-α), apoptosis-related factor caspase-3 and Bax were detected by real-time PCR, Western blot or immunohistochemistry. The results showed that anesthesia alone caused weak cognitive dysfunction on the first day after general anesthesia. Cognitive function in mice with splenectomy under general anesthesia was significantly exacerbated at the first and third days after surgery, and was significantly improved by dexmedetomidine administration. Splenectomy increased the expression of IL-1β, TNF-α, Bax and caspase-3 in hippocampus. These changes were significantly reversed by dexmedetomidine. These results suggest that hippocampal inflammatory response and neuronal apoptosis may contribute to POCD, and selective alpha 2 adrenoreceptor excitation play a protective role.

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1. Introduction

Postoperative cognitive dysfunction (POCD) is characterized by progressive deterioration of cognitive function, reductions in self-care, increased hospitalization and delayed recovery. Some POCD cases even develop into permanent cognitive disabilities, such as Alzheimer’s disease and dementia, and finally loss of independent living skills. Surgery, especially major surgery, was frequently reported to cause POCD in aged population. However, the neurobiological basis remains unknown. General anesthetics used in major surgery reveal harmful effect on cognitive function. They alter synaptic function through modulating specific ligand-gated ion channels, principally γ-aminobutyric acid (GABA) type A receptor and the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor (Franks., 2006). These drugs including ketamine, midazolam and isoflurane cause neuronal apoptosis and interfere in neuronal protein expression (Ikonomidou et al., 1999; Young et al., 2005). Co-application of multiple anesthetics (midazolam, nitrous oxide and isoflurane) in 7-day old mice caused widespread apoptosis and degenerative changes in central nervous system and subsequent impairment of learning and memory in their adulthood (Jevtovic-Todorovic et al., 2003). In aged rats, isoflurane anesthesia induced weak and temporary cognitive deficit (He et al., 2012). Surgical trauma plus anesthesia, especially major surgery, causes more severe POCD. Numerous reports showed that splenectomy, which greatly alters circular system and immune system, resulted in severe cognitive dysfunction in elder rats and mice (An et al., 2013; He et al., 2012; Kamer et al., 2012). Thus, splenectomy is frequently used as major surgery trauma in research involving POCD (Wan et al., 2007).

Dexmedetomidine, a selective alpha 2 adrenal receptor agonist, is reported to have anesthesia, analgesia and cerebral protective effect. Dexmedetomidine exhibited significant postconditioning properties against oxygen and glucose deprivation-induced injury (Dahmani et al., 2010). Dexmedetomidine improved neuronal apoptosis caused by isoflurane in newborn mice (Sanders et al., 2005) and increased the
expression of anti-apoptosis protein while reduced the expression of apoptosis-promoting protein in intracerebral hemorrhage rats (Hwang et al., 2013). Experimental and clinical practice have revealed that dexmedetomidine is neuroprotective on delirium, stress and inflammatory response (Yang and Lee, 2014; Bekker et al., 2013; Zhang et al., 2014a, 2014b). Dexmedetomidine treatment converted disordered electroencephalogram waves to normal non-rapid eye movement wave in patients with sleep disorder, an important induction factor of POCD (Huupponen et al., 2008). Dexmedetomidine shortened the onset time of postoperative delirium (Shehabi et al., 2009). These reports suggest that dexmedetomidine play a beneficial role in POCD. Indeed, dexmedetomidine was shown to protect cognitive function after laparoscopic cholecystectomy and laparoscopic surgery for colorectal cancer in elderly patients (Chen et al., 2013; Zhang et al., 2014a, 2014b).

The present study was designed to evaluate the effect of dexmedetomidine on POCD in aged mice, which underwent general anesthesia and major surgical trauma. Systemic inflammatory response following surgical trauma play an important role in the POCD (Godbout et al., 2005; Rosczyn et al., 2008). Many studies have shown that high level of proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) in blood and brain tissue are associated with the occurrence of POCD (Cibelli et al., 2010; Wright et al., 2006). Thus we investigated whether dexmedetomidine interfered in the expression of these proinflammatory cytokines. Our results revealed that dexmedetomidine protected POCD, and reduced expression of proinflammatory factors and apoptosis trend in hippocampus. These results suggest a new clinical intervention for POCD.

2. Materials and methods

2.1. Animals and surgical procedures

Healthy BALB/c mice (20–22 month old, weight 60–70 g) were individually raised at 23 °C in a temperature-controlled room on a 12-h light and 12-h dark cycle with ad libitum access to food and water. After 7–10 days’ adaptation to their environment, the mice were randomly grouped: control group, anesthesia alone group, splenectomy group, dexmedetomidine alone group, and splenectomy plus dexmedetomidine group. The mice in control group were not given any treatment. The mice in anesthesia group were given 1.5% isoflurane under intubation and mechanical ventilation, immediately followed by intraperitoneal injection of ketamine 100 mg/kg. Isoflurane inhalation was continued to maintain anesthesia. The anesthetized mice exhibited surgical analgesia and intact cardiorespiratory function which was reflected by normal arterial blood gas parameters and loss of response to noxious stimuli, respectively. The mice in splenectomy group underwent splenectomy under the same anesthesia as anesthesia group. Briefly, the spleen was exposed through a small incision in the left upper abdominal quadrant, and was then mobilized, isolated and removed before it was infiltrated with 0.25% bupivacaine. All cuts were closed with sterile suture. In dexmedetomidine intervention group, dexmedetomidine (15 or 25 μg/kg) was administered by intraperitoneal injection alone (dexmedetomidine alone group) or 30 min prior to splenectomy described above (splenectomy plus dexmedetomidine group). All experimental procedures were performed in accordance with the Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Cognitive function testing

Hippocampal-dependent place learning and memory were evaluated by Y-maze on days 1 and 3 after different interventions. Y-maze is composed of three arms and one connection. Each arm is laid on a bottom of thin copper rods, which communicate with the stimulating power. The end of each arm is equipped with signal light. Safe zone is indicated when the light is on. Safe zone can be randomly changed. When one arm is a safe zone, the other two arms are power charged. Mice can be trained to actively respond and escape to the safe zone. It is considered correct response if the mice directly escape to the safe zone when subjected to electric shock. Vice versa, it is error response. All mice were adapted to the maze environment for 3–5 min before the experiment began. Then clockwise transform the safe zone and the position of the electric shock. Five seconds after the signal light is lit in the safe zone, charge the non-safe zone. When mice ran to safety, ends one training session. Repeat the training until the result of 9 correct responses in 10 consecutive trials was obtained. The total number of trials was recorded. The fewer trials number means better learning and memory ability.

Mice were sacrificed after Y-maze tests. One part of mice in each group were anesthetized and decapitated. The hippocampal tissues were quickly removed and immediately cooled in liquid nitrogen and stored at −80 °C until RNA and protein measurement. The remaining mice were used for immunohistochemistry. These mice were killed by CO₂ asphyxiation, perfused transcardially with heparinized saline and then PBS solution containing 4% paraformaldehyde. The hippocampus was dissected, fixed in 4% paraformaldehyde solution in PBS, and subsequently embedded in paraffin until use.

2.3. Real-time RT-PCR

Total RNAs of hippocampal tissues were extracted with ultra pure RNA extraction kit (Cat no.: CW0581, CWbio. Co. Ltd.). Total RNA was then reversely transcribed into cDNA using HiFi-MMLVcDNA reverse transcript reagent kit (Cat no.: CW0744, CWbio. Co. Ltd.). The resulting cDNA was detected using an ABI 7500 real-time PCR System with SYBR Green. β-tubulin was used as an internal control. The primers sequences for real-time PCR were as follows: IL-1β (sense: TTTCTCCTTGCTCTCAGT, antisense: GACAGTTCTGCTAAATGTC); TNF-α (sense: GCCTCCTCTCTAGCTT, antisense: ACTTGGCTGGTTGCTACGAC); β-tubulin (sense: AACCTAGGAGACCTGAACATC; antisense: TGAAGAATGGAGACCTGGGAT ). After an initial denaturation at 95 °C for 3 min, the PCR cycling was performed as follows: 95 °C for 12 s and 65 °C for 50 s. Amplification was performed for 40 cycles, and all samples were performed in triplicate. Results were presented as the levels of expression following normalization to β-tubulin using the 2-ΔΔCT method. Dissolved curve, which was measured immediately after amplification, showed single product peak, indicating good product specificity.

2.4. Western blot analysis

Total proteins of hippocampal tissues were extracted with RIPA lysis buffer, quantified using the Bradford method, and separated by SDS-PAGE (12.5%). After transferring to polyvinylidene fluoride membranes, the membranes were blocked by 1–3% BSA or 5% fat dry milk at room temperature or 37 °C for 1–2 h. The membranes were then incubated by primary antibody (1:100–1:3000) overnight at 4 °C. The membranes were incubated with HRP-coupled secondary antibody for 1–2 h at room temperature. The bound proteins were then visualized using ECL and analyzed using Biolmaging Systems.

2.5. Immunohistochemistry

The paraffin-embedded hippocampal tissues were cut into 4-μm sections. Sections were deparaffinized, rehydrated and
antigen repaired. After endogenous peroxidase was quenched with 0.3% H₂O₂ and nonspecific binding was blocked with normal non-immune serum, the sections were incubated with primary antibody for 60 min. Sections were then incubated with biotinylated secondary antibody for 10 min, followed by streptavidin-HRP complex for 10 min. Immunohistochemical reaction was revealed by using 0.05% 3,3-diaminobenzidine and 0.03% H₂O₂ as chromogen. After each incubation, sections were thoroughly washed with PBS. The sections were stained by hematoxylin (Cat no.: CW0127) for 3–7 min, and were dehydrated with gradient alcohol, cleared with xylene, dried and covered with glass before microscopy.

2.6. Statistical analysis

All data were analyzed with SPSS 17. Statistical data are expressed as mean ± sem. Group comparisons were performed using one-way ANOVA followed by a Bonferroni comparison. P < 0.05 is considered statistically significant.

3. Results

3.1. Cognitive function evaluation

Y-maze test, a widely used method in evaluation of spatial learning and memory, was applied to evaluate the effects of interventions on cognitive function. In this test, when 9 correct escape responses appeared in 10 consecutive trials, the total trial number was recorded. The fewer trials number means better escape responses. The fewer trials number was recorded and shown when 9 correct responses were gained in 10 consecutive trials. Con: control. D15, D25: dexmedetomidine treatment (15, 25 μg/kg).

3.2. IL-1β expression

We next examined whether the change of cognitive function was reflected by inflammatory response in hippocampus. The level of proinflammatory factor IL-1β in hippocampus was measured by real-time PCR and Western blot. As shown in Fig. 2, anesthesia alone did not change the mRNA and protein levels of IL-1β at two time points on day 1 and 3 after anesthesia, as compared to the control. Splenectomy significantly increased IL-1β mRNA expression on day 1 (P < 0.01) and 3 (P < 0.05) after surgery (Fig. 2A). Splenectomy also significantly increased IL-1β protein expression on day 1 (P < 0.01, Fig. 2B). The splenectomy-elevated mRNA and protein expression of IL-1β on day 3 was significantly decreased by dexmedetomidine at 15 and 25 μg/kg (P < 0.05) to the levels almost same as those in control group.

3.3. TNF-α expression

Another important proinflammatory factor TNF-α was measured to further decide the role of inflammatory response. Consistent with the results of IL-1β, anesthesia itself did not alter TNF-α mRNA and protein levels in hippocampus at two time points, as compared to the controls. However, splenectomy significantly increased the mRNA and protein expression of TNF-α (P < 0.01), which was significantly inversed by dexmedetomidine pretreatment. These results of IL-1β and TNF-α demonstrate that dexmedetomidine improve inflammatory response caused by surgical trauma (Fig. 3).

3.4. Expression of caspase 3 and Bax

We next elucidated whether the protective effect of dexmedetomidine is related with neuronal apoptosis. Pro-apoptosis related factor caspase-3 and Bax in hippocampus were measured by Western blot and immunohistochemistry. In Western blot analysis, anesthesia alone and anesthesia plus splenectomy significantly increased caspase 3 level (Fig. 4A, P < 0.01), the effect in anesthesia plus splenectomy groups was more markedly. The increased caspase 3 expression by splenectomy was significantly reduced by dexmedetomidine (P < 0.05 and < 0.01 by 15 and 25 μg/kg, respectively). In immunohistochemistry, there were sporadic caspase 3 positive neurons in control group (43 ± 14, Fig. 4A). The numbers were increased 100 ± 31 and 75 ± 18 (P < 0.05) on days 1 and 3 after anesthesia, respectively. Splenectomy further increased the number of caspase 3 positive cells to 190 ± 55 and 150 ± 31 on day 1 and 3, respectively (P < 0.01). Dexmedetomidine
treatment significantly decreased the increased number of positive neurons by splenectomy to 76 ± 21 (P < 0.05) and 50 ± 18 (P < 0.01) on day 3 at 15 and 25 μg/kg, respectively.

A similar change pattern was seen in Bax (Fig. 5). Anesthesia alone slightly increased the number of Bax positive neurons. Splenectomy markedly increased the number. Dexmedetomidine treatment significantly decreased the increased number of positive neurons by splenectomy. These results suggest dexmedetomidine decrease neuronal apoptosis caused by anesthesia and surgical trauma.

4. Discussion

In the present study, we observed that anesthetics and surgical trauma triggered cognitive dysfunction in aged mice. We also found anesthetics and surgical trauma caused increased expression of proinflammatory cytokine and neuronal apoptosis factors in the hippocampus. The results demonstrate that anesthetics plus surgery contribute to cognitive dysfunction. Our results also showed that dexmedetomidine, a selective α2 adrenergic receptor agonist, improved the anesthetics plus surgery-induced POCD and attenuated inflammatory response and neuronal apoptosis in hippocampus. Thus, these results suggest a protective role of dexmedetomidine in POCD in elderly.

In our study, exposure of aged mice to the anesthetic ketamine and isoflurane induced neuronal apoptosis as reflected by Bax and caspase 3 positive immunostaining and increased expression in Western blotting analysis. This means that anesthetics themselves may result in the deleterious effects on learning and memory function. General anesthetics caused profound depression of neuronal activity by manipulating a specific ligand-gated ion channel, primarily GABAA receptor and NMDA receptor, thereby altering the function of synapses and synaptic plasticity (Franks., 2006). Meanwhile general anesthetics caused abnormal central neurotransmitter and receptor systems and affected neurotransmitter generation, storage, release and disappearing procedures, which can lead to postoperative changes of learning, memory and other cognitive functions (Culley et al., 2003, 2004). GABAA and NMDA receptors and their agonists are essential for migration of neurons to the appropriate regions, dendritic filopodia stabilization, synaptic development, and stabilization (Komuro and Rakic.,...
Any interventions including anesthetics affecting GABA<sub>A</sub> and NMDA-mediated synaptic transmission are not favorable for normal neuronal development, especially for aging whose central nervous system is experiencing degenerative changes. The modulation of NMDA receptor and GABA receptor has the potential in inducing apoptosis (Ma et al., 2007). Possibly, these modulatory effects may induce changes in neurotrophin activation, and in turn, dysregulate the intracellular proapoptotic and antiapoptotic balance, such as the Bax/Bcl-2 ratio (Ma et al., 2005). This hypothesis is supported by a previous study in which constant ketamine administration to monkey cerebral cortex slices led to apoptotic neurodegeneration (Wang et al., 2006). Low-dose of ketamine also resulted in continuous memory and working memory impairment (Morgan et al., 2004; Honey et al., 2003; Pfenninger et al., 2002). Furthermore, cellular ionic and neurotrophin homeostasis is also crucial for normal neuronal survival. Isoflurane could activate the endoplasmic reticulum membrane 1,4,5-trisphosphate receptor (Wei et al., 2005).
al., 2008), cause excessive calcium release and reduce tissue-type plasminogen activator release (Head et al., 2009) to start endo-
genous apoptosis. In the present study, ketamine plus isoflurane increased Bax and caspase-3 expression. This is consistent with previous findings in vitro (Xie et al., 2006) and in vivo (Culley et al., 2004; Bianchi et al., 2008; Valentim et al., 2008), in which isoflurane was revealed to be cytotoxic, and isoflurane inhalation was reported to impair memory in aged rats (Culley et al., 2003).

The increase in proinflammatory cytokines in the hippocampus may be an important risk factor in cognitive decline after surgery, as demonstrated clearly by the present study and other reports (Wan et al., 2007; Rosczyk et al., 2008; Cibelli et al., 2010). Surgical trauma not only activates the immune response of peripheral but also activates that of the central nervous system. It has been postulated that over-expression of central inflammatory cytokines may lead to profound disturbances in sensory-motor coordination and cognition (Barrientos et al., 2006). Considerable evidences show that the release of proinflammatory cytokines within the hippocampus interferes with cognitive function and development of long-term potentiation. POCD is a complicated clinical syn-
drome, and mainly appears in elder patients with massive bleeding, long surgery time and strong stress response in surgery. Sham operation alone is not enough to cause POCD (Rosczyk et al., 2008). Splenectomy under general anesthesia is a major surgery, which was easy to trigger POCD in aged mice (Wan et al., 2007). Thus, splenectomy under general anesthesia was used in this study to examine the effect of dexmedetomidine. Proinflammatory factors, especially IL-1β are p38MAPK signal activation factor. p38MAPK, an important member of the MAPKs family, is involved in cell proliferation, apoptosis and differentiation, and plays an important role in the apoptosis process. In the present study, the increased expression of IL-1β and TNF-α in hippocampal neurons in splenectomized mice may underlie the neuronal apoptosis as reflected by caspase-3 and Bax.

Given the inevitable harm of cognitive function by general anesthesia and major surgical trauma in elderly population, searching an effective treatment or prediction is significant. This study has uncovered a plausible and promising novel drug that significantly attenuated the harm. In the present study, dexmede-
tomidine significantly improved POCD in aged mice, and decreased the expression of IL-1β, TNF-α, caspase-3 and Bax. Especially, the pretreatment of dexmedetomidine significantly improved the detected parameters (cognitive function, proinflam-
matory factors, and apoptosis factors) to the similar levels in control mice. The protective role of dexmedetomidine in cognitive function may be the results of reduction of inflammatory response and neuronal apoptosis. Indeed, dexmedetomidine was reported anti-inflammatory effect through reducing LPS-induced systemic release of TNF-α and IL-1β (Xiang et al., 2014). Further mechanism involving the neuroprotective role of dexmedetomidine is consid-
ered by two. First, dexmedetomidine increases the tyrosine phosphorylation of focal adhesion kinase, a key cellular enzyme, through stimulation of the alpha 2 adrenoceptor subtype and plays a vital role in cell plasticity and survival (Dahmani et al., 2005; Paris et al., 2006). Second, dexmedetomidine increases extracellular signal regulated kinases (ERK)1/2 phosphorylation, a key mitogen-activated protein kinase involved in cell survival and memory by the activation of protein kinase C, and probably imidazoline receptors (Dahmani et al., 2008; Xue et al., 2000). Dexmedetomidine also increases the expression of growth factors such as epidermal growth factor and brain-derived neurotrophic factors, and then participates in neuroprotection. These molecular properties of dexmedetomidine may underlie its anti-inflamm-
atory and anti-apoptotic effect. Indeed, recent reports have shown that dexmedetomidine exerted both preconditioning and postconditioning effects against ischemic injury in hippocampal organotypic slice cultures (Dahmani et al., 2010), and downregu-
lated apoptosis-promoting protein (Engelhard et al., 2003). A large body of experimental models and clinical setting support dexme-
detomidine’s neuroprotective properties, including attenuating delirium, preserving sleep architecture, preserving ventilatory drive and decreasing sympathetic tone and inflammatory response (Yang and Lee, 2014; Bekker et al., 2013; Zhang et al., 2014a, 2014b). Our findings in POCD add dexmedetomidine a new neuroprotective property.

In conclusion, the present study demonstrates a neuroprotective role of dexmedetomidine in POCD in aged mice. This protective role may be resulted from the decreased inflammatory response and neuronal apoptosis in the hippocampus. This study suggests a good choice for clinical prevention of POCD in elderly.

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