

Preventive effects of dexmedetomidine on the development of cognitive dysfunction following systemic inflammation in aged rats

Daiki Yamanaka¹ · Takashi Kawano¹ · Atsushi Nishigaki¹ · Bun Aoyama¹ · Hiroki Tateiwa¹ · Marie Shigematsu-Locatelli¹ · Fabricio M. Locatelli¹ · Masataka Yokoyama¹

Received: 23 August 2016 / Accepted: 2 October 2016 / Published online: 13 October 2016
© Japanese Society of Anesthesiologists 2016

Abstract

Purpose In the present study, we examined whether and by what mechanisms dexmedetomidine (DMED) prevents the development of systemic inflammation (SI)-induced cognitive dysfunction in aged rats.

Methods Animals received a single intraperitoneal (i.p.) injection of either 5.0 mg/kg lipopolysaccharide (LPS) or vehicle. LPS-treated rats were further divided into three groups: early DMED, late DMED, or midazolam (MDZ) treatment ($n = 12$ each). Seven days after LPS injection, cognitive function was evaluated using a novel object recognition task, followed by measurement of hippocampal levels of proinflammatory cytokines and Toll-like receptor 4 (TLR-4) expression. For ex vivo experiments, microglia were isolated from the hippocampus for assessment of cytokine response to LPS.

Results LPS-treated rats showed memory deficits, hippocampal neuroinflammation, and TLR-4 upregulation as compared to saline-treated animals. However, early DMED treatment was able to attenuate these SI-induced neurocognitive changes, whereas no benefits were observed in the MDZ and late DMED treatment groups. In ex vivo experiments, early DMED treatment prevented the development of SI-induced excessive microglial hyperactivation, which was blocked by the nonspecific α_2 -adrenergic receptor

(AR) antagonist atipamezole or the specific α_{2A} -AR antagonist BRL-44408, but not by the specific $\alpha_{2B/C}$ -AR antagonist ARC-239. On the other hand, neither DMED nor MDZ had a direct effect on LPS-induced release of pro-inflammatory cytokines from hippocampal microglia at clinically relevant concentrations.

Conclusion Our findings highlight that treatment with DMED during, but not after, peripheral SI can prevent subsequent hippocampal neuroinflammation, overexpression of TLR-4 in microglia, and cognitive dysfunction, as mediated by the α_{2A} -AR signaling pathway.

Keywords Dexmedetomidine · Neuroinflammation · Microglia · Cognitive function

Introduction

Clinical and preclinical evidence demonstrates that systemic inflammation (SI) can induce long-lasting cognitive complications via immune-to-brain communication, especially in the aged and vulnerable brain [1–5]. This impact even includes acute SI episodes such as aseptic surgical trauma or systemic infections, otherwise known as postoperative [6] or septic-associated cognitive dysfunction [7], respectively. Longitudinal studies suggest that these conditions are associated with subsequent disability and mortality [7–10]; therefore, effective preventive and treatment strategies, as well as molecular targets, need to be developed. Although the precise mechanisms remain to be elucidated, there is increasing evidence that neuroinflammation characterized by increased proinflammatory cytokines derived from activated microglia plays a crucial role in the development of acute SI-induced cognitive dysfunction [1, 2, 11–13].

Electronic supplementary material The online version of this article (doi:10.1007/s00540-016-2264-4) contains supplementary material, which is available to authorized users.

✉ Takashi Kawano
takashika@kochi-u.ac.jp

¹ Department of Anesthesiology and Intensive Care Medicine, Kochi Medical School, Kohasu, Oko-cho, Nankoku, Kochi 783-8505, Japan

In rodents, lipopolysaccharide (LPS, an endotoxin), the major component of Gram-negative bacteria, is widely used as an experimental model of SI [14]. We previously reported that a single systemic injection of LPS in aged rats results in long-lasting hippocampal neuroinflammation and related cognitive deficits [15]. As a major determinant of cognitive vulnerability, microglia are known to be primed during normal aging to produce greater proinflammatory responses to subsequent SI events [16, 17]. Primed microglia exhibit an upregulation of cell surface receptors such as Toll-like receptor 4 (TLR-4), which influences changes in their function and phenotype [18]. Recently, we determined that primed microglia can trigger an exaggerated release of proinflammatory cytokines, which contribute to prolonged neuroinflammation after SI induced by abdominal surgery [3]. In particular, proinflammatory cytokines, tumor necrosis factor- α (TNF- α), and interleukin-1 β (IL-1 β) released from hippocampal microglia play a pathogenic role in neurodegenerative disease-related cognitive dysfunction [19, 20].

Dexmedetomidine (DMED), an α_2 -adrenoceptor agonist, has been widely used in clinical practice as a perioperative sedative agent [21, 22]. Evidence has shown that DMED can reduce the risk of perioperative cognitive outcomes in critical care or postoperatively [23–30]. In support of these observations, preclinical studies have shown that DMED displays neuroprotective properties in a variety of in vitro and in vivo settings [31–34]. In addition, DMED has exhibited peripheral anti-inflammatory effects in patients with severe sepsis [35]. However, DMED is yet to be linked to the SI-induced neurocognitive impairment directly through experimental evidence of its underlying mechanism.

In the present study, we investigated the association and underlying mechanisms involved in DMED-induced neurocognitive protection using the LPS model of SI in aged rats. Specifically, the effects of DMED were compared to those of another commonly utilized benzodiazepine sedative, midazolam (MDZ). In addition, in order to examine which adrenoceptor subtype(s) mediate this effect, antagonist experiments were also performed.

Materials and methods

All experimental protocols were approved by the Kochi University Animal Experiment Committee. Male Sprague–Dawley rats aged 19–23 months were used in this study. Animals were housed in a temperature- and humidity-controlled room under a 12-h light/dark cycle with food and water provided ad libitum. In order to assess baseline locomotor, exploratory, and anxiety-like behaviors, all rats were evaluated in an open-field test 7 days before randomization according to our previous study [36].

Experimental protocol and drug administration

SI was induced by a single intraperitoneal (i.p.) injection of lipopolysaccharide (*Escherichia coli* LPS; 0111:B4, Sigma–Aldrich, St. Louis, MO, USA) at a dose of 5.0 mg/kg, as previously described [15]. This dose mimics a mild infection, producing small changes (<1 °C) in core body temperature. Two independent protocols, a drug-specific and an antagonist experiment, were conducted. For the drug-specific experiment, rats were randomly divided into five treatment groups to concomitantly receive either vehicle alone (group C), LPS alone (group L), LPS injection simultaneously followed by DMED (10 μ g/kg i.p., every 3 h \times 4 times; the early DMED group), midazolam (MDZ, 100 μ g/kg i.p., every 3 h \times 4 times; the MDZ group), or DMED 24 h later (10 μ g/kg i.p., every 3 h \times 4 times; the late DMED group) ($n = 12$ in each group). The DMED treatment regimen was determined based on findings from our previous study, and corresponds to the period of elevated circulating proinflammatory cytokines after LPS injection. MDZ was used as a sedative control. Our preliminary study showed that these doses of DMED and MDZ induce observable signs of comparable sedation (see S1 in the Electronic supplementary material, ESM).

For the adrenaline receptor (AR)-related antagonist experiment, rats were first injected with LPS, followed by a high dose of DMED (10 μ g/kg i.p., every 3 h \times 4 times). Next, they were randomly assigned into four treatment groups: saline (the DMED-alone group); the non-specific α_2 -AR antagonist atipamezole (500 μ g/kg; the DMED + atipamezole group), the specific α_{2A} -AR antagonist BRL-44408 (1.5 mg/kg, the DMED + BRL-44408 group), or the specific $\alpha_{2B/C}$ -AR antagonist ARC-239 (50 μ g/kg, the DMED + ARC-239 group) ($n = 8$ in each group). The dose of antagonists was selected based on the previous study [37]. All antagonists were purchased from Sigma–Aldrich and were administered by an i.p. injection 30 min before either the DMED or vehicle treatment.

The level of sedation was evaluated and graded from 0 to 4 according to a previously reported method [38], with modifications: grade 0, spontaneously active; grade 1, resting quietly and intact righting reflex; grade 2, minimum spontaneous activity but intact righting reflex; grade 3, no spontaneous activity or righting reflex; and grade 4, loss of reflex. In order to minimize physical stress, which could interfere with the measured outcomes, minimum noninvasive measurements were conducted: mean arterial pressure (MAP) was measured by tail-cuff plethysmography (BP-98A; Softron, Tokyo, Japan), and arterial oxygen saturation and pulse rate were measured noninvasively using MouseOX Plus (Starr LifeSciences Corp., Oakmont, PA, USA). Invasive measurements of peripheral levels of pro-inflammatory cytokines were performed in satellite animals.

Novel object recognition task

Seven days after LPS injection, cognitive function was evaluated using a novel object recognition task, as previously described [3]. Briefly, each rat was individually habituated to the test chamber in the absence of objects for 5 min on three consecutive days. The experimental apparatus consisted of a Plexiglas open-field box with an open top, and was cleaned with 70 % ethanol between subjects. On the day of testing, each rat was allowed to freely explore the open field arena containing two identical objects for 5 min (familiarization phase). After the hour-long retention interval, the rat was returned to the experimental chamber with a new pair of objects, including one identical and one novel object. The rat was allowed to again explore the objects for 5 min (testing phase). The animal's behavior was monitored by an overhead video camera connected to a computer equipped with the EthoVision tracking software (Noldus, Wageningen, Netherlands). An experimenter blind to group assignment also scored object interaction manually. Object exploration was defined as the time the rat's nose was in contact with or was within a centimeter of the object. Recognition memory was expressed as a novel object preference ratio. This measure is the ratio of time spent exploring either of the two objects during the familiarization phase or the novel object during the test phase relative to the total time spent exploring both objects.

After cognitive testing, all rats were sacrificed by cervical decapitation under terminal anesthesia with pentobarbital (80 mg/kg, i.p.) and then exsanguinated by transcardiac perfusion with ice-cold standard phosphate-buffered saline. The hippocampus was quickly dissected for enzyme-linked immuno sorbent assay (ELISA), RT-PCR, and ex vivo microglia preparation.

Acute isolation of microglia from the hippocampus

Microglia were acutely isolated from the hippocampus as described in our previous study [3]. Briefly, whole hippocampi were rapidly harvested, minced into pieces, and digested with 0.1 % trypsin and Dispase II (3.6 U/ml) for 1 h at 37 °C while shaking (100 strokes/min). Resulting homogenates were centrifuged at 600×g for 10 min at 4 °C. Supernatants were removed and cell pellets were resuspended in 4 ml of 70 % isotonic Percoll and overlaid with equal volumes of 30 and 37 % isotonic Percoll. The gradient was centrifuged at 2000×g for 20 min. Microglia were collected from the interphase between the 70 and 37 % Percoll layers and resuspended in DMEM culture media containing 10 % fetal bovine serum. Culture purity was greater than 95 %, as verified by immunocytochemistry using antibodies to CD68 to identify microglia.

Real-time PCR analysis

Expressions of TLR-4 in hippocampal microglia were measured by real-time PCR using a StepONE Real Time PCR System (Applied Biosystems, Carlsbad, CA, USA). Total RNA was isolated using an RNeasy Mini Kit (QIAGEN, Valencia, CA, USA), following the manufacturer's protocol. One microgram of total RNA was used for the synthesis of cDNA, employing the PrimeScript II, 1st Strand cDNA Synthesis Kit (Takara Bio, Kusatsu, Japan), and primers provided by the kit. The synthesized cDNA was used as a template for subsequent amplification in the ABI (Foster City, CA, USA) Prism 7500 sequence detection system. The primer sets of TLR-4 and β -actin were used as previously described [39]. Quantitative PCR was performed with an initial 10 min denaturation at 94 °C, followed by 35 amplification cycles (94 °C for 30 s and 58 °C for 30 s). All samples were analyzed in triplicate, including negative controls and standards. The abundance of mRNA was calculated in relation to serially diluted standard curves amplified simultaneously with the samples and corrected for β -actin mRNA levels.

ELISA analysis

The dissected hippocampus was homogenized with a polytron homogenizer (Kinematica Inc., Littau, Switzerland) in ice-cold lysis buffer (10 mM NaCl, 1.5 mM MgCl₂, 20 mM HEPES, 20 % glycerol, 0.1 % Triton X-100, 1 mM dithiothreitol, pH 7.4) containing a protease inhibitor cocktail (P8340, Sigma–Aldrich). The homogenates were centrifuged (11,000×g, 20 min, 4 °C) and the supernatant was aliquoted and frozen at –80 °C until required.

For the ex vivo experiment, microglia cells were plated at a density of 10⁴ cells/100 μ l in a 24-well dish in DMEM containing 10 % fetal bovine serum. Prior to cell treatment, the medium was replaced with fresh serum-free medium, followed by stimulation with LPS (0–100 ng/ml) in the absence or presence of the testing drug for 24 h at 37 °C, 5 % CO₂. At the end of the incubation, the medium was collected and stored at –20 °C for subsequent analysis by ELISA.

For quantification by ELISA, commercially available ELISA kits for measuring rat TNF- α (BioLegend, San Diego, CA, USA) and IL-1 β (Thermo Scientific, Vernon Hills, IL, USA) were used according to the instructions provided by the manufacturer.

Statistical analysis

All data were expressed as the mean \pm standard deviation (SD). For each dependent variable, group and/or other main effect(s) were tested with repeated-measures ANOVA.

Whenever ANOVA indicated statistical significance in identifying a significant main effect, post hoc comparisons between the groups were performed in a pairwise manner by using the Wilcoxon–Mann–Whitney test with Bonferroni correction. Correlations between variables were analyzed using Spearman’s correlation test. All data were analyzed using the statistical software SPSS (version 11; SPSS Inc., Chicago, IL, USA), and $p < 0.05$ was considered statistically significant.

Results

Physiologic parameters

The open field tests conducted before randomization showed no difference in spontaneous locomotion between the groups, suggesting that baseline performance among them was comparable. Each treatment was well tolerated; all rats survived and body weight did not differ significantly between LPS-treated and control groups throughout the study. In addition, there was no statistically significant difference in MAP [main effect of group; $F_{(4, 55)} = 3.67$, $p = 0.76$, Fig. 1a] or HR (main effect of group; $F_{(4, 55)} = 4.45$, $p = 0.69$, Fig. 1b) between the groups. The arterial oxygen saturation was maintained at greater than 97 % in all groups at all times. DMED and MDZ produced comparable levels of sedation, both being almost exclusively grade 2. In satellite animals, LPS-induced SI was confirmed by transiently elevated serum levels of TNF- α (Fig. 2a) and IL-1 β (Fig. 2b), which returned to baseline levels by 12 h following LPS injection. Furthermore, neither DMED nor MDZ had an effect on LPS-induced SI.

Novel object recognition performance

During the training phase, there was no evidence of an intrinsic exploratory preference for either of the two objects in both the drug-specific (Fig. 3a) and antagonist (Fig. 3b) experiments. In addition, total exploration time during the training phase did not differ between any of the treatment groups (drug-specific experiment; $F_{(4, 55)} = 6.07$, $p = 0.83$; antagonist experiment; $F_{(3, 44)} = 9.14$, $p = 0.74$), indicating that task motivation and ability during testing was comparable among all groups in both experiments.

During the testing phase in the drug-specific experiment, the saline-treated rats spent more time exploring the novel than the familiar object, indicating intact recognition memory (Fig. 3a). Consistent with previous findings [15], the LPS-treated rats exhibited significantly impaired novel object recognition performance (79.8 ± 8.4 % in group C vs. 55.7 ± 10.8 % in group L; $p < 0.05$). Our preliminary data showed that this LPS-induced cognitive impairment

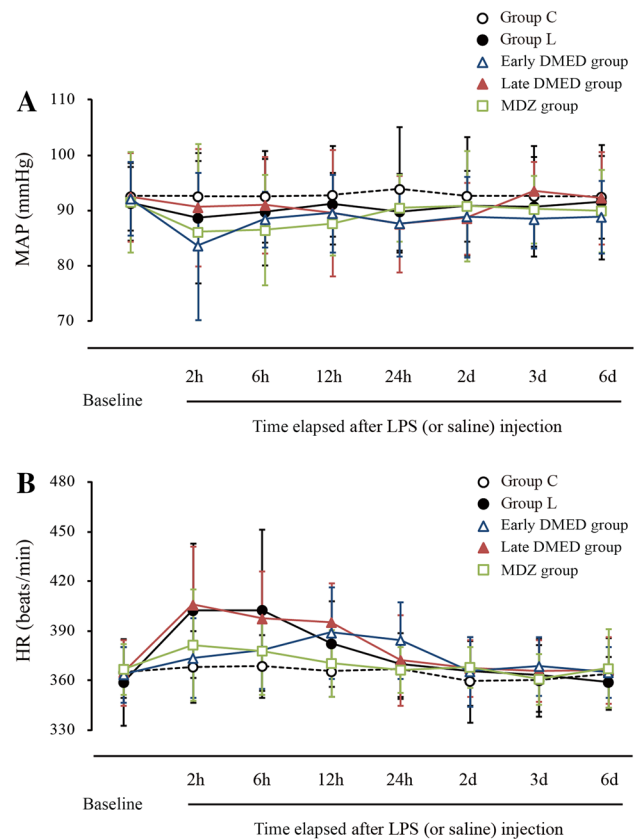


Fig. 1 Time courses of mean arterial blood pressure (MAP, **a**) and heart rate (HR, **b**) before (*baseline*) and after lipopolysaccharide (LPS) injection. The five study groups are as indicated in “Materials and methods” ($n = 12$ in each group). Each point represents the mean \pm SD

was age-specific (S2 in the ESM). In addition, both DMED and MDZ had a significant influence on novel object recognition in control rats (S3 of the ESM). However, the early DMED (72.0 ± 11.6 % in the early DMED group; $p < 0.05$ vs. group L), but not the late DMED group (57.7 ± 7.9 % in the late DMED group; $p = 0.61$ vs. group L), demonstrated improved novel object recognition performance relative to the saline-treated group during the testing phase. In addition, MDZ failed to show any beneficial effects compared to saline (54.6 ± 7.7 % in the MDZ group; $p = 0.77$ vs. group L).

In the antagonist experiment, the early DMED treatment-induced cognitive protection in LPS-treated animals could be blocked by the nonspecific α_2 -AR antagonist atipamezole or the α_{2A} -AR antagonist BRL-44408 (56.1 ± 8.8 % in the DMED + atipamezole group or 59.1 ± 8.8 % in the DMED + BRL-44408 group vs. 77.4 ± 9.7 % in the DMED-alone group; $p < 0.05$) but not by the $\alpha_{2B/C}$ -AR antagonist ARC-239 (75.8 ± 8.3 % in the DMED + ARC-239 group; $p = 0.51$ vs. the DMED-alone group) during the testing phase in Fig. 3b).

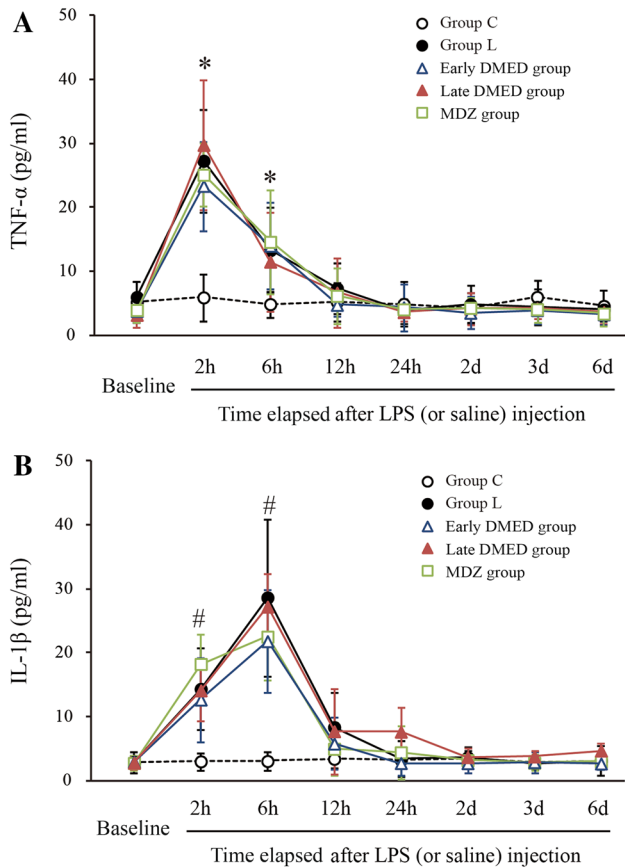


Fig. 2 Time courses of serum TNF- α (a) and IL-1 β (b) levels before (baseline) and after lipopolysaccharide (LPS) injection. The five study groups are as indicated in “Materials and methods” ($n = 12$ in each group). Each point represents the mean \pm SD. * $p < 0.05$: group C vs. all other groups

Levels of hippocampal cytokines after recognition memory testing

Taking all rats in the drug-specific experiment together, novel object recognition performance during the testing phase was inversely correlated with hippocampal levels of both TNF- α ($n = 60$; $R^2 = -0.74$; $p < 0.01$) and IL-1 β ($n = 60$; $R^2 = -0.83$; $p < 0.01$). This relationship suggests that neuroinflammation may play a pivotal role in the cognitive deficits observed after SI in aged rats. In the drug-specific experiment, the average levels of hippocampal TNF- α and IL-1 β in the LPS-treated control group were significantly higher than those in the saline-treated control group (Fig. 4a, TNF- α : 46.0 ± 15.2 pg/ml in group L vs. 11.1 ± 4.3 pg/ml in group C; $p < 0.05$, IL-1 β : 38.1 ± 11.4 pg/ml in group L vs. 6.0 ± 2.5 pg/ml in group C; $p < 0.05$). The LPS-induced increase in both IL-1 β and TNF- α were prevented by early DMED treatment (TNF- α : 17.0 ± 12.1 pg/ml in the early DMED

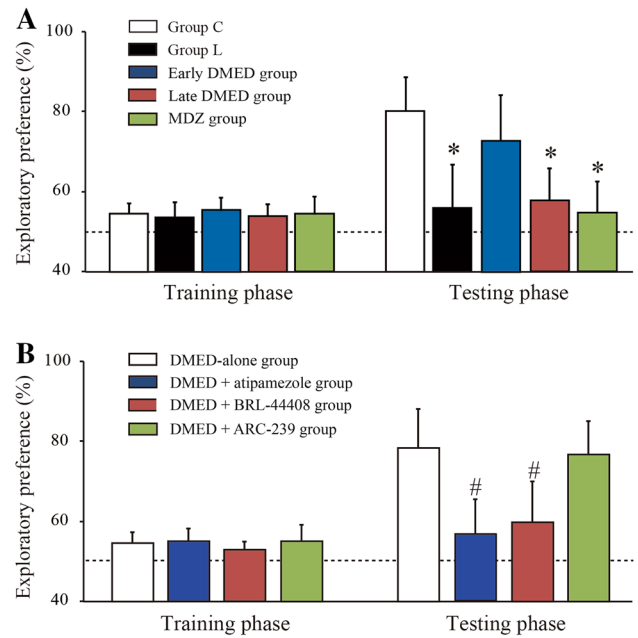


Fig. 3 Cognitive function assessed using the novel object recognition test in aged rats. Two independent protocols, a drug-specific (a) and an antagonist (b) experiment, were conducted. The study groups in each experiment are as indicated in “Materials and methods.” Ratios of preference between two objects in the training and testing phases of the novel object recognition test are shown. Each vertical bar represents the mean \pm SD ($n = 12$ in each group). * $p < 0.05$ vs. group C. # $p < 0.05$ vs. DMED-alone group

group; $p < 0.05$ vs. group L, IL-1 β : 9.1 ± 4.2 pg/ml in the early DMED group; $p < 0.05$ vs. group L), while treatment with neither late DMED (TNF- α : 44.1 ± 21.5 pg/ml in the late DMED group; $p = 0.58$ vs. group L, IL-1 β : 30.6 ± 12.1 pg/ml in the late DMED group; $p = 0.67$ vs. group L) nor MDZ (TNF- α : 44.0 ± 14.5 pg/ml in the MDZ group; $p = 0.65$ vs. group L, IL-1 β : 32.8 ± 12.8 pg/ml in the MDZ group; $p = 0.71$ vs. group L) produced an effect. The antagonist experiment showed that the early DMED treatment-induced anti-neuroinflammatory effect could be attenuated by administration of the nonspecific α_2 -AR antagonist atipamezole or the α_{2A} -AR antagonist BRL-44408 (Fig. 4b, TNF- α : 44.5 ± 22.3 pg/ml in the DMED + atipamezole group or 37.1 ± 19.5 pg/ml in the DMED + BRL-44408 group vs. 14.7 ± 7.8 pg/ml in the DMED-alone group; $p < 0.05$, IL-1 β : 30.8 ± 16.2 pg/ml in the DMED + atipamezole group or 27.0 ± 12.5 pg/ml in the DMED + BRL-44408 group vs. 9.1 ± 5.9 pg/ml in the DMED-alone group; $p < 0.05$) but not by the $\alpha_{2B/C}$ -AR antagonist ARC-239 (TNF- α : 15.8 ± 8.8 pg/ml in the DMED + ARC-239 group; $p = 0.48$ vs. the DMED-alone group, IL-1 β : 10.3 ± 8.2 pg/ml in the DMED + ARC-239 group; $p = 0.52$ vs. the DMED-alone group).

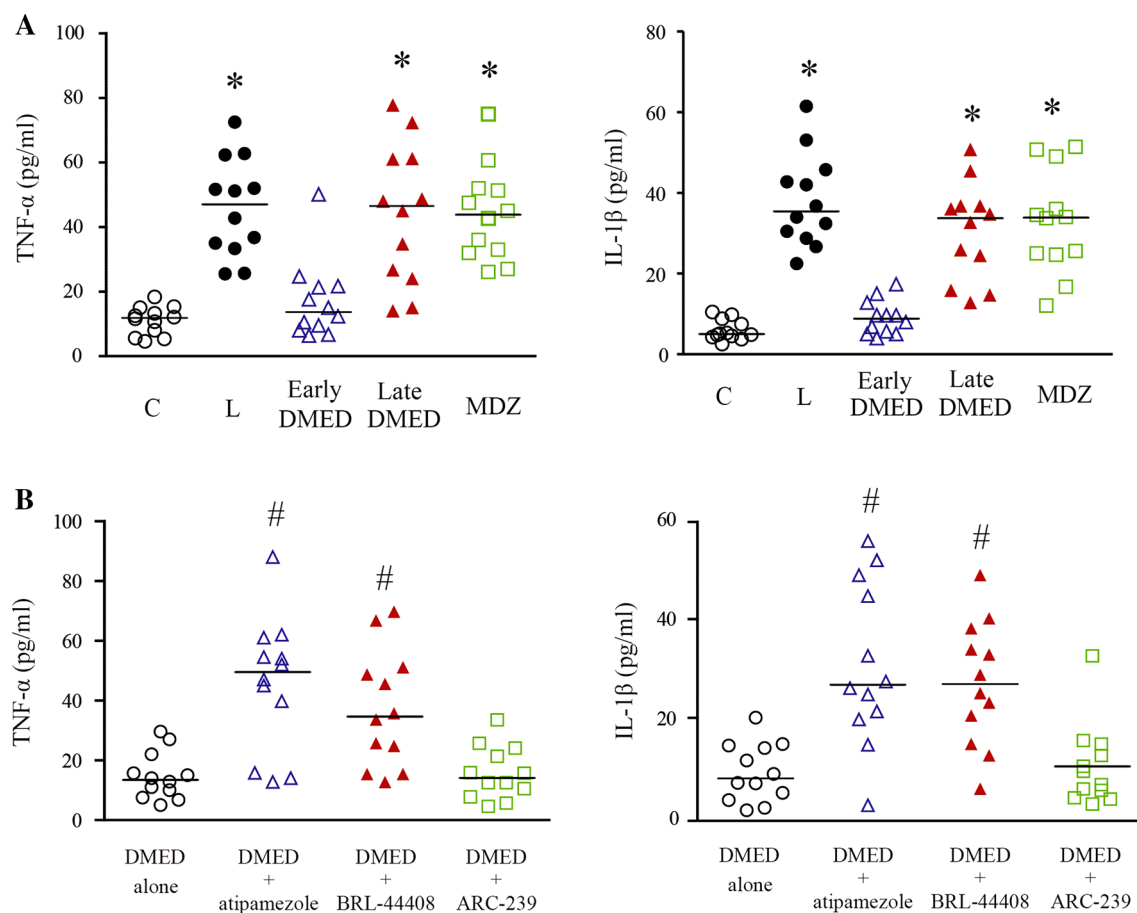


Fig. 4 Levels of pro-inflammatory cytokines in the hippocampus. Two independent protocols, a drug-specific (a) and an antagonist (b) experiment, were conducted. The study groups in each experiment are as indicated in “Materials and methods.” The levels of TNF- α and

IL-1 β for each group are shown. Each point represents one rat, and the line corresponds to the mean of 12 rats per group. * $p < 0.05$ vs. group C. # $p < 0.05$ vs. DMED-alone group

Levels of TRL-4 mRNA of microglia in the hippocampus

To further examine the effects of DMED on the expression of microglial TLR-4, TLR-4 mRNA levels in the hippocampus were measured using RT-PCR. LPS-induced SI significantly elevated microglial expression of TLR-4 compared to the saline-treated group (Fig. 5a, 3.5 ± 1.1 in group L vs. 0.9 ± 0.4 in group C; $p < 0.05$). However, early administration of DMED significantly reduced this expression in the LPS-treated rats (1.6 ± 1.2 ; $p < 0.05$ vs. group L). However, treatment with neither late DMED (3.1 ± 1.5 ; $p = 0.87$ vs. group L) nor MDZ (3.6 ± 1.3 ; $p = 0.91$ vs. group L) affected the LPS-induced upregulation of microglial TLR-4. In the antagonist experiment, the treatment-induced cognitive protection by early DMED among LPS-treated animals was inhibited by the nonspecific α_2 -AR antagonist atipamezole or the α_{2A} -AR antagonist BRL-44408 (Fig. 5b, 3.2 ± 1.8 in the DMED + atipamezole

group or 3.1 ± 1.4 in the DMED + BRL-44408 group vs. 1.4 ± 0.7 in the DMED-alone group; $p < 0.05$) but not by the $\alpha_{2B/C}$ -AR antagonist ARC-239 (1.6 ± 0.9 in the DMED + ARC-239 group; $p = 0.89$ vs. the DMED-alone group).

Ex vivo immunosensitivity of hippocampal microglia following LPS administration

In another experiment with an identical protocol, microglia were acutely isolated from the hippocampus 7 days after LPS injection. In order to investigate whether SI influenced the immunosensitivity of hippocampal microglia and whether this can be modulated by DMED administration, we measured TNF- α release from microglia isolated from the hippocampus following administration of different concentrations of LPS (0–100 ng/ml). As shown in Fig. 6a, a repeated-measures ANOVA revealed a significant main effect of group [$F_{(4, 55)} = 25.14$, $p < 0.05$].

Fig. 5 Expression of TLR-4 mRNA in hippocampal microglia. Two independent protocols, a drug-specific (a) and an antagonist (b) experiment, were conducted. The groups in each experiment are as indicated in “Materials and methods.” The expression of TLR-4 mRNA relative to β -actin mRNA is shown for each group. Each vertical bar represents the mean \pm SD ($n = 12$ in each group). * $p < 0.05$ vs. group C. # $p < 0.05$ vs. the DMED-alone group

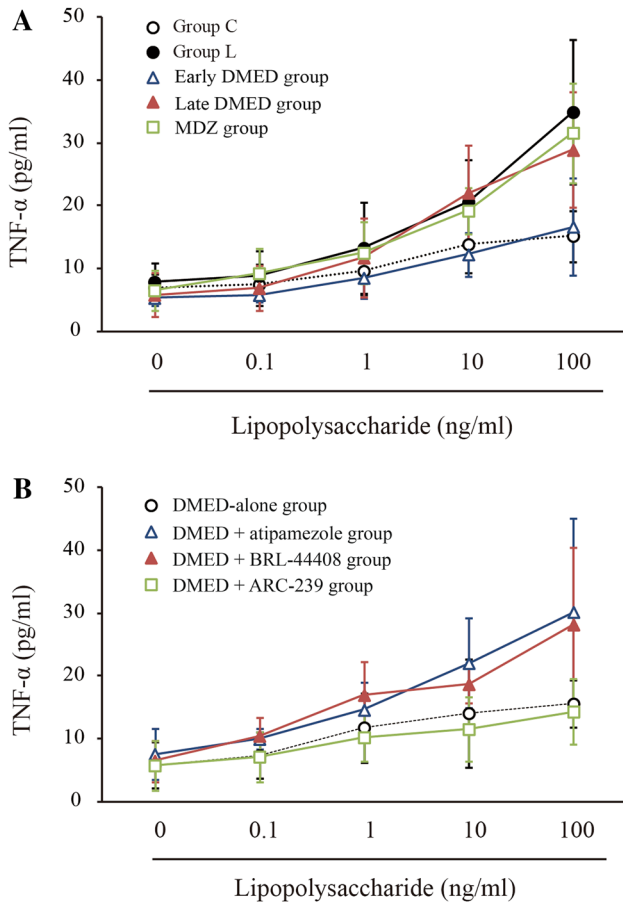
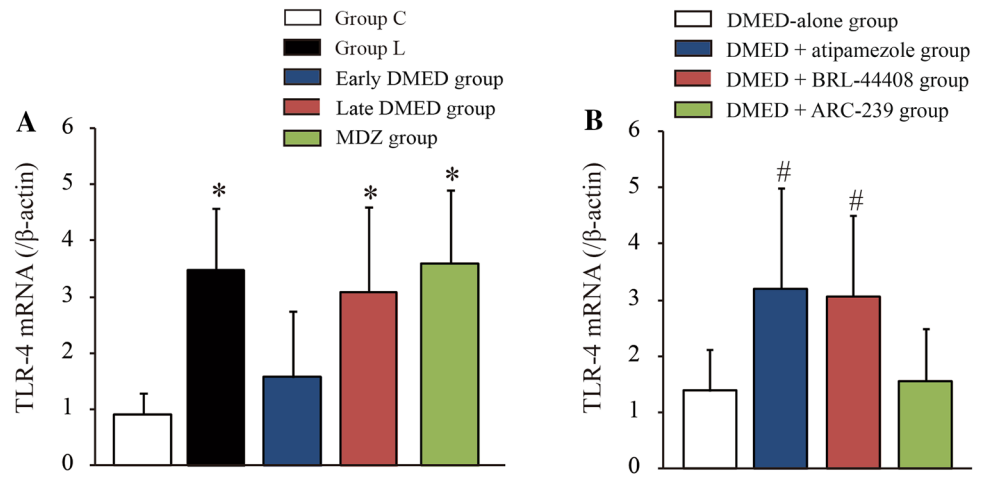


Fig. 6 Concentration–response effects of ex vivo stimulation with lipopolysaccharide (LPS) on the production of TNF- α in cultured microglia. Two independent protocols, a drug-specific (a) and an antagonist (b) experiment, were conducted. The groups in each experiment are as indicated in “Materials and methods.” Cultured microglia were stimulated with 0.1, 1, 10, or 100 ng/ml or the media alone. The levels of TNF- α were determined from supernatants collected 24 h later. Each point represents the mean \pm SD ($n = 12$ in each group)

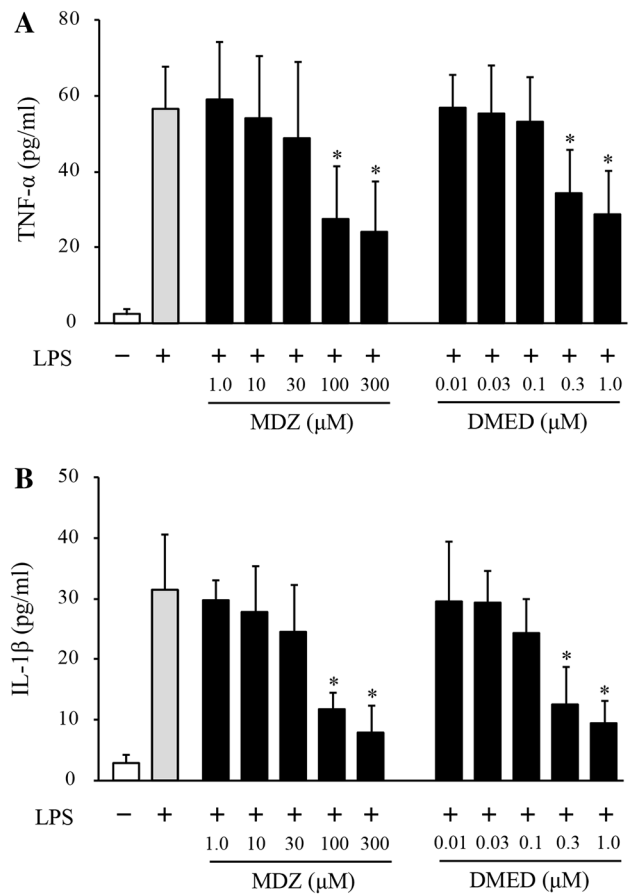


Fig. 7 Effect of anesthetics on lipopolysaccharide (LPS)-stimulated cytokine release from cultured microglia isolated from the aged rat hippocampus. Cultured microglia were pretreated with various concentrations of midazolam (MDZ, 0–300 μ M) or dexmedetomidine (DMED, 0–1.0 μ M) for 1 h prior to treatment with 10 μ g/ml LPS. After 24 h of incubation, the levels of TNF- α (a) and IL-1 β (b) present in the supernatants were measured. Each vertical bar represents the mean \pm SD ($n = 8$ in each group). * $p < 0.05$ vs. LPS alone

Pairwise comparisons using the Bonferroni correction indicated that the 100 ng/ml LPS-induced increase in TNF- α was greater in group L (34.9 ± 11.5 pg/ml) than in group C (15.2 ± 4.0 pg/ml; $p < 0.05$). This LPS-induced microglial reactivity was markedly decreased in the early DMED group. Conversely, the LPS-induced increases of TNF- α in the late DMED and MDZ groups were similar to that of group L. The reduced immunosensitivity after early DMED treatment was prevented by the nonspecific α_2 -AR antagonist atipamezole or the α_{2A} -AR antagonist BRL-44408 (Fig. 6b at 100 ng/ml LPS, 30.2 ± 15.0 pg/ml in the DMED + atipamezole group or 28.2 ± 12.2 pg/ml in the DMED + BRL-44408 group vs. 15.6 ± 3.8 pg/ml in the DMED-alone group; $p < 0.05$), but not by the $\alpha_{2B/C}$ -AR antagonist ARC-239 (14.3 ± 5.2 pg/ml in the DMED + ARC-239 group; $p = 0.91$ vs. the DMED-alone group).

Direct effects of dexmedetomidine and midazolam on hippocampal microglia

The direct effects of DMED or MDZ were evaluated in the release of IL-1 β and TNF- α from LPS-stimulated microglia isolated from the naïve aged hippocampus. As shown in Fig. 7a, b, administration of LPS alone (10 ng/ml) resulted in markedly increased levels of TNF- α (56.7 ± 11.1 pg/ml vs. 2.4 ± 1.3 pg/ml at baseline; $p < 0.05$) and IL-1 β (31.5 ± 9.3 pg/ml vs. 2.9 ± 1.4 pg/ml at baseline; $p < 0.05$) relative to controls. The bath application of either MDZ (1.0–300 μ M) or DMED (0.01–1.0 μ M) inhibited levels of TNF- α (Fig. 7a) and IL-1 β (Fig. 7b) in a concentration-dependent manner.

Discussion

Our findings confirm the hypothesis that treatment with DMED counteracts the pathogenesis of acute SI-induced cognitive dysfunction. Specifically, a sedative dose of DMED improved the performance of LPS-treated aged rats as compared to controls in the novel object recognition task. In contrast, an equisedative dose of MDZ failed to confer similar beneficial effects on cognitive performance, suggesting that the protective effect of DMED may be induced independent of its sedative action per se.

Indeed, the antagonist experiment showed that the effects of DMED were at least partially mediated by α_2 -AR and were associated with the suppressed upregulation of microglial TLR-4. Furthermore, improved cognitive performance was observed when DMED was administered during ongoing, but not after, peripheral inflammation. Taken together, these results indicate that DMED could be

effective in the prevention but not the treatment of acute SI-induced cognitive dysfunction.

The α_2 -AR subtypes, currently classified into α_{2A} , α_{2B} , α_{2C} , and α_{2D} (α_{2D} species variation of the human α_{2A}), are widely distributed in the central and peripheral nervous system [40, 41]. Specifically, microglia have been reported to express α_2 -AR receptors [42]. In this study, we used different α_2 -AR antagonists to elucidate the unique contributions of the α_2 -AR subtypes in the therapeutic effects exhibited by DMED. In particular, the nonspecific α_2 -AR antagonist atipamezole and the α_{2A} -AR antagonist BRL-44408, but not the α_{2B} -AR antagonist ARC-239, were able to block the protective effects of DMED. This suggests that DMED-induced cognitive protection was mediated by the α_{2A} -AR. However, large species differences in α_2 -AR subtype density, expression distribution, and antagonist sensitivity have been reported [40, 41]. Therefore, our findings must be carefully evaluated before being extrapolated to humans.

Recent evidence suggests that acute SI is closely associated with an increased risk of developing or exacerbating cognitive deficits [1–7]. SI interacts with the central nervous system (CNS) by both hematogenous and neural pathways, leading to substantial alterations in neurocognitive functioning [43]. The hippocampus has a high density of proinflammatory cytokine receptors and appears to be particularly vulnerable to an inflammation-induced disruption in cognitive processing [44, 45]. These changes could affect long-term behavioral and cognitive outcomes, especially among the more aged and ill [1, 2]. It has been established that acute SI can trigger an episode of delirium and that the occurrence of this phenomenon is associated with a higher risk of dementia [1, 46, 47]. Another prospective study further showed that acute SI events among elderly subjects with Alzheimer's disease were associated with a twofold increase in the rate of cognitive decline over time [5].

In the present study, we also demonstrated that LPS-induced neuroinflammation was observed even after the disappearance of peripheral inflammation. These results suggest that peripheral immune activation may be a vulnerable period in the development of neurocognitive pathology or cognitive impairment later in life. This theory may also support the hypothesis that administration of DMED during acute SI could prevent the development of SI-induced cognitive dysfunction.

Several studies suggest that DMED exerts a peripheral anti-inflammatory effect after acute SI [48–50]. However, in the present SI model, peripheral cytokine levels were slightly lower in the DMED-treated group than the control group. This divergent finding may be due to the different levels of SI severity used in this study relative to others. Specifically, previous studies examined cytokine levels during severe sepsis-induced SI, whereas our data used a nonlethal or moderate

level of SI. However, consistent with previous studies [14, 15], low-grade SI was sufficient to bring about neuroinflammation-related cognitive deficits. More importantly, our findings showed that DMED administration exerted a profound inhibitory effect on proinflammatory cytokines in the hippocampus. Considering its lack of peripheral action, the DMED-induced anti-neuroinflammatory effects observed in this study may be mediated by the hippocampus itself rather than peripheral immune-to-brain signals.

Neuroinflammation is now widely accepted to be a key pathogenetic element in a number of cognitive disorders [1, 2, 11–13]. Activated microglia are the principal regulator of neuroinflammation in the CNS through the excessive de novo production and release of proinflammatory cytokines [16, 17]. Specifically, early response cytokines TNF- α and IL-1 β are associated with neuronal apoptosis and decreased long-term potentiation [19, 20]. Over time, these effects may result in accelerated neurocognitive pathogenesis. In support of this suggestion, recent positron emission tomography studies of patients with mild cognitive impairment demonstrated a positive correlation between microglial activation and early cognitive deficits [51]. Therefore, inhibition of excessive cytokine release from microglia is currently being evaluated as a potential therapeutic strategy for the alleviation of cognitive deficits. In this vein, we found that DMED could inhibit LPS-induced proinflammatory cytokine release from isolated hippocampal microglia in a concentration-dependent manner (Fig. 7). However, the sedative plasma concentration of DMED has been reported to range between 0.01 and 0.03 μM [52] in healthy young volunteers, and below 2.5 ng/ml (approximately 0.01 μM) in adult rats [53]. Further taking into account the possible age-related change in the pharmacodynamics of DMED, our results suggest that a supraclinical concentration of DMED is necessary to significantly inhibit proinflammatory cytokine release from the hippocampal microglia (greater than 0.3 μM). MDZ was also able to inhibit the release of microglial cytokines at high (>100 μM) but not therapeutically relevant (0.8–1.5 μM) concentrations [54]. These findings suggest that the direct suppression of microglial neuroinflammation is not involved in DMED-induced cognitive protection after acute SI.

Increased age has consistently been reported to be the most important risk factor for SI-induced cognitive dysfunction [1, 6, 7]. In this regard, experimental evidence demonstrates that microglia develop a more proinflammatory phenotype with age, a phenomenon described as “microglial priming” [16, 17]. Priming makes the microglia susceptible to a subsequent immune challenge, which can then trigger a prolonged inflammatory response. Our results are consistent with this hypothesis, as we found that the aged (primed) but not the young (naive) microglia produced an excess of proinflammatory cytokines within

the hippocampus following a peripheral immune challenge, inciting a long-lasting cognitive impairment. Prolonged microglial activation is associated with increased expression of inflammatory-related receptors on microglia [16–18]. In particular, TLR-4 was rapidly upregulated after microglial activation, inducing aberrant neuroinflammation and exacerbating neurocognitive function. In this study, we found that during a peripheral immune challenge, treatment with DMED but not MDZ could prevent the upregulation of microglial TLR-4. This process may involve the underlying mechanisms of DMED-induced neurocognitive protection after acute SI.

DMED is widely used as a sedative during the perioperative period and among the critically ill in intensive care units [21, 22]. Several studies have documented that DMED inhibits the peripheral immune response, reducing complications and mortality in both experimental and clinical settings [23–30, 48–50]. Our findings permit a greater understanding of the effects of DMED in the CNS, and indicate that its administration is advantageous for cognitive outcomes in this SI model. Further investigation is warranted to confirm its clinical efficacy.

This study has several limitations that need to be addressed in the future. First, we focused on the hippocampus because of its high microglial density, vulnerability to aging, and critical role in cognition. However, cognitive function is associated with more complex brain networks, and other brain areas may be involved too [55]. Second, we used the novel object recognition task for the cognitive assessment. Unlike other well-established tests such as the radial-arm maze and Morris water maze [56], this task can be conducted without the need for food deprivation or forced swimming and with minimum stress to the animal, which may make it suitable for an aged rat SI model. However, in order to better evaluate cognitive function, a multiple-test regimen may be preferable and should be considered in the future. Third, since previous studies have shown that both TNF- α and IL-1 β may play important roles in impaired hippocampus-dependent cognitive function [19, 20], we chose to measure these two cytokines. However, receptors for other type of cytokines, such as IL-1 α , IL-6, IL-10, and IL-18, are reported to be constitutively expressed in the rodent hippocampus and to be associated with hippocampal function [57, 58]. Therefore, we cannot rule out possible contributions of these cytokines to the pathogenesis of SI-related cognitive dysfunction.

Conclusions

In conclusion, the present study showed that treatment with a sedative dose of DMED during, but not after, peripheral immune activation can prevent SI-induced hippocampal

neuroinflammation, overexpression of TLR-4 in microglia, and cognitive dysfunction. These effects of DMED were mediated by the α_{2A} -AR. Our findings indicate that treatment with DMED can prevent SI-induced cognitive dysfunction.

Acknowledgments This work was supported in part by a Grant-in-Aid for Scientific Research (C): 15K10538 from the Japan Society for the Promotion of Science, Tokyo, Japan.

References

- Cunningham C, Hennessy E. Co-morbidity and systemic inflammation as drivers of cognitive decline: new experimental models adopting a broader paradigm in dementia research. *Alzheimers Res Ther.* 2015;7:33.
- Hovens IB, Schoemaker RG, van der Zee EA, Absalom AR, Heineman E, van Leeuwen BL. Postoperative cognitive dysfunction: involvement of neuroinflammation and neuronal functioning. *Brain Behav Immun.* 2014;38:202–10.
- Kawano T, Eguchi S, Iwata H, Tamura T, Kumagai N, Yokoyama M. Impact of preoperative environmental enrichment on prevention of development of cognitive impairment following abdominal surgery in a rat model. *Anesthesiology.* 2015;123:160–70.
- Murray C, Sanderson DJ, Barkus C, Deacon RM, Rawlins JN, Bannerman DM, Cunningham C. Systemic inflammation induces acute working memory deficits in the primed brain: relevance for delirium. *Neurobiol Aging.* 2012;33(603–16):e3.
- Holmes C, Cunningham C, Zotova E, Woolford J, Dean C, Kerr S, Culliford D, Perry VH. Systemic inflammation and disease progression in Alzheimer disease. *Neurology.* 2009;73:768–74.
- Moller JT, Cluitmans P, Rasmussen LS, Houx P, Rasmussen H, Canet J, Rabbitt P, Jolles J, Larsen K, Hanning CD, Langeron O, Johnson T, Lauen PM, Kristensen PA, Biedler A, van Beem H, Fraidakis O, Silverstein JH, Beneken JE, Gravenstein JS. Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD investigators. *International Study of Post-Operative Cognitive Dysfunction.* *Lancet.* 1998;351:857–61.
- Iwashyna TJ, Ely EW, Smith DM, Langa KM. Long-term cognitive impairment and functional disability among survivors of severe sepsis. *JAMA.* 2010;304:1787–94.
- Ely EW, Shintani A, Truman B, Speroff T, Gordon SM, Harrell FE Jr, Inouye SK, Bernard GR, Dittus RS. Delirium as a predictor of mortality in mechanically ventilated patients in the intensive care unit. *JAMA.* 2004;291:1753–62.
- Thomason JW, Shintani A, Peterson JF, Pun BT, Jackson JC, Ely EW. Intensive care unit delirium is an independent predictor of longer hospital stay: a prospective analysis of 261 non-ventilated patients. *Crit Care.* 2005;9:R375–81.
- Steinmetz J, Christensen KB, Lund T, Lohse N, Rasmussen LS, ISPOCD Group. Long-term consequences of postoperative cognitive dysfunction. *Anesthesiology.* 2009;110:548–55.
- Dilger RN, Johnson RW. Aging, microglial cell priming, and the discordant central inflammatory response to signals from the peripheral immune system. *J Leukoc Biol.* 2008;84:932–9.
- Steinman L. Modulation of postoperative cognitive decline via blockade of inflammatory cytokines outside the brain. *Proc Natl Acad Sci USA.* 2010;107:20595–6.
- Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain Behav Immun.* 2011;25:181–213.
- Hoogland IC, Houbolt C, van Westerloo DJ, van Gool WA, van de Beek D. Systemic inflammation and microglial activation: systematic review of animal experiments. *J Neuroinflammation.* 2015;12:114.
- Kawano T, Morikawa A, Imori S, Waki S, Tamura T, Yamanaka D, Yamazaki F, Yokoyama M. Preventive effects of multisensory rehabilitation on development of cognitive dysfunction following systemic inflammation in aged rats. *J Anesth.* 2014;28:780–4.
- Perry VH, Holmes C. Microglial priming in neurodegenerative disease. *Nat Rev Neurol.* 2014;10:217–24.
- Norden DM, Godbout JP. Review: microglia of the aged brain: primed to be activated and resistant to regulation. *Neuropathol Appl Neurobiol.* 2013;39:19–34.
- Li W, Gao G, Guo Q, Jia D, Wang J, Wang X, He S, Liang Q. Function and phenotype of microglia are determined by Toll-like receptor 2/Toll-like receptor 4 activation sequence. *DNA Cell Biol.* 2009;28:493–9.
- Wilson CJ, Finch CE, Cohen HJ. Cytokines and cognition—the case for a head-to-toe inflammatory paradigm. *J Am Geriatr Soc.* 2002;50:2041–56.
- Lynch MA. Age-related neuroinflammatory changes negatively impact on neuronal function. *Front Aging Neurosci.* 2010;1:6.
- Carollo DS, Nossaman BD, Ramadhyani U. Dexmedetomidine: a review of clinical applications. *Curr Opin Anaesthesiol.* 2008;21:457–61.
- Hoy SM, Keating GM. Dexmedetomidine: a review of its use for sedation in mechanically ventilated patients in an intensive care setting and for procedural sedation. *Drugs.* 2011;71:1481–501.
- Li Y, He R, Chen S, Qu Y. Effect of dexmedetomidine on early postoperative cognitive dysfunction and peri-operative inflammation in elderly patients undergoing laparoscopic cholecystectomy. *Exp Ther Med.* 2015;10:1635–42.
- Guo Y, Sun L, Zhang J, Li Q, Jiang H, Jiang W. Preventive effects of low-dose dexmedetomidine on postoperative cognitive function and recovery quality in elderly oral cancer patients. *Int J Clin Exp Med.* 2015;8:16183–90.
- Liu Y, Ma L, Gao M, Guo W, Ma Y. Dexmedetomidine reduces postoperative delirium after joint replacement in elderly patients with mild cognitive impairment. *Aging Clin Exp Res.* 2016;28:729–36.
- Ding L, Zhang H, Mi W, Wang T, He Y, Zhang X, Ma X, Li H. Effects of dexmedetomidine on anesthesia recovery period and postoperative cognitive function of patients after robot-assisted laparoscopic radical cystectomy. *Int J Clin Exp Med.* 2015;8:11388–95.
- Chen W, Liu B, Zhang F, Xue P, Cui R, Lei W. The effects of dexmedetomidine on post-operative cognitive dysfunction and inflammatory factors in senile patients. *Int J Clin Exp Med.* 2015;8:4601–5.
- Man Y, Guo Z, Cao J, Mi W. Efficacy of perioperative dexmedetomidine in postoperative neurocognitive function: a meta-analysis. *Clin Exp Pharmacol Physiol.* 2015;42:837–42.
- Djaiani G, Silverton N, Fedorko L, Carroll J, Styra R, Rao V, Katznelson R. Dexmedetomidine vs. propofol sedation reduces delirium after cardiac surgery: a randomized controlled trial. *Anesthesiology.* 2016;124:362–8.
- Pasin L, Landoni G, Nardelli P, Belletti A, Di Prima AL, Taddeo D, Isella F, Zangrillo A. Dexmedetomidine reduces the risk of delirium, agitation and confusion in critically ill patients: a meta-analysis of randomized controlled trials. *J Cardiothorac Vasc Anesth.* 2014;28:1459–66.
- Schoeler M, Loetscher PD, Rossaint R, Fahlenkamp AV, Eberhardt G, Rex S, Weis J, Coburn M. Dexmedetomidine is neuroprotective in an in vitro model for traumatic brain injury. *BMC Neurol.* 2012;12:20.

32. Li Y, Zeng M, Chen W, Liu C, Wang F, Han X, Zuo Z, Peng S. Dexmedetomidine reduces isoflurane-induced neuroapoptosis partly by preserving PI3K/Akt pathway in the hippocampus of neonatal rats. *PLoS One*. 2014;9:e93639.
33. Degos V, Charpentier TL, Chhor V, Brissaud O, Lebon S, Schwendimann L, Bednarek N, Passemard S, Mantz J, Gresens P. Neuroprotective effects of dexmedetomidine against glutamate agonist-induced neuronal cell death are related to increased astrocyte brain-derived neurotrophic factor expression. *Anesthesiology*. 2013;118:1123–32.
34. Sanders RD, Xu J, Shu Y, Januszewski A, Halder S, Fidalgo A, Sun P, Hossain M, Ma D, Maze M. Dexmedetomidine attenuates isoflurane-induced neurocognitive impairment in neonatal rats. *Anesthesiology*. 2009;110:1077–85.
35. Memiş D, Hekimoğlu S, Vatan I, Yandım T, Yüksel M, Süt N. Effects of midazolam and dexmedetomidine on inflammatory responses and gastric intramucosal pH to sepsis, in critically ill patients. *Br J Anaesth*. 2007;98:550–2.
36. Chi H, Kawano T, Tamura T, Iwata H, Takahashi Y, Eguchi S, Yamazaki F, Kumagai N, Yokoyama M. Postoperative pain impairs subsequent performance on a spatial memory task via effects on *N*-methyl-D-aspartate receptor in aged rats. *Life Sci*. 2013;93:986–93.
37. Chi X, Wei X, Gao W, Guan J, Yu X, Wang Y, Li X, Cai J. Dexmedetomidine ameliorates acute lung injury following orthotopic autologous liver transplantation in rats probably by inhibiting Toll-like receptor 4-nuclear factor kappa B signaling. *J Transl Med*. 2015;13:190.
38. Lee VC, Moscicki JC, DiFazio CA. Propofol sedation produces dose-dependent suppression of lidocaine-induced seizures in rats. *Anesth Analg*. 1998;86:652–7.
39. Wang P, You SW, Yang YJ, Wei XY, Wang YZ, Wang X, Hao DJ, Kuang F, Shang LX. Systemic injection of low-dose lipopolysaccharide fails to break down the blood–brain barrier or activate the TLR4-MyD88 pathway in neonatal rat brain. *Int J Mol Sci*. 2014;15:10101–15.
40. Sinclair MD. A review of the physiological effects of alpha2-agonists related to the clinical use of medetomidine in small animal practice. *Can Vet J*. 2003;44:885–97.
41. Calzada BC, de Artiñano AA. Alpha-adrenoceptor subtypes. *Pharmacol Res*. 2001;44:195–208.
42. Gyoneva S, Traynelis SF. Norepinephrine modulates the motility of resting and activated microglia via different adrenergic receptors. *J Biol Chem*. 2013;288:15291–302.
43. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*. 2008;9:46–56.
44. Mattson MP, Magnus T. Ageing and neuronal vulnerability. *Nat Rev Neurosci*. 2006;7:278–94.
45. Jurgens HA, Johnson RW. Dysregulated neuronal–microglial cross-talk during aging, stress and inflammation. *Exp Neurol*. 2012;233:40–8.
46. Witlox J, Eurelings LS, de Jonghe JF, Kalisvaart KJ, Eikelenboom P, van Gool WA. Delirium in elderly patients and the risk of postdischarge mortality, institutionalization, and dementia: a meta-analysis. *JAMA*. 2010;304:443–51.
47. Davis DH, Muniz Terrera G, Keage H, Rahkonen T, Oinas M, Matthews FE, Cunningham C, Polvikoski T, Sulkava R, MacLulich AM, Brayne C. Delirium is a strong risk factor for dementia in the oldest-old: a population-based cohort study. *Brain*. 2012;135:2809–16.
48. Taniguchi T, Kurita A, Kobayashi K, Yamamoto K, Inaba H. Dose- and time-related effects of dexmedetomidine on mortality and inflammatory responses to endotoxin-induced shock in rats. *J Anesth*. 2008;22:221–8.
49. Taniguchi T, Kidani Y, Kanakura H, Takemoto Y, Yamamoto K. Effects of dexmedetomidine on mortality rate and inflammatory responses to endotoxin-induced shock in rats. *Crit Care Med*. 2004;32:1322–6.
50. Zhang J, Wang Z, Wang Y, Zhou G, Li H. The effect of dexmedetomidine on inflammatory response of septic rats. *BMC Anesthesiol*. 2015;15:68.
51. Okello A, Edison P, Archer HA, Turkheimer FE, Kennedy J, Bullock R, Walker Z, Kennedy A, Fox N, Rossor M, Brooks DJ. Microglial activation and amyloid deposition in mild cognitive impairment: a PET study. *Neurology*. 2009;72:56–62.
52. Ebert TJ, Hall JE, Barney JA, Uhrich TD, Colino MD. The effects of increasing plasma concentrations of dexmedetomidine in humans. *Anesthesiology*. 2000;93:382–94.
53. Bol CJ, Vogelaar JP, Mandema JW. Anesthetic profile of dexmedetomidine identified by stimulus-response and continuous measurements in rats. *J Pharmacol Exp Ther*. 1999;291:153–60.
54. Bjelland TW, Klepstad P, Haugen BO, Nilsen T, Dale O. Effects of hypothermia on the disposition of morphine, midazolam, fentanyl, and propofol in intensive care unit patients. *Drug Metab Dispos*. 2013;41:214–23.
55. Bressler SL, Menon V. Large-scale brain networks in cognition: emerging methods and principles. *Trends Cogn Sci*. 2010;14:277–90.
56. Vorhees CV, Williams MT. Assessing spatial learning and memory in rodents. *ILAR J*. 2014;55:310–32.
57. Wang WY, Tan MS, Yu JT, Tan L. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. *Ann Transl Med*. 2015;3:136.
58. Vasconcelos AR, Yshii LM, Viel TA, Buck HS, Mattson MP, Scavone C, Kawamoto EM. Intermittent fasting attenuates lipopolysaccharide-induced neuroinflammation and memory impairment. *J Neuroinflammation*. 2014;11:85.