Am J Transl Res 2023;15(4):2634-2644
www.ajtr.org /ISSN:1943-8141/AJTR0148134

Original Article

Effect of dexmedetomidine on postoperative cognitive dysfunction and the T helper 17/regulatory T cell balance in geriatric patients undergoing orthopedic surgery: a randomized controlled study

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Received November 30, 2022; Accepted February 22, 2023; Epub April 15, 2023; Published April 30, 2023

Abstract: Objectives: To investigate the effect of dexmedetomidine (DEX) on alleviating postoperative cognitive dysfunction (POCD) and T helper 17 (Th17)/regulatory T cell (Treg) imbalance in geriatric patients undergoing orthopedic surgery. Methods: A total of 82 geriatric patients undergoing lower extremity joint replacement surgery were enrolled and randomized into two groups. Patients in the experimental group received a loading dose of 0.5 μg/kg DEX for 10 min and then a maintenance dose of 0.5 μg/kg/h DEX until 30 min before the end of the surgery, while patients in the control group were administered with an equal volume of saline. The mini-mental state examination (MMSE) was used to evaluate the patients' cognitive function levels. The enzyme-linked immunosorbent assay (ELISA) was used to detect the protein levels of S100 calcium-binding protein B (S-100β), matrix metalloproteinase 9 (MMP9), interleukin-10 (IL-10) and interleukin-17A (IL-17A). The quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect and compare the mRNA levels of retinoic acid-related orphan receptor gamma-t (RORγt) and forkhead box P3 (Foxp3), the ratio of which reflected Th17/Treg balance. Results: Compared to the control group, the MMSE scores in the DEX group were obviously higher at 24 and 72 h after the surgery, and the incidence of POCD was lower in the DEX group. Simultaneously, DEX significantly decreased the level of S100β, MMP9, and the ratio of RORγt/Foxp3 mRNA at the end of the surgery and one day after surgery. Furthermore, IL-10 was upregulated, while the IL-17A and the IL-17A/IL-10 ratio were downregulated at the end of the surgery and one day after surgery in the DEX group. Conclusions: DEX could reduce the incidence of POCD in elderly orthopedic patients, which might be related to the attenuation of inflammatory response and blood-brain barrier (BBB) damage through modulating the Th17/Treg imbalance.

Keywords: Dexmedetomidine, T helper 17 cell, regulatory T cell, postoperative cognitive dysfunction, matrix metalloproteinase 9, randomized controlled study

Introduction

Postoperative cognitive dysfunction (POCD) is defined as the patient's cognitive function decline following surgery and anesthesia, which mainly manifests as inattention, memory decline, sleep disorders, and decreased language or learning abilities [1]. Long duration of anesthesia and severe surgical trauma will significantly increase the risk of POCD, especially in elderly patients [2]. In addition, the incidence of POCD depends on the type of surgery, for example, patients undergoing orthopedic surgery are vulnerable to developing POCD with an incidence of 41 to 75% at 7 days and 18 to 45% at 3 months after surgery [3]. POCD causes additional healthcare expenditure, prolonged hospitalization, and increased mortality in patients after orthopedic surgery [1]. Therefore, it is critical for anesthetists to determine proper perioperative prevention measures to reduce the incidence of POCD in elderly orthopedic patients.

Dexmedetomidine (DEX), an α2 adrenergic receptor agonist, has various therapeutic effects, including sedation, hypnosis and analgesia effects with low respiratory depression;

therefore, there are increasing studies on the clinical applications of DEX, especially in neuroprotection [4]. A previous study has shown that DEX is effective in treating sevoflurane-induced neurocognitive impairment in rats by decreasing neuroapoptosis, neuroinflammation and oxidative stress [5]. In addition, several studies have revealed that DEX could reduce the occurrence of POCD by alleviating oxidative stress, inflammation, microglia activation, extracellular amyloid β-protein (Aβ) accumulation, and mitochondrial dysfunction [6-8], however, the precise mechanisms underlying the functions of DEX, particularly the neuroprotective effects of DEX, remain to be fully elucidated.

Recently, there has been increasing attention on the relationship between deregulated immune response and POCD [9]. T helper (Th) cells, an essential component of the adaptive immune system, can exacerbate or alleviate brain damage depending on the infiltrating immune cell subpopulations. Pro-inflammatory subpopulations, including Th1 and Th17, are the main sources of pro-inflammatory cytokines that lead to endothelial cell damage, whereas anti-inflammatory subpopulations, mainly including Th2 and regulatory T (Treg) cells, attenuate the inflammatory response and modulate the function of Th1 and Th17 cells [10, 11]. The role of peripheral Th cells in the process of cognitive impairment has been demonstrated in multiple neurodegenerative diseases [12]. In line with this notion, a previous study demonstrated that vitamin D could improve POCD by regulating the balance of Th17/Treg cell [13]. Another study reported that α7 nicotinic acetylcholine receptor (α7nAchR) could improve POCD by regulating the Th17 response [14]. Together, these results demonstrate that the Th17/Treg imbalance plays an essential role in the development of POCD. In addition, Treg cell might play a major role in alleviating ischemia stroke-induced blood-brain barrier (BBB) damage [15]. It has been reported that matrix metalloproteinases (MMPs) cause BBB damage and are involved in the pathological process of many diseases, such as Alzheimer's disease (AD) and cerebral ischemia-reperfusion injury [16, 17], and the elevated MMP-9 levels might lead to the occurrence of POCD in patients after surgery [18].

Nevertheless, there are no reports on whether DEX could hamper the disruption of BBB integrity by Th cells to reduce the incidence of POCD in clinical studies. In this study, we hypothesized that DEX was involved in the neuroprotection against POCD by regulating the Th17/Treg imbalance to alleviate subsequent BBB impairment and inflammatory response in elderly patients undergoing orthopedic surgery.

Materials and methods

Clinical data

Eighty-two patients scheduled to undergo lower extremity joint replacement surgery were enrolled in this study. This study was written according to the CONSORT 2010 checklist (Supplementary File 1). This trial was approved by the Ethics Committee of the Hebei General Hospital, China (ethics approval No. 2019-48) (Supplementary File 2) and it was registered at clinicaltrials.gov (No. ChiCTR2200055802). All the patients signed the informed consent.

Inclusion and exclusion criteria

The inclusion criteria were: (1) elderly patients aged ≥ 60 years; (2) with ASA scale I-III; (3) have scheduled for lower extremity joint replacement surgery.

The exclusion criteria were: (1) patients who declined to be included in this study; (2) with a history of spinal trauma; (3) have participated in other clinical trials within one month; (4) with the Mini-Mental State Examination (MMSE) scores < 21; (5) patients who were taking tranquillisers or antidepressants; (6) with severe hearing and vision impairment; (7) with contraindications to anesthesia; (8) withdrawal from the surgery.

Anesthesia procedure

All patients who underwent routine preoperative preparations were given combined spinal-epidural anesthesia (CSEA) and received 2 L/min oxygen through a nasal cannula during the surgery to prevent hypoxia. After being connected to monitors, the patients were turned to the lateral position, and 1% lidocaine was subcutaneously administered followed by 2-2.5 ml of 0.5% bupivacaine injection into the subarachnoid space, through a puncture site between lumbar vertebrae 2 and 3. An epidural catheter was placed. The sensory block level was controlled below T8 and the Ramsay Sedation Scale score was 2-3 during the surgery. In order to reduce perioperative bleeding, we used blood conservation strategies, including a tour-

niquet inflated to 300 mmHg for a maximum of 90 min and 10-15 mg/kg tranexamic acid for IV use. At the end of the surgery, the epidural catheter was removed and the patients were transferred to the general ward.

DEX treatment procedure

Patients were randomized into two groups at a 1:1 ratio. Randomization was conducted based on the Microsoft Excel random number generator by an investigator not involved in participant registration and data collection. And the allocation sequences were packed in sealed envelopes with identical shapes and sizes and were not disclosed to ensure concealment until the completion of this study. Based on the allocation sequence, a research assistant prepared the study agents. The study agents (dexmedetomidine 200 μg/2 mL or normal saline 2 mL) were diluted into 50 mL with normal saline. The syringes were given a number and marked as "study medicine" for double-blinding.

The patients, responsible anesthesiologists, and outcome assessors were blinded to the allocation until the completion of the study. The anesthesiologist was not involved in postoperative assessments and the postoperative assessors were not involved in the intraoperative management.

The experimental (DEX) group: patients received a loading dose of 0.5 μg/kg DEX for 10 min and then a maintenance dose of 0.5 μg/kg/h DEX until 30 minutes before the end of surgery.

The control group: patients received the same treatment procedure except that saline instead of DEX was given.

In case that the patient's blood pressure drops more than 20% of the baseline, 6 mg ephedrine will be given intravenously each time, and if bradycardia occurs (25% reduction from baseline), 0.5 mg atropine will be injected. For patients with SpO_2 < 90%, ventilatory support will be provided such as jaw thrust. Patients with persistent hypoxia, failure of spinal anesthesia, or serious complications were excluded from this study.

Measurements

We examined and collected test results from each patient at five-time points: (1) T0: before the surgery; (2) T1: 30 minutes into the surgery; (3) T2: at the end of the surgery; (4) T3: one day post-surgery; and (5) T4: three days post-surgery.

MMSE scores

The MMSE scores included delayed memory, immediate memory, time-oriented ability, position-oriented capacity, language, attention, visuospatial, and computational capacity, which were performed at T0, T3, and T4. The total MMSE scores were 30, and higher scores were associated with better cognitive function. The main outcome was the incidence of POCD, and a 2 points reduction in MMSE scores after surgery was considered as POCD [19].

The enzyme-linked immunosorbent assay (ELISA)

We collected the patient's blood samples at T0, T2, and T3. The serum concentrations of matrix metalloproteinase 9 (MMP9, ELH-MMP9-1, RayBio, China), S100 calcium-binding protein B (S-100β, EH0543, FineTest, China), interleukin-17A (IL-17A, EH3267, FineTest, China) and interleukin-10 (IL-10, EH0173, FineTest, China) at T0, T2, and T3 were detected using ELISA kits according to the manufacturers' instructions and calculated using the standard curve provided with the kits.

The quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from the peripheral blood samples of the patients was extracted with Trizol total RNA extraction reagent (MF034-01, Mei5 Biotechnology, China), quantified by Nanodrop Spectrophotometer (Nanodrop 2000, Thermo Scientific) and reverse transcribed into cDNA using PrimeScript RT reagent kit (RR047A, TaKaRa, China). Based on the manufacturer's recommendations, RT-qPCR thermal cycling was performed, and the relative transcript levels of the forkhead box P3 (Foxp3) and the retinoic acid receptor-related orphan receptor gamma t (RORγt) were quantified using the $2^{-\Delta\Delta Ct}$ method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal control. The primer sequences were listed in **Table 1**.

Statistical analysis

The PASS 15.0 software was utilized to determine the sample size required for this study.

Table 1. Primer sequences used for the qRT-PCR

Gene	Sequence (5'-3')	PCR product (bp)
Foxp3-F	GCCGAGATCTTCGAGGCGG	100
Foxp3-R	GCCACCATGACTAGGGGCAG	
RORγt-F	AGTCGGAAGGCAAGATCAGA	100
RORγt-R	CAAGAGAGGTTCTGGGCAAG	
GAPDH-F	GACCTGACCTGCCGTCTA	83
GAPDH-R	AGGAGTGGGTGTCGCTGT	

qRT-PCR: real-time polymerase chain reaction; F: forward; R: reverse; Foxp3: forkhead box P3; RORγt: retinoicacid-related orphan receptor gamma-t; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

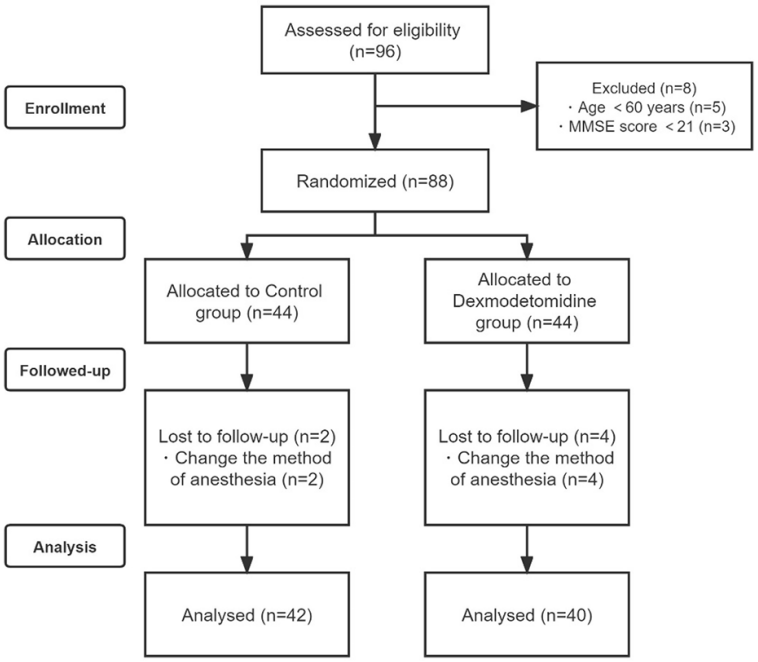

Figure 1. Patient enrollment flow chart.

Based on the previous study [3], we hypothesized that the incidence of POCD was 45%. And in our pilot study, we found that the incidence of POCD in the DEX group was 20% (3 of the 15 patients were diagnosed with POCD). Hence, 82 patients could ensure a power of 0.80 to detect the differences at a significance level of 0.05.

Considering an estimated drop rate of 20%, we planned to include 103 patients. Finally, we included 96 patients in this study and the actual drop rate was 15% (the number of drop out was 14 patients).

SPSS 27.0 (SPSS Inc.) was used for statistical analysis. Mean ± standard deviation (SD) or median (interquartile range, IQR) was used to represent continuous data, while parametric or non-parametric t-test was used for comparison. Normal distribution data were analyzed using the Shapiro-Wilk test, whereas the repeated measures ANOVA followed by the post hoc Bonferroni test was applied to analyze the multi-time points data. Chi-square (or Fisher's exact) test was used to analyze categorical variables. Two-sided $P < 0.05$ was considered statistically significant.

Results

Demographic and intraoperative characteristics

96 patients were evaluated for eligibility, and 82 participants completed the trial ultimately (**Figure 1**). The patients' baseline characteristics indicated that these two groups were balanced in demographic characteristics (**Table 2**). As for intraoperative characteristics, the length of hospital stays was shorter in the DEX group than that in the control group ($P = 0.002$), but other indicators were similar between the two groups (**Table 3**).

Perioperative hemodynamic changes

As shown in **Table 4**, compared to T0, there was a significant decrease in heart rate (HR) and mean arterial pressure (MAP) in both the DEX and control groups at T1 ($P < 0.001$) and T2 ($P < 0.001$), however the reduction in HR and MAP at the T1 was more significant in the DEX group than that in the control group (HR, $P = 0.001$; MAP, $P = 0.003$).

The effects of DEX on POCD

The MMSE scores were used to evaluate patients' cognitive levels at T0, T3, and T4. As shown in **Figure 2**, there was no difference in the preoperative MMSE scores between the two groups ($P = 0.946$). Nevertheless, when

Table 2. Baseline patient characteristics

Characteristics	Control group	Dexmedetomidine group	*P* value
Total patients	42	40	
Age (years)	69.71±7.25	70.93±7.23	0.451
BMI (kg/m^2)	24.72±4.72	24.14±3.55	0.537
Sex, n (%)			0.582
Male	15 (35.71)	12 (30.00)	
Female	27 (64.29)	28 (70.00)	
ASA classification, n (%)			0.126
II	27 (64.29)	19 (47.50)	
III	15 (35.71)	21 (52.50)	
Educational level, n (%)			0.445
Primary	2 (4.76)	5 (12.50)	
Junior high school	21 (50.00)	18 (45.00)	
Senior high school	17 (40.48)	13 (32.50)	
College degree	2 (4.76)	4 (10.00)	
Smoker, n (%)	17 (40.48)	21 (52.50)	0.275
Comorbidities, n (%)			
Hypertension	19 (45.24)	20 (50.00)	0.666
CHD	5 (11.90)	1 (2.50)	0.102
Diabetes	3 (7.14)	8 (20.00)	0.088
Cerebral infarction	3 (7.14)	6 (15.00)	0.255
Baseline laboratory			
Leukocyte (10^9/L)	6.85 (5.53, 8.17)	6.40 (4.79, 8.82)	0.784
Platelet (10^9/L)	238.33±64.34	231.68±58.33	0.626
Hemoglobin (g/L)	127.52±13.58	124.75±13.78	0.361
Creatinine (μmol/L)	58.90 (53.85, 68.75)	62.20 (53.00, 73.50)	0.525
Albumin (g/L)	39.15 (34.73, 41.60)	37.80 (34.83, 40.48)	0.507
Blood glucose (mmol/L)	5.28 (4.89, 6.00)	5.66 (4.88, 6.69)	0.349
D-dimer (mg/L)	1.38 (0.51, 3.43)	2.18 (0.67, 8.05)	0.113

The categorical variables are expressed as n (%). Normally distributed data are given as mean ± SD, whereas non-normally distributed data are expressed as median (25th percentile, 75th percentile). ASA: American Society of Anesthesiologists; BMI: body mass index; CHD: coronary heart disease.

comparing the MMSE score at different testing times, we observed that the MMSE scores were lower at T3 and T4 than that at T0 in the control group ($P < 0.001$), but the MMSE scores at T3 and T4 were improved in the DEX group ($P < 0.001$). Meanwhile, the incidence of POCD was lower in the DEX group than that in the control group (17.50% vs 38.10%, $P = 0.038$).

The effects of DEX on neuronal damage biomarker

We examined the S100β level to explore the beneficial effect of DEX on the central nervous system. As shown in **Figure 3**, there was no difference in the level of S-100β protein between the two groups at T0 ($P = 0.736$). However, the S100β protein level was upregulated at T2 and T3 compared to that at T0 in the control group ($P < 0.001$), while DEX significantly downregulated the level of S100β protein at T2 ($P < 0.001$) and T3 ($P = 0.020$).

The effects of DEX on the inflammatory response

We explored the modulatory effects of DEX on the inflammation and found that the serum IL-17A and IL-10 levels as well as the IL-17A/IL-10 ratio were upregulated in the two groups at T2 ($P < 0.001$) and T3 (control group, IL-17A, $P < 0.001$; IL-10, $P < 0.001$; IL-17A/IL-10 ratio, $P < 0.001$; DEX group, IL-17A, $P < 0.001$; IL-10, $P < 0.001$; IL-17A/IL-10 ratio, $P = 0.004$, **Figure**

Table 3. Perioperative characteristics

Variables	Control group	Dexmedetomidine group	*P* value
Type of surgery, n (%)			0.462
Unilateral hip replacement	23 (54.76)	22 (55.00)	
Unilateral knee replacement	16 (38.10)	12 (30.00)	
Bilateral knee replacement	3 (7.14)	6 (15.00)	
Duration of anaesthesia (min)	230.38±60.97	244.63±82.65	0.376
Duration of surgery, min	143.07±57.16	148.65±65.10	0.681
Bleeding (ml)	40.00 (20.00, 70.00)	50.00 (30.00, 100.00)	0.146
Urine output, median (ml)	150.00 (87.50, 200.00)	200.00 (100.00, 300.00)	0.125
Intraoperative hypotension, n (%)	6 (14.29)	8 (20.00)	0.492
Intraoperative bradycardia, n (%)	3 (7.14)	7 (17.50)	0.152
Resting VAS at 24 h after surgery	3.00 (2.00, 4.00)	3.00 (2.00, 3.75)	0.303
Complications, n (%)			
Postoperative shivering	7 (16.67)	6 (15.00)	0.836
PONV	5 (11.90)	3 (7.50)	0.502
Length of stay (days)	10.43±1.50	9.26±1.82	0.002*

The categorical variables are expressed as n (%). Normally distributed data are given as mean ± SD, whereas non-normal ly distributed data are expressed as median (25th percentile, 75th percentile). PONV: postoperative nausea and vomiting; VAS: visual analogue scale. Intraoperative hypotension defined as systolic BP < 30% of baseline, intraoperative bradycardia defined as HR < 25% of baseline. *$P < 0.05$ indicates a significant difference.

Table 4. Perioperative MAP and HR

Variables	Control group	Dexmedetomidine group	*P* value
MAP (mmHg)			
T0	104.29±14.47	107.15±15.86	0.395
T1	94.12±11.81	85.65±13.29	0.003*
T2	93.38±9.85	89.23±12.16	0.092
HR (beat/minute)			
T0	86.86±12.53	86.98±9.05	0.961
T1	80.24±10.56	73.20±8.48	0.001*
T2	79.52±9.83	77.05±7.29	0.198

Normally distributed data are given as mean ± SD. MAP: mean arterial pressure; HR: heart rate. *$P < 0.05$ indicates a significant difference.

4). Compared with the control group, the intraoperative use of DEX significantly increased the serum IL-10 level at T2 and T3 ($P < 0.001$, **Figure 4A**) but decreased the serum IL-17A level at T2 ($P = 0.003$, **Figure 4B**), as a result, the IL-17A/IL-10 ratio was decreased at T2 and T3 ($P < 0.001$, **Figure 4C**).

The effects of DEX on BBB permeability

With increasing permeability of the BBB, inflammation results in the development of POCD [20]. Because of the disruptive effect of MMP9 on the BBB, we measured the serum MMP9 level to determine the influence of DEX on BBB disruption. As expected, the level of serum MMP9 was significantly increased in the two groups at T2 and T3 ($P < 0.001$, **Figure 5**), and this effect was markedly reduced by DEX ($P < 0.001$, **Figure 5**).

The effects of DEX on Th17/Treg balance

The immune cells play a prominent role in the inflammatory response and BBB integrity [9]. In order to investigate how DEX mitigated inflammatory response and BBB disruption, we further explored the effects of DEX on immune cells. In particular, we investigated the expression levels of RORγt and Foxp3, which are critical transcription factors involved in the immune response of Th17 and Treg cells. In the control group, we found that the Foxp3 mRNA level was significantly decreased at T2 ($P < 0.001$) and T3 ($P < 0.001$, **Figure 6A**), whereas the level of RORγt mRNA was significantly increased at T2 ($P < 0.001$) and T3 ($P < 0.001$, **Figure 6B**). Importantly, DEX significantly elevated the level of Foxp3 mRNA at the T2 ($P < 0.001$, **Figure 6A**). When we compared the ratio of RORγt/Foxp3 mRNA, we found that DEX significantly downregulated this ratio, indicating that DEX modulated the Th17/Treg balance ($P < 0.001$, **Figure 6C**).

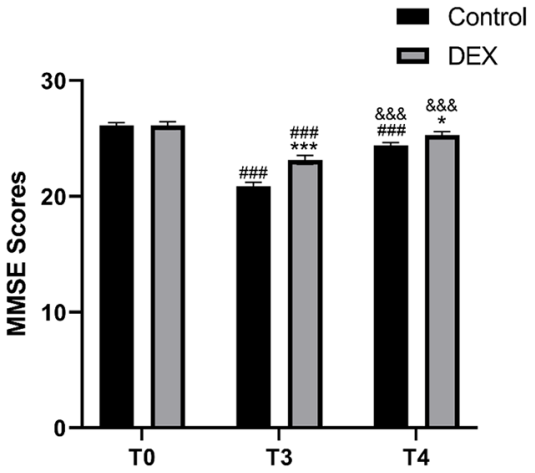

Figure 2. Comparison of the MMSE scores between the two groups. Data are presented as mean ± standard deviation (SD). MMSE: mini-mental state examination. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ versus T0, $^{\&}P < 0.05$; $^{\&\&}P < 0.01$; $^{\&\&\&}P < 0.001$ versus T3, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus control group. T0: before the surgery; T3: 24 hours after the surgery and T4: 72 hours after the surgery.

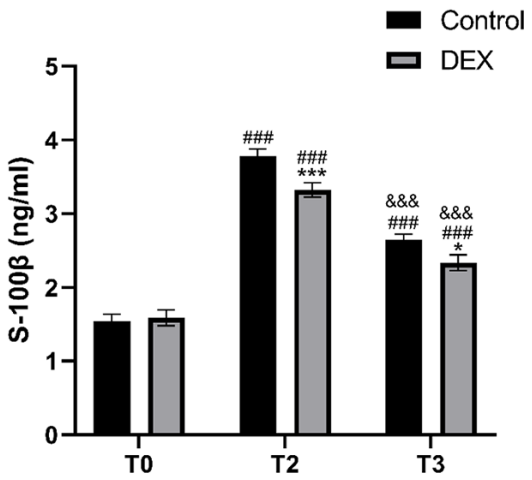

Figure 3. Comparison of the S-100β between the two groups. Data are presented as mean ± standard deviation (SD). S-100β: S100 calcium-binding protein B. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ versus T0, $^{\&}P < 0.05$; $^{\&\&}P < 0.01$; $^{\&\&\&}P < 0.001$ versus T2, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus control group. T0: before the surgery; T2: at the end of the surgery; T3: 24 hours after the surgery.

Discussion

In this study, we discovered that DEX significantly improved the MMSE scores in elderly orthopedic patients at 24 and 72 hours after surgery and reduced the incidence of POCD. Our results also suggested a potential mechanism underlying the effect of DEX on POCD which might involve the attenuation of inflammatory response and BBB disruption through modulating the Th17/Treg imbalance.

POCD, as a common postoperative complication, refers to the decline in nervous system function, which might eventually lead to permanent brain damage [21]. With the increasing trend of the aging population, more geriatric patients are undergoing surgery. It is well known that geriatric patients are more vulnerable to developing POCD, which severely impairs their quality of life and leads to a heavy burden on families. In our study, the incidence of POCD in the elderly undergoing lower extremity joint arthroplasty was 38.10%, which is similar to the finding from another clinical study in which the incidence of POCD was 40.00% in geriatric patients undergoing total hip arthroplasty [22]. Although the perioperative mortality was greatly reduced by the improvement in medical technology, the prevalence of POCD was not markedly improved.

Multifactor synergy contributes to the occurrence of POCD, undoubtedly, anesthetic factors play a significant role in the occurrence of POCD [2]. Surgical and anesthetic trauma cause the systemic inflammatory response, which leads to neuronal damage and even neuronal death [23]. An animal study found that sevoflurane led to cognitive impairment by reducing recombinant glucose transporter 3 (GLUT3) expression in mice [24]. Additionally, general anesthetics can suppress the transmission of cholinergic neurons in patients, which is essential for cognitive function [25]. Thus, it is of great importance to select appropriate anesthetic drugs and explore the potential mechanisms to reduce the occurrence of POCD in geriatric patients. In this study, we found that perioperative application of DEX could reduce the incidence of POCD, which finding was similar to the result of previous studies [26, 27].

We also investigated the potential mechanisms involved in DEX activity. The dysregulated inflammatory response has been reported to contribute to the occurrence of POCD [23, 26]. In our study, we observed that the serum IL-17A and IL-10 levels were increased at the end of the surgery and 24 h post-surgery. Although the serum IL-10 level was increased, the serum IL-17A level and the IL-17A/IL-10 ratio were significantly decreased after DEX treatment. As

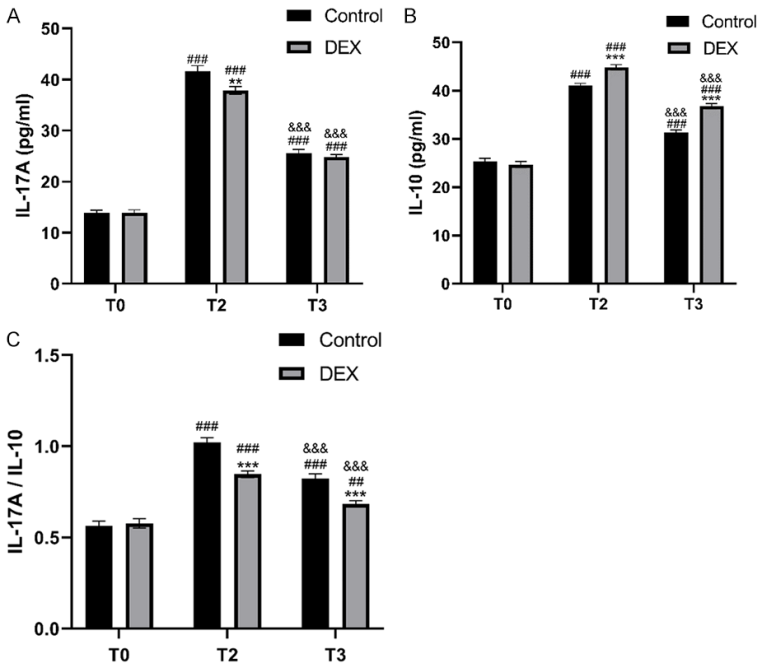

Figure 4. Comparison of the level of IL-17A (A), IL-10 (B) and IL-17A/IL-10 (C) between the two groups. Data are presented as mean ± standard deviation (SD). IL-17A: interleukin-17A; IL-10: interleukin-10. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ versus T0, $^{\&}P < 0.05$; $^{\&\&}P < 0.01$; $^{\&\&\&}P < 0.001$ versus T2, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus control group. T0: before the surgery; T2: at the end of the surgery; T3: 24 hours after the surgery.

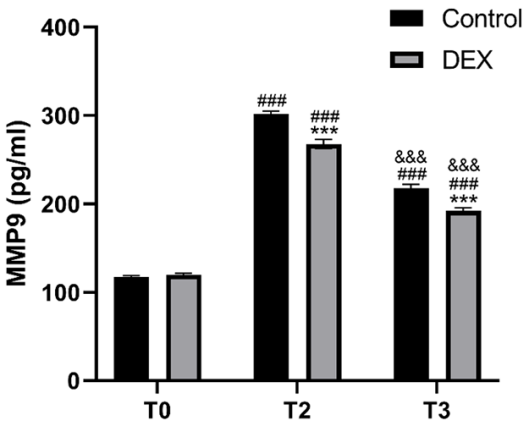

Figure 5. Comparison of the MMP9 between the two groups. Data are presented as mean ± standard deviation (SD). MMP9: matrix metalloproteinase 9. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ versus T0, $^{\&}P < 0.05$; $^{\&\&}P < 0.01$; $^{\&\&\&}P < 0.001$ versus T2, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus control group. T0: before the surgery; T2: at the end of the surgery; T3: 24 hours after the surgery.

we know, IL-17A is mainly secreted by Th17 cells and is associated with many neurodegenerative diseases [28]. On the other hand, IL-10 is an anti-inflammatory cytokine and may be a potential therapy for AD [29]. Therefore, the inflammatory response might exacerbate cognitive impairment, and DEX might be a potential therapeutic agent for POCD by modulating the inflammatory response [20].

Furthermore, the damage of BBB by inflammatory response has been regarded as an essential process for the occurrence of POCD [30]. After the breakdown of the BBB, numerous inflammatory factors and immune cells enter the brain, thus forming a vicious circle that causes long-term brain damage and the disruption of neurological function [20]. The major components of the BBB include astrocytes, pericytes, endothelial cells, and the extracellular matrix (ECM), which offers functional and structural support for the BBB. ECM, one of the important components of BBB, is degraded by MMPs. Hence, MMP-9 plays a pivotal role in the development of neurodegenerative diseases like AD [31]. Significantly, in our study, we observed that DEX markedly suppressed the activity of MMP-9, which was similar to result of the previous study [32].

Notably, we discovered that the immunomodulatory effects of DEX might be related to its neuroprotective effects in this paper. The dysregulated immune responses, which are closely associated with inflammatory response and the disruption of the BBB, are regarded as the main cause of the development of cognitive dysfunction [9, 33]. Previous studies have shown that DEX has immunomodulatory effects in both patients and healthy people [34, 35]. Treg cell can protect BBB integrity by maintaining immune tolerance, counteracting over-activated immune responses, and regulating cerebral endothelial function. Importantly, Treg cell has been shown to play a neuroprotective role in stroke [36], which is related to the secretion of transforming growth factor β (TGF-β), interleukin-10 (IL-10) and interleukin-35 (IL-35) [37].

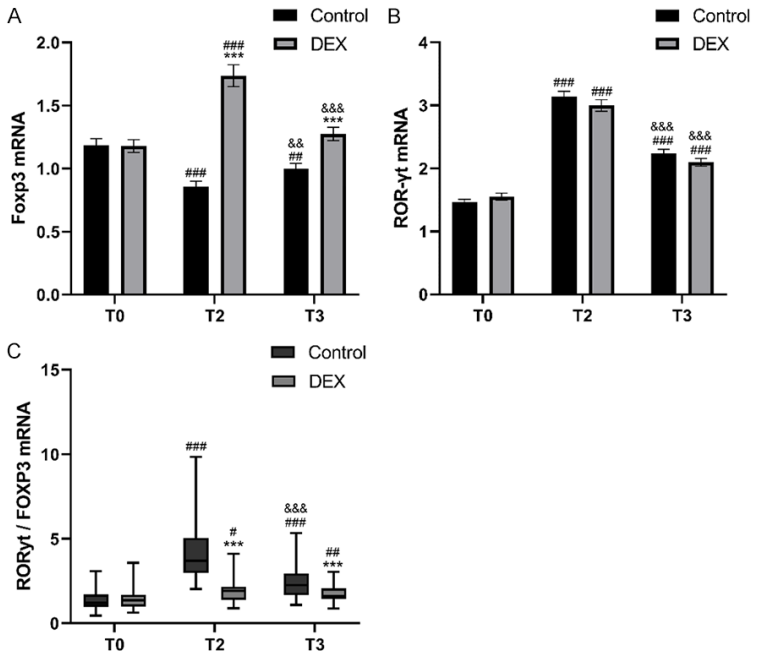

Figure 6. Comparison of the Foxp3 mRNA expression (A), RORγt mRNA expression (B) and Foxp3/RORγt mRNA (C) between the two groups. (A and B) Data are presented as mean ± standard deviation (SD), (C) Data are reported as median (interquartile range). Foxp3: Forkhead box P3; RORγt: Retinoicacid-related orphan receptor gamma-t. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ versus T0, $^{\&}P < 0.05$; $^{\&\&}P < 0.01$; $^{\&\&\&}P < 0.001$ versus T2, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus control group. T0: before the surgery; T2: at the end of the surgery; T3: 24 hours after the surgery.

However, the neuroprotective effect of Treg cell could be diminished by Th17 cell. In our study, we found that DEX alleviated the Th17/Treg imbalance by upregulating the transcription factor level of Treg while downregulating the transcription factor level of Th17. In accordance with our findings, a previous study showed that DEX alleviated cognitive impairment by modulating Th1/Th2/Th17 polarization and reducing the disruption of BBB in a mouse model of sepsis [38]. It should be noted that one study reported that DEX shifted the Th17/Treg cytokine balance toward Th17 after surgery and anesthetic stress [39]. The reasons for the opposite results might be related to the study population and the small sample size. Although findings from this study suggested that DEX played an important role in modulating immune responses to improve POCD, further investigation is needed to clarify the precise role and mechanism of DEX in the immune response.

This study had several limitations. First, this study was a single-center clinical trial with a relatively small sample size, which might bias the conclusions. Second, since the cognitive function of patients was only observed within three days after surgery, the long-term influence of DEX on POCD of geriatric orthopedic patients remains to be further confirmed. Third, we did not use flow cytometry to measure the intracellular cytokine levels to accurately investigate the Th17/Treg balance.

Conclusion

DEX could decrease the occurrence of POCD in elderly orthopedic patients, which might be related to the attenuation of inflammatory response and BBB disruption through modulating the Th17/Treg imbalance. Thus, our study provided evidence of the efficacy and safety of DEX on POCD in geriatric orthopedic patients.

Acknowledgements

The work was supported by the Key Research and Development Program of Hebei Province (Grant No. 19277714D).

Disclosure of conflict of interest

None.

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Supplementary File 1. CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	3
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	5-6
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	8-9
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	Figure 1
Randomisation			
Sequence generation	8a	Method used to generate the random allocation sequence	6
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	6
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	6
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6
	11b	If relevant, description of the similarity of interventions	8-9
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	9
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	9
	14b	Why the trial ended or was stopped	6
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	8
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Figure 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	8-10
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	8-10

Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	8-10
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	15
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12-15
Other information			
Registration	23	Registration number and name of trial registry	3
Protocol	24	Where the full trial protocol can be accessed, if available	3
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	NA

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

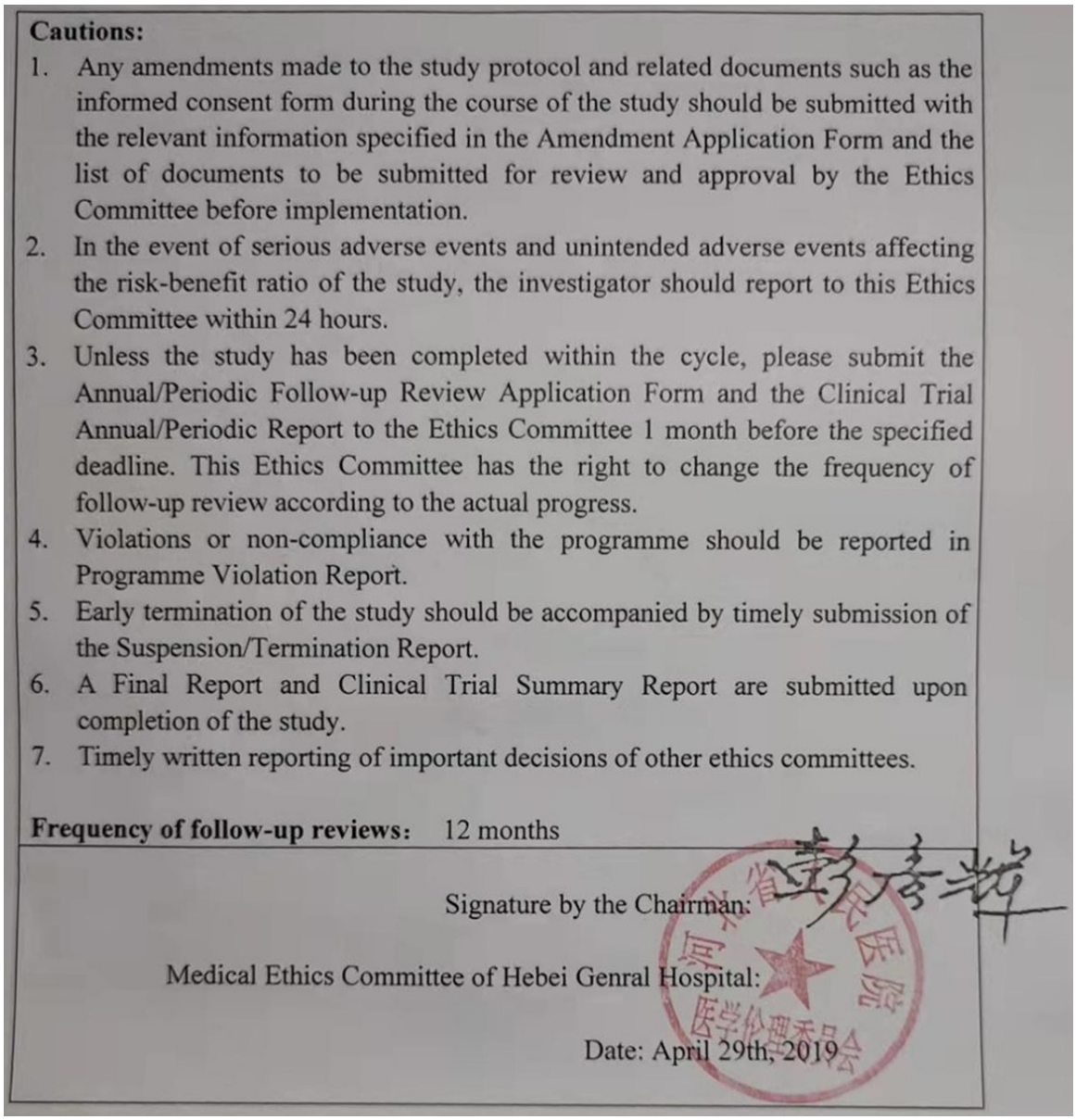
Examination and Approval of Clinical Medicine by

Medical Ethics Committee of Hebei General Hospital

Ethics approval No. 2019–48

Review Project Name	Researches on key techniques of anesthesia for elderly patients under the concept of ERAS				
Funding	Subject application				
Approval Number of CFA	NA	Leading units	Yes		
The Application Side	NA				
Application Department	Department of Anesthesiology	Project Leader	Jianli Li	The Unit of Leader	Hebei General Hospital
The Way of Ethical Review	Quick review in April 29th, 2019	Reviewer	Yang Lu		
Declaration Document	1. Resumes of major researchers in hospital 2. Research Programmes 3. Informed Consent Form				
Audit Result	Agree				
Validity of Approval	NA	Meeting Venue	NA		
The Number of Members in Ethics Committee	Suppose to attending numbers (NA)	Number of avoidance (NA)	Number of abstentions (NA)		
Voting Result	Agree (1 vote)	Consent after necessary modification (0 vote)	Revision and retrial (0 vote)	Disagree (0 vote)	Termination / Suspension (0 vote)

Review Statement : The composition and working procedures of this Ethics Committee are in accordance with the ethical principles of the Code of Practice for the Quality Management of Pharmaceutical Clinical Trials implemented by the State Food and Drug Administration of China, the ICH-GCP, the Measures for the Review of Biomedical Research Involving Human Beings (Trial), and the Declaration of Helsinki issued and the International Ethical Guidelines for Human Biomedical Research issued by the International Committee for the Organization of Medical Sciences.



Cautions:

1. Any amendments made to the study protocol and related documents such as the informed consent form during the course of the study should be submitted with the relevant information specified in the Amendment Application Form and the list of documents to be submitted for review and approval by the Ethics Committee before implementation.
2. In the event of serious adverse events and unintended adverse events affecting the risk-benefit ratio of the study, the investigator should report to this Ethics Committee within 24 hours.
3. Unless the study has been completed within the cycle, please submit the Annual/Periodic Follow-up Review Application Form and the Clinical Trial Annual/Periodic Report to the Ethics Committee 1 month before the specified deadline. This Ethics Committee has the right to change the frequency of follow-up review according to the actual progress.
4. Violations or non-compliance with the programme should be reported in Programme Violation Report.
5. Early termination of the study should be accompanied by timely submission of the Suspension/Termination Report.
6. A Final Report and Clinical Trial Summary Report are submitted upon completion of the study.
7. Timely written reporting of important decisions of other ethics committees.

Frequency of follow-up reviews: 12 months

Signature by the Chairman:

Medical Ethics Committee of Hebei Genral Hospital:

Date: April 29th, 2019

Supplementary File 2. The ethics approval report.

Supplementary File 3. The editing certificate.