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Effects of dexmedetomidine on the expression of inflammatory factors in children with congenital heart disease undergoing intraoperative cardiopulmonary bypass: A randomized controlled trial

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ABSTRACT

Importance: Dexmedetomidine inhibits the inflammatory response associated with cardiopulmonary bypass (CPB) and protects neural function. However, the mechanism of dexmedetomidine's anti-inflammatory pathway is unclear.

Objective: To investigate the effect of dexmedetomidine on the cognitive level and expression of inflammatory factors in children with congenital heart disease undergoing intraoperative CPB.

Methods: Ninety children with congenital heart disease were recruited and randomly divided into 3 groups of 30 children in each. In Group 1, a 1.0 $\mu g \cdot kg^{-1} \cdot h^{-1}$ intravenous bolus of dexmedetomidine was administered 10 minutes after induction of anesthesia, followed by a 0.2 $\mu g \cdot kg^{-1} \cdot h^{-1}$ infusion until the surgical incision. In Group 2, a 0.5 μg/kg intravenous bolus of dexmedetomidine was administered 10 minutes after induction of anesthesia, followed by a 0.1 $\mu g \cdot kg^{-1} \cdot h^{-1}$ infusion until the surgical incision. The control group was given physiological saline using the same method as in Groups 1 and 2. The serum levels of nuclear factor-kappa B (NF-κB), S-100β protein, neuron-specific enolase (NSE), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were measured before the surgery (T1), at the end of CPB (T2), 2 hours after CPB (T3), 6 hours after CPB (T4), and 24 hours after CPB (T5). The Wechsler Intelligence Scale for children (WISC) was measured before the operation and at 3, 6, and 12 months after the operation to evaluate the neurodevelopmental state of the children.

Results: The levels of the NF-κB, S-100β protein, NSE, TNF-α, IL-6 were significantly higher at T2, T3, or T4 than before the surgery (T1) in the control group or the dexmedetomidine groups. However, the increases of NF-κB, TNF-α, IL-6, S-100β and NSE levels were significantly smaller in the dexmedetomidine groups than those in the control group ($P < 0.017$). The WISC scores were similar among the three groups before or after the operation.

Interpretation: The increases in NF-κB, TNF-α, and IL-6 levels indicated aggravation of the inflammatory reaction and the increase S-100β protein and NSE levels indicated that the nervous system was damaged. Administration of dexmedetomidine to children with congenital heart disease undergoing intraoperative CPB can inhibit the inflammatory response and may ameliorate the neurodevelopmental damage caused by CPB.

KEYWORDS
Cardiopulmonary bypass, Children, Dexmedetomidine, Inflammatory factor, Nerve injury

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INTRODUCTION

Surgical correction can improve the quality of life of children with congenital heart disease. However, 42% to 67% of these children still have central nervous dysfunction early in the postoperative period,[1] and their long-term intellectual development may even be affected.[2] Previous studies have suggested that the systemic inflammatory response to cardiopulmonary bypass (CPB) leads to acute neuronal damage. The main mechanism of secondary brain injury involves activation of reactive immune cells by the inflammatory response in the initial stage, leading to release of a large number of immune mediators that aggravate brain damage. In this cascade of activation, nuclear factor-kappa B (NF-κB) plays a key role in the inflammatory response.[3] Almost all cytokines and adhesion molecules have binding sites for NF-κB, and this binding action is important for the regulation of transcription and expression of NF-κB. It is related to the development of systemic inflammatory response syndrome because NF-κB can initiate the transcription of interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).[4-7]

Dexmedetomidine is an α_2 adrenergic receptor (α_2-AR) agonist. A previous study suggested that the anti-inflammatory effect of dexmedetomidine mainly occurs through combination with the α_2-AR subtype.[8] It inhibits the inflammatory response associated with CPB and protects neural function.[9-10] However, the mechanism of dexmedetomidine's anti-inflammatory pathway is still unclear, and few studies have focused on the effect of dexmedetomidine on NF-κB expression. The present study was performed to investigate the effect of α_2-AR agonists on the expression of NF-κB and brain damage in children with congenital heart disease undergoing operations with CPB.

METHODS

Ethical approval

This study was approved by the ethics committee of Zhengzhou Children's Hospital (No. 2018003). Written informed consent was obtained from all the children's guardians.

Study design and patients

From January 2018 to May 2019, children with congenital heart disease were selected as the study candidates. The enrollment criteria were an age of 6 to 12 years, diagnosis of congenital heart disease, planned surgery with CPB, completion of a cognitive function examination, and consent to join the study.

The exclusion criteria were traumatic brain injury, intracranial space-occupying lesion, hypoxic–ischemic encephalopathy, neuroblastoma, neurological damage before the surgery, and a CPB time of > 90 minutes. In total, 101 children were recruited, 11 children did not meet the enrollment criteria, and finally 90 children were enrolled. These 90 children with congenital heart disease were recruited and randomly divided into 3 groups of 30 children each. The selected patients had congenital ventricular septal defects and congenital atrial septal defects, which were balanced in each group.

The eligible children were randomly assigned into three groups using the digital table method. In Group 1, a 1.0 μg/kg bolus of dexmedetomidine was administered 10 minutes after induction of anesthesia, followed by a 0.2 $\mu g \cdot kg^{-1} \cdot h^{-1}$ infusion until the surgical incision. In Group 2, a 0.5 μg/kg bolus of dexmedetomidine was administered 10 minutes after induction of anesthesia, followed by a 0.1 $\mu g \cdot kg^{-1} \cdot h^{-1}$ infusion until the surgical incision. The control group was given physiological saline using the same method as in Groups 1 and 2.

Intraoperative monitoring and dexmedetomidine administration

Continuous monitoring of each patient's electrocardiogram, heart rate, blood pressure, oxygen saturation, and bispectral index was begun soon after the patients entered the operation room. The anesthesia apparatus was connected after the trachea cannula to maintain breathing. After establishment of anesthetic maintenance, the arterial blood pressure was continuously monitored by radial artery catheterization and the central venous pressure was continuously monitored by internal jugular vein catheterization. The bispectral index was maintained at 40 to 60.

In Group 1, dexmedetomidine (Hengrui Pharmaceutical, Shanghai, China) was intravenously injected at 1 μg/kg (concentration of 0.5 μg/mL) for at least 10 minutes, followed by a 0.2 $\mu g \cdot kg^{-1} \cdot h^{-1}$ intravenous infusion that was maintained until the end of the surgery. In Group 2, dexmedetomidine was intravenously injected at 0.5 μg/kg (concentration of 0.25 μg/mL) for at least 10 minutes, followed by a 0.1 $\mu g \cdot kg^{-1} \cdot h^{-1}$ intravenous infusion that was maintained until the end of the surgery. Patients in the control group were given physiological saline solution using the same method and at the same volume. The performing anesthetists were blind to the patients' grouping information. A micro-perfusion pump (Smiths Medical, Beijing, China) was used to control the rate and volume of administration. A Stöckert CPB unit (TERUMO Advanced Perfusion System 1; Terumo Cardiovascular Systems Corporation, Ann Arbor, MI, USA) was used during the operation under CPB.

Operative data and blood sample collection

Arterial blood samples were collected before surgery (T1), after CPB (T2), 2 hours after CPB (T3), 6 hours after CPB

(T4), and 24 hours after CPB (T5) for analysis of S-100β protein, neuron-specific enolase (NSE), TNF-α, IL-6, and NF-κB by anesthesiologists who were blinded to the grouping information of the study. We also recorded the heart rate and mean arterial pressure at each time point as well as the operative duration, CPB duration, aortic occlusion duration and minimum temperature for analysis.

Laboratory examinations

The serum concentrations of S-100β [S-100β enzyme-linked immunosorbent assay (ELISA); R&D Systems, Minneapolis, MN, USA], NSE (NSE ELISA; Sigma Co., Ltd., USA), TNF-α (TNF-α ELISA; R&D Systems, Minneapolis, MN, USA), IL-6 (IL-6 ELISA; Sigma Co., Ltd., USA) were determined by ELISA. Determination of the neutrophil nuclear factor activity in peripheral blood was performed as follows: 8 mL of venous blood was added to a test tube containing 0.2 mL of dimethyl sulfoxide, and the tube was then cryopreserved in a refrigerator at −70 °C. The blood samples were centrifuged at 4 °C and 1728 g for 15 minutes, and the supernatant was collected and stored at −70 °C until analysis. The NF-κB expression in neutrophils of venous blood was then determined by flow cytometry (FACSCalibur; Becton Dickinson, Franklin Lakes, NJ, USA) according to the manufacturer's instructions.

Neuropsychological evaluation

To evaluate the children's neuropsychological status, the Wechsler Intelligence Scale for Children (WISC) and standardized clinical neurological function tests were performed 1 day preoperatively and 3, 6, and 12 months postoperatively. The standardized neural function tests included a consciousness test, cranial nerve test, visual acuity and retinal test, optic papilla size test, normal morphology test, neat edge test, uplift movement test, sensation test, reflection and autonomic functional test, motion system and reflection of the upper and lower limbs test, sensory and autonomic nervous system test, aphasia test, apraxia test, and agnosia cerebral cortex dysfunction test. Children with abnormal test results were followed up by a skull scan, magnetic resonance imaging, and electroencephalography.[11] For the WISC, we separately added the scale score of each subtest in the verbal test and the scale score of each subtest in the performance test, thus obtaining the scale score of the verbal test and the scale score of the performance test. The scale score of the verbal test and the scale score of the performance test were added to obtain the full-scale score, which was converted to the total intelligence quotient.

Statistical analysis

Outcome data were analyzed in the intention-to-treat population. The Shapiro–Wilk test and Anderson–Darling test were used to test for a normal distribution. Normally distributed data are reported as mean ± standard deviation. Non-normally distributed and ordered data are reported as median (Q1, Q3), and categorical data are reported as number (percentage). Continuous variables with a normal distribution were compared using one-way analysis of variance, and continuous variables with a non-normal distribution were compared using the non-parametric Kruskal–Wallis test. Tests for pairwise comparisons were performed when significant differences were found by the Mann–Whitney *U*-test and Bonferroni-adjusted multiple tests (three comparisons). Bonferroni-adjusted *P* values of < 0.017 (0.05/3) were considered statistically significant. Changes in the S-100β protein, NSE, TNF-α, IL-6, and NF-κB levels over time in the three groups were compared using repeated-measures analysis of variance. Categorical variables were analyzed using the χ^2 test or Fisher's exact test. The Spearman rank correlation coefficient was used to assess the correlation between the changes in S-100β protein, NSE, TNF-α, IL-6, and NF-κB in the three groups at each time point. The statistical analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). A *P* value of < 0.017 was considered statistically significant. Bonferroni adjustment was performed to control for type I errors in multiple tests.

RESULTS

General characteristics of the three study groups

The clinical information and preoperative characteristics of the patients in each group were comparable (Table 1). There was no significant difference in sex, age, weight, operative duration, CPB time, or aortic occlusion time among all three groups (all $P > 0.05$).

TABLE 1 Demographic, clinical and preoperative characteristics of patients in the three groups.*

Variables	Control Group (*n* = 30)	Group 1 (*n* = 30)	Group 2 (*n* = 30)
Sex (male/female)	14/16	16/14	13/17
Age (years)	9.4 ± 1.7	8.9 ± 2.3	8.6 ± 1.9
Weight (kg)	24 ± 6	23 ± 4	25 ± 3
Operative duration (min)	86 ± 15	87 ± 18	91 ± 17
CPB time (min)	47 ± 15	44 ± 13	45 ± 11
Aortic occlusion time (min)	24 ± 8	23 ± 6	25 ± 7

Data are presented as *n* or mean ± standard deviation. *There were no significant differences ($P > 0.05$) between the groups in demographic, clinical and preoperative characteristics. CPB, cardiopulmonary bypass.

Changes in biomarkers in the three groups at each time point

At T1, there was no significant difference in S-100β protein, NSE, TNF-α, IL-6, or NF-κB among the groups ($P > 0.05$). In the control group, the S-100β and NSE levels were significantly higher at T2, T3 and T4 than at T1 (Figure 1A, B). Since the levels of S-100β protein

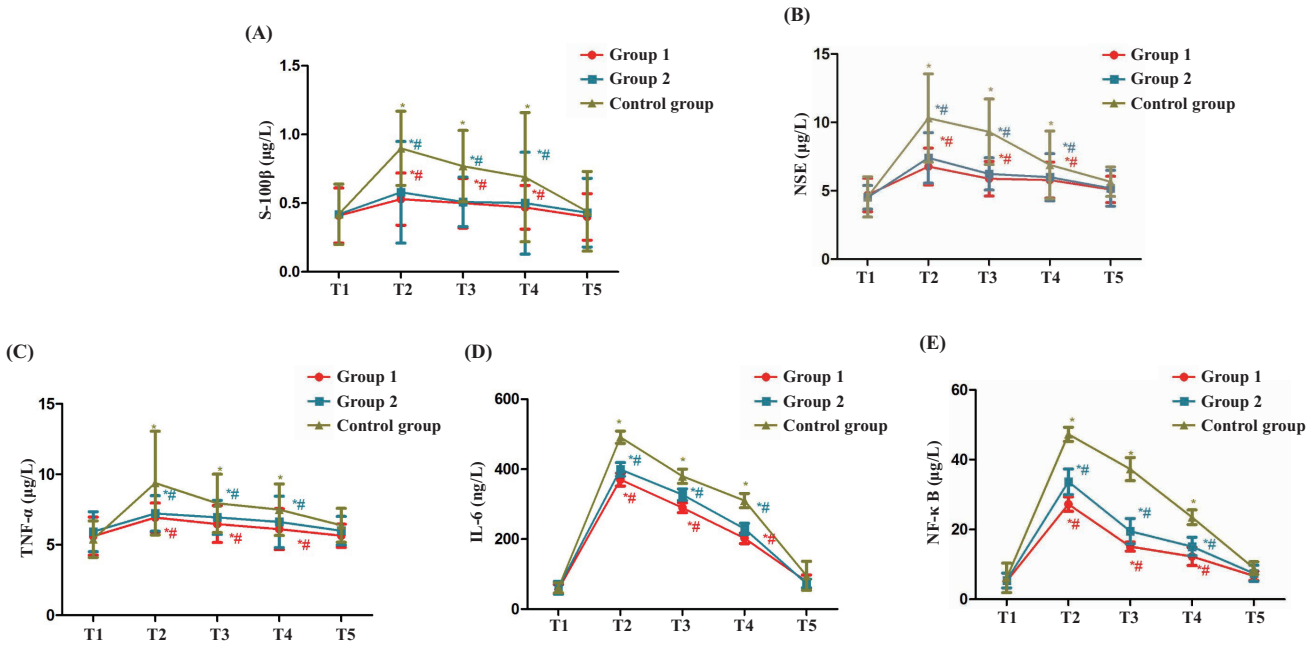

FIGURE 1 Comparison of level of serum S-100β protein, NSE, TNF-α, IL-6, and NF-κB of children at different time points before and after cardiopulmonary bypass surgery among the three groups. The increases in serum S-100β protein, NSE, TNF-α, IL-6, and NF-κB levels from T1 to T2, T3, and T4 in the dexmedetomidine groups or control group were statistically significant. At T2, T3 and T4 the levels of S-100β protein, NSE, TNF-α, IL-6, and NF-κB were lower in Groups 1 and 2 than in the control group ($P < 0.017$). $^{*}P < 0.017$, compared with preoperative value; $^{\#}P < 0.017$, intervention groups compared with control group at corresponding time point. NSE, neuron-specific enolase; TNF-α, tumor necrosis factor-α; IL, interleukin; NF-κB, nuclear factor-kappa B.

and NSE increased after CPB, neurological injury was indicated. In the dexmedetomidine Group 1 or Group 2, the levels of S-100β protein, NSE, TNF-α, IL-6, and NF-κB from T1 to T2, T3, and T4 were also significantly increased (denoted as * in Figure 1). However, at T2, T3 and T4 the levels of S-100β protein, NSE, TNF-α, IL-6, and NF-κB in the dexmedetomidine groups were significantly lower than those in the control group (denoted as # in Figure 1), suggesting that the control group had the most severe inflammatory reaction and that the nervous system was damaged in these patients.

The expression level of NF-κB in peripheral blood neutrophils was well correlated with the level of S-100β protein, NSE, TNF-α, and IL-6 in serum. The correlation coefficients were $r = 0.675$ (0.324–0.931, $P = 0.003$), $r = 0.733$ (0.392–0.992, $P = 0.004$), $r = 0.696$ (0.408–0.897, $P = 0.007$), and $r = 0.691$ (0.395–0.903, $P = 0.005$), respectively.

WISC scores at different time points and during follow-up

As shown in Table 2, the patients' intelligence scores were comparable among the three groups before surgery ($P > 0.05$), and the scores in each group did not change significantly at 3, 6, and 12 months after surgery ($P > 0.05$). However, in two patients of the control group, the speech comprehension index decreased after surgery, and in one patient, the intuitional reasoning index increased on the first day after surgery. However, all scores were restored to the preoperative levels at 3 months postoperatively.

TABLE 2 Comparison of intelligence scores among the three groups during the perioperative period.*

Time of measurement	Control group ($n = 30$)	Group 1 ($n = 30$)	Group 2 ($n = 30$)
1d before surgery	99.83 ± 3.60	100.27 ± 3.05	98.55 ± 2.47
3m after surgery	98.28 ± 2.09	98.92 ± 2.69	98.78 ± 3.83
6m after surgery	99.58 ± 4.82	99.47 ± 3.30	99.39 ± 3.68
12m after surgery	99.39 ± 2.77	99.72 ± 2.72	99.64 ± 3.50

Data are presented as mean ± standard deviation. *There were no significant differences ($P > 0.05$) between the groups in any of the comparisons.

DISCUSSION

The current study showed that at the end of CPB, the NF-κB, TNF-α, IL-6, S-100β protein, and NSE levels increased in all three groups of patients. This result indicates damage to the central nervous system accompanied by upregulation of NF-κB and inflammatory cytokines.

Overactivation of NF-κB can upregulate the expression of a variety of inflammatory response-related genes and increase the number of inflammatory mediators

and cytokines.[12] NF-κB is a key factor in the synthesis of inflammatory mediators and the cascade signal transduction pathway, it plays an important role in regulation of the cytokine network as well as expression of genes that are involved in the inflammatory response, stress response, and immune response.[13,14]

The main mechanism of secondary brain injury involves the inflammatory reaction in the initial stage, by which immune cells are activated and large numbers of immune mediators are released. This cascade aggravates traumatic brain injury. The inflammatory mediators that are closely related are mainly TNF-α, IL-6, and IL-1β.[15] However, overexpression of TNF-α can cause swelling, degeneration, and necrosis of nerve cells, thus aggravating the secondary brain damage.[16,17] Therefore, we evaluated both TNF-α and IL-6 in the inflammatory response of the brain in this study. Because the half-lives of S-100β protein and NSE are different, we measured both of them in the present study to monitor the dynamic changes in brain damage.[18-21]

Dexmedetomidine is a highly selective central α_2-AR agonist. In addition to its sedative, analgesic, and anxiolytic effects, dexmedetomidine also protects brain tissue and has a significant neuroprotective effect.[7] The present study showed that the expression levels of NF-κB, TNF-α, IL-6, S-100β protein, and NSE were lower in Groups 1 and 2 than in the control group, and Group 2 had the lowest expression levels. This result suggests that the α_2-AR agonist dexmedetomidine can decrease the NF-κB expression level, inhibit the release of the serum inflammatory factors TNF-α and IL-6, and reduce the production of S-100β protein and NSE during CPB, which may reduce neuronal damage and secondary brain damage. Dexmedetomidine acts as a neuroprotective agent by binding to the α_2-AR subtype. The mechanism of activity of dexmedetomidine is related to its anti-inflammatory activity.[7] Dexmedetomidine can excite the presynaptic α_2-AR of central neurons and inhibit calcium influx and phospholipase C activity by G protein. Dexmedetomidine can also reduce the plasma catecholamine concentration, decrease sympathetic tone and enhance the parasympathetic effect, thereby downgrading the expression of NF-κB and inhibiting the inflammatory response. Dexmedetomidine has an anti-apoptotic effect and can improve the blood–brain barrier permeability, therefore reducing the brain damage induced by CPB.

The heart rate and blood pressure of the patients in all three groups were within the normal range during the perioperative period in this study, indicating that the administration of α_2-AR agonists did not have severe adverse effects on the children's hemodynamics. The neuropsychological evaluation showed that the α_2-AR agonists did not affect the intelligence of the children undergoing CPB. Additionally, during follow-up, three children in the control group developed transient neuropsychiatric symptoms, while no children in Group 1 or 2 developed such symptoms. These findings indicate that α_2-AR agonists may have long-term protective value in this setting.

This was a single-center study with a relatively small sample size, which limits the power of the results. The associated factors and underlying mechanisms need to be further explored.

In conclusion, dexmedetomidine inhibits the inflammatory response in children undergoing surgery with CPB and decreases the elevation of serum S-100β protein and NSE. It also alleviates nervous system damage and provides hemodynamic stability without significant effects on cognitive function. Further studies are needed to explore the long-term effects of dexmedetomidine in children undergoing CPB.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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