Original Research

Protective effect of dexmedetomidine on intestinal mucosal barrier function in rats after cardiopulmonary bypass

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Impact statement

During CPB, changes in the morphology of the mucosa and its permeability are the underlying causes of inflammation, which directly reflect the degree of intestinal mucosal barrier function damage, and it is needed to be addressed in clinic. DEX can reduce the expression of inflammatory molecules. Our research found that DEX pretreatment could relieve intestinal microcirculation, attenuate intestinal damage, and lead to inhibition of the inflammatory response in CPB model rats, demonstrating that in CPB-induced damage of intestinal mucosal barrier function, DEX pretreatment plays a protective role through the inaction of TLR4/JAK2/STAT3-mediated inflammatory pathway.

Abstract

Cardiopulmonary bypass can result in damage to the intestines, leading to the occurrence of systemic inflammatory response syndrome. Dexmedetomidine is reported to confer anti-inflammatory properties. Here, the purpose of this study is to investigate the effect of dexmedetomidine on the intestinal mucosa barrier damage in a rat model of cardiopulmonary bypass. It was observed that cardiopulmonary bypass greatly decreased the levels of hemodynamic parameters than SHAM group, whereas dexmedetomidine pretreatment in a cardiopulmonary bypass model rat prevented this reduction. Also, it showed that compared with control animals, cardiopulmonary bypass caused obvious mucosal damage, which was attenuated in dexmedetomidine + cardiopulmonary bypass group. The above findings were in line with that of dexmedetomidine pretreatment, which increased the expression of tight junction proteins, but it decreased the levels of DAO, D-LA, FABP2, and endotoxin. Moreover, the results demonstrated that due to pre-administration of dexmedetomidine, the level of pro-inflammatory factors was decreased, while the level of anti-inflammatory cytokine was increased. Also, it showed that dexmedetomidine suppressed TLR4/JAK2/STAT3 pathway that was activated by cardiopulmonary bypass. Together, these results revealed that dexmedetomidine pretreatment relieves intestinal microcirculation, attenuates intestinal damage, and inhibits the inflammatory response of cardiopulmonary bypass model rats, demonstrating that in CPB-induced damage of intestinal mucosal barrier function, dexmedetomidine pretreatment plays a protective role by inactivating TLR4/JAK2/STAT3-mediated inflammatory pathway.

Keywords: Dexmedetomidine, intestinal mucosal barrier function, cardiopulmonary bypass, protective effect

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Introduction

As modern medical technology develops, during cardiac surgery, cardiopulmonary bypass (CPB) is often used to maintain the patient's circulatory function. Studies have found that more and more patients undergo heart surgery under CPB.[1] However, due to non-pulsatile blood flow, hypotension, blood dilution, or other non-physiological conditions, CPB can cause insufficient perfusion of the peripheral blood flow.[2] Also, it causes the intestinal mucosal barrier function to be impaired and eventually leads to the translocation of intestinal bacteria and endotoxins.[3] Previous studies have shown that the intestine plays an important role in the sustained occurrence and development of systemic inflammatory response syndrome and multiple organ dysfunction syndrome.[4,5] The incidence of gastrointestinal complications after CPB heart surgery does not exceed 3%, while the mortality rate could be as high as 90%.[6,7] Therefore, it is an urgent need to find an effective treatment for intestinal mucosal barrier damage. Many measures have been applied to alleviate this problem. For example, it inhibits inflammation and increases intestinal perfusion and provides oxygen to protect the intestinal barrier function during CPB, but the effect is not ideal.[8–10] Therefore, reducing the number of pathogenic bacteria

and endotoxin levels in the intestinal tract, thereby preventing intestinal damage during the peri-intestinal circulation, is of great significance for reducing the incidence of complications and mortality in cardiac surgery patients.

α2-adrenoceptors mediate many physiological responses to catecholamines, adrenaline, and noradrenaline.[11] For example, α2-adrenergic receptors may be involved in regulation of gastric acid secretion and gastric mucosal protection against different types of mucosal damage.[12,13] In addition, α2-adrenergic receptors have been known to modulate the inhibition of a number of gastrointestinal functions including gastrointestinal secretion and motility. For example, clonidine was reported to possess the ability to inhibit gastrointestinal transit and colonic motility.[14,15]

Dexmedetomidine (DEX) has been deemed to a potent and selective α2 adrenergic receptor agonist, which mostly applied to different clinical settings for sedative or analgesic requirements.[16] In 1999, it was approved by the Food and Drug Administration for usage in patients 24 h before mechanical ventilation in the intensive care unit. Recent research results showed that DEX could reduce the expression of inflammatory molecules such as TNF-α and IL-6, and exert anti-inflammatory effects.[17–19] It was also found that DEX can modulate the inflammatory response induced by lipopolysaccharide (LPS) in mouse macrophages.[20] Moreover, it has been proved by some researches that DEX could inhibit oxidative stress in LPS-induced liver injury by acting on α2 adrenergic receptor,[21] indicating that it has a potential protective effect on oxidative stress-related diseases. It is also reported that pretreatment with DEX can effectively inhibit the activation of astrocytes.[22] Besides, DEX can significantly inhibit cell apoptosis, which plays an important role in preventing sepsis and protecting organs.[23] However, there has been no research about whether DEX pre-treatment can protect intestinal barrier function during CPB by reduce inflammatory response.

As one of family of transmembrane proteins, toll-like receptors (TLRs) could perform the functions of signal transduction. Toll-like receptor 4 (TLR4) has long been recognized as the main sensor for identifying pathogen-associated molecular patterns (PAMPs),[24] which are detected by the immune system. Signal transducer and activator of transcription 3 (STAT3) is one of the key regulators in the process of inflammation. As a downstream signaling mediator of interleukin (IL)-6, STAT3 is involved in the occurrence of many inflammations.[25] Under the stimulation of extracellular signals, the intracellular Janus kinase (JAK) causes the activation of STAT3, and it was found that the JAK2 inhibitor AG490 can block the constitutive activation of STAT3.[26] Studies have showed that after CPB-induced brain injury, DEX can protect the nerves through the regulation of JAK2/STAT3 pathway.[27] Also, the results of this study indicated that by inhibiting the phosphorylation of JAK2/STAT3 signaling pathway, Ginkgolide B can significantly inhibit inflammatory response mediated by TLR4.[28]

Therefore, a postoperative intestinal barrier injury model in rats that received CPB was established in this study. We also designed to investigate the multiple effects of pretreatment with DEX on intestinal barrier function injury and inflammation, as well as the expression of TLR4/JAK2/STAT3 signaling pathway-associated proteins in CPB rats. Further aims were to have an in-depth insight into the potential protective effect and mechanism of DEX. It not only provides a reference for the protection of the intestinal mucosal barrier after CPB but also supplies theoretical evidence for clinical application.

Materials and methods

Experimental animals

A total of 50 adult clean-grade healthy male Sprague Dawley (SD) rats from the Experimental Animal Center of the Third Military Medical University, with a body weight of 350–500 g. All rats are in good health and given 22–26°C automatic 12 h/12 h dark-light cycle, accompanied by a relative humidity of about 40–60% throughout the entire experiment. The rats were fasted for one day before surgery and were given standard dry food and tap water for the rest of the time. According to the principle of experimental animals, all animals were given human care. All animal procedures were approved by the Ethics Committee of Academic Committee of Hebei North University (HBNU2019113018). All the operations of the experiment were carried out in accordance with the "Guidelines for the Care and Use of Laboratory Animals," which was issued by the Ministry of Science and Technology of the People's Republic of China in 2006 [398]).

Experimental groupings

Fifty rats were randomly separated into three groups as follows: (i) Sham operation (SHAM group, n = 10); (ii) CPB surgery (CPB group, n = 20); (iii) (DEX + CPB) surgery ((DEX + CPB) group, n = 20). Except for the SHAM group, CPB was used to establish a CPB rat model for the rats. In SHAM group, the animals were cannulated and connected to primed CPB circuit but did not undergo CPB, which were considered sham controls. In CPB group, the rats received only CPB surgery but did not receive any other treatments, and were called CPB controls. DEX (5 μg/kg) has been demonstrated to play a protective role before CPB in rats in many reports.[27,29] Therefore, an intravenous injection of 5 μg/kg DEX (diluted with 0.9% saline, and from Jiangsu Hengrui Medicine Co. Ltd, China) was administered in (DEX + CPB) group using a microinfusion pump 15 min prior to the bypass. The same DEX dose was maintained during CPB procedure.

Preparation of CPB model

The establishment of the CPB model was referred to as mentioned above,[30,31] and some modifications have been made on this basis. Briefly, 30 mg/kg pentobarbital sodium (Shanghai Ziyuan Pharmaceutical Co., Ltd) was injected intraperitoneally, which was employed for the purpose of anesthetizing rats. The TKR-200 C ventilator (Teli Anesthesia Breathing Equipment Company, China) was

used for mechanical ventilation to maintain the end-tidal carbon dioxide, ranging from 35 mmHg to 45 mmHg. In addition, the ventilator was connected to a monitor, which was conducive to observing heart rate, oxygen saturation, and rectal temperature. The concentration of oxygen inhalation was 21%, respiratory rate was 60 beats/min, inspiratory/expiratory ratio was 1:2, and tidal volume was 16–20 ml/kg.

A 22 G trocar was inserted into the left femoral vein and then connected it to a mini infusion pump, which is beneficial to the injection of heparin sodium (300 IU/kg). In addition, a 24 G trocar was placed in the left femoral artery and connected to the monitor through a pressure sensor device to continuously monitor the arterial pressure. Right internal jugular vein catheterization (18 G) and coccygeal artery catheterization (22 G) were both used to drain blood during CPB. A membrane oxygenator was used for the supply of oxygen in CPB. The low-flow CPB velocity was approximately 35 ml/kg/min, and it gradually increased until 100–120 ml/kg/min at full-flow bypass. After starting CPB, mechanical ventilation was transferred to oxygenator for oxygen supply (FiO_2=100%), and the ratio of oxygen flow to perfusion flow was maintained between 0.8 and 1.0. When CPB was over, the flow rate was slowly reduced to keep the hemodynamics stable. In order to maintain the mean arterial pressure and hematocrit within the range of 70–85 mmHg and 20%–25%, respectively, we used a rectal probe central to monitor body temperature and maintained it at 36.5°C ± 1°C for each rat covered with a heat lamp during the experiment. The CPB surgery was kept for 2 h. All rats awoke after 60–90 min of anesthesia. Measurements of hemodynamic parameters, including heart rate (HR), left ventricular diastolic pressure (LVDP), mean arterial pressure (MAP), hemoglobin (HB) and the positive maximal rate of change of pressure development ($+dP/dt_{max}$), the negative maximal rate of change of pressure development ($-dP/dt_{max}$) were recorded through Datex-Ohmeda S/5 Entropy Module (DRE, Inc.). Rats in all groups were sacrificed after CPB by cervical dislocation under sevoflurane anesthesia. In addition, all experimental data were analyzed blindly.

Pathological observation of intestinal tissue

After rats were sacrificed, the collected jejunum intestinal samples were immersed in 10% neutral formalin and then fixed at regular temperature for 48 h. Then the intestinal tissues were embedded with paraffin and sectioning. Paraffin sections were cut into many slices with a thickness of about 4 μm and then they were stained with hematoxylin and eosin (H&E) staining kit (Sigma-Aldrich, St. Louis, MO) for staining analysis. Finally, pathological changes of the intestine were observed under a light microscope (with 200× magnification; Nikon Eclipse Ti-E light microscope, Tokyo, Japan) and evaluated by an experienced pathologist.

Blood sample collection

Venous blood specimens from all rats of each group after sacrifice of the animals were collected. The plasma supernatants were prepared by centrifugation at 1000*g* for 20 min at 4°C and stored at –80°C for further analysis.

Biochemical analysis of blood

The inflammatory factors IL-6, IL-1β, IL-10, and tumor necrosis factor (TNF)-α, as well as other factor, fatty acid-binding protein 2 (FABP2) in rat blood serum were determined using enzyme-linked immunosorbent assay (ELISA) kits (R&D System Europe Ltd, UK) on basis of the manufacturer's instructions. In addition, the level of intestinal injury markers D-lactic acid (DLA) in serum, as well as diamine oxidase (DAO) was detected by a spectrophotometric assay by kits, which were from Wuhan USCN Business Co., Ltd. The tests were carried out following the methods as described by the manufacturer. Besides, a limulus lysate test kit (Yihua Clinical Technology, Inc., China) was employed to detect the level of endotoxin concentration in serum. The experiment was conducted according to the manufacturers' protocol.

Western blot analysis

After homogenization of frozen rat intestinal tissues, the lysates were treated with ice-cold lysis buffer for half an hour. Total protein was collected and then the bicinchoninic acid (BCA) method was employed in order to determine the concentration of the protein solution. A total of 30 μg proteins were loaded to wells and then they were separated by a 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE), followed by transferring to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% fat-free milk for half an hour at room temperature. After that, the PVDF membrane was incubated with primary antibody (e.g. anti-zonula occludens-1 (ZO-1) antibody, anti-claudin-1 antibody, anti-occludin antibody, anti-IL-6 antibody, anti-TNF-α antibody, anti-IL-1β antibody, anti-IL-10 antibody, anti-TLR4 antibody, anti-JAK2 antibody, anti-STAT3 antibody, anti-Caspase-3 antibody, anti-Bax antibody, anti-HIF-1α antibody, and anti-β-actin antibody, all from Abcam, Cambridge, MA, USA) for overnight at 4°C. The membranes were washed for three times with tris-buffered saline with 0.2% Tween-20 for 10 min each time, and then the blot was incubated with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies for an hour at room temperature. After that, the membranes were washed again three times in Tris-buffered saline with 0.2% Tween-20 for 10 min each time. Lastly, the bands were detected by an enhanced chemiluminescence advance detection system (Amersham, Bucks, UK), and the band densities were tested by ImageJ software (National Institutes of Health, Bethesda, MD).

Statistical analysis

The experiments data are expressed with mean ± standard deviation (SD), and each experiment *in vitro* was repeated for at least three times. SPSS 25.0 software (Chicago, IL) was used as the statistical software. Multiple comparisons were analyzed using one-way analysis of variance (ANOVA)

followed by Student-Newman-Keuls (SNK)-q test. *$P < 0.05$ indicates that the difference is statistically significant.

Results

Changes in rat hemodynamics

After all rats recovered from anesthesia for 60–90 min, they were subject to hemodynamic analysis. The basal levels of HR (199.41 ± 9.32 beats/min), MAP (67.86 ± 6.39 mmHg), HB (78.23 ± 9.04 g/L), LVDP (60.54 ± 8.61 mmHg), $+dP/dt_{max}$ (876.18 ± 21.01 mmHg/s), and $-dP/dt_{max}$ (-776.29 ± 29.71 mmHg/s) were more significantly decreased in the CPB model rats than that of the SHAM group (HR: (305.63 ± 15.38 beats/min), MAP: (143.12 ± 9.65 mmHg), HB: (151.18 ± 10.21 g/L), LVDP: (132.76 ± 9.09 mmHg), $+dP/dt_{max}$: (1987.23 ± 43.24 mmHg/s) and $-dP/dt_{max}$: (-1668.02 ± 41.06 mmHg/s), respectively) ($P < 0.05$, Figure 1; the detailed statistical analysis data could be seen in Supplementary Information). However, following pre-treatment with DEX to the rats undergoing CPB, these parameters in DEX + CPB group (HR: (256.35 ± 12.46 beats/min), MAP: (102.32 ± 8.09 mmHg), HB: (111.07 ± 8.18 g/L), LVDP: (96.12 ± 6.24 mmHg), $+dP/dt_{max}$: (1325.09 ± 34.37 mmHg/s) and $-dP/dt_{max}$: (-1125.36 ± 46.81 mmHg/s), respectively) exhibited a significant increase when compared with the CBP group ($P < 0.05$, Figure 1; the detailed statistical analysis data could be seen in Supplementary Information). Thus, it demonstrated that pretreatment with DEX sharply increased left ventricular function of rat hearts during CPB.

Pretreatment with DEX alleviates intestinal damage in CPB model rats

We analyzed the pathological changes of HE-stained intestinal tissue (jejunum section) in rat by light microscopy. Pathological examinations of the degree of intestinal mucosal damage were performed and they were shown in Figures 2(a) to (c). In the SHAM group, it was observed that intestinal mucosa, villi, and brush borders were normal (Figure 2(a)). Also, intact intestinal epithelial structure could be observed, and no obvious pathological changes were seen (Figure 2(a)). In the CPB group, it was shown that the villi were loose, and the intestinal wall was damaged (Figure 2(b)). In addition, we could also observe pathological changes including intestinal mucosal edema, infiltration of neutrophils and lymphocytes, partial atrophy and shedding of the villus, and filling of flaky capillaries. However, treatment with DEX prior to the bypass increased the length of the intestinal villi (Figure 2(c)). The intestinal mucosal injury in DEX + CPB group appeared to be attenuated than that of the CPB group. The histopathological characteristics were significantly improved with less inflammatory infiltration. HIF-1α is reported to play a crucial role in the cellular adaptation to hypoxia and ischemia followed by regulating apoptosis, which is involved in activation of Caspase-3 and Bax.[32,33] To test whether ischemia and apoptosis were related to intestinal barrier dysfunction, Western blot analysis was used to determine the levels of Caspase-3, Bax, and HIF-1α in intestinal tissue of each group. The results showed that compared with the SHAM group, the levels of Caspase-3 and Bax, as well as HIF-1α were significantly increased in CPB group ($P < 0.05$, Figure 2(d) and (e); the detailed statistical analysis data could be seen in Supplementary Information), while pretreatment with DEX made them reduced in DEX+CPB group ($P < 0.05$, Figure 2(d) and (e); the detailed statistical analysis data could be seen in Supplementary Information). These observations demonstrated that pretreatment with DEX had obvious beneficial effects against intestinal mucosal damage induced by CPB, which was due to the reduction of ischemia and apoptosis.

DEX prevented the decline of tight junction proteins expression caused by CBP

It is well known that tight junctions could regulate intestinal paraepithelial pathways, playing a vital role in intestinal permeability in health and disease conditions.[34]

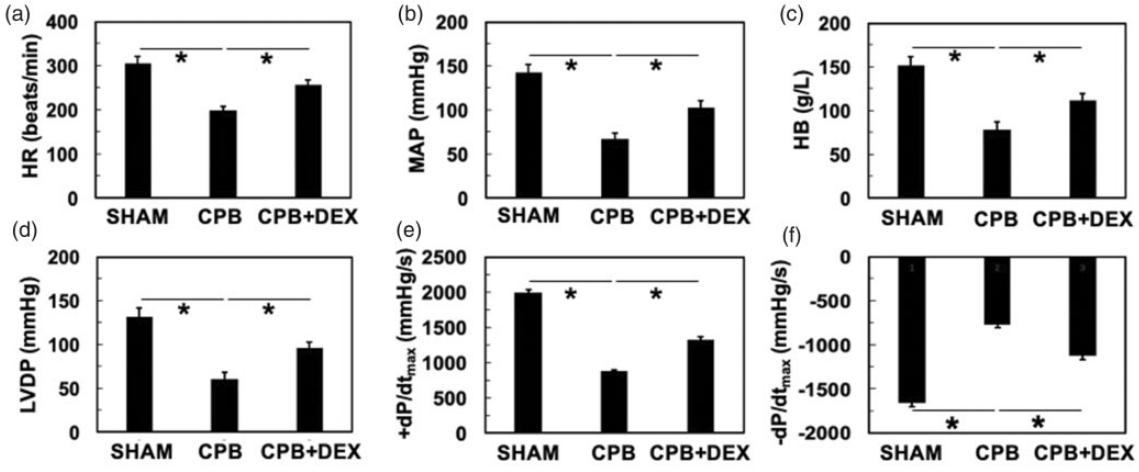

Figure 1. Altered hemodynamics in a rat CPB model with and without DEX pretreatment. Hemodynamic changes exhibited in the SHAM, CPB, and DEX + CPB groups were categorized as (a) HR, (b) MAP, (c) HB, (d) LVDP, (e) $+dP/dt_{max}$, and (f) $-dP/dt_{max}$. Data between two groups were compared using one-way ANOVA followed by SNK-q test: *$P < 0.05$.

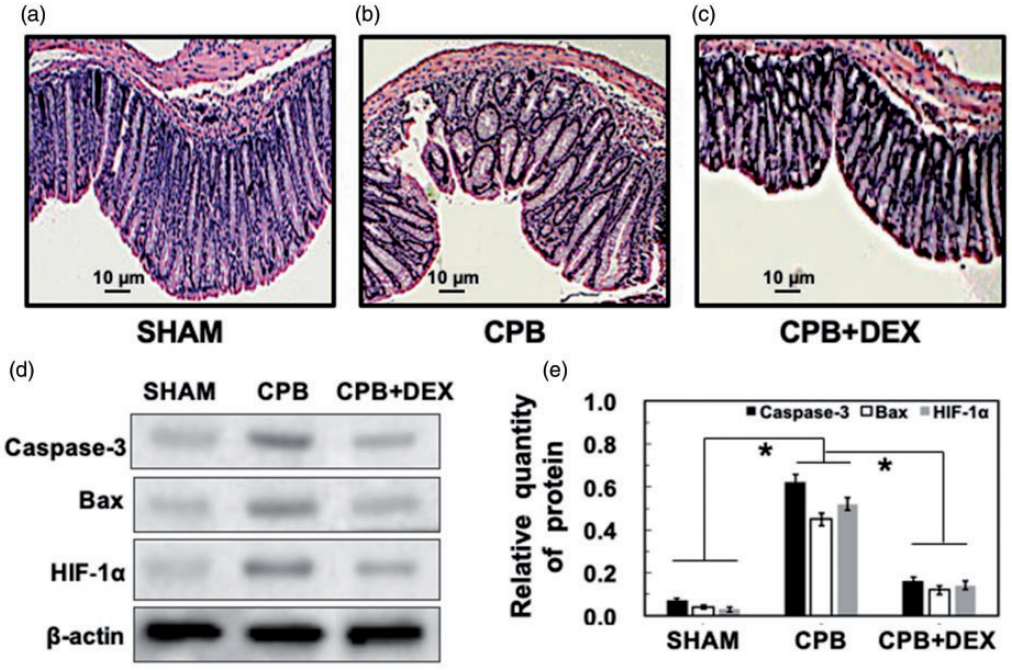

Figure 2. DEX alleviates intestinal mucosal damage induced by CPB due to the reduction of ischemia and apoptosis. To study the effect of DEX on the intestinal mucosal damage of SD rat undergoing CPB, the intestinal tissue (jejunum section) was obtained. Intestinal injuries of rats were observed by H&E staining. Bar: 10 μm. (a) Tissue from the SHAM group; (b) Tissue from the DEX group; (c) Tissue from the DEX+CPB group. (d) Western blot was used to determine the levels of Caspase-3, Bax, and HIF-1α in intestinal tissue of each group. β-actin was used as loading control. (e) The graph represents the relative band densities. Values are mean ± SD ($n = 3$). *$P < 0.05$ according to one-way ANOVA followed by SNK-q test. (A color version of this figure is available in the online journal.)

The level of tight junction proteins expression is essential for normal intestinal ultrastructure.[35] ZO-1, occludin, and claudin-1 are all the intestinal tight junction proteins markers.[36] In order to further assess the function of intestinal mucosal barrier among those three groups, the expressions of ZO-1, occludin, and claudin-1 in the intestinal tissue of SD rat were determined by Western blot analysis. As shown in Figure 3(a), the protein expression levels of ZO-1, occludin, and claudin-1 were highly lower in groups of CPB and (DEX+CPB) than SHAM group in the current study. However, the expression of the above three proteins in group DEX+CPB was dramatically higher than that in the CBP group. These differences were both statistically significant ($P < 0.05$, Figure 3(b); the detailed statistical analysis data could be seen in Supplementary Information). The result confirmed that pretreatment with DEX upregulated the expression of ZO-1, occludin, and claudin-1, showing that DEX was essential for preventing intestinal mucosal damage caused by CPB, and it may improve the function of intestinal mucosal barrier in CPB rats.

DEX improves intestinal mucosal function in CPB model rats

Markers of intestinal permeability include DAO, D-LA, FABP2, and endotoxin, etc.[37] CPB-induced intestinal mucosal dysfunction leads to the release of high activity DAO from intestinal epithelial cells into the blood, also increasing the metabolism of DLA via gastrointestinal bacterial fermentation, as well as serum levels of FABP2.[38] The changes in the levels of these factors in the serum of rats were investigated using ELISA in the current study. The results demonstrated that the activity of DAO and D-LA (Figure 4(a) and (b)), and the levels of FABP2 and endotoxin (Figure 4(c) and (d)) in serum were greatly higher in group CPB as compared to the control SHAM group ($P < 0.05$, Figure 4; the detailed statistical analysis data could be seen in Supplementary Information). However, all values were significantly lower in group DEX+CPB compared with those in group CPB ($P < 0.05$, Figure 4; the detailed statistical analysis data could be seen in Supplementary Information). The above results indicated that CPB could cause damage to the intestinal mucosa of rats, and DEX may mitigate this kind of damage, also showing that intestinal permeability was much improved by pretreatment of the CPB rat model with DEX.

DEX inhibits inflammatory response in CPB model rats

As we all know, the level of inflammatory cytokines, pro-inflammatory factors such as IL-6, TNF-α, IL-1β, and anti-inflammatory factors such as IL-10, play an important role in the process of intestinal injury.[39] TNF-α has always been regarded as the initial regulator of sepsis.[40] The expressions IL-6 and IL-1β were immediately induced by pathogens in epithelial cells.[41] Also, IL-10 can remarkably inhibit the production of single cell pro-inflammatory cytokines, which indicates its anti-inflammatory activity *in vivo*.[42] In terms of intestinal injury caused by CPB, the serum inflammation-related factors (IL-6, TNF-α, IL-1β, and IL-10) have been identified in rats. The ELISA data showed that the levels of inflammatory factors expression (IL-6, TNF-α, and IL-1β) were much higher in the CPB

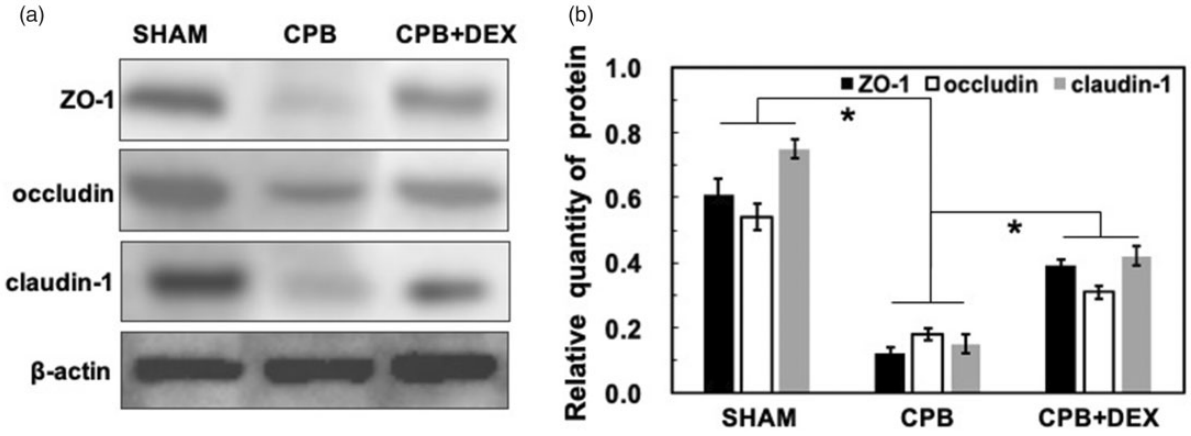

Figure 3. DEX prevented the decline of tight junction proteins expression caused by CBP. (a) Western blot analysis of ZO-1, occludin, and claudin-1 in samples from each of the three groups. β-actin was used as an internal standard. (b) Data were analyzed by Modifit software. Data are expressed as mean ± SD of at least three independent experiments. *$P < 0.05$ according to one-way ANOVA followed by SNK-q test.

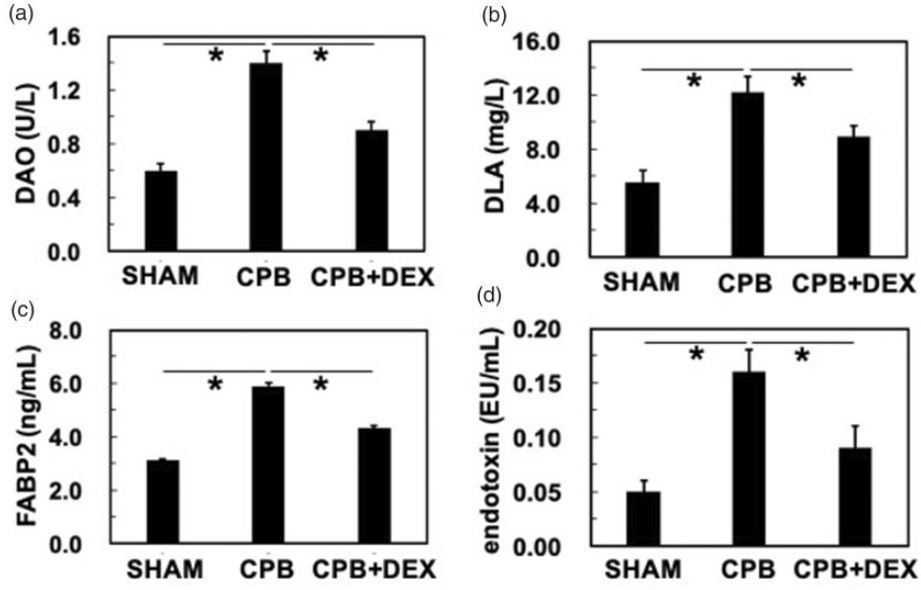

Figure 4. DEX improves intestinal mucosal function in a rat model of CPB. The activity of DAO (a) and D-LA (b) and the levels of FABP2 (c) and endotoxin (d) in serum were detected by ELISA. Data corresponds to the mean ± SD of at least three independent experiments. Comparison of different biochemical indices among the three groups including SHAM, CPB, and DEX+CPB groups. Statistical significance was calculated by one-way ANOVA followed by SNK-q test: *$P < 0.05$.

group ($P < 0.05$, Figure 5(a) to (c); the detailed statistical analysis data could be seen in Supplementary Information), while the level of IL-10 was much lower than that of SHAM group ($P < 0.05$, Figure 5D; the detailed statistical analysis data could be seen in Supplementary Information). However, we pretreated rats which received CPB with DEX, and it strikingly demonstrated that the levels of IL-1β, IL-6, and TNF-α were significantly reduced in DEX+CPB group with increased level of IL-10 than that in CPB group ($P < 0.05$, Figure 5; the detailed statistical analysis data could be seen in Supplementary Information). Results of ELISA analyses were consistent with those obtained by the Western blot assay. It was an interesting observation that the trend changes of the above inflammatory factors in protein expression level were same with that in the serum of rats between SHAM group and CPB group, as well as between DEX+CPB group and CPB group ($P < 0.05$, Figure 6(a) and (b); the detailed statistical analysis data could be seen in Supplementary Information). Therefore, the results suggested that CPB triggered inflammatory response in CPB model rats, and pretreatment with DEX could reverse the response.

DEX attenuates inflammatory reaction by suppressing TLR4/JAK2/STAT3 signaling pathway

More and more evidence indicate that JAK2/STAT3 pathway activates inflammation. In addition, some studies have also demonstrated that STAT3 is involved in IL-6-mediated inflammation.[43] Tissue or cell-derived inflammatory factors can activate Toll-like receptors.[44] Recent studies have also confirmed that TLR4 promotes inflammation.[45] The TLR4 ligation facilitates the activation of complex signal

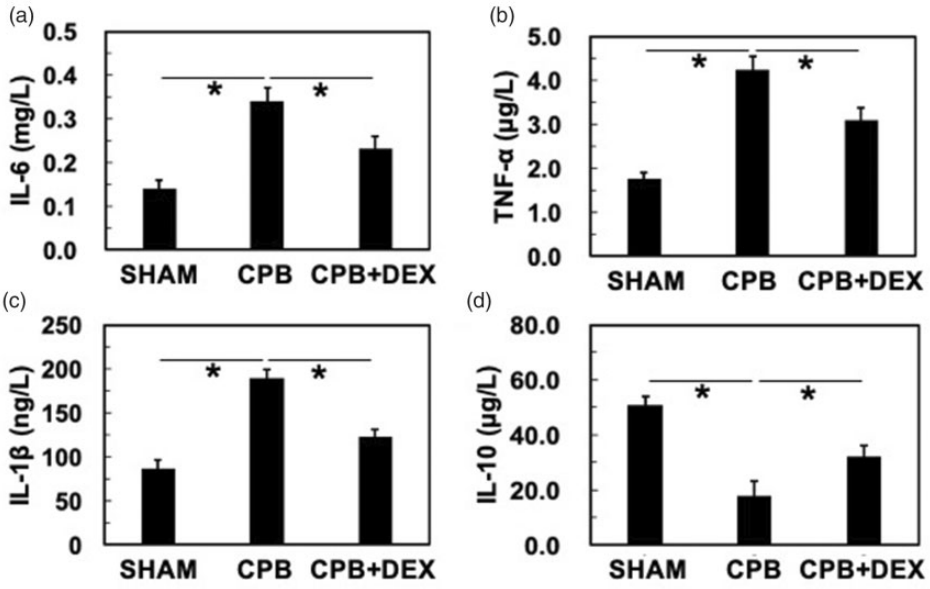

Figure 5. DEX attenuates secretion of inflammatory factors in CPB rats. Following DEX pretreatment, the serum of rats was collected, and ELISA analysis was performed to determine the expression levels of the following inflammatory factors (a) IL-6, (b) TNF-α, (c) IL-1β, and (d) IL-10. Data among the three groups including SHAM, CPB, and DEX+CPB groups were compared using one-way ANOVA followed by SNK-q test: *$P < 0.05$, which was considered as statistically significant.

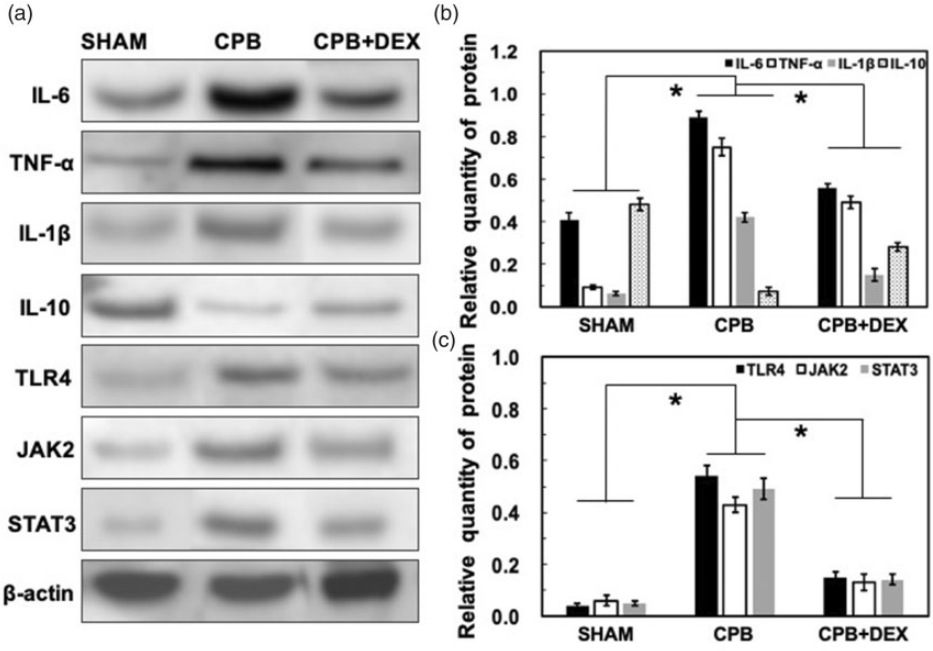

Figure 6. DEX reduces inflammatory reaction in CPB model rats by suppressing TLR4/JAK2/STAT3 signaling pathway. (a) Protein expression levels of the TLR4/JAK2/STAT3 signaling pathway, as well as inflammatory factors (IL-6, TNF-α, IL-1β, and IL-10) in intestinal tissue were determined by western blot analysis. (b) The data presented are the mean ± SD ($n = 3$), and *$P < 0.05$ by one-way ANOVA followed by SNK-q test, showing significant difference between two groups.

pathways, such as signal transduction mediated by JAK2/STAT3, and then exerts its function in the inflammatory response.[28,46] In order to further clarify the mechanism of DEX inhibiting inflammation in CPB model rats, we used Western blot analysis to study the expression levels of related proteins in the TLR4/JAK2/STAT3 signaling pathway in intestinal tissues. In current work, the results revealed that TLR4, JAK2, and STAT3 levels were up-regulated in the CPB group than SHAM group (Figure 6(a)). However, the expression levels of TLR4, JAK2, and STAT3 in the group DEX+CPB were significantly decreased than that in the CPB group (Figure 6(a)). Moreover, statistically significant difference was observed in the above changes of TLR4/JAK2/STAT3 expression levels of intestinal tissue in those three groups ($P < 0.05$, Figure 6(b); the detailed statistical analysis data could be seen in Supplementary Information). These present findings suggested that the TLR4/JAK2/STAT3 signaling pathway played a striking

role in DEX-mediated inhibition of inflammatory reaction in CPB model rats.

Discussion

CPB mainly connects the body's circulatory system with the heart-lung machine through artificial channels,[47] making cardiovascular surgery safer and more practical. However, due to the presence of pulseless blood flow, hypotension and other non-physiological conditions, it can also lead to insufficient peripheral blood perfusion.[38] Despite excellent improvements, more and more evidence show that in clinical and experimental studies, CPB is associated with extensive inflammation and visceral edema formation.[48,49] Therefore, the importance of CPB-related complications has become more pronounced.

The intestinal mucosa is the main anatomical and functional barrier, which can separate potentially harmful intraluminal elements (e.g. bacteria and endotoxins) from extra-intestinal tissues and systemic circulation.[50] It is reported that CPB makes the intestinal susceptible to hypoperfusion, hypoxia damage, and increased intestinal permeability, which causes endotoxin from the gut to rise in the circulation and provokes a systemic inflammatory response.[51,52] Generally speaking, changes in mucosal permeability and morphology during CPB are important reasons for inflammation, which also reflects the degree of damage to the intestinal mucosal barrier function.[53]

As an α2-adrenergic receptor agonist, DEX has high selectivity, and it has been widely used in clinical sedation and anesthesia in the intensive care unit.[54] Also, it is demonstrated that DEX can reduce endotoxin-induced inflammation in septic rats.[55] Furthermore, it is well known that DEX can significantly inhibit the inflammatory response, which was associated with ischemia-reperfusion injury during CPB, and it may be also related to the inhibition of nuclear factor kappa B activity.[56] Besides, DEX could reduce the nervous system damage in CPB model rats by inhibiting inflammation or apoptosis,[57] and it showed that DEX plays a neuroprotective role by improving postoperative neurocognitive function after CPB in rats, which is involved in inactivation of the JAK2-STAT3 pathway.[27] In addition, it was demonstrated that DEX could alleviate CPB-related myocardial injury by inhibiting inflammatory reactions and myocardial apoptosis, in which JAK2/STAT3 pathway also plays an important role.[29] What is more, DEX plays a stable role in CPB priming solution, which could effectively control blood pressure level and reduce myocardial injury, and therefore it is conducive to rapid postoperative recovery.[58] However, there are very few studies that have specifically examined the impact of DEX on the intestinal mucosal barrier function in CPB.

In this study, a CPB rat model with or without DEX pretreatment was established, and then the intestinal microcirculation was evaluated, and the intestinal tissue was also examined by H&E staining. Firstly, we found that CPB markedly down-regulated the levels of hemodynamic parameters including HR, MAP, HB, LVDP, +dP/dtmax, and –dP/dtmax than that in SHAM group ($P < 0.05$, Figure 1). However, pretreatment with DEX in a rat CPB model prevented this reduction ($P < 0.05$, Figure 1). In addition, it also showed that compared with the control animals, CPB group caused obvious mucosal damage, such as villi fracturing, epithelial shedding, edema, mucosal atrophy, and villus shortening. However, the intestinal mucosa damage was attenuated in the DEX+CPB group due to the reduction of ischemia and apoptosis (Figure 2), also suggesting that DEX, as α2-adrenergic receptor agonist may influence blood vessel vascular tone especially as it relates to perfusion of the gastrointestinal tract. Surgical stress and pain are both stimulating factors, which will activate the sympathetic nervous system and form microthrombosis in several small vessels in intestinal muscles, resulting in a significant decrease in the density of perfused small vessels.[59] DEX may reduce sympathetic activity and cause vasodilation of small vessels. The recovery of intestinal microcirculation, including the normalization of overall hemodynamics, helps to reduce intestinal ischemia and intestinal mucosal epithelial cell apoptosis.[60] Besides, it was also supported by the fact that DEX pretreatment was conducive to the increase of tight junction protein levels including ZO-1, occludin, and claudin-1 in the intestinal tissue ($P < 0.05$, Figure 3), as well as that DEX+CPB group could decrease the levels of DAO, D-LA, FABP2, and endotoxin ($P < 0.05$, Figure 4). Moreover, results from ELISA and Western blot analyses demonstrated that preadministration of DEX could decrease the level of proinflammatory factors (IL-6, TNF-α, and IL-1β) and increase the level of anti-inflammatory cytokine (IL-10) ($P < 0.05$, Figures 5 and 6). Together, the results of the present study revealed that pretreatment with DEX could relieve intestinal microcirculation, attenuate intestinal damage, and significantly inhibit the inflammatory response in CPB model rats, thereby protecting the intestinal barrier function. Next, we made in-depth research on possible molecular mechanisms of DEX. The occurrence of inflammation is related to the activation of many different signal pathways, such as the signal pathway mediated by TLR4.[46] It was also reported that TLR4/JAK2/STAT3 signaling pathway is involved in cell activation, such as inflammation.[28] The current results demonstrated that DEX may suppress TLR4/JAK2/STAT3 pathway that was activated by CPB ($P < 0.05$, Figure 6).

However, our research has a number of limitations. The study did not establish the efficacy of DEX treatment after CPB, and it is essential to evaluate the effects of postoperative administration of DEX in future work. Besides, further research is needed to determine the optimal time for DEX administration. Moreover, additional work is required to assess its safety in order to develop standard guidelines for the application of DEX. The level of lactate is increased by CPB, which shows the imbalance between tissue oxygen supply and utilization indirectly.[35] Whether these changes will aggravate intestinal mucosal barrier dysfunction and interfere with the protective effect of DEX remains to be elucidated.

Therefore, we could conclude that DEX, acting at the α2-adrenergic receptor, is likely to affect the level of proinflammatory factors to relieve intestinal microcirculation and attenuate intestinal damage. Moreover, DEX, with its

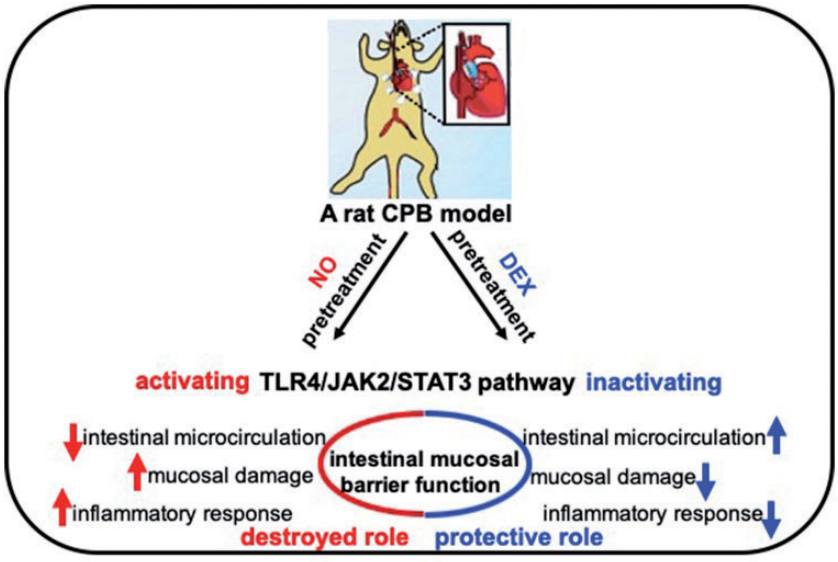

Figure 7. Model to account for the protective role of DEX pretreatment in intestinal mucosal barrier dysfunction response induced by CPB. (A color version of this figure is available in the online journal.)

wide effect on inflammatory responses, has certain protective properties for the gastrointestinal tract especially in patients with atherosclerosis or have increased risk of mesenteric ischemia. Importantly, DEX pretreatment played a protective role in intestinal mucosal barrier dysfunction response induced by CPB partly through the inaction of TLR4/JAK2/STAT3-mediated inflammatory pathway (Figure 7).

These findings provide us with new insights and also help us understand in detail the protective effect and mechanism of DEX pretreatment on the intestinal mucosal barrier function of CPB model rats. Also, the results of this study provide a rationale for human studies to evaluate the protective effects of DEX on the gastrointestinal tract, which may be a valuable clinical property to protect intestinal mucosal barrier function during CPB. These findings should be followed by a detailed investigation utilizing CPB model to identify the effect of DEX on the tissue from other parts of the body.

DEX decreased blood flow to most organs, but the largest decrease occurred in skin, spleen, and in arteriovenous shunts. Perfusion of vital organs such as the heart, brain, and kidneys was only moderately reduced. It showed that DEX causes considerable redistribution of blood flow, predominantly reducing blood flow to less vital organs and shunt flow.[61] Therefore, it may be feasible that DEX could be used for hemodynamically unstable patients. Certainly, we will also continue to investigate the potentially effect of DEX on hemodynamic instability to contribute to a better understanding of protective role of DEX on intestinal mucosal barrier function, which will be reported in due course.

Considering that those patients who received DEX in CPB priming solution and during bypass had subdued hemodynamics and better cognitive function,[58] this method of treatment will also be studied in our group to focus on more protective roles of DEX during CPB. In addition, its side effects will be discussed in future.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies and analysis of the data and review of the article, TJ and GL designed the research and wrote the article, TJ, ZX, HW, and GL conducted the experiments and performed the data analysis, GL reviewed and edited the article.

DECLARATION OF CONFLICTING INTERESTS

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DATA AVAILABILITY

The data will be made available upon reasonable request.

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SUPPLEMENTAL MATERIAL

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