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ORIGINAL ARTICLE

Preliminary study on the safety and efficacy of a new polymyxin B-immoblized resin column in treatment of LPS-induced sepsis beagles

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Abstract

Background: This study aims to assess the safety and efficacy of direct hemoperfusion using a new polymyxin B-immobilized resin column (disposable endotoxin adsorber, KCEA) in an endotoxin/ lipopolysaccharide (LPS)-induced sepsis model.

Methods: Eighteen beagles were randomized into 1 intervention group (KCEA group, $n = 6$) and 2 control groups (sham group and model group, $n = 6$ each). Sepsis was induced by continuous intravenous application of 0.5 mg/kg body weight of endotoxin for 60 min. An extracorporeal hemoperfusion device made with KCEA for endotoxin adsorption was used. Model group beagles received standard treatment with fluids and vasoactive drugs, KCEA group beagles received standard treatment and direct hemoperfusion of KCEA for 2 h, and sham group beagles were treated with standard treatment and direct hemoperfusion of a sham column for 2 h.

Results: Good blood compatibility of KCEA was confirmed by assessing clinical parameters. Blood endotoxin peak levels in the KCEA group were significantly lower, resulting in a significant suppression of IL-6, TNF-α and procalcitonin, which improved mean arterial pressure and significantly lowered vasopressor demand, thereby protecting organ function and improving survival time and rate. In the KCEA group, MAP was significantly higher over 6 h than those recorded both in the sham group and model group. The 7-day survival rates of the KCEA, sham and model groups were 50%, 0% and 0%, respectively.

Conclusion: KCEA hemoadsorption was effective at detoxifying circulatory endotoxin and inflammatory mediators and contributed to the decreased mortality rate in the sepsis beagles.

KEYWORDS
endotoxin, hemoadsorption, polymyxin B sulfate, resin, sepsis

Yonggui Li and Zhenggen Yang contributed equally to this work.

1 | INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infections.[1] It is a leading cause of mortality in the intensive care unit (ICU), especially when a patient's condition progresses to septic shock, with a mortality rate as high as 42%.[2] Bacterial endotoxin is a gram-negative lipopolysaccharide (LPS) containing a lipid A moiety as a part of the entire molecule. Its biological effects have been the subject of extensive studies.[3–5] It is believed to be primarily responsible for the clinical phenomena observed in gram-negative bacteremia, causing septic shock in humans and resulting in a high rate of mortality.[6] Using a logistic regression model, multiple organ failure occurred 10.3 times more frequently and depression of left ventricular ejection fraction (≤45%) occurred 4.8 times more frequently in endotoxemic patients. In patients with positive blood cultures, endotoxemia was associated with a high mortality.[7]

Treatment of endotoxemia and sepsis should involve treating or preventing the translocation of enteric endotoxin. Currently, the treatment of sepsis includes early resuscitation, anti-infection and hormone balancing regimes, mechanical ventilation, nutritional support, immune regulation, symptomatic management, etc. However, mortality has not improved significantly.[8,9] In recent years, extracorporeal blood purification has been widely used to control mediators in the blood as an adjunctive therapy for improving the pathological condition of septic shock. Over the past decade, there has been an increasing interest in selective removal of endotoxin from blood to reduce the burden of sepsis.[10,11]

To date there have been few randomized controlled trial (RCT) reports showing that marketed products can improve sepsis patients. PMX-F (Toraymyxin) has a good ability to clear endotoxin, and in the latest EUPHRATES trial conducted in North America, PMX showed improvements in mean arterial pressure and ventilator-free days. However, the mortality rate was not significantly different between groups.[12] One explanation was that it does not reduce the level of inflammatory cytokines.[13] Cytosorb, a synthetic adsorption column composed of highly porous biocompatible polymer beads, is only able to clear inflammatory mediators.[14] oXiris is a hollow fiber prepared from AN69 membrane modified by heparin and PEI. A small clinical study showed it can clear endotoxin and inflammatory mediators to some extent, but there have not yet been any of large-scale randomized controlled clinical studies.[15,16]

We hypothesized that KCEA can improve hemodynamics, oxygenation, etc. by simultaneously removing the endotoxin and inflammatory mediators during the process of sepsis and septic shock. To test this hypothesis, we established an endotoxin-induced sepsis beagle model to explore KCEA's therapeutic effect by using a KCEA resin column.

2 | METHODS

2.1 | Animals

This study was approved by the Ethics Committee of Guangdong Medical Laboratory Animal Center (No.: B202008-8). All experiments were performed according to the Declaration of Helsinki conventions for the use and care of animals. Eighteen healthy beagles of both sexes were chosen from a local stock routinely used for experimental research. The average weight of the dogs used in this experimental procedure was 11.75 ± 0.77 kg.

2.2 | Randomization

Sepsis was induced by continuous intravenous application of lipopolysaccharide for 60 min. The animals were randomly assigned to 3 groups, with 3 males and 3 females in each group.

The model group ($n = 6$) of dogs were treated with *Escherichia coli* endotoxin infusion and standard treatment to sustain hemodynamic stability, based on the surviving sepsis campaign guidelines.[17]

The treatment group ($n = 6$) beagles were treated with endotoxin + direct hemoperfusion (DHP) with KCEA. Fifteen minutes before the start of *Escherichia coli* endotoxin infusion, DHP with KCEA was started at a flow rate of 50 ml/min and performed for 2 h.

The sham group ($n = 6$) of beagles were treated with endotoxin + DHP without KCEA. The beagles were infused with endotoxin and received DHP with a sham column filled with the same NaCl injection volumes. Endotoxin infusion and DHP were performed in the same manner as for Treatment group.

In all groups, compound sodium chloride injection (5 ml/kg/h) and norepinephrine (0.05–1 μg/kg/min) were administered to achieve a mean arterial pressure (MAP) ≥70 mmHg. Observation was continued for 6 h.

2.3 | KCEA column-directed hemoadsorption

The extracorporeal circuit consisted of a hemoperfusion machine (Guangzhou Jihua Medical Device Co., Ltd), extracorporeal circulation blood lines (Dalian JMS medical appliance Co., Ltd) and a hemoperfusion column (KCEA, Guangzhou KONCEN BioScience Co., Ltd.). The KCEA column is a positively charged resin coupled with sulfate polymyxin B (PMB). Fixed PMB was estimated to interact with the lipid A portion of endotoxin through hydrophobic and ionic interactions. KCEA is a powerful new weapon in the clearance of endotoxin and prevention of the 'cytokine storm' that occurs in sepsis. Hemoadsorption was performed from the right femoral artery to the femoral vein using of Y-type 18 G venous indwelling needles. Heparin was used as the systemic anticoagulant. Heparin was injected 15 min before hemoperfusion at a dose of 62.5 U/kg. Direct hemoperfusion over a polymyxin B-immobilized resin column begun 15 min before the endotoxin infusion and continued for 2 h at a blood flow rate of 50 ml/min. KCEA-sham treatment was also performed to exclude the effect of the extracorporeal circuit alone, without hemoadsorption, on the hemodynamics, for comparison with KCEA treatment. We used a column filled with saline instead of KCEA to form a closed extracorporeal circuit. Sham treatment was carried out in the same way as for KCEA treatment. To produce sepsis, 0.5 mg/kg body weight of lipopolysaccharide was infused intravenously.

2.4 | Anesthesia

After 16 h fasting and receiving water ad libitum, beagles were anesthetized with 0.2 ml/kg serazine hydrochloride (Dunhua Shengda Animal Drug Co., Ltd, Jilin, China) and 0.5 ml/kg 3% sodium pentobarbital (Sigma-Aldrich (Shanghai) Trading Co., Ltd.) injected into the arm muscle successively. During treatment, beagles were given additional anesthesia if signs of awakening were observed. When the animals showed signs of resuscitation and muscle tone had obviously recovered, an intramuscular injection with a supplement of 0.5 ml serazine hydrochloride was given to maintain anesthesia. When a beagle showed signs of waking up, but muscle tone was not obvious, the beagle was subcutaneously injected with 0.5 ml 3% pentobarbital sodium to maintain anesthesia. If a beagle suddenly awoke and subcutaneous injection of pentobarbital sodium plus anaesthesia failed to sedate the animal immediately, an intravenous injection of 0.5 ml 3% pentobarbital sodium solution was used to maintain anaesthesia. During anesthesia, approximately 60% of the anesthetic dose was injected for initial anesthesia, and the beagle's anesthetic state was observed and the appropriate anesthetic supplement was added.

2.5 | Instrumentation and hemodynamic measurements

First, the left femoral artery was separated and a Y-type 18 G intravenous indwelling needle was inserted for the recording of blood pressure and heart rate. The right femoral artery and femoral vein of beagles were separated, and Y-type 18 G venous indwelling needles were inserted in each for hemoadsorption access. The beagles in the model group did not need to be perfused and the right femoral artery and femoral vein were not separated. 20 G intravenous indwelling needles were inserted into the veins of the two forelimbs of the beagle for intravenous injection. A suprapubic bladder catheter (B. Braun, Melsungen, Germany) was inserted for the recording of urine output.

Measured hemodynamic variables included heart rate (HR) and mean arterial pressure (MAP). Blood gas analysis was assessed on femoral arterial and mixed venous blood samples (2 ml) using a blood gas analyzer (GEM Premier 3000, Instrumentation Laboratory, Bedford, MA, USA) for correction of oxygen partial pressure, correction of carbon dioxide partial pressure, blood oxygen saturation, the ratio of arterial partial pressure per inspiratory fraction of oxygen (oxygenation index, PaO_2/FIO_2) and lactate.

2.6 | Endotoxin measurement

Kinetic turbidimetric analysis was performed using an LKM kinetic tube reader (LKM-02-64, Lab Kinetics Ltd, England) to measure endotoxin with a sensitivity of 0.005 EU/ml. In brief, 1–2 ml heparinized blood was centrifuged (400 *g* for 10 min) to separate out the plasma, which was stored at 2–8°C within 4 h, or otherwise at below −20°C for at most 30 days. Plasma samples were diluted 10-fold with pyrogen-free water and heated to 75°C for 10 min to overcome assay inhibition by plasma.

2.7 | Concentrations of inflammatory mediators in circulation

A 2 ml heparinized blood sample was centrifuged (400 *g* for 10 min) to separate out the plasma.

Plasma levels of interleukin-6 (IL-6), anti-tumor necrosis factor α (TNF-α) and procalcitonin (PCT) were measured by standard enzyme-linked immunosorbent assay (ELISA) kits (CUSABIO Co., Ltd, Shanghai, China) according to the manufacturer's instructions.

2.8 | Clinical variables

We used routine laboratory tests to assess blood data (white blood cells, WBC; red blood cells, RBC; hemoglobin, HGB; platelet count, PLT), blood biochemistry (alanine transaminase, ALT; aspartate aminotransferase, AST; total bilirubin, TBIL; Creatinine,CRE; blood urea nitrogen, BUN; total Protein, TP; albumin, ALB; globulin, GLB), electrolytes (K^+, Na^+, Cl^-, Ca^{2+}, Mg^{2+}, P)and coagulation index (prothrombin time, PT; activated partial thromboplastin time, APTT; fibrinogen, FIB).

All data were collected at 0, 30, 60, 90, 120, 180, 240, 300 and 360 min from the start of treatment.

2.9 | Statistical analysis

Continuous variables are described as means and standard deviations, unless otherwise specified, and data were processed using Micro Office, SPSS 21.0 and SigmaPlot 14 Software. Data for the same group at different time points were compared and analyzed by double-tailed *t* test ($\alpha = 0.05$). For the same time point between different groups, when the data met normal distribution and the overall variance was equal, one-way analysis of variance was used for analysis. Otherwise, a non-parametric test was used for analysis ($\alpha = 0.05$).

3 | RESULTS

Eighteen beagles were randomized into 1 intervention group (KCEA group, $n = 6$) and 2 control groups (sham group and model group, $n = 6$ each).

To produce sepsis (Figure 1), beagles were given lipopolysaccharide (0.5 mg/kg) as a drip infusion. Direct hemoadsorption was performed using KCEA for 6 of 18 beagles. The adsorption was performed between the right femoral artery and femoral vein via Y-type

18 G venous indwelling needles. KCEA hemoadsorption is effective at detoxifying circulatory endotoxin and inflammatory mediators, and contributes to decreased mortality rate in the sepsis beagles.

3.1 | Survival

The survival rate at 7 days after endotoxin infusion of each group was 50% in the KCEA group, and 0% (no survivors) both in the sham group and model group. This survival data is summarized in Table 1.

3.2 | The effect of KCEA on endotoxin clearance

As depicted in Figure 2, in comparison with model and sham groups, KCEA beagles had a lower endotoxin peak concentration at later time points. Compared with the model group, the endotoxin clearance rate of KCEA group was 12.3% at 120 min and 35.3% at 360 min. Compared with sham group, the endotoxin clearance rate of KCEA group was 33.5% at 120 min and 38.3% at 360 min.

3.3 | The effect of KCEA on systemic inflammatory cytokines

As shown by ELISA assay in Figure 3A,B, there were no differences in the baseline concentration of plasma IL-6 (Figure 3A) and PCT (Figure 3B) among the model, sham and KCEA groups. All of these increased rapidly after endotoxin infusion, but IL-6 and PCT levels in the KCEA group were significantly lower than in the model and sham groups throughout the 6 h ($p = .013$ for IL-6, $p = .003$ for PCT at 120 min).

3.4 | The effect of KCEA on hemodynamics (circulatory index)

Figure 4A depicts the dynamics of mean arterial pressure among study groups. There was a significant similar change in mean arterial pressure ($p < .05$) over time in both model and sham groups, whereas there were no effects associated with the use of KCEA, and no significant change over time.

The use of KCEA hemoperfusion decreased vasopressor requirements (Figure 4B), and the changes reached statistical difference when compared with the model group and sham group ($p = .001$ at 120 min).

3.5 | The effect of KCEA on pulmonary function and resuscitation index

Compared with the baseline value, there was no significant change for the oxygenation index of the KCEA groups at each observation time point (Figure 5A, $p > .05$). The changes reached statistical difference when compared with model group at 300 and 360 min ($p = .018$ at 300 min, $p = .039$ at 360 min).

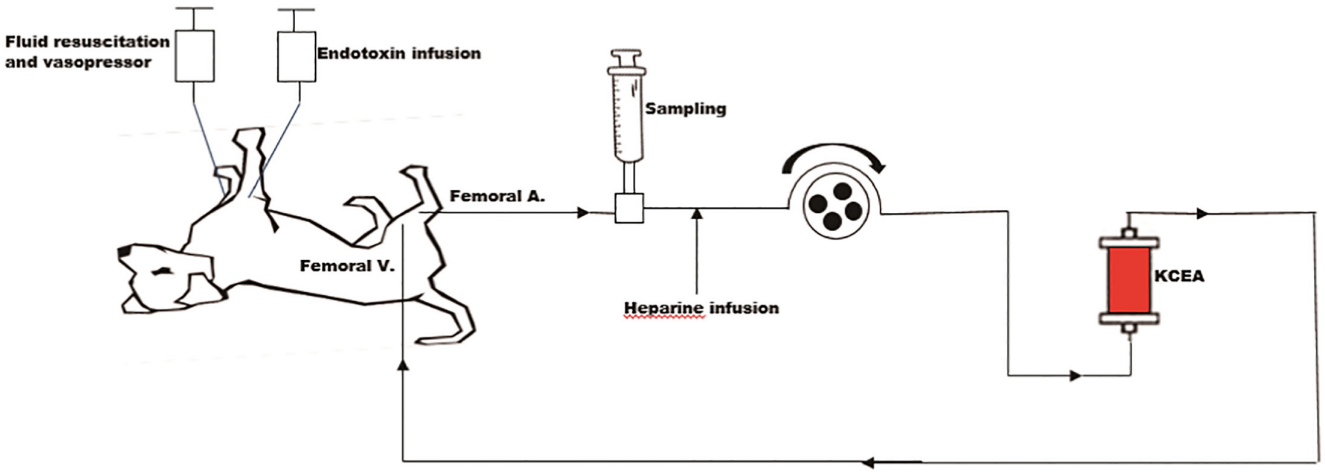


FIGURE 1 Direct hemoperfusion with KCEA in Beagles

TABLE 1 Survival

	No. of animals						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Model	1	1	0	0	0	0	0
Sham	2	2	1	1	1	1	0
KCEA	4	4	4	4	3	3	3

Note: Values are no. of animals surviving for each group at baseline (day 0, i.e., day of endotoxin infusion) and on each study day (days 1–6).

Compared with the model group, the LACT concentration of KCEA group was at a lower level throughout the treatment time, and the changes reached statistical difference (Figure 5B, $p < .05$). Compared with the baseline value, the changes were not statistical different at each time point ($p > .05$).

3.6 | The effect of KCEA on liver function index

We also examined whether KCEA can affect the function of the kidney and liver. Compared with the baseline value, there was no significant changes for the ALT and AST in the KCEA group

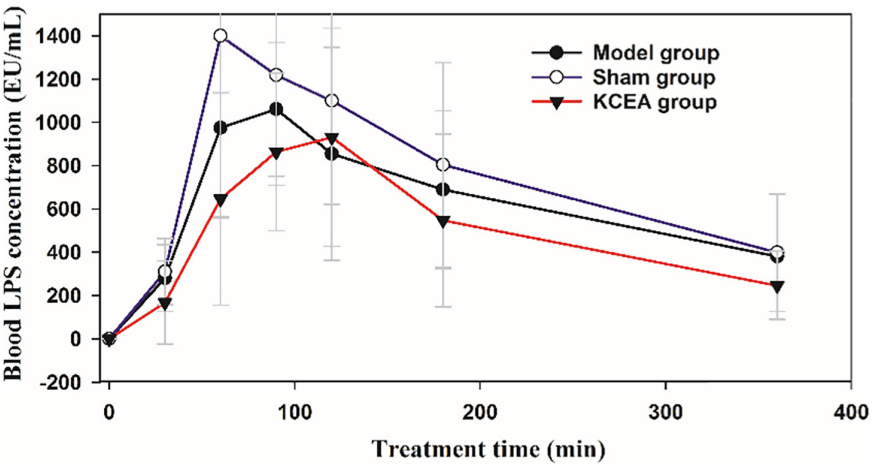


FIGURE 2 Differences in endotoxin among the Model, Sham and KCEA groups during the whole duration

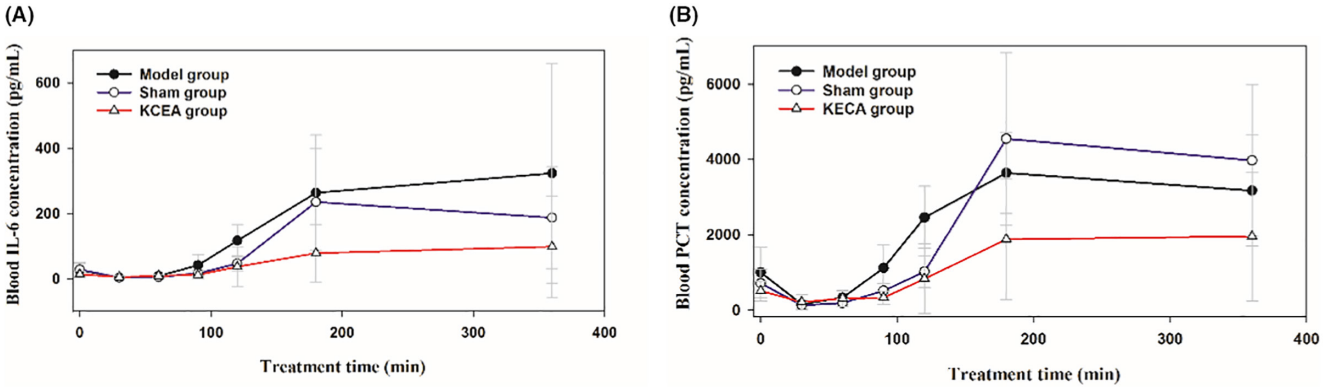


FIGURE 3 The effect of KCEA on systemic inflammatory cytokines (A) Differences in IL-6 among the Model, Sham and KCEA group during the whole duration (B) Differences in PCT among the Model, Sham and KCEA groups during the whole duration

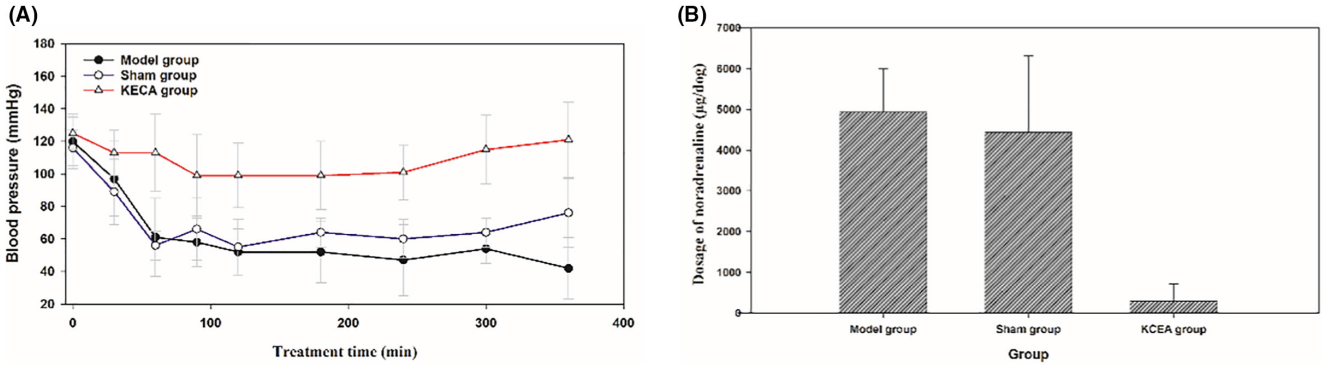


FIGURE 4 The effect of KCEA on hemodynamics (circulatory index) (A) Differences in MAP among the Model, Sham and KCEA groups during the whole duration (B) Differences in dosage of noradrenaline among the Model, Sham and KCEA groups during the whole duration

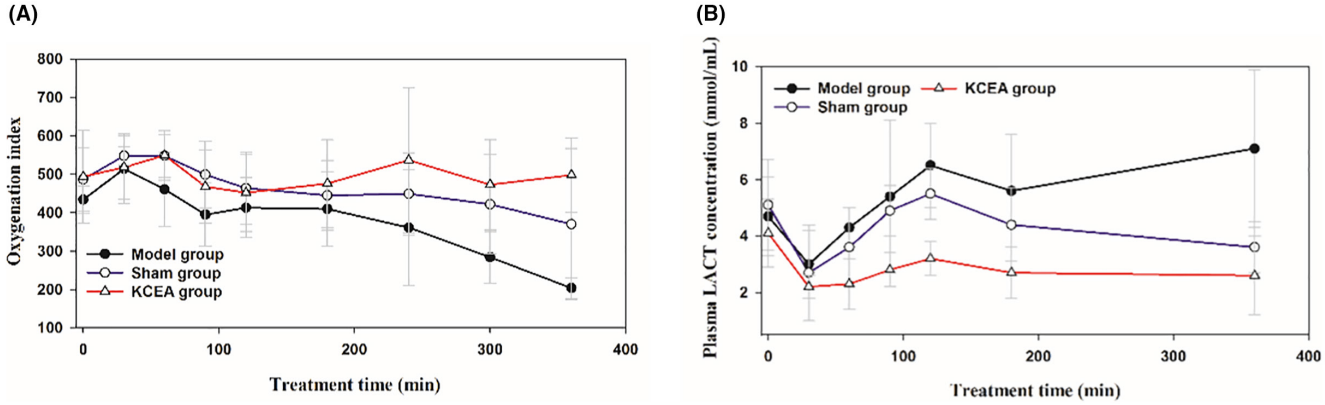


FIGURE 5 The effect of KCEA on pulmonary function and resuscitation index (A) Differences in oxygenation index among the Model, Sham and KCEA groups during the whole duration (B) Differences in LACT among the Model, Sham and KCEA groups during the whole duration

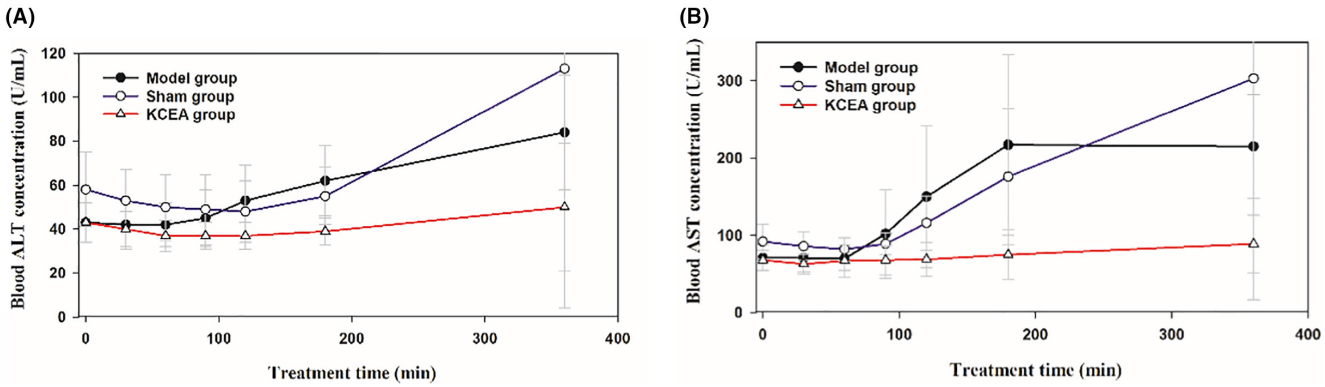


FIGURE 6 The effect of KCEA on liver function index (A) Differences in ALT among the Model, Sham and KCEA groups during the whole duration (B). Differences in AST among the Model, Sham and KCEA groups during the whole duration

at each observation time point (Figure 6A,B, $p > .05$), while the changes reached statistical difference at 180 and 360 min for ALT and AST, respectively, in the model group (ALT: $p = .046$ at 180 min, $p = .042$ at 360 min; AST: $p = .014$ at 180 min, $p = .046$ at 360 min).

3.7 | The effect of KCEA on indicators of renal function

Compared with the baseline value, there was no significant change in CRE and BUN in the KCEA group at each observation time point (Figure 7A,B, $p > .05$). There were statistical differences for CRE between the KCEA group and the model group at 180 and 360 min ($p = .037$ at 180 min, $p = .028$ at 360 min). Compared with the model and sham groups, BUN in the KCEA group did not increase significantly at 360 min, and the difference between groups was statistically significant ($p = .008$).

Compared with model and sham groups, the urine output of KCEA group increased significantly at from 180 to 360 min, and the changes reached statistical significance ($p < .05$).

3.8 | The effect of KCEA on clinical parameters

We also determined the effect of the blood compatibility of KCEA. Figure 8 depicts clinical parameters throughout the study time. During the course of treatment, the white blood cell (WBC) and blood platelet counts of each group decreased significantly (Figure 8A,B). Compared with the model group, WBC in KCEA group was statistically different at 30 min ($p = .037$) while no significant differences were observed at other time points ($p > .05$). Compared with the baseline value, there was no significant change in the hemoglobin content of the KCEA group at each observation time point (Figure 8C, $p > .05$).

The plasma prothrombin time (PT) of the KCEA group did not show obvious fluctuation during the course of treatment. Compared with the baseline value, there was no significant difference at each

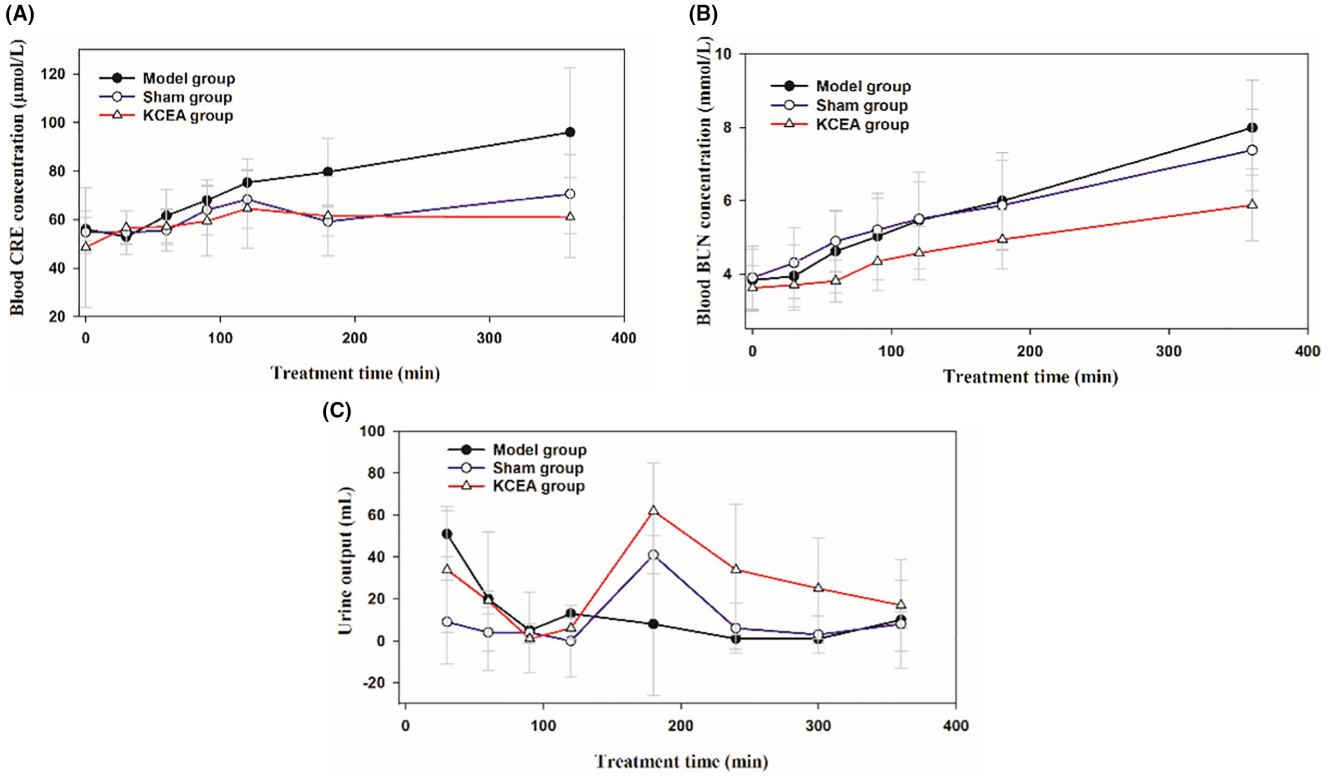


FIGURE 7 The effect of KCEA on indicators of renal function (A) Differences in CRE among the Model, Sham and KCEA groups during the whole duration (B) Differences in BUN among the Model, Sham and KCEA groups during the whole duration (C) Differencesin Urine output among the Model, Sham and KCEA groups during the whole duration

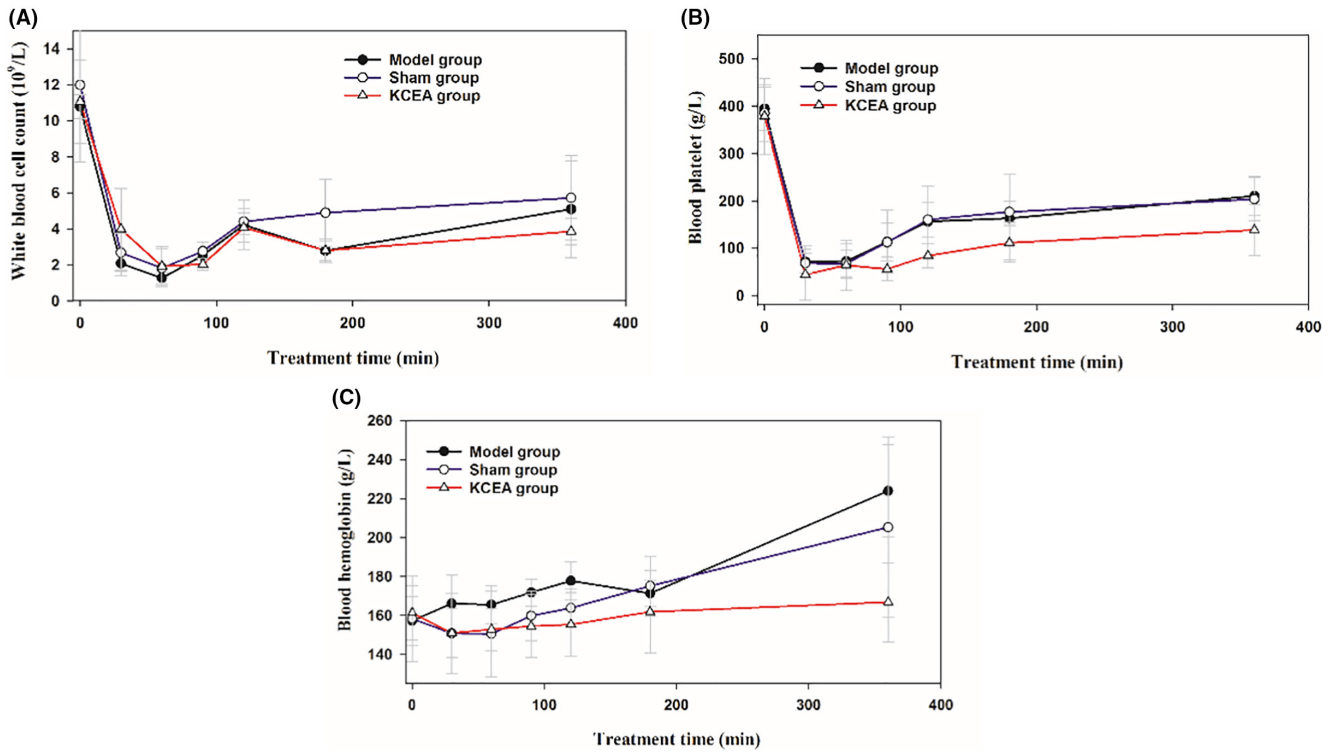


FIGURE 8 The effect of KCEA on clinical parameters WBC, PLT and Hb (A) Differences in WBC among the Model, Sham and KCEA groups during the whole duration (B) Differences in PLT among the Model, Sham and KCEA groups during the whole duration(C) Differences in Hb among the Model, Sham and KCEA groups during the whole duration

observation time point ($p > .05$). Compared with the model group, the PT of the KCEA group was shortened significantly at 360 min (Figure 9A, $p = .041$).

The plasma activated partial thrombin time (APTT) of the KCEA group increased during the course of treatment. Compared with the model and sham groups, it increased significantly at 90 min, and the differences were statistically significant (Figure 9B, $p < .05$).

The fibrinogen (FIB) of the KCEA group did not show obvious fluctuation during the course of treatment. Compared with the baseline value, there was no significant difference at each observation time point (Figure 9C, $p > .05$).

The total protein (TP) of the KCEA group did not show obvious fluctuation during the course of treatment. Compared with the baseline value, there was no significant difference at each observation time point (Figure 10, $p > .05$).

4 | DISCUSSION

LPS has been shown to cause hepatic insufficiency, gut disorders, pancreatitis, peritonitis, systemic inflammatory response syndrome, septic shock, nonseptic shock and other diseases.[18–20] Therefore, it

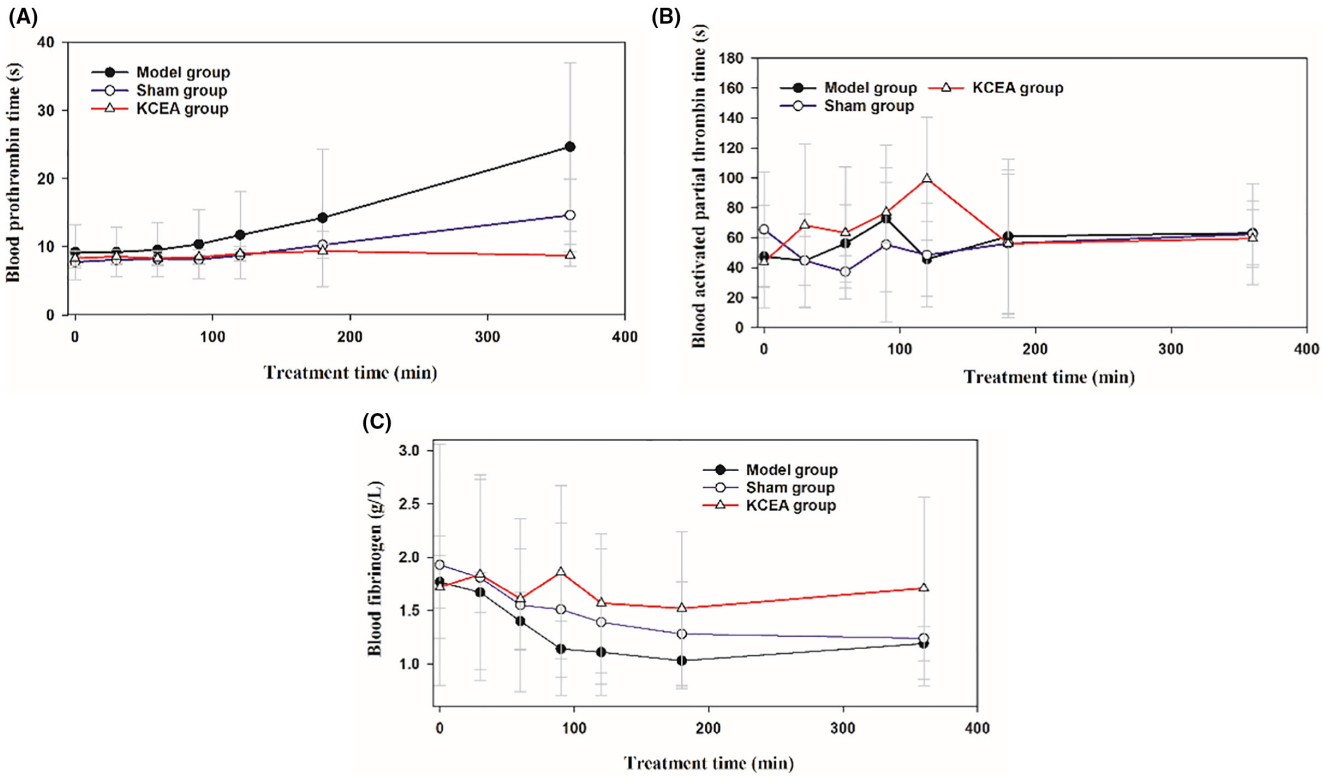
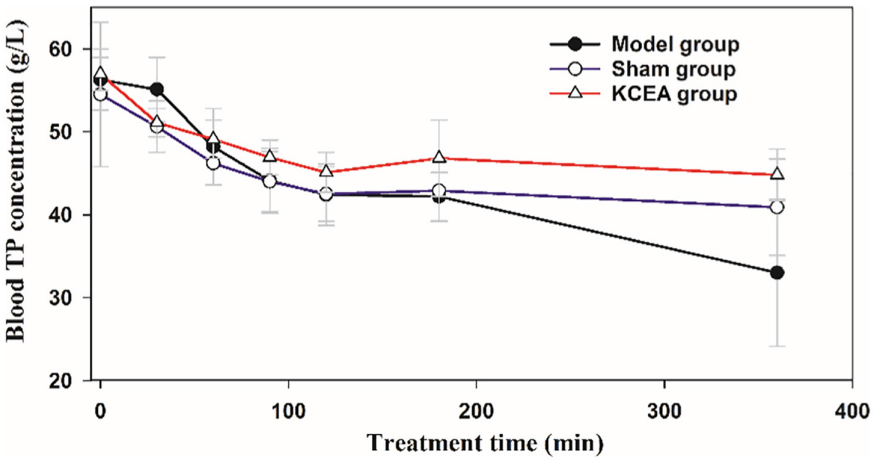


FIGURE 9 The effect of KCEA on clinical parameters PT, APTT and FIB (A) Differences in PT among the Model, Sham and KCEA groups during the whole duration (B) Differences in APTT among the Model, Sham and KCEA groups during the whole duration (C) Differences in FIB among the Model, Sham and KCEA groups during the whole duration

FIGURE 10 Differences in TP among the Model, Sham and KCEA groups during the whole duration



seems to be important to eliminate LPS from the blood during critical illness. In our vitro experiment, KCEA effectively removed endotoxin and inflammatory mediators from the blood, plasma and water. This study investigated the effect of an extracorporeal device which was designed to specifically adsorb LPS and inflammatory mediators from the blood.

In this study, we tested the efficacy of 'sorbent-strategy'-based KCEA on a beagle sepsis model and found that KCEA reduced circulating levels of endotoxin and pro-inflammatory cytokines, improved oxygenation and tissue perfusion, greatly reduced the use of noradrenaline, effectively protected organ function, and effectively improved the survival time of beagles and reduced their mortality over 7 days. This provides some evidence that a KCEA column may be a novel potential weapon in the fight against endotoxin and the 'cytokine storm'.

We demonstrated a significant efficacy of hemoperfusion over KCEA in experimental sepsis. The efficacy of blood perfusion over KCEA was more than that of sham and models, with the levels of plasma endotoxin and IL-6 in the KCEA group significantly lower than those in the sham and model groups. It might be speculated that detoxification of endotoxin by KCEA caused suppression of the release of IL-6 from macrophages and that IL-6 release was upregulated by the circulating endotoxin level. Recent studies on sepsis have focused attention on cytokines such as interleukin 1 (IL-1), IL-6, interleukin 1β (IL-1β) and TNF-α.[21,22] These studies have shown that interleukins are released during bacterial infection and mediate many systemic and organic disorders. Of the cytokines, IL-6 is an important member of the cytokine network, and it can be used to more quickly diagnose early inflammation and warn of sepsis. In this study, we demonstrated the significant suppression and clearance of IL-6 release by KCEA perfusion. We can extrapolate from our data that the endotoxin detoxification by KCEA suppressed IL-6 release and reduced IL-6 levels and improved morbidity and mortality.

The most commonly used large-animal models of sepsis include endotoxin infusion, cecal ligation and puncture (CLP), colon ascendens stent peritonitis (CASP), etc.[23]

To reproduce the most well-known risk factors and etiology for sepsis,[24] we systemically administrated LPS to mimic clinically relevant sepsis. Rats, rabbits, and dogs showed strong tolerance to LPS attacks, requiring much higher doses, tens to hundreds of times larger than those of sheep, to develop sepsis and organ damage. Once infected, they may rapidly enter shock and die.[25] In our study, 0.5 mg/kg of LPS was used to develop sepsis, which required rapid fluid resuscitation (5 ml/kg/h of saline) and the use of vasopressor infusion (0.05–1 μg/kg/min noradrenaline).

Therefore, we performed subsequent studies to explore the therapeutic effect of hemoperfusion on the well-established sepsis model. KCEA hemoperfusion was performed using a polymyxin B column modified with microporous resin specially designed for the adsorption of endotoxin and medium-sized inflammatory cytokines. First, we found that KCEA treatment can keep MAP at a high level, and at the same time greatly reduce the dosage of noradrenaline required, and reduce the probability of arrhythmia (Figure 4A,B). The improvement in hemodynamics was attributed to the KCEA adsorption of endotoxin and medium-sized inflammatory cytokines. We also found that animals treated with or without KCEA-based hemoperfusion differed in 7-day survival (Table 1). The surviving animals could stand up, move slowly and eat a little food in the first 3 days, and then returned to normal in the following 4 days, with their body weight basically returning to normal. We assumed that oxygenation improvement demonstrated effective improvement of the lung function injury caused by modeling procedures, and LACT reduction was due to the decrease of noradrenaline (NE) used in the early stage, which significantly improved the acidosis caused by tissue hypoperfusion induced by modeling procedures (Figure 5A,B). The effective improvement of the lung function injury could be partly due to a reduction of alveolar fluid leakage after clearing the peak concentration of alveolar endotoxin and cytokines, corresponding with the 'peak concentration hypothesis' for the extracorporeal blood purification (EBP) modality.[26] Taken together, and in line with a recent study,[27] by removing endotoxin and inflammatory mediators, KCEA rapidly controlled the 'cytokine storm' process of sepsis and septic shock, and restored immunologic balance at a much lower set-point.

We found that early effective endotoxin adsorption therapy intervention can reduce inflammation, ensure effective tissue perfusion, and thereby reduce the dosage of NE needed to effectively protect the function of the kidney and liver (Figures 6 and 7).

Notably we found that modeling had an effect on hematologic indexes and the trend was the same as the change of EBP in clinical situations. Compared with the baseline value, there was no significant change in the hemoglobin, FIB and TP content for the KCEA group at each observation time point ($p > .05$) (Figures 8, 9 and 10).

In the present study, we designed the controls for sham and model groups to exclude the influence of modeling and cardiopulmonary bypass on hematologic indexes. In addition, we assessed the efficacy of KCEA on an experimental sepsis model by removing the endotoxin and inflammatory mediators at the same time. However, our studies also have some limitations. This is a small sample study. Also, the effect of KCEA directed hemoadsorption should be further tested using other sepsis models. Given the high reproducibility of continuous endotoxin in infusion models, this may not necessarily mimic the situation of controlled infection in a clinical setting, although continuous application of live bacteria has been shown to have nearly identical time courses of hemodynamic and inflammatory parameters.[28] Evaluation of KCEA adsorption in different models, for example, cecal ligation and puncture (CLP),[29] colon ascendens stent peritonitis (CASP),[30] *E. coli* infusion,[31] implantation of Klebsiella pneumoniae-infected fibrin clot,[32] etc. may be a topic for futher investigation. Third, the experimental data cannot be used directly in real-world clinical practice; a randomized controlled trial (RCT) for sepsis patients is urgently needed to confirm the safety and efficacy of KCEA column further.

5 | CONCLUSIONS

In summary, in a progressive LPS-induced beagle sepsis model, KCEA therapy significantly lowered the plasma LPS levels and inflammatory mediator levels, which improved MAP and significantly lowered vasoactive drug needs, thereby protecting organ function and causing improvement of survival time and rate. In addition, good blood compatibility of KCEA was confirmed through clinical parameters. Thus, KCEA improved the pathophysiologic events of septic shock in an LPS-induced sepsis model and is worthy of further study for application in therapy of sepsis.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHOR CONTRIBUTIONS

Yonggui Li designed, made, analyzed the experiments and drafted the manuscript. Zhenggen Yang participated in the design of the experiments, the analysis of the data, the ciritical revision of the article and final approval of the version to be published. Jialiang Hu participated in the performance of the experiments and the analysis of the data. Zhennan Lin participated in the performance of the experiments. All of the authors contributed to the acquisition of data, reviewed, discussed, and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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