Fluid Resuscitation via Colon Alleviates Systemic Inflammation in Early Stage of Rats With Severe Acute Pancreatitis

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Abstract

Fluid Resuscitation Via Colon (FRVC) is a complementary therapy for severe acute pancreatitis (SAP) in early stage. The expression of intestinal dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) has been found to regulate systemic inflammation. The present study aimed to investigate the effect of FRVC on the expression of DC-SIGN in the colon tissue of SAP rats and its effect on the early response of systemic inflammatory and multiple organ injury. SAP rats were induced by retrograding injection of sodium taurocholate into biliopancreatic duct. The expression of DC-SIGN was observed in the proximal and distal colons. Histological characteristics and inflammatory cytokines were examined to compare the effect of FRVC and intravenous fluid resuscitation (IVFR) treatment. The results showed that the expression of DC-SIGN in the proximal colon increased in a time-dependent manner in early stage of SAP rats. FRVC inhibits the expression of DC-SIGN in the proximal colon. Both FRVC and IVFR treatment alleviates histological injury of pancreas and colon. However, FRVC had an advantage over IVFR in alleviating lung injury and reducing Serum TNF-α, IL-6 and LPS. These results suggest that FRVC treatment might be helpful in suppressing systemic inflammation and preventing subsequent organ failure in early stage of SAP rats. The mechanism might be to inhibit the expression of DC-SIGN protein in the proximal colon.

Introduction

Severe acute pancreatitis (SAP) is a severe systemic disease characterized by acute inflammation and necrosis of the pancreas and peripancreatic tissues[1]. It is a subtype of acute pancreatitis, accompanied by multiple organ dysfunction syndrome (MODS) and systemic inflammatory response syndrome (SIRS)[2]. The incidence of SAP is increasing in many countries and different between different socio-economic population groups[3, 4]. Although its mortality rate is gradually decreasing with the improvement of treatment methods[5], it is still a lethal disease with a mortality rate between 20% and 40%[1]. Fluid resuscitation is the main measure for the treatment of SAP to prevent hypovolemia and organ hypoperfusion. Although the classic intravenous fluid resuscitation (IVFR) can significantly reduce the mortality of patients with SAP[6, 7], the body is in a state of passively receiving fluid infusion. The absorption of fluid by the colon is an active process. Research showed that Fluid Resuscitation Via Colon (FRVC) can not only significantly improve hemodynamics, but also reduce the pathological damage of lung and liver in rats with SAP[8]. However, up to now, the mechanism is still unclear.

Dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) is a DC-specific C-type lectin-like cell-surface receptor that binds intercellular adhesion molecule-3 (ICAM-3) and intercellular adhesion molecule-2 (ICAM-2) on T cells, promoting the adhesion of DCs to naive T cells[9]. It plays an important role in the intestinal immune response[10]. Intestinal epithelial cells were found to undergo epithelial-dendritic cell transformation (EDT) by expressing DC-SIGN[11]. The previous study showed that DC-SIGN inhibition could reduce the intestinal and systemic inflammatory responses as well as the mortality of septic mice[12].

In view of this background, we hypothesized that FRVC could regulate the early inflammatory response in SAP rats by affecting the expression of colon DC-SIGN. The purpose of paper is to investigate the effect of FRVC on the expression of DC-SIGN in the colon tissue of rats with severe acute pancreatitis and its effect on the early response of systemic inflammatory and multiple organ injury.

Results

DC-SIGN expression in proximal colon of SAP rats increased with the time of SAP modeling

The protein expression of DC-SIGN was detected both in the proximal and distal colon of SAP rats. The results showed that DC-SIGN expression was almost undetectable in the proximal colon tissues of sham group and significantly increased after 12h and 24h of SAP model building ($P < 0.05$) (Fig. 1A), while the expression in the distal colon tissues of sham group was very low and not time-dependent (Fig. 1B). The immunohistochemical staining results demonstrated that DC-SIGN expression was mainly observed in colonic epithelial cells of SAP rats (Fig. 1C-D and Fig. 1E-F).

FRVC inhibits the expression of DC-SIGN in the proximal colon of SAP rats in early stage

The results of Western Blot showed that the expression of DC-SIGN in the proximal colon increased in the IVFR group compared with the NFR group, but did not reach a statistical difference. FRVC treatment significantly inhibited the expression of DC-SIGN in the proximal colon compared with the NFR treatment ($P < 0.05$) (Fig. 2A). A similar trend was observed in the distal colon, but no significant statistical difference was found (Fig. 2B). The immunohistochemistry results also showed that the DC-SIGN expression in proximal colon epithelium cells increased markedly after IVFR treatment and reduced after FRVC treatment (Fig. 2C-D), while the changes in the distal colon were not obvious (Fig. 2E-F).

FRVC alleviates organ damage of SAP rats in early stage.

Observed by HE staining, the histological damage of the pancreas in the NFR group significantly was found to be worsened compared with the SHAM group after the induction of SAP at 24 h ($P < 0.05$) (Fig. 3A). However, after IVFR or FRVC treatment, the histological damage of the pancreas was significantly relieved ($P < 0.05$). But there was no significant difference between the two treatment groups. The same situation happened on the proximal and distal colon tissue. The histological damage of the proximal and distal colon was significantly different between the SHAM group and the NFR group ($P < 0.05$) (Fig. 3B-C). Both IVFR and FRVC treatment improved the histological damage of the proximal and distal colon ($P < 0.05$). Obvious histological damage was observed in the lung with HE staining ($P < 0.05$) (Fig. 3D). But the IVFR treatment did not reduce the damage. In comparison, FRVC treatment significantly improved the lung injury compared with the NFR and IVFR group ($P < 0.05$). The histological damage of liver and kidney was mild and no significant difference was found between the four groups (Fig. 3E-F).

FRVC reduced systemic cytokines levels of SAP rats in early stage.

The serum levels of TNF-α, IL-1β, IL-6 and LPS were greatly increased in NFR group compared to sham group ($P < 0.05$) (Fig. 4). After IVFR treatment, the serum levels of TNF-α, IL-1β and LPS of SAP rats were found to decrease significantly ($P < 0.05$). All of the four systemic cytokines decreased significantly compared between the NFR and FRVC groups ($P < 0.05$). Moreover, the serum levels of TNF-α, IL-6 and LPS of FRVC groups decreased significantly compared to the IVFR groups ($P < 0.05$).

Discussion

To our knowledge, this is the first study to investigate how FRVC reduces the inflammatory response in SAP rats in early stage. Our present study showed that the increase of DC-SIGN expression in the proximal colon of SAP rats was time dependent. In addition, FRVC could down-regulate the expression of DC-SIGN in the proximal colon of SAP rats and reduce inflammation to attenuate lung injury.

EDT in rat intestine epithelial cells can be induced by hemorrhagic hypotension[11]. Under chronic inflammation, the expression of DC-SIGN in epithelial cells of the stomach, intestine and renal tubules promote the differentiation of CD4 + T cell to Th1 or Th2 cell secrete cytokines[13, 14]. Recent study[12] reported that the expression of DC-SIGN in intestinal epithelial cells can also be up-regulated in septic mice. In the present study, the increase of DC-SIGN expression was observed in the proximal colon of SAP rats. Moreover, this expression was time dependent within 24 hours after induction of SAP. intestinal epithelial cells play an antigen presentation function after DC-SIGN expression to regulate the immune inflammatory response.

FRVC, evolved from enema, is to inject liquid directly and continuously into the colon. Large intestine enema, as an adjuvant therapy for acute pancreatitis, was believed to reduce the frequency of infectious complications by reducing bacterial translocation[15] and allow the active ingredients of certain drugs to be absorbed into the blood circulation[16, 17]. However, the clinical application of FRVC has not been promoted. When performing enema for SAP rats in early stage, that the colon could quickly absorb a large amount of fluid and improve hemodynamics were accidentally found[8]. Further study[18] showed that the mechanism of FRVC to improve hemodynamics might be through regulating the expression of aquaporins in the colon of rats in early stage of SAP. In our study, the expression of DC-SIGN in the proximal colon of SAP rats was mainly observed in colonic epithelial cells and could be inhibited by FRVC treatment. However, it is not clear whether the source of DC-SIGN is the migration of immature DC from the circulatory system to the inflammatory tissue or the occurrence of EDT in colon epithelial cells. Zeng et al[19] showed that the colon epithelial cells play an antigen presentation function after DC-SIGN expression to promote T cell proliferation and regulate the immune inflammatory response. Using siRNA to inhibit the expression of DC-SIGN in intestinal epithelial cells can significantly inhibit the intestinal immune inflammatory response and protect multiple organ functions[12]. Therefore, we speculate that while FRVC inhibits the expression of DC-SIGN in the proximal colon of SAP rats, it may also inhibit the intestinal immune response.

Gut immune response and microbiota play an important role in the occurrence and development of pancreatitis[20, 21]. For a long time, the role of the intestine in critical illness has attracted much attention and been regarded as the motor of MODS[22]. Colon epithelial cells are in a huge number and cooperate with intestinal immune cells (Paneth cells, lymphocytes, etc.) and intestinal flora to regulate the steady-state of the intestinal local immune response[23]. Previous studies paid more attention to the role of immune cells in the intestinal immune inflammatory response, and ignored the role of colon epithelial cells. Classical theory believes that intestinal epithelial cells are the lining cells of the intestinal lumen, which mainly perform absorption and barrier functions[24]. However, several reports[25–27] have shown that intestinal epithelial cells have unique immune regulation functions and participated in the intestinal immune inflammatory response. A large number of inflammatory factors produced by the uncontrolled intestinal immune inflammatory response enter the blood circulation through the lymphatic pathway and lead to SIRS and MODS. Intravenous infusion of mesenteric lymph from severe intraperitoneal infection rats induce lung injury in healthy rats[28] while mesenteric lymph duct ligation attenuates lung injury after intraperitoneal injection of endotoxin in rats[29]. Weakened DC-SIGN or DC-SIGN/TLR-4 complex activation might be beneficial in acute kidney injury[30]. All these suggest that inhibiting the uncontrolled intestinal immune inflammatory response is one of the effective measures to block SIRS and MODS. And as expected, our findings are in concordance with the previous study[8]. The histological damage of pancreas, proximal colon and distal colon can be improved by IVFR or FRVC treatment. The link between bowel injury, damage of gut barrier, bacterial translocation, and organ failure is very close[31]. The lung histological injuries were only significantly improved in FRVC group. But the same phenomenon failed to reproduce in the histological manifestations of liver and kidney. The possible explanation for this might be that sample size was small.

The present study also demonstrated that FRVC reduced systemic inflammatory factors in the serum at the same time. Although IVFR and FRVC treatment were helpful in reducing serum TNF-α, IL-1β, and LPS, FRVC treatment reduces serum TNF-α and LPS more. LPS interacts with Toll-like receptor 4 (TLR4) to initiate a complex signal pathway and results in a proinflammatory response that damages the lungs[32]. This further explained the cause of lung histological damage. Both TNF-α and IL-6 mediate injury in acute pancreatitis[33]. The level of IL-6 is an important indicator for predicting SAP[34] and was significantly decreased in FRVC group. These findings suggest that FRVC treatment has a greater effect on reducing early response of systemic inflammatory of SAP rats.

There are some limitations in this study. First, since the establishment of the SAP rat model of FRVC requires continuous anesthesia for up to 12 hours, in order to eliminate the influence of anesthesia, the rats in the SHAM group were also continuously anesthetized for 12 hours. It might affect the pathophysiology of the rat. Second, it is very difficult to calculate how much normal saline is absorbed by the colon. When the catheter used for fluid resuscitation was removed, some normal saline was discharged from the intestine. Also, normal saline could be excreted when the rats were awake. Third, the present study did not further explorer the relationship between the expression of DC-SIGN in the colon of

SAP rats and lung injury. Further research is needed to investigate the effect of colon DC-SIGN expression on lung injury.

Conclusion

In summary, this is the first study to show the mechanism of FRVC treatment to reduce SIRS and MODS in SAP rats in early stage. We demonstrated that inhibiting the expression of DC-SIGN in proximal colon of SAP rats by FRVC treatment reduced the histological damage of the pancreas, colon and lungs of SAP rats, and reduces the concentration of systemic inflammatory factors of SAP rats in an early stage. These results indicated that FRVC, as a supplementary method for intravenous fluid resuscitation, might be helpful in suppressing SIRS at early stage and preventing subsequent organ failure. Yet, the relationship between DC-SIGN and organ damage still needs further study.

Materials And Methods

Animals

Clean grade healthy Sprague-Dawley (SD) rats (male, 6–7 weeks, 250–300g) were purchased from Zhejiang Vital River Laboratory Animal Technology Company Limited. All rats were housed in an air-conditioned environment (ambient temperature controlled at 25±0.5℃, humidity controlled at 50%–60%) with a 12/12-h light/dark cycle in animal experiment center of Ruijin Hospital. Food and water were freely accessible by rats. The study was performed in accordance with the Principles of Laboratory Animal Care (NIH publication no.85Y23, revised 1996) and in compliance with the ARRIVE guidelines. All experiments were approved by the Animal Ethics Committee of Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine and carried out according to the institutional guidelines.

SAP modeling and experimental design

In the first stage, twenty-four rats were randomly assigned into four groups (n=6 in each group) to observe the expression of DC-DIGN in colon tissue: sham group, SAP 6h group, SAP 12h group and SAP 24h group. After screening out the time point with the highest expression of DC-SIGN in colon tissues, another twenty-four rats were divided into four groups according to the random number table, namely sham group, non-fluid resuscitation (NFR) group, IVFR group and FRVC group.

SAP model was established according to the method described by Aho et al.[35]. The rats were anesthetized by using isoflurane (RWD Life Science, Shenzhen, China). After the pancreatic duct at the tail of the pancreas was blocked by the vascular clip, closed venous indwelling needle (BD Company, Shanghai, China) was retrogradely penetrated into the pancreatic duct through the duodenal intestinal wall. 5% sodium taurocholate solution (0.1 mL/100 g body weight, Sigma, United States) was injected into the pancreatic duct with a microinjection pump. Before pulling out the needle, maintaining the pressure in the pancreatic duct for 5 minutes.

IVFR and FRVC operation of SAP rats were performed according to the previous study[8] after the SAP models were completed. In IVFR model of SAP rats, normal saline was continuously infused at a rate of 4ml/kg/h for 12 hours with a microinjection pump through a Y-type trocar implanted in the right femoral vein. The FRVC operation method was to insert a Swan-Ganz floating catheter from the anus, inject about 0.5ml of air into the balloon to fix the catheter, and inject saline at the same speed as the IVFR model. In the sham group, the abdominal cavity of rat was opened and closed without other operation. The serum samples and tissues were immediately isolated and stored at -80°C until analysis.

Western blot

The protein concentration was measured using a BCA protein assay kit (Servicebio, Wuhan, China).20 μg of the protein samples were loaded onto 10% sodium dodecyl sulfate–polyacrylamide gel for electrophoresis and transferred to polyvinylidene fluoride membranes (Millipore, Temecula, Calif). After being blocked with 5% skim milk at room temperature for 1 h, membranes were incubated overnight at 4℃ with rabbit primary antibodies of DC-SIGN (1:2000, Thermo Fisher scientific, Shanghai, China) and with HRP-conjugated secondary antibodies for 1 hour at room temperature. The blots were detected by chemiluminescence using the ECL reagent (Servicebio, Wuhan, China). The results were visualized using darkroom development techniques. The bands were analyzed with the AlphaEaseFC software and compared with GAPDH.

Immunohistochemical

For immunohistochemical detection of DC-SIGN expression, tissue sections on glass slides were placed in the citrate antigen retrieval solution (PH 6.0) retrieve the antigen. After being treated with endogenous peroxidase and nonspecific protein blocking, the sections were overnight incubated with DC-SIGN primary antibody (1:100, A01025-2, Boster Biological Technology Co., Ltd., Calif) at 4℃ and then washed three times for 5 min each with PBS. After being incubated with biotinylated secondary antibody (1:200, GB23303, Servicebio, Wuhan, China) for 1 h at room temperature, the sections were stained by diaminobenzidine for microscopic examination.

Histological analysis

The pancreas, colon, lung, liver, and kidney tissues were fixed in 4% paraformaldehyde for 24 hours. The target tissues were dehydrated and waxed. Then, the tissues were embedded and cut into 4-μm thick slices for hematoxylin and eosin (HE) staining. Two senior pathologists separately made evaluation on the tissues through an optical microscope. All of the histopathology evaluations were performed on six fields per section under 100× magnification and scored according to the previous study[36-38].

Enzyme-linked immunosorbent assays

Enzyme-linked immunosorbent assays (ELISAs) were performed with the serum samples of rats using commercial rat-specific kits for tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6)

and Lipopolysaccharide (LPS) (Multi Sciences Biotech Co., Ltd. Hangzhou, China) according to the product specifications.

Statistical Analysis

The clinical data were analyzed by SPSS 19.0 statistical software (SPSS, Inc., Chicago, IL) and GraphPad Prism 6 (GraphPad Software, San Diego, Calif). Differences between groups were analyzed using a one-way ANOVA and Mann-Whitney test. Data are expressed as mean ± standard error of mean (SEM). $P<0.05$ was considered statistically significant.

Declarations

Data Availability

The data used to support the findings of the study are available from the corresponding author upon request.

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Author Contributions

E.M., Y.C. and E.C conceived the experiments, reviewed and revised the manuscript. T.N., L.X., S.S. and Y.W. carried out the experiments and wrote the manuscript; N.N. and L.M. analyzed data. W.Z. and B.Z. provided comments and technical advice; All authors have read and approved the submitted manuscript.

Additional Information

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Figures

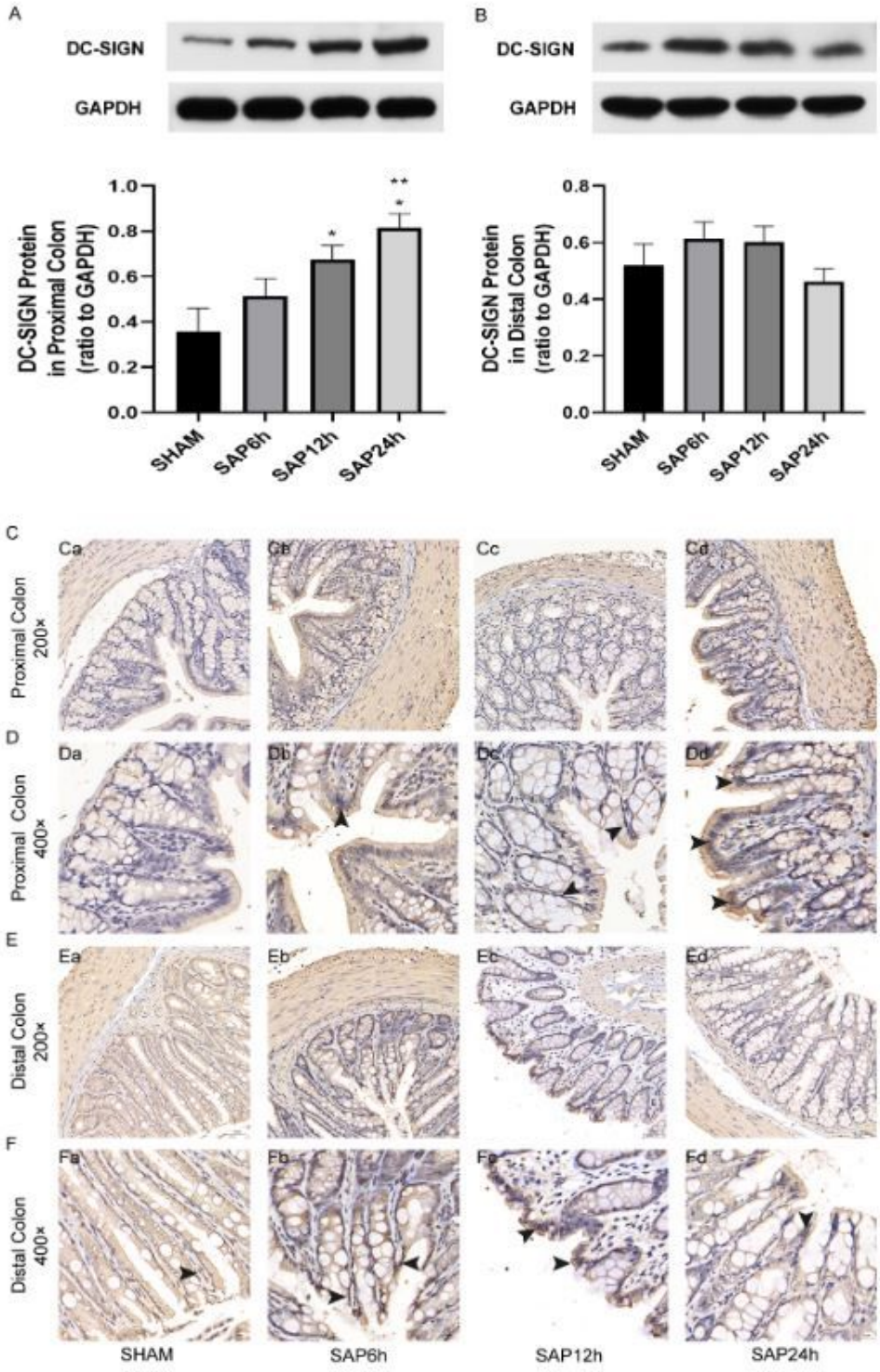

Figure 1

The expression of DC-SIGN in proximal and distal colons of SAP rats at 6, 12, and 24 h. (A) Western blot analysis of DC-SIGN expression in proximal colon. (B) Western blot analysis of DC-SIGN expression in distal colon. (C-D) Immunohistochemical staining of DC-SIGN expression in proximal colon. (E-F) Immunohistochemical staining of DC-SIGN expression in distal colon. *$p < 0.05$ compared to SHAM, **$p < 0.05$ compared to SAP6h.

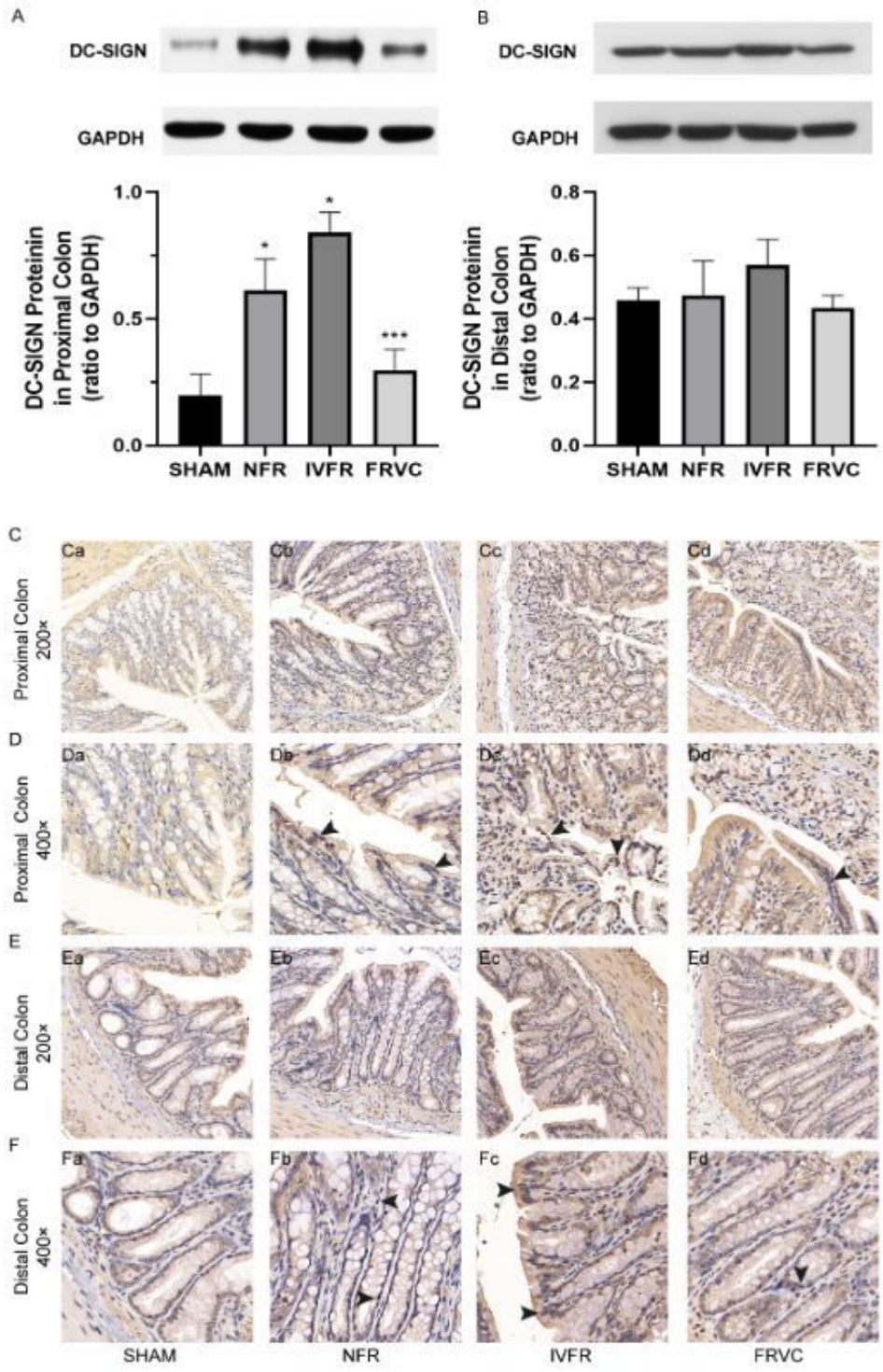

Figure 2

FRVC inhibits the expression of DC-SIGN in the proximal colon of SAP rats. (A) Western blot analysis of DC-SIGN expression in proximal colon. (B) Western blot analysis of DC-SIGN expression in distal colon. (C-D) Immunohistochemical staining of DC-SIGN expression in proximal colon. (E-F) Immunohistochemical staining of DC-SIGN expression in distal colon. *p < 0.05 compared to SHAM, **p < 0.05 compared to NFR; ***p < 0.05 compared to IVFR. SHAM: the rats treated with sham operation and no

fluid resuscitation; NFR: the rats treated with the SAP operation and no fluid resuscitation; IVFR: the rats treated with the SAP operation and intravenous normal saline infusion; FRVC: the rats treated with the SAP operation and normal saline infusion via colon.

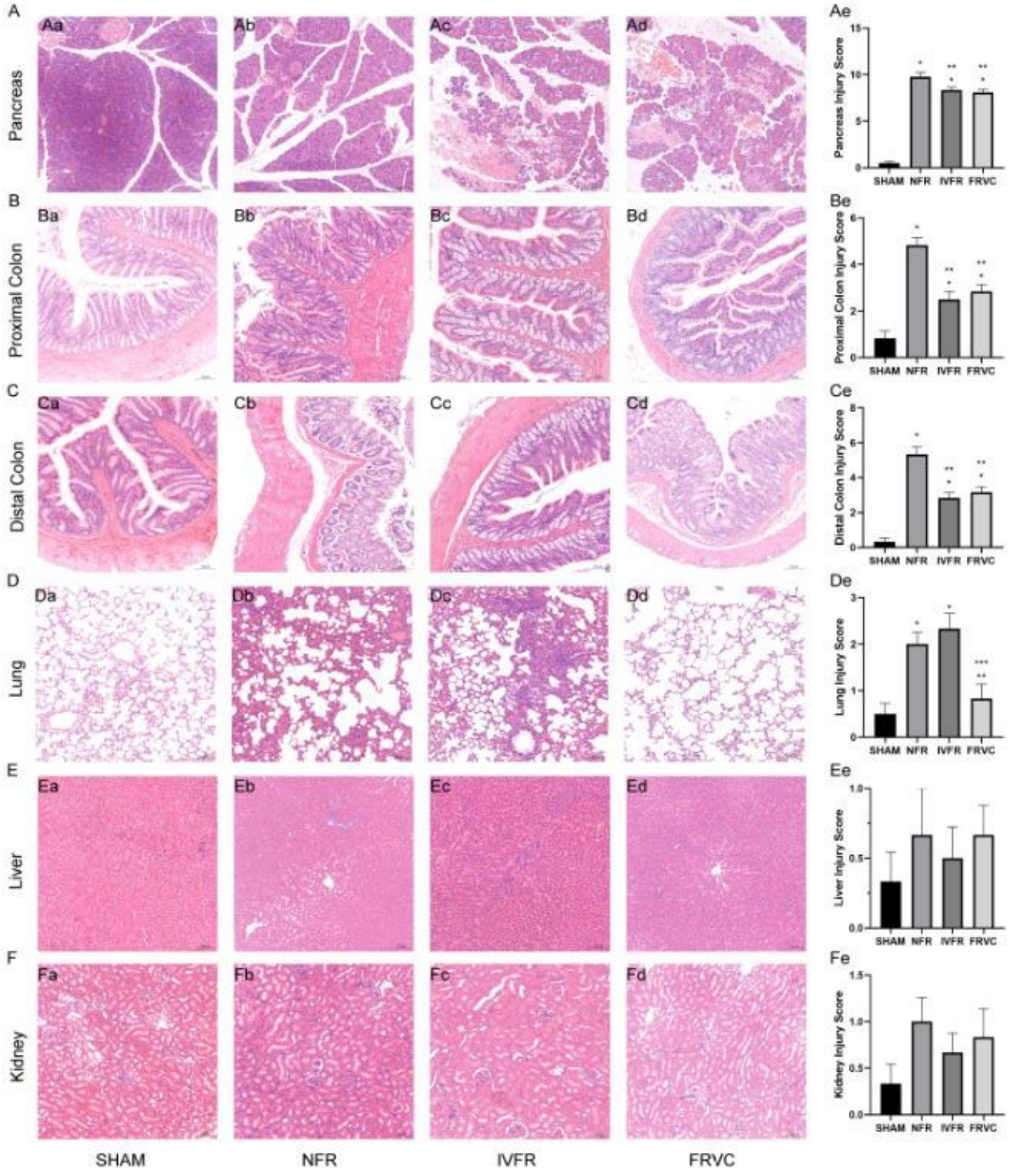

Figure 3

Organ damage of SAP rats and SAP rats treated with IVFR or FRVC. (A–F) The severity of pancreas, proximal colon, distal colon, lung, liver, and kidney injury was assessed by HE staining and histological

scores. Magnification: 100×. Scale bar: 100 μm. *p < 0.05 compared to SHAM, **p < 0.05 compared to NFR; ***p < 0.05 compared to IVFR. SHAM: the rats treated with sham operation and no fluid resuscitation; NFR: the rats treated with the SAP operation and no fluid resuscitation; IVFR: the rats treated with the SAP operation and intravenous normal saline infusion; FRVC: the rats treated with the SAP operation and normal saline infusion via colon.

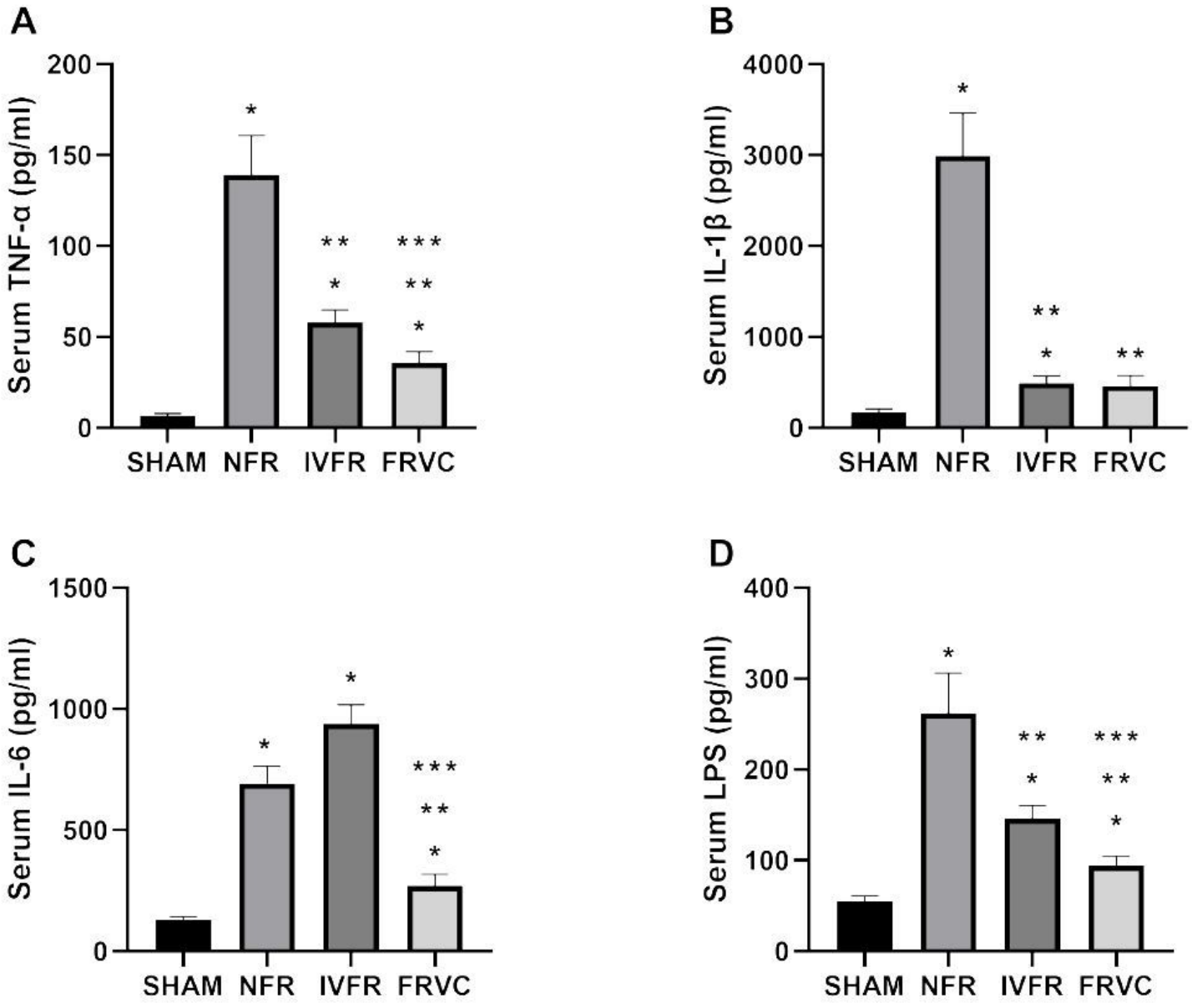

Figure 4

Changes of systemic cytokines levels in SAP rats and SAP rats treated with IVFR or FRVC. (A-D) The levels of TNF-α, IL-1β, IL-6 and LPS in serum were analyzed by ELISA. *p < 0.05 compared to SHAM, **p < 0.05 compared to NFR; ***p < 0.05 compared to IVFR. SHAM: the rats treated with sham operation and no fluid resuscitation; NFR: the rats treated with the SAP operation and no fluid resuscitation; IVFR: the rats treated with the SAP operation and intravenous normal saline infusion; FRVC: the rats treated with the

SAP operation and normal saline infusion via colon. TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; LPS, lipopolysaccharide.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInfoFile.pdf