

# Alterations of intestinal immune function and regulatory effects of L-arginine in experimental severe acute pancreatitis rats

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## Abstract

**AIM:** To discuss the changes of intestinal mucosal immune function in rats with experimental severe acute pancreatitis (SAP) and the regulatory effect of L-arginine.

**METHODS:** Male adult Wistar rats were randomly divided into pancreatitis group, sham-operation group, and L-arginine treatment group. Animals were killed at 24, 48, and 72 h after SAP models were developed and specimens were harvested. Endotoxin concentration in portal vein was determined by limulus endotoxin analysis kit. CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup> T lymphocytes in intestinal mucosal lamina propria were examined by immunohistochemistry. Secretory immunoglobulin A (SIgA) in cecum feces was examined by radioimmunoassay.

**RESULTS:** Compared to the control group, plasma endotoxin concentration in the portal vein increased, percentage of CD3<sup>+</sup> and CD4<sup>+</sup> T lymphocyte subsets in the end of intestinal mucosal lamina propria reduced significantly, CD4<sup>+</sup>/CD8<sup>+</sup> ratio decreased, and SIgA concentrations in cecum feces reduced at 24, 48, and 72 h after SAP developed. Compared to SAP group, the L-arginine treatment group had a lower level of plasma endotoxin concentration in the portal vein, a higher CD3<sup>+</sup> and CD4<sup>+</sup> T lymphocyte percentage in the end of intestinal mucosal lamina propria, an increased ratio of CD4<sup>+</sup>/CD8<sup>+</sup> and a higher SIgA concentration in cecum feces.

**CONCLUSION:** Intestinal immune suppression occurs in the early stage of SAP rats, which may be the main reason for bacterial and endotoxin translocation. L-arginine can improve the intestinal immunity and reduce bacterial and endotoxin translocation in SAP rats.

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**Key words:** Acute pancreatitis; Immunity; Intestinal mucosa; L-arginine

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## INTRODUCTION

Infection is a common and usually lethal complication during severe acute pancreatitis (SAP). In previous studies, the prevalence of infection in SAP varies between 40% and 70%, and most of the deaths of patients are related with infection. The gastrointestinal tract may be the origin of bacterial translocation during SAP, and SAP may influence the gut barrier function, leading to the occurrence of gut origin sepsis. In this study, we discussed the changes of intestinal immunity in SAP rats.

## MATERIALS AND METHODS

### *Animals and materials*

Male Wistar rats weighing 250-300 g; L-arginine (Sigma Chemical Co.); sodium taurocholate (Sigma Chemical Co.); mouse anti-rat anti-CD3, CD4, CD8 mAb (Santa Cruz Co.); SIgA radioimmunoassay kit (Institute of Atomic Energy Physics Chinese Academy of Sciences, Beijing); limulus endotoxin analysis kit (Shanghai Medical Laboratory) were used in the study.

### *SAP model and experimental groups*

Rats were fasted for 14 h before the experiment. Animals were anesthetized by intraperitoneal injection of 10% chloral (3 mL/kg) and then SAP models were developed as previously described<sup>[1]</sup>. Under sterile conditions, a middle laparotomy was performed. The SAP rat model was developed by pumping 3% sodium taurocholate (TCA) into the pancreatic tract at a rate of 18 mL/h (1 mL/kg). Fifty-four rats were randomly divided into SAP group ( $n = 18$ ), sham group ( $n = 18$ ), and L-arginine treatment group ( $n = 18$ ). The rats were allowed to drink 1% L-arginine water for 5 d before SAP model was developed. At 24, 48, and 72 h after the model was developed, animals from each experimental group were killed and specimens were harvested.

### *Analysis of serum endotoxin in portal vein by limulus methods*

The serum endotoxin concentration in portal vein was assayed by chromogenic limulus analysis kit according to the manufacturer's protocol.

### Immunohistochemistry

Intestinal tissue fragment (3 cm from cecum) was cut and fixed by immersing in 4% paraformaldehyde immediately. The specimens were embedded in regular paraffin wax and cut into 4- $\mu$ m-thick sections. Tissue sections were deparaffinized and rehydrated in PBS. Endogenous peroxidase activity was blocked by incubation with 3% H<sub>2</sub>O<sub>2</sub>/PBS for 10 min. After being immersed in 1% BSA/PBS at 37 °C for 1 h, the sections were incubated with mouse anti-rat anti-CD3, CD4, CD8 mAb at 4 °C overnight. The specimens were incubated with peroxidase-conjugated goat anti-rat secondary antibody for 2 h at room temperature. Finally, the specimens were immersed in DAB for 10 min. Brown staining cells were defined as positive cells and counted in four high microscope view, the percentage of positive cells and CD4+/CD8+ ratio were calculated.

### Analysis of SIgA content by radioimmunoassay methods

Cecum feces were diluted with water to double volumes. Samples were shaken at 4 °C overnight and then examined by SIgA radioimmunoassay test kit.

### Statistical analysis

Data were analyzed by *t* test and expressed as mean $\pm$ SD. *P*<0.05 was considered statistically significant.

## RESULTS

### Changes of serum endotoxin in portal vein of rats

Compared to the control group, plasma endotoxin concentration in the portal vein increased significantly 24, 48, and 72 h after SAP model was developed (*P*<0.01). The L-arginine treatment group had a lower level of endotoxin concentration than SAP group (*P*<0.01, Table 1).

**Table 1** Changes of serum endotoxin in rat portal vein (U: Eu/mL)

Group	24 h	48 h	72 h
NS	0.041 $\pm$ 0.011	0.039 $\pm$ 0.007	0.042 $\pm$ 0.015
SAP	0.157 $\pm$ 0.024 <sup>b</sup>	0.150 $\pm$ 0.018 <sup>b</sup>	0.146 $\pm$ 0.014 <sup>b</sup>
L-Arg	0.087 $\pm$ 0.011 <sup>d</sup>	0.079 $\pm$ 0.016 <sup>d</sup>	0.082 $\pm$ 0.013 <sup>d</sup>

<sup>b</sup>*P*<0.01 vs NS; <sup>d</sup>*P*<0.01 vs SAP.

**Percentage of CD3+, CD4+, CD8+ T lymphocytes in intestinal mucosal lamina propria** Percentage of CD3+, CD4+ T lymphocytes subsets in the end of intestinal mucosal lamina propria in SAP group reduced significantly and the CD4+/CD8+ ratio decreased. Compared to the SAP group, the L-arginine treatment group had a higher CD3+, CD4+ T lymphocyte percentage and a higher ratio of CD4+/CD8+ (Table 2).

**Changes of SIgA content in cecum feces** SIgA concentrations in cecum feces reduced significantly 24, 48, and 72 h after SAP was developed. SIgA concentrations in cecum feces increased in the L-arginine treatment group (Table 3).

## DISCUSSION

SAP is a very common disease in clinical surgery. Though the diagnosis and therapy have been improved, its mortality

**Table 2** Percentage of CD3+, CD4+, CD8+ T lymphocytes in intestinal mucosal lamina propria (mean $\pm$ SD)

Group		24 h	48 h	72 h
CD3+	NS	20.54 $\pm$ 3.46	22.32 $\pm$ 4.78	21.24 $\pm$ 5.32
	SAP	11.24 $\pm$ 3.06 <sup>b</sup>	12.07 $\pm$ 4.24 <sup>b</sup>	11.07 $\pm$ 3.16 <sup>b</sup>
	L-Arg	14.36 $\pm$ 2.12 <sup>c</sup>	17.45 $\pm$ 3.52 <sup>c</sup>	15.42 $\pm$ 4.18 <sup>c</sup>
CD4+	NS	12.78 $\pm$ 3.39	11.56 $\pm$ 3.14	12.47 $\pm$ 2.53
	SAP	7.23 $\pm$ 1.46 <sup>a</sup>	8.34 $\pm$ 1.69 <sup>a</sup>	9.06 $\pm$ 2.94 <sup>a</sup>
	L-Arg	9.85 $\pm$ 2.51	9.22 $\pm$ 2.75	9.42 $\pm$ 2.72
CD8+	NS	9.69 $\pm$ 1.65	8.89 $\pm$ 1.95	9.41 $\pm$ 2.57
	SAP	9.73 $\pm$ 2.75	9.12 $\pm$ 2.47	8.12 $\pm$ 3.59
	L-Arg	8.72 $\pm$ 2.63	9.05 $\pm$ 3.95	7.87 $\pm$ 1.79
CD4+/CD8+	NS	1.32	1.30	1.35
	SAP	0.74	0.91	0.96
	L-Arg	1.13	1.02	1.07

<sup>a</sup>*P*<0.05 vs NS; <sup>b</sup>*P*<0.01 vs NS; <sup>c</sup>*P*<0.05 vs SAP.

**Table 3** Changes of SIgA content in cecum feces (mean $\pm$ SD,  $\mu$ g/mL)

Group	24 h	48 h	72 h
NS	8.70 $\pm$ 2.69	8.52 $\pm$ 1.84	9.24 $\pm$ 1.73
SAP	4.75 $\pm$ 1.07 <sup>a</sup>	4.45 $\pm$ 0.94 <sup>a</sup>	4.69 $\pm$ 1.28 <sup>a</sup>
L-Arg	7.57 $\pm$ 1.69 <sup>c</sup>	7.55 $\pm$ 1.45 <sup>c</sup>	8.42 $\pm$ 2.17 <sup>c</sup>

<sup>a</sup>*P*<0.05 vs NS; <sup>c</sup>*P*<0.05 vs SAP.

is 10-20%. Secondary pancreatic infection and multiple organ dysfunction syndrome (MODS) are major causes of death in patients with SAP. Infection is the most common clinical syndrome at present and the morbidity in SAP patients is about 40-70%<sup>[2]</sup>. Infection is also an important factor for SAP followed by MODS<sup>[3]</sup>. Bacterial infection is devastating for the pancreas and other tissues, and increases the mortality of acute pancreatitis patients. Clinical data and animal experimental studies have shown that most bacteria-associated pancreatic and peripancreatic infection are of enteric origin<sup>[4]</sup>, and the gut seems to be the principal source of the infection<sup>[5]</sup>. The gastrointestinal tract is regarded as the largest immune organ in the body containing diverse immunocytes to prevent bacterial and endotoxin translocation from indigenous gut flora. Viable enteric bacteria must pass through the intestinal mucosal barrier to extraintestinal sites, leading to gut-associated infection. During the course of SAP, gastrointestinal tract may be attacked and gut-barrier function is damaged, allowing a large amount of bacteria and endotoxin to enter into the systemic circulation, leading to bacteremia and endotoxemia that may cause more severe complications. Prevention of gut bacterial translocation is most important in avoiding extraintestinal infection and improving the prognosis of SAP patients.

SIgA plays an important role in intestinal mucosal defense and is the first line of defense on intestinal and extra-intestinal mucosal surfaces. SIgA can prevent bacterial adherence and subsequent invasion of the mucosal surface, which is considered crucial in initiating mucosal invasion and infection. SIgA deficiency results in bacterial overgrowth, adherence and translocation<sup>[6]</sup>. Our results showed that SIgA concentration in cecum feces of the SAP group reduced significantly, indicating that SIgA secretion from intestinal mucosa to the intestinal tract was decreased in SAP rats. SIgA deficiency may increase dissociative bacteria and

endotoxin concentration in the intestinal tract, and their migration through the intestinal mucosa and entry into the portal vein blood<sup>[7]</sup>.

CD3 molecules are expressed on the surface of all mature T lymphocytes and can be used to measure the total number of mature T lymphocytes. In our study, the number of CD3+, CD4+ T lymphocytes in intestinal mucosal lamina propria reduced significantly in SAP rats and remained at the lower level at 72 h. The ratio of CD4+/CD8+ T lymphocytes markedly decreased, indicating that mature T lymphocytes are decreased in intestinal mucosa and immune function is suppressed in SAP rats, while CD8+ T lymphocytes do not change significantly. Because the majority of T lymphocytes in intestinal mucosal lamina propria are CD4+ T lymphocytes, change of CD8+ T lymphocytes is not the main trend<sup>[8]</sup>. Further experiments are needed to validate the results. Since the number of CD4+ T lymphocytes reduces, cytokines including IL-2, IL-4, IL-6, IL-7, IL-12, IFN- $\gamma$  are secreted by CD4+ T helper (T<sub>H</sub>) lymphocytes and the number of active effector Tc cells reduces accordingly. Thus, the intestinal immune function decreases significantly in SAP rats.

L-arginine is important for the proliferation and maturation of CD3+ T lymphocytes. Dietary arginine may increase the number of CD4+ and CD8+ T lymphocytes as well as the IgG and IgA content<sup>[9-11]</sup>. Our results have confirmed that oral supplement of L-arginine increases the number of CD3+ and CD4+ T lymphocytes as well as the ratio of CD4+/CD8+ T lymphocytes in intestinal mucosal lamina propria and SIgA concentration in cecum feces in SAP rats, suggesting that L-arginine can improve the immune responsibility of intestinal mucosa and decrease the bacterial and endotoxin translocation in SAP rats.

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