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Original Article

Peritoneal lavage with ulinastatin reduced the expression levels of P-selectin protein in severe acute pancreatitis rats

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Abstract: Objective: To observe the effect and mechanisms of peritoneal lavage with ulinastatin on the inflammatory reaction and expression and staining intensity of P-selectin in SAP rats. Methods: Severe acute pancreatitis (SAP) model of rat was made by retrograde infusion of 5% sodium taurocholate into the pancreatic duct. After establishment of SAP model, intraperitoneal lavage was performed immediately in saline lavage group and peritoneal lavage with ulinastatin group, ulinastatin injection was also performed immediately in ulinastatin injection group. At 3 h after induction of SAP in all groups, TNF-α, and IL-10 and the expression levels, staining intensity of P-selectin protein of multiple organs in each group were detected. Results: Group IU and UL reduced the level of serum TNF-α and increased IL-10 effectively, Group UL exert the better effect. In Group SL, IU and UL, the expression levels and the staining intensity of P-selectin protein in the multiple organs were significantly lower than those in Group SAP and Group UL exert the best effect. Conclusions: Inflammatory condition could be well improved by intraperitoneal lavage with ulinastatin and may through inhibiting P-selectin protein expression in multiple organs.

Keywords: Intraperitoneal lavage, severe acute pancreatitis, ulinastatin, P-selectin

Introduction

Multiple organ dysfunction syndrome (MODS) may easily happen during the early stage of SAP due to the excessive expression of inflammatory mediators may cause systemic inflammatory response syndrome (SIRS). P-selectin is a glycoprotein involved in the inflammatory reaction through mediating the adhesion of activated platelets and leukocytes which plays a crucial role in the development and progression of inflammation in SAP [1, 2]. Studies reported that anti-P-selectin antibody would reduce leukocytes infiltration in multiple organs so that prevent the tissue injury in SAP [3]. Therefore, suppressing the expression of P-selectin can effectively mitigate the degree of inflammation [4]. Ulinastatin, a serine protease inhibitor that is found in human urine and blood, has been widely used in acute inflammatory disorders such as acute pancreatitis [5, 6]. Ulinastatin may inhibits inflammatory proteases through anti-inflammatory properties apart from blocking of protease pathway [6]. To clarify the direct contributions of ulinastatin to inflammatory condition in peritoneal lavage way, the present study would comparing the serum levels of TNF-α and IL-10 to explored the therapeutic effects on inhibiting inflammatory mediators and comparing the expression levels of P-selectin protein in multiple organs to found the underlying mechanism.

Methods

Experimental animals and animal model

Experiments were performed on 50 male Wistar rats weighing 300 ± 15 g. Animals were supplied by the Experimental Animal Center of General Hospital of the PLA. All experimental rats received humane care.

The rats were anesthetized by intraperitoneal injection of chloral hydrate (10%, 3 ml/kg); then we used the microinfusion pump for continuous transfusion (5% sodium taurocholate 0.6 ml,

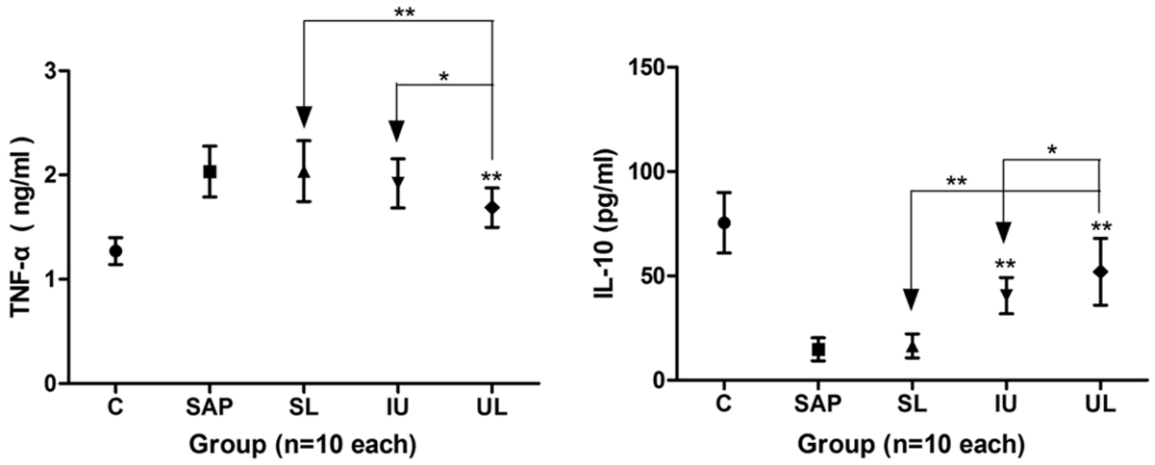

Figure 1. Effect of peritoneal lavage with ulinastatin on TNF-α and IL-10. TNF-α in plasma of Group SAP were significantly increased and IL-10 was significantly decreased than Group C. Group SL, IU and UL could reduce TNF-α and rise IL-10 compared with Group SAP. Moreover, Group UL exert the best effect than Group SL and IU. (*P<0.05, **P<0.01).

0.2 ml/min) retrograde into the pancreatic duct to established a high mortality rate SAP model (> 80% within 12 h). The method is referred to a published work [7].

Two catheters (A and B) with five lateral outlets were inserted into the abdomen in all groups before closing. Adjacent to the pancreas placed Catheter A and pelvic cavity placed Catheter B.

Reagents

Chloral hydrate were purchased from Shanghai Yingxin Laboratory Equipment Co., Ltd., China; Sodium taurocholate were purchased from Shanghai Hufeng Biotechnology Co., Ltd., China; Ulinastatin were purchased from Guangdong Tianpu Biochemical Pharmaceutical Co., Ltd., China; IL-10 and TNF-α assay kit were purchased from Shanghai Hengyuan Biological Technology Co., Ltd., China. Rat soluble P-selectin ELISA Kit were purchased from Beijing Fangcheng Biological Technology Co., Ltd., China.

Experimental groups

50 male Wistar rats were randomly divided into the following groups with 10 rats in each: normal control group (Group C), after undergoing abdomen opening, only pancreas and duodenum turning over was performed; model group (Group SAP), only induction of SAP; saline lavage group (Group SL), induction of SAP and saline lavage for 3 hours immediately; intravenous ulinastatin group (Group IU), induction of SAP and intravenous ulinastatin at 2500 U/100 g from caudal vein immediately, which approximately to the Group UL total dose of ulinastatin; ulinastatin lavage group (Group UL), induction of SAP and ulinastatin lavage in 62.5 U/ml for 3 hours immediately.

Peritoneal lavage

Following the induction of SAP model, Group SL and UL were performed peritoneal lavage immediately. Block catheter B and input 37°C lavage fluid into catheter A at 80 ml/h for 15 min. Then, block catheter A and opened catheter B allowed fluid to flow out for 15 minutes. Thus each lavage procedure lasted 30 minutes and the whole procedure was performed 6 times lasted 3 hours. Volume input and output were balanced. The ulinastatin lavage fluid consisted of saline and ulinastatin at 62.5 U/ml which can exert the best therapeutic effect [8]. After lavage, both catheters were blocked.

Observation indexes

Blood samples were collected immediately when the rats were mercifully killed at 3 h after operation (10 rats in each group). The level of TNF-α and IL-10 in serum were determined using the ELISA technique. And specimens of multiple organs (including pancreas, lung, liver

Table 1. Effect of different kinds of treatment on TNF-α, and IL-10 at 3 h

	C	SAP	SL	IU	UL
TNF-α (ng/ml)	1.271 (0.129)	2.033 (0.244)*	2.037 (0.291)*	1.921 (0.236)*	1.688 (0.189)*,+
IL-10 (pg/ml)	75.44 (14.55)	14.82 (5.51)*	16.44 (5.73)*	40.47 (8.70)*,+	51.97 (16.01)*,+

*P<0.05, compared with Group C; +P<0.05, compared with Group SAP.

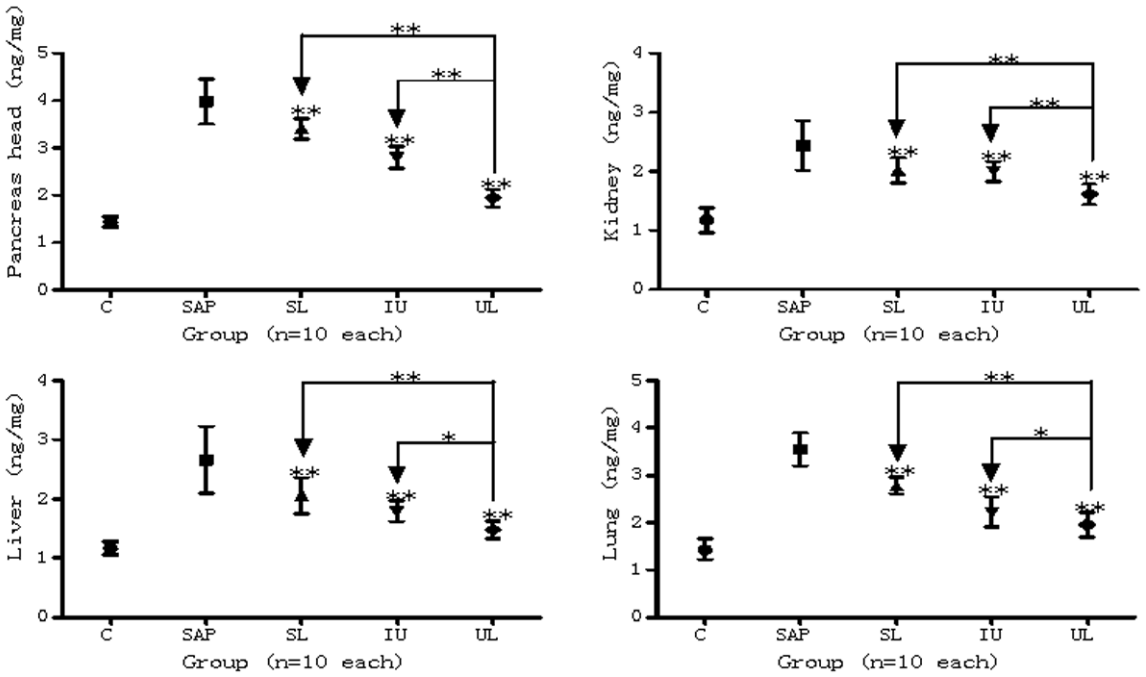

Figure 2. Comparison of different treatments on the expression levels of P-selectin protein in multiple organs. The expression levels of P-selectin protein in multiple organs greatly increased in Group SAP than C. Group SL, IU and UL could significantly decrease the expression levels of P-selectin protein in multiple organs compared with Group SAP and Group UL exert the best effect than other groups. (*P<0.05, **P<0.01).

and kidney) were prepared for expression levels, staining intensity of P-selectin protein.

Statistical analysis

All values were reported as mean ± standard using the SPSS 19.0 software package performed the analyses. Differences were analyzed by analysis of variance, Kruskal-Wallis H test, Bonferroni correction test and chi-squared test. Significance was assigned to *P* values less than 0.05.

Results

Effect of peritoneal lavage with ulinastatin on TNF-α and IL-10

The level of TNF-α in plasma of Group SAP was significantly greater increased than Group C. Compared with Group SAP, the level of TNF-α in both Group SL and IU did not have a statistically significant result but in Group UL were significantly reduced. Group UL exert the better effect than Group SL and IU (**Figure 1**). The level of IL-10 in plasma of Group SAP was significantly greater reduced than Group C. Compared with Group SAP, the level of IL-10 in Group SL did not have a statistically significant result but in Group IU and UL were significantly increased. Group UL exert the better effect than Group SL and IU (**Figure 1**). All the results are summarized in **Table 1**.

The expression levels of P-selectin protein in multiple organs

The expression levels of P-selectin protein of pancreas, liver, lung and kidney in Group SAP were all significantly greater increased than

Table 2. Effect of different treatment on the expression levels of P-selectin protein in multiple organs at 3 h

	C	SAP	SL	IU	UL
Pancreas (ng/mg)	1.437 (0.104)	3.984 (0.478)*	3.405 (0.218)*,+	2.795 (0.232)*,+	1.941 (0.185)*,+
Liver (ng/mg)	1.168 (0.117)	2.659 (0.562)*	2.059 (0.304)*,+	1.792 (0.181)*,+	1.478 (0.148)*,+
Kidney (ng/mg)	1.174 (0.215)	2.440 (0.420)*	2.021 (0.213)*,+	2.000 (0.169)*,+	1.617 (0.175)*,+
Lung (ng/mg)	1.430 (0.222)	3.550 (0.356)*	2.783 (0.171)*,+	2.390 (0.7709)*,+	1.963 (0.263)*,+

*P<0.05, compared with Group C; +P<0.05, compared with Group SAP.

Table 3. Comparison of staining intensity of P-selectin protein in multiple organs at 3 h

	C	SAP	SL	IU	EUL
Pancreas	0.0 (0.0)	3.0 (0.0)*	2.0 (1.0)*,+	2.5 (1.0)*,+	1.0 (1.0)*,+
Liver	0.0 (0.0)	3.0 (1.0)*	2.0 (1.0)*,+	1.0 (2.0)*,+	1.0 (0.25)*,+
Kidney	0.0 (0.0)	3.0 (0.0)*	2.0 (0.0)*,+	2.0 (1.0)*,+	1.0 (0.0)*,+
Lung	0.0 (0.0)	2.0 (0.0)*	1.0 (1.0)*,+	1.0 (1.0)*,+	1.0 (0.2)*,+

Values are median (interquartile range); *P<0.05, compared with Group C; +P<0.05, compared with Group SAP.

Group C. The expression levels of P-selectin protein of pancreas in Group SL, IU and UL were significantly reduced compared with Group SAP. Group UL exert the better effect than Group SL and IU (**Figure 2**). The expression levels of P-selectin protein of liver in Group SL, IU and UL were significantly reduced compared with Group SAP. Group UL exert the better effect than Group SL and IU (**Figure 2**). The expression levels of P-selectin protein of lung in Group SL, IU and UL were significantly reduced compared with Group SAP. Group UL exert the better effect than Group SL and IU (**Figure 2**). The expression levels of P-selectin protein of kidney in Group SL, IU and UL were significantly reduced compared with Group SAP. Group UL also exert the better effect than Group SL and IU (**Figure 2**). Therefore, Group SL, IU and UL all could reduce the expression levels of P-selectin protein in multiple organs but the way of adding ulinastatin in peritoneal lavage could get the best effect. All the results are summarized in **Table 2**.

The products of the staining intensity and positive rate of P-selectin protein in multiple organs

The products of the staining intensity and positive rate of P-selectin protein at 3 h in the pancreas, liver, kidney and lung in the Group SAP were significantly higher than the Group C. The products of the staining intensity and positive rate of P-selectin protein at 3 h in the pancreas, liver, kidney and lung in the Group SL, IU and UL were significantly lower than the Group SAP and Group SAP exerts the best effect. All the results are summarized in **Table 3**.

Discussion

SAP is characterized by the SIRS and MODS and the development is very fast with high mortality [9-11]. Ulinastatin is a multivalent Kunitz-type serine protease inhibitor that is found in human urine and blood, which has been widely used in acute inflammatory disorders such as SAP and with a significant therapeutic effect [5, 6, 12, 13]. However, ulinastatin were used in intravenous way in these studies. Many studies reported that protease inhabitor added into the peritoneal lavage would improve the outcome of experimental SAP [7, 14]. Studies also reported ulinastatin inhibits neutrophil elastase activity in vitro and trypsin activity in patients with pancreatitis [13]. Therefore, ulinastatin would exert better effects in peritoneal lavage way. In the early study, we have presented the first evidence that ulinastatin in peritoneal lavage way could bring the better therapeutic effect in SAP [8, 15]. We also found that ulinastatin in peritoneal lavage way significantly reduced the level of IL-1 and IL-6 in plasma and the expression levels of TNF-α and NF-κB in multiple organs [16].

It was well known that TNF-α are significant inflammatory mediators which play a critical role in the pathogenesis of acute pancreatitis (AP) by driving the subsequent inflammatory response and IL-10 is an anti-inflammatory factor which could inhibit the synthesis and release of other proinflammatory cytokine [17, 18]. The results of this study showed that the contents

of TNF-α in the blood of Group SAP were significantly higher than those in the sham-operated group (Group C) but the contents of IL-10 was converse. The results indicated that the contents of inflammatory mediators in the blood of SAP model were greatly elevated and the protective factor of IL-10 was greatly decreased. After the treatment with different methods, the contents of TNF-α and IL-10 in the Group UL were significantly improved than those in the Group SAP and much better than Group SL and IU, proving that ulinastatin through peritoneal lavage way was able to effectively lower the contents of inflammatory mediators and higher the anti-inflammatory factor in the blood. We further proved that ulinastatin has a greatly anti-inflammatory effect through higher the level of IL-10 so that effectively inhibit the release of inflammatory mediators, thereby improving the SIRS and prognosis.

P-selectin is a platelet activation-dependent glycoprotein that mediates the adhesion of activated platelets and leukocytes in various inflammatory conditions [2]. P-selectin is not expressed or is expressed at a low level on multiple organs in the normal physiological conditions [4]. In the early pathophysiology of SAP, P-selectin plays a central role to mediate the adhesion of both activated platelets and leukocytes as well as various other inflammatory conditions [19]. The Inhibition of P-selectin could reduce leukocyte infiltration in multiple organs and improve tissue damage in experimental acute pancreatitis [19]. The results of our experiment showed that the expression of P-selectin protein in multiple organs of Group SAP was greater than Group C. After the treatment of different groups, the expression levels of P-selectin protein in pancreas, lung, liver, and kidney of the Group SL, IU and UL were significantly lower than those in the Group SAP, but Group UL exerted the best effect. It is indicated that saline lavage and the use of ulinastatin could mitigate the extent of inflammation through reduce the expression levels of P-selectin protein and the peritoneal lavage with ulinastatin downregulate the expression levels of P-selectin protein in multiple organs more effectively.

Therefore, ulinastatin added to the peritoneal lavage could more effectively mitigate the extent of inflammation because of its inhibiting the release of inflammatory mediators in the blood and the expression levels of P-selectin protein in multiple organs, thereby greatly improving the inflammatory condition, multiple organs damage and the prognosis of SAP.

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Disclosure of conflict of interest

None.

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