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Therapeutic effect of ultrasound interventional perirenal catheter-assisted early peripancreatic lavage of protease inhibitor on severe acute pancreatitis in miniature pigs

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ABSTRACT

Objectives: To study the therapeutic effect of early peripancreatic lavage of ulinastatin on severe acute pancreatitis(SAP).
Methods: Sixteen pigs were divided into 4 groups: model(SAP), saline lavage(SL), ulinastatin lavage(UL), intravenous ulinastatin(IU). UL and SL group were given peripancreatic lavage of ulinastatin by ultrasound-guided perirenal catheterization and IU group was intravenously instilled with ulinastatin. The multi-organ functions and the inflammatory factors were observed.
Results: UL group has the best therapeutic effect. The changes of multi-organ functions and the inflammatory factors were compared with SAP group as follows. In time window of treatment: amylase ($p < 0.01$), lipase ($p < 0.01$), ALT ($p > 0.05$), AST ($p < 0.05$), CR ($p < 0.01$), UR ($p < 0.01$), IL-6 ($p < 0.01$), IL-10 ($p < 0.01$). In post-treatment phase: amylase ($p < 0.01$), lipase ($p < 0.01$), ALT ($p < 0.01$), AST ($p < 0.01$), CR ($p < 0.05$), UR ($p > 0.05$), IL-6 ($p < 0.01$), IL-10 ($p < 0.01$).
Conclusions: Early peripancreatic lavage of ulinastatin in SAP could effectively improve the multi-organ functions and inflammatory response.

Introduction

SAP is a critically ill disease with acute onset, rapid development, many complications and high mortality. The acute response period is an important treatment window for SAP. Trypsin plays a key role in the pathogenesis of SAP. Activated trypsin can damage the pancreas and surrounding tissues, stimulate the body's inflammatory response, release a large amount of inflammatory mediators. Acute response period can lead to the accumulation of a large amount of peritoneal effusion around the pancreas, which could increase intra-abdominal pressure, induce abdominal compartment syndrome, damage tissue and organs, aggravate systemic inflammatory response, affect the function of important organs. Therefore, focusing on trypsin and early intervention for peripancreatic effusion is one of the key points of SAP treatment and affecting prognosis.

The problem of peripancreatic effusion is difficult to be solved well in a short time with a conservative method of systemic intravenous drugs. Surgical treatment has been less considered in the early stage of SAP because of its heavier injury and more complications. Therefore, minimally invasive intervention is needed and becoming the first choice. At present, ultrasound intervention technology has been able to provide minimally invasive and satisfactory drainage therapy for SAP patients. It can be performed at the bedside in the earliest time after the patient's admission to the hospital so as to achieve early minimally invasive

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intervention in SAP, which significantly improved the patient's condition and prognosis.

Ulinastatin is currently the clinically preferred drug for SAP treatment and has important therapeutic value. However, due to pancreatic microcirculation disturbance during the early stage of SAP, the effect of intravenous administration is not remarkable. Peritoneal lavage is one of the effective measures to eliminate harmful substances in the abdominal cavity. It not only can dilute pancreatic enzymes but also eliminate toxic substances in the peripancreatic effusion, which could reduce damage to the pancreas and other important tissue and organs.

Therefore, this study aimed to achieve peripancreatic lavage of protease inhibitor by ultrasound interventional technique, which to provide technical and theoretical support for the clinical trial and application.

Methods

Ethics

The experimental protocol was approved by the Ethics Committee for Animal Research from the General Hospital of the PLA and all experimental pigs received humane care.

Experimental animals

A total of 16 healthy male miniature pigs that weighing 10±1 kg were provided from the Experimental Animal Center of the PLA General Hospital(Beijing, China).

Drugs and reagents

Drugs and reagents were purchased from the following companies: Pentobarbital sodium (National Pharmaceutical Group Chemical Reagent Co., Ltd. China); heparin sodium injection (Shanghai First Biochemical Pharmaceutical Co., Ltd. China); ulinastatin (Guangdong Tianpu Biochemical Pharmaceutical Co., Ltd. China); amylase, lipase,IL-6 and IL-10 assay kit (RANDOX company).

Main experimental equipments

Color Doppler Ultrasound Scanner (GE US General Electric LOGIQ E9 XD Clear). Automatic biochemical analyzer (Beckman Coulter, USA) -AU5800). Fully automatic microplate reader (BIOTEK, USA).

Experimental groups

Pigs were randomly divided into four groups. 1) Model group (n = 4, SAP): Induction of SAP without peripancreatic lavage or intravenous injection but with a ultrasound-guided perirenal catheterization. 2) Saline lavage group (n = 4, SL): Induction of SAP with ultrasound-guided perirenal catheterization and peripancreatic lavage of saline. 3) Intravenous ulinastatin group (n = 4, IU): Induction of SAP with continuous intravenous ulinastatin(25000U/kg) and ultrasound-guided perirenal catheterization but without peripancreatic lavage. 4) Ulinastatin lavage group (N = 4, UL): Induction of SAP with ultrasound-guided perirenal catheterization and peripancreatic lavage of ulinastatin(62.5U/ml).

Animal model

The pigs were fasted for 12 h and had no access to water for 4 h prior to undergoing surgery. Pigs were anesthetized by intramuscular injection of 3% pentobarbital sodium(30 mg/kg). Surgically exposed pancreas, a syringe needle was inserted into the opening of the duodenal bile duct. Then, 5% sodium taurocholate solution (freshly prepared in saline solution, 3 ml/kg), was retrogradely injected into the pancreatic bile duct at a constant rate of 0.2 ml/min. After 5 min, the puncture needle was removed and the abdomen was closed by surgical method.

Perirenal catheterization under ultrasound guidance

One hour after modeling, all experimental groups were scanned with ultrasound under normal condition on the region of left kidney. The body surface position at the deepest point of the effusion was set as the puncture point and the direction of the puncture guide line was adjusted. Then, percutaneously punctured into the perirenal effusion area with 16G puncture needle under real-time ultrasound guidance. When the needle core was removed, dark red peritoneal effusion can be seen to flow out. After insert the guide wire and remove the puncture needle, the 10F drainage tube was placed along the guide wire.

Peripancreatic lavage method

After perirenal catheterization in SL and UL group, peripancreatic lavage was performed by intermittent lavage method. Firstly, the pig was placed at the head low position, then the lavage solution (warm bath to 37 °C) was injected from the perirenal drainage tube (80 ml/h) for 15 min. After 15 min, the infusion of the lavage solution was stopped and the pig was restored to the supine position, which the peritoneal effusion was discharged from the perirenal drainage tube for 15 min. Therefore, a lavage cycle is 30min and 6 cycles of intermittent lavage were carried out for a total of 3 h that as the window of treatment for SL and UL group. At the end of the peripancreatic lavage, the perirenal drainage tube was clamped. The input and output volume was monitored during the intermittent lavage. When the pig was placed at the head low position, peripancreatic effusion was found gradually increased by ultrasound. It was proved that the lavage solution could be accumulated in the peripancreatic area and exerted therapeutic effect (Fig. 1).

Intravenous injection method

The IU group received intravenous ulinastatin 25000U/kg(freshly prepared in 500 ml of saline solution) from the internal jugular vein after perirenal catheterization. The dose of intravenous ulinastatin was approximately equal to the total dose of UL group and the instillation was continued for 3 h that as the window of treatment for IU group.

Assays and calculations

The entire experiment lasted 6 h, including 1 h of model preparation(the 0 h~1 h of the experiment), 3 h of the time window of treatment(the 1 h~4 h of the experiment) and 2 h of post-treatment phase(the 4 h~6 h of the experiment). Blood samples from internal jugular vein in SAP, SL, IU and UL group were collected for multi-organ functions: amylase, lipase, aspartate aminotransferase(ALT), alanine aminotransferase(AST), creatinine(CR), urea(UR),) and inflammatory factors(IL-6 and IL-10). The time points for collecting blood samples respectively were: 1) before the model preparation(the 0 h of the experiment); 2) before the peripancreatic lavage and intravenous injection (the 1 h of the experiment); 3) the end of the peripancreatic lavage(the 4 h of the experiment); 4) 2 h after the peripancreatic lavage(the 6 h of the experiment).

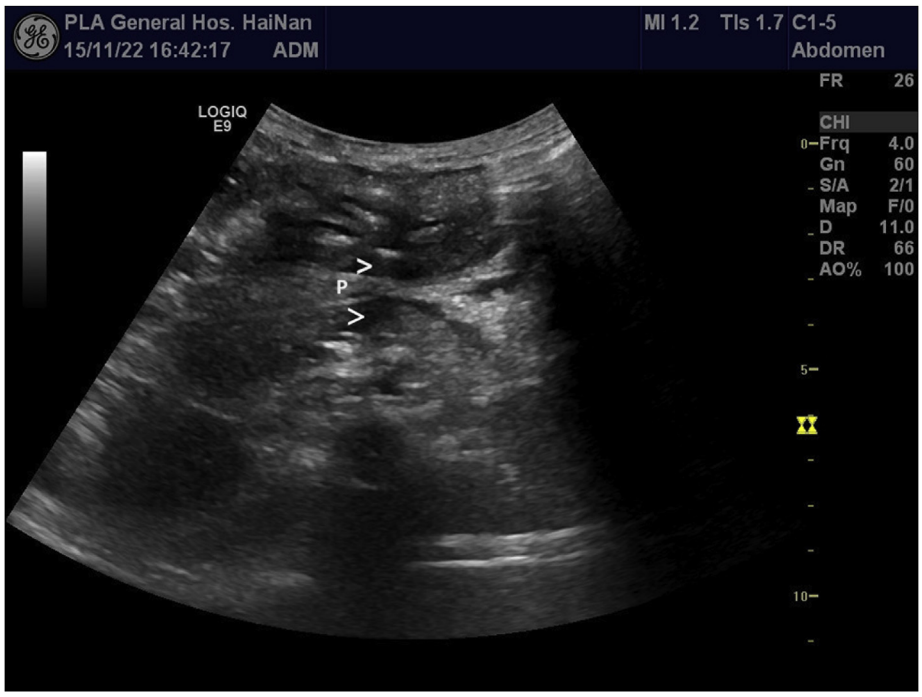

Fig. 1. When the pigs were placed at the head low position, the lavage solution could be accumulated in the peripancreatic area after injection from the perirenal tube. "P": pancreas, ">": lavage fluid.

Statistical analysis

Results are expressed as mean ± standard deviation, using SPSS 19.0 statistical software. Repeated measures were analyzed of variance. Comparisons of multiple independent samples were performed using the rank sum test (Kruskal-Wallis H test). $P < 0.05$ indicates a statistical difference, and $p < 0.01$ indicates a significant difference.

Results

Pancreatic function

The analysis of the pancreatic function showed that the activity of amylase and lipase in the plasma of pigs in SAP group gradually increased with the passage of time, and reached the highest value at the 6 h of the experiment. In the time window of treatment(the 1 h~4 h of the experiment), the UL group could significantly reduce the activity of amylase and lipase compared with that in SAP group and with the greater reduction observed than SL group and IU group. The reduction of amylase activity in IU group is better than SL group. In the post-treatment phase(the 4 h~6 h of the experiment), the UL group could also significantly reduce the activity of amylase and lipase compared with that in SAP group. The reduction of amylase activity was most marked in group UL. The reduction of lipase activity in UL and IU groups were both significantly compared with that in SAP group, but no significant difference was found between UL and IU group. The reduction of lipase activity in IU group is better than SL group (Table 1).

Liver function

The analysis of the liver function showed that the activity of ALT and AST in the plasma of pigs in SAP group gradually increased with the passage of time, and reached the highest value at the 6 h of the experiment. In the time window of treatment(the 1 h~4 h of the experiment), no significant difference about the reduction of ALT activity was found among the UL, SL and IU group compared with SAP group. However, UL, SL and IU group could significantly reduce the activity of AST compared with that in SAP group and with the greatest reduction observed in IU group. In the post-treatment phase(the 4 h~6 h of the experiment), the UL group could significantly reduce the activity of ALT and AST compared with that in SAP group. The reduction of ALT was most marked in group UL. The reduction of AST among UL, SL and IU groups was significantly compared with that in SAP group, and no significant difference was found between UL and SL group. The reduction of AST both in UL and SL group were better than IU group (Table 1).

Kidney function

The analysis of the kidney function showed that the activity of CR and UR in the plasma of pigs in SAP group gradually increased with the passage of time, and reached the highest value at the 6 h of the experiment. In the time window of treatment(the 1 h~4 h of the experiment), the UL, SL and IU group could significantly reduce the activity of CR and UR compared with that in SAP group and no significant difference was found among them. In the post-treatment phase(the 4 h~6 h of the experiment), the UL group could significantly reduce the activity of CR compared with that in SAP group and was better than SL and IU group. However, there is no significant difference about the reduction of UR was found among the UL, SL and IU group compared with SAP group (Table 1).

Inflammatory factors

The analysis of the inflammatory factor showed that the activity of IL-6 in the plasma of pigs in SAP group gradually increased with the passage of time, and reached the highest value at the 6 h of the experiment. The activity of IL-10 in the plasma of pigs in SAP group

Table 1
Changes of amylase, lipase, ALT, AST, CR, UR, IL-6 and IL-10 in different observation intervals.

	SAP	UL	SL	IU
1 h~4 h of the experiment:				
Amylase (U/L)	1487.50(48.13)	737.75(77.23)**	1533.50(123.85)##	1315.50(245.60)##+
Lipase (U/L)	124.73(21.80)	41.60(11.36)**	105.18(16.37)##	106.58(20.31)##
ALT (U/L)	6.22(6.55)	4.50(3.12)	2.90(1.34)	1.65(3.19)
AST (U/L)	73.48(22.89)	47.33(0.85)*	49.73(11.07)*	23.53(3.26)**#+
Cr (umol/L)	9.25(0.96)	3.50(1.29)**	5.75(3.30)*	4.75(1.50)**
Ur (mmol/L)	1.10(0.01)	0.45(0.13)**	0.53(0.26)**	0.43(0.22)**
IL-6(pg/ml)	0.08(0.01)	−0.07(0.03)**	0.03(0.03)**##	−0.05(0.01)**++
IL-10(pg/ml)	−0.64(0.27)	0.67(0.25)**	0.16(0.05)**##	0.50(0.05)**+
4 h~6 h of the experiment:				
Amylase (U/L)	1592.25 (535.18)	364.75 (66.89) **	1509.75 (421.89) ##	1466.50 (394.75) ##
Lipase (U/L)	155.90(15.20)	98.68(24.01)**	136.65(20.22)##	108.90(2.81)**+
ALT (U/L)	8.60(3.42)	3.78(2.42)**	7.25(0.78)#	6.68(1.36)
AST (U/L)	61.95(5.62)	28.35(9.34)**	34.35(1.73)**	52.35(3.23)*##++
Cr (umol/L)	7.75(2.22)	3.50(1.29)*	7.50(3.11)#	8.25(2.06)##
Ur (mmol/L)	0.80(0.40)	0.93(0.22)	0.80(0.27)	0.75(0.30)
IL-6(pg/ml)	0.09(0.01)	0.03(0.01)**	0.07(0.02)	0.09(0.04)##
IL-10(pg/ml)	−0.40(0.05)	0.02(0.06)	−0.29(0.13)	−0.63(0.18)

UL,SL and IU groups compared with SAP group : *p < 0.05, **p < 0.01 ; .
SL and IU groups compared with UL group : #p < 0.05 , ##p < 0.01 ; .
SL group compared with IU group : + p < 0.05 , ++ p < 0.01.
Data are presented as the mean (standard deviation).

gradually decreased with the passage of time, and reached the lowest value at the 6 h of the experiment. In the time window of treatment(the 1 h~4 h of the experiment), the UL, SL and IU groups could significantly reduce the activity of IL-6 and raise the activity of IL-10 compared with that in SAP group. The UL and IU group got better effect on IL-6 and IL-10 than SL group and there was no significant difference between them. In the post-treatment phase(the 4 h~6 h of the experiment), the UL group could significantly reduce the activity of IL-6 compared with that in SAP group and was better than IU group. However, the UL, SL and IU group could not significantly raise the activity of IL-10 compared with SAP group and there is no difference was found among them (Table 1).

Discussion

SAP has a high mortality rate in the acute response period, accounting for more than 60% of deaths [1,2], which is an important time window for SAP treatment. Previous studies have found that peritoneal lavage of ulinastatin in rats has important therapeutic value in early intervention of SAP [3–5]. However, in order to avoid peripancreatic infection, it was not recommended to perform early directly invasive treatment of pancreas in SAP patients and conservative treatment is still the first choice. Peripancreatic invasive intervention usually waits until a stable cystic parcel formed around the pancreas and after formation of a safe puncture path under CT guidance. Therefore, there is a certain lag in the timing of treatment and the treatment is not minimally invasive [6,7].

Ultrasound intervention has been able to provide minimally invasive and satisfactory drainage therapy for SAP patients. A large amount of active trypsin, inflammatory mediators and toxic metabolites can be excreted from the body, which could reduce resorption and damage to the abdominal tissue and organs [8]. It can be performed at the bedside in the earliest time after the patient's admission to the hospital. However, it is necessary to find a better way to achieve early peripancreatic lavage avoiding the early directly invasive intervention of the pancreas and solve the technical limitation of deep catheterization in ultrasound intervention.

In the early stage of SAP, not only a large amount of fluid accumulation occurred in the peripancreatic area, but also these fluids could flow through the peritoneal passage to other cavities, which is one of the mechanisms of ultrasound intervention for early drainage of peritoneal effusion [9]. Therefore, through these peritoneal fluid drainage pathways, liquid perfusion also could be reversed to achieve peripancreatic drug lavage, not only avoid the early directly invasive intervention of pancreas and can also take the advantage of minimally invasive intervention by ultrasound. The kidney and the pancreas are same as the retroperitoneal organs. The left perirenal area was often used as an important treatment pathway for minimally invasive surgery for pancreas. Therefore, this study firstly gave the early minimally invasive left perirenal catheterization by ultrasound and then performed peripancreatic lavage of protease inhibitor. Through changing body position, the drug could be accumulated around the pancreas to achieve peripancreatic lavage therapy.

The experimental results showed that the UL group better than SL and IU group in protecting the pancreatic function. The possible mechanism was that due to early microcirculation disturbance of SAP, the concentration of ulinastatin was not high in the pancreas through intravenous administration. The peripancreatic lavage of ulinastatin can increase the local and systemic concentration, which could effectively inhibit the digestive effect of pancreatic enzymes and control the further development of the disease. In addition, peripancreatic lavage of ulinastatin can directly inhibit or dilute on pancreatic enzyme in the abdominal cavity.

In terms of liver function, peripancreatic lavage of ulinastatin may control the development of SAP in the time window of treatment, which further effectively reduced the activity of ALT in the post-treatment phase. However, the intravenous ulinastatin has limited therapeutic effect on pancreas in the time window of treatment and it was unable to effectively control the progression of the disease. Therefore, a sharp increase of AST occurred in the post-treatment phase of IU group. In addition, AST changed remarkably throughout the experiment and may be more sensitive and accurate than ALT in early assessment of liver function damage in SAP.

In terms of renal function, early damage to kidney function was mainly related to less renal blood perfusion caused by microcirculatory disorder and systemic inflammatory response in SAP. Peripancreatic lavage of ulinastatin or saline could rely on peritoneal osmosis to improve the renal function by increasing blood volume. On the other hand, ulinastatin could also effectively inhibit the inflammatory response and the activity of trypsin, which can

effectively control the development of SAP and further reduce the damage to the kidney.

IL-6 and IL-10 are representative proinflammatory and protective factors in the inflammatory response and play an important role in the pathophysiology of SAP [10–13]. The results of this experiment showed that SL and IU group could only control the changes of IL-6 and IL-10 in the time window of treatment. The probable reason was that peripancreatic lavage of saline could control the changes of IL-6 and IL-10 by reducing the reabsorption of inflammatory factors in peritoneal effusion. However, in the post-intervention period, due to the limited anti-inflammatory effects of lavage of saline and the infusion of ulinastatin, the systemic inflammatory response was further aggravated without. The peripancreatic lavage of ulinastatin can effectively control the increase of IL-6 and the decrease of IL-10 in the time window of treatment, fully exert the local anti-inflammatory and anti-digestive effects of ulinastatin. Through peritoneal osmosis, the concentration of ulinastatin in the circulation and local pancreas may be increased and the anti-inflammatory effect could be exerted.

Conclusion

Ultrasound-guided minimally invasive perirenal catheterization in SAP combined with postural changes can achieve peripancreatic drug lavage. Early peripancreatic lavage of ulinastatin could effectively protect important multi-organ functions and improve the inflammatory response.

Conflicts of interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to subject of this article.

Contributors

All authors have contributed to and agree with the contents of the manuscript. Tanshi Li, Cong Feng and Li Chen designed the study. Yongzhi Zhai, Lu Gan, Qinrui Xing, Sai Huang and Xuan Zhou did experiment. Lili Wang helped with data collection and statistical analysis. We certify that the submission is original work and is not under review at any other publication.

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Ethical approval

The experimental protocol was approved by the Ethics Committee for Animal Research from the General Hospital of the PLA and all experimental rats received humane care.

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