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Early local drug therapy for pancreatic contusion and laceration

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ABSTRACT

Objectives: To study the therapeutic effect of early local drug therapy on pancreatic contusion and laceration.
Methods: Twenty pigs were divided into 4 groups: model(PL), 1 ml of saline; medical protein glue (EC), 1 ml of medical protein glue; ulinastatin (UL), 50000U of ulinastatin; combined treatment (UE), 1 ml of medical protein glue and 50000U of ulinastatin. 30 min after model establishment, different groups received different local drug treatments. The pancreatic function, peritoneal effusion and pancreatic pathology were observed.
Results: The UE group got the best therapeutic effect. The changes of pancreatic function and the peritoneal effusion were compared with PL group as follows. 0-6h: amylase ($p < 0.01$), lipase ($p > 0.05$), effusion ($p < 0.01$); 6-12h: amylase ($p > 0.05$), lipase ($p < 0.01$), effusion ($p < 0.01$); 12-24h: amylase ($p < 0.01$), lipase ($p < 0.01$), effusion ($p < 0.01$).
Conclusions: Early local drug therapy in pancreatic contusion and laceration could effectively control the development of the disease and improve the prognosis.

Introduction

Pancreatic contusion and laceration, even a small wound would activate and produce digestive effects. Pancreatic juice containing high concentrations of digestive enzymes could be extravasated from the laceration to the pancreatic interstitial or peripancreatic tissue and then activated and digests the pancreas and peripancreatic tissue, causing mild pancreatic laceration to become a serious "secondary pancreatic rupture" and following the severe complications: the main pancreatic duct broken and the surrounding large blood vessels ruptured.

When pancreatic capsule is not ruptured, the pancreatic capsule plays a key role of "tightening" and aggravates the dysfunction of circulation of pancreatic tissue, which forming a vicious circle. In addition, self-injury repair is more difficult, leading to the occurrence of traumatic pancreatitis. More over, due to the overactivation effect of pancreatic enzyme after pancreatic contusion and laceration, the incidence of postoperative pancreatic fistula is high. Therefore, early intervention is the key to the treatment of pancreatic contusion and laceration and affect prognosis.

In this study, we use medical protein glue and protease inhibitors for early local drug intervention in pancreatic contusion and laceration to observe the therapeutic effects in different methods, providing new research evidence of early local drug therapy for pancreatic contusion and laceration.

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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 20 healthy male miniature pigs weighing 10±1 kg were provided by the Experimental Animal Center of the PLA General Hospital(Beijing, China).

Drugs and reagents

Pentobarbital sodium (National Pharmaceutical Group Chemical Reagent Co., Ltd. China), heparin sodium injection (Shanghai First Biochemical Pharmaceutical Co., Ltd. China), medical protein glue EC type (Guangzhou Baiyun Medical Adhesive Co., Ltd. China), ulinastatin (Guangdong Tianpu Biochemical Pharmaceutical Co., Ltd. China), amylase and lipase assay kit (RANDOX company).

Main experimental equipments

Automatic biochemical analyzer (Beckman Coulter-AU5800, USA), fully automatic microplate reader (BIO-TEK, USA).

Experimental groups

Pigs were randomly divided into four groups. 1) Model group (n = 5, PL): Induction of PL with sham early local drug therapy of 1 ml of saline. 2) Medical protein glue EC type group (n = 5, EC): Induction of PL with early local drug therapy of medical protein glue (1 ml). 3) Ulinastatin group (n = 5, UL): Induction of PL with early local drug therapy of ulinastatin (50000U, 1 ml). 4) Combined treatment group (n = 5, UE): Induction of PL with early local drug therapy of medical protein glue (1 ml) and ulinastatin (50000U, 1 ml).

Animal model

The pigs were fasted for 12h 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). After successful anesthesia, the animals were placed on the bench in the supine position and heparin (300 IU/kg) was given intravenously to resist the coagulation system.

According to Song Qing method to prepare the pancreatic contusion and laceration model. Surgically exposed pancreas and prepared one site of pancreatic contusion and laceration of about 1.5 cm in length by hemostatic forceps on the surface of the body of the pancreas [1]. The depth of the contusion and laceration is less than 1/2 of the thickness of the pancreas to avoid damage to the pancreatic duct. The contusion and laceration wound is rough, the pancreatic capsule is ruptured and there is persistent bloody fluid exuding from the crack, which is a successful model of pancreatic contusion and laceration. This model meets the classification criteria of scale II for pancreatic contusion and laceration in AAST [2,3]. After the model was successfully constructed for 30 min, the early local drug therapy was started.

Early local drug therapy method

Early local drug therapy for different groups: 30 min after model establishment, different drug treatments were started. In PL group, 1 ml of saline was injected into the wound margin tissue. In EC group, the medical protein glue EC-type (1 ml) was used to cover the wound of laceration. In UL group, the ulinastatin (50000U,1 ml) was injected into the wound of laceration. In the EU group, the wound of laceration was injected ulinastatin (50000U,1 ml) first and covered medical protein glue EC-type (1 ml). After the intervention of each group, the abdomen was closed by surgical method.

Measurement of peritoneal effusion under ultrasound

At 6 h, 12 h, and 24 h after model establishment, the peritoneal effusion was observed at the left kidney area and the maximum depth of peritoneal effusion was measured and recorded. At each time point, the measure area was fixed and the model preparation and ultrasound examination were performed by different physicians without communication of each other.

Pathological observation of pancreatic contusion and laceration

At the 24th hour after model establishment, pancreatic contusion and laceration tissue for histology was fixed in formalin, subjected to conventional dehydration, embedded in paraffin and then sectioned into 5-μm sections, for subsequent staining by hematoxylin and eosin. Examination by light microscopy was performed by the same professional pathologist without knowing the groups. 200 high-power field of vision was selected for each slice and pancreatic pathology scores were analyzed based on histology according to the Schmidt method [4].

Assays and calculations

The entire experiment lasted 24 h. Blood samples from internal jugular vein in PL, EC, UL and EU groups were collected for pancreatic functions: amylase, lipase. Measurements of peritoneal effusion were observed for PL development evaluation. Pathological pathology observation of pancreatic contusion and laceration was for local drug treatment evaluation. The time points for collecting blood samples and the measurement of peritoneal effusion were: 1) before the model preparation(the 0 h of the experiment); 2) 6 h after the model establishment; 3) 12 h after the model establishment; 4) 24 h after the model establishment (the end of the experiment).

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 PL group gradually increased with the passage of time and reached the highest value at the 24h of the experiment. In the time window 0h~6h of the experiment, the UE, UL and EC groups could significantly reduce the activity of amylase compared with that in PL group and UE group had the greater reduction observed than UL group and EC group. However, the UE, UL and EC groups could not significantly reduce

the activity of lipase in this time window. In the time window 6h~12h of the experiment, the UE, UL and EC groups could significantly reduce the activity of lipase compared with that in PL group and UE group had the greater reduction observed than other two groups. Meanwhile, the UE, UL and EC groups could not significantly reduce the activity of amylase in this time window. In the time window 12h~24h of the experiment, all the UE, UL and EC groups could significantly reduce the activity of both amylase and lipase compared with that in PL group and UE group had the greater reduction observed than other two groups. Moreover, the UL group could reduce the activity of lipase better than EC group in this time window (Table 1).

The depth of the peritoneal effusion

The analysis showed that the depth of effusion in the peritoneal of pigs in PL group gradually increased with the passage of time and reached the highest value at the 24h of the experiment. In the time window 0h~6h of the experiment, all the UE, UL and EC groups could significantly reduce the depth of effusion compared with that in PL group. UE and EC groups had the greater reduction observed than UL group. In the time window 6h~12h of the experiment, UE and EC groups could significantly reduce the depth of effusion compared with that in PL group. UL group could not significantly reduce the depth of effusion in this time window. In the time window 12h~24h of the experiment, only UE group could significantly reduce the depth of effusion compared with that in PL group. UL and EC groups could not significantly reduce the depth of effusion in this time window (Table 1).

Pancreatic pathology

In PL group, pancreatic cell necrosis area <20%, inflammatory cell infiltration area <20%, parenchymal hemorrhage area <25%, pancreatic cell edema is not obvious. In EC group, the area of pancreatic cell necrosis was significantly reduced compared with the PL group. The inflammatory cell infiltration was not significantly improved compared with the PL group, and the pancreatic cell edema was not obvious. In UL group, the necrotic area of pancreatic cells was not significantly improved compared with the PL group. The inflammatory cell infiltration was significantly less than that of the PL group, and the edema of the pancreatic cells was not obvious. In UE group, the necrotic area of pancreatic cells was significantly reduced compared with the PL group. The inflammatory cell infiltration was significantly less than that of the PL group, and the edema of the pancreatic cells was not obvious. The results of pancreatic pathology scores based on histology from the laceration areas in different groups are shown in Table 2.

Discussion

Although pancreatic trauma accounts for only a small part (2%–5%) of abdominal closed injury, the prognosis is extremely poor and the mortality rate is extremely high [5]. Pancreatic contusion and laceration, even a small contusion (not easily visible), pancreatic juice containing high concentrations of digestive enzymes can be extravasated from the contusion and laceration to the pancreatic interstitial or peripancreatic tissue. It is then activated and produced digestive effects on the pancreas and peripancreatic tissues. This self-digestive mechanism and pathophysiological processes can cause mild pancreatic contusion and laceration to become a serious “secondary pancreatic rupture”. When the pancreas is contused and the pancreatic capsule is not ruptured, the pancreatic tissue is swollen after injury, and the pancreatic capsule plays a role of “tightening”, which aggravates the circulation disorder of the pancreas, thereby forming a vicious circle, and it is more difficult to repair the damage itself and take the occurrence of traumatic pancreatitis. In addition, due to the over-activation effect of pancreatic enzyme after pancreatic contusion and laceration, even if the postoperative surgery effectively treats the traumatic foci, the incidence of postoperative pancreatic fistula is as high as 40%, leading to severe local and systemic complications, even life-threatening [4]. Therefore, early intervention for pancreatic trauma is the key to the treatment of pancreatic contusion and laceration and affect prognosis.

Ulinastatin can inhibit the activity of various digestive enzymes in pancreatic enzymes, stabilize lysosomal membranes, inhibit the release of various inflammatory mediators, and has a good effect on reducing tissue and organ damage and improving immune status. The basis of the research on the treatment of pancreatic diseases, especially various types of pancreatitis, is one of the ideal choices for early minimally invasive drug intervention in pancreatic contusion and laceration [2,3,6–8]. Medical protein glue is a biomedical special functional adhesive. In addition to its usual

Table 2
The results of pancreatic pathology scores based on histology from the laceration areas in different groups.

	PL	EC	UL	UE
Pancreatic pathology score	5.0(0.5)	4.0(0.5)++	4.0(1.0)*+	3.0(0.5)**

Comparison with PL group: *p < 0.05,**p < 0.01.
Comparison with UE group: + p < 0.05,+ + p < 0.01.

Table 1
Changes in the amylase, lipase and peritoneal effusion in different observation intervals.

	PL	UL	EC	UE
0–6 h:				
Amylase (U/L)	535.6(79.69)##	38.00(9.14)**#	88.20(22.43)**##	−40.80(46.28)**
Lipase (U/L)	4.14(0.48)	3.60(0.60)	3.86(0.60)	3.68(0.77)
Peritoneal effusion (cm)	4.46(0.55)##	3.32(0.19)**#	2.78(0.44)**+	2.68(0.36)**
6 ~ 12 h:				
Amylase (U/L)	5.60(13.50)	6.40(14.29)	19.20(10.06)	0.40(39.69)
Lipase (U/L)	7.36(0.68)##	0.92(0.46)**##	2.60(0.72)**##+	−1.42(1.55)**
Peritoneal effusion (cm)	0.76(0.09)##	0.80(0.14)##	0.48(0.18)**++	0.34(0.17)**
12 ~ 24 h:				
Amylase (U/L)	956.00(466.44)##	342.40(155.80)**##	588.00(236.57)*##	−189.80(52.05)**
Lipase (U/L)	11.40(0.99)##	4.84(0.94)**##	7.54(1.06)**##++	0.26(0.71)**
Peritoneal effusion (cm)	1.10(0.24)##	1.06(0.25)##	0.92(0.13)##	0.44(0.13)**

Comparison with PL group:*p < 0.05, **p < 0.01.
Comparison with UE group: #p < 0.05, ##p < 0.01.
Comparison between UL group and EC group: + p < 0.05, + +p < 0.01.

bonding function and mechanical function, it also has biomedical functions. It is widely used in various surgical operations, with hemostasis, sealing, adhesion and blocking, leakage and other effects. In the early treatment of abdominal parenchymal organs (liver, spleen, kidney), medical protein glue has been effectively combined with minimally invasive interventional techniques assisted by contrast-enhanced ultrasound to form a percutaneous transection of abdominal parenchymal organ trauma [9–11].

In terms of the effect on blood amylase, the effect of inhibiting the increase of amylase content in the UE group was the best, which not only inhibited the increase of amylase content in the whole process, but also reduced the amylase content of the 24th hour compared with 0 h. The possible mechanism is that the wound is effectively treated in the early stage by medical protein glue, and the effects of hemostasis, sealing, adhesion and plugging are achieved. The injection of local tissue of the ulinastatin is effective to inhibit the exudation of pancreatic enzyme activity and reduce the self-digestion. Although the UL group can effectively inhibit trypsin activity and reduce self-digestion in the early stage, due to the failure of effective treatment of the wound, there are still bleeding, pancreatic juice extravasation, etc. When the drug concentration it is lowered, it could not effectively inhibit the activity of trypsin, and the self-digestion is started again. Although the EC group effectively treated the wound at the initial stage, the exuded pancreatic juice has also been activated, and the self-digestion has not been effectively controlled. The enzyme content showed an increasing trend throughout the process.

In terms of the effect on blood lipase, the overall trend was different compared with amylase. The content of blood lipase in the PL group increased overall, and the interval from 6 h to 12 h was significantly increased. In other experimental groups, the UE group had the best effect of inhibiting the increase of lipase content. After 6 h, it could effectively inhibit the increase of lipase. The possible mechanism was still effective protection of early wounds and the effective inhibition of ulinastatin. The UL group was able to effectively inhibit the activity of pancreatic enzyme. The increase of lipase was slowed down after the 6th hour. However, due to the failure of effective protection of the wound, the lipase increased again after the 12th hour. The EC group can effectively protect the wound in the early stage, but it can't effectively inhibit the trypsin activity, so it could not effectively prevent the further development of the disease course. The lipase content increases in the range of 6 h–12 h and did not change the overall trend of the rise. It is worth nothing that after early intervention on pancreatic trauma, lipase showed different elevations in the 6 h–12 h interval. The more effective the intervention, the smaller the tendency of lipase to increase. But after 12 h, the trend became higher.

Pancreatitis can produce pancreatic fluid accumulation in the acute reaction period, forming pancreatic effusion [12], pancreatic contusion and laceration is not exception. With the progress of the disease, bleeding, traumatic pancreatitis and other complications would happened, the liquid will accumulate more and more. The depth of the peritoneal effusion increased gradually in the PL group, but the increase in the depth of the peritoneal effusion in the 0 h–12 h interval was not obvious, and it appeared sharply increasing after the 12th hour. The possible mechanism for the progression of pancreatic contusion and laceration is further developed. The UE group had the best effect of inhibiting the increase of the depth of the peritoneal effusion. The possible mechanism was still effective in protecting the wound and inhibiting the self-digestion in the early stage. UE group inhibit peritoneal effusion depth effect is the best. Its possible mechanism is still the early effectively protects the wound and inhibit their digestive function.

Conclusion

The early local drug therapy of combined medical protein glue and ulinastatin could effectively control the development of the disease and improve the prognosis.

Potential 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.

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Contributors

All authors have contributed to and agree with the contents of the manuscript. Tanshi Li, Faqin Lv and Li Chen designed the study. Cong Feng, Hao Yang, Sai Huang and Xuan Zhou did experiment. Xiang Cui and 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.

Ethical approval

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.

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