

Basic Investigations

Synergetic Effect of Yihuo Qingyi Decoction (益活清胰汤) and Recombinant Staphylokinase in Treatment of Severe Acute Pancreatitis of Rats

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Objective: To investigate the effect of recombinant staphylokinase (r-Sak) and the Chinese medicine Yihuo Qingyi Decoction (Herbal decoction for severe acute pancreatitis) in the treatment of the severe acute pancreatitis (SAP) in rats, and to observe the synergistic effect of the two. **Methods:** One hundred and sixty-two adult male SD rats with the body mass of 250–280 g were randomly divided into the following 5 groups: sham operation group ($n=18$), control group ($n=36$), Yihuo Qingyi Decoction treatment group ($n=36$), r-Sak treatment group ($n=36$), and Yihuo Qingyi Decoction plus r-Sak treatment group ($n=36$). The SAP rat model was prepared by retrograde injection of 5% sodium taurocholate into the cholangiopancreatic duct. Two days before modeling, Yihuo Qingyi Decoction was intragastrically administered, and r-Sak was intraperitoneally injected. The survival rate within 18 h after modeling was determined. The pancreatic blood flow, the weight of ascites, and the serum amylase and lipase were investigated at 6 h, 12 h, and 18 h after modeling, and the pancreatic tissue was examined under light microscopy to see its pathological change. **Results:** The 18 h survival count of group A, B, C, D and E rats was 9, 2, 6, 7 and 8 respectively. After r-Sak and Yihuo Qingyi Decoction intervention, the serum amylase and lipase and the weight of ascites were significantly decreased, especially in group E. 18 h after modeling, the level of the serum amylase and lipase and the weight of ascites in group E was $1100 \pm 118 \text{ U} \cdot \text{L}^{-1}$, $1000 \pm 150 \text{ U} \cdot \text{L}^{-1}$ and $13.40 \pm 1.80 \text{ g}$ respectively, obviously lower than that of group B ($P < 0.05$). After SAP was induced, the pancreatic blood flow showed a tendency to decrease, but the decrease extent in the treatment groups was smaller than that in the control group. 18 h after modeling, the pancreatic blood flow in group B and group E was $30.16 \pm 8.96 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$, and $129.10 \pm 42.58 \text{ mL} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ respectively, there was significant difference ($P < 0.05$). The pathological change of the pancreatic tissue was alleviated in the treatment groups. **Conclusion:** Both r-Sak and Yihuo Qingyi Decoction play a beneficial role in the treatment of rat SAP and there is a synergistic effect between the two.

Keywords: *Yihuo Qingyi Decoction (Herbal decoction for severe acute pancreatitis); Recombinant staphylokinase (r-Sak); Severe acute pancreatitis (SAP)*

The definite pathogenesis of severe acute pancreatitis (SAP) is still not clear, and the effect of conventional treatments is not satisfactory enough. In recent years, the studies showed that the pathological change of pancreatic microcirculation plays an important role in attack of acute pancreatitis. Local disorder of pancreatic microcirculation, microvascular thrombosis, and blood flow blockage can cause acute pancreatitis.¹⁻³ The pancreatic ischemia is believed to be possibly the key factor causing hemorrhage, necrosis, and autolysis of the pancreatic tissue in pancreatitis of acute necrotic type.⁴⁻⁷ Based on the studies of pathogenesis of acute pancreatitis, the authors propose a new thinking for treatment of SAP with specific thrombolytic agents. In this study, the effect of recombinant staphylokinase (r-Sak) and Yihuo Qingyi Decoction (益活清胰汤 Herbal decoction for severe acute pancreatitis) on SAP of rats and the synergistic effect between the two were investigated in the hope of providing experimental evidence for treatment of SAP with TCM and western medicine in combination.

MATERIALS AND METHODS

Animals

One hundred and sixty-two adult male SD rats with the body mass of 250–280 g were provided by the

Experimental Animal Center of Chengdu University of Traditional Chinese Medicine. Clean grade, animal experiment licence: SCXK (CHUAN) 2006-11.

Drugs and Reagents

Standard r-Sak was provided by China National Institute for the Control of Pharmaceutical and Biological Products. 45% sodium taurocholate (STC) was purchased from Sigma Company, USA. Yihuo Qingyi Decoction composed of Dai Huang (Radix et Rhizoma Rhei) 50 g (decocted later), Mang Xiao (Natrii Sulphas) 50 g, Huang Qin (Radix Scutellariae) 15 g, Bai Shao (Radix Paeoniae Alba) 15 g, Mai Dong (Radix Ophiopogonis) 15 g, Mu Xiang (Radix Aucklandiae) 9 g, Chai Hu (Radix Bupleuri) 12 g, Bing Lang (Semen Arecae) 12 g, Ku Lian Pi (Cortex Meliae) 10 g, Shi Jun Zi (Fructus Quisqualis) 12 g, Chuan Xiong (Rhizoma

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Chuanxiong) 12 g, Dan Shen (*Salviae Miltiorrhizae*) 15 g, Huang Qi (*Radix Astragali*) 15 g, and Ren Shen (*Radix Ginseng*) 10 g was decocted in water into a 100% decoction containing crude ingredient of 1 g/mL.

Modeling and Grouping of Animals

The SD rats were randomly divided into the following 5 groups: sham operation group (group A, $n=18$), control group (group B, $n=36$), Yihuo Qingyi Decoction treatment group (group C, $n=36$), r-Sak treatment group (group D, $n=36$), and Yihuo Qingyi Decoction plus r-Sak treatment group (group E, $n=36$). The rats fasted for 12 h but were freely access to water before experiment.

The rats were anaesthetized with intraperitoneal injection of 3% pentobarbital sodium (35 mg/kg). After a conventional disinfection and towel spreading, an incision along the white line of abdomen was conducted, and the retrograde pressurizing injection of 5% sodium taurocholate solution through the cholangiopancreatic duct (1.5 mL/kg) was adopted. About 10 min later, swelling and petechial hemorrhagic necrosis appeared in the pancreas in the rats of group B and C. In group A, only the pancreas was turned. In group C, 2 days before modeling, Yihuo Qingyi Decoction was intragastrically administrated, twice a day, 2 g each time. In group D, 2 days before modeling, r-Sak was administrated by intraperitoneal injection, 1.5 mg/kg, twice daily. In group E, 2 days before modeling, intragastric administration of Yihuo Qingyi Decoction (twice/d, 2 g/time) and intraperitoneal injection of r-Sak (1.5 mg/kg, twice/d) were carried out. In group B, equal volume of 0.85% NaCl was given by intragastric administration. And no drug was given in group A. After modeling, both intragastric administration and intraperitoneal injection were continuously performed until the rats were killed. Nine rats were randomly selected from each group for determining the survival rate within 18 h after modeling. Nine rats were killed by decapitation at 6 h, 12 h, and 18 h after modeling, and the arterial blood and pancreatic tissue were collected.

Determination of Serum Amylase and Lipase

Serum amylase and lipase were detected by a Hitachi 7170A full-automatic biochemical analyzer.

Detection of Ascites Weight

After the rats were killed and the abdomen was open, dry gauze was placed into the abdominal cavity to absorb the ascites. The weight difference between the wet gauze and the dry gauze was regarded as ascites weight.

Measurement of Pancreatic Blood Flow

Intraperitoneal anesthesia (3% pentobarbital sodium, 30 mg/kg), conventional disinfection, towel spreading, and abdominal incision were performed at 6 h, 12 h, and 18 h after modeling respectively. After the abdomen was incised, the electrolytic electrode of LS III tissue blood flow meter was penetrated into the cavity 1.0 cm towards the right posterior upper part from the spleen side end of

pancreas. Twenty minutes later the measurement started and the values were recorded in an interval of 10 min. The tissue blood flow meter was automatically balanced and traced by a computer. The data was calculated and showed automatically by RCBF Bench software.

Pathological Examination

Pathological examination of pancreas was performed with the conventional method. The pancreatic tissue was stained with HE and examined under a light microscopy.

Statistical Analysis

All data were expressed as mean \pm standard deviation ($\bar{x} \pm s$) and the t-test and χ^2 test were used for statistics. The data was analyzed by using SPSS computer software Version 6.0. Level of significance was fixed at 0.05.

RESULTS

Survival Rats within 18h after Modeling

In group A, B, C, D and E, 9, 2, 6, 7, and 8 rats survived respectively.

Pathological Change of Pancreas and Myocardium

The obvious pathological change of pancreas was not found in group A, but the hemorrhagic necrosis, mostly the coagulative necrosis, appeared in group B with the isolated island-like distribution of the acinus. In group C, D and E, the pathological change was significantly alleviated, the pancreatic cells had mild degeneration and edema with the interstitial vasodilatation and hyperemia, the interlobular septum widened, and a small amount of fat had necrosis. The myocardial tissue in group A had no significant pathological change. In group B, the myocardial cells had the swelling with spotty and piecemeal necrosis which connected each other into pieces sometimes, and there was infiltration of inflammatory cells and formation of cell contraction band. The myocardial cells in group C, D and E had a mild swelling, the cross striations were clear, no myocardial necrosis focus was found, the arrangement and structure of the myocardial cell were basically normal, and there was a small amount of inflammatory cell infiltration.

Changes of Amylase, Lipase, and Weight of Ascites

At each time point, the amylase and lipase and the weight of ascites in group A, C, D and E significantly decreased as compared with group B ($P<0.05$), those in group C decreased as compared with group D ($P<0.05$), and those in group E decreased as compared with group C and D ($P<0.05$), as shown in Table 1.

Change of Pancreatic Blood Flow

The pancreatic blood flow in all groups showed a tendency to decrease after SAP was induced, especially in group B. The pancreatic blood flow in group B significantly decreased as compared with group A at 6h, 12 h, and 18 h after modeling ($P<0.05$). The pancreatic blood flow in group C, D and E was also significantly

decreased ($P<0.05$), but the decrease extent was smaller than in group B, in group D was smaller than in group C,

and in group E was the smallest, as showed in Figure 1.

Table 1. Effect of r-Sak and Yihuo Qingyi Decoction on serum amylase and lipase and weight of ascites in SAP rats ($\bar{X} \pm s, n=9$)

Group	Time points (h)	Amylase/(U·L ⁻¹)	Lipase/(U·L ⁻¹)	Weight of ascites (g)
A		520±105	2,000±300	0.25±0.01
B	6	11,000±500 ^a	8,080±800 ^a	12.70±3.00 ^a
	12	14,000±460 ^a	8,500±500 ^a	14.50±4.00 ^a
	18	12,000±345 ^a	8,000±470 ^a	16.80±3.20 ^a
C	6	4,500±210 ^{abd}	2,700±200 ^{abd}	7.50±1.20 ^{abd}
	12	4,000±150 ^{abd}	2,800±190 ^{abd}	8.80±1.10 ^{abd}
	18	2,800±170 ^{abd}	2,500±140 ^{abd}	10.90±1.30 ^{abd}
D	6	4,800±180 ^{ab}	3,500±300 ^{ab}	10.50±1.40 ^{ab}
	12	4,500±130 ^{ab}	3,200±170 ^{ab}	11.20±1.30 ^{ab}
	18	3,500±120 ^{ab}	3,000±120 ^{ab}	13.40±1.80 ^{ab}
E	6	2,700±110 ^{abcd}	1,140±88 ^{abcd}	5.00±2.00 ^{abcd}
	12	1,200±120 ^{abcd}	1,400±210 ^{abcd}	6.20±2.50 ^{abcd}
	18	1,100±118 ^{abcd}	1,000±150 ^{abcd}	13.40±1.80 ^{ab}

Notes: a. $P<0.05$, compared with group A; b. $P<0.05$, compared with group B; c. $P<0.05$, compared with group C; d. $P<0.05$, compared with group D.

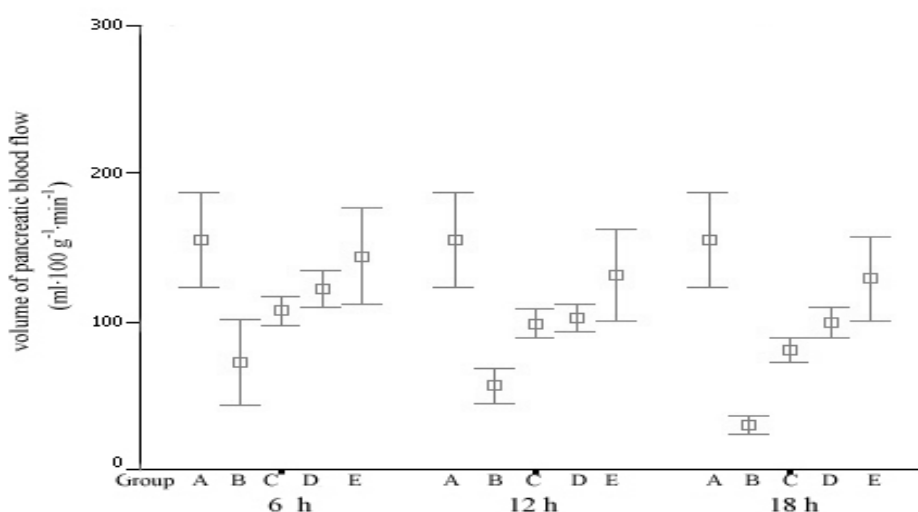


Figure 1. Effect of r-Sak and Yihuo Qingyi Decoction on the pancreatic blood flow in SAP rats

DISCUSSION

The recombinant Sak is a very effective thrombolytic agent with fibrin specificity. Because it does not activate the systematic fibrinolysis, with only few side effects but high thrombolytic activity (especially the arterial thrombosis containing rich blood platelet), it is believed to be one of the most promising thrombolytic drugs. At present, in the treatment of myocardial infarction, cerebral thrombosis, and thrombotic diseases with r-Sak, more studies on its effect on the blood flow of coronary artery and cerebral artery have been carried out.⁸⁻¹¹

However, any report on r-Sak for treatment of pancreatitis as well as its effect on blood flow of pancreatic tissue has not been found yet. In the very study, the therapeutic effect of r-Sak on SAP as well as its influence on blood flow of pancreatic tissue were studied for the first time.

Treatment of SAP with TCM and western medicine in combination has a long history in China. Qingyi Decoction (清胰汤 Decoction for acute pancreatitis), composed of the Chinese herbs with functions of promoting blood circulation and supplementing *qi*, clearing heat and toxic material, and purgation, is proved to be the effective prescription for AP in clinic.¹²⁻¹⁵ The Chinese herbs with the effect of promoting blood circulation to relieve blood stasis can improve the microcirculation dysfunction and stabilize the function of cell membrane in SAP, which have attracted a great attention.¹⁶⁻¹⁸ In the Yihuo Qingyi Decoction used in this experiment, Ren Shen, Mai Dong and Huang Qi to supplement *qi* and nourish yin, and Chuan Xiong and Dan Shen to promote blood circulation and remove blood stasis were added to the traditional Qingyi

Decoction for the purpose to increase the effect of promoting blood circulation to remove blood stasis.

The result of this study shows that the single use of r-Sak or Yihuo Qingyi Decoction can significantly improve the pathological state in serum enzyme, ascites, and blood flow of pancreatic tissue, indicating that either of them has therapeutic effect on rat SAP. In the present study, it is proved that r-Sak is stronger in improving ischemia of pancreatic tissue, while Yihuo Qingyi Decoction is better in decreasing serum enzyme and relieving ascites. Their combination is more effective in improving all the above indices, indicating that Yihuo Qingyi Decoction and r-Sak have a synergistic action in treatment of SAP, although the mechanism needs to be further explored.

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