Ligustrazine alleviates acute renal injury in a rat model of acute necrotizing pancreatitis

Jian-Xin Zhang, Sheng-Chun Dang, Jian-Guo Qu, Xue-Qing Wang

Abstract

AIM: To evaluate the effect of ligustrazine, a traditional Chinese medicine, on renal injury in a rat model of acute necrotizing pancreatitis (ANP).

METHODS: A total of 192 rats were randomly divided into three groups: control (C group), ANP without treatment (P group), and ANP treated with ligustrazine (T group). Each group was further divided into 0.5, 2, 6, 12 h subgroups. All rats were anesthetized with an intraperitoneal injection of sodium pentobarbital. Sodium taurocholate was infused through the pancreatic membrane to induce ANP. T group was infused sodium taurocholate as above, and 0.6% ligustrazine was then administered via the femoral vein. Serum urea nitrogen (BUN) and creatinine (Cr) concentrations were measured for the evaluation of renal function. The effects of ligustrazine on the severity of renal injury were assessed by renal function, TXA\textsubscript{2}/PGI\textsubscript{1} and histopathological changes. Renal blood flow was determined by the radioactive microsphere technique (RMT).

RESULTS: Compared with control group, the renal blood flow in P group was decreased significantly. Serious renal and pancreatic damages were found in P group, the BUN and Cr levels were elevated significantly, and the ratio of TXA\textsubscript{2} to PGI\textsubscript{1} was increased at 2, 6 and 12 h. Compared with P group, the blood flow of kidney was elevated significantly at 6 and 12 h after induction of ANP, the renal and pancreatic damages were attenuated, and the BUN and Cr levels were decreased significantly, and the ratio of TXA\textsubscript{2} to PGI\textsubscript{1} was decreased at 6 and 12 h in T group.

CONCLUSION: Microcirculatory disorder (MCD) is an important factor for renal injury in ANP. Ligustrazine can ameliorate the condition of MCD and the damage of pancreas and kidney.

INTRODUCTION

Acute pancreatitis complicated by multiple organ dysfunctions is still a life-threatening disease\textsuperscript{[4-6]}, although the precise mechanism by which such local inflammation in the pancreas progresses to systemic illness is still unclear. Recently, this systemic inflammatory response syndrome (SIRS) has become a widely accepted disease state\textsuperscript{[8,9]}, which could lead to the failure of distant organ systems, such as the lungs, intestine, stomach and kidneys\textsuperscript{[10-12]}.

Acute pancreatitis (AP) is often complicated by renal injury. However, its pathogenesis remains unclear. Recent studies indicate that during the pathogenesis of acute necrotizing pancreatitis (ANP), the change of microcirculation plays an important role in the worsening of pancreatitis\textsuperscript{[7]}.

Pharmacologic studies have demonstrated that ligustrazine, an intravenous drug made from traditional Chinese herbs, is able to inhibit release of intracellular calcium and to scavenge oxygen free radicals\textsuperscript{[13,14]}. Ligustrazine has been widely applied in the treatment of vascular diseases in China due to its significant efficacy on cerebral ischemia and reperfusion injury. However, its role and mechanism in treatment of renal injury have not been extensively studied. The effect of ligustrazine on renal injury was observed in this study based on the established model of ANP.

MATERIALS AND METHODS

Animals

One hundred and ninety-two adult Sprague-Dawley rats (250-300 g) were provided by the Laboratory Animal Center of Jiangsu University, China. The animals were kept in rooms at 21 ± 1°C in a 12 h light/dark cycle for 1 wk to acclimate to the surrounding with free access to water and standard laboratory chow. Prior to experiment, the rats

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Key words: Pancreatitis; Microcirculation; Ligustrazine; Renal injury

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were fasted overnight with access to water.

**Experimental design**

The animals were randomly divided into three groups: control (n = 64, C group), ANP without treatment (n = 64, P group), and ANP treated with ligustrazine (n = 64, T group). Each group was further divided into 0.5, 2, 6, 12 h subgroups. All rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Sodium taurocholate (50 g/L, 4 mL/kg, Ward Blen Kinsop CO., UK) was infused through the pancreatic membrane to induce ANP as previously described. After 5-10 min, pancreatic edema and dotted bleeding occurred. T group was infused sodium taurocholate as above, 6 g/L ligustrazine (Seventh Pharmaceutical Factory, Wuxi, China; batch number: 0008241) was then administered via the femoral vein (10 mL/kg) as previously described. C group received isovolumic infusion of 9 g/L physiological saline solution using the same method. The abdominal wounds were closed and all the rats were sent back to their cages. Half of the animals in each subgroup were sacrificed at 0.5, 2, 6 and 12 h after infusion for further examination. Left kidneys were removed immediately and fixed in paraformaldehyde solution for 12-24 h and paraffin-embedded for routine histopathologic analysis. The histopathologist was blinded to routine histopathologic analysis. The blood was obtained from eight rats in each group via superior mesenteric vein for determination of serum blood urea nitrogen (BUN), creatinine (Cr), TXA2 and PGI2. The remaining rats in each group were used for kidney blood flow determination by the radioactive microsphere technique (RMT). The left kidney was also removed and weighed at 0.5, 2, 6 and 12 h after infusion for subsequent radioactive measurement.

**Blood flow measurements**

At 0.5, 2, 6 and 12 h after the infusion, renal blood perfusion values were determined by RMT as previously described. "Tc"-labeled microspheres ("Mo-"Tc" generator preparation was provided by Chinese Institute of Nuclear Power) with a specific activity of 74 MBq/mL were used for measurement of blood flow. The right carotid artery was catheterized with placement of the tip of the tubing in the left ventricle for infusion of "Tc"-labeled microspheres. One milliliter "Tc" radioactive microspheres (approximately 50,000 microspheres) was injected for 10 s via the catheter with its tip in the aortic ventricle of the heart. A reference blood sample was obtained from the femoral artery catheter for 60 s at a constant rate of 1 mL/min with a continuous-withdrawal pump. The animals injected microspheres were killed by intra-arterial injection of 2 mL 100 g/L KCL. The whole left kidney was removed, weighed, cut into small pieces and placed in a 1-μm section, and mounted. After removed from the paraffin, the tissues were stained with hematoxylin and eosin. The severities of renal injury were quantified using a histological scoring system as previously described. Histopathologic analysis of renal specimens was performed and scored as 0-III (Table 1). Twenty fields per kidney were examined, a mean of the total score was compared between the groups. The renal sections were also analyzed with a HPIAS-1000 multimedia color analysis system (Huahai Co., Shanghai). Five fields (0.265 mm × 0.2 mm) of each section were read. Average values of neutrophil infiltration were calculated and recorded. All examinations were performed in a blind fashion by an experienced pathologist.

**Statistical analysis**

All data were analyzed by the SPSS 11.0 software. The results were expressed as mean ± SD except for data on the grading of renal lesions. Differences in grading of renal lesion were determined using the non-parametric Mann-Whitney test. Statistical analysis was performed with post-hoc test. P < 0.05 was considered statistically significant.

### RESULTS

**Renal blood flow**

Blood flow in the P group was significantly lower than that in the C group. It began to decrease at 0.5 h and became
the lowest at 12 h. However, the blood flow in the T group was significantly higher than that in the P group at 6 and 12 h, showing no significant difference from the C group (Table 2).

**Serum levels of BUN, Cr, TXA₂ and PGI₁**

Compared with the control group, the BUN and Cr levels in the P group were elevated significantly (P < 0.01 or P < 0.05), and the ratio of TXA₂ to PGI₁ was increased at 2, 6 and 12 h (P < 0.01). Compared with the P group, the BUN and Cr levels were decreased significantly (P < 0.01 or P < 0.05), and the ratio of TXA₂ to PGI₁ was decreased at 6 and 12 h in the T group (P < 0.01 or P < 0.05) (Table 3, Table 4, Table 5).

**Pathological examination**

After induction of ANP model, the pancreas showed mild edema and congestion. At 0.5 h, typical pathological changes of ANP were found, such as a large number of inflammatory cells, necrosis of the adjacent fat tissues, interstitial edema, parenchyma hemorrhage and necrosis, large amount of ascites. The changes became severer with the prolongation of time. The renal pathological changes were aggravated significantly in the P group. Histopathologic scores were higher in the P group than in the C group throughout the experiment (P < 0.01) and lower in the T group than in the P group (Table 6). Under the light microscope, different swelling denaturation and necrosis of renal tubular epithelial cells were observed. Simultaneously, interstitial congestion, edema and infiltration of inflammatory cells were also observed. While in the ligustazine-treated group, the outward appearance of the kidney was normal. The mean number of neutrophils infiltrated increased in × 400 field increased from 0.5 h in the T group, while decreased significantly from 2 h in the P group (Table 7).

**Correlated analysis**

Correlated analysis showed that there was a negative correlation between renal blood flow and serum Cr (r = -0.931, P < 0.01) and TXA₂/PGI₁ (r = -0.977, P < 0.05), as well as between renal blood flow and pathologic score (r = -0.948, P < 0.05).

**DISCUSSION**

Acute renal injury is a major cause of morbidity in ANP. Our experimental study in rats demonstrated that microcirculatory disorder (MCD) of rats resulted in a sequence of events that ultimately caused renal injury. Although the renal injury occurring in ANP has been well described, the underlying mechanism remains unclear. The rat model of renal injury in this study resulted in a dramatic decrease in renal blood flow as evidenced by

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**Table 2** Renal blood flow in groups C, P and T (mean ± SD, n = 8)

| Group | 0.5 h | 2 h | 6 h | 12 h |
|-------|-------|-----|-----|------|
| C     | 7.33 ± 0.35 | 7.63 ± 0.43 | 7.46 ± 0.67 | 7.55 ± 0.67 |
| P     | 5.67 ± 0.51* | 6.64 ± 0.68* | 5.81 ± 0.67* | 5.16 ± 0.72* |
| T     | 7.17 ± 0.72 | 7.22 ± 0.82 | 7.22 ± 0.73* | 7.31 ± 1.12* |

*P < 0.01 vs C group; **P < 0.01 vs P group.

**Table 3** Serum Cr level in groups C, P and T (mean ± SD, mmol/L)

| Group | 0.5 h | 2 h | 6 h | 12 h |
|-------|-------|-----|-----|------|
| C     | 9.80 ± 1.36 | 10.31 ± 1.50 | 10.05 ± 0.87 | 10.21 ± 1.33 |
| P     | 11.99 ± 2.08* | 12.73 ± 1.72* | 14.71 ± 2.08* | 15.16 ± 2.73* |
| T     | 10.12 ± 1.23* | 10.53 ± 2.25* | 11.52 ± 2.21* | 11.71 ± 1.31* |

*P < 0.05, **P < 0.01 vs C group; ***P < 0.01 vs P group.

**Table 4** Serum BUN level in groups C, P and T (mean ± SD, mmol/L)

| Group | 0.5 h | 2 h | 6 h | 12 h |
|-------|-------|-----|-----|------|
| C     | 1.18 ± 0.15 | 1.22 ± 0.11 | 1.24 ± 0.15 | 1.23 ± 0.16 |
| P     | 1.23 ± 0.16 | 1.50 ± 0.21* | 1.61 ± 0.19* | 1.86 ± 0.28* |
| T     | 1.19 ± 0.14 | 1.31 ± 0.14 | 1.31 ± 0.17* | 1.45 ± 0.24* |

*P < 0.05, **P < 0.01 vs C group; ***P < 0.01 vs P group.

**Table 5** Serum level of TXA₂/PGI₁ in groups C, P and T (mean ± SD)

| Group | 0.5 h | 2 h | 6 h | 12 h |
|-------|-------|-----|-----|------|
| C     | 5.5 ± 1.2 | 6.5 ± 1.2 | 6.7 ± 1.3 | 6.9 ± 1.4 |
| P     | 13.0 ± 1.6* | 15.0 ± 1.9* | 18.0 ± 1.7* | 21.1 ± 3.0* |
| T     | 6.9 ± 1.4 | 10.8 ± 1.4* | 13.0 ± 1.6* | 15.0 ± 2.0* |

*P < 0.01 vs C group; **P < 0.01 vs P group.

**Table 6** Renal tissue injury in groups C, P and T

| Group | 0.5 h | 2 h | 6 h | 12 h |
|-------|-------|-----|-----|------|
| C     | 0 | I | II | III | 0 | I | II | III | 0 | I | II | III |
| P     | 0 | 1 | 3 | 4* | 0 | 0 | 3 | 5* | 0 | 0 | 8* |
| T     | 4 | 4 | 0 | 0* | 0 | 3 | 5 | 0* | 1 | 2 | 3 | 4 |

*P < 0.01 vs C group; **P < 0.05, ***P < 0.01 vs P group.

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Ligustrazine could intervene in hemorheological events, such as blood flow, erythrocyte deformation, leukocyte adhesion, platelet aggregation and thrombolyis. It was reported that ligustrazine could inhibit pulmonary hypertension by decreasing the mRNA expression of endothelin-1, oxygen free radical level, lipid peroxidation and adjusting TXA2/PGI2 imbalance in pulmonary arterioles.

To investigate the protective effects of ligustrazine against renal injury, the influence of ligustrazine injection on BUN, Cr and TXA2/PGI2, as well as changes of morphology of renal tubules, were studied in a rat kidney model during ANP. Ligustrazine improved renal microcirculation, suggesting that the protective effects of ligustrazine against renal injury may be attributable to improving microcirculation and further preventing accumulation of neutrophils.

In conclusion, MCD plays an important role in the development of renal injury. The early use of ligustrazine seems to be effective. This provides further evidence for ligustrazine as a therapeutic strategy against renal injury during ANP.

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In the present study, ligustrazine improved renal microcirculation, suggesting that ligustrazine can protect against renal injury by improving microcirculation and further preventing accumulation of neutrophils. The early use of ligustrazine seems to be effective. This provides further evidence for ligustrazine as a therapeutic strategy against renal injury during ANP.

**Terminology**
Radiolabeled microsphere technique (RMT): Microspheres containing radioactive substances are infused into the circulatory system to measure perfusion rates in tumors and normal tissues, cerebral blood flow, tissue oxygenation, cardiovascular function, regional vascular resistance, and the effect of various substances on these parameters. In the present study, Tc-labeled microspheres were used for measurement of blood flow.

**Peer review**
This is a very interesting study demonstrating the effects of ligustrazine on the pathophysiology of acute pancreatitis. However, some limitations and additional data should be provided.

**COMMENTS**

**Background**
Acute pancreatitis (AP) is often complicated by renal injury. However, its pathogenesis remains unclear. The significant efficacy of ligustrazine on cerebral ischemia and reperfusion injury was confirmed in this study. However, the role and mechanisms of ligustrazine in treatment of renal injury have not been extensively studied.

**Research frontiers**
To investigate the protective effects of ligustrazine against renal injury, the influence of ligustrazine injection on BUN, Cr, and TXAs/PGLs, as well as changes of morphology of renal tubules, were studied in a rat kidney model during ANP.

**Innovations and breakthroughs**
The effect of ligustrazine on renal injury was observed in this study based on the established model of ANP. The radioactive microsphere technique was used to analyze the blood flow. Although it is not commonly used and has major disadvantages, it can analyze the blood flow in the pancreas and extrapancreatic vital organs simultaneously.

**Applications**
In the present study, ligustrazine improved renal microcirculation, suggesting that ligustrazine can protect against renal injury by improving microcirculation and further preventing accumulation of neutrophils. The early use of ligustrazine seems to be effective. This provides further evidence for ligustrazine as a therapeutic strategy against renal injury during ANP.

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