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• *BRIEF REPORTS* •

Changes of gastric and intestinal blood flow, serum phospholipase A_2 and interleukin-1β in rats with acute necrotizing pancreatitis

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Abstract

AIM: To explore the relationship between gastric and intestinal microcirculatory impairment and inflammatory mediators released in rats with acute necrotizing pancreatitis (ANP).

METHODS: A total of 64 rats were randomized into control group and ANP group. ANP model was induced by injection of 5% sodium taurocholate under the pancreatic membrane. Radioactive biomicrosphere technique was used to measure the gastric and intestinal tissue blood flow at 2 and 12 h after the induction of ANP, meanwhile serum phospholipase A_2 (PLA_2) activities and interleukin-1β levels were determined. Pathologic changes in pancreas, gastric and intestinal mucosae were studied.

RESULTS: The gastric blood flow in ANP group (0.62±0.06 and 0.35±0.05) mL/(min·g) was significantly lower than that in control group (0.86±0.11 and 0.85±0.06) mL/(min·g) (P<0.01) at 2 and 12 h after induction of ANP. The intestinal blood flow in ANP group (0.80±0.07 and 0.50±0.06) mL/(min·g) was significantly lower than that in control group (1.56±0.18 and 1.61±0.11) mL/(min·g) (P<0.01). Serum PLA_2 activities (94.29±9.96 and 103.71±14.40) U/L and IL-1β levels (0.78±0.13 and 0.83±0.20) μg/L in ANP group were higher than those in control group (65.27±10.52 and 66.63±9.81) U/L, (0.32±0.06 and 0.33±0.07) μg/L (P<0.01). At 2 and 12 h after introduction of the model, typical pathologic changes were found in ANP. Compared with control group, the gastric and intestinal mucosal pathologic changes were aggravated significantly (P<0.01) at 12 h after induction of ANP. Gastric and intestinal mucosal necrosis, multiple ulcer and hemorrhage occurred.

CONCLUSION: Decrease of gastric and intestinal blood flow and increase of inflammatory mediators occur simultaneously early in ANP, both of them are important pathogenic factors for gastric and intestinal mucosal injury in ANP.

Key words: Acute necrotizing pancreatitis; Interleukin-1; Phospholipase A_2; Microcirculation

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INTRODUCTION

The change of microcirculation play important roles in the progression of acute necrotizing pancreatitis (ANP) and the damage of extrapancreatic vital organs. Serum levels of proinflammatory cytokines, including interleukin-1β (IL-1β) and phospholipase A_2 (PLA_2), are significantly higher in severe acute pancreatitis. The purpose of this study was to explore the relationship between gastric and intestinal microcirculatory impairment and serum PLA_2 and IL-1β in rats with ANP.

MATERIALS AND METHODS

Animals

Sixty-four adult Sprague-Dawley rats (male or female), weighing 250-300 g, were obtained from the Laboratory Animal Center of Jiangsu University and used throughout the study. The rats were divided randomly into ANP group and control group (32 in each group).

Reagents and instruments

Sodium taurocholate (Ward Blen Kinsop Co.), PLA_2 kit (Cayman Co.), IL-1β kit (Center of Science Technique Development, General Hospital of PLA), sodium pyrophosphate and stannous chloride for injection (PYP kit) was from Jiangsu Institute of Nuclear Medicine, ^{99m}Tc (^{99}Mo-^{99m}Tc) generator preparation was provided by Chinese Institute of Nuclear Power, GC-1200 gamma radioimmunoassay counter was from USTC Chuangxin Co., Ltd, 753 UV-Vis spectrophotometer was a product of Shanghai Optical Instrument Factory.

Experimental design

The rats were fasted for 12 h with free access to water. All

the rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg). Sodium taurocholate (5% 1.0 mL) was infused to ANP rats via the pancreatic membrane as previously described[1]. After 5-10 min, pancreatic edema and dotted bleeding occurred. Then 0.9% normal solution was infused to the pancreatic membrane of control rats, and no abnormality was noticed 2 and 12 h after the operation. The blood of eight rats in each group was obtained via superior mesenteric vein for determination of serum PLA_2 activities and IL-1β levels. The other eight rats in each group were used for gastric and intestinal blood flow determinations by the intravenous radioactive microsphere technique (RMT).

Blood flow measurements

At 2 and 12 h after the infusion, organ blood perfusion values were determined with a RMT as previously described in detail[2] and modified by Chen and Dai[3]. The right carotid artery was catheterized with placement of the tip of tubing in the left ventricle for infusion of ^{99m}Tc-labeled microspheres, 1 mL ^{99m}Tc radioactive microspheres was injected for 10 s via the catheter with its tip in the aortic ventricle of 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 microsphere animals were killed by intra-arterial injection of 2 mL 10% KCl. The whole pancreas and parts of the stomach, distal ileum were removed, weighed and cut into small pieces, and placed in a γ counter to determine the radio-activity (cpm).

The blood flow values were calculated according to the following formula[4]:

$$Q_{org}\ [mL/(min \cdot g)] = \frac{Q_{ref}\ (mL/min) \times N_{org}\ (cpm)}{N_{ref}\ (cpm) \times weighing\ (g)}$$

where Q_{org} denotes organ blood flow (mL/min·g), Q_{ref} is withdrawal rate of the reference sample (mL/min), N_{org} is the number of microspheres in the organ (cpm) and N_{ref} is the number of microspheres in the reference sample.

Table 1 Histological grading of gastric mucosal damage

Grade	Histological appearance
0	No lesion
1	Lesion of mucosal surface, undamaged gastric pit cells
2	Damage involving gastric pits, undamaged gastric gland cells
3	Lesion of gastric gland cells
4	Partial mucosal necrosis, multiple linear ulcer and hemorrhage
5	Total mucosal necrosis

Table 2 Histological grading of intestinal mucosal damage

Grade	Histological appearance
0	Normal
1	Subepithelial edema, partial separation of apical cells
2	Epithelial cell slough from tips of villi
3	Progression of slough to base of villi
4	Partial mucosal necrosis of lamina propria
5	Total mucosal necrosis

Determination of serum PLA_2 activity and IL-1β levels

IL-1β levels were determined with ELISA. Serum PLA_2 activity was assayed by colorimetry.

Pathological examination

Rats were killed by intra-arterial injection of 2 mL 10% KCl. The whole pancreas and parts of the stomach, distal ileum were obtained and preserved in 40 g/L formaldehyde fluid for further studies. Paraffin-embedded tissue sections (2-3-mm thick) were stained with hematoxylin and eosin. Histological evaluation was performed with light microscopy by two blinded observers on two separate occasions.

Gastric mucosal damage was evaluated under microscope according to a method of Lo *et al*[5]. Severity of the gastric mucosal damage was graded (Table 1).

The grading of intestinal mucosal damage was described by Chiu *et al*[6], and modified by Oldham *et al*[7], (Table 2).

Statistical analysis

All data were analyzed with the SPSS software. Results were expressed as mean±SD except for date of the grading of gastric and intestinal mucosal damage. Statistical analysis was performed with *t*-test between control and ANP groups. $P<0.05$ was considered statistically significant. Differences in grading of gastric and intestinal mucosal damage between the two groups were determined using the non-parametric Mann-Whitney test.

RESULTS

Gastric and intestinal mucosal blood flow

Gastric and intestinal mucosal blood flow was 28% and 49%, 2 h after injection of 5% sodium taurocholate and 59% and 69%, 12 h after injection of 5% sodium taurocholate as compared with control group ($P<0.01$, Table 3).

Serum PLA_2 activities and IL-1β levels

At 2 h after injection of 5% sodium taurocholate, serum PLA_2 activity and IL-1β level in the samples from mesentery vein in ANP group were higher than those in control group. After 12 h, there was a significant difference between ANP group and control group ($P<0.01$, Table 4).

Table 3 Change of gastric and intestinal blood flow [mL/(min·g)] (mean±SD, $n = 8$)

Group	Stomach		Intestine	
	2 h	12 h	2 h	12 h
Control	0.86±0.11	0.85±0.06	1.56±0.18	1.61±0.11
ANP	0.62±0.06[b]	0.35±0.05[b]	0.80±0.07[b]	0.50±0.06[b]

[b]$P<0.01$ *vs* control group.

Table 4 PLA_2 activity and IL-1β level in each group (mean±SD, $n = 8$)

Group	PLA_2(U/L)		IL-1β (ng/mL)	
	2 h	12 h	2 h	12 h
Control	65.27±10.52	66.63±9.81	0.32±0.06	0.33±0.07
ANP	94.29±9.96[b]	103.71±14.40[b]	0.78±0.13[b]	0.83±0.20[b]

[b]$P<0.01$ *vs* control group.

Table 5 Gastric and intestinal mucosal injury in each group (n = 8)

Group	Stomach												Intestine											
	2 h						12 h						2 h						12 h					
	0	I	II	III	IV	V	0	I	II	III	IV	V	0	I	II	III	IV	V	0	I	II	III	IV	V
Control	8	0	0	0	0	0	8	0	0	0	0	0	6	2	0	0	0	0	6	2	0	0	0	0
ANP	0	0	2	3	3	0[b]	0	0	0	1	3	4[b]	0	0	2	4	2	0[b]	0	0	0	0	3	5[b]

[b]P<0.01 *vs* control group.

Pathologic examination of gastric and intestinal mucosa and pancreas

After induction of ANP model, pancreas showed mild edema and congestion. At 2 and 12 h after introduction of the model, typical pathologic changes were found in ANP, such as a large number of inflammatory cells, necrosis of adjacent fat tissues, interstitial edema, parenchyma hemorrhage and necrosis, large amount of ascites. Compared with control group, the gastric and intestinal mucosal pathological changes were aggravated significantly (P<0.01) at 12 h after induction of ANP. Gastric and intestinal mucosal necrosis, multiple ulcer and hemorrhage occurred (Table 5).

DISCUSSION

Microcirculatory mechanisms are involved in the development of acute pancreatitis[8,9]. The change of microcirculation is an important factor for the development of ANP. It can damage the pancreas and extrapancreatic vital organs[10,11] leading to a series of changes including vasoconstriction, ischemia, increased vascular permeability, impairment of nutrient tissue perfusion, ischemia/reperfusion, leukocyte adherence. The RMT clearly provides an efficient method for estimating blood flow to various organs in the body. Our results revealed that, at the early stage of ANP, gastric and intestinal mucosal blood flow decreased significantly 2 and 12 h after injection of 5% sodium taurocholate as compared with control group (P<0.01). Gastric and intestinal mucosal injury occurred at the same time which might result from ANP and release of inflammatory mediators. A series of changes of the nerve endocrine system bring about redistribution of viscera blood flow, producing a sharp decrease in gastric and intestinal blood flow. Gastric and intestinal mucosa is sensitive to the shortage of blood and oxygen. Due to the further decrease of circulatory blood amount and over activation of inflammatory mediators, gastric and intestinal blood flow becomes much lower to further damage the mucosa suggesting that microcirculation disturbances may contribute to gastric and intestinal mucosal damage.

Except for microcirculatory impairment, proinflammatory cytokines play another role in acute pancreatitis[12,13]. Serum PLA_2 and IL-1β are important inflammatory mediators in the process of ANP. In this study, retrograde infusion of sodium taurocholate into the pancreatic membrane resulted in increase of PLA_2 activity and IL-1β level 2 and 12 h after pancreatitis. The serum PLA_2 activities and IL-1β levels in ANP group were far significantly higher than those in control group (P<0.01). Overactivation of the inflammatory mediators may contribute to gastric and intestinal mucosal damage.

Recently, IL-1 has been shown to be a significant cytokine for the development of ANP[14]. The production of IL-1 occurs rapidly in the pancreas and specific end organs such as the lung and liver once pancreatitis is induced. IL-1β plays a crucial role in the release of other members of the inflammatory mediators such as PLA_2. With the increase of IL-1β levels, IL-1β stimulate the gene expression of PLA_2[15], and leads to direct endothelium injury with an increase in microvessel permeability resulting in poor flow conditions. IL-1β can also injure the tissue by activating inflammatory mediators such as prostaglandin, leukotrienes, platelet activating factor, *etc*.

PLA_2 is synthesized and secreted by pancreatic acinar cells. Although its mechanism is unknown, PLA_2 has long been considered to be one of the important digestive enzymes that destroy pancreatic tissue, resulting in acute pancreatitis. In the present study, amylase activity in plasma was noticeably increased even 1 h after the induction of pancreatitis[16]. The results suggest that PLA_2 plays a role in the injury of gastric and intestinal mucosa.

At the early stage of ANP, serum PLA_2 activity and IL-1β level increase rapidly with the aggravation of ANP. In this study, we found that gastric and intestinal mucosal blood flow decreased in ANP group and serum PLA_2 activity and IL-1β level in ANP group increased. In conclusion, gastric and intestinal mucosal ischemia/reperfusion can lead to tissue cell damage to free inflammatory mediators including PLA_2 and IL-1β, which interact with each other to cause gastric and intestinal mucosal injury.

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