Changes of gastric and intestinal blood flow, serum phospholipase A₂ and interleukin-1β in rats with acute necrotizing pancreatitis

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

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 A₂ (PLA₂) activities and interleukin-1β levels were determined. Pathologic changes in pancreas, gastric and intestinal mucosa 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 PLA₂ 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.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

Key words: Acute necrotizing pancreatitis; Interleukin-1; Phospholipase A₂; Microcirculation

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 A₂ (PLA₂), 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 PLA₂ 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.), PLA₂ 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, ⁹⁹ᵐTc 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 of pentobarbital (50 mg/kg). Sodium taurocholate (5% in the left ventricle for infusion of artery was catheterized with placement of the tip of tubing in detail. Values were determined with a RMT as previously described. At 2 and 12 h after the infusion, organ blood perfusion 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 and modified by Chen and Dai. The right carotid artery was catheterized with placement of the tip of tubing in the left ventricle for infusion of Tc labeled microspheres, 1 mL 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:

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

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 PLA₂, activity and IL-1β levels**
IL-1β levels were determined with ELISA. Serum PLA₂ 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., and modified by Oldham et al. (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 PLA₂, activities and IL-1β levels**
At 2 h after injection of 5% sodium taurocholate, serum PLA₂ activity and IL-1β level in the samples from mesentry 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*                   | 0.35±0.05*                    | 0.80±0.07*   | 0.50±0.06*          |

*P<0.01 vs control group.

| Table 4 | PLA₂ activity and IL-1β level in each group (mean±SD, n=8) |
|---------|---------------------------------------------------------------|
| Group   | PLA₂ (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*                  | 103.71±4.40*                 | 0.78±0.13*   | 0.85±0.20*          |

*P<0.01 vs control group.
DISCUSSION

Microcirculatory mechanisms are involved in the development of acute pancreatitis\cite{8,9}. The change of microcirculation is an important factor for the development of ANP. It can damage the pancreas and extrapancreatic vital organs\cite{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 sodium taurocholate 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\cite{12,13}. Serum PLA$_2$ and IL-1$\beta$ 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-1$\beta$ levels 2 and 12 h after pancreatitis. The serum PLA$_2$ activities and IL-1$\beta$ 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\cite{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-1$\beta$ plays a crucial role in the release of other members of the inflammatory mediators such as PLA$_2$. With the increase of IL-1$\beta$ levels, IL-1$\beta$ stimulate the gene expression of PLA$_2$\cite{15}, and leads to direct endothelium injury with an increase in microvessel permeability resulting in poor flow conditions. IL-1$\beta$ 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\cite{10}. 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-1$\beta$ 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-1$\beta$ 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-1$\beta$, which interact with each other to cause gastric and intestinal mucosal injury.

REFERENCES

1. Xue JG, Wang Y, Xu WD. An improved model of acute hemorrhagic necrotizing pancreatitis in roots. Zhonghua Shiyan Waike Za Zhi 1994; 11: 313
2. Wang JX, Yu ZJ, Jin HZ, Wang QX, Wang FC, Yang MG, Cao J, Zhou XY. Estimation of regional blood flow in animals using 51Cr- and 99mTc-bimicrospheres. Zhongguo Yaoli Xuebao 1985; 6: 248-251
3. Chen JZ, Dai ZB. The relationship between microcirculation and bacterial translocation in severe acute pancreatitis. Zhonghui Waike Za Zhi 1998; 36: 47-49
4. Xu D, Qi L, Guifordy D, Cruz N, Berg R, Deitch EA. Mechanisms of endotoxin-induced intestinal injury in a hyperdynamic model of sepsis. J Trauma 1993; 34: 676-682; discussion 682-683
5. Lo SK, Leung FW, Guth PH. Protection against absolute-ethanol-induced gastric antral and corpus mucosal injury. A gross and histologic study. Dig Dis Sci 1988; 33: 1403-1408
6. Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. Arch Surg 1970; 101: 478-483
Oldham KT, Guice KS, Gore D, Gourley WK, Lobe TE. Treatment of intestinal ischemia with oxygenated intraluminal perfluorocarbons. Am J Surg 1987; 153: 291–294

Kaska M, Pospisilova B, Slizova D. Pathomorphological changes in microcirculation of pancreas during experimental acute pancreatitis. Hepatogastroenterology 2000; 47: 1570-1574

Johansson M, Carlsson PO, Jansson L. Caerulein-induced pancreatitis and islet blood flow in anesthetized rats. J Surg Res 2003; 113: 13–20

Hotz HG, Foitzik T, Rohweder J, Schulzke JD, Fromm M, Runkel NS, Buhr HJ. Intestinal microcirculation and gut permeability in acute pancreatitis: early changes and therapeutic implications. J Gastrointest Surg 1998; 2: 518-525

Foitzik T, Eibl G, Hotz B, Hotz H, Kahrau S, Kasten C, Schneider P, Buhr HJ. Persistent multiple organ microcirculatory disorders in severe acute pancreatitis: experimental findings and clinical implications. Dig Dis Sci 2002; 47: 130-138

Osman MO, Gesser B, Mortensen JT, Matsushima K, Jensen SL, Larson CG. Profiles of pro-inflammatory cytokines in the serum of rabbits after experimentally induced acute pancreatitis. Cytokine 2002; 17: 53-59

Bidarkundi GK, Wig JD, Bhatnagar A, Majumdar S. Clinical relevance of intracellular cytokines IL-6 and IL-12 in acute pancreatitis, and correlation with APACHE III score. Br J Biomed Sci 2002; 59: 85-89

Fink GW, Norman JG. Intrapancreatic interleukin-1 beta gene expression by specific leukocyte populations during acute pancreatitis. J Surg Res 1996; 63: 369-373

McHowat J, Liu S. Interleukin-1 beta stimulates phospholipase A2 activity in adult rat ventricular myocytes. Am J Physiol 1997; 272: C450-C456

Zhang H, Li Y. Role of phospholipase A2 in injury of lung complicated by acute pancreatitis and the therapeutic effect and mechanism of verapamil on it. Zhongguo Weizhongbing Jiji Yixue 2003; 15: 418-421

Science Editor Wang XL and Guo SY  Language Editor Elsevier HK