Pancreatic microcirculatory impairment in experimental acute pancreatitis in rats

Zong-Guang Zhou, You-Dai Chen, Wei Sun, Zhong Chen

INTRODUCTION
Etiopathology of acute pancreatitis (AP) is not fully understood. Microcirculatory impairment has long been recognized as one of the etiological factors of acute pancreatitis. Pancreatic microcirculatory disturbance may act as initiating factor or aggravating/continuing factor. However, the mechanism of microcirculatory impairment in acute pancreatitis is complex; there are questions concerning local pancreatic microcirculatory change in acute pancreatitis and the features of pancreatic microcirculatory disturbance in various stages of AP remain subject to further study. To investigate the feature of the pancreatic microcirculatory impairment in the early-stage of caerulein-induced experimental acute pancreatitis, dynamic method of microcirculatory research combined with static method had been carried out in this study.

MATERIALS AND METHODS
Animals
48 adult male Wistar rats, weighing 250-350 g, were randomly assigned to 4 groups: (1) control group (group 1, n=12). (2) intravitral study group, pancreatic microcirculation observed with FITC-labeled RBC and intravital fluorescence microscope (group 2, n=12). (3) light microscopy and scanning electron microscopy study group, pancreatic microvasculature perfused with ink and methylmethacrylate (group 3, n=12). (4) histocellular study group (group 4, n=12).

Experimental pancreatitis
Caerulein used to induce acute pancreatitis was obtained from Sigma Co.. All experimental groups were injected caerulein subcutaneously 5.5 and 7.5 µg. kg⁻¹ 1 and 2 h after the beginning of experiment respectively, while control group was injected physiological saline solution subcutaneously. All groups were observed 4 after the beginning of the experiment.

Erythrocytes labeling
Erythrocytes were labeled by fluorescein isothiocyanate (FITC, purchased from Sigma Co.) using a combined approach of the procedures of Klar (1995). The labeled cells were stored a maximum of 24h before use.

In vivo microscopy
The pancreas of the studied animal was exteriorized on a stage, then FITC-labeled RBC was intravenously injected and intravitral fluorescence microscope (Olympus X-70) were used to dynamically observe the pancreatic microcirculatory indices, and the images were simultaneously picked up by high-resolution video cassette recorder.

Morphology of microvasculature
Thoracic aortas of the studied animal were cannulated for perfusion. After flushing the vessels with warmed heparinized physiological saline solution, a diluted resin mixture or China ink was injected through the cannula with an injection pressure of 12-16kPa, until the portal vein and inferior vena cava was filled with the injected resin or ink. The pancreas of resin-injected animal was corroded overnight or longer in a hot 300-400 g. L⁻¹ KOH solution, washed in running water and rinsed again several times in distilled water.
The pancreas of ink-injected animal was fixed overnight or longer in Bouins solution, cleared in trichloromethane, embedded in paraffin, serially sectioned (thin sections of 5-7 µm for observation of the relationship between capillaries and cells, thick sections of 50-100 µm for observation of the vessel continuation), and observed with an Olympus X-60/50 light microscope. Serial reconstruction was carried out, camera lucida tracings of photographs were made at x330 final magnification on transparent sheets and superimposed for analysis.

Statistical analysis
The results were expressed in mean ± standard deviation, and t-test was used to evaluate differences between control and AP groups. Difference was considered significant at the P<0.05 level.

RESULTS
Pancreatic edema
Gross appearance of pancreatic tissue of control group remained normal, and presented 72 % of water content. In comparison, pancreatic edema gradually appeared in Group2, 3 and 4 four hours after subcutaneous injection of caerulein, in parallel with an increase in pancreatic tissue volume. Edema of pancreatic head and body was much prominent, and the water content increased to 75 %. Inflammatory exudate accumulated in the anterior pararenal space and lesser omental sac in 50 % cases.

Morphology
Injury of intralobular arteriolar sphincter became visible 4 h after animal model established, and numerous cytoplasmic vacuoles formed; massive interstitial edema and inflammatory cell infiltration gradually emerged at 6 h. While in control group, pancreatic acini, tubules and blood vessels were normal microscopically.

Serum amylase
Serum amylase measurement in control group presented normal level (20.8 µkat, L⁻¹). Serum amylase in all AP groups showed hyperamylasemia (45.0 µkat, L⁻¹), significant higher than that of control group (P<0.01).

Light microscopy and scanning electron microscopy
Animals in the caerulein-treated group showed constriction of intralobular arteriolar sphincter 4 h after beginning of the experiment, presence of vacuoles in all the layers of sphincter, gross irregularity in capillary network of acini, reduction of capillary density, and blebs protruded from the surface of casts reflecting a substantial increase in capillary permeability.

In vivo fluorescence microscopy
Comparing with the control group, 4 hours after the start of experiment in AP groups the pancreatic microcirculatory in the caerulein-treated group showed the reduction of the velocity of FITC-labeled RBC, decrease of pancreatic capillary blood flow (P<0.01, Table 1), reduction of functional capillary density and arterioles diameter (P<0.05), and irregular intermittent perfusion of capillary network (P<0.05). Arterioles of pancreatic lobules and capillary density experienced significant changes at 6 h. The calibers of venules and capillaries showed no marked change in 6 h, while there was significant change by 8 h (P<0.05, Table 2).

| Group | d(FITC-RBC)/vel. (×10⁶ cells/L) | Capillary blood flow (nl.min⁻¹) | Microcirculatory perfusion |
|-------|---------------------------------|-------------------------------|---------------------------|
| Control | 113±5                           | 86±3                          | 0.28±0.01                 | 0.88±0.06                 |
| AP     | 85±9                            | 43±2                          | 0.12±0.03                 | 0.56±0.09                 |
| 6 Control | 104±4                           | 81±4                          | 0.33±0.02                 | 0.99±0.07                 |
| AP     | 68±7                            | 36±5                          | 0.09±0.03                 | 0.45±0.12                 |
| 8 Control | 96±6                            | 84±5                          | 0.29±0.04                 | 0.91±0.06                 |

DISCUSSION
In 1862 Panum demonstrated that acute hemorrhagic pancreatitis could be induced with wax droplets injected into pancreatic arteries. From then on, the etiological role which pancreatic ischaemia and tissue hypoperfusion plays in AP has been extensively discussed[29]. Many researches suggested that local microcirculatory disturbance, not insufficient blood flow in peripheral circulation, was responsible for perfusion failure of pancreatic tissue. In recent years, various animal models such as hemorrhagic shock, embolization of pancreatic microvasculature by minute particles and ligation of pancreatic arteries, have been used to verify that microcirculatory impairment of pancreas is the initial stage of AP. But the following questions haven’t been answered conclusively: whether all types of AP are initiated by pancreatic microcirculatory impairment? What are the characteristics of early-stage pancreatic microcirculatory change? And what are the features of pancreatic microcirculatory disturbance in the natural process of AP? Insights into all these areas are crucial to the development of prevention and treatment measures.
Animal model
Sodium taurocholate-induced experimental pancreatitis was used by many authors to investigate microcirculatory change of AP; this model can reflect soundly the pathological features of acute necrotizing pancreatitis. Since direct injury to pancreatic ductules, acini and blood vessels may happen in several minutes, the gradual evolution of early-stage pathological change of pancreas in AP cannot be explored. In addition, modulation of intraductal pressure in the process of retrograde pancreateobiliary injection of sodium taurocholate also poses a real challenge. In this study, caerulein-induced experimental pancreatitis was chosen to investigate the features of early-stage pancreatic microcirculatory change. Subcutaneous administration of caerulein is easy to operate, and can result in acute edematous pancreatitis similar to that induced by intravenous injection of caerulein. In this model, the pathological changes develop slowly and gradually, the microcirculatory and histological changes of pancreas become prominent 4 h after the beginning of experiment, and pancreatic edema reaches its zenith by 8 h. This gradual development course allows us to study the triggering factor and the features of early-stage pancreatic microcirculatory impairment without haste.

Study techniques
For decades, pancreatic microcirculatory study heavily depended on the following techniques: injection of minute particles, Indian ink and methylthionine chloride into pancreatic arteries; measurement of pancreatic blood flow through pancreateoduodenal arteries and veins; measurements of relative flow blood and tissue perfusion of pancreas with intravenous injection of nuclide Rb-86, etc. Since acute necrotizing pancreatitis is characterized with progressive regional or focal necrosis of pancreatic tissue, observation with a single method has the following disadvantages: (1) dynamic and direct observation of local microcirculatory change of pancreas is impossible, since the animal must be sacrificed at a specific time; (2) observation of local blood flow of pancreas and tissue perfusion cannot be made simultaneously on the same specimen; (3) as to traditional intravital observation of pancreatic microcirculation, quantitative study cannot be effective due to dim image. This experiment has solved the above problems by developing a new approach; this approach combined intravital microcirculation observation technique, using selective blood element fluorescent marker, with another technique-maintaining dynamic and tissue message on static specimen.

Pancreatic microcirculatory impairment in AP
In recent years, applied basic researches on the morphology of pancreatic microcirculation revealed that the blood supply of pancreatic lobule, in most cases, is provided by a single intralobular arteriole. This arteriole sends forth tree-like branches when entering pancreatic lobule; it has no anastomosis with adjacent intralobular arterioles and their branches, and can be considered end-artery[8]. This characteristic suggested that pancreatic lobules are susceptible to ischaemic injury due to spasm of intralobular arterioles, embolization of arterioles by emboli, formation of microthrombi or compression by interstitial edema. However, causative factors of early-stage ischaemia and the precise triggering factor of local microcirculatory disturbance are not evident.

This study showed that, manifested as lasting spasm of arteriolar sphincter and multiple cytoplasmic vacuoles within smooth muscle cells of sphincter, the main feature of early-stage pancreatic microcirculatory impairment of AP is injury of sphincter of pancreatic intralobular arteriole. This experiment also demonstrated that among many factors causing early-stage ischaemia, the key one is injury and spasm of sphincter of pancreatic intralobular arteriole. In this study, injury of arteriolar sphincter occurred earlier than microcirculatory impairment, which reflected that injury of intralobular arteriolar sphincter was the initial stage of pancreatic perfusion failure and local microcirculatory disturbance. Microcirculatory hypoperfusion happened almost simultaneously with injury and spasm of arteriolar sphincter, indicating that pancreatic tissue is highly sensitive to ischaemic stress and has no compensatory reserve. Since sphincter of pancreatic intralobular arteriole serves as main lockgate to control blood flow to pancreatic lobule, and intralobular arteriole has characteristics of end-artery, even sphinter spasm of very short time will quickly evoke obvious pancreatic microcirculatory impairment. Other factors causing ischaemia[31-37], such as compression from interstitial edema, microemboli or obstruction due to thrombosis, tend to be secondary ones, which may happen gradually in the course of pathological change of AP. These traumatic factors help to sustain and aggravate pancreatic microcirculatory impairment. To clarify relationship between traumatic factors of pancreatic microcirculatory impairment and pathological evolution of AP has guiding value in making treatment plans for clinical AP cases of various development stages. Features of early-stage microcirculatory change of experimental pancreatitis suggested that early adoption of spasm relieving and counter-injury measures are of vital importance in prevention and treatment of local microcirculatory disturbance of AP.

REFERENCES
1. Chen QP. Enteral nutrition and acute pancreatitis. World J Gastroenterol 2001; 7: 185-192
2. Fleischer F, Dabew R, Göke B, Wagner ACC. Stress kinase inhibition modulates acute experimental pancreatitis. World J Gastroenterol 2001; 7: 259-265
3. Zhang WZ, Han TQ, Tang YQ, Zhang SD. Rapid detection of sepsis complicating acute necrotizing pancreatitis using polymerase chain reaction. World J Gastroenterol 2001; 7: 289-292
4. Luo Y, Yuan CX, Peng YL, Wei PL, Zhang ZD, Jiang JM, Dai L, Hu YK. Can ultrasound predict the severity of acute pancreatitis early by observing acute fluid collection? World J Gastroenterol 2001; 7: 293-295
5. Slavin J, Ghaneh P, Sutton R, Hartley M, Rowlands P, Garvey C, Hughes M, Neoptolomos J. Management of necrotizing pancreatitis. World J Gastroenterol 2001; 7: 476-481
6. Wu XN. Current concept of pathogenesis of severe acute pancreatitis. World J Gastroenterol 2000; 6: 32-36
7. Xia Q, Jiang JM, Gong X, Chen GF, Li L, Huang ZW. Experimental study of “Tong Xia” purgative method in ameliorating lung injury in acute necrotizing pancreatitis. World J Gastroenterol 2000; 6: 115-118
8. Tiscornia OM, Hamamura S, Lehmann ES, Otero G, Waisman H, Tiscornia Wasserman P, Bank S. Glucose intolerance in acute pancreatitis: a review. World J Gastroenterol 2000; 6: 157-168
9. Wu XJ, Xu JY, Yuan YZ. Effect of emodin and sandostatin on metabolism of eicosanoids in acute necrotizing pancreatitis. World J Gastroenterol 2000; 6: 293-294
10. Qin RY, Zou SQ, Wu ZD, Qiu FZ. Influence of splanchnic vessel inflammation on the content of endotoxins in plasma and the translocation of intestinal bacteria in rats with acute hemorrhage necrosis pancreatitis. World J Gastroenterol 2000; 6: 577-580
11. Wu XN. Treatment revisited and factors affecting prognosis of severe acute pancreatitis. World J Gastroenterol 2000; 6: 633-635
12. Chen DL, Wang WZ, Wang JY. Epidermal growth factor
prevents gut atrophy and maintains intestinal integrity in rats with acute pancreatitis. World J Gastroenterol 2000; 6:762-765

13 Wu XN. The mechanism of actions of Octreotide, Bupleurum, Peony Cheng Qi decoction and Dan Shan in severe acute pancreatitis. World J Gastroenterol 1999; 5: 249-251

14 Pezzilli R, Mancini F. Assessment of severity of acute pancreatitis: a comparison between old and most recent modalities used to evaluate this perennial problem. World J Gastroenterol 1995; 1: 263-265

15 Yuan YZ, Lou KX, Gong ZH, Tu SP, Zhai ZK, Xu JY. Effects and mechanisms of emodin on pancreatic tissue EGF expression in acute pancreatitis in rats. Shijie Huaren Xixia Zazhi 2001; 9: 127-130

16 Xia SH, Zhao XY, Guo P, Da SP. Hemorocirculatory disorder in dogs with severe acute pancreatitis and intervention of platelet activating factor antagonist. Shijie Huaren Xixia Zazhi 2001; 9: 550-554

17 Wu CT, Li ZL. Effect of DAO on intestinal damage in acute necrotizing pancreatitis in dogs. Shijie Huaren Xixia Zazhi 1999; 7: 64-65

18 Gong ZH, Yuan YZ, Lou KX, Tu SP, Zhai ZK, Xu JY. Effects and mechanisms of somatostatin analogues on apoptosis of pancreatic acinar cells in acute pancreatitis in mice. Shijie Huaren Xixia Zazhi 1999; 7: 964-966

19 Qin RY, Zou SQ, Wu ZD, Qiu FZ. Effect of splanchnic vascular perfusion on production of TNF α and OFR in rats with acute hemorrhagic necrotic pancreatitis. Huaren Xixia Zazhi 1998; 6: 831-833

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

21 Hirano T, Hirano K. Thromboxane A2 receptor antagonist prevents pancreatic microvascular leakage in rats with caerulein-induced acute pancreatitis. Int Surg Investig 1999; 1: 203-210

22 Bhatia M, Saluja AK, Singh VP, Frossard JL, Lee HS, Bhagat L, Gerard C, Steer ML. Complement factor C5a exerts an anti-inflammatory effect in acute pancreatitis and associated lung injury. Am J Physiol Gastrointest Liver Physiol 2001; 280: G974-978

23 Bhatia M, Brady M, Zagorski J, Christmas SE, Campbell F, Neoptolemos JP, Slavin J. Treatment with neutralising antibody against cytokine induced neutrophil chemoattractant (CINC) protects rats against acute pancreatitis associated lung injury. Gut 2000; 47: 838-844

24 Leung PS, Chan WP, Nobiling R. Regulated expression of pancreatic renin-angiotensin system in experimental pancreatitis. Mol Cell Endocrinol 2000; 166: 121-128

25 Gomez-Cambronero L, Camps B, de La Asuncion JG, Cerda M, Pelín A, Pallardo FV, Calvetej, Swery JH, Mann GE, Vina J, Sastre J. Pentoxifylline ameliorates cereulain- induced pancreatitis in rats: role of glutathione and nitric oxide. J Pharmacol Exp Ther 2000; 293: 670-676

26 al-Eryani S, Payer J, Huerka M, Duris I. Etiology and pathogenesis of acute pancreatitis. Bratisl Lek Listy 1998; 99: 303-311

27 Sunamura M, Yamauchi J, Shibuya K, Chen HM, Ding L, Takeda K, Kobari M, Matsuura S. Pancreatic microcirculation in acute pancreatitis. J Hepatobiliary Pancreat Surg 1998; 5: 62-68

28 Pluszczky T, Rathgeb D, Westermann S, Feipel G. Somatostatin attenuates microcirculatory impairment in acute sodium taurocholate-induced pancreatitis. Dig Dis Sci 1998; 43: 575-585

29 Pluszczky T, Westermann S, Besral B, Menger M, Feipel G. Temporary pancreatic duct occlusion by ethibloc: cause of microcirculatory shutdown, acute inflammation, and pancreas necrosis. World J Surg 2001; 25: 432-437

30 Zhou Z, Zeng Y, Yang P, Cheng Z, Zhao J, Shu Y, Gao X, Yan L, Zhang Z. Structure and function of pancreatic microcirculation. Shangwu Yixue Gongchengxue Zazhi 2001; 18: 195-200

31 Klär E, Werner J. New pathophysiological knowledge about acute pancreatitis. Chirurg 2000; 71: 253-264

32 Skoromnyy AN, Starosek VN. Hemodynamic changes in the liver, kidney, small intestine and pancreas in experimental acute pancreatitis. Klin Khir 1998; 12: 46-48

33 Obermaier R, Benz S, Kortmann B, Benthues A, Ansorge N, Hopt UT. Ischemia/ reperfusion-induced pancreatitis in rats: a new model of complete normothermic transplantation. J Gastrointest Surg 1999; 3: 162-166

34 von Dobschuetz E, Hoffmann T, Messmer K. Inhibition of neutrophil proteinases by recombinant serpin Lex032 reduces capillary no-reflow in Ischemia/ reperfusion-induced acute pancreatitis. J Pharmacol Exp Ther 1999; 290: 782-788

35 Vollmar B, Janata J, Yamachi J, Wolf B, Heuser M, Menger MD. Exocrine, but not endocrine, tissue is susceptible to microvascular ischemia/ reperfusion injury following pancreas transplantation in the rat. Transplantation 1999; 293: 50-55

36 Benz S, Schnabel R, Morgenroth K, Weber H, Pfeffer F, Hopt UT. Ischemia/ reperfusion injury of the pancreas: a new animal model. J Surg Res 1998; 75: 109-115

Edited by Pagliarini R