PO Box 2345, Beijing 100023, China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2004;10(21):3185-3187
World Journal of Gastroenterology
 ISSN 1007-9327

• BRIEF REPORTS •

Changes of cytosolic [Ca^{2+}]i in neutrophils in pancreatic microcirculation of rats with caerulein-induced acute pancreatitis under fluid shear stress

Zong-Guang Zhou, You-Qin Chen, Xu-Bao Liu, Wei-Ming Hu, Bo-Le Tian, Huai-Qing Chen

Zong-Guang Zhou, You-Qin Chen, Xu-Bao Liu, Wei-Ming Hu, Bo-Le Tian, Department of General Surgery & Institute of Gastroenteric Surgery, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China
Huai-Qing Chen, Institute of Biomedical Engineering, West China Center of Medical Sciences, Sichuan University, Chengdu 610041, Sichuan Province, China
Supported by the National Natural Science Foundation of China, No. 39770722 and the Key Project of National Outstanding Youth Foundation of China, No. 39925032
Correspondence to: Professor Zong-Guang Zhou, Department of General Surgery & Institute of Gastroenteric Surgery, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China. zhou767@21cn.com
Telephone: +86-28-85422484 **Fax:** +86-28-85422484
Received: 2004-03-27 **Accepted:** 2004-04-05

Abstract

AIM: To investigate the fluid shear stress induced changes of [Ca^{2+}]i in neutrophils in pancreatic microcirculation of experimental acute pancreatitis (AP).

METHODS: Wistar rats (n = 36) were randomized into three groups. A model of AP was established by subcutaneous injection of caerulein. Low-shear 30 viscometer was used to provide steady fluid shear stress on separated neutrophils. The mean fluorescent intensity tested by flow cytometry was used as the indication of [Ca2+]i quantity.

RESULTS: Under steady shear, cytosolic [Ca^{2+}]i showed biphasic changes. The shear rate changed from low to high, [Ca^{2+}]i in different groups decreased slightly and then increased gradually to a high level (P<0.05). A close correlation was observed between the cytosolic [Ca^{2+}]i level and the alteration of fluid shear stress in regional microcirculation of AP.

CONCLUSION: The increase of [Ca^{2+}]i is highly related to the activation of neutrophils, which contributes to neutrophil adhesion to endothelium in the early phase of AP. The effect of fluid shear stress on [Ca^{2+}]i may play a crucial role in pancreatic microcirculatory failure of AP.

Zhou ZG, Chen YQ, Liu XB, Hu WM, Tian BL, Chen HQ. Changes of cytosolic [Ca^{2+}]i in neutrophils in pancreatic microcirculation of rats with caerulein-induced acute pancreatitis under fluid shear stress. *World J Gastroenterol* 2004; 10(21): 3185-3187
http://www.wjgnet.com/1007-9327/10/3185.asp

INTRODUCTION

Studies have confirmed the hypothesis that microcirculatory derangements play a pivotal role in the pathogenesis of acute pancreatitis (AP), including the process of conversion from edematous to necrotizing injury[1-3]. Although several pathophysiological sequences, such as protease activation, free radical generation, and inflammatory mediator release, have been described in acute pancreatitis, the precise mechanism by which acute pancreatitis is initiated is unknown[4-7]. Cellular calcium, a key physiological signaling element in cell function and also a crucial pathological intracellular messenger in cell injury, appears to be involved in the initiation and development of acute pancreatitis[8-12]. Previous studies have suggested that AP is frequently associated with sequestration of inflammatory cells, particularly neutrophils, within pancreas, and it is generally believed to be an early and important event in the evolution of pancreatitis[13,14]. Recently, considerable attention has been directed at identifying the chemoattractant substances responsible for leukocyte sequestration within these tissues and the factors released from these inflammatory cells that contribute to progression of AP[15-21]. Cytosolic free Ca^{2+}, a well-known second messenger, takes part in many cellular reaction processes and regulates the activity of many enzymes[8]. Caerulein-induced AP has been shown to cause cytosolic free Ca^{2+} transient increase[9-12]. Increase in leukocyte cytosolic [Ca^{2+}]i might be involved in intercellular adhesion by regulating the affinity of surface adhesion molecules or by facilitating transendothelial leukocyte migration, which might lead to increased leukocytic infiltration and tissue damage during AP[9-12].

Previous studies mainly concentrated on the peripheral blood neutrophils during AP under static state due to limitation of experimental methodology. However, to the authors' knowledge, no reports are available about local pancreatic microcirculatory neutrophils during AP under flow state. Although the number of neutrophils in circulation is much less compared with the total mature neutrophils in the body, these circulating cells move with blood in the whole body, and execute disinfectant functions. The stored neutrophils replenish at a certain rate into the blood to maintain a dynamic balance between circulation pool and bone marrow storage pool[22,23]. When it is necessary, bone marrow can release more neutrophils to enhance the defense efficacy. So blood flow is an important physiological environment for neutrophils[22,23]. We first undertook to investigate the change of [Ca^{2+}]i in local pancreatic microcirculatory neutrophils during AP under fluid sheer stress. The results of this study might provide a new insight into the pathogenesis of AP.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighting 250-350 g were purchased from Center of Experimental Animals, Sichuan University, Chengdu, China. All animals were starved for 24 h prior to experimentation. All animal experiments were conducted according to the guidelines of the Local Animal Use and Care Committees and the National Animal Welfare Law.

Induction of acute pancreatitis

All rats of the experimental groups were injected with 5.5 μg/kg and 7.5 μg/kg of acaerulein (Sigma Co., USA) subcutaneously

at 0 and 1 h after the beginning of experiment respectively, while the rats of the control group were subcutaneously injected with normal saline solution.

Experimental protocol

Experimental animals were divided into three groups, with 12 rats each group. Group 1: normal control, group 2 (AP- I): rats at 2 h after the induction of AP , and group 3 (AP- II): rats at 4 h after the induction of AP. Rats in each experimental group were killed to obtain blood by splenic vein puncture at 2 and 4 h after first caerulein injection.

Preparation of neutrophils

Rat polymorphonuclear cells (PMNs) were isolated according to the technique described by Hjorth *et al*.[24] for human PMNs. Blood was immediately mixed with heparin (50 U/mL) and centrifuged in a discontinuous Percoll gradient to yield a fraction of approximately 97% purity. Cell viability, as assessed by trypan blue exclusion, was above 96% under all experimental conditions.

Shear stress action on neutrophil suspension

Low-shear 30 viscometer (Switzerland) was used to provide low shear rate (5.96/s, 14.98/s, 51.2/s, 94.5/s and 128.5/s), then 37 g/L formaldehyde was added to fix neutrophils after sheared for 1 min.

Measurement of [Ca^{2+}]i

Cells were loaded (5×10^6/mL) with the Ca^{2+}-sensitive fluorescent dye fluo-3/AM (2 μmol/L; Molecular probes, Eugene OR) at 37 ℃ for 30 min in 145 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L $MgCl_2$, 10 mmol/L glucose, 4 mmol/L probenecid, 10 mmol/L HEPES, pH 7.4. Fluorescence intensity was then measured with flow cytometry. The intensity of fluorescence correlated with the concentration of [Ca^{2+}]i. Therefore, the mean fluorescence intensity (MFI) indicated the level of [Ca^{2+}]i.

Statistical analysis

The results were expressed as mean±SD. The mean of MRI between groups was compared by a two-tailed Student's *t* tests. $P\leqslant0.05$ was considered statistically significant.

RESULTS

Under steady shear, cytosolic [Ca^{2+}]i had biphasic changes. When the shear rate was very low, [Ca^{2+}]i decreased slightly. With the increase of shear rate, [Ca^{2+}]i increased gradually. When the shear rate was increased higher than 50/s, [Ca^{2+}]i in the experimental group was higher than that in the control group. With the shear rate changed from low to high, cytosolic [Ca^{2+}]i gradually increased to a significantly high level compared with the stationary control ($P<0.05$). After treated with a shear rate of 128.5/s, the cytosolic [Ca^{2+}]i of AP-II group was significantly induced compared with AP-I group ($P<0.05$) (Figure 1).

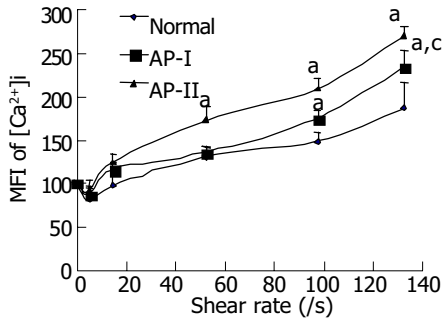

Figure 1 Effects of fluid shear stress on cytosolic [Ca^{2+}]i in neutrophils in pancreatic microcirculation of rats with AP. $^aP<0.05$ *vs* normal; $^cP<0.05$ *vs* AP- II.

DISCUSSION

Acute pancreatitis remains a clinical challenge because it is difficult to predict whether the disease is mild or runs a severe course with a possibly fatal outcome in a given patient[25]. In recent years, researches on the morphology of pancreatic microcirculation have revealed that the blood supply of pancreatic lobules in most cases is provided by a single intralobular arteriole[26]. This arteriole sends forth the tree-like branches when entering pancreatic lobule, and has no anastomosis with adjacent intralobular arterioles and their branches, and could be considered as an end-artery[26]. This characteristic suggested pancreatic lobules were susceptible to ischemic injury due to spasm of intralobular arterioles, embolization of arterioles, formation of microthrombi or compression by interstitial edema [1-3]. However, causative factors of early-stage ischemia and the precise triggering factors of local microcirculatory disturbance remain obscure[3].

Cellular calcium, a key physiological signaling element in cell function and also a crucial pathological intracellular messenger in cell injury, appears to be involved in the initiation and development of acute pancreatitis[7-12]. The present study provided several lines of evidence supporting this suggestion. We investigated whether calcium was involved in the priming response encountered during AP. We found that cytosolic [Ca^{2+}]i showed a biphasic changes when the neutrophil suspension was under a steady shear. When the shear rate changed from low to high, cytosolic [Ca^{2+}]i decreased slightly under a very low shear rate (5.96/s) and then increased gradually to a high level. From this study we could also infer that cytosolic [Ca^{2+}]i might be an identifying marker for AP.

Activation of neutrophils is one of the critical roles in pathologic process of AP, and cytosolic [Ca^{2+}]i has a close relation with neutrophil activation[27,28]. Our study demonstrated the fluid shear stress induced changes of cytosolic [Ca^{2+}]i in neutrophils in pancreatic microcirculation of rats with caerulein-induced AP. These lines of evidence indicate that altered intracellular calcium might play an important role in the initiation and development of AP. In conclusion, cellular calcium may be an important factor in the pathogenesis of cerulein-induced acute pancreatitis.

REFERENCES

1 **Vollmar B,** Menger MD. Microcirculatory dysfunction in acute pancreatitis. A new concept of pathogenesis involving vasomotion-associated arteriolar constriction and dilation. *Pancreatology* 2003; **3**: 181-190
2 **Chen HM,** Sunamura M, Shibuya K, Yamauchi JI, Sakai Y, Fukuyama S, Mikami Y, Takeda K, Matsuno S. Early microcirculatory derangement in mild and severe pancreatitis models in mice. *Surg Today* 2001; **31**: 634-642
3 **Menger MD,** Plusczyk T, Vollmar B. Microcirculatory derangements in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2001; **8**: 187-194
4 **Gomez-Cambronero LG,** Sabater L, Pereda J, Cassinello N, Camps B, Vina J, Sastre J. Role of cytokines and oxidative stress in the pathophysiology of acute pancreatitis: therapeutical implications. *Curr Drug Targets Inflamm Allergy* 2002; **1**: 393-403
5 **Frossard JL.** Pathophysiology of acute pancreatitis: a multistep disease. *Acta Gastroenterol Belg* 2003; **66**: 166-173
6 **Makhija R,** Kingsnorth AN. Cytokine storm in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2002; **9**: 401-410
7 **Weber CK,** Adler G. From acinar cell damage to systemic inflammatory response: current concepts in pancreatitis. *Pancreatology* 2001; **1**: 356-362
8 **Sutton R,** Criddle D, Raraty MG, Tepikin A, Neoptolemos JP, Petersen OH. Signal transduction, calcium and acute pancreatitis. *Pancreatology* 2003; **3**: 497-505
9 **Parekh AB.** Calcium signaling and acute pancreatitis: specific

response to a promiscuous messenger. *Proc Natl Acad Sci U S A* 2000; **97**: 12933-12934

10 **Raraty MG,** Petersen OH, Sutton R, Neoptolemos JP. Intracellular free ionized calcium in the pathogenesis of acute pancreatitis. *Baillieres Best Pract Res Clin Gastroenterol* 1999; **13**: 241-251

11 **Kruger B,** Albrecht E, Lerch MM. The role of intracellular calcium signaling in premature protease activation and the onset of pancreatitis. *Am J Pathol* 2000; **157**: 43-50

12 **Niederau C,** Luthen R, Klonowski-Stumpe H, Schreiber R, Soika I, Sata N, Bing H, Haussinger D. The role of calcium in pancreatitis. *Hepatogastroenterology* 1999; **46**: 2723-2730

13 **Acioli JM,** Isobe M, Kawasaki S. Early complement system activation and neutrophil priming in acute pancreatitis: participation of trypsin. *Surgery* 1997; **122**: 909-917

14 **Frossard JL,** Past CM. Experimental acute pancreatitis: new insights into the pathophysiology. *Front Biosci* 2002; **7**: d275-d287

15 **Bhatia M,** Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J Pathol* 2004; **202**: 145-156

16 **de Dios I,** Perez M, de La Mano A, Sevillano S, Orfao A, Ramudo L, Manso MA. Contribution of circulating leukocytes to cytokine production in pancreatic duct obstruction-induced acute pancreatitis in rats. *Cytokine* 2002; **20**: 295-303

17 **Rau B,** Baumgart K, Kruger CM, Schilling M, Beger HG. CC-chemokine activation in acute pancreatitis: enhanced release of monocyte chemoattractant protein-1 in patients with local and systemic complications. *Intensive Care Med* 2003; **29**: 622-629

18 **Frossard JL,** Saluja AK, Mach N, Lee HS, Bhagat L, Hadenque A, Rubbia-Brandt L, Dranoff G, Steer ML. *In vivo* evidence for the role of GM-CSF as a mediator in acute pancreatitis-associated lung injury. *Am J Physiol Lung Cell Mol Physiol* 2002; **283**: L541-L548

19 **Bhatnagar A,** Wig JD, Majumdar S. Expression of activation, adhesion molecules and intracellular cytokines in acute pancreatitis. *Immunol Lett* 2001; **77**: 133-141

20 **Lundberg AH,** Granger N, Russell J, Callicutt S, Gaber LW, Kotb M, Sabek O, Gaber AO. Temporal correlation of tumor necrosis factor-alpha release, upregulation of pulmonary ICAM-1 and VCAM-1, neutrophil sequestration, and lung injury in diet-induced pancreatitis. *J Gastrointest Surg* 2000; **4**: 248-257

21 **Berney T,** Gasche Y, Robert J, Jenny A, Mensi N, Grau G, Vermeulen B, Morel P. Serum profiles of interleukin-6, interleukin-8, and interleukin-10 in patients with severe and mild acute pancreatitis. *Pancreas* 1999; **18**: 371-377

22 **Kantor AB,** Stall AM, Adams S, Watanabe K, Herzenberg LA. De novo development and self-replenishment of B cells. *Int Immunol* 1995; **7**: 55-68

23 **Suratt BT,** Young SK, Lieber J, Nick JA, Henson PM, Worthen GS. Neutrophil maturation and activation determine anatomic site of clearance from circulation. *Am J Physiol Lung Cell Mol Physiol* 2001; **281**: L913-L921

24 **Hjorth R,** Jonsson AK, Vretblad P. A rapid method for purification of human granulocytes using percoll. A comparison with dextran sedimentation. *J Immunol Methods* 1981; **43**: 95-101

25 **Beger HG,** Rau B, Isenmann R. Prevention of severe change in acute pancreatitis: prediction and prevention. *J Hepatobiliary Pancreat Surg* 2001; **8**: 140-147

26 **Zhou ZG,** Gao XH. Morphology of pancreatic microcirculation in the monkey: light and scanning electron microscopic study. *Clin Anat* 1995; **8**: 190-201

27 **Petty HR.** Neutrophil oscillations: temporal and spatiotemporal aspects of cell behavior. *Immunol Res* 2001; **23**: 85-94

28 **Davies EV,** Hallett MB. Cytosolic Ca2+ signalling in inflammatory neutrophils: implications for rheumatoid arthritis (Review). *Int J Mol Med* 1998; **1**: 485-490

Edited by Kumar M and Wang XL **Proofread by** Xu FM