Mol Biol Rep (2012) 39:3557–3563
DOI 10.1007/s11033-011-1129-z

Vasoactive intestinal peptide promotes gut barrier function against severe acute pancreatitis

Lu Zhongkai · Ye Jianxin · Chen Weichang

Received: 9 October 2010 / Accepted: 22 June 2011 / Published online: 3 July 2011

Abstract To explore the influence of vasoactive intestinal peptide (VIP) on the gut barrier function in severe acute pancreatitis (SAP). Fifty four SD rats were randomly divided into three groups: sham operated (SO) group, SAP group and VIP intervention group. Each group was further divided into three time points: 1, 6 and 12 h after operation with 6 rats for each treatment point. SAP models were induced by retrograde injection of 4% sodium taurocholate into the bili-pancreatic duct. VIP intervention group was made by 5 nmol VIP intraperitoneal injection within 5 min after SAP model successfully obtained. The VIP in plasma and intestinal homogenate were detected with ELISA. The endotoxin in plasma of all groups was also tested. The expression levels of TLR4, TNF-α, IL-6, and IL-10 in gut mucosa were measured by RT-PCR. Meanwhile intestinal samples were harvested for pathological examination. Compared to SO group, the VIP in plasma and intestinal homogenate of SAP group were significantly decreased at 1 h after induction, and then gradually increased to beyond the level of SO group at 12 h. The endotoxin of SAP group was continually increased. The mRNA levels of TLR4, TNF-α, IL-6, and IL-10 were also increased with obvious pathological injuries in the intestine. In the VIP group, endotoxin in plasma was obviously decreased compared to SAP group. The expressions of TNF-α, IL-6 mRNA were suppressed while IL-10mRNA was increased. The intestinal pathological injuries were also markedly alleviated. These results suggested that VIP had protective effects on SAP gut barrier function through inhibiting intestinal mucosal inflammatory responses.

L. Zhongkai · Y. Jianxin · C. Weichang (✉)
Department of Gastroenterology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, China
e-mail: weichangchen@126.com

Keywords Vasoactive intestinal peptide · Severe acute pancreatitis · Gut barrier · Inflammation

Introduction

Severe acute pancreatitis (SAP) is a serious systemic disease as its mortality remains high due to its fulminate course and severe complications [1]. Progression from severe acute necrotizing pancreatitis to infected pancreatic necrosis results in high mortality. Although the exact mechanism is not clear, inflammatory mediators have been shown to play an important role in the pathogenesis of SAP [2, 3]. Among the pro-inflammatory factors, endotoxin plays a pivotal role in triggering inflammation reaction locally as well as systematically. It has been shown that endotoxin comes mainly from the intestine [4–7]. Intestine is a multifunctional organ. It not only plays roles in nutrient absorption and metabolism, but also functions as barrier to protect against bacterial and other infections. When intestinal barrier structure and function are compromised, excessive endotoxin accumulates and translocates to promote or to aggregate acute pancreatitis. Intestinal barrier dysfunction often occurs with SAP, in which endotoxin shock and inflammatory cytokine surge might cause "second hit" resulting in severe pathological manifestation [8]. Prevention of intestinal barrier dysfunction may hold the key in improving the outcome of SAP.

Vasoactive intestinal peptide (VIP) is originally characterized as a brain-gut hormone that regulates the proliferation and maintenance of intestinal epithelial cells, intestinal fluid secretion, and intestinal motility [9]. It has recently been shown produced by neurons, endocrine cells, and immune cells (reviewed in Ref [9]). VIP actions in immunity include anti-inflammation, regulating Th1/Th2

balance, inducing regulatory T cells, and generating tolerogenic dendritic cells [10]. Although several research reported that some materials such as probiotics and galactooligosaccharide had protective effect on intestinal barrier function in SAP [11, 12], there has not been study on the influence of VIP on the gut barrier function in SAP so far. Kojima et al. [13] reported VIP attenuated the severity of experimental pancreatitis through inhibiting the cytokine production including IL-6 and TNF-alpha in monocytes from the spleen. However, the influence of VIP on intestinal inflammation-related cytokine and intestinal barrier in SAP is still unknown. Toll-like receptors (TLRs) were identified as essential components of the innate immune system, they have been the subjects of intensive investigation on their role in the inflammatory process. Sawa et al. [14] reported that TLR2 and 4 might be implicated in the mechanism of endotoxin/bacterial translocation in SAP. Regulation of TLR expression by VIP can further provide a possible mechanism to improve SAP treatment by preserving intestinal barrier function.

Here we demonstrated that VIP inhibited TLR4 expression, reduced endotoxin and inflammatory cytokine production by intestine, and alleviated pathologic injury, thus relieved SAP through promoting gut barrier function.

Materials and methods

Animal and reagents

Sprague–Dawley rat with weight about 250–300 grams were provided by Suzhou University animal center, were housed in cages under standard conditions, on a commercial pellet chow, at room temperature (25°C) and 55% humidity with a 12-h light–dark cycle.

The sources of major reagents are: sodium taurocholate from Sigma Company, St. Louis, USA; VIP from Phoenix Pharmaceutical, USA; VIP ELISA kit from Shanghai Xitang Biotechnology Inc, China; Endoxin Assay kit from Beijing Jingshanchuan Science and Development Inc, China; Trizol Reagent from Invittrogen, USA; Reverse-transcription kit from Takara, Japan; and oligonucleotide primers were synthesized by Shanghai Biotechnology Inc., China.

Animal model

SAP model was created essentially according to the previously described procedure [15]. Briefly, Rats were fasted for 12 h prior to operation. Rats were anesthetized with sodium pentobarbital (80 mg/kg, i.p.). 2% sodium taurocholate (dissolved in normal saline) was injected into pancreatic and bile duct retrogradely with micro-pump set at a speed of 0.1 ml/min and volume of 1 ml/kg body weight. The abdomen was then closed. For the sham operation group, the abdomen was opened and the pancreatic and bile duct were flipped and 1 ml/kg body weight normal saline was injected into colon cavity before closing abdomen. Five minutes after model creation, VIP treatment group were injected 5 nmol of VIP (dissolved in normal saline) into abdomen cavity, sham and SAP groups were injected the same volume of saline i.p., separatively. A group of six rats were sacrificed 1, 6, and 12 h after operation to collect samples.

All experimental procedures were performed in accordance with the National Institutes of Health's Guide in China for the Care and Use of Laboratory Animals with the approval from the Scientific Investigation Board of Suzhou University.

Serological tests

Blood samples were obtained by cardiac puncture. The serum levels of endotoxin and VIP were measured using commercially available clinical assay kits.

RNA isolation

Intestinal tissues from SD rats were isolated with Trizol reagent in accordance with the manufacture's protocol (Invitrogen, USA), the detailed procedure is as follows: 1 mg of intestinal tissue in 1 ml of Trizol reagent was homogenated, 200 μl of chloroform was used for phase separation, total RNA precipitated from the aqueous phase using isopropyl alcohol was washed and re-dissolved in double distilled water treated with DEPC, and stored at −20°C for detecting gene expression using RT-PCR.

RT-PCR

Total intestinal RNA was reverse-transcripted into cDNA using kit (Invitrogen, USA). The operations were done strictly in accordance with to the manufacturer's protocol. The expression levels of TLR4, TNF-α, IL-6, and IL-10 were analyzed by semi-quantitative RT-PCR with β-actin as internal control. Primers for RT-PCR were as following: TLR4 forward 5′-gccggaaagttattgtggtgg-3′ and reverse 5′-atgggttttaggcgcagagttt-3′; TNF-α forward 5′-gccaatggcatggatctcaaag-3′ and reverse 5′-cagagcaatgactccaaagt-3′; IL-6 forward 5′-gactgaatgttgttgacagccactgc-3′ and reverse 5′-tagccactccttctgtgactctaact-3′; IL-10 forward 5′-gctcagcactgctatgttgc-3′ and reverse 5′-ttcatggccttgtagacacc-3′; β-actin forward 5′-cacgatggaggggccggactcatc-3′ and reverse 5′-taaagacctctatgccaacacagt-3′. After 30 cycles of amplification, 5 μl of amplification product was used for gel electrophoresis.

For quantitative analysis of the stained gels, the software program Gel-Pro Analyzer 4.0 (Media Cybernetics, United States) was used. The integrated optical density (IOD) of the bands on digitized images was measured. All RT-PCR reactions were done 3 times for each sample. TLR4, TNF-α, IL-6 or IL-10 gene expression was expressed as the ratio of TLR4, TNF-α, IL-6 and IL-10 over β-actin, respectively. The ratio between the PCR amplified gene and the amplified standard was obtained for each sample.

Immunohistochemical analysis of TLR4

Immunohistochemistry was then performed using a standard 3-stage indirect immunoperoxidase technique [16]. Briefly, formalin fixed intestine sections were deparaffinized and rehydrated in graded alcohols and then rinsed in a running water bath, with endogenous peroxidase activity quenched by preincubating slides in 3% hydrogen peroxide in. After rinsing with PBS, slides were incubated for 10 min in blocking solution [5% skim milk (v/v) and 0.15% H_2O_2 (v/v) in deionized water]. The slides were rinsed in PBS, 50 μl rabbit polyclonal anti-TLR4 antibody (Santa Cruz, CA) applied at a 1:250 dilution, and the tissue incubated for 1 h in a humidity chamber. After rinsing again with PBS, the tissues were incubated with biotinylated anti-rabbit IgG (DAKO) for 10 min. After rinsing with PBS, the slides were incubated with streptavidin conjugated to horseradish peroxidase (DAKO) for 10 min and rinsed with PBS. Incubating slides with Liquid DAB Substrate–Chromogen System (DAKO) for the indicated period of time (2 min unless otherwise specified) identified bound antibody. After a final wash in PBS and distilled water, the slides were counterstained with a 50% dilution of Gill's hematoxylin for the indicated period of time (usually 1 min unless otherwise specified), dehydrated in graded alcohols, and mounted with a coverslip using Permount.

Expressions of TLR4 were determined by the semi-quantitative method. Ten versions per slice were selected randomly under 400× microscopes, then the proportion of TLR4-positive cells was scored in each version. The semi-quantitative score was determined according to the proportion of stained cells. The proportions of stained cells and the corresponding scores are as follows: ≤5%, 0; 5–25% (including 25%), 1; 25–50% (including 50%), 2; 50–75% (including 75%), 3; >75%, 4.

Histopathology

Intestine was harvested and treated sequentially by 10% formalin fixation, paraffin imbedding after 72 h. Intestinal ultra structure was examined on a Hitachi H600 transmission electron microscope (Japan) after conventional fixation, staining, and sectioning.

Statistical analysis

Data were expressed as mean ± SD. Statistical analyses were performed with SPSS13.0 software (SPSS, Inc., Chicago, IL, USA). One-way ANOVA with bonferroni post-tests was used to determine the differences among groups. *T*-test was used to determine the differences between groups. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

The VIP levels in SAP rats

Serum and intestinal VIP levels of SAP rats were significantly changed at each time point post-operation comparing to control SO rats (Fig. 1a, b). While VIP levels of SO rats remained within a narrow range for both serum (55–59 pg/ml) and intestine (104–108 pg/g), they were reduced in SAP rats to about 75–85% of SO rats levels in first 6 h but rebound significantly 12 h after operation.

Changes of serum endotoxin levels

Serum endotoxin level of SO rats did not change within 12 h after operation but that of SAP rats significantly increased just 1 h post-operation and further elevated 6 and 12 h after operation. SAP rats with VIP treatment had higher level of serum endotoxin than SO rats but lower than SAP rats (Fig. 2).

Expression of TLR4 and cytokines in intestinal mucosa

TLR4, TNF-α, IL-6, and IL-10 were expressed at a very low level in SO rat intestinal mucosa but significantly increased in SAP rats (Figs. 3a, 4). TNF-α mRNA level peaked 1 h after operation and IL-6 expression peaked 6 h post-operation while IL-10 and TLR4 expression increased continuously (Fig. 4). TLR4 mRNA level was positively correlated with serum endotoxin level ($r = 0.900$, $P < 0.01$). Comparing to SAP rats, VIP treated rats had significantly lower TLR4, TNF-α, and IL-6 but higher IL-10 expression level in intestinal mucosa (Fig. 4). Immunohistochemistry staining showed massive TLR4 positive cells with high expression level in intestinal epithelia of SAP rats while TLR4 was almost undetectable in SO rats. VIP treatment significantly reduced both the number of TLR4 positive cells and TLR4 expression level in intestinal epithelia of SAP rats (Table 1; Fig. 3b).

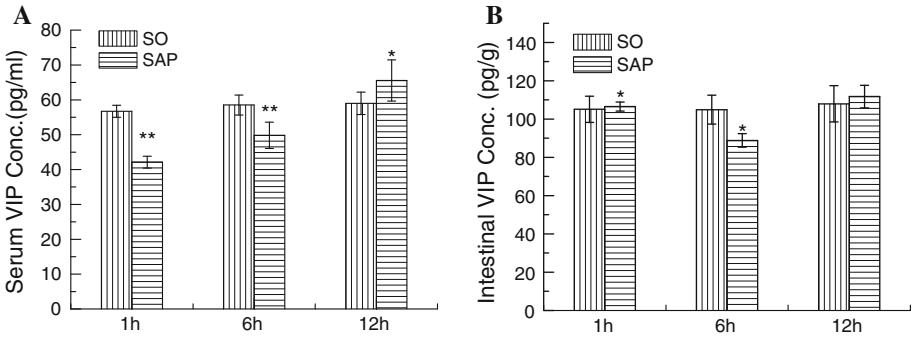

Fig. 1 VIP levels in serum and intestine**.** Compared to control animals, SAP rats had lower serum (**a**) and intestinal (**b**) VIP levels at 1 and 6 h but rebounded significantly at 12 h time after operation. $*P < 0.05$; $**P < 0.01$ compared to control

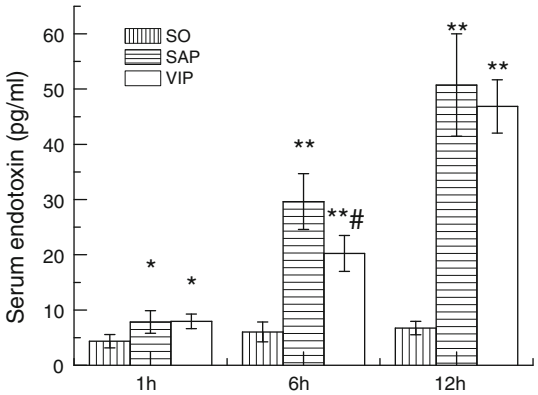

Fig. 2 Serum endotoxin levels. At all time points, SAP rats had significantly higher serum endotoxin levels than control. VIP treatment reduced serum endotoxin level of SAP rats. The reduction at 6 h was significant. $*P < 0.05$; $**P < 0.01$ compared to control, $\#P < 0.05$ compared to SAP

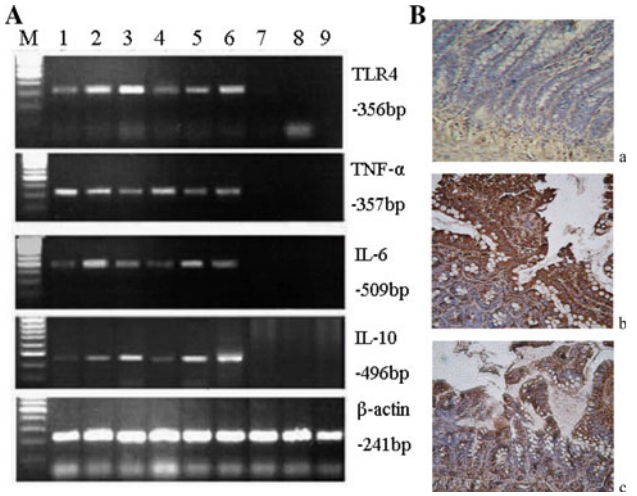

Fig. 3 TLR4 and inflammatory cytokine expression in intestinal mucosa. **a** mRNA levels of TLR4, TNF-α, IL-6, and IL-10 were significantly higher in SAP rats at all time points compared to control. VIP treatment reduced the expression level of all those genes except IL-10, which was increased by VIP. *Lane 1–3* SAP 1, 6, and 12 h; *lane 4–6* VIP 1, 6, and 12 h; *lane 7–9* control 1, 6, and 12 h, respectively. **b** TLR4 positive cell number and intensity were significantly higher in intestine of SAP rats (*b*) than controls (*a*). VIP treatment reduced both TLR4 positive cell number and expression level (*c*)

Pathological changes of intestinal structure of SAP rats

Transmission electron microscopic analysis provided details to the pathological changes of SAP rat intestinal structure. The intestinal epithelia of SO rats had well-organized microvillus layer and tight-junction structure (Fig. 5a). On the other hand, the intestine of SAP rats almost completely lost epithelial structure and microvilli with destructed organelle and tight-junctions (Fig. 5b). Treatment with VIP partially attenuated the pathological changes of SAP rats. In those rats, microvilli still presented although with breakage, and cellular structure and tight-junction were visible (Fig. 5c).

Discussion

SAP is a severe systematic disease which can fulminate to multiple organs dysfunction syndrome (MODS) or multiple organs failure (MOF) in early stages [17]. In turn pancreatic injury during SAP is further complicated by injury of multiple organs including the liver, lung, kidney, ileum, brain and heart [17]. Therefore, interventions reducing infection and/or inflammation at early stage of acute pancreatitis may prevent pathological progression of SAP. In our study, VIP intervention decreased blood endotoxin level, reduced expression of inflammatory cytokines and toll-like receptor 4 in intestine, and improved pathological outcomes in SAP rat model.

Gut barrier dysfunction often occurs in SAP [18, 19]. Ryan et al. [20] showed that intestinal permeability to macromolecular substance in pancreatitis correlated with the severity of the disease in rats. Ammori et al. [21] found that intestinal permeability to macromolecules in SAP increased significantly compared to controls and mild pancreatitis. The change of intestinal permeability could be found 72 h after the onset of pancreatitis [22]. The increased intestinal permeability was an indication of gut barrier dysfunction [23], which resulted in the translocation

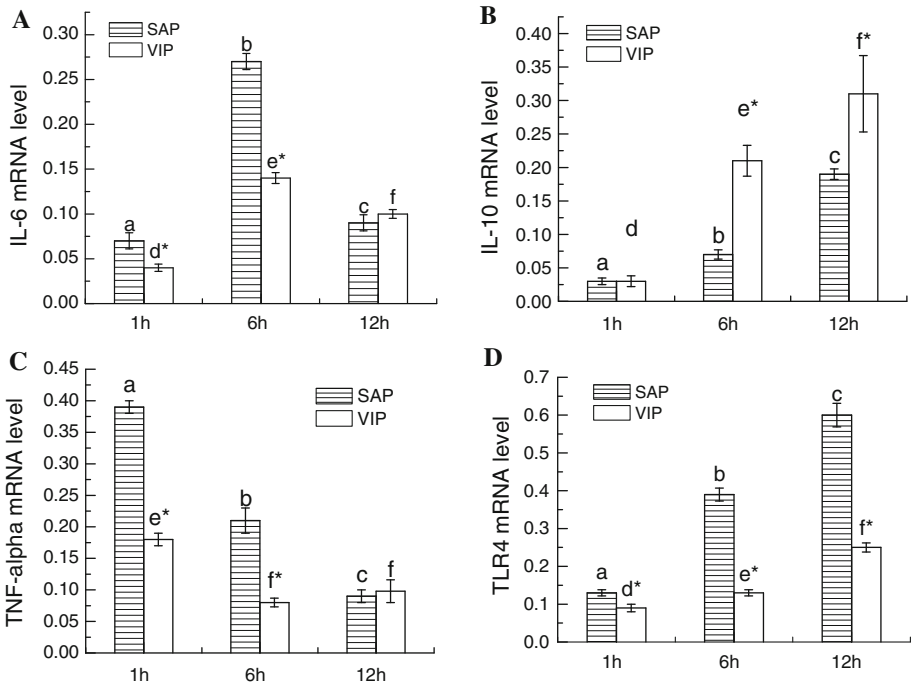

Fig. 4 The relative mRNA level of intestinal IL-6 (**a**), IL-10 (**b**), TNF-α (**c**) and TLR4 (**d**) calculated from Gel-Pro Analyzer 4.0. Different lowercase letter above the columns in the same group means different significantly ($P < 0.05$), *$P < 0.05$ compared to SAP

Table 1 The expressions of TLR4 in intestines by immunohistochemistry

Group	SO	SAP	VIP
1 h	0.083 ± 0.279	2.167 ± 0.693[a]	1.667 ± 0.951[a]
6 h	0.100 ± 0.354	3.017 ± 0.813[a]	2.050 ± 0.594[a,b]
12 h	0.117 ± 0.415	3.517 ± 0.537[a]	3.367 ± 0.736[a]

TLR4 Toll-like receptor 4; *SO* sham operated group; *SAP* group with severe acute pacreatitis; *VIP* group treated with vasoactive intestinal peptide

[a] Compared to SO group; [b] Compared to SAP; $P < 0.05$

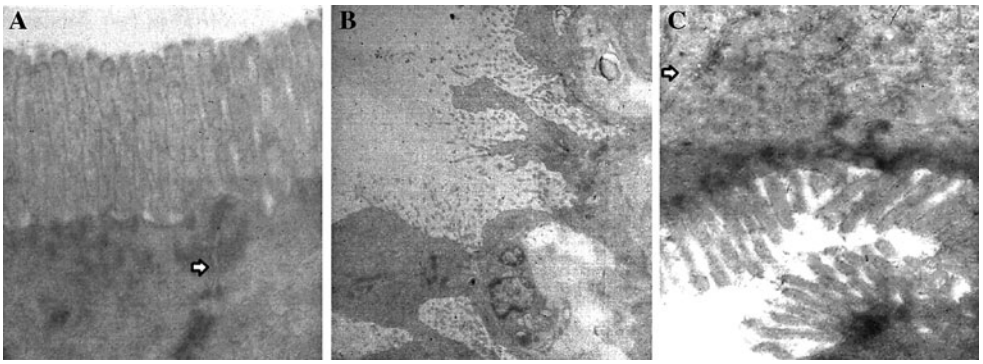

Fig. 5 Intestinal mucosa electron microscopic changes (×10,000) **a** Control mucosa showing long and tight microvilli and intact tight junction (*arrowhead*); **b** Pancreatitis intestine lost all microvilli and tight junctions; **c** VIP intervened SAP intestine showing shortened microvilli with visible tight junction (*arrowhead*)

of the enteric microbial population and/or endotoxin into other organs. The incidence of enteric microbial/endotoxin translocation was about 50–60% and it mainly came from the intestine [24]. The endotoxin level in peripheral blood has become the important index indicating the gut barrier function. Here we found serum endotoxin levels in the SAP rats induced by sodium taurocholate were significantly higher than SO rats and increased continuously, which indicated the gut barrier function of SAP rats were damaged obviously. The transmission electron microscopic figure of SAP rat intestine also showed the disrupted intestinal barrier.

Many researches showed dexamethasone have been shown beneficial effects in mitigating SAP caused damage to pancreas and other organs [25–27]. However, dexamethasone treatment induced ileum epithelial cell apoptosis [26], and intrajejunal delivery needs complicated operation. Therefore, more effective and convenient reagent need to be developed. VIP has been shown to regulate a wide range of biological functions, participating in the control of immune system homeostasis [28]. The effects of VIP on intestinal barrier function of SAP model is not very clear**.** Our experiment showed that serum VIP and intestinal VIP in SAP rats increased continuously during 12 h after induction, serum endotoxin also showed the continuous increase, which suggested VIP was related to intestinal barrier function. Colin et al. [29] reported that VIP ameliorated intestinal barrier disruption associated with Citrobacter rodentium-induced colitis. Whether VIP can ameliorate intestinal barrier disruption induced by SAP? Here our test for serum endotoxin and intestinal mucosa electron microscopic figure in VIP intervention group showed VIP treatment significantly alleviated gut barrier dysfunction by preserving intestinal mucosa structure, which would protect pancreas and other organs from infection by intestinal bacteria.

VIP actions in immunity can be found in the regulation of innate and adaptive immunity including anti-inflammatory actions, regulation of Th1/Th2 switch, induction of regulatory T cells, and generation of tolerogenic dendritic cells. Inflammatory factors also play a key role in the development of SAP [30] and inflammatory conditions of the intestinal mucosa result in compromised barrier function independent of epithelial cell apoptosis [31]. It is well known that excessive inflammatory factors together with the immune system trigger systemic inflammatory response syndrome (SIRS) and MODS in the development of acute pancreatitis (SAP) [32]. Studies have shown that inflammatory factors such as TNF-α, IL-6, ICAM-1 were regulated by the NF-κB nucleus transcription factor [33]. The overexpression of NF-κB and the activation of its downstream gene aggravate SAP [34]. Many research showed VIP could prevent NF-κB activation [10, 35, 36], which suggested VIP might attenuate the severity of SAP through inhibiting inflammation cytokines. Kojima et al. [13] reported VIP attenuated the severity of experimental pancreatitis through inhibiting the cytokine production in monocytes from the spleen. There is still no study on the influence of VIP on the intestinal cytokine. In intestine, whether VIP ameliorated the intestinal barrier disruption of SAP also by inhibiting inflammation cytokines? Our results gave the positive answer: VIP inhibited the expressions of TLR-4, TNF-α and IL-6 which increased in SAP group. All TLRs activate a common signaling pathway, leading to the activation of several transcription factors, such as nuclear-kappaB (NF-κB), a master switch for inflammation that regulates the transcription of inflammatory cytokines involved immunity and inflammation [14]. Recent studies have suggested that pancreatic enzymes can induce SIRS though TLR4 signaling pathway [37, 38]. Li et al. [39] reported that pancreatic and lung injury in rats was attenuated by downregulating TLR4, our experiment in intestine also showed the consistent results. Besides, TLR4 expression was positively correlated with serum endotoxin level, implying that TLR4 was involved in taurocholate-induced SAP. Study of Kojima et al. [13] showed VIP might inhibit inflammation cytokines through VIP receptor signaling pathway. We found the expression of TLR4 increased in SAP group, and decreased in VIP intervention group, TNF-α, IL-6 indicated the consistent trends, suggesting that VIP might inhibit the expressions of TNF-α, IL-6 through TLR4 signaling pathway, which included inhibition of NF-κB activation, and possibly co-worked with VIP receptor signaling. Unexpectedly, IL-10 expression increased in VIP intervention group compared to SAP group induced by sodium taurocholate, whereas, in the study of Kojima et al. [13], VIP also did not reduce the expression of IL-10 elevated in SAP induced by cerulean and LPS, the two results suggested that IL-10 expression was not regulated by VIP signal, and there might be other regulation pathway.

In summary, the translocation of gut bacteria and gut derived endotoxin due to intestinal barrier dysfunction induced by SAP may be the aggravating cause of SAP. The upregulation of TLR4, TNF-α and IL-6 may contribute to intestine mucosa damage. VIP intervention was able to improve the pathological outcome by protecting gut barrier function, which might be obtained by reducing inflammatory responses through TLR-4 signal pathway co-worked with VIP receptor pathway. Our study may provide an experimental basis for preventing SAP aggravation in clinical practice.

Acknowledgments We thank the support of the First Affiliated Hospital of Soochow University.

References

1. Mann DV, Hershman MJ, Hittinger R, Glazer G (1994) Multicentre audit of death in acute pancreatitis. Br J Surg 81:890–893
2. Norman J (1998) The role of cytokines in the pathogenesis of acute pancreatitis. Am J Surg 175:76–83
3. Shi C, Zhao X, Lagergren A, Sigvardsson M, Wang X, Andersson R (2006) Immune status and inflammatory response differ locally and systemically in severe acute pancreatitis. Scand J Gastroenterol 41:472–480
4. Swank GM, Deitch EA (1996) Role of the gut in multiple organ failure: bacterial translocation and permeability changes. World J Surg 20:411–417
5. MacFie J, O'Boyle C, Mitchell CJ, Buckley PM, Johnstone D, Sudworth P (1999) Gut origin of sepsis: a prospective study

investigating associations between bacterial translocation, gastric microflora, and septic morbidity. Gut 45:223–228
6. Matheus AS, Coelho AM, Sampietre S, Patzina R, Jukemura J, Cunha JE, Machado MC (2007) Effect of inhibition of prostaglandin E2 production on pancreatic infection in experimental acute pancreatitis. HPB (Oxford) 9:392–397
7. Leveau P, Wang X, Sun Z, Börjesson A, Andersson E, Andersson R (2005) Severity of pancreatitis-associated gut barrier dysfunction is reduced following treatment with the PAF inhibitor lexipafant. Biochem Pharmacol 69:1325–1331
8. Ammori BJ (2003) Role of the gut in the course of severe acute pancreatitis. Pancreas 26:122–129
9. Delgado M, Abad C, Martinez C, Juarranz MG, Arranz A, Gomariz RP, Leceta J (2002) Vasoactive intestinal peptide in the immune system: potential therapeutic role in inflammatory and autoimmune diseases. J Mol Med 80:16–24
10. Chorny A, Gonzalez-Rey E, Varela N, Robledo G, Delgado M (2006) Signaling mechanisms of vasoactive intestinal peptide in inflammatory conditions. Regul Pept 137:67–74
11. Besselink MG, van Santvoort HC, Renooij W, de Smet MB, Boermeester MA, Fischer K et al (2009) Intestinal barrier dysfunction in a randomized trial of specific probiotic composition in acute pancreatitis. Ann Surg 250:712–719
12. Zhang Y, Cai D, Cai W, Geng S, Chen L, Han T (2009) Protective effect of galactooligosaccharide-supplemented enteral nutrition on intestinal barrier function in rats with severe acute pancreatitis. Clin Nutr 28:575–580
13. Kojima M, Ito T, Oono T, Hisano T, Lgarashi H, Arita Y, Kawabe K, Coy DH, Jensen RT, Nawata H (2005) VIP attenuation of the severity of experimental pancreatitis is due to VPAC1 receptor-mediated inhibition of cytokine production. Pancreas 30:62–70
14. Sawa H, Ueda T, Takeyama Y, Yasuda T, Shinzeki M, Matsumura N, Nakajima T, Kuroda Y (2008) Expression of toll-like receptor 2 and 4 in intestinal mucosa in experimental severe acute pancreatitis. Hepatogastroenterology 55:2247–2251
15. Lankisch PG, Ihse I (1987) Bile-induced acute experimental pancreatitis. Scand J Gastroenterol 22:257–260
16. Jasani B, Schmid KW (1993) Immunocytochemistry in diagnostic histopathology, 1st edn. Churchill Livingstone, Edinburgh
17. Zhang XP, Ye Q, Jiang XG, Ma ML, Zhu FB, Zhang RP, Cheng QH (2007) Preparation method of an ideal model of multiple organ injury of rat with severe acute pancreatitis. World J Gastroenterol 13:4566–4573
18. Xu GF, Lu Z, Gao J, Li ZS, Gong YF (2006) Effect of ecoimmunonutrition supports on maintenance of integrity of intestinal mucosal barrier in severe acute pancreatitis in dogs. Chin Med J (Engl) 119:656–661
19. Liu ZH, Peng JS, Li CJ, Yang ZL, Xiang J, Song H, Wu XB, Chen JR, Diao DC (2009) A simple taurocholate-induced model of severe acute pancreatitis in rats. World J Gastroenterol 15:5732–5739
20. Ryan CM, Schmidt J, Lewandrowski K, Compton CC, Rattner DW, Warshaw AL, Tompkins RG (1993) Gut macromolecular permeability in pancreatitis correlates with severity of disease in rats. Gastroenterology 104:890–895
21. Ammori BJ, Leeder PC, King RF et al (1999) Early increase in intestinal permeability in patients with severe acute pancreatitis: correlation with endotoxemia, organ failure, and mortality. J Gastrointest Surg 3:252–262
22. Juvonen PO, Alhava EM, Takala JA (2000) Gut permeability in patients with acute pancreatitis. Scand J Gastroenterol 35: 1314–1318
23. Ding LA, Li JS, Li YS, Zhu NT, Liu FN, Tan L (2004) Intestinal barrier damage caused by trauma and lipopolysaccharide. World J Gastroenterol 10:2373–2378
24. Kazantsev GB, Hecht DW, Rao R, Fedorak IJ, Gattuso P, Thompson K, Djuricin G, Prinz RA (1994) Plasmid labeling confirms bacterial translocation in pancreatitis. Am J Surg 167:201–207
25. Xiping Z, Ruiping Z, Binyan Y, Li Z, Hanqing C, Wei Z, Rongchao Y, Jing Y, Wenqin Y, Jinjin B (2010) Protecting effects of a large dose of dexamethasone on spleen injury of rats with severe acute pancreatitis. J Gastroenterol Hepatol 25:302–308
26. Zhang X, Chen L, Luo L, Tian H, Feng G, Cai Y, Xu R, Wang K, Wang Z (2008) Study of the protective effects of dexamethasone on ileum mucosa injury in rats with severe acute pancreatitis. Pancreas 37:e74–e82
27. Xiping Z, Li C, Miao L, Hua T (2007) Protecting effects of dexamethasone on thymus of rats with severe acute pancreatitis. Mediator inflamm 2007:72361
28. Smalley SG, Barrow PA, Foster N (2009) Immunomodulation of innate immune responses by vasoactive intestinal peptide (VIP): its therapeutic potential in inflammatory disease. Clin Exp Immunol 157:225–234
29. Colin VS, Wu X, Nguyen C, Dai C, Vallance BA, Buchan AM, Boyer L, Jacobson K (2009) Vasoactive intestinal peptide ameliorates intestinal barrier disruption associated with Citrobacter rodentium-induced colitis. Am J Physiol Gastrointest Liver Physiol 297:G735–G750
30. de Campos T, Deree J, Martins JO, Loomis WH, Shenvi E, Putnam JG, Coimbra R (2008) Pentoxifylline attenuates pulmonary inflammation and neutrophil activation in experimental acute pancreatitis. Pancreas 37:42–49
31. Bruewer M, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, Nusrat A (2003) Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. J Immunol 171:6164–6172
32. Rahman SH, Ammori BJ, Holmfield J, Larvin M, McMahon MJ (2003) Intestinal hypoperfusion contributes to gut barrier failure in severe acute pancreatitis. J Gastrointest Surg 7:26–36
33. Wang X, Gong Z, Wu K, Wang B, Yuang Y (2003) Gastrointestinal dysmotility in patients with acute pancreatitis. J Gastroenterol Hepatol 18:57–62
34. Wang YL, Zheng YJ, Zhang ZP, Su JY, Lei RQ, Tang YQ, Zhang SD (2009) Effects of gut barrier dysfunction and NF-kappaB activation on aggravating mechanism of severe acute pancreatitis. J Dig Dis 10:30–40
35. Delgado M, Ganea D (2001) Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit nuclear factor-kappa B-dependent gene activation at multiple levels in the human monocytic cell line THP-1. J Biol Chem 276:369–380
36. Chrivia JC, Kwok RP, Lamb N, Hagiwara M, Montminy MR, Goodman RH (1993) Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature 365:855–859
37. Johnson GB, Brunn GJ, Platt JL (2004) Cutting edge: an endogenous pathway to systemic inflammatory response syndrome (SIRS)-like reaction through Toll-like receptor-4. J Immunol 172:20–24
38. Hietaranta A, Mustoneu H, Puolakkainen P, Haapiainen R, Kemppainen E (2004) Proinflammatory effects of pancreatic elastase are mediated through TLR-4 and NF-κB. Biochem Biophys Res Commun 323:192–196
39. Li Z, Xia X, Zhang S, Zhang A, Bo W, Zhou R (2008) Up-regulation of Toll-like receptor 4 was suppressed by emodin and baicalin in the setting of acute pancreatitis. Biomed Pharmacother 63:120–128