

Influence of NF- κ B-Vasoactive Intestinal Peptide on Intestinal Dysmotility in Severe Acute Pancreatitis

Hanlin Gong¹, Zhiyu He^{1,2}, Wenfu Tang^{1*},
Guangyuan Chen^{1*}, Qing Xia¹ and Xi Huang¹

¹Department of Integrated Traditional Chinese and Western Medicine, West China Hospital, Sichuan University, Chengdu, Sichuan Province - 610 041, PR China.

²Department of Integrated Traditional Chinese and Western Medicine, Nanchong Central Hospital, Nanchong, Sichuan Province - 637 000, PR China.

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To investigate the influence of NF- κ B-vasoactive intestinal peptide on intestinal dysmotility in severe acute pancreatitis (SAP). 24 male SD rats were randomly divided into 3 groups: sham-operation (SO) group, SAP group and pyrrolidine dithiocarbamate (PDTC) group. To calculate intestinal propulsion index, we measured length of the small intestine and intestinal canal labeled by glucan; Vasoactive intestinal peptide (VIP) was detected by enzyme linked immunosorbent assay (ELISA). We used RT-PCR (Real Time-polymerase chain reaction) and used TaqMan fluorescent probes in this experiment. The intestinal propulsion rate (IPR) in SAP group is significantly lower than that in SO group and PDTC group ($P < 0.05$). Serum VIP in SAP group is significantly higher than that in SO group ($P = 0.000$). Serum VIP in SAP group is lower than that in PDTC group ($P = 0.021$). Compared to SO group and PDTC group, VIP of intestinal tissue is significantly low in SAP group ($P < 0.05$). There was a negative correlation between IPR and VIP. VIP mRNA expression was higher in SAP group than that of SO group ($P = 0.019$). VIP mRNA expression in PDTC group was lower than that of in SAP group ($P = 0.006$). When SAP occurred, activation of NF- κ B exists in the neuroendocrine cells of secreting VIP, NF- κ B inhibits intestinal motility through the promotion of VIP expression. Intestinal motility may be more closely related to intestinal tissue VIP concentration.

Key words: Severe acute pancreatitis; Intestinal propulsion rate; Vasoactive intestinal peptide; Nuclear factor-kappa B.

Acute pancreatitis is a common clinical acute abdomen, but the severe cases will endanger the patient's life. Acute pancreatitis, especially severe acute pancreatitis, is often associated with paralytic ileus¹⁻³. And the mechanism of intestinal motility disorders in acute pancreatitis is not fully clarified.

Generally speaking, gastrointestinal motility is mainly affected by neural and endocrine regulation, which is closely related to each other. A number of gastrointestinal peptide hormones

* To whom all correspondence should be addressed.
Tel.: +86-28-85422556; Fax: +86-28-85423373
E-mail: wftang900@gmail.com

such as vasoactive intestinal peptide (VIP), by acting on the specific VIP2 receptors on the surface of gastrointestinal smooth muscle, plays an important role in the regulation of gastrointestinal motility⁴⁻⁶.

NF- κ B, as a ubiquitous transcription factor, plays a central role in a considerable number of gene's transcription regulation. Also, it plays a broad and important role in immunity, inflammation, cell survival, proliferation, differentiation and apoptosis. In the AP, inflammatory mediators TNF- α , IL-6, IL-1 β and IL-8 not only can induce NF- κ B's activation, but also subject to the regulation of NF- κ B⁷⁻¹⁰. Once the NF- κ B is activated, it will activate the inflammatory mediator genes, and produce inflammatory mediators's positive feedback amplification effect¹¹⁻¹⁴.

Studies have shown that: When we suffer from AP, especially SAP, a large number of inflammatory cells will be activated and then a variety of inflammatory mediators will increase. NF- κ B is highly expressed in a variety of organizations, including the pancreas and the intestine, while the concentrations of VIP elevates in serum and intestinal tissue¹⁵. So far, it has not been clear that how NF- κ B impact for cells of secreted VIP. We assume that NF- κ B can promote the transcription of VIP and suppress intestinal motility.

Pyrrolidine dithiocarbamate (PDTC) is an important inhibitor of NF- κ B, which is proved both in vitro and in vivo studies¹⁶⁻¹⁷. It is not only a way to reduce acute and chronic inflammatory response, but also a powerful tool to prove the causality between NF- κ B-mediated inflammatory response and other events¹⁸⁻²⁰. We use immunohistochemical approach to determine the existence of NF- κ B activation in neuroendocrine cells, which secrete VIP. We use PDTC to inhibit NF- κ B's activation, in order to understand the expression of VIP and the changes in the rate of intestinal propulsion, which will help us to verify this assumption.

MATERIALS AND METHODS

Animals

Twenty-four male clean-grade, healthy Sprague-Dawley rats (body weight, 320 ± 25 g and

90 ± 5 days of age) were used. They were born, housed, fed and handled according to the University Guidelines and the Animal Ethics Committee Guidelines of the Animal Facility of the West China Hospital. The rats were maintained in air-conditioned animal quarters under the following conditions: temperature, $22 \pm 2^\circ\text{C}$; relative humidity, $65 \pm 10\%$; free access to water; feeding with laboratory rodent chow (Chengdu, China). The animals were acclimatized to the facilities for 10 days. Rats were then subjected to a fast with free access to water for 24 h prior to experiments.

Reagents

PDTC and anti an NF- κ B subunit P65 immunohistochemistry kit were purchased from Sigma-Aldrich Inc. (Saint Louis, USA). VIP kit (double-antibody sandwich ELISA) was obtained from Adlitteram diagnostic laboratories (USA).

Induction of acute pancreatitis in rats

Under ether anesthesia, Anesthesia administration during the operative procedures and subsequent postoperative care were consistent with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (1985). We use retrograde cholangiopancreatic injection with sodium taurocholate (STC) as described in our previous study²¹⁻²³.

Sham-operated model making

We use retrograde cholangiopancreatic with SAP group and no injection with sodium taurocholate.

Experimental groups and sample collection

Rats were randomly divided into the SAP group, SO group, and PDTC group ($n=8$). Blood samples (3 ml) were collected in heparinized eppendorf tube via cutting off the neck subsequently at 8h after the success in modeling. After centrifuging at 3000 rpm for 15min, the plasma samples were obtained and frozen at -80°C until analysis.

After the success in modeling 8 hours to cut off the neck to take 3ml of blood into a pre-installed with 10% EDTA- Na_2 (90ul) and aprotinin 750u (90ul) of a test tube, 4°C under 1500r/min centrifugal 30min, the supernatant obtained liquid, -80°C to save³⁸. Preparation of measurement VIP.

Detection index

Intestinal propulsion index, serum VIP and intestinal tissue VIP.

Detection of serum VIP and tissue VIP

Using double-antibody sandwich ELISA.

Fluorescence quantitative using the PCR

In order to understand the expression of VIP mRNA each group, we were used RT-PCR (Real Time-polymerase chain reaction) and used TaqMan fluorescent probes in this experiment. Under the power curve, we read the Ct value and converted to relative gene expression levels ($2^{-\Delta\Delta Ct}$).

Statistical Methods

All measurement data were expressed as arithmetic mean \pm standard deviation (SD), using analysis of variance (significance level $\alpha = 0.05$). We analyze the difference of intestinal propulsion rate and VIP parameters using correlation analysis. Statistical software used SPSS13.0 for Windows.

RESULTS

Comparison of intestinal propulsion rate

It was shown that the rate of intestinal propulsion of SAP group were significantly lower than that of SO group ($P=0.000$). The intestinal propulsion rate of PTDC group is higher than that of SAP group ($P=0.02$) (Table 1, Fig. 1).

Comparison of Serum VIP and comparison of intestinal tissue VIP in groups

It was shown that the serum VIP in SAP group was significantly higher than that of SO group ($P = 0.000$); the serum VIP in SAP group was lower than that of PTDC group ($P = 0.021$). Tissue VIP in SAP group was higher than that of SO group ($P = 0.003$); Tissue VIP in PTDC group than that of in SAP group ($P = 0.000$) (Table 2, Fig. 2).

Comparison of intestinal tissue VIP mRNA expression level

It is shown that VIP mRNA expression was higher in SAP group than that of SO group ($P=0.019$). VIP mRNA expression in PTDC group was lower than that of in SAP group ($P=0.006$) (Table 3, Fig. 3).

Related analysis of VIP and RIP

The RIP was negatively correlated with intestinal tissue ($r = -0.83$, $P = 0.000$) (Table 4; Fig. 5).

Table 1. Comparison of intestinal propulsion rate (%),* VS SO group,^aVS SAP group

Group	n	IPR
SO	8	32.09 \pm 6.65
SAP	8	18.30 \pm 3.82*
PTDC	8	27.69 \pm 6.49 ^a

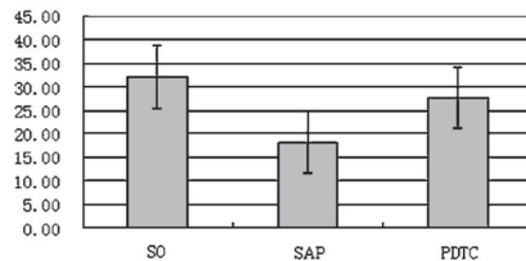


Fig. 1. Comparison of intestinal propulsion rate (%)

Table 2. Comparison of Serum VIP and comparison of intestinal tissue VIP in groups (ng/ml) ^aVS SO group; ^bVS SAP group; ^cVS SO group; ^d VS SAP group

Group	n	Serum VIP	Tissue VIP
SO	8	10.19 \pm 2.25	2.1 \pm 0.65
SAP	8	18.75 \pm 3.84 ^a	3.03 \pm 0.74 ^c
PTDC	8	22.1 \pm 2.3 ^b	1.74 \pm 0.51 ^d

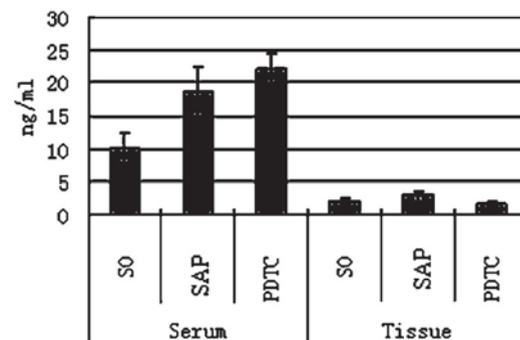
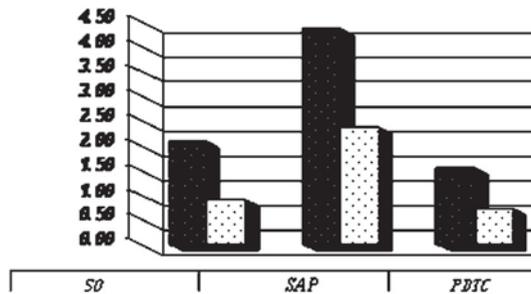


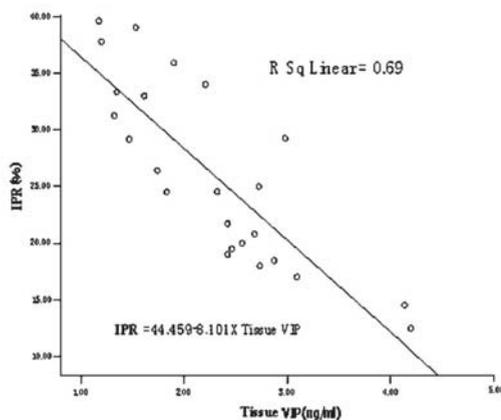
Fig. 2. Comparison of Serum VIP and comparison of intestinal tissue VIP in groups

Table 3. Comparison of intestinal tissue VIP mRNA expression level ($2^{-\Delta\Delta CT}$)* VS SO group;^A VS SAP group

Group	n	VIP mRNA
SO	6	2.06±0.90
SAP	6	4.35±2.34*
PTDC	6	1.53±0.71 ^A

**Fig. 3.** Intestinal tissue VIP mRNA ($2^{-\Delta\Delta Ct}$)**Table 4.** Correlation analysis of intestinal tissue VIP and IRP

	IRP	intestinal tissue VIP
Pearson Correlation IRP intestinal tissue VIP	1.000	-.830
Sig.(1-tailed) IRP intestinal tissue VIP	.	.000
N		
IRP	24	24
intestinal tissue VIP	24	24

**Fig. 4.** Scatter of IPR and intestinal tissue VIP correlation analysis

DISCUSSION

Currently, the study of NF- κ B's effect on nerve cells has been mostly confined to the central nervous system. And there has been no reports about the NF- κ B's activation in the enteric nervous system. After human gastric mucosa has been infected with *Trichinella spiralis*, myenteric nerve plexus's self-expression of IL1- β , IL-6 and TNF- α , which are inflammatory cytokines and be regulated by NF- κ B, might mediate the release of neurotransmitter in the past studies²⁵. The latest evidence shows that the TNF- α , IL1- β 's activation of MAPK and protein kinase C will significantly increases mRNA's expression of gastrin in G cells in the study of intestine endocrine cells²⁶. The combination of endotoxin, Toll-like receptor-4 the activation of NF- κ B and MAPK will induce calcium influx and increase the secretion of CCK, which suggests that NF- κ B's activation may exist in intestinal neuroendocrine cells²⁷. In this experiment, by using fluorescence immunohistochemistry, we have detected the VIP's expression in the cytoplasm, NF- κ B's expression in the cytoplasm or nuclear. And these positive cells presence in the SAP rat's jejunal muscularis and submucosa. It shows that in the condition of AP, the NF- κ B's activation exist in neuroendocrine cells, which secret VIP.

We use PDTC to inhibit the activation of NF- κ B to observe the expression of VIP as well as changes in the rate of intestinal propulsion. And the result shows that jejunal VIP mRNA expression of SAP group is significantly higher than that of control group. Compared with the SAP group, VIP mRNA expression of PDTC treatment group is downregulate, and at the same time, the VIP level in intestinal tissue is obviously lower than in SAP group, which improves gastrointestinal motility significantly. It shows that the antagonism of NF- κ B's activation will down-regulate the expression of VIP mRNA, reduce VIP's secretion and improve gastrointestinal motility.

This experiment confirms that under the condition of AP, NF- κ B's activation up-regulates the expression of VIP mRNA and increases VIP's secretion, which will suppress gastrointestinal motility. In addition, this study also finds that, compared with SAP group, the VIP level of PDTC treatment group is significantly higher in serum,

while lower in intestinal tissue. And the possible reason is that the antagonism of NF- κ B's activation promotes the intestinal tissue's releasing of VIP, which needs further studies to be confirmed.

In summary, when SAP occurred, activation of NF- κ B exists in the neuroendocrine cells of secreting VIP, NF- κ B inhibit intestinal motility through the promotion of VIP expression. Intestinal motility may be more closely related to intestinal tissue VIP concentration.

REFERENCES

1. Wang XP, Gong Z, Wu K, Wang B, Yuang Y. Gastrointestinal dysmotility in patients with acute pancreatitis. *Gastroenterol Hepatol*. 2003; **18**(1): 57-62.
2. Uhl W, Isenmann R, Curti G, Vogel R, Beger HG, Büchler MW. Influence of etiology on the course and outcome of acute pancreatitis. *Pancreas*. 1996; **13**(4): 335-343.
3. Seerden TC, De Winter BY, Van Den Bossche RM, Herman AG, Pelckmans PA, De Man JG. Regional differences in gastrointestinal motility disturbances during acute necrotising pancreatitis. *Neurogastroenterol Motil*. 2005; **17**(5): 671-679.
4. Schulz S, Röcken C, Mawrin C, Weise W, Höllt V, Schulz S. Immunocytochemical identification of VPAC1, VPAC2, and PAC1 receptors in normal and neoplastic human tissues with subtype-specific antibodies. *Clin Cancer Res*. 2004; **10**(24): 8235-8242.
5. Schmidt CJ, Hamer DH. Cell specificity and an effect of ras on human metallothionein gene expression. *Proc Natl Acad Sci U S A*. 1986; **83**(10): 3346-3350.
6. Vittoria A, Costagliola A, Carrese E, Mayer B, Cecio A. Nitric oxide-containing neurons in the bovine gut, with special reference to their relationship with VIP and galanin. *Arch Histol Cytol*. 2000; **63**(4): 357-368.
7. Chen X, Ji B, Han B, Ernst SA, Simeone D, Logsdon CD. NF- κ B activation in pancreas induces pancreatic and systemic inflammatory response. *Gastroenterology*. 2002; **122**(2): 448-457.
8. Chan EL, Murphy JT. Reactive oxygen species mediate endotoxin-induced human dermal endothelial NF- κ B activation. *Surg Res*. 2003; **111**(1): 120-6.
9. Mercurio F, Manning AM. Multiple signals converging on NF- κ B. *Curr Opin Cell Biol*. 1999; **11**(2): 226-232.
10. Neurath MF, Becker C, Barbulescu K. Role of NF- κ B in immune and inflammatory responses in the gut. *Gut*. 1998; **43**(6): 856-860.
11. Beg AA, Finco TS, Nantermet PV, Baldwin AS Jr. Tumor necrosis factor and interleukin-1 lead to phosphorylation and loss of I κ B α : a mechanism for NF- κ B activation. *Mol Cell Biol*. 1993; **13**(6): 3301-3310.
12. Finco TS, Baldwin AS. Mechanistic aspects of NF- κ B regulation: the emerging role of phosphorylation and proteolysis. *Immunity*. 1995; **3**(3): 263-272.
13. DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M. A cytokine-responsive I κ B kinase that activates the transcription factor NF- κ B. *Nature*. 1997; **388**(6642): 548-554.
14. Jobin C, Sartor RB. The I κ B/NF- κ B system: a key determinant of mucosal inflammation and protection. *American Journal of Physiology - Cell Physiology*. 2000; **278**(3): C451-462.
15. Gukovsky I, Gukovskaya AS, Blinman TA, et al. Early NF- κ B activation is associated with hormone-induced pancreatitis. *Am J Physiol*. 1998; **275** 6Pt1 :G1402-G1414.
16. Peeters TL, Bormans V, Vantrappen G. Comparison of motilin binding to crude homogenates of human and canine gastrointestinal smooth muscle tissue. *Regul Pept*. 1988; **23**(2): 171-182.
17. Luiking YC, Akkermans LM, Peeters TL, Cnossen PJ, Nieuwenhuijs VB, Vanberge-Henegouwen GP. Effects of motilin on human interdigestive gastrointestinal and gallbladder motility, and involvement of 5HT₃ receptors. *Neurogastroenterol Motil*. 2002; **14**(2): 151-159.
18. Vantrappen G, Janssens J, Peeters TL, Bloom SR, Christofides ND, Hellemans J. Motilin and the interdigestive migrating motor complex in man. *Dig Dis Sci*. 1979; **24**: 497-500.
19. Ludtke FE, Muller H, Golenhofen K. Direct effects of motilin on isolated smooth muscle from various regions of the human stomach. *Pflugers Arch* 1989; **414**: 558-563.
20. Portincasa P, Peeters TL, van Berge-Henegouwen GP, van Solinge WW, Palasciano G, van Erpecum KJ. Acute intraduodenal bile salt depletion leads to strong gallbladder contraction, altered antroduodenal motility and high plasma motilin levels in humans. *Neurogastroenterol Mot*. 2000; **12**: 421-430.
21. Ren YY, Gong HL, Tang WF, Wan MH, Zhao JL, Huang X. Dachengqi Decoction induces

- pancreatic acinar cell apoptosis in experimental acute pancreatitis in rats. *J Chin Integr Med.* 2009; **7**(7): 651-656.
22. Qiu Y, Li YY, Li SG, Song BG, Zhao GF. Effect of Qingyitang on activity of intracellular Ca²⁺-Mg²⁺ - ATPase in rats with acute pancreatitis. *World J Gastroenterol.* 2004; **10**(1): 100-104.
23. Gong HL, Tang WF, Yu Q, Xia Q, Huang X, Liang MZ. Effect of severe acute pancreatitis on pharmacokinetics of components from *Da-Cheng-Qi decoction*. *World Journal of Gastroenterology*, 2009; **15**(47): 5992-5999
24. Nieuwenhuijs VB, Verheem A, van Duijvenbode-Beumer H, Visser MR, Verhoef J, Gooszen HG, Akkermans LM. The role of interdigestive small bowel motility in the regulation of gut microflora, bacterial overgrowth and bacterial translocation in rats. *Ann Surg.* 1998; **228**(2): 188-193.
25. Khan I, Collins SM. Expression of cytokines in the longitudinal muscle myenteric plexus of the inflamed intestine of rat. *Gastroenterology.* 1994; **107**(3): 691-700.4957.
26. Suzuki T, Grand E, Bowman C, Merchant JL, Todisco A, Wang L, Del Valle J. TNF-alpha and interleukin 1 activate gastrin gene expression via MAPK- and PKC-dependent mechanisms. *Am J Physiol Gastrointest Liver Physiol.* 2001; **281**(6): G1405-1412.
27. Bogunovic M, Davé SH, Tilstra JS, Chang DT, Harpaz N, Xiong H, Mayer LF, Plevy SE. Enteroendocrine cells express functional Toll-like receptors. *Am J Physiol Gastrointest Liver Physiol.* 2007; **292**(6): G1770-1783.