Nuclear factor-kappaB activation on the reactive oxygen species in acute necrotizing pancreatitic rats

Jin Long, Na Song, Xi-Ping Liu, Ke-Jian Guo, Ren-Xuan Guo

AIM: To investigate the potential role of nuclear factor kappa-B (NF-κB) activation on the reactive oxygen species in rat acute necrotizing pancreatitis (ANP) and to assess the effect of pyrrolidine dithiocarbamate (PDTC, an inhibitor of NF-κB).

METHODS: Rat ANP model was established by retrograde injection of 5% sodium taurocholate into biliopancreatic duct. Rats were randomly assigned to three groups (10 rats each): Control group, ANP group and PDTC group. At the 6th h of the model, the changes of the serum amylase, nitric oxide (NO), malondialdehyde (MDA), superoxide dismutase (SOD) and pancreatic morphological damage were observed. The expressions of inducible nitric oxide (iNOS) were observed by SP immunohistochemistry. And the expressions of NF-κB p65 subunit mRNA were observed by hybridization in situ.

RESULTS: Serum amylase and NO level decreased significantly in ANP group as compared with PDTC administrated group [(7 170.40±1 308.63) U/L vs (4 074.10±1 719.78) U/L, P<0.05], [(76.95±9.04) μmol/L vs (65.18±9.02) μmol/L, P<0.05] respectively. MDA in both ANP and PDTC group rose significantly over that in control group [(9.88±1.52) nmol/L, (8.60±1.41) nmol/L, vs (6.04±1.78) nmol/L, P<0.05], while there was no significant difference between them. SOD levels in both ANP and PDTC group underwent a significant decrease as compared with that in control [(3 214.59±297.74) NU/mL, (3 260.62±229.44) NU/mL, vs (3 977.80±309.09) NU/mL, P<0.05], but there was no significant difference between them. Though they were still higher than those in Control group, pancreas destruction was slighter in PDTC group, iNOS expression and NF-κB p65 subunit mRNA expression were lower in PDTC group as compared with ANP group.

CONCLUSION: We conclude that correlation among NF-κB activation, serum amylase, reactive oxygen species level and tissue damage suggests a key role of NF-κB in the pathogenesis of ANP. Inhibition of NF-κB activation may reverse the pancreatic damage of rat ANP and the production of reactive oxygen species.

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

Key words: Pancreatitis; Acute necrotizing; Nuclear factor-kappaB; Reactive oxygen species

Long J, Song N, Liu XP, Guo KJ, Guo RX. Nuclear factor-kappaB activation on the reactive oxygen species in acute necrotizing pancreatitic rats. World J Gastroenterol 2005; 11(27): 4277-4280
http://www.wjgnet.com/1007-9327/11/4277.asp

INTRODUCTION

Acute pancreatitis is clinically classified into mild and severe forms. Mild or edematous acute pancreatitis is a self-limiting disease with a low complication and mortality rate. However, ANP has an unacceptably high morbidity and mortality rate. Multiple therapeutic modalities have been suggested for acute pancreatitis, but none has been unambiguously proven to be effective yet. The major problem is that the pathophysiology of the disease is not fully understood[1,2].

Oxygen free radicals are molecules produced continuously in cells by several mechanisms. The generation of oxygen free radicals is physiologic. In most circumstances, oxygen free radicals are neutralized immediately by enzymatic scavengers. But when formation of oxygen free radicals overwhelms radical neutralization in cells, oxidative stress occurs. As they are very reactive, they react well with all biological substances such as proteins, polysaccharides, and nucleic acids, resulting in tissue injury. It has been suggested that oxygen free radicals are responsible for a wide variety of diseases or conditions, for they play an important role in the pathogenesis of pancreatitis in some experimental models, and they are involved in the initiation of pancreatitis. Also, it was reported that oxygen free radicals acted as important mediators of tissue damage in experimental acute pancreatitis[3,4].

Nuclear factor kappa-B (NF-κB) is a sequence-specific transcription factor known to be involved in inflammatory and immune responses. It plays an important role in physiologic and pathologic conditions as an inducible nuclear factor. NF-κB is able to mediate a variety of inflammatory mediators involved in acute pancreatitis, including cytokines and adhesion molecules, as well as specific inducible isoform of nitric oxide synthase enzymes. Recent experimental studies appeared to have shed some light on the intracellular signaling...
pathway in the inflammatory cascade in acute pancreatitis. Hence, the role of NF-κB in acute pancreatitis has attracted more and more attention.

Therefore, this study was conducted to evaluate the role of NF-κB in experimental model of rat ANP and to analyze the role of NF-κB activation on nitric oxide (NO) and other reactive oxygen species in the pathogenesis of ANP.

**MATERIALS AND METHODS**

**Experimental groups and models**

We randomized 30 male Wistar rats (weighing 250-300 g) to three groups, Control group, ANP group, and PDTC group. After having fasted for 24 h before the experiment, and allowed only drinking water freely, all rats were intraperitoneally infused with 2.5% pentobarbital sodium. When the abdominal cavity was opened through the median incision, the common bile duct and the pancreatic duct were found. After intubation from the end of pancreatic duct, the pancreatic duct was shut both at the duodenal ampulla and near the hepatic hilum transiently to prevent regurgitation of the infusion into the liver or duodenum. The ANP and PDTC group were induced by slow and even infusion of 5% sodium taurocholate (Sigma, St. Louis, MO, USA) into the pancreatic duct. The Control group received infusion of saline of the same amount instead of sodium taurocholate. In addition to sodium taurocholate, PDTC group received intravenous infusion of PDTC (Sigma, St. Louis, MO, USA) 10 mg/kg, while Control and ANP group received the same amount of saline instead.

**Tested parameters**

At the 6th h of the model, blood was collected from the abdominal aorta of rats, and the pancreas was removed according to the following measurement: (1) Serum amylase detected by HITACHI 7170 automatic biochemical analyzer; (2) Ratio of NO2 to NO3 determined by copper-zinc-cadmium reductive chromatometry, which reflects NO level; (3) Malondialdehyde (MDA) detected by TBA chromatometry; (4) Superoxide dismutase (SOD) detected by hydroxylamine chromatometry; (5) Morphological damage to pancreas observed microscopically after fixation in 10% neutral formaldehyde solution and HE stain, morphological alterations were graded using a scale of the degree of inflammation, necrosis and edema; (6) Expressions of iNOS in pancreatic tissue detected by iNOS immunohistochemical kits (Beijing Zhongshan Biotechnology Co., Ltd. Beijing, China) iNOS antibody was diluted 50 fold, tested with SP method, and cells stained with brown-yellow granules were considered to be positive; (7) Expressions of NF-κB p65 subunit mRNA in pancreatic tissue: After fixation in 4% citromint and 1% DEPC, expression of NF-κB p65 subunit mRNA in situ kits (Wuhan Boster Bioengineering Co., Ltd. Wuhan, Hubei, China) Plasma and nucleus of pancreatic tissue detected by iNOS immunohistochemical method, and cells stained with brown-yellow granules were considered to be positive. All immunohistochemistry or in situ images were analyzed and processed using MetaMorph v4.6 software (Universal Imaging Corp.). The intensity of expression was represented by average gray value, and the difference of average gray value reflected the difference of expression.

Two pathologists assessed all of histopathologic sections in 20 fields/organ, and they were not aware as to which groups the sections belonged.

**Statistical analysis**

SASS 6.12 statistical analytic software was applied to process the data collected, and P<0.05 was considered statistically significant.

**RESULTS**

**Change of serum amylase, NO, MDA, and SOD (Table 1)**

After the above treatment, serum amylase in ANP group rose rapidly. Although it was significantly higher than that in control group (P<0.05), serum amylase of PDTC group was still significantly lower than that in ANP group (P<0.05). NO levels in both ANP and PDTC group increased. The former, however, was significantly higher than the latter (P<0.05). MDA in both ANP and PDTC group rose significantly over that in control group (P<0.05), while there was no significant difference between the two results themselves. SOD levels in both ANP and PDTC group underwent a significant decrease as compared with that in control group (P<0.05), but there was no significant difference between either of them.

**Morphological change of the pancreatic tissue (Figure 1)**

The only gross change in the abdominal cavity in control group was the mildly edematous pancreas. No structural damage was found microscopically in the pancreatic tissue, except for some local interstitial edema. In ANP group, bloody ascites, necrotic foci in pancreas, and fat necrosis in mesentery and omentum were found grossly. Interstitial lobular inflammatory infiltrations were observed in pancreas microscopically, as well as diffusive bleeding and piecemeal necrosis. In PDTC group, ascites and fat necrosis diminished notably compared with those in ANP group. Microscopically, slight bleeding, mild acinar degeneration and mild structure damage to lobules were observed together with declining inflammatory infiltration. The damage of ANP group is obviously grave than PDTC group [Histopathologic score: (5.76±1.12) vs (4.00±1.50), P<0.05].

| Group   | Amylase (U/L) | NO (umol/L) | MDA (nmol/L) | SOD (NU/mL) |
|---------|--------------|-------------|--------------|-------------|
| Control | 990.20±189.32 | 56.97±13.31 | 6.04±1.78    | 3977.80±309.09 |
| ANP     | 7170.40±1308.63 | 76.95±9.04  | 9.88±1.52    | 3214.59±297.74 |
| PDTC    | 4074.10±179.78 | 65.18±9.02  | 8.60±1.41    | 3260.62±229.44 |

*P<0.05 vs ANP group; /P<0.05 vs control group.*
Expressions of iNOS in pancreatic tissue (Figure 2)

Expression of iNOS was negative in control group while positive in both ANP and PDTC group, mainly found in endotheliums and smooth muscle cells. The iNOS expression is significantly lower with PDTC group than ANP group [Average gray value: (80.43±10.48) vs (64.26±9.18), P<0.05].

Expressions of NF-κB p65 subunit mRNA in pancreatic tissue (Figure 3)

In control group, expression of NF-κB was negative in all acinar nuclei, and positive only in some plasma. In ANP group, however, the expression was positive in both nucleus and plasma of pancreatic acinar. And the expression in nucleus and plasma decreased significantly in PDTC group [Average gray value: (104.25±19.08) vs (67.28±8.95), P<0.05].

DISCUSSION

One of the most severe complications of ANP is multiple system organ failure (MSOF) in early stage. The early systemic complication is the major cause of death in ANP, which leads to a mortality rate of 20%. Although it has been indicated that trypsin activation, auto-digestion of pancreas, cytokines, endotoxin, reactive oxygen species, and arachidnate[8-11] play an important role in the progression of MSOF in ANP, the mechanisms of the development of this disease remain obscure.

Like what happens in other inflammatory diseases, reactive oxygen species are generated in the early stage of ANP, and they play quite a vital role in the onset and development of ANP[10]. During ANP, activated neutrophiles are attached to endothelial cells, infiltrate into tissues, and produce large amount of reactive oxygen species and a kind of cytokines, which may cause severe damage to the pancreatic tissue. NO, in addition to potent function of vasodilation, can also inhibit the adherence, infiltration, and activation of white blood cells, and affect the production of reactive oxygen species[11,12]. NO is generated by two classes of nitric oxide synthase (NOS): One that is constitutive, Ca²⁺-dependent and physiologically activated (cNOS) and the other is inducible (iNOS). cNOS produces temperate amount of NO relieving ANP, whereas iNOS produces excess NO exacerbating the damage of ANP to the body[12].

Excessive production of NO causes vasodilatation and hypotension leading to organ hypoperfusion, edema, and organ dysfunction. Moreover, the reaction of NO with superoxide causes the formation of peroxynitrite, which is a powerful oxidant and cytotoxic agent and may play an important role in the cellular damage associated with the overproduction of NO. The spontaneous reaction of peroxynitrite with proteins makes the nitration of tyrosine residues to form nitrotyrosine, which is a specific nitration product of peroxynitrite and a marker for peroxynitrite-induced oxidative tissue damage. In this study, we found the concentration of SOD as an antioxidant decreased and that of MDA as the lipid peroxide increased, indicating the role of NO on the free radical reaction and oxidation response could intensify ANP.
NF-κB is a kind of pleiotropic regulative protein of transcription. Its activation takes part in the pathogenesis of ANP. Inhibition of the action can ameliorate the rat transcription. Its activation takes part in the pathogenesis production of reactive oxygen species, thus ameliorate the study that NF-κB cause damage to the pancreatic and extra-pancreatic tissues in ANP. NF-κB activation does relate to the reactive oxygen species in ANP.

Our study provides evidence that the injection of sodium taurocholate can cause ANP, with manifestation of the rise of serum amylase and NO, MDA level, damage to pancreas, inflammatory infiltration, and the decrease of SOD. Hybridization in situ and immunohistochemical results suggest that NF-κB is activated immediately at the onset of ANP, accompanied by high expression of iNOS. And there is otherwise no expression of NF-κB and iNOS under physiological conditions. The lower expression of NF-κB p65 subunit mRNA in PDTC group indicates that the administration of PDTC may inhibit the NF-κB activation. PDTC also leads to lower increase in serum amylase and slighter histological damage to pancreas. All these results are consistent with previous studies, substantiating that NF-κB activation does relate to the reactive oxygen species in ANP.

We may finally draw the conclusion from the above study that NF-κB activation in rat ANP may reduce over-production of reactive oxygen species, thus ameliorate the severity of ANP, all of which is achieved by inhibiting iNOS expression. The drug that can inhibit the activation of NF-κB may become a way of the therapy of ANP.

REFERENCES

1. Yousaf M, McCallion K, Diamond T. Management of severe acute pancreatitis. Br J Surg 2003; 90: 407-420
2. Banks PA. Practice guidelines in acute pancreatitis. Am J Gastroenterol 1997; 92: 377-386
3. Schoenberg MH, Birk D, Beger HG. Oxidative stress in acute and chronic pancreatitis. Am J Clin Nutr 1995; 62 (6 Suppl): 1306S-1314S
4. Sweiry JH, Mann GE. Role of oxidative stress in the pathogenesis of acute pancreatitis. Scandinav J Gastroenterol Suppl 1996; 219: 10-15
5. Jaffray C, Yang J, Carter G, Mendez C, Norman J. Pancreatic elastase activates pulmonary nuclear factor kappa B and inhibitory kappa B, mimicking pancreatitis-associated adult respiratory distress syndrome. Surgery 2000; 128: 225-231
6. Frossard JL, Pastor CM, Hadengue A. Effect of hyperthermia on NF-kappaB binding activity in cerulein-induced acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2001; 280: G1157-1162
7. Algut H, Tando Y, Schneider G, Weidenbach H, Adler G, Schmid RM. Acute Experimental Pancreatitis and NF-kappaB/Rel Activation. Pancreatology 2002; 2: 503-509
8. Karne S, Gorelick FS. Etiopathogenesis of acute pancreatitis. Surg Clin North Am 1999; 79: 699-710
9. Denham W, Norman J. The potential role of therapeutic cytokine manipulation in acute pancreatitis. Surg Clin North Am 1999; 79: 767-781
10. Norman J. The role of cytokines in the pathogenesis of acute pancreatitis. Am J Surg 1998; 175: 76-83
11. Schulz HU, Niederau C, Konowski-Stumpe H, Halangk W, Luthen R, Lippert H. Oxidative stress in acute pancreatitis. Hepatogastroenterology 1999; 46: 2736-2750
12. Jaworek J, Jachimeczak B, Tomaszewski R, Konturek PC, Pawlik WW, Sendur R, Hahn EG, Stachura J, Konturek SJ. Protective action of lipopolysaccharides in rat caerulein-induced pancreatitis: role of nitric oxide. Digestion 2000; 62: 1-13
13. Gukovsky I, Gukovskaya AS, Blinnman TA, Zaninovic V, Pandol SJ. Early NF-kappaB activation is associated with hormone-induced pancreatitis. Am J Physiol 1998; 275(6 Pt 1): G1402-1414
14. Dunn JA, Li C, Ha T, Kao RL, Browder W. Therapeutic modification of nuclear factor kappa B binding activity and tumor necrosis factor-alpha gene expression during acute biliary pancreatitis. Am Surg 1997; 63: 1036-1043
15. Abraham E. NF-kappaB activation. Crit Care Med 2000; 28: N100-104