BASIC RESEARCH

Effect of BN52021 on NF- κ Bp65 expression in pancreatic tissues of rats with severe acute pancreatitis

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Abstract

AIM: To investigate dynamic changes and significance of expression of NF- κ Bp65 in pancreatic tissues of rats with severe acute pancreatitis (SAP), as well as BN52021 effects.

METHODS: Wistar male rats were randomly divided into negative control group (NC group, $n = 60$), SAP-model group (SAP group, $n = 60$), and BN52021-treated group (BN group, $n = 60$), and each of the above groups was respectively divided into 6 subgroups at different time points after operation (1 h, 2 h, 3 h, 6 h, 12 h, and 24 h) ($n = 10$). By RT-PCR and Western blot, NF- κ Bp65 mRNA and its protein expression in pancreatic tissues of rats were detected respectively.

RESULTS: The expression of NF- κ Bp65 mRNA dynamically changed in both SAP groups and BN groups. The mRNA level was higher in SAP groups than NC groups at 2 h, 3 h, 12 h, and 24 h after operation ($P < 0.05$), higher in BN groups than NC groups at all time points ($P < 0.05$), and higher in BN groups than SAP group at 1 h ($P < 0.05$). The NF- κ Bp65 protein level was higher in SAP groups than NC groups at 1 h, 3 h, and 6 h ($P < 0.01$), and 2 h, 12 h, and 24 h ($P < 0.05$), higher in BN groups than NC groups at all time points ($P < 0.05$), and lower in BN groups than SAP groups at 1 h, 3 h, and 6 h ($P < 0.05$).

CONCLUSION: The expression of NF- κ Bp65 in pancreatic tissues is dynamically changed and the changes play an important role in pathogenesis of SAP. BN52021 exerts therapeutic effects through reducing the expression level of NF- κ Bp65 protein in the early stage of SAP.

INTRODUCTION

Up to now, the precise pathogenesis of Severe acute pancreatitis (SAP) with a high mortality has not been completely elucidated although the theories of self-digestion, leukocyte over-activation, microcirculatory disorder, bacterial shifting and secondary infection, i.e. the second attack, immune functional change, cell apoptosis and oxygen free radicals etc. have explained the pathogenesis of SAP from different angles^[1,2].

NF- κ Bp65 is a type of protein that can bind many kinds of cytokines and adhesion molecules at the κ B site of their gene promoters to enhance transcription of the genes. It plays an important role in cellular signal transduction in different theories of SAP pathogenesis^[3]. For example, the activation of NF- κ Bp65 is the decisive factor in many pathological states and it especially has a close relationship with the occurrence and pathophysiological process of severe infections^[4]. In SAP pathogenesis, the clinical course of SAP is dependent on the over-activated inflammatory cells and inflammatory factors expressed, in which platelet activating factor (PAF) is a crucial transmitter for systematic inflammatory reaction of SAP. PAF was found to have the ability to activate NF- κ B in 1994^[5], and the studies since then have shown that PAF receptor (PAF-R) is a target gene of NF- κ B^[6]. All these findings have attracted researchers to further study the relationship between PAF and NF- κ B.

BN52021 (ginkgolide B) is a specific antagonist to PAF-R. In recent years, studies at home and abroad have showed that it has significant physiological activities, such as platelet aggregation inhibition, anti-inflammation, and anti-shock, etc. BN52021 has significant effects in treatment of animals with SAP^[7,8]. But the exact pathogenesis of BN52021 on SAP is unknown. This study was aimed at dynamically investigating the changes and significance of the expression of NF- κ Bp65 mRNA and its protein in pancreatic tissues and effects of BN52021 in rats with SAP.

MATERIALS AND METHODS

Main reagents and apparatus

BN52021 and sodium taurocholate were purchased from Sigma (USA), amylase kit was from Beijing Kemei Reagent Co. (Beijing, China), trizol and diethyl pyrocarbonate (DEPC) were from Invitrogen Company (USA), RT kit was from IBM Fermentas Company (Lithuania), dNTPs and the RNA enzyme inhibitor were from TaKaRa Company (Japan), DNA Tag enzyme was from Promega (USA), Primary antibody of NF-κBp65 rabbit-anti-rat serum and the enhanced chemiluminescence (ECL) system were from Santa Cruz Biotechnology (USA), secondary antibody of sheep-antirabbit was from Beijing Dingguo Biotechnology Co., Ltd. (Beijing, China), polyvinylidene fluoride (PVDF film) was from Millipore Corp. (USA), prestained marker was from Beijing Tianwei Time Biotechnology Co., Ltd. (Beijing, China), β-actin was from Beijing Zhongshan Biotechnology Co., Ltd. (Beijing, China), DYY-12 electrophoresis system and electric trans-blot SD were from Beijing Liuyi Instrument Factory (Beijing, China), type-2720 PCR apparatus was from ABI Company (USA), and gel scanning & imaging system and vertical electrophoresis system were from Bio-Rad (USA).

Animal grouping and model preparation

One hundred and eighty Wistar male rats (weighing 0.20-0.23 kg, 6-8 wk old, Grade II, Certificate SCXK 2002-001) were provided by Laboratory Animal Center of PLA Academy of Military Medical Sciences, China. All rats were maintained in an environment of controlled temperature (22°C-25°C), humidity (55%-58%), and lighting (12 h light/12 h dark), with free access to tap water and regular chow diet. They were randomly divided into the negative controlled group (NC group, $n = 60$), SAP-modeled group (SAP group, $n = 60$), and BN52021-treated group (BN group, $n = 60$), and each of the above groups was respectively divided into 6 subgroups at different time points after operation (1 h, 2 h, 3 h, 6 h, 12 h, and 24 h) ($n = 10$). SAP models were prepared according to the method by Aho *et al*^[9]. Wistar male rats were weighed, marked and fasted for 24 h before the operation, with free access to water. Anaesthesia was conducted by abdominal cavity injection of 0.4% sodium pentobarbital (40 mg/kg). Rats were fixed in dorsal decubitus. The skin was prepared and sterilized. And a 2-cm incision was made along the middle line of the upper belly and the abdominal cavity was entered. The duodenum and pancreaticobiliary duct were searched, the hepatic end of the pancreaticobiliary duct was clipped with a non-invasive vascular clip, pancreaticobiliary duct retrograde centesis was conducted with an obtuse (pointless) needle through duodenum seromuscular layer, and then 5% sodium taurocholate (0.1 mL/100 g) was injected in the retrograde direction of pancreaticobiliary duct with a micro-syringe, at an injection rate of 0.20 mL/min. After the injection of the drug, the part of pancreaticobiliary duct entering the duodenum was clipped with a non-invasive vascular clip for 10 min, and then the vascular clip was removed. After making sure that there was no active bleeding in the abdominal cavity, the abdomen was closed in two layers, and the wound was covered with sterile gauze. For the rats in NC group, the

Table 1 Scoring standard of pathological change for pancreatic tissue of rats with SAP

Pathological grading	Pathological change	Scores
Edema	Inter-lobule local edema, widened pleura	1
	Inter-lobule diffuse edema, widened intra-lobule clearance	2
	Increased intra-lobule clearance, alveolus swollen, and separated	3
Inflammatory	White cells < 20/visual field under high-power microscope	1
Cell infiltration	White cells 20-50/visual field under high-power microscope	2
	White cells > 30/visual field under high-power microscope, or micro-abscess occurs	3
Hemorrhage	Parenchymal hemorrhage < 20%	1
	Parenchymal hemorrhage 20%-50%	2
	Parenchymal hemorrhage > 50%	3
Necrosis	Necrosis area < 20%	1
	Necrosis area 20%-50%	2
	Necrosis area > 50%	3

duodenum was merely stirred and pancreas was touched several times after opening the abdomen, and then the abdomen was closed. For the BN group, BN52021 (5 mg/kg; dissolved with Me₂SO) was injected intravenously within 15 min after the operation; and for the groups of NC and SAP, the same volume of physiological saline (0.9% NaCl) was injected through femoral vein.

Sample collection and storage

The rats in each group received anaesthesia at respective time points after the operation (1 h, 2 h, 3 h, 6 h, 12 h, and 24 h), and venous blood was collected from the right atrium. After a 10 min water bath at 37°C, and then a centrifugation for 10 min at 3000 g/min, the supernatant of the blood was respectively placed into sterilized EP tubes, and stored in a refrigerator at -20°C for determination of serum amylase. Meanwhile, two portions of pancreatic tissues of each group were treated differently; one portion was placed in liquid nitrogen overnight, and then frozen in a refrigerator at -80°C for further use, and another portion was fixed with 40 g/L neutral buffer formaldehyde, embedded with paraffin wax, cut into slices, and then HE stained for pathological observation and scoring.

Determination of serum amylase

Determination of serum amylase was conducted using a fully automatic biochemical apparatus and an amylase kit.

Pathological observation and scoring of pancreas

Pathological observation and scoring for pancreatic tissue samples (Table 1)^[10]: 10 visual fields under a high-power microscope (HE stain, × 400) were randomly selected, and pathological changes of each item in the table were graded and scored, with a score of 0 for pathological changes of items not included in Table 1.

Primers

NF-κBp65 primer was prepared according to the previous

method as reported^[11]. The NF- κ Bp65 and β -actin primers were synthesized by Shanghai Yingjun Biotechnology Co. Ltd. (Table 2).

Determination of NF- κ Bp65 mRNA expression by RT-PCR

First, the total RNA of pancreatic tissues was extracted with Trizol Reagent for each group. Then, the integrity of the total RNA was examined by agarose electrophoresis, its concentration and purity were determined by a UV spectrophotometer, and the total RNA concentration of the sample was calculated. According to instructions of the RT reagent kit, 1 μ g of the total RNA was used, and OligdT18 was used as a primer to produce 20 μ L of the reaction system. PCR reaction was conducted by using 4 μ L cDNA-RT product, 3 μ L 10 $\times$ PCR buffer, 3 μ L dNTPs of 200 μ mol/L, 3 μ L upstream primer of 10 pmol/ μ L, 3 μ L downstream primer of 10 pmol/ μ L, 1 μ L Taq enzyme, and 13 μ L deionized double-distilled water. Thermal cycle conditions were as follows: Pre-degeneration for 4 min at 94°C, 45 s at 94°C, 1 min at 72°C; after 35 cycles, an extension was conducted for another 5 min at 72°C. For each PCR reaction, a negative control with the same volume of deionized double-distilled water instead of cDNA was used, and meanwhile, amplification was conducted to control DNA contamination. The housekeeping gene was amplified, i.e. β -actin, as internal reference for gene quantitative expression. The sequence of PCR product was determined by Shanghai Yingjun Biotechnology Co., Ltd., and the results were in accordance with the corresponding mRNA. To 5 μ L of RT-PCR product, 1 μ L of supernatant buffer (containing 0.2 mg/mL ethidium bromide) was added, electrophoresis was conducted on gels containing 1.5% agarose at 80 V for 45 min, and photographed with Quantity One Gel Imaging System. A semiquantitative analysis of the experimental results was conducted.

Determination of NF- κ Bp65 protein expression by Western blot

The prepared total protein was added to gel-added buffer at a ratio of 1:2 and then heated in water at 100°C for 5 min. For its vertical plating electrophoresis, the added volume was 15 μ L. Each sample was palced on 2 parallel glue plates, one of which was used for staining and the other for membrane transferring. The voltage and time of electrophoresis were 80 v, 10 min and 120 v, 60 min for concentrating glue and isolating glue, respectively. The gels and membranes were placed among 6 sheets of filter paper and then between 2 foamy pads and put into electrical transfer tank containing buffer. After precooling for 10 min in icy water, the gel and membrane were galvanized for trans-printing for 4 h at a current of 1 mA/cm². The trans-printed gel was stained to determine whether the trans-printing was complete. The trans-printed product was put into a solution containing 0.5% bovine serum albumin, slightly shaken and enclosed for 1 h. NF- κ Bp65 antibody (diluted in 1:400) was added and placed at 4°C overnight. After the labeled PVDF membrane was washed with 0.05% Tween-20 buffer (TBST) for 5 min three times, it was incubated with IgG labeled with HRP (diluted

Table 2 PCR primer series, position and segment length of NF- κ Bp65 and β -actin.

Gene	Primer series (5'-3')	Gene No.	Position	Length
NF- κ Bp65	GAAGAAGCGAGACCTGGAG TCCGGAACACAATGGCCAC	NM_199267	424-442 821-803	398 bp
β -actin	TCCTAGCACCATGAA GATC AAACGCAGCTCAGTAACAG	NM_031144	1044-1062 1233-1215	190 bp

in 1:200) at 4°C overnight. The membrane was washed with 0.05% TBST again for 5 min three times. The liquid of ECL A and that of ECL B were mixed at an equal volume (0.125 mL liquid/cm² membrane). The PVDF membrane was immersed in the mixed liquid for 1 min. Then the membrane was put on the freshness-retaining membrane. The trans-printing membrane was fixed in the developing tray and pictured using medical film for 30 s to 1 min, developed for 1-3 min and fixed for 1 min. Finally, the membrane was cleaned with water and dried up. The PVDF membrane was put into the desorption liquid, shaken at 50°C for 30 min, enclosed, added with β -actin antibody (diluted in 1:200) and placed at 4°C overnight. Then the above-mentioned process was repeated again for imaging. The results were photographed, saved in a computer, analysis was conducted by using Image-Pro Plus 4.5 image-analyzing software, and the integrated optical density (IOD) was calculated. The optical density ratio of target protein and β -actin in the same membrane was taken as the value of the final experimental results.

Statistical analysis

The above experiments were repeated 3 times, the average values were calculated as the final data, and expressed as mean $\pm$ SEM. Data were processed with SPSS11.5 statistical software, normal test was conducted using *t* test and single-factor analysis of variance. Results were considered statistically significant when $P < 0.05$ or $P < 0.01$.

RESULTS

Serum amylase

It showed that the serum amylase in the SAP groups and the BN groups significantly increased at each time point than those in the NC groups ($P < 0.05$); however, the values in the BN groups significantly decreased at 3 h, 6 h, and 24 h than those in the SAP groups ($P < 0.05$) (Table 3).

Pathological observation and scoring

The pathological results showed that there was no obvious abnormality in abdominal cavity at all time points, and the pancreatic structure was almost normal in NC groups; in the SAP groups, hemorrhagic ascites occurred, necrosis foci were present in the pancreas, a number of saponifying spots occurred in the mesentery and greater omentum, and inflammatory cells infiltrated in pancreatic stroma and glandular lobule, and diffuse bleeding and piecemeal necrosis occurred and over time, the pathological changes were exacerbated; in the BN groups, the pathological changes were less serious than those in the SAP groups. It

Figure 1 Pathological change of pancreatic tissues at 6 h after operation in each group. **A** and **B**: In NC group, the pancreatic structure was almost normal; **C** and **D**: In SAP group, inflammatory cells infiltrated, diffuse bleeding and piecemeal necrosis were occurred; **E** and **F**: In BN group, the pathological changes were less serious than those in SAP group.

Table 3 Level of serum amylase at each time point in each group after operation (U/L) $n = 10$, mean $\pm$ SD

Groups	Time points					
	1 h	2 h	3 h	6 h	12 h	24 h
NC	1835.6 ± 613.2	1491.5 ± 507.0	1530.4 ± 247.0	1400.2 ± 447.5	2153.2 ± 236.3	337 ± 43.7
SAP	2560.5 $\pm 121.5^a$	2810.5 $\pm 147.2^a$	4799.3 $\pm 107.0^a$	4919.7 $\pm 139.6^a$	3486.3 $\pm 181.8^a$	2283 $\pm 127.0^a$
BN	2214.5 $\pm 109.1^b$	3331.7 $\pm 196.4^b$	4185 $\pm 147.8^{bc}$	3784.7 $\pm 124.1^{bc}$	3454 $\pm 264.1^b$	1360.4 $\pm 161.4^{bc}$

^a $P < 0.05$, ^b $P < 0.05$ vs NC group; ^c $P < 0.05$ vs SAP group.

was shown that the scores in the SAP groups and the BN groups significantly increased at all time points than those in the NC groups ($P < 0.05$); however, the scores in the BN groups markedly decreased at 3 h, 6 h, and 24 h than those in the SAP groups ($P < 0.05$) (Figure 1, Table 4).

NF-κBp65 mRNA expression in the pancreatic tissues and effects of BN52021 on it

The expression of NF-κBp65 mRNA was dynamically changed in both SAP groups and BN groups in a dual-peak manner. The two peaks of the expression appeared at 1 h and 24 h in the SAP groups and at 1 h and 12 h in the BN groups and then the expression reached its lowest level at 6 h. The mRNA level was higher in SAP groups than NC groups at 2 h, 3 h, 12 h, and 24 h after operation ($P < 0.05$), higher in BN groups than NC groups at all time points ($P < 0.05$), and higher in BN group than SAP group at 1 h ($P < 0.05$) (Figure 2, Table 5).

Table 4 Scores of pancreatic tissues at each time point in each group after operation $n = 10$, mean $\pm$ SD

Groups	Time points					
	1 h	2 h	3 h	6 h	12 h	24 h
NC	0.12 $\pm$ 0.05	0.11 $\pm$ 0.06	0.12 $\pm$ 0.05	0.13 $\pm$ 0.04	0.12 $\pm$ 0.06	0.13 $\pm$ 0.05
SAP	4.82 $\pm$ 0.35 ^a	7.65 $\pm$ 0.40 ^a	8.85 $\pm$ 0.39 ^a	9.15 $\pm$ 0.55 ^a	10.10 $\pm$ 0.65 ^a	11.75 $\pm$ 0.25 ^a
BN	5.55 $\pm$ 0.30 ^b	6.65 $\pm$ 0.42 ^b	5.95 $\pm$ 0.19 ^{bc}	5.55 $\pm$ 0.36 ^{bc}	6.72 $\pm$ 0.30 ^{bc}	9.95 $\pm$ 0.58 ^b

^a $P < 0.05$, ^b $P < 0.05$ vs NC group; ^c $P < 0.05$ vs SAP group.

NF-κBp65 protein expression in the pancreatic tissue and effects of BN52021 on it

The NF-κBp65 protein level was markedly higher in SAP groups than NC groups at 1 h, 3 h, and 6 h ($P < 0.01$), and 2 h, 12 h, and 24 h ($P < 0.05$), also higher in BN groups than NC groups at all time points ($P < 0.05$), but lower in BN group than SAP groups at 1 h, 3 h, and 6 h ($P < 0.05$) (Figure 3, Table 6).

DISCUSSION

Studies in recent years confirmed that cytokines and adhesion molecules play an important role in pathogenesis of SAP. NF-κBp65, which is a type of protein that can bind many kinds of cytokines and adhesion molecules at the κB site of their gene promoters to enhance transcription of the genes, plays an important role in the occurrence and development of SAP^[4,11]. Meanwhile, it is one member of the family of transcription-regulating protein and a homologous or heterogenous dimer that contains 5 subunits of p50, p52, p65, cRel and RelB.

Figure 2 Expression of NF-κBp65 mRNA at all time points in each group after operation. **A:** NC group; **B:** SAP group; **C:** BN group. 1: Marker, 2: 1 h, 3: 2 h, 4: 3 h, 5: 6 h, 6: 12 h, 7: 24 h.

Table 5 Absorbance values of NF-κBp65 mRNA in pancreatic tissue at each time in each group after operation $n = 10$, mean $\pm$ SD

Groups	Time points					
	1 h	2 h	3 h	6 h	12 h	24 h
NC	220.01 $\pm$ 94.51	186.30 $\pm$ 51.52	167.19 $\pm$ 51.55	114.91 $\pm$ 55.96	123.91 $\pm$ 54.78	131.08 $\pm$ 66.84
SAP	340.47 $\pm$ 119.14	269.03 $\pm$ 66.03 ^a	229.45 $\pm$ 50.34 ^a	197.07 $\pm$ 96.02	307.42 $\pm$ 100.62 ^a	376.97 $\pm$ 0.22 ^a
BN	470.80 $\pm$ 122.78 ^{b,c}	268.70 $\pm$ 87.64 ^b	267.95 $\pm$ 49.66 ^b	277.64 $\pm$ 83.37 ^b	337.21 $\pm$ 103.02 ^b	340.24 $\pm$ 0.44 ^b

^a $P < 0.05$, ^b $P < 0.05$ vs NC group; ^c $P < 0.05$ vs SAP group.

Figure 3 Expression of NF-κBp65 protein at all time points in each group after operation. **A:** NC group; **B:** SAP group; **C:** BN group. 1: 1 h, 2: 2 h, 3: 3 h, 4: 6 h, 5: 12 h, 6: 24 h.

Under normal condition, it binds to its inhibiting protein single IκB (including IκBα, IκBβ and IκBε) and has no activity. Upon stimulation of cells by such activating signals as endotoxin or TNF-α, it separates from IκB and enters the cellular nucleus and binds with specific κB sequence of

Table 6 Absorbance values of NF-κBp65 protein in pancreatic tissue at each time point in each group after operation. $n = 10$, mean $\pm$ SD

Groups	Time points					
	1 h	2 h	3 h	6 h	12 h	24 h
NC	0.05 $\pm$ 0.00	0.09 $\pm$ 0.01	0.07 $\pm$ 0.01	0.05 $\pm$ 0.01	0.06 $\pm$ 0.01	0.10 $\pm$ 0.02
SAP	1.43 $\pm$ 0.06 ^a	0.80 $\pm$ 0.05 ^d	1.58 $\pm$ 0.02 ^a	1.38 $\pm$ 0.03 ^a	0.87 $\pm$ 0.09 ^d	0.90 $\pm$ 0.05 ^d
BN	1.04 $\pm$ 0.02 ^{b,c}	0.72 $\pm$ 0.02 ^b	1.01 $\pm$ 0.04 ^{b,c}	0.95 $\pm$ 0.03 ^{b,c}	0.75 $\pm$ 0.02 ^b	0.76 $\pm$ 0.01 ^b

^a $P < 0.05$, ^b $P < 0.05$, ^d $P < 0.05$ vs NC group; ^c $P < 0.05$ vs SAP group.

DNA to enhance transcription of genes of inflammatory media and cytokines. In 1997, Dunn *et al*, for the first time, found that activation of NF-κBp65 is an important early event in the occurrence of acute pancreatitis (AP) through establishing bile-originated AP by ligation of pancreatic duct. Blinman *et al* showed that the activity of NF-κBp65 is significantly enhanced and the expression of IL-6 and KC increases in the pancreatic tissues in the early stage of edematous pancreatitis in rats induced by litorin. Therefore, they believe that the activation of NF-κBp65 plays an important role in pathogenesis of AP; IL-1, IL-6, IL-8, IL-10 and TNF are associated with AP. Since the production of these inflammatory cytokines is regulated by NF-κBp65 and activator protein 1, it is believed that NF-κBp65 plays the major role in the inflammatory media regulating network^[12,13].

In this study, we found that the expression of NF-κBp65 mRNA and protein were significantly higher in SAP groups than in NC groups at all time points. Moreover, the expression markedly increased in the early stage, dynamically changed in dual-peak and time-dependent

manners. These findings confirm that NF- κ Bp65 plays an important role in the occurrence and development of SAP. The mechanisms include: SAP causes severe endotoxemia, and LPS and endotoxin component bind with their respective binding protein and CD14 molecule on cell membrane to form an LPS-LBP-CD14 complex to activate the signal transduction of Toll-like receptor 4, and the signal transduction further activates gene transcription of cytokines (such as IL-1, IL-6 and IL-8)^[14]. In SAP, the pancreatic elastase is activated to activate NF- κ Bp65 to exert its inflammation-inducing effects through the TLR4/NF- κ B pathway. Consequently, damage of pancreatic self-digestion function and injuries of other organs outside of pancreas occur^[15]. In SAP, PLA2 is activated to activate expression of NF- κ Bp65 of target cells in tissues^[16]. NF- κ Bp65 is firstly activated in the pancreatic tissue to release cytokines. The cytokines start the “waterfall reaction” of inflammatory media through the “triggering role”. The inflammatory media such as PAF are released into the blood. Though the activation of NF- κ B in the pancreatic tissues can be partly inhibited by intra- and extracellular pathways of negative feedback of NF- κ Bp65, the inflammatory media can again activate NF- κ Bp65 in other organs outside of pancreas (neutrophils, Kuffer cells, liver, lung and intestines, etc.) to produce more inflammatory media. As a result, they participate in the development of SAP and aggravate the injuries of pancreas and other organs or even cause SIRS, ARDS and MOF^[4].

BN52021, one of the effective components of Chinese medicine ginkgo biloba leaf and a strong antagonist against the inflammatory medium of PAF, can not only block the signal transduction of PAF but also decrease blood concentration of PAF to exert its biological effect^[17]. This study showed that BN52021 could decrease serum amylase level and alleviate pathological changes in SAP, which is consistent with that reported by other researchers^[18,19]. The expression of NF- κ Bp65 mRNA was significantly higher in SAP and BN groups than in NC group at all time points after operation. However, it was markedly higher in BN groups than in SAP group at 1 h after the operation. The reason for the latter might be that the MeSO used for dissolution of BN52021 can aggravate inflammatory reaction in the early stage of SAP, resulting in enhancement of NF- κ Bp65 mRNA expression, however, the mechanism needs to be further elucidated. Our results suggest that BN52021 does not significantly affect the expression of NF- κ Bp65 mRNA. The expression of NF- κ Bp65 protein was significantly lower in BN groups than in SAP groups at 1, 3 and 6 h after operation. However, it was still markedly higher in BN groups than in NC groups at all time points after operation. These findings suggest that BN52021 can inhibit the expression of NF- κ Bp65 protein in the early stage, to some extent, which might be one mechanism of its therapeutic effects on SAP. However, there was difference in the effect of BN52021 on NF- κ Bp65 mRNA expression and its protein expression. We believe that BN52021 causes downstream changes in signal transduction, leading to the difference, the mechanism of which also needs to be further studied.

In summary, the increase of NF- κ Bp65 expression

plays an important role in pathogenesis of SAP. BN52021 can decrease serum level of amylase and alleviate pathological changes in SAP. Meanwhile, it can decrease expression of NF- κ Bp65 protein in pancreatic tissues in the early stage of SAP to exert its therapeutic effects.

COMMENTS

Background

BN52021 (ginkgolide B) is a specific antagonist to platelet activating factor receptor (PAF-R). In recent years, studies at home and abroad have shown that it has significant physiological activities, such as platelet aggregation inhibition, anti-inflammation, and anti-shock. BN52021 has significant effects in treatment of animals with severe acute pancreatitis (SAP). But the exact pathogenesis of BN52021 on SAP is unknown. This study was aimed to dynamically investigate the changes and significance of the expression of NF- κ Bp65 mRNA and its protein in pancreatic tissues and effects of BN52021 in rats with SAP.

Research frontiers

To explore molecule mechanism of BN52021 on severe acute pancreatitis.

Innovations and breakthroughs

BN52021 has remarkable curative effects in acute pancreatitis. But the mechanism of BN52021 hasn't been researched extensively. The study explored the significance of NF- κ Bp65 in platelet activating factor receptor signal transduction.

Applications

The results may provide theoretic and experimental evidence for study, and application of BN52021, and new approaches for therapy of severe acute pancreatitis.

Terminology

NF- κ Bp65 is a type of protein that can bind many kinds of cytokines and adhesion molecules at the κ B site of their gene promoters to enhance transcription of the genes. It plays an important role in cellular signal transduction in different theories of pathogenesis of severe acute pancreatitis. BN52021, namely code of ginkgolide B, one of the effective components of Chinese medicine ginkgo biloba leaf and a strong antagonist against the inflammatory medium of platelet activating factor (PAF), can not only block the signal transduction of PAF but also decrease blood content of PAF to exert its biological effect. It has significant physiological activities, such as platelet aggregation inhibition, anti-inflammation, and anti-shock.

Peer review

The results show that the expression of NF- κ Bp65 in pancreatic tissues dynamically changed and the changes played an important role in pathogenesis of severe acute pancreatitis (SAP). BN52021 exerted some kind of therapeutic effect through reducing the expression level of NF- κ Bp65 proteins in the early stage of SAP.

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