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• BASIC RESEARCH •

Effect of resveratrol on activation of nuclear factor kappa-B and inflammatory factors in rat model of acute pancreatitis

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Abstract

AIM: To observe the effect of resveratrol on nuclear factor Kappa-B (NF-κB) activation and the inflammatory response in sodium taurocholate-induced pancreatitis in rats.

METHODS: Seventy-two male SD rats were randomly divided into three groups: sham operation group (control), severe acute pancreatitis (SAP) group, and severe acute pancreatitis group treated with resveratrol (RES). A SAP model was established by injecting 4% sodium taurocholate 1 mL/kg through puncturing the pancreatic duct. In Res group, Res was given at 30 mg/kg b.m. intraperitoneally after the SAP model was successfully established. Eight animals from each group were sacrificed at 3, 6 and 12 h after modeling. The expression of NF-κB activation of pancreas was detected by immunohistochemical staining, whereas the levels of TNF-α and IL-8 in pancreatic tissues were estimated by radioimmunoassay. The pathological changes of pancreas and lungs were examined microscopically.

RESULTS: Much less hyperemia, edema, dust-colored necrotic focus and soaps were noticed in pancreas in RES group than in SAP group. In RES group, hemorrhage, exudates and infiltration of inflammatory cells in pancreas and interstitial edema, destruction of alveolar wall in lung were significantly less than in SAP group. In the SAP group, the activation of NF-κB in pancreatic tissues was enhanced significantly at any measure point compared with control group (64.23±10.72% *vs* 2.56±0.65%, 55.86±11.34% *vs* 2.32±0.42%, 36.23±2.30% *vs* 2.40±0.36%, $P<0.01$), TNF-α, IL-8 were also increased and reached their peak at 6 h and then declined. The activation of NF-κB and the levels of TNF-α and IL-8 in RES group were significantly lower than those in SAP group ($P<0.01$): activation (52.63±9.45% *vs* 64.23±10.72%, 40.52±8.40% *vs* 55.86±11.34%, 29.83±5.37% *vs* 36.23±2.30%), TNF-α (132.76±15.68 pg/mL *vs* 158.36±12.58 pg/mL, 220.32±23.57 pg/mL *vs* 247.67±11.62 pg/mL, 175.68±18.43 pg/mL *vs* 197.35±12.57 pg/mL) and IL-8 (0.62±0.21 μg/L *vs* 0.83±0.10 μg/L, 1.10±0.124 μg/L *vs* 1.32±0.18 μg/L, 0.98±0.16 μg/L *vs* 1.27±0.23 μg/L).

CONCLUSION: The activation of NF-κB is involved in the inflammatory response of rats with SAP. Resveratrol could effectively inhibit the expression of NF-κB activation, alleviate the severity of SAP through its anti-inflammatory effects and regulate the inflammatory mediators.

Key words: Acute pancreatitis; Resveratrol; NF-κB; Inflammatory factors

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INTRODUCTION

Organ failure is common in patients with severe acute pancreatitis (SAP), and patients with multiple organ dysfunction (MODF) and acute respiratory distress syndrome (ARDS) have a higher mortality rate. Prevention and active treatment of organ failure can improve the outcome of patients with SAP[1]. Recent studies have demonstrated that SAP complicated by ARDS and MODF is hardly elucidated by the traditional concepts of pancreatic enzyme activation and autodigestion, but closely associated with some cytokines such as TNFα,IL-1 and, IL-8[2,3]. At present, researchers pay more attention to the relationship between nuclear factor kappa-B (NF-κB), inflammatory cytokines and acute pancreatitis (AP)[2,4]. NF-κB is a protein that can bind to its consensus sequence on the promoter-enhancer region of multiple genes for activating gene transcription. It has been found that inflammatory genes associated with inflammatory and immune responses have the NF-κB-binding domain and NF-κB plays a critical role in the expression of these genes[5]. Its activation may enhance recruitment of inflammatory cells and production of proinflammatory mediators like IL-1, IL-6, IL-8, and TNF-α[6,7] .

As a polyphenol compound existing mainly at high levels in grapes, Eranthis hyemalis and giant knotweed rhizome, resveratrol (3,5,4'-trihydroxystilbene, Res) etc, has been found to have anti-inflammatory, antioncogenic, antioxidant, immunity-modulatory properties[8-15]. Recent *in vitro* studies have shown that Res can suppress NF-κB activation. In the present research, we investigated the effect of Res on NF-κB activation and the inflammatory response in sodium taurocholate-induced severe acute pancreatitis in rats.

MATERIALS AND METHODS

Reagents

NF-κB P65 monoclonal antibody was from SantaCruz. The immunohistochemistry kit was provided by Beijing Zhongshan Biotechnology Company. Kits of TNF-α and IL-8 assay were purchased from China Institute of Atomic Energy. Sodium taurocholate and Res were from Sigma.

Animals and experimental groups

Healthy male SD rats weighing 250-300 g were provided by the Experimental Animal Center of Medical School of Xi'an Jiaotong University. All animals were randomly divided into three groups with 24 rats each, including ASP group, RES group and control (sham operation) group.

Methods

Preparation of animal models Rats were starved but allowed free access to water for 12 h before initiation of the experiments. Rats were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and fixed. The biliopancreatic duct was cannulated through the duodenum and the hepatic duct was closed by means of a clip placed at the hilum of the liver. A SAP model was established by injecting 4% sodium taurocholate 1mL/kg through puncturing the pancreatic duct. After the SAP model was successfully established in Res group, Res was given at 30 mg/kg b.m. intraperitoneally. A control group was established by turning over the duodenum. Animals in control group were only turned over duodenum. Animals from all groups were injected with normal saline solution 5 mL subcutaneously on the back. Eight animals from each group were sacrificed at 3, 6 and 12 h after modeling. Tissue samples from the cauda pancreatis and lungs were taken and fixed in 4% paraformaldehyde, embedded in paraffin wax and sectioned for immunohistochemical staining. Tissue samples from the border between the body and the tail of pancreas were also collected and frozen in liquid nitrogen for the measurement of TNF-α and IL-8.

Histopathological examination of pancreas and lung Changes in pancreas were observed macroscopically and microscopically. Specimens from the pancreas and lung were fixed by immersion in 4% paraformaldehyde at 4 ℃ and cut into 4 μm thick sections. After stained with hematoxylin and eosin, the sections were observed under a microscope.

Assay of the activated NF-κB in pancreas Sections embedded in paraffin wax were de-waxed, rehydrated in gradient alcohol and endogenous peroxidase activity was blocked with 3% H_2O_2 for 5 min, and then incubated in normal goat serum and followed by using monoclonal NF-κB p65 (F-6) antibody (diluted 1:100) that could recognize the amino-terminal sequences of the p65 subunit overnight at 4 ℃. Then, sections were incubated with secondary antibody using goat anti-rabbit IgG. At last, the sections were visualized by incubating with diaminobendizine (DAB) solution and weakly counter-stained with hematoxylin. Positive signals were detected as cytoplasm and nucleus staining presenting yellow color. According to the staining intensity, the staining was graded as follows : (-) = negative, (+) = weak, (++) = moderate, (+++) = strong .The positive cells and total cells were counted in 4 random high power fields under a light microscope and the positive rate was calculated by the formula: the positive rate = the number of positive cells/ the number of total cells.

Assay of TNF-α, IL-8 One hundred mg sample tissue from the border between the body and tail of pancreas was used and homogenated with 1mL phosphate-buffered solution. The supernatant was collected and radioimmunoassay was carried out according to the kit application protocol.

Statistical analysis

Data were represented as mean±SD. ANVOA was used to analyze the data with the SPSS10.0 software. $P<0.05$ was considered statistically significant.

RESULTS

Pathological observation of pancreas

In SAP group, hyperemia, edema, dust-colored necrotic focus and soaps were noticed in pancreas, and hemorrhage was found in peripancreatic fat. It showed that the region of hyperemia and necrosis extended with time passsing. Although pathologic region expanded with time in the initial 6 h in RES group, but it was alleviated significantly after 6 h by the treatment of Res.

Histopathology under light microscope

In SAP group, the edematous pancreas was infiltrated with numerous inflammatory cells and scattered foci and a large scale of hemorrhage and necrosis. Microthrombi could be found at small vessels in the peripheral part of necrosis foci. Whereas, the histological changes, such as hemorrhage, exudates and infiltration of inflammatory cells were significantly less in RES group than in SAP group.

Remarkable changes were observed in the lungs of SAP groups: alveolar septal thickening, interstitial edema, and infiltration of numerous inflammatory cells, destruction of alveolar wall, and numerous erythrocytes locating in the alveolar space. The aforementioned histological abnormalities were not found in RES group.

NF-κB expression in pancreas

The activated NF-κB, which could hardly be found in normal pancreatic cells was located in nuclei and mainly in the cytoplasm of pancreatic acinar cells. The expression of NF-κB activation was the highest at 3 h. But it reduced slightly at 6 h and 12 h, and still maintained high. In RES group, the expressions of NF-κB activation at 3 h, 6 h, and 12 h were lower than those in the other groups respectively (Tables 1-2)

Table 1 Activation extent of NF-kB in pancreas (mean±SD, %)

Group	Number of animal	3 h	6 h	12 h
Control	8	2.56±0.65	2.32±0.42	2.40±0.36
SAP	8	64.23±10.72[b]	55.86±11.34[b]	36.23±2.30[b]
RES	8	52.63±9.45[a]	40.52±8.40[a]	29.83±5.37[a]

[b]$P<0.01$ *vs* control group; [a]$P<0.05$ *vs* control group.

TNF-α and IL-8 examination

In SAP group, the level of TNF-α was higher than in RES group at 3 h and reached its peak at 6 h. After reaching its highest level, the level of TNF-α in RES group dropped gradually and a

Table 2 Expression level of active NF-κB in pancreas in three group (n = 8)

Group	3 h				6 h				12 h			
	(-)	(+)	(++)	(+++)	(-)	(+)	(++)	(+++)	(-)	(+)	(++)	(+++)
Control	8	0	0	0	8	0	0	0	8	0	0	0
SAP	0	0	2	6	0	2	6	0	0	5	3	0
RES	0	1	5	2	1	4	3	0	2	5	1	0

significant decrease was found at 6 h and 12 h compared to SAP group (P<0.05) (Tables 3 and 4).

Table 3 Concentration of TNF-α in pancreas (mean±SD, pg/mL)

Group	Number of animal	3 h	6 h	12 h
Control	8	43.32±1.21	44.58±2.10	44.22±1.58
SAP	8	158.36±12.58[b]	247.67±11.62[b]	197.35±12.57[b]
RES	8	132.76±15.68	220.32±23.57[a]	175.68±18.43[a]

[b]P<0.01 *vs* the control group; [a]P<0.05 *vs* the control group.

Table 4 Concentration of IL-8 in pancreas (mean±SD, μg/L)

Group	Number of animal	3 h	6 h	12 h
Control	8	0.20±0.03	0.22±0.013	0.18±0.014
SAP	8	0.83±0.10[b]	1.32±0.18[b]	1.27±0.23[b]
RES	8	0.62±0.21[a]	1.10±0.124[a]	0.98±0.16[a]

[b]P<0.01 *vs* control group; [a]P<0.05 *vs* control group.

DISCUSSION

Acute necrotic pancreatitis is one of the frequent abdominal emergencies[16]. Its pathogenesis still remains unclear. Presently, researchers have payed more attention to the relation between NF-κB and AP[2]. Some studies have shown that it could modulate the expression of numerous genes, such as genes of cytokines, adhesion molecules, enzymes, and that it could play a pivotal role in the initiation, promotion, progression of inflammatory response[17].

NF-κB is a general transcription factor, which belongs to the REL family and exists as a heterodimer or a homodimer formed by polypeptides P50 and P65. Its physiological activity relies mainly on the P50-65 heterodimer, which exists in the cytoplasm of the majority of cell types at a high level. In the state of unactivation, NF-κB binds to its inhibitor I-κB as an incompetence complex is "imprisoned" in cytoplasm and does not exert its function. In response to the extracellular stimuli, the activated protein kinase degrades I-κB and then induces NF-κB to separate from the NF-κB/I-κB complex. The free NF-κB translocates rapidly from cytoplasm to nuclei. In the nuclei, the NF-κB dimmer binds to its specific site-κB sequences and promotes the corresponding gene expression. Some could produce inflammatory mediators (IL-1β, TNF-α, *etc*), and inversely augment the activation of NF-κB to produce more inflammatory mediators and exacerbate inflammatory response[18].

In the early phase of sodium taurocholate-induced SAP in our experiments, NF-κB was markedly activated to improve the corresponding gene transcription and to synthesize TNF-α and IL-8, which is consistent with the studies of Vaquero *et al*.[19] and Rakonczay *et al*.[20]. We also observed that the activation of NF-κB was a dynamic course from a lower level to a higher level and the higher level to the lower level with the time passing, and it reached the highest level at 3 h after the induction of pancreatitis. After animals were treated with Res , the activation of NF-κB was significantly inhibited and the expression of TNF-α and IL-8 decreased markedly. The results demonstrate that Res could modulate the expression of cytokines such as TNF-α, IL-8, by inhibiting the NF-κB activation and suppressing the inflammatory response of pancreatitis.

Resveratrol has a significant antioxidant and antiinflammatory effect. It can protect cells and tissues by eliminating oxidants, blocking oxidant production, modulating lipoprotein metabolism and inhibiting lipid peroxidation. It can also inhibit the production of COX, reduce the production of leukotriene, TXAB2, 5-HETE, endodermis attachment proteins (ICAM-1, VCAM-1)[21] and increase CAMP levels in neutrophils and suppress NF-κB activation[21]. The mechanism underlying the NF-κB activation is to decrease the production of reactive oxygen species (ROS). ROS can activate NF-κB[22], inhibit the I κB kinase activation and P65 subunit phosphorylation, which can effectively suppress the NF-κB binding ability[23], inhibit the I κB phosphorylation and degradation and the NF-κB activity[5].

In conclusion, imbalance in cytokine network plays a critical role in AP. There is a close relation between SIRS, MODS and the high expression of cytokines. Res might be a potential candidate for therapy of AP.

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