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Application of Tissue Microarrays to Study the Influence of Dexamethasone on NF-κB Expression of Pancreas in Rat with Severe Acute Pancreatitis

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Abstract To discuss the influence of dexamethasone on NF-κB expression of pancreas in rat with severe acute pancreatitis (SAP). Ninety rat SAP models were divided into the model group and dexamethasone treatment group with 45 rats in each group; another healthy 45 rats were selected to be the sham operation group. The groups were divided into the 3, 6 and 12 h group with 15 rats in each group. The survivals, pancreas pathological changes were observed 3, 6 and 12 h after operation. The changes in expression levels of NF-κB protein of pancreas tissue microarray were observed. The treatment group was significantly lower than the model group at 3 and 6 h ($P < 0.05$) and than the model group at 12 h in pancreas pathological scores ($P < 0.01$). The expression level of NF-κB protein of pancreas head of the treatment group was significantly less than that of the model group at 3 h ($P < 0.01$). The alleviation of pancreatic tissue injury by dexamethasone during SAP might be closely related to its role in inhibiting NF-κB expression and regulating cytokines. The advantages of tissue microarrays in pancreatitis pathological examination include time and energy savings, high efficiency and representative results.

Keywords Severe acute pancreatitis (SAP) · Dexamethasone · NF-κB · Inflammatory mediators · Pancreas · Tissue microarrays

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Introduction

The pathogenesis of severe acute pancreatitis (SAP), as a common clinical acute abdomens with a relatively high mortality rate, has been the focus of researchers for some time. As inflammatory mediators play important roles in the onset and progression of SAP, a inflammatory mediator antagonist can lower the severity and mortality of SAP [1, 2]. Studies show one or multiple κB sequences whose generation is regulated by the nuclear factor-kappa B (NF-κB), a genetic transcription-regulating factor in the promoters and enhancers of manifold inflammatory mediator genes [3–5]. It is generally believed that dexamethasone is capable of improving microcirculation, protecting against inflammation and anaphylaxis, inhibiting enzymes and inflammatory mediators, etc. for application in SAP treatment, although its exact mechanism remains unclear. This

experiment adopts the improved Aho method to prepare the SAP rat model, observes the influence of dexamethasone on NF-κB expression level of pancreatic tissue of SAP rats by tissue microarrays, and provides a new theoretical basis for dexamethasone treatment of SAP and the application of tissue microarrays in pathological examination of pancreatitis.

Methods

Animal grouping

Ninety clean grade healthy male SD rats were prepared into SAP models via the improved Aho method and randomly divided into a model (45 rats) and treatment group (45 rats). Another 45 were selected to be the sham operation group. In the next step, these groups were randomly divided into 3, 6 and 12 h groups, with 15 rats in each group. The treatment group was injected with dexamethasone via vena caudalis at a dose iof 0.5 mg/100 g body weight with a single administration 15 min after successful preparation of the SAP model. In the sham operation group the abdomen was opened, the pancreas and duodenum were turned over, and the abdomen was closed. The sham operation and model groups were injected with saline of the same volume via vena caudalis 15 min after the operation.

Animal model preparation

Fasting with restricted water was imposed on all rat groups 12 h prior to the operation. The rats were anesthetized by intraperitoneal injection of 2% sodium pentobarbital (0.25 ml/100 g) after which they were lain and fixed, and routine shaving, disinfection, and draping were performed.

Model group

After entering the abdomen via a median epigastrium incision, confirming the bile-pancreatic and hepatic hilus common hepatic ducts, uncovering the pancreas, and identified the duodenal papilla inside the duodenum duct wall, a no. 5 needle was used to drill a hole into the mesenterium avascular area. A segmental eqidural catheter was inserted into the duodenum cavity via the hole and in a retrograde direction to the bile-pancreatic duct in the direction of the papilla. The microvascular clamp was used to nip the catheter head temporarily while another microvascular clamp was used to temporarily occlude the common hepatic duct at the confluence of the hepatic duct. After connecting the epidural catheter end with the transfusion converter, 3.5% sodium taurocholate 0.1 ml/100 g was by retrograde transfused via the microinjection pump

at a speed of 0.2 ml/min. Four minutes after injection and the microvascular clamp and epidural catheter were removed. After checking for bile leakage, the hole in the duodenum lateral wall was sutured. A disinfected cotton ball was used to absorb the anesthetic in the abdominal cavity and the abdomen was closed [6].

Preparation of pancreatic tissue microarrays

(1) The tissue sample in neutral formalin was prepared in a routine paraffin block (called the donor block). The donor block was chopped into 5 μm thick tissue sections and routine hematoxylin-eosin staining and morphological observation under a microscope were carried out. The required representative area was selected, and the location on the HE section and the location on the corresponding part of the donor block were marked.

(2) A blank recipient block with a size of 45 × 20 × 15 mm was prepared.

(3) The recipient block with the tissue microarray sections was drilled (2.0 mm diameter needle, Beecher Instruments, USA).

(4) The donor block tissue chips were obtained as follows. Another drilling needle (with an inner diameter equal to the outer diameter of the former drilling needle) was used to drill the marked location on the paraffin block and collect the tissue chip. Its length was about 0.1 mm shorter than the depth of the hole. The tissue chip collecting method was the same as that for drilling of the recipient block. After pushing out the tissue chip, it was inserted either directly or with forceps into the hole of recipient block. After pressing the tissue chip downwards with a common glass slide, a distance adjuster was used to move the drilling needle the correct distance forward and backward or right and left. Tens of tissue chips can be inserted into the recipient block by repeating this process. Finally, three glass slides were piled up to compress the tissue chips and give the prepared tissue microarray section blocks a flat smooth surface.

(5) The prepared tissue microarray section blocks were placed in the paraffin block again to make the mold, and heated in an oven at 60°C for 1 h, to melt together the paraffin of the tissue chip and recipient block. The mold was removed from the oven gently, and the half-melted paraffin was cooled to room temperature (about 30 min) and further cooled in a refrigerator at –20°C for 6 min. Later, the tissue microarray section block was taken out of the mold and preserved in a refrigerator at 4°C for later use.

(6) *Sectioning*: the standby paraffin block was removed and rapidly nipped on a sectioning machine for

correction until all the tissue chips were on the same plane. An ice block was then placed onto the paraffin block for about 5 min, rapidly sectioned about 10–30 5 µm thick slices in succession while the ice block was used to freeze the paraffin block. This process was repeated until all of the tissue was sectioned. The sections were successively floated in cool water and allowed to spread naturally. Ophthalmic elbowed forceps and a glass slide were used to separate the sections, during which the first section of the head part of the successive sections can be stuck to the glass slide, fixed, and separated with the forceps to avoid loss of the tissue chip sample due to leakage during separation and to increase the separation speed. The sections were then transferred into warm (45°C) water to spread for 1 min to ensure full spreading without scattering. The sections were backed by the glass slide, which was processed with a 10% apurinic/apyrimidinic endonuclease (APES) acetone solution for staining. The prepared tissue microarrays section were placed in an oven at 60°C for 1 h, and then cooled at room temperature and plaecd in a refrigerator at –20°C for later use.

NF-κB p65 immunohistochemical staining (supersensitive S-P method)

(1) The section was baked at 60°C for 16 h and dewaxed in a routine fashion.
(2) Antigen retrieval in 0.01 M CB (pH 6.0) at high temperature and pressure was carried out for 2 min.
(3) Reagent A was added to block the endogenous peroxidase, followed by incubatation at room temperature for 10 min and washing with distilled water thrice.
(4) Biotin blocking reagent A was added at room temperature for 10 min, followed by PBS washes for 5 min twice.
(5) Biotin blocking reagent B was added at room temperature for 10 min, followed by PBS washes for 5 min twice.
(6) Normal goat serum blocking liquid was dropped onto the sample, incubated at room temperature for 20 min and excess liquid was removed.
(7) The primary antibody was added, and the sample was put aside overnight at 4°C, followed by a PBS wash for 5 min thrice.
(8) The secondary antibody marked by biotin, was added at room temperature for 10 min, followed by a PBS wash for 5 min thrice.
(9) Streptomycete antibiotin–peroxydase solution was dropped onto the sample, which was left at room temperature for 10 min, followed by a PBS wash for 5 min four times.
(10) Freshly prepared DAB solution was added for coloration, with the sample observed under a microscope and washed with distilled water.
(11) Hematoxylin counterstain was added with a water wash, and washed fully with water after differentiation until the return of the blue colour; routine dehydration and transparence was carried out, followed by neutral gum mounting.

Explanation

(1) Dilution with the primary antibody NF-κB p65 1:100 was carried out, followed by incubation at 4°C overnight.
(2) PBS was used to replace the negative control; the positive control section provided by the kit was used.
(3) Tissue was processed using 3% H_2O_2 to block endogenous peroxydase, goat serum to block non-specific site, and biotin blocking reagent to block endogenous biotin.

Statistical methods

Statistical analysis was conducted using the SPSS v. 11.5 software. The Kruskal–Wallis test or variance analysis was applied to the three group comparison. The Bonfferoni test was applied to the two-group comparison, and the likelihood ratio χ^2-test was applied to the survival comparison. Statistical significances was determined at the $P \leq 0.05$ level.

Observation index

Survival rate

Rat mortality was determined 3, 6, and 12 h after operation and the survival rate was calculated.

Pancreatic tissue pathological changes

After sacrificing the rats in batches, gross samples of pancreas head and tail tissue were collected and observed. Fixing them according to the related requirements, the pathological changes of pancreas were observed after HE staining and pancreas pathological score performed according to the self-made standards (Zhang's standard) [7].

NF-κB p65 protein expression

Tissue microarrays were applied to prepare pancreas head and tail microarray sections using the adopted SP method

for immunohistochemical staining. The NF-KB p65 protein expression of pancreas head and tail under light microscopy was observed and a comprehensive assessment according to the positive cell percentage was carried out into the following categories: positive cell count <10% means (—); positive cell count 10–20% means (+); positive cell count 20–50% means (++); positive cell count >50% means (+++).

Results

Survival rate

The 3, 6 and 12 h mortality of the model group were 0% (0/15), 0% (0/15), and 13.33% (2/15), respectively; the overall survival rate was 86.67%, while the survival rate of the sham operation and treatment groups at all time points was 100%. The mortality at all time points was not significantly different between the model and treatment groups ($P > 0.05$).

Pathological changes of pancreatic tissue

Gross pathological changes of pancreas

Sham operation group: no apparent abnormality of pancreas, peripancreatic, and epiploon at any time point.

Model group: the gross pathological changes of pancreas tail were more apparent than that of pancreas head. The severity of the overall pathological change increased with time after modeling. At 3 h, a small amount of hemorrhagic ascites could be observed with the naked eye with apparent changes of pancreas hyperemia and edema, hemorrhage and necrosis; at 6 and 12 h, hemorrhagic ascites increased as well as the scope of edema, hemorrhage and necrosis, and a more-saponified spot could be seen on the peripancreatic epiploon and peritoneum.

Treatment group: at 3 h, the degree of pancreas hyperemia and edema, hemorrhage and necrosis was milder than that of the model group with a decrease of ascitic fluid; at 6 and 12 h the pancreatic hemorrhage, necrosis area and degree were milder than those of the model group with an apparent decrease of ascitic fluid and saponified spot area.

Pancreas pathological changes under light microscope

Sham operation group: mild interstitial edema occurred in very few cases, neutrophil infiltration was occasional; no acinar cell and fat necrosis and hemorrhage was observed (see Fig. 1).

Model group: the severity of the pathological change increased with time after modeling. At 3 h, pancreas
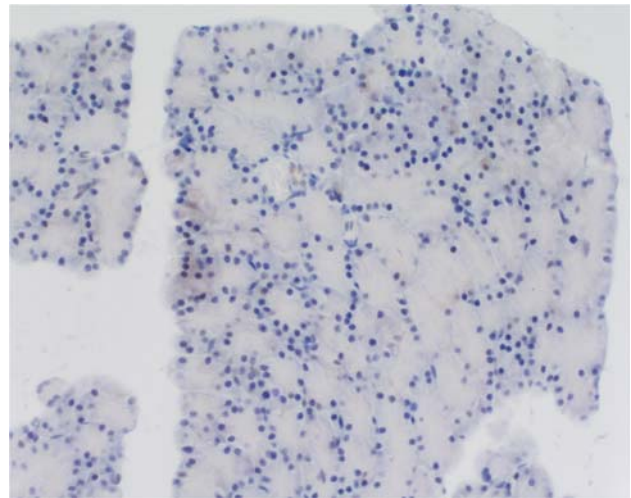

Fig. 1 NF-κB x200 times (negative) sham operation group (3 h) pancreas head

interstitial hyperemia, edema, a small amount of inflammatory cell infiltration, focal necrosis, and interstitial hemorrhage occurred with some lamellar hemorrhage and necrosis; at 6 h, interstitial edema, hemorrhage, more inflammatory cell infiltration, and focal and lamellar hemorrhage and necrosis occurred; at 12 h there was a large area of hemorrhage and necrosis, lobule outline damage, and a large amount of inflammatory cell infiltration (see Figs. 2 and 3).

Treatment group: the scope and degree of the pathological change in most cases were milder than those of the model group at the corresponding time. Only a few showed lamellar hemorrhage and necrosis, but the scope of hemorrhage and necrosis decreased and inflammatory cell infiltration was apparently alleviated (see Fig. 4).
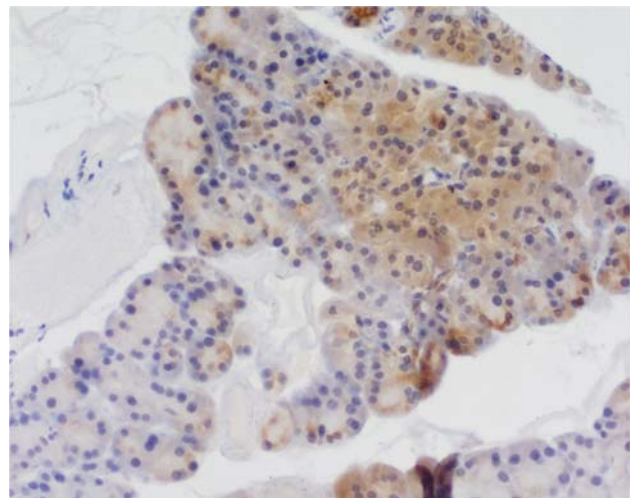

Fig. 2 NF-κB x200 times (+++) model group (3 h) pancreas head
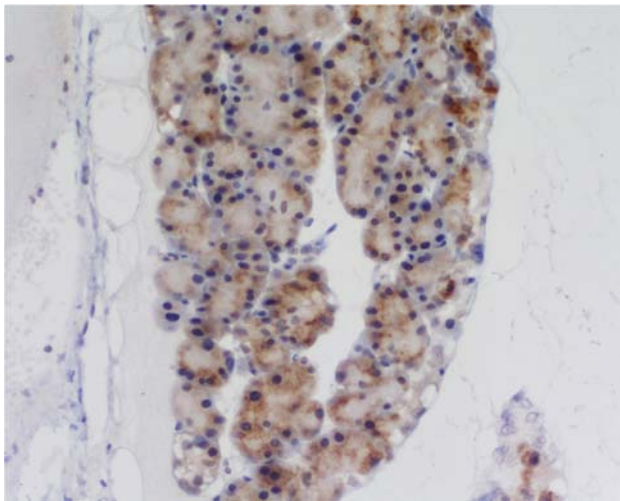
575

Fig. 3 NF-κB x200 times (+++) model group (3 h) pancreas head

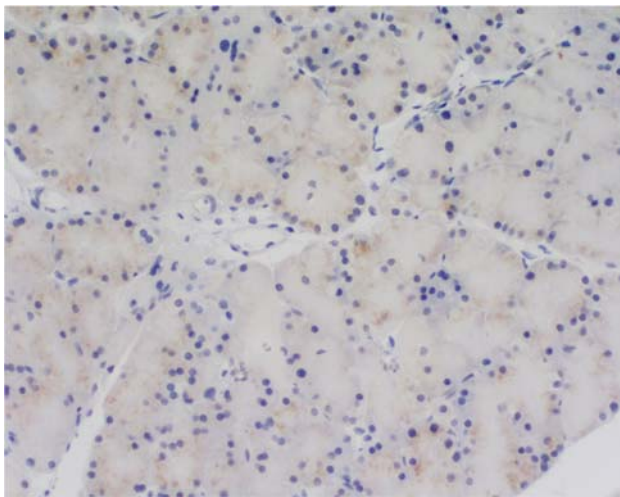**Fig. 4** NF-κB x200 times (+) treatment group (3 h) pancreas head

Pathological score of pancreatic tissue

Pathological score standard of pancreas

HE staining was applied and the pathohistological score standard referred to the improved Schmidt score (named

Zhang's standard by myself) made by myself. Two chief physicians of pathology department have adopted the blind method for score. The method is as follows (see Table 1).

Comparison of pathological score of pancreatic tissue

The pathological score for both the model and dexamethasone group exceeded the sham operation group at different time points ($P < 0.01$). The score for the dexamethasone group was significantly lower than that for the model group at 3 and 6 h ($P < 0.05$). The score for the dexamethasone group was also significantly lower than that of the model group at 3 and 6 h ($P < 0.01$) (see Table 2).

Comparison of the expression level of NF-κB protein of pancreas head

The expression level in the model group exceeded that of the sham operation group at 3 and 6 h ($P < 0.01$) and that of the sham operation group at 12 h ($P < 0.05$). The expression level in the treatment group exceeded that of the sham operation group at 3 and 6 h ($P < 0.05$) and there was no significant difference between the treatment and sham operation groups at 12 h ($P > 0.05$). The expression level of the treatment group was less than the model group at 3 h ($P < 0.001$); the expression levels of NF-κB protein of the treatment group at 6 and 12 h were lower than those of the model group, but statistics showed no marked difference between the treatment and model groups at 6 and 12 h ($P > 0.05$) (see Tables 3 and 4).

Comparison of expression level of NF-κB protein of pancreas tail

The expression level of both the model and treatment groups exceeded that of the sham operation group at 3 and 6 h ($P < 0.01$). The expression levels of NF-κB protein of the treatment group at all time points were lower than those of the model group, but statistics showed no significant difference between the treatment and model groups at any time point ($P > 0.05$) (see Tables 5, 6).

Table 1 Zhang's standard of pathological scoring of pancreas

Edema		Acinar	Necrosis (Necrotic cells/HPF)	Inflammation and perivascular infiltrate (intralobular or perivascular leukocytes/high power field (HPF))		Hemorrhage and fat necrotis (focus)	
0	Absent	0	Absent	0	0–1	0	Absent
1	Focal expansion of interlobular septae	1	1–4	1	2–10	1	1–2
2	Same as 1 + diffuse expansion of interlobar septae/diffuse expansion of interlobar septae	2	5–10	2	11–20	2	3–4
3	Same as 2 + expansion of interacinar septae	3	11–16	3	21–30	3	5–6
4	Same as 3 + expansion of intercellular spaces	4	>16	4	>30/microabscesses	4	>7

Table 2 Comparison of the pancreas pathological score in each group $[M(Q_R)]$

Group (time/h)	3 h*	6 h**	12 h***
Sham operation	0.0000 (1.0000)	0.0000 (1.0000)	0.0000 (1.0000)
Model	8.0000 (2.0000)	9.0000 (2.0000)	11.0000 (1.5000)
Treatment	7.0000 (2.0000)	8.0000 (2.0000)	8.0000 (1.0000)

* $\chi^2 = 32.522$, $P = 0.000$;   ** $\chi^2 = 32.958$, $P = 0.000$;
*** $\chi^2 = 35.541$, $P = 0.000$

Table 3 Expression of NF-κB protein in pancreas head

Group	Time/h	Cases	Pathologic grade			
			−	+	++	+++
Sham operation	3	15	15			
	6	15	15			
	12	15	15			
Model	3	15	0	4	6	5
	6	15	6	5	4	
	12	13	9	3	1	
Treatment	3	15	8	6	1	
	6	15	10	4	1	
	12	15	14	1		

Table 4 Comparison of NF-κB protein in the head of pancreas $[M(Q_R)]$

Group (time/h)	3 h*	6 h**	12 h***
Sham operation	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Model	2.0000 (2.0000)	1.0000 (2.0000)	0.0000 (1.0000)
Treatment	0.0000 (1.0000)	0.0000 (1.0000)	0.0000 (0.0000)

* $\chi^2 = 32.407$, $P = 0.000$;   ** $\chi^2 = 12.594$, $P = 0.002$;
*** $\chi^2 = 6.897$, $P = 0.032$

Table 5 Expression of NF-κB protein in the tail of pancreas

Group	Time/h	Cases	Pathologic grade			
			−	+	++	+++
Sham operation	3	15	15			
	6	15	15			
	12	15	15			
Model	3	15	5	4	2	4
	6	15	9	2	1	3
	12	13	9	3	1	
Treatment	3	15	6	7	2	
	6	15	8	5	1	1
	12	15	12	3		

Table 6 Comparison of NF-κB protein in the tail of pancreas $[M(Q_R)]$

Group (time/h)	3 h*	6 h**	12 h***
Sham operation	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Model	1.0000 (3.0000)	0.0000 (2.0000)	0.0000 (1.0000)
Treatment	1.0000 (1.0000)	0.0000 (1.0000)	0.0000 (0.0000)

* $\chi^2 = 15.888$, $P = 0.000$; ** $\chi^2 = 8.669$, $P = 0.013$; *** $\chi^2 = 5.047$, $P = 0.080$

Discussion

Acute pancreatitis (AP) especially SAP, which is a relatively hazardous acute disease of digestive system, can cause systemic inflammatory response syndrome (SIRS) in the early stage and result in multiple organ dysfunction syndrome (MODS) [8, 9], with a mortality rate as high as 20–30%. Although researchers have not yet idenitified the complete pathogenesis of AP, recognition has developed from the traditional pancreatin autodigestion theory to an inflammatory mediator or cytokine theory, microcirculation disturbance theory, NO and oxygen free-radical (OFR) injury theory, bacteria translocation theory, calcium overload theory, etc. In 1997, Dunn et al. [10] first studied the role of NF-κB in AP by ligation of the pancreatic duct to prepare an AP model. They found a significant rise of NF-κB activity in pancreatic tissue 4 h after inducing AP, lasting for 24 h, while amobarbital inhibited the expression of NF-κB and TNF-α, alleviating AP. Blinman et al. [11] also found a rise of NF-κB activity and IL-6/KC (an IL-8 analogue) expression in pancreatic tissue during AP. Natural killer cell, an NF-κB inhibitor, can inhibit the activation and excessive expression of IL-6 and KC mRNA, suggesting that the activation of NF-κB participates in AP onset and plays an important role in the progression of AP pathological changes. Regulation of NF-κB can inhibit inflammation and protect tissue cells, providing an important theoretical basis for clinical treatment of AP. In the past decade, the large worldwide empirical studies have further proved the close relationship between NF-κB and onset, progression, and prognosis of AP. The role of NF-κB as an important nuclear factor in SAP can be summarized into two aspects:

(1) Cytokine imbalance plays an extremely important role in the progression of pancreatitis, while the nuclear factor NF-κB is core to the cytokine unbalance process. Activated NF-κB can promote and regulate the transcription of genes participating in a manifold inflammatory reaction, encode cytokine, adhesion molecules, etc., further amplify inflammatory signals through the extracellular positive-feedback path, cause cytokine waterfall cascade reactions as well as

injury to organ structures and functions, and lead to pathological and physiological changes such as SIRS and MODS. Altavilla et al. [12], who have applied a mouse model with NF-κB gene knock-out (KO) to study the role of NF-κB in the course of AP, found that the serum amylase level, lipase level, lipid peroxide malonaldehyde level, myeloperoxidase activity, NF-κB activity, pancreas TNF-α expression level and pancreatic tissue pathological score of the KO group were markedly lower than those of the control group but that the glutathione level was markedly higher than in the control group, indicating the close correlation between AP course and NF-κB activation and the aggravation of AP by enhancing oxidative stress. Chen et al. [13], who injected the adenovirus vector with the active NF-κB subunit RelA/p65 into the pancreatic-bile duct of the pancreas to infect the pancreatic acinar cells, found that the NF-κB activation level, NF-κB expression level, and degree of inflammatory reaction in the pancreatic tissue were markedly higher than those of the control group and in addition observed the increased neutrophil infiltration of lung and pancreatic tissue as well as injury of pancreatic acinar cells; the specific inhibiting IκB-alpha of the active NF-κB subunit RelA/p65 can effectively antagonize these reactions, indicating that the activation of NF-κB in pancreatic tissue is sufficient to cause the inflammatory reaction state. These studies show that the activation of NF-κB can activate the expression of inflammatory factors and lead to SIRS, which is important in the course of AP.

(2) NF-κB, with certain anti-apoptosis effects, can lead to the onset of SAP. Animal experiments have proved that a NF-κB inhibitor can inhibit the expression and release of inflammatory factors, increase pancreatic apoptosis, reduce necrotic cells, alleviate the state of SAP animal models, and lower the mortality of animals in the experimental group during AP [14, 15]. Pei et al. [16, 17] found that NF-κB participated in the onset of edematous and necrotic pancreatitis through animal experiments with rats, and that NF-κB expression of edematous pancreatitis was obviously weaker than that of necrotic pancreatitis, which was believed to be possibly caused by the protective mechanism of apoptosis during edematous pancreatitis.

While understanding the pathogenesis of AP, safe and effective measures for prevention and treatment are also keys to reduce AP complications and lower mortality. In 1952 Stephensen et al. [18] first reported the effect of glucocorticoid in AP treatment. Later, many empirical studies have proven that glucocorticoid can improve the survival of animals with pancreatitis, and lower the mortality of animal models by 1/3–2/3; the exact mechanism remains unclear but is possibly related to effects including improved circulatory failure, anti-inflammation action, inhibition of enzymes and anti-anaphylaxis. Meanwhile some researchers also believe that glucocorticoid may be a causative factor of AP [19, 20]. After years of studies, most scholars now agree that glucocorticoid (represented by dexamethasone) treatment has effects on AP. Its mechanism includes inflammatory mediation, anti-endotoxin, improving microcirculation [21], elimination of OFR [22], inhibiting NO and NF-κB [23–25], etc. It is now believed that glucocorticoid can inhibit NF-κB in two ways in order to inhibit the expression of manifold inflammatory genes: (1) by combing the activated glucocorticoid receptors and their expression regulating transcripton (activated NF-κB subunit); (2) by upregulating the expression level of IκB-transcriptional in certain cells to stop the combination of nuclear transfer and DNA of NF-κB. Pei et al. [16], who have used the improved Pfetter method (common bile duct ligation plus bile injection) to prepare a SAP model and study the NF-κB expression in pancreatic tissue, found that the activation of NF-κB in the SAP group was time dependent with a weak-strong-weak trend, reaching a peak at 3 h, but was higher than that of the dexamethasone treatment group at all time points. This indicates that dexamethasone can inhibit the activation of NF-κB and that the selection of the proper time to inhibit the activation of NF-κB could be a new therapeutic strategy.

This study found that, although the rat survival rate of the treatment group was higher than that of the model group after adopting the improved Aho method to prepare the SAP models, there was no significant difference between the two groups ($P < 0.05$). However, whether observed gross or under light microscopy, the treatment group had obviously milder pathological changes of cell inflammation in pancreatic tissue than the model group at all time points as well as reduced bleeding and necrosis range; the pathological changes of pancreatic tissue of the treatment group, observed gross or under light microscopy, were milder than those of the model group at all time points. It was found by comparing the pancreas pathological scores that both the model and treatment groups significantly exceeded the sham operation group at different time points ($P < 0.001$); the score in the treatment group was significantly less than that in the model group at 3 and 6 h ($P < 0.05$) and tham the the model group at 12 h ($P < 0.01$). There was no NF-κB protein expression observed in the pancreas head and tail of the sham operation group, indicating uneven NF-κB protein expression in both the model and treatment groups, and no NF-κB protein expression in normal tissues. The expression level of

NF-κB protein of pancreas head of the model group significantly exceeded the level in the sham operation group at different time points ($P < 0.05$), the expression level of NF-κB protein of pancreas tail of both the model group and groups significantly exceeded that in the sham operation group at 3 and 6 h ($P < 0.01$). The expression level of NF-κB protein of pancreas head of the treatment group was obviously less than that of the model group at 3 h ($P < 0.01$). The statistical results showed no marked difference between the treatment and model groups in terms of expression level of NF-κB protein of pancreas head at 6 and 12 h, and pancreas tail at all time points ($P > 0.05$). However, compared to the model group, the expression levels of NF-κB protein of the treatment group all dropped by different degrees. After observing the positive rate changes of NF-κB protein expression of the model group at all time points, we find the highest positive rate at 3 h, lower rates at 6 and 12 h, indicating the changing weak-strong-weak trend of NF-κB protein expression, namely peak expression at 3 h. The positive rate of NF-κB protein expression in pancreas head of the treatment group at 3 h was siginificantly lower than that of the model group ($P < 0.01$), suggesting statistical significance; the positive rates of the treatment group at all time points dropped gradually, which indicates that dexamethasone can inhibit NF-κB protein expression and that the effect continues with time. There was no significant difference between the treatment and model groups in their positive rate of NF-κB protein expression in pancreas head at 6 and 12 h, and pancreas tail at all time points ($P > 0.05$). However, it could be found by careful observation of the results obtained that the NF-κB protein of pancreas head at 6 and 12 h as well as the pancreas tail of the treatment group dropped by different degrees, indicating the decline of NF-κB by various degrees after treatment. Such a statistical result in this experiment could be attributed to experimental errors due to sample deficiency, or a decrease of NF-κB expression at that time due to a lower inhibiting effect of dexamethasone compared to that at 3 h, also suggesting the use of dexamethasone at an early stage to achieve better therapeutic effects. This experiment proves that dexamethasone can slow the rate of pancreas pathological changes and protect pancreatic cells by downregulation of NF-κB and inhibition of the expression of manifold inflammatory mediators. NF-κB protein expression is positively correlated with the severity of SAP. However, this experiment has not directly shown the overall trend that dexamethasone influences on the inflammatory reactions of pancreatitis by regulating NF-κB protein expression.

In addition, this experiment for the first time applied tissue microarrays to the pathological examination of pancreatitis. Tissue microarrays (TMA), also called tissue chips, were first proposed by Kononen et al. [26] in 1998. The tissue chip technique has been greatly developed in recent years. In brief, a tissue chip is a section made by arranging tens or even over a 1,000 tissue samples of different individuals on a slide in a preset order [27]. Its advantages include high throughput, multiple samples, economy, time saving, low error rates, convenience for experimental control design, the capability of being combined with other biological techniques, and broad application [26–32]. Currently the study of various tumors by combing immunohistochemical and in situ hybridization techniques with the tissue chip approach has become relatively mature [33–47]. Factors including chip preparation, staining and examination have limited the application of the technique in nontumor diseases. There was no study report on the application of tissue microarrays to the pathological examination of pancreatitis before this experiment, which has for the first time applied this technique to the pathological examination of SAP. We used the tissue microarray section (Beecher Instruments, USA) to drill a hole 2.0 mm in diameter in a recipient block and combined the supersensitive S-P immunohistochemical method to examine NF-κB protein expression in pancreatic cells. This satisfying result indicates that 2.0 mm diameter tissue chips can achieve reliable experimental results that are representative, save time, energy and reagents, and can convenient be used for controls. Therefore, it is also worthwhile to apply tissue microarrays to the pathological examination and analysis of nontumor diseases such as pancreatitis. The choice of the 2.0 mm point on the primitive block during the experiment proves the sound representativeness of tissue microarrays. The experimental results show a positive correlation between the level of NF-κB protein expression and inflammatory reactions of pancreatitis. The tissue microarrays can aid the study of the molecular biological mechanisms of SAP [6].

The AP pathophysiological process consists of the acute reaction period, the systemic infection period, and the residual infection period. The purpose of using dexamethasone intervention at an early stage is to solve bile-pancreatic duct obstruction due to the reversible factor, inflammatory edema with the positive effect of protecting tissue cells such as in the pancreas, and preventing SIRS or even MODS. However, after the occurrence of MODS, even large doses of dexamethasone cannot have a good effect. Dong et al. [48] used a rat model to observe the effect of dexamethasone treatment on SAP at different times and at different dosage. After using dexamethasone, the blood inflammatory mediator level dropped significantly. A large dose can have a better effect than a smaller one. If the doses are the same, early-stage use can have a better effect than delayed use. Although dexamethasone possesses a number of advantageous effects such as

cooling, antitoxin, anti-inflammation, enzyme inhibiting, antishock, etc., from the viewpoint of clinical experience, issues such as indications, dosage, and treatment course should be identified to prevent hormonal side-effects [49, 50]. Dexamethasone should be used at an early stage, at full dose, over a short course to cure patients relatively rapidly with the assistance of other basic treatments. We hope that the discussion of this experiment on the mutual relationship between dexamethasone, apoptosis of acinar cells in pancreas, and SAP can offer a more-theoretical foundation and train of through for hormone therapy of SAP.

This experiment, assisted by tissue microarrays, studied the inhibition of NF-κB protein expression of pancreatic tissue of rat with SAP using dexamethasone, alleviating pancreatic pathological changes and lowering rat mortality, providing a more-theoretical basis for dexamethasone treatment of SAP.

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