Effect of early administration of exogenous basic fibroblast growth factor on acute edematous pancreatitis in rats

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AIM: To observe the therapeutic effect of early administration of exogenous Basic fibroblast growth factor (bFGF) on acute edematous pancreatitis (AEP) in rats.

METHODS: Thirty male Sprague-Dawley rats were randomly divided into three \((n = 10)\): normal control group (group I), AEP group (group II) and AEP with bFGF treatment group (group III). AEP was induced by subcutaneous injection of cerulein (5.5 \(\mu\)g/kg and 7.5 \(\mu\)g/kg) at 1 h interval into rats of groups II and III. Three hours after induction of AEP, 100 \(\mu\)g/kg bFGF was administrated intraperitoneally for 1 h to group III rats. For test of DNA synthesis in acinar cells, 5-bromo-2'-deoxyuridine (BrdU) labeling solution was intraperitoneally injected into the rats of groups II and III 24 h after bFGF treatment. The changes in serum amylase, lipase, pancreatic tissue wet/dry ratio were detected.

RESULTS: In bFGF treatment group, there was a significant decrease in the volume of serum amylase, lipase and the pancreatic wet/dry weight ratio\((1383.0 \pm 94.6\) U/L, 194.0 \(\pm\) 43.6 U/L, 4.32 \(\pm\) 0.32) compared to AEP group \((3464 \pm 223.7\) U/L, 456 \(\pm\) 68.7 U/L, 6.89 \(\pm\) 0.47) \((P < 0.01)\), and no significant difference was found between bFGF treatment and control group \((1289 \pm 94.0\) U/L, 171 \pm 23.4 U/L, 4.12 \(\pm\) 0.26, \(P > 0.05)\). The inflammatory changes such as interstitial edema, polymorphonuclear neutrophils (PMNs) and vacuolization were significantly ameliorated compared to AEP group \((P < 0.01)\). A small number of BrdU-labeled nuclei were observed in acinar cells of AEP rats \((1.8 \pm 0.3\) nuclei/microscopic field, \(n = 10)\) while diffuse BrdU-labeled nuclei were found in bFGF-treated rats \((18.9 \pm 1.4\) nuclei/microscopic field, \(n = 10)\) \((P < 0.01)\). Immunohistochemical study showed increased DNA synthesis in pancreatic acinar cells.

CONCLUSION: Early administration of exogenous bFGF has significant therapeutic effect on cerulein-induced acute edematous pancreatitis in rats. Its mechanism is related to the amelioration of inflammation and facilitation of pancreatic regeneration.

INTRODUCTION

Basic fibroblast growth factor (bFGF) is one of the mitogens that facilitate cellular proliferation, maturation and regeneration. Hoshi et al.\(^{[8,\ 9]}\) reported that bFGF exerts direct trophic effects on rat pancreatic acinar cells \(in vitro\). Some studies\(^{[3-6]}\) showed that FGF is involved in the process of acute pancreatic exocrine regeneration during recovery of acute pancreatitis. Acute edematous pancreatitis (AEP) rats were selected as the animal model to investigate the therapeutic effect of exogenous bFGF on the early acute pancreatitis (AP) and its mechanism.

MATERIALS AND METHODS

Animal model

Thirty male healthy Sprague-Dawley rats weighing 400-450 g were purchased from the Animal Center, Academy of Zhejiang Medical Sciences. All rats were randomly divided into three groups \((n = 10)\), with 10 rats each group. Group I (normal control group), group II (AEP group), and group III (AEP with bFGF treatment group). AEP was induced in rats of groups II and III by injection of 5.5 \(\mu\)g/kg and 7.5 \(\mu\)g/kg of caerulein (Sigma Chemical Co., USA) subcutaneously at 0 and 1 h after the beginning of experiment respectively, while the rats of the control group were subcutaneously injected with normal saline solution.

bFGF treatment and 5-bromo-2'-deoxyuridine (BrdU) label

All animals were anesthetized with 3% thiopental sodium
(0.3 µL/g). In group III bFGF (100 µg/kg) was administered by continuous intraperitoneal infusion of 10 µg/mL bFGF solution (Essex Co., China) for 1 h, while normal saline solution was administered to all other rats in groups I and II as the contrast. Twenty-four hours after treatment, 200 µg/mL BrdU-labeling solution (10 mL/kg) was intraperitoneally injected into the rats of groups II and III.

**Blood and tissue sample collection**

Two hours after BrdU-labeling solution injection, venous blood was taken from all rats in each experimental group to obtain blood samples. Then all rats were killed and pancreatic tissue was divided into 3 portions from each rat and quickly removed.

**Histological examination**

A portion of the pancreas was fixed overnight in 10% neutral formaldehyde solution and embedded in paraffin. Tissue slices were subjected to hematoxylin and eosin staining and histologic study under light microscope. Slides were coded and examined blindly by the pathologist for the grading of histologic alterations. Histological changes were evaluated according to Kyogoku et al.[9]. Interstitial edema was scored as 0 = absent, 1 = expanded interlobular septa, 2 = expanded intralobular septa, 3= separated individual acini. Polymorphonuclear neutrophil (PMN) infiltration was scored as 0 = absent, 1 < less than 20 PMNs per IPF, 2 = 20 PMNs-50 PMNs per IPF and 3 = more than 50 PMNs per IPF. The grading of vacuolization was based on the percentage of acinar cells with cytoplasmic vacuoles per IPF: 0 = absent, 1 = less than 20%, 2 = 20%-50%, 3 = more than 50%. Hemorrhagic and parenchymal necrosis changes were absent and therefore not scored.

**Immunohistochemistry examination (S-P method)**

DNA synthesis of pancreatic acinar cells was determined as the uptake of Brdu in the cells. Another portion of the pancreas was fixed in 10% formalin, embedded in paraffin, and sectioned at 3 mm. After dewaxing, the tissue was then incubated with the monoclonal antibodies against BrdU for 1 h at room temperature in a humidified chamber. The tissue was washed three times with 0.01 mol/L phosphate-buffered saline (PBS) and incubated in peroxidase-conjugated anti-mouse immunoglobulin G (IgG) for 30 min at room temperature. The tissue was again washed three times with 0.01 mol/L PBS, and immunoreactivity of BrdU was detected using 3, 3’-diaminobenzidine tetrahydrochloride (DAB) with nickel chloride as a chromogen. DAB (10 mg) was dissolved in 0.05 mol/L phosphate buffer (30 mL), and three drops of nickel chloride (3%) and hydrogen peroxide (3%) solution were also added. The DAB solution was applied to the tissue for 5 min at room temperature. The BrdU-labeled nuclei were stained purplish blue.

**Statistical analysis**

All data were expressed as mean ± SD. Histologic scores were compared using the Mann-Whitney test, while statistical differences of serum amylase were performed using paired Student’s t test. P < 0.05 was considered statistically significant.

### RESULTS

**Serum amylase, lipase and pancreatic tissue wet/dry weight ratio**

When SEP model was established and bFGF treatment was taken after 24 h, serum amylase and lipase activity as well as pancreatic tissue wet/dry weight ratio of group III (bFGF treatment group) (1383.0 ± 94.6 U/L, 194.0 ± 43.6 U/L, 4.32 ± 0.32) were significantly lower than those of group II (AEP group) (3464 ± 223.7 U/L, 456 ± 68.7 U/L and 6.89 ± 0.47) (P < 0.01), with no significant difference compared with group I (normal control group) (1289 ± 94.0 U/L, 171 ± 23.4 U/L and 4.12 ± 0.26) (P > 0.05). There was a significant difference between group I and II (P < 0.01) (Table 1).

**Histological staining**

In the histological examination of pancreatic tissue stained with HE, pancreatitis changes such as angioectasia and inflammatory cell infiltration, tissue edema and vacuolization of acinar cells in bFGF treatment group (group III) were remarkably reduced compared with AEP group (group II) (Table 2 and Figures 1-3).

**Immunohistochemical analysis**

A small number of BrdU-labeled nuclei were observed in acinar cells of AEP rats (group II) (1.8 ± 0.3 nuclei/ microscopic field, n = 10) (Figure 4A). The BrdU-labeled nuclei were diffusely distributed in bFGF treated rats (group III) (18.9 ± 1.4 nuclei/microscopic field, n = 10) (P < 0.01) (Figures 4B).
DISCUSSION

Some factors can repair the injured tissues or cells\textsuperscript{[10-14]} and can be used to treat acute pancreatitis. Recent studies indicate that various growth factors such as bFGF\textsuperscript{[15-17]}, TGF-β\textsuperscript{[18-19]}, HGF\textsuperscript{[6, 20]}, EGF\textsuperscript{[18, 21-24]}, and CTGF\textsuperscript{[25]} are involved in pancreatic repair and tissue remodeling in humans and rats with AP. It was reported that ischemia/reperfusion (I/R) injury of intestine and other internal organs reduces the expression of endogenous basic fibroblast growth factor (bFGF)\textsuperscript{[26-29]}, and intravenous administration of exogenous bFGF could induce the expression of endogenous bFGF and improve the physiological function of some internal organs after I/R injury\textsuperscript{[30-33]}. So there may be a bright future to accelerate the “positive repair” of internal organs’ I/R injuries by using growth factors based on all these results.

The present study used acute edematous pancreatitis rats as a model to observe the therapeutic effect of exogenous bFGF on acute edematous pancreatitis in rats. Early treatment with exogenous bFGF drastically ameliorated all the pathological changes both in histology and in serum enzymes, and showed significant therapeutic effect on ce-
rulein-induced acute edematous pancreatitis in rats. Immunohistochemical examination showed that DNA synthesis was increased in pancreatic acinar cells of bFGF-treated rats, suggesting that bFGF treatment can promote pancreatic cell regeneration and accelerate rebuilding the integration of injured pancreatic tissue. The beneficial effects of bFGF appear to depend, at least in part, on amelioration of pancreatic inflammation and increase of pancreatic cell growth. Results of the present study have proved that early usage of exogenous bFGF can prevent the development of acute pancreatitis, and may play an important role in limiting its progression to severe pancreatitis.

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S- Editor Wang J  L- Editor Wang XL  E- Editor Zhang Y