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Effect of recombinant human basic fibroblast growth factor on acute inflammation in mice and rats

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KEY WORDS recombinant proteins; basic fibroblast growth factor; carrageenan; inflammation; dexamethasone

ABSTRACT

AIM: To investigate the anti-inflammatory effects of recombinant human basic fibroblast growth factor (rh-bFGF). **METHODS**: Several inflammation models such as croton oil-induced ear swelling, carrageenan-induced hind paw edema, and acute peritonitis in rats or mice were prepared. Superoxide dismutase (SOD) activity was measured by hydroxyamine method, nitric oxide (NO) concentration by Griess reaction assay, nitric oxide synthase (NOS) activity by NADPH-diaphoras stain assay, *N*-acetyl-β-*D*-glucosaminidase (NAG) activity by colorimetry, prostaglandin E_2(PGE_2) production by radioimmunoassay (RIA), malondialdehyde (MDA) content by thiobarbituric acid (TBA) fluorescence technique, and protein content by Coomassie brilliant blue method in peritoneal exudate in rats. **RESULTS**: Recombinant human bFGF 2, 4 kU/kg im inhibited croton oil-induced ear swelling and carrageenan-induced paw edema in mice. In addition, rh-bFGF 2, 4 kU/kg im reduced neutrophil counts in the rat peritoneal exudate, and lessened protein content in peritoneal exudate in rats and mice. In the rat peritonitis induced by carrageenan, rh-bFGF 4 kU/kg decreased the MDA and NO levels, inhibited the NOS activity, augmented the SOD activity, and lowered the production of PGE_2 in exudate. However, rh-bFGF had no effect on NAG content. **CONCLUSION**: Recombinant human bFGF has an anti-inflammatory effect and its mechanisms are related to the inhibition of NOS activity, reduction of NO, MDA, and PGE_2 content, and increase of SOD activity.

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INTRODUCTION

Basic fibroblast growth factor (bFGF) is a potent growth factor that stimulates endothelial cells, fibroblast and epithelial cells proliferation, migration, and differentiation to form neovasculature and promotes the synthesis of collagen and fibronectin[1]. The therapeutic efficacy of recombinant human bFGF (rh-bFGF) has been demonstrated by a number of experiments in laboratories and clinical applications, such as ulcer healing[2,3], treatment of burn injury, and wound repair promoting[4,5]. There is evidence that endogenous bFGF is involved in inflammation. To date, no information is available concerning the potential effects of rh-bFGF at therapeutic dosage on acute inflammation. The effect of rh-bFGF on acute anti-inflammation and its mechanism remains little known. The objective of the investigation was to explore the effects of rh-bFGF at therapeutic dosage[3] on acute inflammatory models like croton oil-induced ear swelling, carrageenan-induced hind paw edema, and acute peritonitis in rats and mice *in vivo*.

MATERIALS AND METHODS

Recombinant human bFGF was provided by Torita Bio-Pharma Co Ltd (Zhuhai, China), is kept under –20 ℃, and diluted with normal saline (NS) just before it is used. Carrageenan (Car) was the product of Liaoning Institute of Materia Medica (Shenyang, China). Dexamethasone (Dex) was purchased from Industry and Commerce Company of Ebei (Guangshui, China). Superoxide dismutase (SOD), nitric oxide (NO), nitric oxide synthase (NOS), and *N*-acetyl-β-*D*-glucosaminidase (NAG) detection kits were obtained from Jiancheng Institute of Biotechnology (Nanjing, China). Prostaglandin E_2(PGE_2) RIA kit was the product of Department of Thrombus, Suzhou Medical College, China. All other reagents were of analytical grade.

Kunming mice were from the Animal Center of Lanzhou Institute of Biological Products, Ministry of Public Health (Grade Ⅱ, Certificate № 14-001). Wis-

tar rats (♂, weighing 180 g ± s 20 g) were from Experimental Animal Center of Gansu Academy of Medical Sciences (Grade Ⅱ, Certificate № 14-004).

Croton oil-induced ear edema in mice Kunming mice (n = 50, weighing 26 g ± s 2 g, ♂♀) were randomly divided into 5 groups. Recombinant human bFGF, dexamethasone or normal saline was injected im and injected im again after 12 h, and not until one hour later, croton oil was used to induce ear edema. Croton oil mixture (2 % croton oil, 20 % anhydrous alcohol, 73 % ethyl ether, and 5 % redistilled water) was applied (0.1 mL) topically in the right ear pinna of mice. The left pinna was used as a control. The mice were killed 4 h later. Disks from the pinnae were taken with a 6.5 mm-diameter punch. The edema extent was expressed in terms of the weight difference between the inflamed disks and the control disks.

Carrageenan-induced hind paw swelling in mice[6] The 5 groups of Kunming mice (n = 45, weighing 22 g ± s 2 g, ♂♀) were given im rh-bFGF 2, 4, and 8 kU/kg, dexamethasone 2 mg/kg, or equal volume of solvent (in control) and were given im again after 12 h, respectively, in their left hind legs. Then, one hour later, 1 % carrageenan (0.04 mL) was injected sc in the right hind paws of the same 5 groups of mice to induce hind paws swelling. After 1 h sc Car, the mice were sacrificed and both hind paws were cut from the ankle joint. The hind paw swelling was obtained by the weight difference between the inflamed hind paws and the control hind paws.

Carrageenan-induced peritonitis in mice[7] Kunming mice (n = 78, weighing 20 g ± s 2 g, ♂♀) were randomized into 6 groups. Recombinant human bFGF or Dex was injected im and was injected im again after 12 h. One hour later, carrageenan 25 mg/kg was given ip to induce the inflammation. At 4 h the mice were decapitated. The abdomen was washed with ice-cold D-Hanks' solution 2.5 mL and the exudates were collected and centrifuged (4 ℃, 2500 × g, 5 min). The supernatant was used to determine the content of protein.

Acute peritonitis in rats[7,8] Rat peritonitis was induced by injecting ip carrageenan 25 mg/kg. Six hours after inducing peritonitis, rats were decapitated and were injected ip with D-Hanks' solution 5 mL immediately. After each abdomen was massaged for 2 min, it was incised and all the diluted exudate in peritoneal cavity was drawn out. The exudate was centrifuged (4 ℃, 2500 × g, 5 min). The supernatant from each abdomen was separately kept at −20 ℃ until it is analyzed for protein, MDA, SOD, NO, NOS, PGE_2, and NAG. The cells were centrifuged twice with D-Hanks' solution (2500 × g, 10 min). An aliquot of cell suspension was taken for trypan-blue test and its results consistently showed 98 % cellular viability rate. Neutrophils (Neu) accounted for 95 % of the cells as demonstrated by staining classification. The therapeutic drugs or normal saline in control group was given im and given again after 12 h, respectively, before peritonitis was induced.

Analysis methods Protein content of samples were measured by the Coomassie brilliant blue G-250 method with bovine serum albumin (BSA) as a standard[9].

MDA was determined according to the method previously described[10] with some modification. The samples (0.5 mL) were mixed with 2 mL HCl (0.6 mol/L) and 1 mL TBA reagent [1:1 (v/v) mixture of 0.57 % TBA and acetic acid]. The reaction mixture was heated at 95 ℃ for 1 h. After the reaction mixture got cool, 4 mL n-butanol was added, and the reaction mixture was shaken vigorously for 1 min. After its being centrifuged at 3000 × g for 10 min, the reaction mixture was placed at 4 ℃ overnight. The n-butanol layer was fluorometrically measured with λ_{ex} 520 nm and λ_{em} 546 nm, using a fluorescence spectrophotometer. The value of fluorescence was calculated by comparing with standards prepared from 1,1,3,3-tetraethoxypropane (Fluka).

SOD activity was measured by hydroxyamine method, NO concentration by Griess reaction assay, NOS activity by NADPH-diaphoras stain assay, and NAG activity by colorimetric method. The detecting method was taken according to the protocol enclosed in the detection kit.

Statistics Data were expressed as $\bar{x} \pm s$ and assessed by t-test.

RESULTS

Effect of rh-bFGF im on ear edema, hind paw swelling, and protein content in peritoneal exudate in mice Recombinant human bFGF 2, 4, 8 kU/kg and dexamethasone 2 mg/kg inhibited the ear edema induced by croton oil as compared with control with the inhibition of 21.8 %, 25.0 %, 19.4 %, and 59.7 %, respectively. As compared with control, hind paw swelling induced by carrageenan in mice was greatly lessened by rh-bFGF 2, 4 kU/kg and dexamethasone 2 mg/kg, as well as the protein content in the peritoneal

exudate. Recombinant human bFGF 8 kU/kg induced significant inhibition neither in the hind paw swelling model nor in the peritonitis model in mice (Tab 1).

Tab 1. Effect of rh-bFGF im on ear edema induced by croton oil (n = 10 mice), hind paw swelling induced by Car (n = 8 - 10 mice), and protein content in peritoneal exudate of mice with peritonitis induced by Car (n = 11 mice). $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control group. $^fP<0.01$ *vs* normal saline.

Groups	Weight difference of ear pinnae/mg	Weight difference of hind paw/mg	Protein in peritoneal exudate/ $g \cdot L^{-1}$
Normal	-	-	1.2±1.0
Control	14.4±1.4	49±14	5.7±1.9^f
rh-bFGF 8 kU/kg	11.3±1.9^b	39±10^a	4.4±1.5^a
rh-bFGF 4 kU/kg	10.8±1.7^c	30±7^c	3.6±1.3^b
rh-bFGF 2 kU/kg	11.6±2.5^b	36±12^b	3.7±1.1^b
Dex 2 mg/kg	6±3^c	36±9^b	3.7±1.1^c

Effect of rh-bFGF on carrageenan-induced peritonitis in rats After ip carrageenan, the protein content and Neu counts in the peritoneal exudate in control group were greatly increased as compared with normal saline group. Recombinant human bFGF 2, 4 kU/kg and Dex reduced the protein content and Neu counts in the peritoneal exudate (Tab 2). NO concentration, NOS activity, PGE_2 content, MDA content, and NAG release were raised while SOD activity was reduced in the peritoneal exudate of control group. Recombinant human bFGF 4 kU/kg and Dex 2 mg/kg decreased NO concentration, NOS activity, and PGE_2 content in the peritoneal exudate. However, Dex failed to affect SOD activity and MDA concentration in contrast with the effect of rh-bFGF 4 kU/kg on SOD activity and MDA concentration in our experiments. Dex decreased NAG release in peritoneal exudate, but rh-bFGF had no such effect (Tab 2, 3).

DISCUSSION

Several other investigations have revealed that endogenous bFGF level increases in patients on CAPD (continuous ambulatory peritoneal dialysis) and bFGF overexpresses after cerulein-induced acute pancreatitis in rats. This suggests that endogenous bFGF participates in the inflammatory reaction and is potentially involved in the development of inflammation. bFGF induced the mRNA expression of MCP-1 (monocyte chemoattractant protein-1), RANTES (regulated upon activation, normal T cell expressed and secreted), and IL-8 (interleukin-8)

Tab 2. Effect of rh-bFGF and Dex on neutrophil counts, protein content, NOS activity and NO concentration in rats peritoneal exudate in Car-induced peritonitis. n = 9 - 12. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* Car group. $^fP<0.01$ *vs* normal saline.

Drugs	$10^9 \times$ Neutrophils$\cdot L^{-1}$	Protein content/$g \cdot L^{-1}$	NOS/$U \cdot mL^{-1}$	NO/$\mu mol \cdot L^{-1}$
Normal	2.8±0.6	2.9±0.6	2521±439	3.4±1.1
Car	10±4^f	8.0±1.2^f	3132±161^f	8.8±2.6^f
Car + rh-bFGF 4 kU/kg	5±3^c	5.3±2.4^c	2553±707^b	5.8±2.2^c
Car + rh-bFGF 2 kU/kg	5.5±2.7^c	6.4±2.2^b	2829±529^a	6.7±2.0^a
Car + Dex 2 mg/kg	4.8±1.2^c	5.9±1.5^b	2842±192^c	2.5±1.7^c

Tab 3. Effect of rh-bFGF and Dex on Car-induced peritonitis in rats. n = 8 - 10. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* Car group. $^fP<0.01$ *vs* normal saline.

Drugs	SOD/$U \cdot mg^{-1}$ protein	MDA/$\mu mol \cdot L^{-1}$	PGE_2/$ng \cdot L^{-1}$	NAG/$U \cdot L^{-1}$
Normal	22±3	0.87±0.23	8.5±1.5	134±108
Car	12.2±1.6^f	2.5±1.4^f	13±4^f	336±21^f
Car + rh-bFGF 4 kU/kg	25±9^b	1.5±0.4^b	6.3±1.7^c	309±184^a
Car + rh-bFGF 2 kU/kg	14±4^a	1.6±0.3^b	-	348±82^a
Car + Dex 2 mg/kg	12±4^a	1.8±0.6^a	4.4±1.9^c	242±99^c

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in human mesangial cells, implying that endogenous bFGF plays an important role in the selective recruitment of different leukocyte populations to the sites of inflammation. Meanwhile, bFGF indirectly promoted production of inflammatory mediators PGE_2, NO, and free radicals[11-16]. On the other hand, lots of papers have reported that rh-bFGF can protect brain and heart against ischemic damage, and the results of using rh-bFGF in clinical wound therapy are satisfactory. Inflammatory response may be involved in these pathophysiological events. Therefore, it is necessary to investigate and demonstrate the possible actions of exogenous rh-bFGF at therapeutic dosage on acute inflammation.

The present study demonstrated that rh-bFGF 2 and 4 kU/kg im obviously lessened the ear edema, hind paw swelling, and protein content in the peritoneal exudate in mice, and reduced protein content and Neu counts in the peritoneal exudate in rats, indicating that exogenous rh-bFGF had significant anti-inflammatory effects on acute inflammation. This first demonstrated that exogenous rh-bFGF at therapeutic dosage has an inhibitory action on acute inflammation.

That rh-bFGF reduced protein content in the peritoneal exudate showed that rh-bFGF played a role in reducing capillary permeability at inflammatory sites. Meanwhile, rh-bFGF decreased Neu counts in the peritoneal exudate, suggesting that it is capable of suppressing Neu emigration to the inflammatory sites. However, rh-bFGF did not inhibit NAG release, implying that rh-bFGF has no action on stabilizing the lysosome membrane. Oxygen free radicals, NO and PGE_2 are considered as inflammatory factors, and play important roles in damage of tissues by inflammation. The present experiment showed that rh-bFGF enhanced SOD activity and reduced MDA concentration in rat peritonitis, suggesting that rh-bFGF has an oxygen free radicals scavenging action. Recombinant human bFGF lessened NOS activity and NO content in rat peritonitis, implying that its inhibiting action on NO biosynthesis and lessening of PGE_2 production in inflammatory fluid may also contributes towards its action of lowering capillary permeability and reducing inflammatory exudation.

On the basis of these data, it can be concluded that rh-bFGF at therapeutic dosage inhibits acute inflammation, and its inhibition of the PGE_2 and NO levels and its scavenging action of oxygen free radicals were important anti-inflammatory mechanisms of rh-bFGF. However, the effects observed with endogenous bFGF and exogenous rh-bFGF on acute inflammation are different.

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重组人碱性成纤维细胞生长因子对大鼠和小鼠急性炎症的影响

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关键词 重组蛋白质类；碱性成纤维细胞生长因子；角叉菜胶；炎症；地塞米松

目的：研究重组人碱性成纤维细胞生长因子的抗炎作用. **方法**：用巴豆油致小鼠耳肿，角叉菜胶致小鼠足肿，致小鼠、大鼠急性腹膜炎模型，观察 rh-bFGF 对小鼠耳肿胀、足肿胀、腹腔渗液中蛋白含量以及大鼠腹腔渗液中白细胞数、蛋白含量、超氧化酶歧化酶活性、β-*N*-乙酰氨基葡萄糖苷酶释放、一氧化氮含量、一氧化氮合酶活性、丙二醛和前列腺素 E_2 生成的影响. **结果**：rh-bFGF 2，4 kU/kg im 明显抑制小鼠耳肿、足肿和腹腔中蛋白渗出；rh-bFGF 4 kU/kg im 明显抑制大鼠腹腔中白细胞游走、蛋白渗出，降低腹腔渗液中 MDA 含量，升高 SOD 活性，明显降低 NOS 活性、NO 和 PGE_2 含量. 但 rh-bFGF 对腹腔渗液中 NAG 的升高无明显影响. **结论**：rh-bFGF 肌注有抗炎作用，可能与降低毛细血管通透性，抑制白细胞游走，降低 NO、MDA 和 PGE_2 含量，清除自由基有关.

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