P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2003;9(6):1312-1317
World Journal of Gastroenterology
 ISSN 1007-9327

• BASIC RESEARCH •

Rapid mitogen-activated protein kinase by basic fibroblast growth factor in rat intestine after ischemia/reperfusion injury

Xiao-Bing Fu, Yin-Hui Yang, Tong-Zhu Sun, Wei Chen, Jun-You Li, Zhi-Yong Sheng

Xiao-Bing Fu, Yin-Hui Yang, Tong-Zhu Sun, Wei Chen, Jun-You Li, Zhi-Yong Sheng, Wound Healing and Cell Biology Laboratory, Burns Institute, 304 Hospital, Trauma Center of Postgraduate Medical College, Beijing 100037, China
Supported in part by the National Basic Science and Development Programme (973 Programme, No.G1999054204), Grant for National Distinguished Young Scientists No. 39525024, Grant for National Natural Science Foundation of China, No. 39900054, 30170966
Correspondence to: Professor Xiao-Bing Fu, Wound Healing and Cell Biology Laboratory, 304 Hospital, Burns Institute, Trauma Center of Postgraduate Medical College, 51 Fu Cheng Road, Beijing 100037, China. fuxb@cgw.net.cn
Telephone: +86-10-66867396 **Fax:** +86-10-88416390
Received: 2003-03-04 **Accepted:** 2003-04-01

Abstract

AIM: Previous studies showed that exogenous basic fibroblast growth factor (bFGF or FGF-2) could improve physiological dysfunction after intestinal ischemia/reperfusion (I/R) injury. However, the mechanisms of this protective effect of bFGF are still unclear. The present study was to detect the effect of bFGF on the activities of mitogen-activated protein kinase (MAPK) signaling pathway in rat intestine after I/R injury, and to investigate the protective mechanisms of bFGF on intestinal ischemia injury.

METHODS: Rat intestinal I/R injury was produced by clamping the superior mesenteric artery (SMA) for 45 minutes and followed by reperfusion for 48 hours. Seventy-eight Wistar rats were used and divided randomly into sham-operated group (A), normal saline control group (B), bFGF antibody pre-treated group (C), and bFGF treated group (D). In group A, SMA was separated without occlusion. In groups B, C and D, SMA was separated and occluded for 45 minutes, then, released for reperfusion for 48 hours. After the animals were sacrificed, blood and tissue samples were taken from the intestine 45 minutes after ischemia in group A and 2, 6, 24, and 48 hours after reperfusion in the other groups. Phosphorylated forms of p42/p44 MAPK, p38 MAPK and stress activated protein kinase/C-Jun N-terminal kinase (SAPK/JNK) were measured by immunohistochemistry. Plasma levels of D-lactate were examined and histological changes were observed under the light microscope.

RESULTS: Intestinal I/R injury induced the expression of p42/p44 MAPK, p38 MAPK, and SAPK/JNK pathways and exogenous bFGF stimulated the early activation of p42/p44 MAPK and p38 MAPK pathways. The expression of phosphorylated forms of p42/p44 MAPK was primarily localized in the nuclei of crypt cells and in the cytoplasm and nuclei of villus cells. The positive expression of p38 MAPK was localized mainly in the nuclei of crypt cells, very few in villus cells. The activities of p42/p44 MAPK and p38 MAPK peaked 6 hours after reperfusion in groups B and C, while SAPK/JNK peaked 24 hours after reperfusion. The activities of p42/p44 MAPK and p38 MAPK peaked 2 hours after reperfusion in group D and those of SAPK/JNK were not changed in group B. D-lactate levels and HE staining showed that the intestinal barrier was damaged severely 6 hours after reperfusion; however, histological structures were much improved 48 hours after reperfusion in group D than in the other groups.

CONCLUSION: The results indicate that intestinal I/R injury stimulates the activities of MAPK pathways, and that p42/p44 MAPK and p38MAPK activities are necessary for the protective effect of exogenous bFGF on intestinal I/R injury. The protective effect of bFGF on intestinal dysfunction may be mediated by the early activation of p42/p44 MAPK and p38 MAPK signaling pathways.

Fu XB, Yang YH, Sun TZ, Chen W, Li JY, Sheng ZY. Rapid mitogen-activated protein kinase by basic fibroblast growth factor in rat intestine after ischemia/reperfusion injury. *World J Gastroenterol* 2003; 9(6): 1312-1317
http://www.wjgnet.com/1007-9327/9/1312.asp

INTRODUCTION

Previous studies have shown that intestinal ischemia/reperfusion (I/R) injury reduce the expression of endogenous basic fibroblast growth factor (bFGF) in rats, and the intravenous administration of exogenous bFGF could induce the expression of endogenous bFGF and improve the physiological functions of the intestine, lung, kidney, and other internal organs after I/R injury[1-6]. However, the protective mechanisms of bFGF on intestinal I/R injury remain unknown.

Mitogen-activated protein kinase (MAPK) cascade, a cytoplasmic protein kinase that requires dual phosphorylation on specific threonine and tyrosine residues for their activation, can transmit mitogen or differentiation signals from the cell surface into the nucleus, thus regulating the gene expression[7-10]. P42/p44 MAPK, p38MAPK and stress activated protein kinase/C-Jun N-terminal kinase (SAPK/JNK) are three important members of the MAPK family. The purpose of the present study was to detect the activities of mitogen-activated protein kinase (MAPK) signaling pathway in rat intestine after administration of bFGF, and to investigate the protective mechanisms of bFGF on intestinal (I/R) injury.

Rat intestinal I/R injury was produced by clamping the superior mesenteric artery (SMA) for 45 minutes and by different durations of reperfusion[11]. The activities of p42/p44 MAPK, p38 MAPK, and SAPK/JNK were measured after administration of bFGF or bFGF monoclonal antibody. The results indicate that the early activation of p42/p44 MAPK and p38 MAPK is necessary for the protective effect of bFGF on intestinal I/R injury.

MATERIALS AND METHODS

Animal model

Seventy-eight healthy Wistar rats weighing 220±20 g (Animal Center, Academy of Military Medical Sciences, Beijing) were used. All animals were housed in the laboratory and given free access to food and water for 1 week before being used. The animal was under anesthesia by 3 % sodium pentobarbital (40 mg/kg), a middle incision was made. The superior mesenteric artery (SMA) was identified and freed by blunt dissection. A microvascular clamp was placed at the root of SMA to cause complete cessation of blood flow for 45 minutes, and thereafter the clamp was loosened to form reperfusion injury[1, 11].After 2, 6, 24 and 48 hours reperfusion, the animals were sacrificed and blood samples and intestinal tissue biopsies were taken. Blood samples were centrifuged and serum was frozen to measure plasma D-lactate. Tissue biopsies were fixed with 4 % paraformaldehyde.

In this study, all operations were performed under aseptic conditions. The animal experiments were approved by the local animal management committee.

Experimental design

The animals were randomly divided into four groups: sham-operated (A), normal saline control (B), bFGF monoclonal antibody (Sigma, St. Louis, MO, USA) pre-treated (C) and bFGF (Sigma, St. Louis, MO, USA) treated groups (D). In group A, SMA was freed but without occlusion and blood samples and tissue biopsies were taken 45 minutes after exposure of the SMA. In groups B and D, 0.15 ml saline or 0.15 ml saline plus bFGF (2 μg/rat) was injected immediately 45 minutes after SMA occlusion from the tail vein. In group C, 0.15 ml saline plus bFGF monoclonal antibody (25 μg/rat) was injected right before SMA occlusion from tail vein for pre-treatment.

Measurement of phosphorylated forms of p42/p44 MAPK, p38 MAPK and SAPK/JNK

Formalin-fixed, paraffin-embedded small intestinal tissues were used to measure the expression of phosphorylated forms of p42/p44 MAPK, p38 MAPK, and SAPK/JNK by immunohistochemistry. Immunohistochemical staining was performed according to the instructions of the PowerVision™ kit (Santa Cruze, USA). Briefly, sections (5 μm) were dewaxed and rehydrated in graded alcohols. Endogenous peroxidase activity was quenched, and antigen retrieval was performed by heating for 20 minutes at 100 ℃ in 0.01 mol/L sodium citrate. The primary monoclonal antibodies for p42/p44 MAPK, p38 MAPK and SAPK/JNK (Cell Signaling Technology, Inc., USA) were diluted to 1:100 in buffer and incubated for 40 minutes at 37 ℃. The sections were then incubated with HRP-conjugated secondary antibodies (Santa Cruz, USA) for 20 minutes at 37 ℃. Positive expression was detected with diaminobenzidine (DAB) (Sigma, St. Louis, MO, USA). The sections were lightly counterstained with hematoxylin, dehydrated in graded alcohol, and mounted. For negative control, the sections were processed similarly but PBS was used as primary antibodies instead of the MAPKs monoclonal antibodies.

The result of positive staining was semi-quantitatively defined as -, +, ++ and +++. This was observed under microscope with 10 times eyepiece and 40 times objective. "-" represents no visible positive staining, "+" less than 10 stained cells and "++" 10-30 stained cells, while "+++" represents more than 30 positively stained cells within one high power field.

Measurement of plasma D-lactate

The levels of plasma D-lactate were measured with modified Brandt' s method[12]. Briefly, heparinized blood was centrifuged at 3 200 rpm for 10 min and 2 ml of the plasma was deproteinized with 0.2 ml perchloric acid (PCA) (1/10 vol), mixed and kept in an ice bath for 10 min. The denatured protein solution was centrifuged at 3 200 rpm for 10 min and the supernatant solution was removed. To 1.4 ml of supernatant solution, 0.12 ml KON was added and they were mixed for 20 s. Precipitant $KClO_4$ was removed by centrifugation at 3 200 rpm for 10 min. The supernatant solution and neutralized-protein-free plasma were used to measure the absorbency at 340 nm. Plasma D-lactate concentration was expressed as mmol/L.

Histological observation

Paraformaldehyde fixed, paraffin embedded small intestine samples were also cut 5 μm in thickness, deparaffinized in xylene, rehydrated in graded ethanol, and then stained with haematoxylin-eosin (HE) for histological observation under light microscope (Olympus, Japan).

Statistical analysis

Data were expressed as mean ± standard error. Comparisons between groups of data were analyzed by Student' s *t*-test. *P* values <0.05 were considered statistically significant.

RESULTS

Activities of p42/p44 MAPK and p38 MAPK

Quantitative immunohistochemical results for phosphorylated forms of p42/p44 MAPK and p38 MAPK were evaluated (Tables 1 and 2). The expression of activated p42/p44 MAPK was localized in the cytoplasm and nuclei of villus cells and in the nuclei of crypt cells, mainly in the epithelium and villus cells (Figure 1). Activated p38 MAPK was localized primarily in the nuclei of crypt cells, very few in villus cells (Figure 2). There was a consistent correlation between positive expression levels and the intensity of p42/p44 MAPK and p38 MAPK. The positive expression of p42/p44 MAPK and p38 MAPK was weak in the sham-operated intestinal tissues and ischemic tissues. However, the number of positive staining cells increased with high staining intensity after reperfusion injury. In the normal saline and bFGF antibody pre-treated groups, the number of positive staining cells of p42/p44 MAPK (Figures 1B and C) and p38 MAPK (Figures 2B and C) increased 2 hours after reperfusion, peaked at 6th hours, and decreased from 24 to 48 hours. In the bFGF treated group, however, the number of positive staining cells and the intensity of p42/p44 MAPK and p38 MAPK peaked 2 hours after reperfusion (Figures 1D and 2D) and decreased afterwards, but they were still higher than those in the sham-operated control at 48 hours. Compared with the normal saline and bFGF treated groups, the intensity of p42/p44 MAPK and p38 MAPK positive staining in the bFGF antibody pretreated group was weaker from 2 hours to 48 hours after reperfusion.

Activities of SAPK/JNK

Weak staining of SAPK/JNK was observed in small intestine after I/R injury. Positive staining was localized in the nuclei and cytoplasm of villus and crypt cells. The staining, however, was weak without much difference among the groups (Table 3). The positive staining of SAPK/JNK in bFGF treated group was slightly higher only at 24 hours after reperfusion. Among all the groups, the positive staining of SAPK/JNK was weaker than that of p42/p44 MAPK and p38 MAPK.

Changes of plasma D-lacate levels

Plasma D-lactate levels were measured 2, 6, 24, and 48 hours after reperfusion in all groups. They were elevated 2 hours after reperfusion in all groups, peaked at 6th hour, and decreased to nearly normal 48 hours later (Table 4). The levels at 45 min after ischemia in the sham-operated group were served as controls.

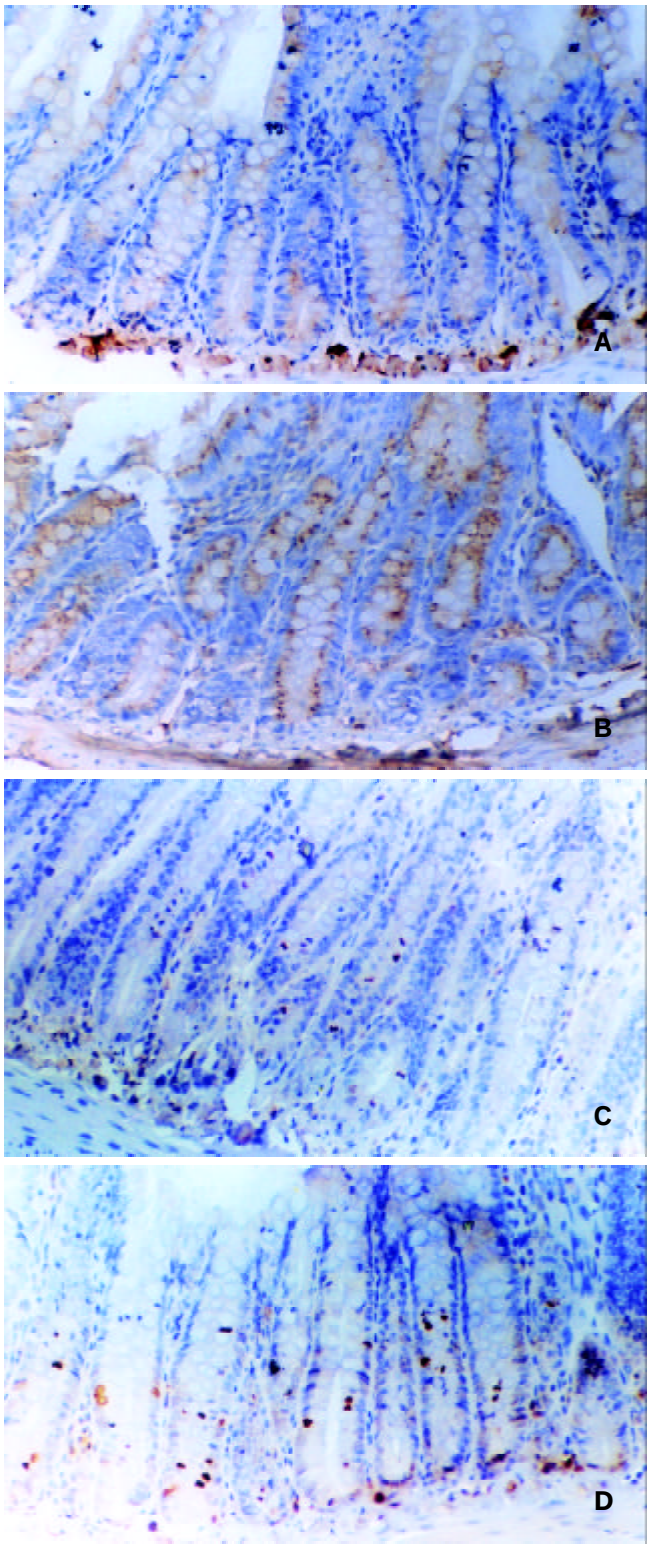

Figure 1 Immunohistochemical staining of phosphorylated p42/p44 MAPK in intestinal biopsies in rats after ischemia/reperfusion injury (SP×200). A: Negative control of p42/p44 MAPK staining. There was no positive expression signal in this group. B: The expression of phosphorylated p42/p44 in intestinal biopsies in the saline control group 2 hours after reperfusion. The activated p42/p44 MAPK expression was localized in the cytoplasm and nuclei of villus cells and in the nuclei of crypt cells, mainly in the epithelium and villus cells. C: Phosphorylated p42/p44 staining in the bFGF antibody pre-treated group. The number of positive cells and intensity in this group were weaker compared with those in the saline control and bFGF treated groups. D: The expression of phosphorylated p42/p44 in the bFGF treated group 2 hours after reperfusion. The activated p42/p44 MAPK expression was localized in the cytoplasm and nuclei of villus cells and in the nuclei of crypt cells, mainly in the epithelium and villus cells. The number of positive cells in this group was more than that in the bFGF antibody pre-treated group. ISH×400.

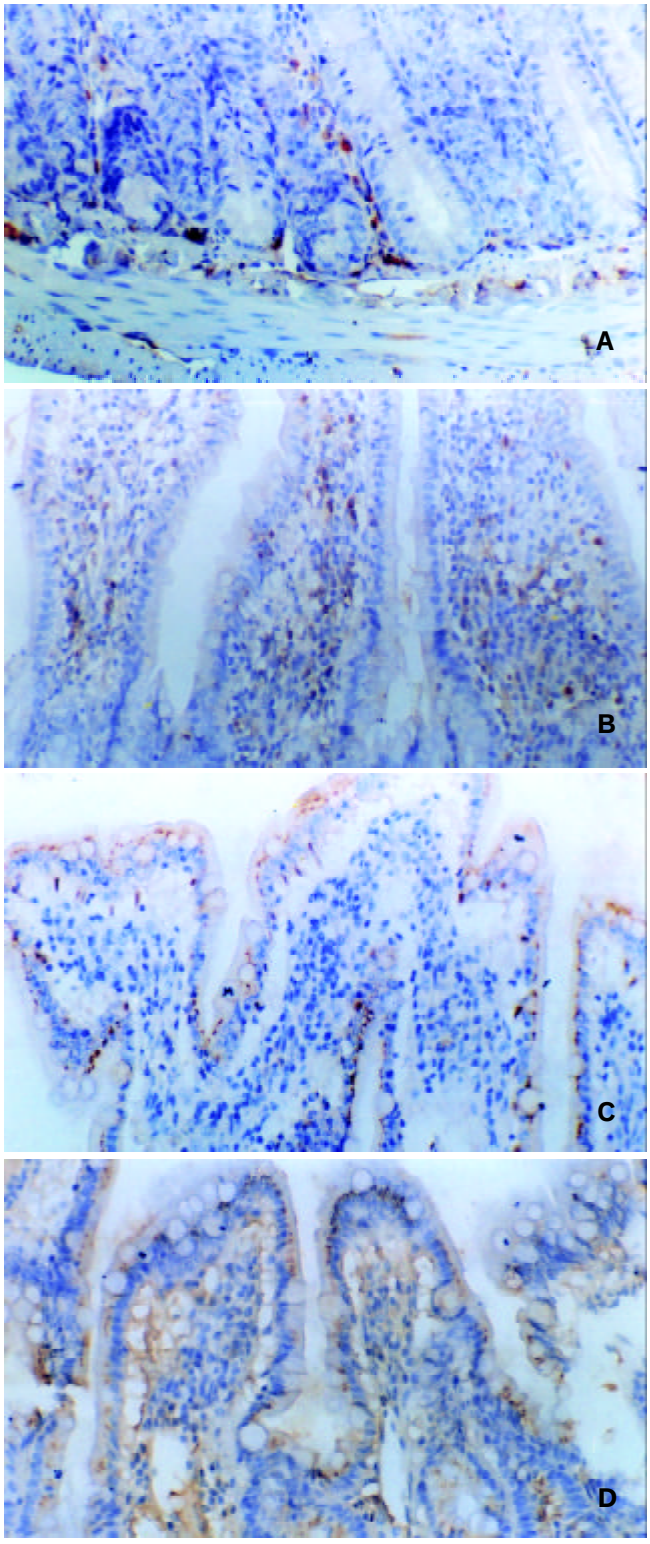

Figure 2 Immunohistochemical staining of phosphorylated p38 MAPK in intestinal biopsies in rats after ischemia/reperfusion injury (SP×200). A: Negative control of p38 MAPK staining. There was no positive expression signal in this group. B: Phosphorylated p38 MAPK staining in the saline control group 2 hours after reperfusion. Few p38 MAPK positive expression were localized in the cytoplasm and nuclei of villus cells and in the nuclei of crypt cells, mainly in the

Table 1 Semi-quantitative results of immunohistochemical staining for phosphorylated forms of p42/p44 MAPK in different groups

Groups	Pre-injury	2 hrs post-injury	6 hrs post-injury	24 hrs post-injury	48 hrs post-injury
Group B	-	++	++	+	++
Group C	-	+	++	+	+
Group D	-	+++	+++	+	+

"-" represents no visible positive staining, "+" less than 10 stained cells and "++" 10-30 stained cells, while "+++" represents more than 30 positively stained cells within one high power field.

Table 2 Semi-quantitative results of immunohistochemical staining for phosphorylated forms of p38 MAPK in different groups

Groups	Pre-injury	2 hrs post-injury	6 hrs post-injury	24 hrs post-injury	48 hrs post-injury
Group B	-	++	++	+	++
Group C	-	+	++	+	+
Group D	-	+++	++	+	+

"-" represents no visible positive staining, "+" less than 10 stained cells and "++" 10-30 stained cells, while "+++" represents more than 30 positively stained cells within one high power field.

Table 3 Semi-quantitative results of immunohistochemical staining for phosphorylated forms of SAPK/JNK in different groups

Groups	Pre-injury	2 hrs post-injury	6 hrs post-injury	24 hrs post-injury	48 hrs post-injury
Group B	-	+	+	++	+
Group C	-	+	+	+	+
Group D	-	+	+	++	+

"-" represents no visible positive staining, "+" less than 10 stained cells and "++" 10-30 stained cells, while "+++" represents more than 30 positively stained cells within one high power field.

Table 4 The changes of plasma D-lactate levels at different time points in three groups (mmol/L) ($\bar{x}\pm s$)

Groups	Animal numbers	Control	2 hours	6 hours	24 hours	48 hours
Group B	24	0.332±0.132	0.372±0.090	0.397±0.096	0.463±0.147	0.511±0.179
Group C	24	0.332±0.132	0.309±0.079	0.327±0.098	0.415±0.177[a]	0.425±0.208[a]
Group D	24	0.332±0.132	0.369±0.124	0.407±0.089	0.475±0.128	0.537±0.098

[a]$P<0.05$ *vs* compared with control.

epithelium and villus cells. C: P38 MAPK staining in the bFGF antibody pre-treated group. The number of positive cells and localization of p38 MAPK positive cells were similar with those in the saline group. D: Phosphorylated p38 staining in the bFGF treated group 2 hours after reperfusion. Activated p38 MAPK was localized primarily in the nuclei of crypt cells, few in villus cells. The number of positive cells was more than that in the saline control and bFGF antibody pre-treated groups. In the bFGF treated group, the number of positive expression cells of p38 MAPK as well as its intensity peaked 2 hours after reperfusion.

Histological evaluation

Intestinal I/R injury resulted in the damage of intestinal barrier and the increase of mucosal permeability. HE staining showed partial loss of the mucosa 2 hours after reperfusion. 6 hours after reperfusion, however, the damage of intestinal epithelial cells, hemorrhage and necrosis were observed and accompanied by inflammatory cell infiltration into the intestinal wall. Histological structure of the intestinal mucosa was markedly improved after administration of bFGF.

DISCUSSION

Intestinal I/R injury causes release of bacteria and toxin from the gut into the host blood circulation and changes of inflammatory factors, cytokines and growth factors, resulting in damage to the intestinal barrier and other internal organs[1-3,13-17]. We found that administration of exogenous basic fibroblast growth factor (bFGF) could reduce the intestinal injury caused by I/R insult. However, the mechanisms of this protective effect of bFGF are not elucidated. BFGF is expressed in many normal adult tissues and has mitogenic activity in a wide variety of cells of mesenchymal, neuronal, and epithelial origins, and regulates events in normal embryonic development, angiogenesis, wound repair, and neoplasia[18-20]. Also, it can regulate migration and replication of intestinal epithelial cells in culture[21]. Recent studies have shown that L-glutamine, tumor necrosis factor-α and epidermal growth factor (EGF) stimulate proliferation of intestinal crypt cells by activating the MAPK pathway, and that p42/p44 MAPK activities are necessary for both cell cycle progression and differentiation of the intestinal cells[22-25]. In many other cell types, growth factor controls proliferation and differentiation

via the MAPK pathway. MAPK is a common signal pathway to transmit the mitogen or the differentiating signals from the cell surface to the nucleus, and thus ultimately regulates different gene expression[26-28]. Hence, we hypothesized that MAPK activation might be involved in the regulation of bFGF signals in the process of intestinal barrier repair.

To investigate this hypothesis, we evaluated changes of the activated MAPK signal pathway after administration of bFGF and bFGF antibodies. We found that intestinal I/R injury stimulated the activities of phosphorylated forms of the p42/p44 MAPK and p38MAPK pathways, and increased the SAPK/JNK activity slightly. p42/p44 MAPK and p38MAPK activities were increased 2 hours after reperfusion, and peaked at 6 hours. At the same time, the levels of SAPK/JNK increased slightly 24 hours after reperfusion compared with those of the normal control. Phosphorylated forms of p42/p44 MAPK were mainly localized in the nuclei of crypt cells and in the cytoplasm and nuclei of villus cells, whereas those of p38MAPK were primarily localized in the nuclei of crypt cells, few in villus cells. After administration of bFGF, the expression of both p42/p44 MAPK and p38MAPK was quickly stimulated, and the activation of both p42/p44 MAPK and p38MAPK peaked 2 hours after reperfusion, declined gradually to normal at 48 hours. A coherence was noted between the changes of p42/p44 MAPK and p38MAPK and histological findings. These results indicate that intestinal I/R injury induces the activities of the MAPK pathways, and p42/p44 MAPK and p38MAPK activities are necessary for the protective effects of exogenous bFGF on intestinal I/R injury. The early stimulation of the p42/p44 MAPK and p38MAPK signal pathways may mediate the protective effects of bFGF on intestinal dysfunction.

MAPK family is composed of "extracellular signal regulated" p42/p44 MAPK, "stress-regulated" MAPK (SR-MAPKs), stress-activated protein kinases (SAPKs)/c-Jun N-terminal kinases (JNKs) and p38-MAPKs. On stimulation, MAPKs are translated into the nucleus where they may phosphorylate nuclear transcription factors and thus regulate gene expression. The four principal differentiated cell lineages of intestinal epithelium are derived from common multipotent stem cells located near the base of each crypt. These crypt stem cells divide to produce daughter stem cells as well as more rapidly replicating transit cells, which in turn undergo 4-6 rapid cell divisions in the proliferative zone located in the lower half of each crypt[29,30]. Factors determining whether cells continue to proliferate, cease dividing, and begin to differentiate, appear to operate during the first gap phase (G1) of the cell cycle. P42/p44 is activated during G0 to G1 transition, and the activity remains elevated up to S phase entry, implicating this family of protein quinces in the control of G1 progression[31,32]. Activation of p42/p44 MAPK is also necessary for growth factor-dependent proliferation of some cell lines.

We propose the possible mechanisms of the protective effects of bFGF on intestinal I/R injury be involved in the activation of MAPK pathway. First, to protect the survival of intestinal stem cells within crypt and mediate the proliferation and differentiation of these cells. Intestinal epithelium is maintained by continuous and rapid replacement of differentiated epithelial cells by replication of undifferentiated epithelial cells. Exogenous bFGF markedly enhances the survival of crypt stem cells before and after irradiation injury[33]. Microvascular endothelial apoptosis is the primary lesion leading to stem cell dysfunction, while endothelial apoptosis could be inhibited by intravenous bFGF[34]. Second, to regulate the inflammation reactions after I/R injury. The TNF translation by IL-10 is inhibited mainly by inhibiting the activation of the p38 MAPK pathway[35]. This is necessary for maintenance of immune homeostasis in the gut.

In the perfused heart, ischemia/reperfusion activates stress-regulated MAPKs, direct pharmacological activation of p38 triggers delayed preconditioning of the heart, and there is minimal activation of the p42/p44 MAPK subfamily by heart I/R injury[35-37]. Yet phosphorylation of p42/p44 MAPK occurs consistently in the grey matter penumbra of brain tissue after ischemic stroke, and may be associated with neuronal survival and/or angiogenic activity in the recovering brain tissue[38]. The results indicate that the MAPK pathways respond differently to ischemic injury in different sites.

The changes of serum D (-)-lactate were used as a predictor of intestinal I/R injury in this study. D (-)-lactate is the stereoisomer of mammalian L (+)-lactate. Mammalian tissue does not produce D (-)-lactate and only slowly metabolizes it. It is a strict product of bacterial fermentation. Since mammals do not possess the enzyme systems to rapidly metabolize D (-)-lactate[11, 39,40], the released D (-)-lactate will pass through the gut barrier and liver in an unchanged form and appear in the peripheral blood. As intestinal ischemia injury causes mucosal injury and subsequent bacterial proliferation, D (-)-lactate is released from gut into the circulation. In this study, the serum D (-)-lactate level was increased after injury, but in the bFGF treated group, it was not significantly increased as in the control group, indicating that bFGF exerts a positive protective effect on the mucosal barrier and decreases the intestinal permeability.

In summary, intestinal I/R injury induces the activities of the MAPK pathways, and p42/p44 MAPK and p38MAPK activities are necessary for the protective effect of exogenous bFGF on intestinal I/R injury. The protective effect of bFGF on intestinal dysfunction may be mediated by the early stimulation of the p42/p44 MAPK and p38 MAPK signaling pathways.

REFERENCES

1 **Fu XB**, Sheng ZY, Wang YP, Ye YX, Xu MH, Sun TZ, Zhou BT. Basic fibroblast growth factor reduces the gut and liver morphologic and functional injuries after ischemia and reperfusion. *J Trauma* 1997; **42:** 1080-1085

2 **Yang YH,** Fu XB, Sun TZ, Jiang LX, Gu XM. bFGF and TGFβ expression in rat kidneys after ischemic / reperfusional gut injury and its relationship with tissue repair. *World J Gastroenterol* 2000; **6**: 147-149

3 **Fu XB,** Yang YH, Sun XQ, Sun TZ, Gu XM, Sheng ZY. Protective effects of endogenous basic fibroblast growth factor activated by 2, 3 butanedion monocime on functional changes of ischemic intestine, liver and kidney in rats. *Zhongguo Weizhongbing Jijiu Yixue* 2000; **12**: 69-72

4 **Yang YH,** Fu XB, Sun TZ, Jiang LX, Gu XM. The effect of exogenous basic fibroblast growth factor on hepatic endogenous basic fibroblast growth factor and fibroblast growth factor receptor expression after intestinal ischemia-reperfusion injury. *Zhongguo Weizhongbing Jijiu Yixue* 1999; **11**: 734-736

5 **Fu XB,** Yang YH, Sun TZ, Sun XQ, Gu XM, Chang GY, Sheng ZY. Effect of inhibition or anti-endogenous basic fibroblast growth factor on functional changes in intestine, liver and kidneys in rats after gut ischemia-reperfusion injury. *Zhongguo Weizhongbing Jijiu Yixue* 2000; **12**: 465-468

6 **Yang YH,** Fu XB, Sun TZ, Jiang LX, Gu XM. Renal endogenous expression of basic fibroblast growth factor and transforming growth factor β after intestinal ischemia-reperfusion injury. *Zhongguo Weizhongbing Jijiu Yixue* 1999; **11**: 203- 205

7 **Marshall CJ.** Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* 1995; **80**: 179-185

8 **Seger R,** Krebs EG. The MAPK signaling cascade. *FASEB J* 1995; **9:** 726-735

9 **Marais R,** Wynne J, Treisman R. The SRF accessory protein Elk-1 contains a growth factor-regulated transcriptional activation domain. *Cell* 1993; **73:** 381-393

10 **Nishida E,** Gotoh Y. The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem Sci* 1993; **18**: 128-131

11 **Sun XQ,** Fu XB, Zhang R, Lu Y, Deng Q, Jiang XG, Sheng ZY. Relationship between plasma D(-)-lactate and intestinal damage after severe injuries in rats. *World J Gastroenterol* 2001; **7:** 555-558

12 **Qin RY,** Zou SQ, Wu ZD, Qiu FZ. Influence of splanchnic vascular infusion on the content of endotoxins in plasma and the translocation of intestinal bacteria in rats with acute hemorrhage necrosis pancreatitis. *World J Gastroenterol* 2000; **6**: 577-580

13 **Wang XJ,** Luo XD, Luo Q, Yang ZC. Effects of sera from burn patients on human hepatocytic viscoelasticity. *World J Gastroenterol* 1998; **4**: 60

14 **Zhang P,** Yang WM, Shui WX, Du YG, Jin GY. Effect of Chinese herb mixture, shock decoction on bacterial translocation from the gut. *World J Gastroenterol* 2000; **6**(Suppl 3): 74

15 **Zhu L,** Yang ZC, Li A, Cheng DC. Protective effect of early enteral feeding on postburn impairment of liver function and its mechanism in rats. *World J Gastroenterol* 2000; **6**: 79-83

16 **Li YS,** Li JS, Li N, Jiang ZW, Zhao YZ, Li NY, Liu FN. Evaluation of various solutions for small bowel graft preservation. *World J Gastroenterol* 1998; **4**: 140-143

17 **Zhang GL,** Wang YH, Ni W, Teng HJ, Lin ZB. Hepatoprotective role of ganoderma lucidum polysaccharide against BCG in duced immue liver injury in mice. *World J Gastroenterol* 2002; **8:** 728-733

18 **Liu XJ,** Yang L, Mao YQ, Wang Q, Huang MH, Wang YP, Wu HB. Effects of the tyrosine protein kinase inhibitior genistein on the proliferation, activation of cultured rat hepatic stellate cells. *World J Gastroenterol* 2002; **8**: 739-745

19 **Brandt RB,** Siegel SA, Waters MG, Bloch MH. Spectrophotometric assay for D-(-)-Lactate in plasma. *Anal Biochem* 1980; **102**: 39-46

20 **Basilico C,** Moscatelli D. The FGF family of growth factors and oncogenes. *Adv Cancer Res* 1992; **59:** 115-165

21 **Dignass AU,** Tsunekawa S, Podolsky DK. Fibroblast growth factors modulate intestinal epithelial cell growth and migration. *Gastroenterology* 1994; **106:** 1254-1262

22 **Estival A**, Monzat V, Miquel K, Gaubert F, Hollande E, Korc M, Vaysse N, Clemente F. Differential regulation of fibroblast growth factor receptor-1 mRNA and protein by two molecular forms of basic FGF. *J Biol Chem* 1996; **271**: 5663-5670

23 **Nice EC,** Fabri L, Whitehead RH, James R, Simpson RJ, Burgess AW. The major colonic cell mitogen extractable from colonic mucosa ia an N-terminally extended form of basic fibroblast growth factor. *J Biol Chem* 1991; **266**: 14425-14430

24 **Rhoads JM,** Argenzio RA, Chen W, Rippe RA, Westwick JK, Cox AD, Berschneider HM, Brenner DA. L-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. *Am J Physiol* 1997; **272:** G943-G953

25 **Oliver BL,** Sha'afi RI, Hajjar JJ. Transforming growth factor-alpha and epidermal growth factor activate mitogen-activated protein kinase and its substrates in intestinal epithelial cells. *Proc Soc Exp Biol Med* 1995; **210**: 162-170

26 **Goke M,** Kanai M, Lgnch-Devaney K, Podolsky DK. Rapid mitogen-activated protein kinase activation by transforming growth factor alpha in wounded rat intestinal epithelial cells. *Gastroenterology* 1998; **114**: 697-705

27 **Aliaga JC,** Deschenes C, Beaulieu JF, Calvo EL, Rivard N. Requirement of the MAP kinase cascade for cell cycle progression and differentiation of human intestinal cells. *Am J Physiol* 1999; **277**:G631-G641

28 **Gordon JI,** Hermiston ML. Differentiation and self-renewal in the mouse gastrointestinal epithelium. *Curr Opin Cell Biol* 1994; **6**: 795-803

29 **Potten CS,** Booth C, Pritchard DM. The intestinal epithelial stem cell: the mucosal governor. *Int J Exp Pathol.* 1997; **78:** 219-243

30 **Brondello JM,** McKenzie FR, Sun H, Tonks NK, Pouyssegur J. Constitutive MAP kinase phosphatase (MKP-1) expression blocks G1 specific gene transcription and S-phase entry in fibroblasts. *Oncogene* 1995; **10:** 1895-1904

31 **Meloche S,** Seuwen K**,** Pages G, Pouyssegur J. Biphasic and synergistic activation of p44MAPK (ERK1) by growth factors: correlation between late phase activation and mitogenicity. *Mol Endocrinol* 1992; **6**: 845-854

32 **Houchen CW,** George RJ, Sturmoski MA, Cohn SM. FGF-2 enhances intestinal stem cell survival and its expression is induced after radiation injury. *Am J Physio* 1999; **276**: G249-G258

33 **Paris F,** Fuks Z, Kang A, Capodieci P, Juan G, Ehleiter D, Haimovitz-Friedman A, Cordon-Cardo C, Kolesnick R. Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science* 2001; **293:** 293-297

34 **Kontoyiannis D,** Kotlyarov A, Carballo E, Alexopoulou L, Blackshear PJ, Gaestel M, Davis R, Flavell R, Kollias G. Interleukin-10 targets p38 MAPK to modulate ARE-dependant TNF mRNA translation and limit intestinal pathology. *EMBO J* 2001; **20:** 3760-3770

35 **Clerk A,** Fuller SJ, Michael A, Sugden PH. Stimulation of "stress-regulated" mitogen-activated protein kinases (stress-activated protein kinases/c-Jun N-terminal kinases and p38-mitogen-activated protein kinases) in perfused rat hearts by oxidative and other stresses. *J Biol Chem* 1998; **273**: 7228-7234

36 **Zhao TC**, Taher MM, Valerie KC, Kukreja RC. p38 triggers late preconditioning elicited by anisomycin in heart: involvement of NF-KB and iNOS. *Circ Res* 2001; **89**: 915-922

37 **Tong H**, Chen W, London RE, Murphy E, Steenbergen C. Preconditioning enhances glucose uptake is mediated by p38 MAP kinase not by phophatidylinositol 3-kinase. *J Biol Chem* 2000; **275**: 11981-11986

38 **Slevin M,** Krupinski J, Slowik A, Rubio F, Szczudlik A, Gaffney J. Activation of MAP kinase (ERK-1/ERK-2), tyrosine kinase and VEGF in the human brain following acute ischaemic stroke. *Neuroreport* 2000; **11:** 2759-2764

39 **Murray MJ,** Barbose JJ, Cobb CF. Serum D (-)-lactate levels as a predictor of acute intestinal ischemia in a rat model. *J Surg Res* 1993; **54:** 507-509

40 **Murray MJ,** Gonze MD, Nowak LR, Cobb CF. Serum D (-)-lactate levels as an aid to diagnosing acute intestinal ischemia. *Am J Surg* 1994; **167:** 575-578

Edited by Zhang JZ and Zhu LH