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Dexamethasone inhibits basic fibroblast growth factor-stimulated gastric epithelial cell proliferation

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ABSTRACT

Basic fibroblast growth factor (bFGF) is essential for gastric ulcer healing, whereas glucocorticoids delay gastric ulcer healing. We found that dexamethasone inhibited bFGF-stimulated rat gastric epithelial RGM-1 cells proliferation and attempted to elucidate the possible mechanistic pathway. Flowcytometry was used to determine cell proliferation. Western blot and RT-PCR were performed to evaluate changes in signaling pathways. Results showed that bFGF significantly increased mRNA expression of FGF receptor (FGFR)1 and FGFR2 at 10 min and increased expression of phosphorylated extracellular signal-regulated kinase (pERK1/pERK2) but not phosphorylated p38 mitogen-activated protein kinase (MAPK) or phosphorylated phosphatidylinositol 3-kinase (PI3K) within 30 min. This was followed by an increase of cyclooxygenase (COX)-2 mRNA and protein expression at 30 and 240 min, respectively. Mitogen-activated protein kinase kinase (MEK) inhibitor-PD98059 (10^{-5} M) markedly suppressed bFGF-stimulated COX-2 expression and cell proliferation, but neither p38 MAPK inhibitor-SB203580 nor PI3K inhibitor-Wortmannin had any effect. Dexamethasone (10^{-6} M) substantially reduced bFGF-stimulated ERK activation at 10 min, COX-2 mRNA and protein expression at 30 and 240 min, respectively, and prostaglandin E_2 synthesis at 8 h. Dexamethasone (10^{-6} M) also significantly decreased mRNA expression of FGFR1 and FGFR2 at basal and bFGF-stimulated conditions at 10 min. This study indicated that bFGF-stimulated gastric epithelial RGM-1 cells proliferation via up-regulating FGFR1 and FGFR2, activating ERK1/ERK2 signal transduction pathway and COX-2 pathway. Dexamethasone significantly suppresses bFGF-stimulated RGM-1 cells proliferation in part via down-regulation of FGFR1/FGFR2, then decreasing bFGF-stimulated activation of ERK1/ERK2, followed by inhibition of COX-2 activation, and finally DNA synthesis.

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1. Introduction

The repair and healing of gastric ulcer requires the reconstruction of epithelial structures and the underlying connective tissue, including blood vessels and muscle layers [1–3]. Several growth factors have been implicated in ulcer healing process, since they are able to regulate cell proliferation, migration, differentiation, secretion, and degradation of extracellular matrix, all of which are essential during tissue healing [1,4–6]. The expression of these growth factors and their receptors are strongly increased over the ulcer margin or injured gastric mucosa [1]. Among these growth factors, epidermal growth factor, transforming growth factor, and hepatocyte growth factor play a major role in epithelial reconstruction; basic fibroblast growth factor (bFGF), vascular endothelial growth factor, and platelet-derived growth factor are mainly involved in the reconstruction of connective tissue [1,4–8].

Basic fibroblast growth factor belongs to the large family of fibroblast growth factors consisting of at least of 18 members which are produced from a single mRNA encoded by a single copy gene [9]. On the cell surface, bFGF interacts with a set of specific cell membrane receptors belonging to the FGF receptor (FGFR) family. Each receptor has an intracellular domain with intrinsic tyrosine kinase activity [10]. The activation of FGFR tyrosine kinase initiates the intracellular signaling cascade and activates mitogen-activated protein kinase (MAPK), and finally regulates intra-nuclear gene expression [1]. Thus bFGF is able to control several distinct activities via autocrine, paracrine, intracrine, and different signal transduction pathways, depending on its specific cellular localization [11]. The expression of bFGF mRNA has been shown to increase in the submucosal area days after an acetic acid-induced gastric ulcer in rats, with an increase in expression over the ulcer granulation tissue after 1–2 weeks [12]. In human gastric ulcer, bFGF immunoreactivity and its mRNA expression were both increased in smooth muscle fibers, fibroblasts, and microvessels of the granulation tissue [13]. In addition, bFGF is also found to induce cyclooxygenase (COX)-2 expression in different tissues and cell lines [14–16] and COX-2 activation with increased prostaglandin (PG) formation is essential for cell proliferation and ulcer healing [2,3]. Moreover, bFGF may accelerate ulcer healing significantly via increasing the density of microvessels in the ulcerated tissue and via promoting epithelial cell proliferation [4,5,8]. However, the mechanism on how bFGF stimulates gastric epithelial cell proliferation is not clear at present.

Our previous study showed that dexamethasone delayed rat gastric ulcer healing partially due to inhibition of epithelial cell proliferation of the gastric ulcer margin [2]. This in vitro study clearly showed that bFGF significantly stimulated proliferation of rat gastric mucosal epithelial RGM-1 cell and dexamethasone significantly inhibited bFGF-stimulated RGM-1 cell proliferation. This study also further elucidated the possible mechanistic pathways through which dexamethasone-inhibited bFGF-stimulated gastric epithelial cell proliferation.

2. Materials and methods

2.1. Chemicals and preparation of reagents

All study chemicals were purchased from Sigma–Aldrich (Sigma–Aldrich Biotechnology, St. Louis, MO) unless otherwise specified. Glucocorticoid receptor antagonist-mifepristone, mitogen-activated protein kinase kinase (MEK) inhibitor-PD98059, U0126, p38MAPK inhibitor-SB203580, phosphatidylinositol 3-kinase (PI3K) inhibitor-Wortmannin, and COX-2 inhibitor-NS398 were dissolved in dimethylsulfoxide (DMSO). bFGF (recombinant human basic fibroblast growth factor) was purchased from Invitrogen (Grand Island, NY), and 10 μg bFGF was dissolved in 0.01 M Tris–HCl with 0.2% bovine serum albumin (BSA) as a stock solution.

2.2. Cell culture

The RGM-1 rat gastric mucosal cells were established from normal Wistar's rat (RCB-0876 at Riken Cell Bank, Tsukuba, Japan). RGM-1 cells are homogenous epithelial-like cells with positive cytokeratin staining and were negative for Bowie staining for chief cells and succinic dehydrogenase activity for parietal cells. These characteristics indicate that RGM-1 cells are epithelial in origin and like mucous epithelial cells or mucous neck cells [17].

RGM-1 cells were grown in Dulbecco's modified Eagle's medium (D-MEM)/F-12 medium (Gibco-BRL, Grand Island, NY) supplemented with 100 U/ml penicillin G, 100 μg/ml streptomycin, and 20% fetal bovine serum (FBS) (Gibco-BRL) in an incubator at 37 °C and 5% carbon dioxide. Cells were free from mycoplasma contamination as tested by Hoechst 33342 staining (Invitrogen, Grand Island, NY). Cells were detached from the wells using 0.25% trypsin EDTA. Trypan blue exclusion assay was used to assess cell viability. Cell numbers were counted using a Burker hemocytometer (Marienfeld GmbH, Marienfeld, Germany).

2.3. Flowcytometry analysis

Cellular DNA replication was analyzed by flowcytometry as described previously [18]. The two peaks of DNA content corresponding to G1 and G2/M phase cells, respectively, and the intermediate amount of DNA corresponding to S phase cells were counted. RGM-1 cells were seeded in a 10 cm dish at roughly 10^5 cells/ml and were allowed to grow in D-MEM/F-12 medium containing 20% FBS for 24 h. In order to synchronize cells, they were cultured in the same medium with 1% FBS for 24 h and this resulted in a 90% of cells synchronized at G0/G1 phase. The cells were then treated for 3, 6, 12, and 24 h with 1% FBS D-MEM/F-12 medium containing bFGF (10 and 50 ng/ml). For drug treatment, synchronized cells were treated for 24 h with 1% FBS D-MEM/F-12 medium containing either bFGF (50 ng/ml), dexamethasone (10^{-6} M), or mifepristone (10^{-6} M)—a glucocorticoid receptor antagonist. The following combinations were also applied: bFGF (50 ng/ml) with dexamethasone (10^{-8} or 10^{-6} M) in the presence or absence of mifepristone (10^{-6} M); bFGF (50 ng/ml) with MEK inhibitor-PD98059 (10^{-5} M), p38MAPK inhibitor-SB203580 (10^{-5} M), or PI3K inhibitor-Wortmannin (10^{-6} M); bFGF (50 ng/ml) with

COX-2 inhibitor-NS398 (10^{-5} M). After treatment, cells (about 3×10^6 each dish) were trypsinized, pelleted, washed with phosphate buffered saline (PBS), repelleted and resuspended with lysis buffer (0.5% Triton-X 100, 0.2 μg/ml $Na_2EDTA{\cdot}2H_2O$, 1% BSA) for 15 min. The cells were fixed in 80% cold methanol at −20 °C overnight. The fixed cells were then centrifuged, washed with PBS, pretreated with RNase (5 Kunitz U/ml) at 37 °C for 30 min and then reacted with propidium iodide (50 μg/ml). The cells were analyzed using a FACSCalibur flowcytometer (Becton Dickinson, Mountain View, CA). Data were analyzed using ModFit and CellQuest software [18].

2.4. Western blot analysis

After synchronization as described above, the cells were treated with 1% FBS D-MEM/F-12 containing 50 ng/ml bFGF for 5, 10, 30, 60, and 180 min. In a second set of experiments, cells were treated with 1% FBS D-MEM/F-12 containing 50 ng/ml bFGF for 10 min in the presence or absence of dexamethasone (10^{-6} M), or pretreated with dexamethasone (10^{-6} M) for 2 or 4 h. In a third set of experiment, cells were incubated with 50 ng/ml bFGF in the presence or absence of dexamethasone (10^{-6} M), PD98059 (10^{-5} M), U0126 (10^{-5} M), or dexamethasone (10^{-6} M) alone for 4 h. The cells were then collected in radioimmunoprecipitation assay buffer for Western blot analysis. Following sonication and centrifugation, protein concentration was measured using a protein assay kit (BCA™ Protein Assay Kit, Pierce, Rockford, IL). Proteins were separated by SDS-polyacrylamide gel electrophoresis overlaid with a 10% acrylamide stacking gel, and then transferred to Hybond C nitrocellulose membranes (Amersham International Plc, Amersham, UK). Membranes were probed with antibodies against ERK1/2, p38 MAPK, PI3K, COX-1, COX-2, and β-actin (Santa Cruz Biotechnology Inc., Santa Cruz, CA), as well as pERK1/2, pp38 MAPK, and pPI3K, (Cell Signaling Technology, Beverly, MA) overnight at 4 °C and incubated for 1 h with secondary antibodies conjugated with peroxidase. The membrane was developed using the enhanced chemiluminescence system (Amersham Biosciences, Piscataway, NJ) and was exposed to an X-ray film (Fuji Photo Film, Tokyo, Japan). Quantitation was performed using a densitometer (Scan Marker III, Microtek, Carson, NV).

2.5. Measurement of PGE_2 level

After synchronization as described above, cells were treated with 50 ng/ml bFGF in the presence or absence of dexamethasone (10^{-8} and 10^{-6} M), or dexamethasone (10^{-8} and 10^{-6} M) alone for 8 h, cells were homogenized with homogenizing buffer (0.05 M Tris–HCl at pH 7.4, 0.1 M NaCl, 0.001 M $CaCl_2$, 1 mg/ml D-glucose, 28 μM indomethacin to inhibit further PGE_2 formation) for 30 s. After centrifugation at 12,000 rpm for 15 min at 4 °C, the supernatants were assayed by using a commercial available PGE_2 enzyme-linked immunosorbent assay kit (Quantikine, R&D systems Inc., Minneapolis, MN). The assay procedures were in accordance to the protocol suggested in the kit. The optical densities were determined with the MRX microplate reader at 405 nm (Dynex Technologies, Inc. Chantilly, VA). The amount of protein in the sample was determined by a protein assay kit and the intracellular PGE_2 level was expressed as pg/mg protein. The culture media of each group were also harvested for analysis of extracellular PGE_2 level.

2.6. Total RNA isolation and mRNA expression of FGFRs, COXs determined by RT-PCR

After synchronization, cells were treated with 1% FBS D-MEM/F-12 containing 50 ng/ml bFGF for different period in the presence or absence of dexamethasone (10^{-8} and 10^{-6} M). Total RNA was isolated from the cells using the RNeasy Mini kit (Qiagen, AMBION Inc., Austin, Texas). cDNA was synthesized using an Invitrogen Superscrip II First-strand synthesis system (Invitrogen, Grand Island, NY). Reverse transcriptase-polymerase chain reaction (RT-PCR) primer sequences were designed according to the published cDNA sequence for rats from NCBI Gene Bank and the Primer 3 program. The sequences of forward and reverse primers are: FGFR1 5′-CTCTGTGGTGCCTTCTGACA-3′ and 5′-GGAAGTCGCTCTTCTTGGTG-3′, respectively with a product of 572 bp; FGFR2 5′-AATACGCATCGAAAGGCAAC-3′ and 5′-CAGTTCATTGGTGCAGTTGG-3′, respectively with a product of 503 bp; COX-1 5′-GGCGTT GCTCATCCATCTA CTC-3′ and 5′-AGCATCTGTGAGCAGTAC CGG-3′, respectively with a product of 116 bp; COX-2 5′-TTTGTTGAGTCATTCACCAGA CAGAT-3′ and 5′-ACGATGTGTAAGGTT TCAGGGAGAAG-3′, respectively with a product of 169 bp. Concomitantly, amplification of control rat β-actin (forward primer: 5′-GGCATCCTGACCCT-GAAGTA-3′; reverse primer: 5′-TTTGAGACCTTCAACACCCC-3′ with a product of 203 bp) was performed on the same sample to verify RNA integrity. The PCR conditions for COX-1 and COX-2 were 95 °C for 3 min, followed by 26 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 75 °C for 60 s, finally completed by extension at 72 °C for 10 min. The PCR conditions for FGFR1 and FGFR2 were 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, and elongation at 75 °C for 60 s, finally completed by extension at 72 °C for 10 min. Each PCR product was electrophoresed on 2% agarose gel stained with ethidium bromide, and then visualized under UV light. Localization of the predicted PCR product was confirmed using 100-bp ladder as a standard marker. Quantitation of PCR product was performed using Image-J system (NCBI published program).

2.7. Statistical analysis

Results are expressed as means ± S.D. There were four to six samples in each group. Differences between the means were analyzed with the Student's *t*-test when appropriate. Bonferroni correction was performed to adjust for the fact that multiple comparisons were done in each experiment. A *p*-value <0.05 was considered as statistically significant.

3. Results

3.1. Proliferation of RGM-1 cells

Treatment with bFGF 10 and 50 ng/ml for 3 and 6 h had no significant effect on the growth of rat gastric epithelial RGM-1 cells either in cell number or S phase cells (Table 1). Longer

Table 1 – Effect of basic fibroblast growth factor (bFGF) treatment on RGM-1 cell proliferation

Treatment duration (h)	Cell count (10^5 ml^{-1})			Cell in S phase of cell cycle (% change from control)		
	Control	bFGF		Control	bFGF	
		10 ng/ml	50 ng/ml		10 ng/ml	50 ng/ml
3	6.1 ± 0.6	6.0 ± 0.7	6.0 ± 0.6	100 ± 10	100 ± 4	100 ± 9
6	8.0 ± 0.7	8.1 ± 0.6	8.0 ± 0.6	100 ± 9	101 ± 10	100 ± 10
12	18.5 ± 1.5	19.1 ± 1.7	18.9 ± 1.9	100 ± 9	162 ± 14***	150 ± 14***
24	32.2 ± 2.3	44.0 ± 2.8***	40.8 ± 3.4***	100 ± 11	185 ± 13***	161 ± 11***

RGM-1 cells were incubated with 10 or 50 ng/ml bFGF for 3, 6, 12, and 24 h. Cell numbers were counted using a hemocytometer, and the S phase of the cell cycle of the RGM-1 cells was determined by flowcytometry. Data were analyzed using ModFit and CellQuest software. Values are means ± S.D. from six samples per group.***$p < 0.001$ when compared with the control group.

treatment with bFGF for 12 and 24 h significantly stimulated RGM-1 cells proliferation (S phase cells) (Table 1). Therefore, we applied 24-h treatment with 50 ng/ml bFGF for further study and found that dexamethasone (10^{-8} and 10^{-6} M) significantly inhibited bFGF-stimulated cell proliferation (Fig. 1). This is a specific inhibition because mifepristone (10^{-6} M) completely blocked the inhibitory action of dexamethasone (10^{-6} M) on bFGF-stimulated cell proliferation. Neither dexamethasone (10^{-6} M) nor mifepristone (10^{-6} M) alone had impact on cell proliferation compared with that in the control group (Fig. 1).

Further study showed that PD98059 (10^{-5} M) (MEK inhibitor), but not SB203580 (10^{-5} M) (p38MAPK inhibitor) nor Wortmannin (10^{-6} M) (PI3K inhibitor) significantly inhibited bFGF-stimulated RGM-1 cell proliferation (Fig. 2). In addition,

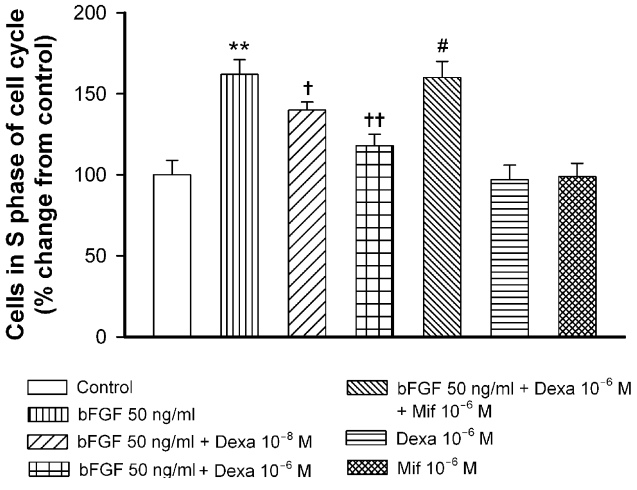

Fig. 1 – Effect of basic fibroblast growth factor (bFGF) and dexamethasone (Dexa) on proliferation of the RGM-1 cells. RGM-1 cells were incubated with 50 ng/ml bFGF, Dexa (10^{-6} M), mifepristone (Mif) (10^{-6} M), or combination of bFGF and Dexa in the presence or absence of Mif for 24 h. The percentage of the S phase RGM-1 cells was determined by flowcytometry. Data were analyzed using ModFit and CellQuest software. Values are means ± S.D. from six samples per group. $^{**}p < 0.01$ when compared with the control group, $^{+}p < 0.05$ and $^{++}p < 0.01$ when compared with the bFGF-treated group, $^{\#}p < 0.05$ when compared with the bFGF + Dexa (10^{-6} M)-treated groups.

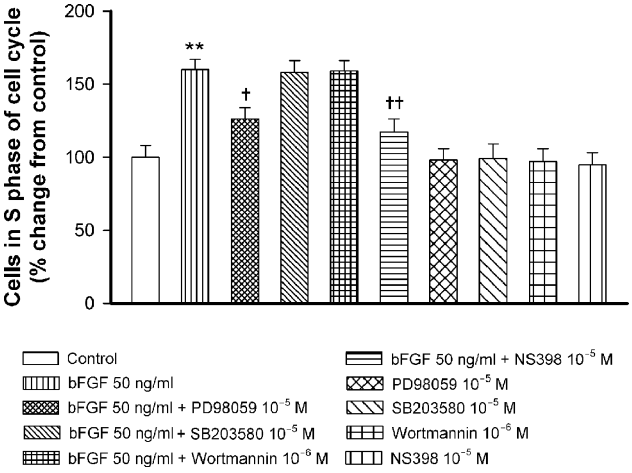

Fig. 2 – Effect of basic fibroblast growth factor (bFGF) and inhibitors of signal transduction pathways on cell proliferation of the RGM-1 cells. RGM-1 cells were incubated with 50 ng/ml bFGF, PD98059 (10^{-5} M), SB203580 (10^{-5} M), Wortmannin (10^{-6} M), NS398 (10^{-5} M), or combination of bFGF with previous inhibitors for 24 h. The percentage of S phase RGM-1 cells was determined by flowcytometry. Data were analyzed using ModFit and CellQuest software. Values are means ± S.D. from six samples per group. $^{**}p < 0.01$ when compared with the control group, $^{+}p < 0.05$, $^{++}p < 0.01$ when compared with the bFGF-treated group.

NS398 (10^{-5} M) (the COX-2 inhibitor) also significantly inhibited bFGF-stimulated RGM-1 cell proliferation. However, PD98059, SB203580, Wortmannin, or NS398 alone treatment had no effect on cell proliferation when compared with that in the control group (Fig. 2).

3.2. *Signal transduction after bFGF treatment*

After cells were treated with 50 ng/ml bFGF for 0 (control), 5, 10, 30, 60, and 180 min, it was found that 50 ng/ml bFGF markedly increased the expression of pERK1/pERK2 from 5, 10, to 30 min with maximal effect at 10 min, but the expression of total ERK1/ERK2 from 5 to 180 min remained the same (Fig. 3). bFGF treatment (50 ng/ml) for 5–180 min did not increase the

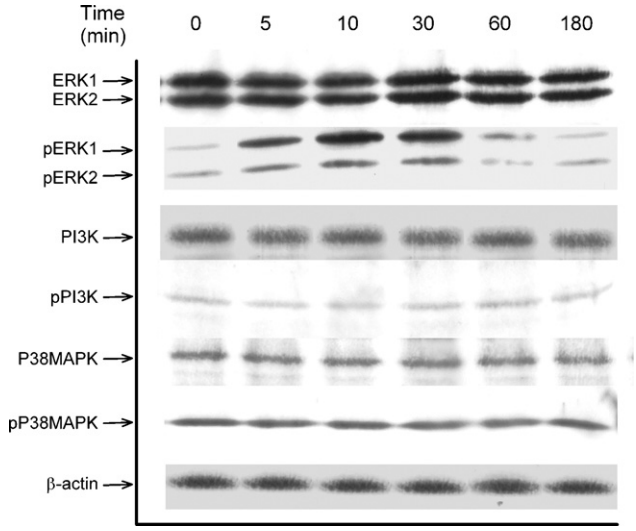

Fig. 3 – The time-course effect of basic fibroblast growth factor (bFGF) on protein expression of signal transduction pathways: extracellular signal-regulated kinase (ERKs), phosphorylated ERK1/ERK2; P38 mitogenic activated protein kinases (MAPK), phosphorylated P38 MAPK (pP38MAPK), phosphatidyl inositol 3-kinase/protein kinase B (PI3K), and phosphorylated PI3K (pPI3K). Cells were incubated with bFGF 50 ng/ml for 0–180 min. Western blot analysis was performed; bands of ERK1/ERK2, pERK1/pERK2, P38 MAPK, pP38MAPK, PI3K, and pPI3K are representative of three separate assays.

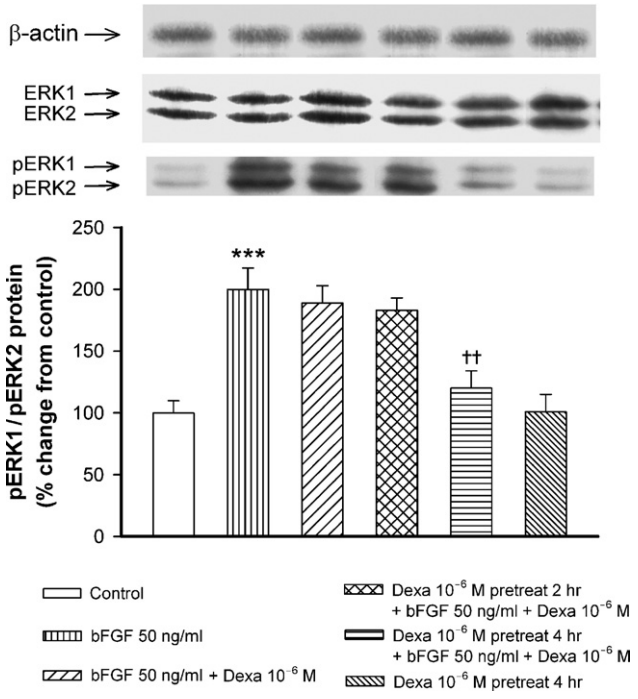

Fig. 4 – The effect of basic fibroblast growth factor (bFGF), bFGF plus dexamethasone (Dexa), and Dexa alone on the expression of ERK1/ERK2 and pERK1/pERK2 in the RGM-1 cells. Cells were incubated with 50 ng/ml bFGF for 10 min in the presence or absence of Dexa (10^{-6} M), with or without pretreatment with Dexa (2 or 4 h), or Dexa alone (10^{-6} M) for 4 h. Western blot analysis was performed; the bands shown ERK1/ERK2, pERK1/pERK2 are representative of three separate assays. Values are means ± S.D. from six samples per group. $^{***}p < 0.001$ when compared with the control group, $^{++}p < 0.01$ when compared with the bFGF-treated group.

expression of P38 MAPK/pP38MAPK, or PI3K/pPI3K when compared with the control group (Fig. 3).

3.3. *Dexamethasone antagonized the bFGF-stimulated signal transduction*

Incubation of RGM-1 cells with bFGF (50 ng/ml) and dexamethasone (10^{-6} M) combined for 10 min did not alter the expression of ERK1/ERK2, pERK1/pERK2 compared with the bFGF-treated group (Fig. 4). However, pretreatment of RGM-1 cells with dexamethasone (10^{-6} M) for 4 h but not for 2 h significantly reduced the bFGF-stimulated expression of pERK1/pERK2 (Fig. 4). On the other hand, incubation of RGM-1 cells with bFGF (50 ng/ml) and dexamethasone (10^{-6} M) combined for 4 h significantly decreased the bFGF-stimulated COX-2 expression but not COX-1 expression when compared with the bFGF group (Fig. 5). We also found that incubation of RGM-1 cells with bFGF (50 ng/ml) and PD98059 (10^{-5} M) or bFGF (50 ng/ml) and U0126 (10^{-5} M) for 4 h significantly decreased the bFGF-stimulated COX-2 expression when compared with the bFGF group (Fig. 5).

3.4. *Effects of bFGF and dexamethasone on PGE_2 level*

bFGF (50 ng/ml) treatment significantly increased intracellular PGE_2 level when compared with the control group (Fig. 6A). Again, dexamethasone treatment (10^{-8} and 10^{-6} M) decreased bFGF-stimulated intracellular PGE_2 level and reached a significant difference in the higher concentration group (10^{-6} M) when compared with the bFGF-treated group (Fig. 6A). A similar trend of the effect of bFGF and dexamethasone on extracellular PGE_2 was observed. bFGF treatment also resulted in an increase of extracellular PGE_2 level and dexamethasone decreased bFGF-stimulated extracellular PGE_2 level, it did not reach a significant difference (Fig. 6B).

3.5. *mRNA expression of FGFRs and COXs after bFGF treatment in the presence or absence of dexamethasone*

Incubation of RGM-1 cells with bFGF (50 ng/ml) for 10 min significantly increased the mRNA expression of FGFR1 and FGFR2 with higher mRNA expression of FGFR2 than FGFR1 when compared with the control group (Fig. 7). Pretreatment but not co-treatment of RGM-1 cells with dexamethasone (10^{-8} and 10^{-6} M) for 4 h significantly reduced the mRNA level of FGFR1 and FGFR2 when compared with the control group. In addition, this pretreatment with dexamethasone also significantly reduced bFGF-stimulated mRNA expression of

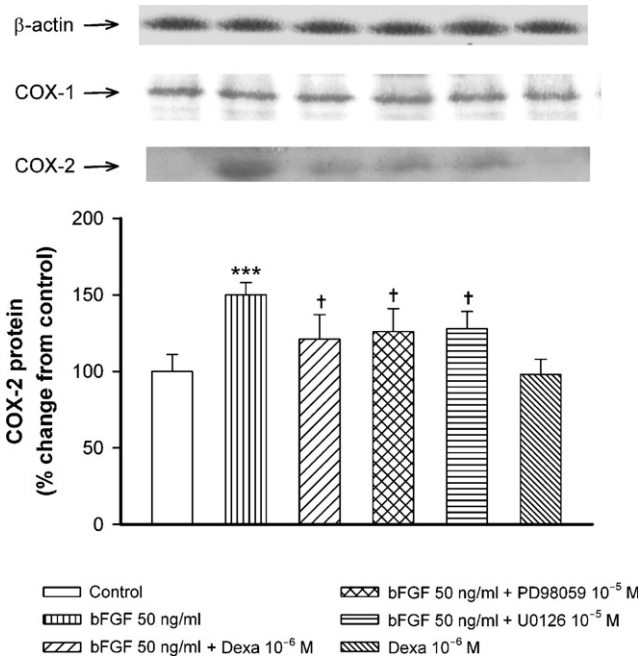

Fig. 5 – The effect of basic fibroblast growth factor (bFGF), bFGF plus dexamethasone (Dexa), bFGF plus MEK inhibitors on the expression of cyclooxygenase (COX-1) and COX-2 in the RGM-1 cells. Cells were incubated with 50 ng/ml bFGF in the presence or absence of Dexa (10^{-6} M), PD98059 (10^{-5} M), U0126 (10^{-5} M), or Dexa alone (10^{-6} M) for 4 h. Western blot analysis was performed; the bands shown COX-1, COX-2 are representative of three separate assays. Values are means ± S.D. from six samples per group. $^{***}p < 0.001$ when compared with the control group, $^{+}p < 0.05$ when compared with the bFGF-treated group.

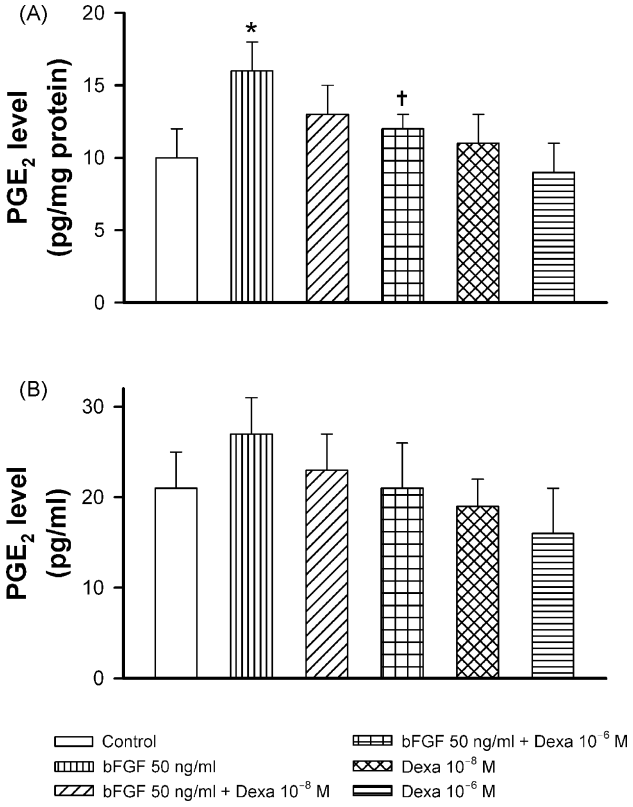

Fig. 6 – Effect of basic fibroblast growth factor (bFGF) and dexamethasone (Dexa) on prostaglandin E_2 (PGE_2) formation in the RGM-1 cells. Cells were incubated with bFGF 50 ng/ml in the absence or presence of Dexa (10^{-8} and 10^{-6} M) or Dexa (10^{-8} and 10^{-6} M) alone for 8 h. (A) Intracellular PGE_2 level was measured by enzyme-linked immunosorbent assay (ELISA) kit and was expressed as pg/mg protein. (B) Extracellular PGE_2 level was measured by ELISA kit from culture medium of each treated group and was expressed as pg/ml. Values are means ± S.D. from four samples per group. $^{*}p < 0.05$ when compared with the control group. $^{+}p < 0.05$ when compared with the bFGF-treated group.

FGFR1 and FGFR2 when compared with the bFGF-treated group (Fig. 7). Furthermore, bFGF treatment also significantly increased the COX-2 mRNA expression after 30 min, and the response persisted up to 180 min (Fig. 8). Pretreatment of RGM-1 cells with dexamethasone (10^{-8} and 10^{-6} M) for 4 h significantly decreased the bFGF-stimulated mRNA expression of COX-2 at 30 min when compared with the bFGF-treated group (Fig. 8).

4. Discussion

This study, for the first time, demonstrates that bFGF stimulates proliferation of gastric epithelial RGM-1 cells proliferation via up-regulation of its receptors, activation of ERK–COX signal transduction pathway. Dexamethasone treatment of RGM-1 cells significantly suppresses bFGF-stimulated cell proliferation via down-regulation of FGFR1/FGFR2 receptor, then decreasing bFGF-stimulated activation of ERK1/ERK2, followed by inhibition of COX-2 activation.

The activation of the FGF receptor tyrosine kinase initiates the intracellular signaling cascade and activates MAPK which are important in regulating cell proliferation, migration and differentiation [19,20]. Previous studies have shown that the COX-2 expression is regulated by MAPK subtypes, such as ERK1/ERK2, p38 MAPK, depending on the types of extracellular stimuli and cells [21,22]. This study clearly showed that bFGF treatment up-regulated the expression of FGFR1 and FGFR2 (Fig. 7) which lead to subsequent activation of ERK1/2 signal transduction pathway within 10 min, but not the P38MAPK and PI3K pathways, then increases the COX-2 expression after 30 min and, finally promotes RGM-1 cells proliferation. In addition, dexamethasone, a glucocorticoid frequently used clinically, suppresses bFGF-induced pERK1/pERK2 (Fig. 4), COX-2 expression (Figs. 5 and 8), PGE_2 synthesis (Fig. 6), and inhibits RGM-1 cells proliferation (Fig. 1). Our experimental findings are different from Tessner's study which showed that bFGF up-regulated COX-2 in intestinal epithelial cell line (I407 cells) through the p38MAPK pathway [16]. One

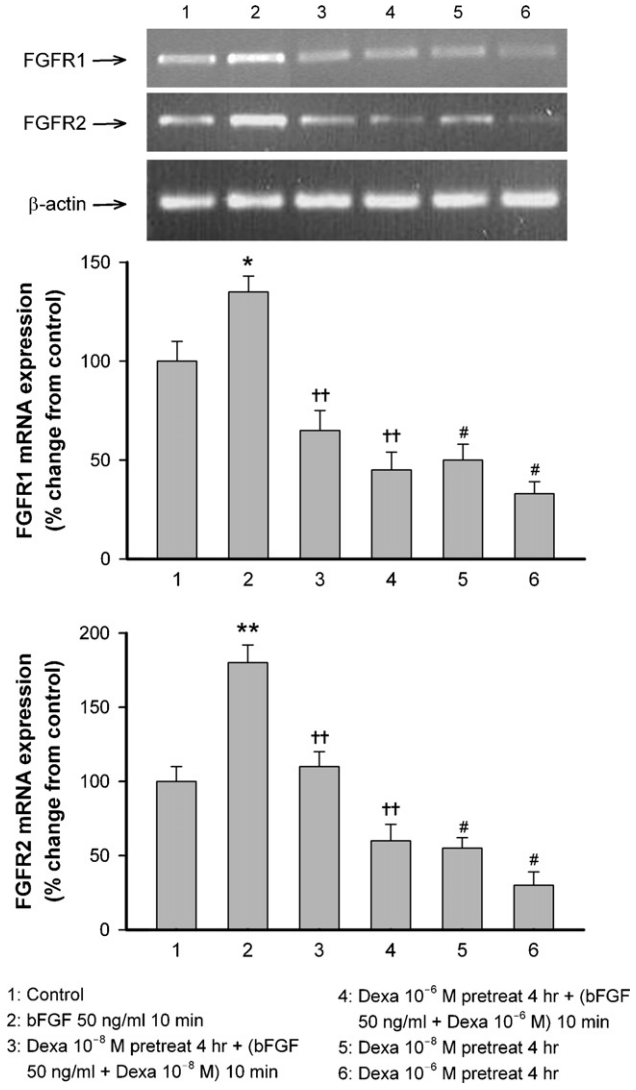

Fig. 7 – The effect of basic fibroblast growth factor (bFGF), bFGF plus dexamethasone (Dexa), or Dexa treatment alone on the mRNA expression of FGF receptors 1 and 2 in the RGM-1 cells. Cells were incubated with 50 ng/ml bFGF for 10 min in the presence or absence of Dexa pretreatment (10^{-8} and 10^{-6} M) for 4 h or Dexa alone (10^{-8} and 10^{-6} M) for 4 h. RT-PCR was performed; the bands shown FGFR1, FGFR2 are representative of two separate assays. Values are means ± S.D. from four samples per group. $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with the control group, $^{++}p < 0.01$ when compared with the bFGF-treated group, $^{\#}p < 0.05$ when compared with the control group.

possible explanation is that the different types of cells respond differently to bFGF. Current experimental findings are corresponded well with results from previous in vivo studies, which demonstrated that ulcerated gastric mucosa-treated with bFGF activated the COX-2 expression [15] and dexamethasone delayed ulcer repair via limiting epithelial cell proliferation at the ulcer margin by suppressing the COX-2 expression and PG synthesis in rat stomachs [2].

This study showed that dexamethasone decreased FGFR1 and FGFR2 expression at basal condition and at bFGF-stimulated condition (Fig. 7). Our experimental findings are different from other studies which revealed transforming growth factor combined with dexamethasone increased FGFR2 mRNA expression in human adult stem cell derived from bone marrow stroma [23]; dexamethasone did not change FGFR1 mRNA expression in PC12 cells derived from adrenal medulla [24]. To the best of our knowledge, there is no data demonstrating that dexamethasone or glucocorticoids down-regulated growth factor receptor and inhibited growth factors-stimulated cell proliferation via down-regulating their receptors in in vivo or in vitro studies. It will be interesting to investigate whether glucocorticoids have an inhibitory action in the transcription of FGFR or other growth factor receptors. Our results also showed that the glucocorticoid-receptor antagonist, mifepristone, significantly and completely reversed the inhibitory action of dexamethasone on bFGF-stimulated cell proliferation (Fig. 1). The finding suggests that the inhibitory action of dexamethasone was glucocorticoid-receptor mediated.

The mucosal bFGF concentration at human gastric ulcer margin which are expressed as pg bFGF per mg wet of biopsy specimen ranged from 10 pg/mg to 400 pg/mg [13,25]. The concentration is around 10–400 ng/ml if one estimates that 1 mg biopsy gastric specimen is about equal to the weight of 0.001 ml water. The bFGF concentrations we used in this study were 10–50 ng/ml which is also similar to other in vitro studies [14,16], and are within the physiological concentration of gastric ulcerated mucosa in humans. The concentration of dexamethasone (10^{-8} and 10^{-6} M) we used in this study was also similar to the pharmacologic concentration found in plasma of patients treated with dexamethasone [26,27]. Therefore, the stimulatory effect of bFGF on gastric epithelial cell proliferation and the inhibitory effect of dexamethasone on bFGF-stimulated gastric epithelial cell proliferation resemble a real life situation.

Our previous study demonstrated that EGF stimulated RGM-1 cell proliferation partially via activation of ERK1/ERK2 signal transduction pathway, followed by COX-2 activation [18]. This study demonstrated that bFGF promotes RGM-1 cell proliferation partially via activation of ERK-COX-2 pathway, but not P38MAPK, nor PI3K pathway. In addition, bFGF significantly upregulated mRNA expression of FGF receptors (Fig. 7) but not EGFR (data not shown). Our preliminary results showed that EGF significantly activated EGFR mRNA expression but not FGFR1 nor FGFR2 mRNA expression (data not shown). These findings suggested that bFGF and EGF activated their own receptors, respectively, to stimulated RGM-1 cell proliferation in part through the common down-stream ERK-COX-2 signal transduction pathways.

In conclusion, we found that bFGF-stimulated gastric epithelial RGM-1 cells proliferation via up-regulating its receptors (FGFR1 and FGFR2), activating ERK1/ERK2, and the COX-2 pathway. Dexamethasone significantly suppressed bFGF-stimulated gastric epithelial cell proliferation at least in part via the down-regulation of FGFR1 and FGFR2, inhibiting activation of ERK1/ERK2, followed by inhibition of COX-2 activation, and finally DNA synthesis.

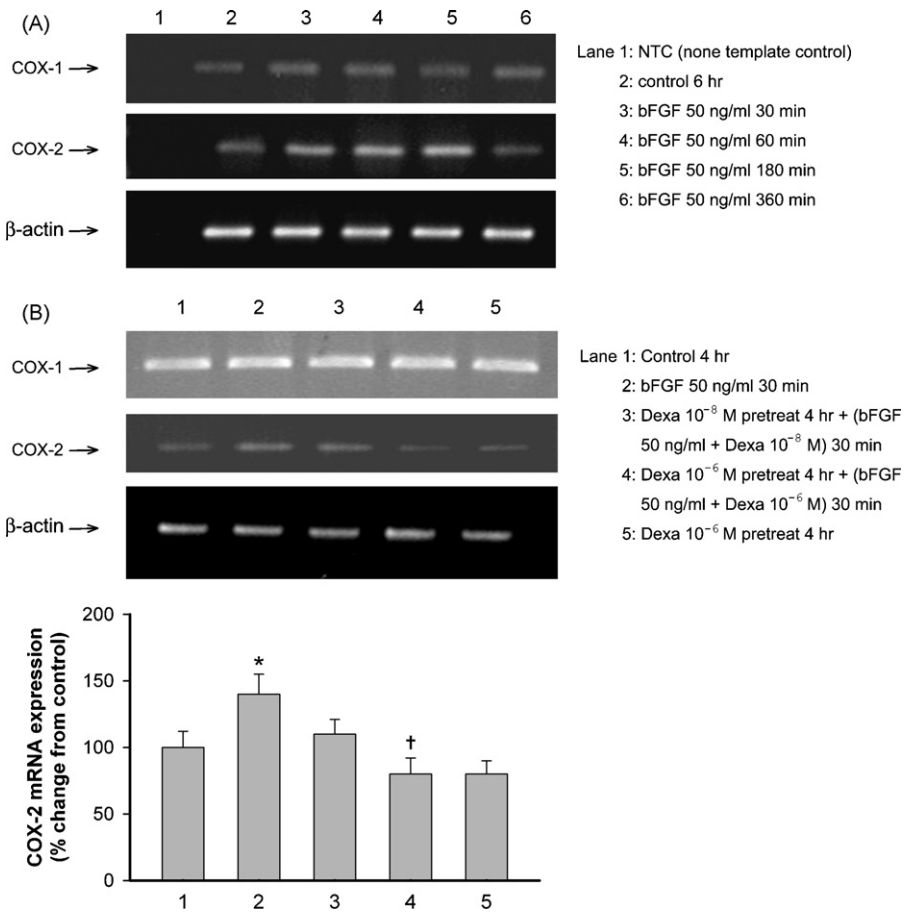

Fig. 8 – The effect of basic fibroblast growth factor (bFGF), bFGF plus dexamethasone (Dexa), or Dexa alone on the mRNA expression of cyclooxygenase (COX-1) and COX-2 in the RGM-1 cells. (A) Cells were incubated with 50 ng/ml bFGF for 30 min, 60 min, 180 min, and 360 min or without bFGF at 6 h as control. RT-PCR was performed; the bands shown COX-1, COX-2 are representative of two separate assays. (B) Cells were incubated with 50 ng/ml bFGF for 30 min in the presence or absence of Dexa pretreatment for 4 h and co-treatment (10^{-8} and 10^{-6} M) or Dexa alone (10^{-6} M for 4 h). RT-PCR was performed; the bands shown COX-1, COX-2 are representative of two separate assays. Values are means ± S.D. from four samples per group. $^{*}p < 0.05$ when compared with the control group, $^{+}p < 0.05$ when compared with the bFGF-treated group.

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