

**PROSTAGLANDIN E2 REGULATES THE EXPRESSION OF BASIC
FIBROBLAST GROWTH FACTOR MESSENGER RNA IN NORMAL
HUMAN FIBROBLASTS**

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KEY WORDS

prostaglandin E2; basic fibroblast growth factor; fibroblast; human; wound healing

ABSTRACT

Prostaglandin E2 (PGE2) has been reported to control angiogenesis and play an important role in wound healing in soft tissues, although the precise mechanism is still unknown. Since basic fibroblast growth factor (bFGF) has been reported to generate neovascularization, PGE2 and bFGF might work closely, or one might control the expression of the other.

In this study, we demonstrate that PGE2 enhances the expression of bFGF in normal human fibroblasts, and that calcium ionophore, A23187, and adenylate cyclase activator, forskolin, also enhances the expression bFGF mRNA. These results suggest that enhancement of bFGF mRNA stimulated by PGE2 is mainly controlled through EP1 or EP2 and EP4 receptor.

In conclusion, our findings suggest that the mechanism of PGE2-induced angiogenesis and wound healing in soft tissue could be mediated by bFGF through EP1 or EP2 and EP4 receptor.

INTRODUCTION

Prostaglandin E2 (PGE2) is produced from arachidonic acid by means of the activity of cyclooxygenase enzyme. Its expression is enhanced at sites of inflammation in soft tissues, and it performs various biological activities. PGE2 has been shown to stimulate new bone formation(31, 33, 34) and its suppression causes gastric mucosal injury(26). In several studies, PGE2 has induced angiogenesis in vivo and in vitro(4, 18, 22, 23). Joyce et al.(23) reported that PGE2 induced angiogenesis in corneal endothelial cells, and they suggested that PGE2 was a mediator of wound repair. PGE2, furthermore, has cytoprotective properties against toxic chemicals in various types of tissues (11, 14, 36, 38). These reports suggest that PGE2 has an ability to control cytoprotection and the repair of tissues. However, the mechanism of tissue repair or angiogenesis by PGE2 is poorly understood.

Recently, it has been reported that PGE2 stimulates hepatocyte growth factors in gastric fibroblasts(39), and enhances the expression of vascular endothelial growth factor and basic fibroblast growth factor (bFGF) in rat Muller cells(12).

PGE2 might control neovascularization or tissue healing via the expression of certain cytokines or growth factors.

Basic Fibroblast growth factor (bFGF), on the other hand, is the prototype of the fibroblast growth factor family, and has been isolated from various normal and malignant tissues(5, 9, 20, 35). This growth factor is one of the most potent inducers of formation of mesenchyme(37), angiogenesis(17, 19, 28), and caused rapid neovascularization in various tissues (13, 16, 21, 35). It stimulates the proliferation of all cell types involved in the wound healing process both in vivo and in vitro(20, 40), and it increases the formation of granulation tissue in vivo(6-8). Therefore, we postulated that PGE2 stimulates angiogenesis and wound healing through the induction of bFGF.

The goal of this study, therefore, was to evaluate the effect of PGE2 on soft tissue repair by detecting the expression of the mRNA of bFGF, which is one of the well-known factors of angiogenesis and tissue repair.

MATERIALS AND METHODS

Cell culture

Normal human WI-38 fibroblasts (Dainippon Seiyaku, Osaka, Japan) were obtained at passage 15 and were used between passages 20-25: They were cultured in Dulbecco's modified eagle's medium (DMEM) (Sigma Chemical Co., St Louis, MO, USA) containing 10% fetal bovine serum (FBS) (Sigma Chemical Co., St Louis, MO, USA) in a humidified

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atmosphere of 5% CO₂ at 37°C. The WI-38 cells were plated at 2 × 10⁵ cells onto 6-well dishes, 3.4 mm in diameter, (FALCON, Becton Dickinson Labware, Franklin Lakes, NJ, USA), and grown for 3 days until confluent. The medium was then replaced by DMEM containing 2% FBS and cultured for another 24 hours. The medium was then replaced again and reagents were added.

Reagents

Cells were treated with following reagents; PGE2 (Sigma Chemical Co., St Louis, MO, USA), forskolin (Research Biochemicals International, Natick, MA, USA) as an adenylate cyclase activator and calcium ionophore, A23187 (Sigma Chemical Co., St Louis, MO, USA). All reagents were dissolved in dimethylsulfoxide (DMSO) as a vehicle

Total RNA Isolation and Determination of b-FGF mRNA by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted directly from cell layer by using TRIzol reagent (GIBCO-BRL, Grand Island, NY, USA). Total RNA was reverse-transcribed with random hexanucleotide primers using a RT-PCR kit (Perkin-Elmer Gene Amp; Roche Molecular Systems, Branchburg, NJ, USA) according to the manufacturer's protocol. Reaction was performed 42 °C for 60 minutes and then consecutively for 5 minutes at 99 °C and at 4 °C, respectively. PCR was carried out using a thermal cycler (Gene Amp PCR System 2400, Perkin-Elmer Corp., Norwalk, Connecticut, USA).

The following primers were used for PCR.

Basic FGF (375bp) (15),

5'-GCTCTTAGCAGACATTGGAAG-3' and 5'-GTGTGTGCTAACCTTACCT-3'

Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (356bp) (1),

5'-GAGATGATGACCCTTTTGGC-3', and 5'-GTGAAGGTCGGAGTCAACG-3'

GAPDH used as an internal control. PCR amplifications for bFGF were performed for 27-30 cycles, and for GAPDH were performed for 25-27 cycles (95°C for 30 seconds, 55°C for 30 seconds, 72°C for 60 seconds). In each case, the last cycle ended with 7 minutes at 72°C. PCR products were visualized using electrophoresis through 1.5% agarose gel.

Semiquantitative image analysis of the PCR products on the gel

Each gel was photographed onto positive/negative Polaroid film under ultraviolet illumination. The negative image was then captured by using an imaging scanner. After the images were recorded in a computer, the band intensities were processed with the NIH image program (Ver.1.62) as described previously(3).

Statistical analysis

Stat View-J 4.5 software was used for all statistical analyses. Data are expressed as mean \pm S.D. Statistical analysis was carried out on all data points with regard to control by means of one-way ANOVA and Fisher's PLSD method. P values under 0.05 were considered statistically significant.

RESULTS

Effect of intensity of PCR products by PCR cycles

For semiquantitative analysis, we showed that the PCR products of bFGF were amplified linearly in 25-35 cycles (Fig.1-A), and those of GAPDH were amplified linearly in 20-30 cycles (Fig.1-B). Therefore the number of PCR cycles used in this study was within the linear range of PCR amplifications.

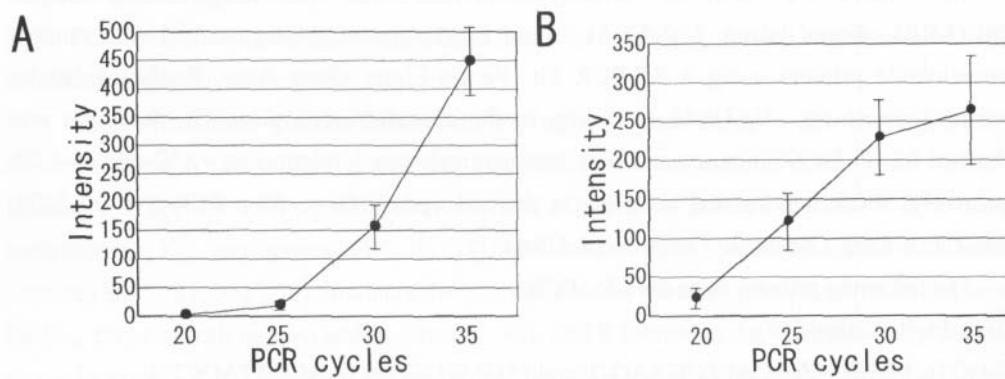


Fig. 1. (A) Effect of PCR cycles on the intensity of the PCR products of bFGF. The PCR products were amplified linearly in 25-30 cycles. (B) Effect of PCR cycles on the intensity of the PCR products of GAPDH. The PCR products were amplified linearly in 20-35 cycles.

Induction of basic fibroblast growth factor mRNA expression by prostaglandin E2

Treatment with PGE2 for 6 hours resulted a dose-dependent increase of bFGF mRNA expression by RT-PCR (Fig.2-A). Semiquantitative data from three experiments are shown in Figure 2-B, demonstrating that 10^{-6} M and 10^{-8} M PGE2 significantly enhanced bFGF mRNA expression. We also determined the time course of the 10^{-6} M PGE2-mediated increase of bFGF mRNA, which appeared to be enhanced after 2 hours from stimulation, with a residual effect found after 24 hours (Fig. 3).

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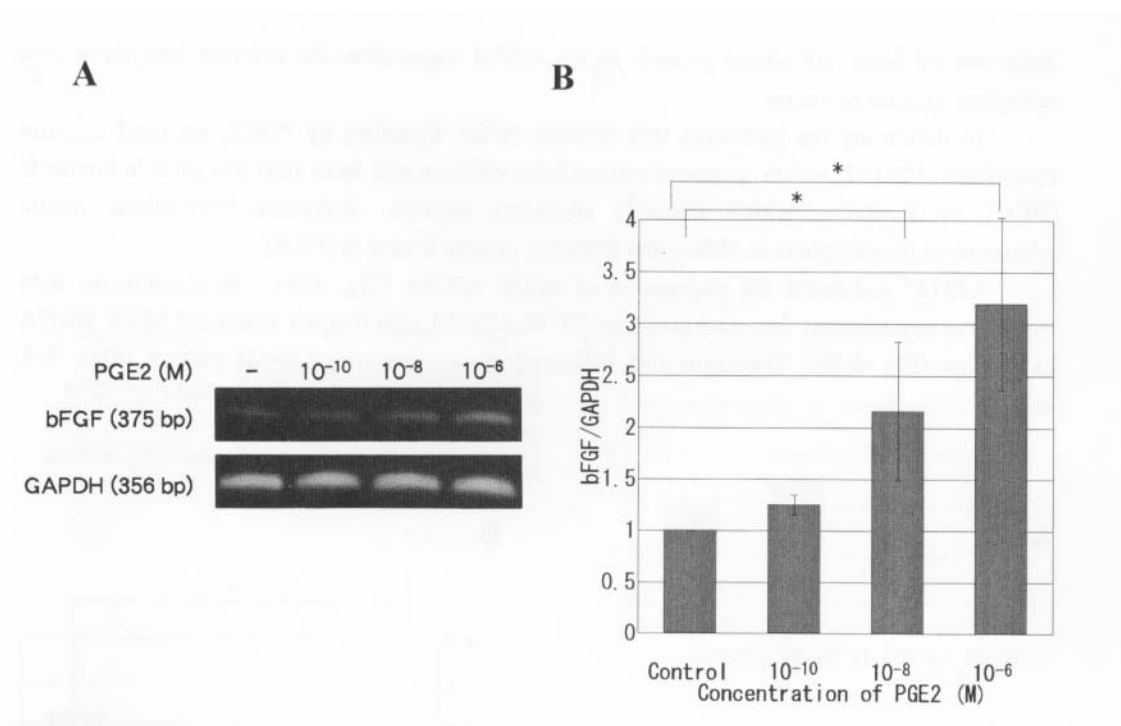


Fig. 2. (A) Effect of PGE2 on the expression of bFGF mRNA by RT-PCR. Normal human fibroblasts were exposed to various concentrations of PGE2 for 6 hours. The size of PCR products of FGF mRNA was 375bp (upper panel) and GAPDH was 356bp (lower panel). (B) Data from three independent experiments were averaged and presented as relative to the control level (mean \pm SD, n=3). *P<0.05 versus the control.

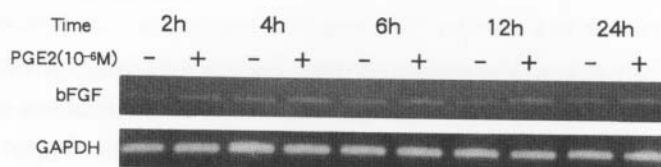


Fig. 3. Time course of the effect of PGE2 (10⁻⁶M) on the expression of bFGF mRNA by RT-PCR. Normal human fibroblasts were cultured for 2-24 hours with or without PGE2.

Induction of basic fibroblast growth factor mRNA expression by calcium ionophore and adenylate cyclase activator

To determine the pathways that mediate bFGF signaling by PGE₂, we used calcium ionophore, A23187, which increases intracellular calcium and later activates protein kinase C (PKC), or forskolin, which activates adenylate cyclase, increases intracellular cyclic adenosine monophosphate (cAMP), and activates protein kinase A (PKA).

A23187 enhanced the expression of bFGF mRNA (Fig. 4-A). Semiquantitative data from three experiments demonstrated that 10⁻⁵M A23187 significantly enhanced bFGF mRNA expression (Fig. 4-B). Forskolin also enhanced the expression of bFGF mRNA (Figs. 5-A and B).

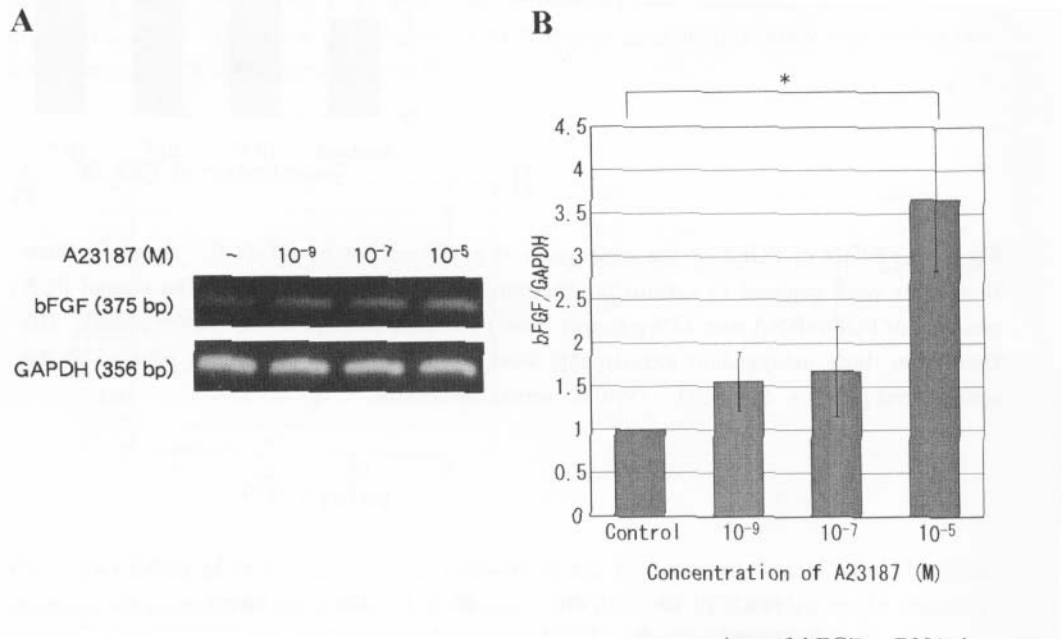


Fig. 4. (A) Effect of calcium ionophore, A23187, on the expression of bFGF mRNA by RT-PCR. Normal human fibroblasts were exposed to various concentrations of A23187 for 6 hours. (B) Data from three independent experiments were averaged and presented as relative to the control level (mean \pm SD, n=3). *P<0.05 versus the control.

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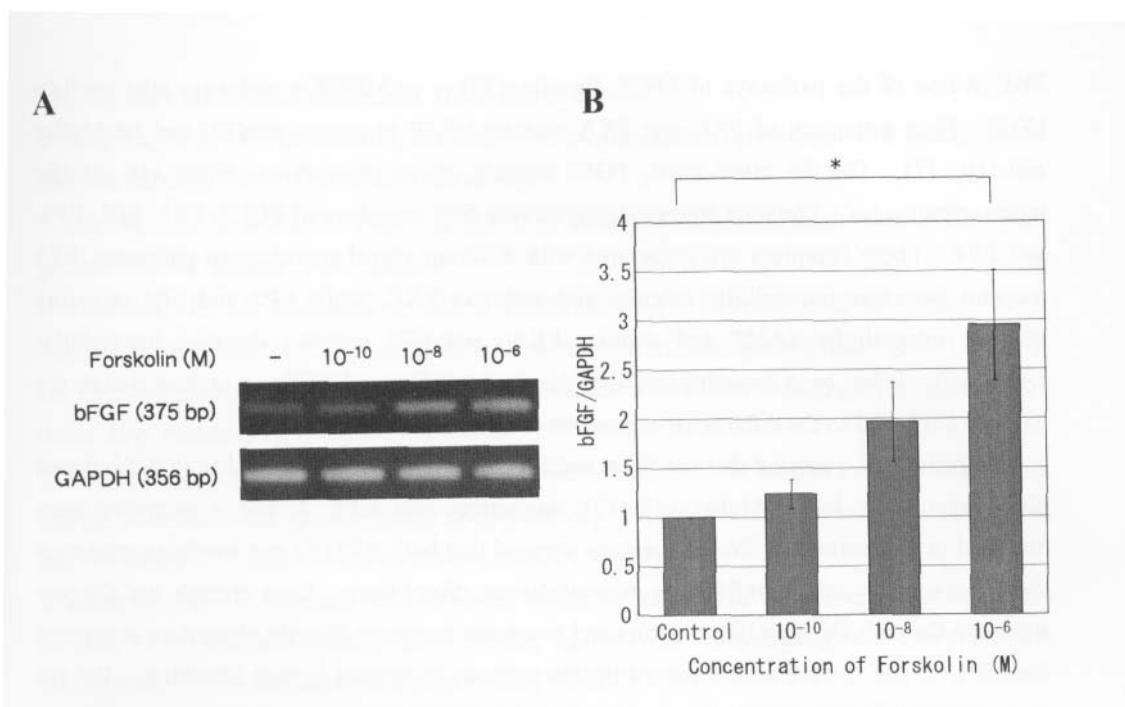


Fig. 5. (A) Effect of adenylate cyclase activator, forskolin, on the expression of bFGF mRNA by RT-PCR. Normal human fibroblasts were exposed to various concentrations of forskolin for 6 hours. (B) Data from three independent experiments were averaged and presented as relative to the control level (mean \pm SD, n=3). *P<0.05 versus the control.

DISCUSSION

We have shown that, in normal human fibroblasts, PGE2 enhanced bFGF mRNA expression in a dose-dependent fashion. Basic FGF stimulates angiogenesis and proliferation of human fibroblasts(20, 35), and has been seen to induce a predominantly angiogenetic response in wounds, increase wound collagenolytic activity, and accelerate healing of soft tissues(32). Therefore, PGE2 may participate in angiogenesis and in the healing mechanism in soft tissues by means of the induction of bFGF.

Previous studies showed that bFGF increased PGE2 production in several kinds of cells(2, 24, 25), and the healing mechanism by bFGF could be through the production of PGE2. However, the mechanism of bFGF production by PGE2 is poorly understood. From our results, PGE2 enhanced the expression of bFGF, suggesting that PGE2 and bFGF regulate each other in positive way for wound healing. Therefore PGE2 and bFGF have been shown that they have similar action in repairing tissues.

Two pathways mediate bFGF signaling. A previous study suggested that activation of

PKC is one of the pathways of bFGF signaling(29). cAMP/PKA pathways also mediate bFGF. Both activation of PKC and PKA mediate bFGF in astrocytes(27) and rat Muller cells(10, 12). On the other hand, PGE₂ usually elicits its various effects via specific receptor-pathways. Previous studies demonstrated four receptors of PGE₂: EP1, EP2, EP3, and EP4. These receptors are associated with different signal transduction pathways: EP1 receptor increases intracellular calcium and activates PKC, while EP2 and EP4 receptors increase intracellular cAMP and activate PKA, and EP3 receptor decrease intracellular cAMP(30). It has been hypothesized that the diverse effects of PGEs in various tissues are partially attributed to the differential expression of EP receptors.

Cheng et al. reported that the PKA and PKC pathways were involved in PGE₂-induced bFGF expression in rat Muller cells (12), suggesting that EP1, 2, and 4 receptors were involved in this pathway. We preliminary showed that both A23187 and forskolin enhanced the expression of bFGF mRNA in normal human fibroblasts. Even though we did not stimulate the cells through EP receptors and block the receptors directly, these data suggested that EP1, 2, and 4 were also involved in this pathway in normal human fibroblast. We are now preparing the experiments to stimulate the EP receptors directly.

Our findings may provide insight into the mechanism of PGE₂-induced angiogenesis and wound healing in soft tissue through the production of bFGF, in which EP1 or EP2 and EP4 receptor might play an important role.

ACKNOWLEDGMENTS

The authors greatly appreciate the technical assistance of Miss Kyoko Tanaka and Miss Masako Nishiumi. And we thank Dr. Pyuichi Saura for the comments on the manuscript and technical guidance in this study.

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