

Topically applied EGF and PDGFs affect positively the co-ordinate expression of EGF and PDGF receptor genes during acute cutaneous wound-healing process

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Daily topical application of epidermal growth factor (EGF) and platelet-derived growth factors (PDGFs) individually resulted in approximately two- to three-fold increase in EGF-R and α - and β -PDGF-R mRNA levels, and combinations of these growth factors produced a 2.5 to four-fold increase. Rapid attainment of maximum level of receptor mRNA on day one and its maintenance at comparatively higher level till day three appears to be essential for faster and better healing. PDGF-AA + EGF inhibited EGF-mediated increase in receptor mRNA levels when applied pre-mixed or within 30 min following EGF application. The results are also suggestive of the possible role of PDGF-AA during later stages of wound healing.

Keywords: Epidermal growth factor, platelet-derived growth factors, receptor mRNA, wound healing.

GROWTH factors are essential to the complex process of wound repair and they have become targets for therapeutic interventions. Various growth factors such as epidermal growth factor (EGF), platelet-derived growth factors (PDGFs), transforming growth factors (TGF) α and β , and insulin-like growth factor-1 (IGF-1), either alone or in combination, have been implicated in the wound healing process in various tissues¹⁻⁵. The regulatory role of EGF in gene expression and development has been well established^{2,6}. It has been shown that EGF is necessary for re-epithelialization during the wound-repair process⁶. EGF is known to stimulate keratinocyte migration, granulation, tissue development and mitogenesis of keratinocytes and fibroblasts⁷. Some of these studies were performed with single or multiple application of growth factors⁶⁻⁸.

Studies have shown that topical application of PDGF enhances wound repair, especially in animals with healing defects⁹⁻¹⁶. Reduced expressions of PDGFs and PDGF-R- α were observed during early phase of impaired wound healing^{10,11,13,14}. Previous studies have shown that PDGF-

treated wounds closed by re-epithelialization and the filling-in with scar, whereas control wounds healed by contraction¹⁷⁻²⁰. We have previously reported that growth factor-treated wounds healed with fine scars compared to control²⁰. The growth factor-treated wounds healed in approximately 5 to 7 days compared to controls, which took 10 to 15 days²⁰. The wound-healing process was further accelerated with the application of a combination of growth factors (EGF + PDGF-BB or PDGF-AB), which took only 3 to 5 days for healing²⁰. Recent reports using gene-transfer techniques where the *PDGF-B* gene was transferred into cultured dermal fibroblasts from normal and diabetic mice, indicated that the *PDGF-B* gene enhanced healing of dermal fibroblast^{14,15,17}. Topical application of PDGF-gels healed wounds with decreased wound depth compared to antibiotic-treated wounds¹⁸. Addition of exogenous PDGF in problematic wounds resulted in increased fibroblasts and collagen content, suggesting that cytokines such as EGF and PDGFs are pivotal molecules that may be deficient in some wound conditions^{2,16}.

Using the mouse model system previously we have shown that EGF, PDGF-AB and PDGF-BB, when topically applied either alone or in combination, accelerated wound healing by 50 to 66% faster with less scar tissue compared to the control²⁰. The best healing effect was obtained with a combination of EGF plus PDGF-BB. PDGF-AA, either alone or in combination with EGF, did not enhance the wound-repair process²⁰. The present study was carried out to determine the effect of optimum concentrations of purified EGF, PDGF-AB, PDGF-BB and PDGF-AA, individually and in various combinations, on the expression of their receptor mRNAs during the acute cutaneous wound-healing process.

Materials and methods

Animals

The experimental protocol was cleared by the institutional Animal Ethics Committee. Random bred male Swiss al-

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bino mice were used as a model system. Three-week-old mice were obtained from the Central Animal Research Facility, National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore and kept for an additional two to three weeks before use. This time period assured that the animals were free from diseases. All the animals were provided ample pelleted animal food (Godrej India Ltd, Mumbai) and water and normal day-night light cycles.

Wounding of mice

The dorso-lateral sides of mice were cleaned with disinfectant and approximately 5 mm × 1.5 cm area was cleared of all hair. Two full-thickness wounds of approximately 1 cm long and 0.3 to 0.4 cm deep were created on the dorso-lateral sides of each mouse, as described previously²⁰.

Number of mice used and collection of tissues

The experiments were repeated thrice to obtain statistically significant data. Fifty mice were kept as untreated external controls where wounds on both sides were left untreated. In the second set of 50 mice, one wound from each mouse was untreated and the other wound was treated with diluent alone and these acted as external controls. In the third set of 50 mice, wound on one side was treated with growth factors and the other wound was treated with diluent alone which acted as internal control. Thus, a group of 50 mice were used for each of the following growth factor treatments, either alone or in combination: (1) EGF, (2) PDGF-AB, (3) PDGF-BB, (4) PDGF-AA, (5) EGF + PDGF-BB, (6) EGF + PDGF-AB or (7) EGF + PDGF-AA. The newly formed tissues at the wound site were collected and pooled from 8 to 10 mice for each time period, i.e. day 1, 3, 5 and 7. After collecting tissues at the specified time points (40 mice) the mice were sacrificed and disposed. The remaining ten mice were kept under observation for approximately 30 days for any abnormal growth or rash. Thus, for each experiment a total of 100 mice were used as external control and 350 mice were used for growth-factor application. Overall, 1350 mice were used for three separate sets of experiment.

Growth factors

Recombinant mouse EGF and recombinant human PDGF-AB, PDGF-BB and PDGF-AA were obtained from Sigma-Aldrich (Saint Louis, USA) as lyophilized powders and diluted according to the manufacturer's instructions in a diluent solution containing 4 mM HCl and 0.1% (w/v) bovine serum albumin (Sigma-Aldrich). Aliquots of appropriate volumes were stored at -20°C and they were thawed only once for each use.

Application of growth factors

The day of creating the wound was counted as day 0 and the next day as day 1. Optimum concentrations of the growth factors in diluent (EGF, 10 ng; PDGF-AB, 15 ng; PDGF-BB, 15 ng or PDGF-AA, 20 ng) were applied directly to the wounds each day²⁰ starting from day 0 to day 7. The growth factors were applied with a micropipette as previously described²⁰. For the studies using combinations of growth factors, 10 ng of EGF was applied first followed by application of 15 ng of PDGF-AB, 15 ng PDGF-BB or 20 ng PDGF-AA within 30 min after EGF application. Alternatively, the growth factors, EGF plus PDGF-AB, EGF plus PDGF-BB and EGF plus PDGF-AA, were premixed and applied to the wounds as described previously²⁰.

Monitoring

The wound-repair process was monitored visually. The time taken for wound repair was scored visually and photographically using the following parameters: (a) wound closure and (b) scar-tissue formation (fine or raised), and these data have been already published²⁰. The newly formed irregular masses of granular tissues were collected and pooled from the wound sites of control and growth factor-treated mice. The pooled granular tissues from approximately 8 to 10 mice were stored at -70°C till they were further processed. The total RNAs were extracted from these granular tissues and used for Northern blot analysis²¹.

Extraction of total RNA

The granular tissues were homogenized in commercially available Tri-reagent (Sigma-Aldrich) and the total RNAs were isolated according to the protocol supplied by the manufacturer.

Northern blot analysis

Equal quantities of approximately 80 µg of total RNAs from the controls and growth factor-treated wounds were denatured in a buffer containing 50% formaldehyde, size-separated on a 1.2% (w/v) denaturing agarose-formaldehyde gel and transferred to nylon membrane (Sigma-Aldrich)²¹. The filters were baked at 80°C for 2 h and stored at 4°C until hybridized²¹.

Probes

Human EGF-R and β -PDGF-R cDNA probes were obtained from American Type Culture Collection (ATCC,

USA). The EGF-R cDNA probe recognized the expected two bands of 10.0 and 5.5 kb. The β -PDGF-R probe recognized the expected two bands of 6.4 kb and a 5.3 kb corresponding to α -PDGF-R and β -PDGF-R respectively, under low-stringency washing condition. However, under high-stringency washing condition, this probe recognized only the 5.3 kb β -PDGF-R mRNA. The internal control probe, β -actin cDNA, was also obtained from ATCC.

Probe labelling and detection

The cDNA probes were labelled with North2South Direct HRP Labelling and Detection kit (Pierce, USA). The Northern filters were probed with chemiluminescent-labelled cDNA probes. The solutions supplied by the manufacturer (Pierce) were used for labelling of the probe, pre-hybridization and hybridization, and the manufacturer's protocol was followed. Pre-hybridization and hybridization were done at 55°C. The filters were first hybridized with chemiluminescent-labelled β -PDGF-R cDNA probe.

These filters were washed under low-stringency condition, and after developing and visualizing the α -PDGF-R and β -PDGF-R bands, the same filters were washed under high-stringency condition and developed again to visualize the β -PDGF-R bands. For low-stringency conditions, the filters were washed in 2X SSC/0.1% (v/v) SDS, three times for 5 min each at 50°C. Then the filters were washed in 2X SSC three times for 5 min each at room temperature. For high-stringency washing conditions the solutions were the same, but the temperature was increased to the hybridization temperature of 55°C. Then the filters were washed three times at room temperature as described. The filters were developed with Luminol (Pierce) and the bands were visualized by exposing the filters to X-ray films according to the manufacturer's (Pierce) protocol. After stripping, these filters were hybridized with EGF-R cDNA probe, washed under a high-stringency condition at 55°C and the bands were visualized as previously described. Finally, the same Northern filters were stripped and re-probed with β -actin cDNA probe and washed under high-stringency wash condition at 55°C.

Quantitation

The levels of mRNAs were calculated by measuring the receptor band intensities in Bio-Rad gel documentation system (Bio-Rad, USA) using Bio-Rad quantity one software. The background values on a blank lane were subtracted from these values. The values were then compared to the corresponding β -actin mRNA levels in the same lanes. The effect of no treatment or diluent treatment on the level of receptor mRNA expression was taken as a base value and then the effect of exogenous addition of

growth factors on the level of mRNA was calculated and is reported here as fold increase. To calculate the level of EGF-R mRNA, intensities of both 10.0 and 5.5 kb mRNA species were measured and the combined value is reported here.

Statistical analysis

Statistical analysis was performed using SPSS software version 13.0. Student's *t* test, χ^2 test and odds ratio were compared. $P < 0.05$ was considered statistically significant. The following parameters were used for statistical correlation: (a) total number of experiments conducted; (b) total number of mice used for each experiment; (c) fold induction of receptor mRNA level to the wound-repair process and (d) standard deviation between the three separate experimental results.

Results

All the internal and external controls showed consistently similar levels of growth factor receptor mRNAs in all the three separate sets of experiment.

Effect of EGF and PDGFs (PDGF-AB or PDGF-BB) on the expression of EGF-R mRNA

The EGF-R cDNA probe recognized the expected two mRNA species, one approximately 10 kb and the other 5.5 kb (Figure 1). The induction level of both species of mRNA appeared to be similar for each treatment (Figure 1). EGF application resulted in a rapid increase of EGF-R mRNA to three-fold on day 1 (Figures 1 and 2). PDGF-BB and PDGF-AB application produced a maximum of 2.5-fold EGF-R mRNA level and it was attained on day 3. The effect of EGF on the EGF-R mRNA level was observed at a more rapid phase and maximum level was attained earlier compared to the effect of PDGF-BB and PDGF-AB on the same receptor mRNA level (Figures 1 and 2).

Effect of EGF and PDGFs (PDGF-AB or PDGF-BB) on the expression of α -PDGF receptor (α -PDGF-R) mRNA

The low-stringency wash conditions produced two bands of approximately 6.4 and 5.3 kb corresponding to the α -PDGF-R and β -PDGF-R respectively. The same filters were washed under high stringency wash conditions and they retained only the bottom band corresponding to β -PDGF-R and this wash condition did not significantly affect the 5.3 kb β -PDGF-R band intensity (data not shown).

Daily topical application of EGF or PDGFs (PDGF-AB or PDGF-BB) increased the level of 6.4 kb mRNA corresponding to α -PDGF-R (top band, Figure 3), but the maximum level (Figure 4) was slightly lower than that of EGF-R (Figure 2) and β -PDGF-R (Figure 5) mRNA levels. Application of PDGF-BB resulted in a rapid increase in the level of α -PDGF-R to a maximum of 2.3-fold on day 1 (Figures 3 and 4). However, application of PDGF-AB and EGF caused a gradual increase in the α -PDGF-R receptor mRNA level, reaching a maximum of approximately two- to 2.3-fold on day 3 (Figures 3 and 4).

Effect of EGF and PDGFs (PDGF-AB or PDGF-BB) on the expression of β -PDGF-receptor (β -PDGF-R) mRNA

Daily topical application of the growth factors resulted in the induction of the 5.3 kb mRNA corresponding to β -PDGF-R (bottom band, Figure 3). Application of PDGF-AB or PDGF-BB resulted in rapid induction of β -PDGF-R to three-fold on day 1, and the level was approximately the same till day 3 (Figures 3 and 5). However,

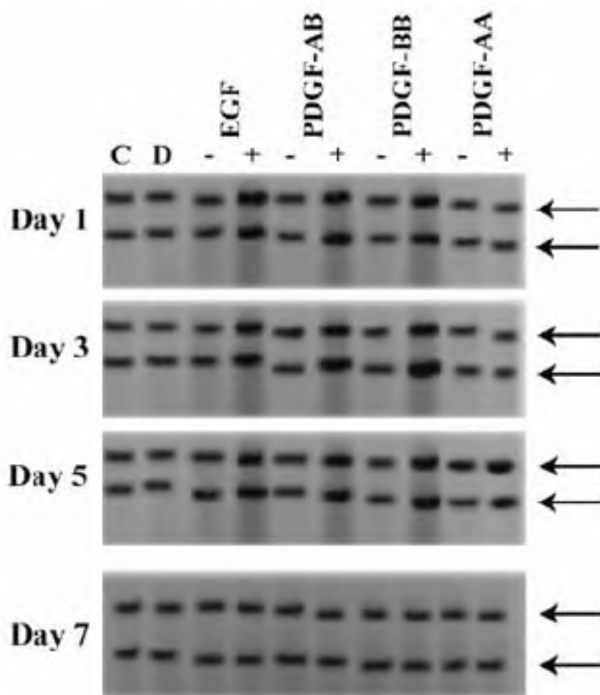


Figure 1. Effect of EGF and PDGFs on the expression of EGF-R mRNA level. EGF-R mRNA levels were compared between the growth factor-treated wounds and the controls. The levels of EGF-R mRNAs were calculated by measuring the band intensities of both the 10.0 kb (top arrow) and 5.5 kb bands (bottom arrow). Details are described in the text. C, Untreated external control; D, Diluent-treated external control. The growth factors used are marked above the lanes. -, Diluent-treated internal control; +, Growth factor treatment. Days of growth factor treatment are marked on the left.

EGF application produced a gradual increase of β -PDGF-R mRNA reaching a maximum level of three-fold on day 3 (Figures 3 and 5).

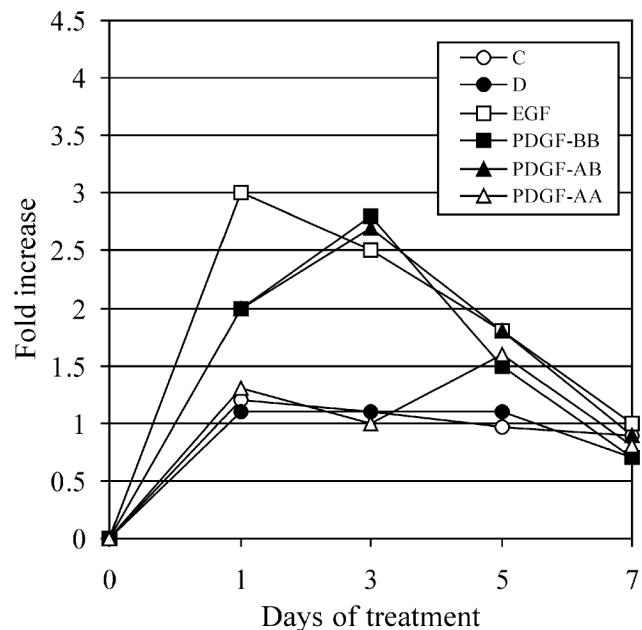


Figure 2. Level of EGF-R expression with EGF and PDGFs. Days of treatment are marked on the X-axis, and the levels of receptor mRNAs are marked on the Y-axis. C, Untreated external control; D, Diluent-treated internal control. The levels of EGF-R mRNA given are the combined value of both 10.0 kb plus 5.5 kb bands. The levels of receptor are the average from three independent experiments.

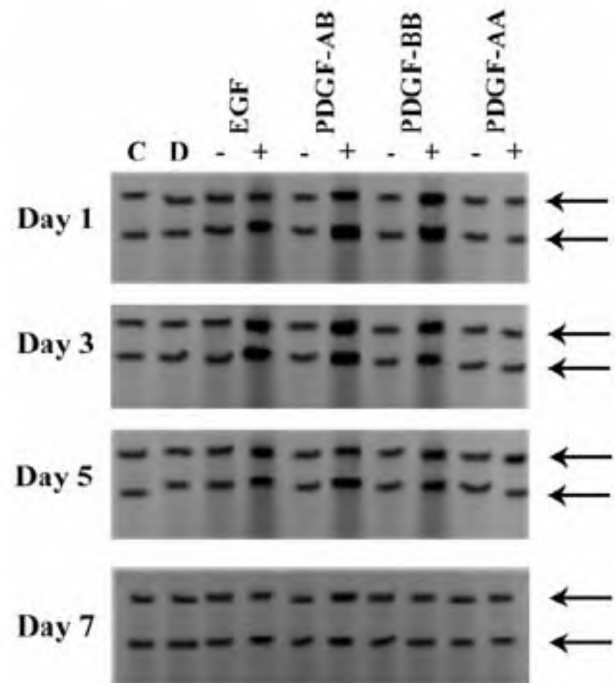


Figure 3. Effect of EGF and PDGFs on the expression of α -PDGF-R (top arrow) and β -PDGF-R (bottom arrow) mRNA levels. The procedures are given in the text. C, Untreated external control; D, Diluent-treated external control; -, Diluent-treated internal control; +, Growth factor treatment. Days of treatment are shown on the left. Top arrow, 6.4 kb α -PDGF-R mRNA; bottom arrow, 5.3 kb β -PDGF-R mRNA.

Effect of PDGF-AA on the expression of α -PDGF-R, β -PDGF-R and EGF-R mRNA levels

Daily topical application of 20 ng of PDGF-AA per wound produced little increase in the levels of all three receptor mRNAs. However, on day 5, a slight increase in the levels of all three receptor mRNAs was observed (Figures 1–5).

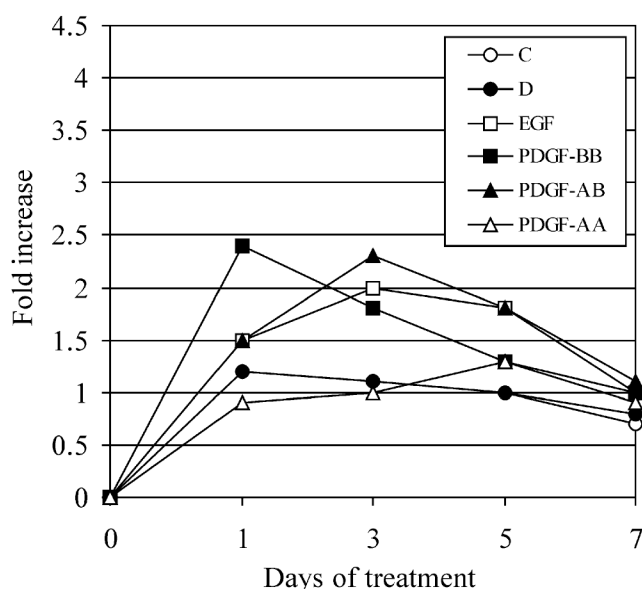


Figure 4. Level of α -PDGF-R mRNA expression in growth factor-treated wounds. Days of growth factor treatment are shown on the X-axis and the levels of receptor mRNA are shown on the Y-axis. C, Untreated external control; D, Diluent-treated internal control. The level of receptor mRNA is the average of three separate experiments.

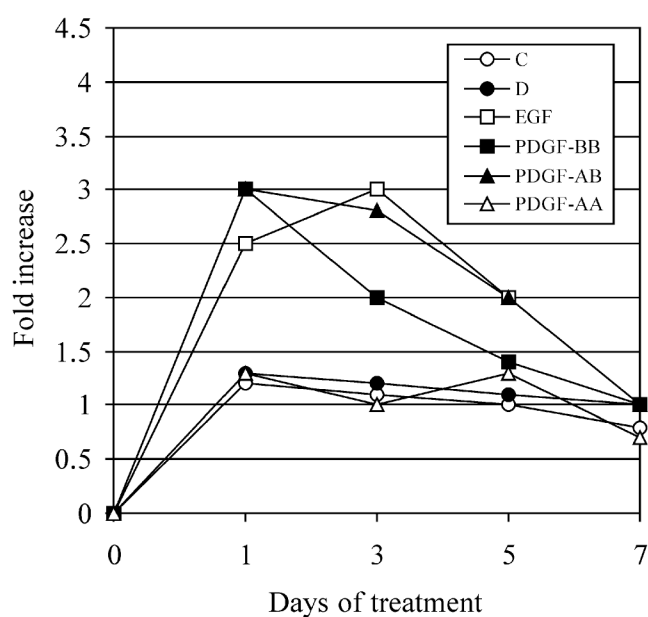


Figure 5. Level of β -PDGF-R mRNA expression in growth factor-treated wounds. Days of growth factor treatment are shown on the X-axis and the receptor mRNA levels are shown on the Y-axis. C, Untreated external control; D, Diluent-treated internal control. The level of receptor mRNA is the average of three separate experiments.

Control

Re-probing the Northern filters with β -actin cDNA probe confirmed that increase in the level of growth factor receptor mRNAs was not due to variation in the loading of the RNA gels (Figure 6).

Effect of combination of EGF plus PDGF-AB and EGF plus PDGF-BB on the level of expression of EGF-R mRNAs

Combination of EGF plus PDGF-BB showed rapid induction of EGF-R mRNA level, reaching a maximum of 3.5-fold on day 1 followed by a slight decrease to three- and 2.8-fold on day 3 and day 5 respectively (Figures 7 and 8). A combination of EGF plus PDGF-AB also resulted in rapid induction of EGF-R mRNA on day 1, but this combination led to only a maximum of 2.8-fold increase. The maximum level of the EGF-R mRNA was not additive with the combination of growth factors to that obtained with individual growth factors (Figures 1–5).

Effect of combination of EGF plus PDGF-AB or EGF plus PDGF-BB on the expression of α -PDGF-R mRNA level

With the combination of EGF + PDGF-AB or EGF + PDGF-BB, the level of α -PDGF-R mRNA also increased

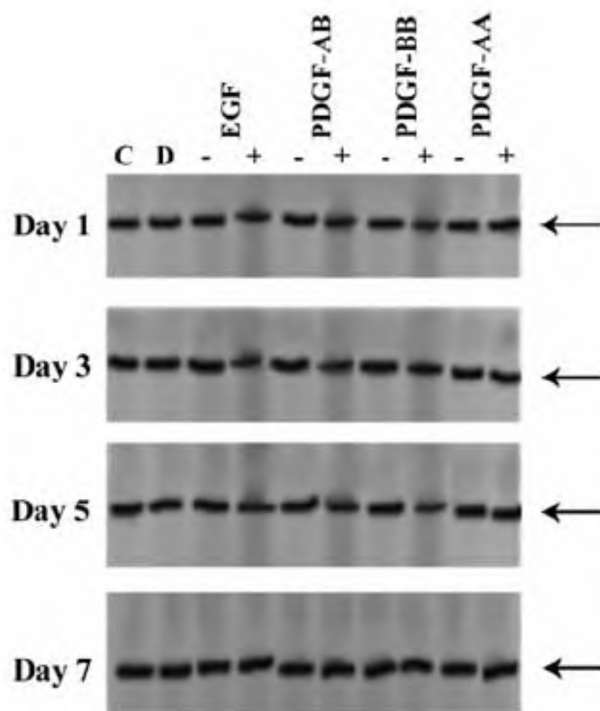


Figure 6. Level of control β -actin mRNA. C, Untreated external control; D, Diluent-treated external control; –, Diluent-treated internal control; +, Growth factor treatment. Days of treatment are shown on the left. Arrows indicates β -actin mRNA.

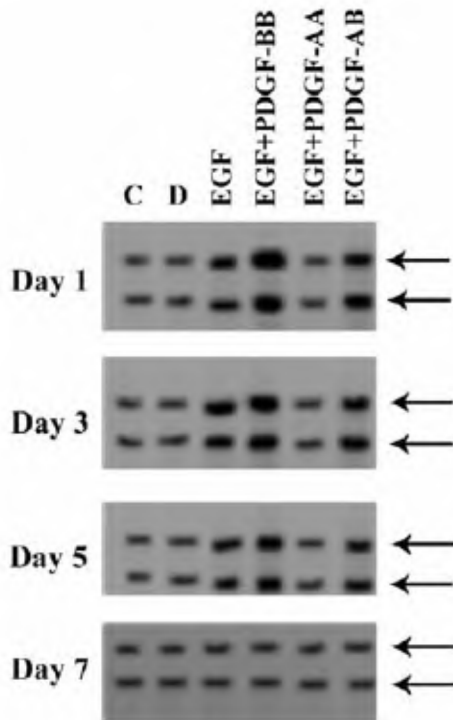


Figure 7. Effect of combination of EGF plus PDGFs on the expression of EGF-R. Arrows indicate EGF-R mRNA. Days of growth factor treatment are given on the left. C, Untreated external control; D, Diluent-treated internal control. As the diluent-treated internal control showed similar results in all the blots, only one control is shown in this blot. Treatment with growth factor as indicated above the lanes. The top (10.0 kb) and bottom arrow (5.5 kb) indicate the two species of EGF-R mRNA band. Days of growth factor treatment are indicated on the left.

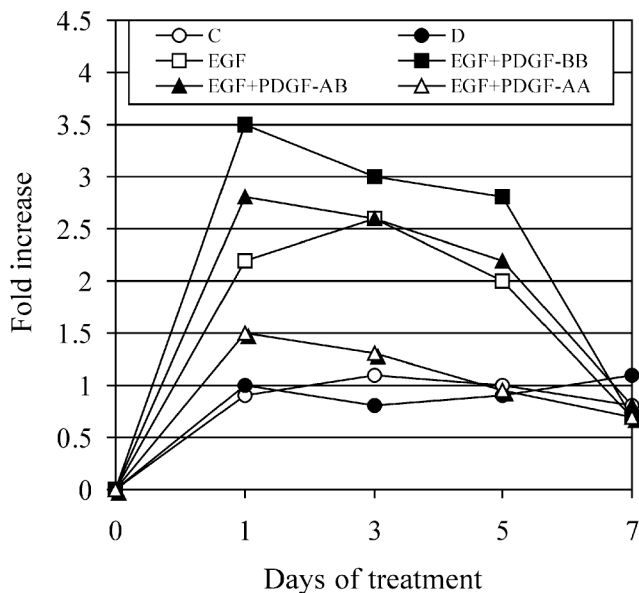


Figure 8. Levels of EGF-R mRNAs in growth factor-treated wounds. Days of growth factor treatments are given on the X-axis and EGF-R mRNA levels are given on the Y-axis. C, Untreated external control; D, Diluent-treated internal control. The levels shown here are the combined values of both 10.0 and 5.5 kb EGF-R mRNA bands. The level of receptor mRNA shown here is the average from three separate experiments.

rapidly, reaching a maximum of 3.5- to four-fold on day 1 (top arrow, Figures 9 and 10) and the maximum levels with these combinations were not additive compared to the individual growth factors.

Effect of combination of EGF plus PDGF-AB or EGF plus PDGF-BB on the expression of β -PDGF-R mRNA level

Maximum level of 3.5- to four-fold β -PDGF-R mRNAs was attained rapidly on day 1 with the application of EGF + PDGF-AB or EGF + PDGF-BB (bottom arrow, Figures 9 and 11). The maximum levels were not additive compared to the levels obtained with individual growth factors.

Effect of combination of EGF plus PDGF-AA on the level of PDGF-R and EGF-R mRNA expression

Combination of EGF plus PDGF-AA produced little effect on the levels of EGF-R (Figures 7 and 8) or PDGF-R

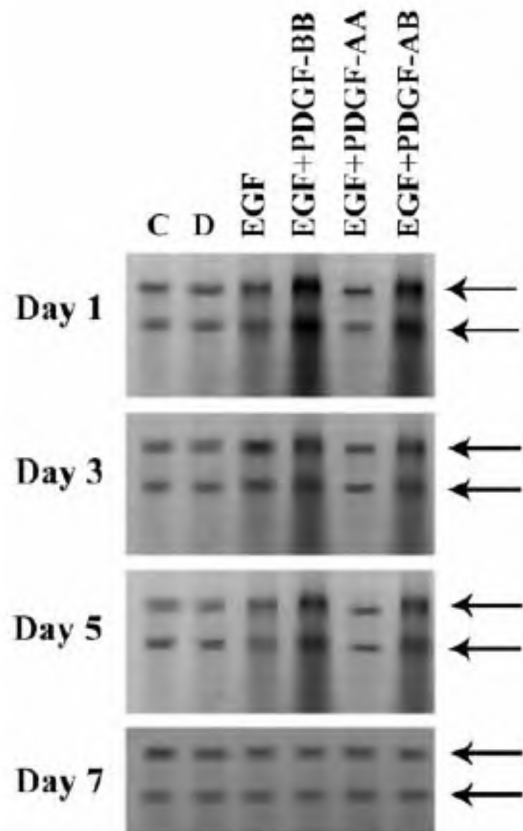


Figure 9. Effect of combination of EGF plus PDGFs on the expression of α -PDGF-R (top arrow) and β -PDGF-R (bottom arrow) mRNA levels. C, Untreated external control; D, Diluent-treated internal control. All diluent-treated internal controls showed similar results, therefore only one lane is loaded with this sample; Treatment with growth factors as indicated above the lanes. Days of treatment are shown on the left.

(Figures 9–11) mRNAs. PDGF-AA in combination with EGF repressed the EGF-mediated increase in the EGF-R and PDGF-R mRNA expression (Figures 7–11). This repression was observed when PDGF-AA was applied within 30 min after the application of EGF. Similar results were obtained when these growth factors were applied pre-mixed (data not shown). However, application of PDGF-AA approximately 30 min after the application of EGF did not affect the EGF-mediated induction of EGF-R or the pleiotropic effect of EGF observed in our experiments (data not shown).

Control

The observed increase in the growth factor receptor mRNAs was not due to unequal loading of the Northern gels as confirmed by re-probing the filters with β -actin cDNA probe (Figure 12).

Statistical analysis

The number of mice used in each experiment was statistically significant. There was a positive significant correlation between the day of attainment of peak level of the receptor mRNA to the rate of wound healing and quality of the scar tissues ($p < 0.05$). In addition, similar positive correlation was also observed between the maximum level of receptor mRNA to that of wound healing and

scar tissue. The standard deviations for the levels of receptor mRNAs between the three different sets of experiments were less than 0.05, confirming the statistical significance of the results from these experiments.

Discussion

A previous report had shown that one to two days after injury, epidermal cells at the wound margin begin to proliferate behind actively migrating cells²². A sustained daily release of 10 to 20 μ g of partially purified, biologically active EGF was found²² to increase the extent and organization of the granulation tissue at day 7. The stimuli for the migration and proliferation of epidermal cells during re-epithelialization have not been determined, but it has been suggested that the presence of growth factor EGF and its receptor may stimulate these processes^{5,22}. We have previously shown that daily topical application of optimum concentration of 10 ng EGF enhanced the acute cutaneous wound-healing process, where the wounds healed in 5 to 7 days compared to the controls which took approximately twice as long, i.e. 10 to 15 days to heal²⁰. Among individual growth factors, the best healing was observed with PDGF-BB and among the combinations EGF + PDGF-BB produced the best results²⁰. This is consistent with other reports for the positive role of EGF during wound healing²², as EGF promoted cell motility⁵ and maximally stimulated keratinocytes and epithelial cells at

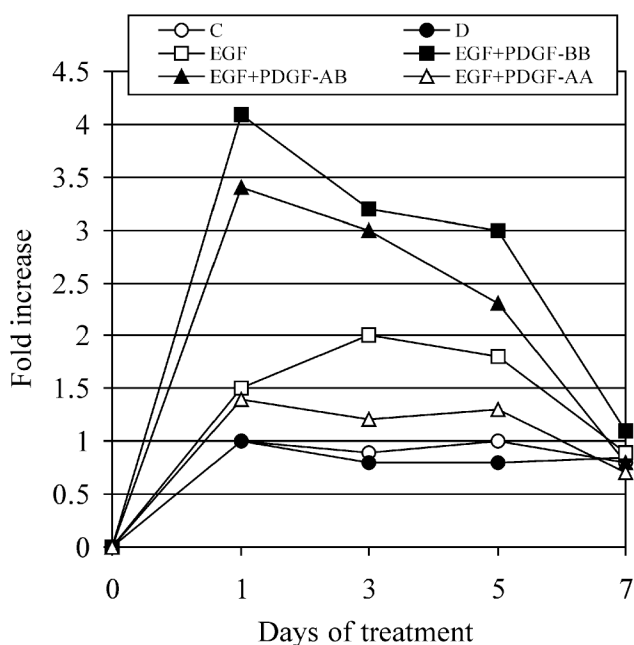


Figure 10. Levels of α -PDGF-R mRNA in growth factor-treated wounds. Days of growth factor treatment are given on the X-axis and levels of α -PDGF-R mRNA are shown on the Y-axis. C, Untreated external control; D, Diluent-treated internal control. The levels of receptor mRNA shown here are the average from three separate experiments.

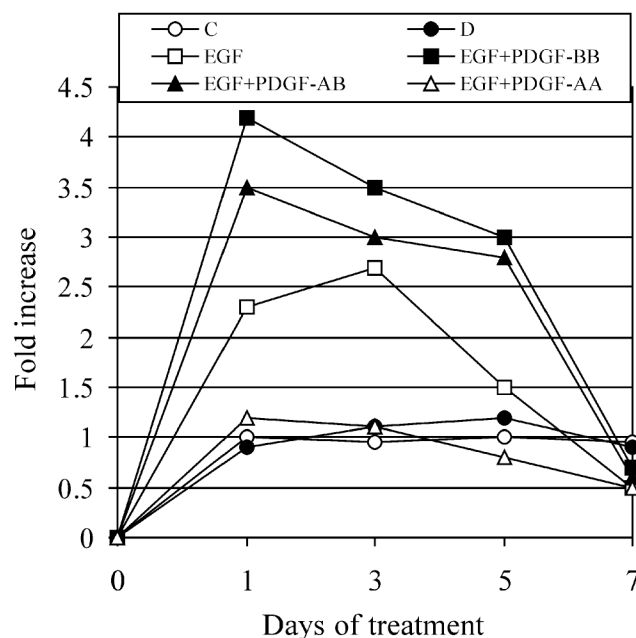


Figure 11. Levels of β -PDGF-R mRNA in growth factor-treated wounds. Days of growth factor treatment are given on the X-axis and levels of β -PDGF-R mRNA are marked on the Y-axis. C, Untreated external control; D, Diluent-treated internal control. The levels of receptor mRNA shown here are the average from three separate experiments.

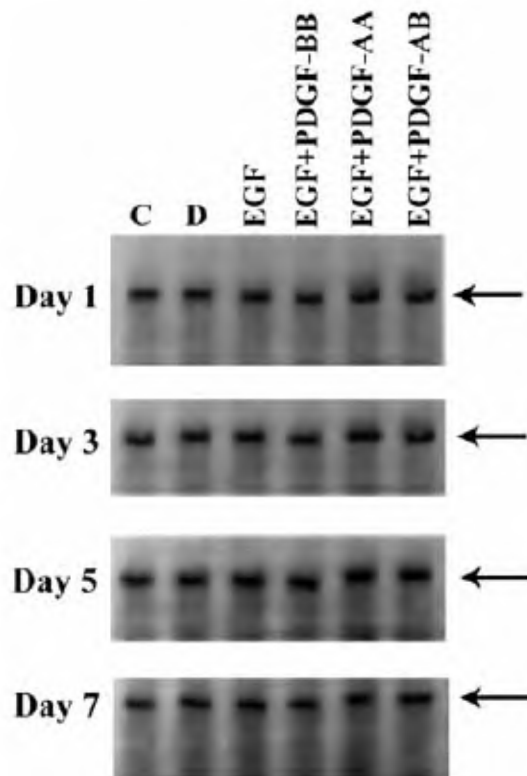


Figure 12. Levels of control β -actin mRNA. C, Untreated external control; D, Diluent-treated internal control. Days of growth factor treatment are given on the left. Arrows indicate β -actin mRNA.

25 ng and 5 ng/ml respectively^{7,8}. The optimum concentration required for EGF to produce its effect on various cell types could depend on the purity of the growth factor and type of wound^{7,8,20}. In addition to inducing the level of its own receptor mRNA (Figures 1 and 2), EGF also induced α -PDGF-R and β -PDGF-R mRNA levels (Figures 3–5), indicating the pleiotropic effect of EGF during the wound-healing process. Delayed appearance of EGF and EGF-R in incisional wounds of aged mice has been reported by other groups^{16,23}. Our results show that in response to daily EGF treatment, the maximum EGF-R level was reached rapidly on day 1 (Figure 2). Thus, it is conceivable that optimum concentration of growth factors that could produce peak levels of receptor mRNAs rapidly could be essential for enhancement of cutaneous wound repair (Figures 3–5).

Similar to EGF, PDGF-AB and PDGF-BB also produced pleiotropic effect by inducing EGF-R mRNA level in addition to inducing PDGF-R mRNAs levels (Figures 1–5). Rapid induction of peak level of β -PDGF-R mRNA by PDGF-BB on day 1 and its maintenance till day 3 (Figures 1 and 5) could be correlated with published data on its better healing ability compared to other growth factors^{18–20}. No significant induction of PDGFs was reported in normal mice after skin injury and their expression levels were found to be similar at all stages of the repair

process²⁴. Our data confirm this report, where the control wounds showed only a slight increase in the receptor mRNA levels (Figures 1–5), perhaps due to the lower levels of PDGFs²⁴. The prolonged time taken for the healing of control wounds²⁰ could also be due to the presence of sub-optimal levels of receptor mRNAs as seen in the present study. Glucocorticoid treatment has been reported to cause severe defect in wound repair and it resulted in decreased α -PDGF-R and β -PDGF-R in the early phase of wound healing²⁴. Reports have shown that daily application of lower doses of 5 to 10 ng of PDGF-AB in rat²⁵ and mouse²⁰ failed to significantly enhance wound repair. In the present study, we found that rapid attainment of peak value of the receptor mRNA expression could be achieved only with the optimum concentrations of growth factors (Figures 1–5). Therefore, the beneficial effect of topically applied PDGF observed by others^{18,19} and by us²⁰ could be due to sustained expression of maximum level of PDGF-R mRNAs at the very early stages (Figures 1, 3 and 4).

Combinations of growth factors were found to be more effective in the wound-healing process compared to single growth factors, perhaps due to their pleiotropic effects on the expression of various genes involved in the regeneration mechanism^{1,5,8,13,20,26,27}. It has been suggested that the levels of receptor could be critical for faster and better wound healing^{27,28}. Statistical analysis of our data showed significant positive correlation between the rapid attainment of peak levels of receptor mRNAs (Figures 1–5 and 7–11) and the time taken for healing and the quality of healed wound (scar) that we reported previously²⁰.

PDGF-AA does not accelerate the rat cutaneous wounds compared to PDGF-AB²⁵, but helped healing of the tympanic membrane²⁹. This is suggestive of the ability of PDGF-AA to heal only certain types of wounds and not others. Our previous study²⁰ and data from the present study suggest that EGF is essential at early stages of wound healing and the long form of PDGF-AA could antagonize the EGF-mediated healing (Figures 7–11). Differential physiologic functions of various forms of PDGFs have been reported previously^{30,31}. The PDGF-B chain (c-sis proto-oncogene) homodimer (PDGF-BB) and v-sis, its viral counterpart, were known to activate both α -PDGF-R and β -PDGF-R and mediate anchorage-independent phenotypic transformation in NIH 3T3 cells³⁰. In contrast, the PDGF-A chain homodimer (PDGF-AA) was known to activate α -PDGF-R only, and it failed to induce phenotypic transformation³⁰. β -PDGF-R activation alone is reported to be sufficient for PDGF-BB-mediated anchorage-independent cell growth and inhibition of α -PDGF-R signalling enhanced PDGF-BB-mediated phenotypic transformations, suggesting that α -PDGF-R antagonized β -PDGF-R-induced transformation³⁰. Further, α - and β -PDGF-R are known to differentially regulate Ras-mitogen-activated protein kinase pathway, and transformation-suppressing activity of α -PDGF-R involves JNK-1

activation³⁰. The role of PDGF-AA was reported to be different from that of PDGF-BB during mitogenesis³¹. It would be interesting to see if the transformation ability is essential for the PDGF-induced wound-healing process. In such a case, it would be crucial to distinguish if lack of such ability reported for PDGF-AA could be deleterious to the repair process of certain types of wounds. Alternatively, PDGF-AA might require other growth factor(s) to function during the cutaneous wound repair. PDGF is reported³² to inhibit angiogenic properties of basic FGF through α -PDGF-R. PDGF-AA is known to bind only to α -PDGF-R and angiogenesis is essential for wound repair.

It has been suggested^{30,31} that PDGF-AA might act through a different molecular pathway compared to PDGF-BB and PDGF-AB. We found a slight increase in the levels of receptor mRNAs with the application of PDGF-AA (Figures 1–11). Also, it might not be sufficient to enhance or accelerate the cutaneous wound repair as it appears that the sustained optimum levels of these mRNAs are essential for the same (Figures 1–11)²⁰. Alternately, the slight increase in the receptor mRNA levels at day 5 produced by PDGF-AA (Figures 1–5) could be indicative of a possible role of PDGF-AA during later stages of wound healing. It has been shown that PDGF-BB, PDGF-AA and foetal bovine serum were mitogenic, and anti-apoptotic, but only PDGF-BB could induce cell migration³³. Therefore, it appears that PDGF-AA lacks the ability to induce cell migration which has been reported to be essential for normal wound healing^{33,34}. Recent reports indicate that various PDGFs act through different signalling pathways that are mediated by various cell types³⁵. It is conceivable that some of these cell types required for the PDGF-AA mediated signalling pathway could be absent at the wound site during the initial stages of wound healing.

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MEETINGS/SYMPOSIA/SEMINARS

IX Convention of Mineralogical Society of India (MSI-2007) and National Seminar on Exploration for Platinum Group Elements (PGE), Gold and Diamonds in India

Date: 19–20 July 2007

Place: Hyderabad

Topics include: PGE in the ultramafic flows of Deccan Volcanic Province (DVP); PGE fractionation in mantle sulfide phases: Finger printing of metasomatic processes; Are all mantle plumes equal in Ni & PGE potential?; Noble metal mineralization in non-traditional geological formations; Determination of PGE, Au and other trace metals in geological samples; Au–Pb–Zn and other base metal mineralizations; Volcanic hosted massive sulfide (VHMS) deposits; Evolution and metallogeny of greenstone belts; Shear zone-related mineralization; Continental crust and associated mineralization; Diamond and associated indicator minerals; Role of trace elements in diamond exploration; Mantle xenoliths in kimberlites; Kimberlites and lamproites as probes of the deep crust/lithosphere; Surface geochemical prospecting; Geochemical baseline mapping; Marine mineral resources.

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