Effects of prostaglandin E₁ on cultured dermal fibroblasts from normal and hypertrophic scarred skin

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Received 7 March 1996; revised 30 August 1996; accepted 10 September 1996

Abstract

To investigate the role of prostaglandin (PG) E₁ in preventing scar formation as well as that of the related cytokines, we cultured fibroblasts from hypertrophic scar tissue (SDF) and normal dermis (NDF) collected from patients with scar contracture. We have compared the type I collagen synthesis, type I collagenase activity, and the production of interleukin (IL)-6, IL-8 and transforming growth factor (TGF)-β₁ in two types of cultured fibroblasts before and after addition of PGE₁. Our results demonstrated that levels of type I collagen and TGF-β₁ production were higher and that type I collagenase activity and IL-8 production were significantly lower in the culture supernatants of SDF. There was no significant difference in IL-6 production between SDF and NDF culture supernatants. On the other hand, PGE₁ significantly increased type I collagenase activity and IL-8 production in the SDF culture supernatants and it increased IL-6 and TGF-β₁ production in both types of fibroblasts. However, there was no effect on synthesis of type I collagen in either group. To further investigate the role of TGF-β₁ in NDF and SDF, exogenous recombinant human (rh) TGF-β₁ was added. In NDF group, rhTGF-β₁ induced a decrease in the type I collagenase/type I collagen ratio, while rhTGF-β₁ had no effect on the same ratio in the SDF group. These results suggest that PGE₁ may have a role in the prevention of hypertrophic scar by increasing the activity of type I collagenase. © 1997 Elsevier Science Ireland Ltd. All rights reserved

Keywords: Transforming growth factor-β₁; Prostaglandin E₁; Collagen; Collagenase; Interleukin-6; Interleukin-8; Scar-derived fibroblasts

1. Introduction

In recent years, it has been shown that certain cytokines, such as transforming growth factor
(TGF)-β1 and interleukin (IL)-6, play important roles in wound healing and are involved in both epithelization and scar formation [1–5]. Prostaglandin (PG) E1 has been therapeutically appraised with respect to its efficacy in the treatment of various diseases due to its effects on vasodilatation and anti-platelet aggregation [6–9]. Therefore, many studies on the prevention of scar formation after wound healing have employed various agents in an attempt to control levels of certain cytokines [4,5]. Application of PGE1 ointment to chronic ulcers and burns was considered to be effective not only in improving the blood circulation but also in increasing the tissue oxygen tension [10,11]. Also, the tendency that PGE1 promotes epithelization of the wound surface and limits the hypertrophic scar formation in human burned ulcers has been described in clinical research [12]. We previously found that PGE1 and PGI1 analogues caused keratinocytes to proliferate in vitro by promoting proliferation of fibroblasts and increasing production of IL-6 from fibroblasts [13,14]. In this study, we examined the effects of PGE1 on extracellular matrix formation as well as the cytokine production in vitro and compared the results obtained from normal dermal fibroblasts (NDF) to those obtained from hypertrophic scar-tissue derived fibroblasts (SDF).

2. Materials and methods

2.1. Patients and materials

Scar tissue was taken from nine patients (five males, four females; age: 24.2 ± 8.6 years; mean ± SE) with hypertrophic scars due to trauma or burns they had received within the past year; normal skin was also obtained from the same patients and used as a control.

2.2. Fibroblast culture and measurement for the collagen synthesis, collagenase activity and cytokines production

Dermis from the hypertrophic scar and normal skin tissues of each patient were cut into small pieces and placed on the center of a Petri dish (35 × 10 mm). Each was covered gently with a glass slide (24 × 24 mm, Matsunami Glass Industry Co., Ltd., Osaka, Japan) and then immersed in 2 ml of Dulbecco's modified Eagle medium (DMEM) (Nihon Seiyaku Co., Ltd., Tokyo, Japan) containing 20% inactivated fetal bovine serum (FBS). Each tissue sample was incubated at 37°C in a CO2 incubator and the culture medium was exchanged every 3 days. The preparation of fibroblasts was carried out as previously described [13,14]. The 3rd or 4th passage was used for study.

Fibroblasts were seeded on a Petri dish (60 × 15 mm) at 4 × 10^5 cells/dish in 4 ml of DMEM containing 10% inactivated FBS. After reaching confluence, the culture medium was changed to DMEM after washing with phosphate-buffered saline (PBS), and PGE1 (Ono Pharmaceutical Co. Ltd., Osaka, Japan) of 10^-6 M or rhTGF-β1 (King Jozo Co., Ltd., Kakogawa, Japan) of 2 ng/ml final concentration were also added. The supernatants were collected 72 h after incubation to measure biological activities of type I collagen synthesis, type I collagenase, and IL-6, IL-8 and TGF-β1 levels. The results were expressed as average figures per 10^6 cells.

2.2.1. Measurement of activity of type I collagen synthesis

The procollagen type I C-peptide concentration was measured in the culture supernatants of fibroblasts by enzyme-linked immunosorbent assay (ELISA) using a procollagen type I C-peptide (PIP) kit (Takara Shuzo Co., Ltd., Kyoto, Japan).

2.2.2. Measurement of type I collagenase activity

After 1 μg of trypsin was added to 100 μl of each culture supernatant which had been harvested from fibroblasts incubation, it was incubated at 37°C for 5 min. A fivefold amount of trypsin inhibitor was then added. Type I collagenase activity was measured using a type I collagenase activity kit (Yagai Inc., Yamagata, Japan). The reagent in the kit which is FITC-labeled is type I collagen dissolved in 0.05 M Tris–hydrochloride buffer (containing NaCl and CaCl2, pH 7.5). The principle of the test is as follows;
when animal collagenase or a culture supernatant is added to the FITC-labeled type I collagen, collagen molecules are cleaved and degraded and the addition of ethanol solution leads to precipitation of only the undigested collagen. After the precipitate was removed by centrifugation, collagenase activity was quantified by measuring the fluorescent intensity of the supernatant.

2.2.3. Measurement of cytokines

TGF-β₁, IL-6 and IL-8 in the culture supernatants were measured by ELISA using IL-6 and IL-8 detection kits (R and D Systems, Minneapolis, MN) and TGF-β₁ kit (Genzyme Corporation, Cambridge, USA).

2.3. Statistical analysis

The results were expressed as means ± SE. Statistical analysis was performed using Fisher’s protected least significant difference test by Statview-J 4.02 (Abacus Concepts Inc, Berkeley, CA). P < 0.05 was regarded as significant.

3. Results

3.1. Effects of PGE₁ on extracellular matrix formation and cytokine production of NDF and SDF

3.1.1. The concentration of procollagen type I C-peptide (PIP) in the culture supernatants of NDF and SDF and the effects of PGE₁ on its synthesis

The amount of PIP produced by SDF was 865.2 ± 53.2 ng/ml/10⁶ cells, which was significantly higher (P < 0.05) than that produced by NDF (788.4 ± 49.2 ng/ml/10⁶ cells). The addition of 10⁻⁶ M PGE₁ had no significant effect on the PIP production of either type of cells (Fig. 1A).

3.1.2. The effect of PGE₁ on type I collagenase activity in NDF and SDF

The amount of type I collagenase produced by SDF was 205 ± 38.4 unit/ml/10⁶ cells, which was significantly lower (P < 0.05) than that produced by NDF (393 ± 84.5 unit/ml/10⁶ cells). With the addition of 10⁻⁶ M of PGE₁, type I collagenase activity of SDF increased to 250 ± 41.8 unit/ml/10⁶ cells, which was approximately 1.3 times greater (P < 0.05) than that of the SDF without PGE₁. In contrast, despite the addition of 10⁻⁶ M of PGE₁, type I collagenase activity in NDF did not significantly increase in comparison to that of the NDF without PGE₁ (Fig. 1B).

3.1.3. Effects of PGE₁ on IL-6 production in NDF and SDF

IL-6 production by NDF and SDF was 0.328 ± 0.074 ng/ml/10⁶ cells and 0.282 ± 0.085 ng/ml/10⁶ cells, respectively. There was no significant difference between two types of cells. With the addition of PGE₁, IL-6 secretion by NDF increased to 0.674 ± 0.084 ng/ml/10⁶ cells (P < 0.01) and also that by SDF increased to 0.552 ± 0.105 ng/ml/10⁶ cells (P < 0.01) (Fig. 1C).

3.1.4. Effects of PGE₁ on TGF-β₁ production by NDF and SDF

TGF-β₁ production by SDF was 0.148 ± 0.039 ng/ml/10⁶ cells, which was significantly higher (P < 0.05) than that by NDF (0.061 ± 0.020 ng/ml/10⁶ cells). With the addition of PGE₁, the amount of TGF-β₁ secreted by NDF increased to 0.184 ± 0.052 ng/ml/10⁶ cells, and TGF-β₁ secreted by SDF increased to 0.260 ± 0.062 ng/ml/10⁶ cells. Both values were significantly higher than those of the fibroblasts without PGE₁ (P < 0.05) (Fig. 1D).

3.1.5. The effects of PGE₁ on IL-8 production by NDF and SDF

IL-8 production by SDF was 0.043 ± 0.007 ng/ml/10⁶ cells, which was significantly lower (P < 0.05) than that by NDF (0.079 ± 0.014 ng/ml/10⁶ cells). With the addition of PGE₁, the amount of IL-8 secreted by NDF increased to 0.227 ± 0.078 ng/ml/10⁶ cells, and IL-8 by SDF significantly increased to 0.142 ± 0.033 ng/ml/10⁶ cells (P < 0.01) (Fig. 1E).
Fig. 1. Effects of $10^{-6}$ M PGE$_1$ on type I collagen production (A), type I collagenase activity (B), IL-6 production (C), and TGF-$\beta_1$ production (D), and IL-8 production (E) of NDF and SDF. Results are expressed as means ± SE ($n = 9$). NDF, normal dermal fibroblasts; SDF, scar-tissue fibroblasts; PGE$_1$, prostaglandin E$_1$. 
3.2. Effects of TGF-β₁ on extracellular matrix formation of NDF and SDF

3.2.1. Effects of TGF-β₁ on PIP concentrations in the culture supernatants of NDF and SDF

PIP production by SDF was 1072.6 ± 58.0 ng/ml/10⁶ cells, which was significantly higher than that of NDF (843.2 ± 108.6 ng/ml/10⁶ cells). With the addition of TGF-β₁, PIP production by NDF increased to 1026.1 ± 63.2 ng/ml/10⁶ cells; however, this was not statistically significant compared with the values obtained before the addition of TGF-β₁. TGF-β₁ had no effect on PIP production by SDF (1070.5 ± 59.0 ng/ml/10⁶ cells) (Fig. 2A).

Fig. 2. Effects of TGF-β₁ on type I collagen production (A), and type I collagenase activity (B) of NDF and SDF. Results are expressed as means ± SE (n = 9).

3.2.2. Effects of TGF-β₁ on the type I collagenase activity of NDF and SDF

The type I collagenase activity of SDF was significantly lower than that of NDF (P < 0.05). With the addition of TGF-β₁, the activity in both NDF and SDF decreased slightly from 259.5 ± 37.7 unit/ml/10⁶ cells to 222.6 ± 47.3 unit/ml/10⁶ cells and 198.9 ± 31.4 unit/ml/10⁶ cells to 169.1 ± 29.9 unit/ml/10⁶ cells, respectively. All these decreases, however, were not statistically significant (Fig. 2B).

3.3. Effects of PGE₁ or TGF-β₁ on the collagenase/PIP ratios of NDF and SDF

In order to illustrate further the effects of PGE₁ and TGF-β₁ on extracellular matrix formation in
both NDF and SDF, we calculated the type I collagenase/PIP ratio (Fig. 3A,B). In SDF, this ratio was 0.25 ± 0.05, significantly lower \((P < 0.05)\) than that of NDF \((0.45 ± 0.01)\). Regarding the effects of PGE\(_1\) and TGF-\(\beta_1\) on the ratios of collagenase/PIP for both types of cells, PGE\(_1\) significantly increased the ratio of SDF \((0.25 ± 0.05 \text{ vs. } 0.31 ± 0.06, P < 0.05)\) (Fig. 3A), whereas TGF-\(\beta_1\) significantly decreased the ratio of NDF \((P < 0.05)\) (Fig. 3B) (Table 1).

4. Discussion

Hyperproliferation of fibroblasts and their hyper synthesis of collagen have been considered to be two causes in keloid and hypertrophic scar formation [15–17]. In recent years, PGE\(_1\) has been found not only to have a highly curative effect on wound healing [10,11] but also to prevent hypertrophic scar formation [12]. These findings indicate that PGE\(_1\) may act directly on fibroblasts to limit hypertrophic scar formation in addition to its well known effects on the vascular system. In this paper, the effects of PGE\(_1\) on fibroblasts in preventing hypertrophic scar formation were studied.

There are three pharmacologically defined subtypes of the PGE receptor(s) (EP), EP\(_1\), EP\(_2\) and EP\(_3\) [18]. Recently, the nucleotide and deduced amino acid sequences of these receptor cDNA have been reported [18–21]. PGE\(_1\) and PGE\(_2\) have the same ability to bind with the EP\(_2\) and EP\(_3\) receptor [19,20], but in comparison with PGE\(_2\), the ability of PGE\(_1\) to bind with the EP\(_1\) receptor is poor [21]. Thus, the activation of the EP\(_1\) receptor is associated with a rise in inositol triphosphate and intracellular calcium, and the activation of the EP\(_2\) receptor is associated with an increase in intracellular cAMP, and in contrast the activation of the EP\(_3\) receptor is associated with a decrease in intracellular cAMP [18]. But the action of these receptors of PGE in fibroblast surface has not been reported. We have previously shown that PGE\(_1\) increases the intracellular cAMP level in cultured fibroblasts and keratinocytes [13,14], suggesting that fibroblasts have PGE receptors which result in intracellular signal transduction.

We have also shown that PGE\(_1\) promotes epithelization of the wound through the proliferation of fibroblasts and increases IL-6 production by fibroblasts which facilitates the growth of the keratinocytes in vitro [13]. In this study, we first compared the in vitro production of extracellular matrix and cytokines by NDF and SDF collected from the same patients. The results showed that both TGF-\(\beta_1\) and PIP production by SDF were significantly higher than those by NDF, whereas type I collagenase activity in SDF was significantly lower than that in NDF, suggesting that the collagenase/PIP ratio of SDF was significantly lower than that of NDF. We have also showed in a previous report that the growth rate of SDF in DMEM was higher than that of NDF [22]. In addition, once the procollagens are secreted, they are converted to collagen by proteolytic cleavage of both the amino- and carboxyl-terminal propeptide extensions [23]. Kanayama, Raghow and co-workers reported detecting changes of type I collagen in tissues by measuring PIP [24,25].

| Treatment | Normal | | Scar |
|-----------|--------|--------|--------|--------|--------|--------|--------|
| PIP       | Collagenase | IL-6 | IL-8 | TGF-\(\beta_1\) | PIP       | Collagenase | IL-6 | IL-8 | TGF-\(\beta_1\) |
| Control   | 788.4 ± 43.2 | 393 | 328 | 86.5 ± 0.02 | 865.2 ± 50.1* | 205 | 282 | 40.9 | 0.148 |
| ±43.2     | ±84.5 | ±73.6 | ±21.1 | ±0.02 | ±50.1* | ±38.4* | ±85.7 | ±7.7* | ±0.039* |
| PGE\(_1\) | 849.3 ± 73.6 | 419 | 674 | 313.1 ± 0.184 | 893.4 ± 37.9 | 250 | 552 | 187.4 | 0.26 |
| ±73.6     | ±78.9 | ±83.7* | ±99.8 | ±0.052* | ±37.9 | ±41.8* | ±105.6* | ±36.2* | ±0.066* |
| TGF-\(\beta_1\) | ↑ | ↓ | \(\downarrow\) | \(\downarrow\) | | | | | |

*\(P<0.05\); up arrows, increase; down arrows, decrease; backslashes, not measured; dashes, no difference.
therefore, we have regarded the PIP as an activity of type I collagen synthesis. Furthermore, we studied the effects of the rhTGF-β1 on PIP concentration and type I collagenase activity in NDF and SDF. The results revealed that 2 ng/ml of rhTGF-β1 significantly decreased the type I collagenase/PIP ratio of NDF, but had no effect on that of SDF, suggesting that this could be due to the autocrine activity of TGF-β1 in SDF. These findings suggest that SDF produce TGF-β1, which might proliferate themselves in an autocrine manner, conferring a high capacity for collagen synthesis.

It was reported that the levels of PGE2 as a PGE1 isomer rose when gastric mucosal protective agents were applied to cutaneous wounds for treatment in an experimental model [26,27] and that their levels also rose when keratinocytes were injured by ultraviolet B irradiation [28]. Diaz et al. have reported that fibroblasts stimulated by TGF-β produce PGE2 which might be related to an auto-regulatory mechanism to limit the effects of TGF-β on connective tissue protein synthesis [29]. The evidence which supports the above-mentioned speculation is that the production of collagen and PGE2 synthesis by fibroblasts stimulated with TGF-β has been enhanced when pretreated with indomethacin as a PG inhibitor. However, it is not known whether the exogenous PGE has any effect on extracellular matrix formation and cytokine production in fibroblasts.

Cortical steroid hormones are often used in treating hypertrophic scars and keloids as a means of inhibiting collagen production. Recently, we have demonstrated that Tranilast (Rizaben, Kissei Pharmaceutical Co., Matsumoto, Japan) an anti-allergic agent, has an effect on treating keloid and hypertrophic scars due to its inhibition of TGF-β1 production by SDF [22]. In order to investigate the effects of PGE1 on wound healing processes especially on scar formation, we further studied its action on NDF and SDF in vitro. The results showed that PGE1 significantly increased type I collagenase activity in SDF, although it did not directly influence activity of type I collagen synthesis. Decreased activity of collagenase in SDF may be responsible for excessive accumulation of collagen in hypertrophic scars. PGE1 is thought to enhance the activity of type I collagenase which regulates the level of type I collagen in SDF, so PGE1 not only promotes epithelization but also prevents scar formation.

On the other hand, as IL-8 production by SDF was significantly lower than by NDF, even taking into account the PGE1-increased IL-8 production by SDF, IL-8 may play an important role in the increase of collagenase production. It was reported that IL-8 was able to stimulate collagenase activity and to decrease the collagen concentration in various cells [30–33]. Furthermore, PGE1 increased both TGF-β1 and IL-8 levels about threefold in NDF, whereas in SDF, the level of TGF-β1 was increased only 1.7-fold, and the level of IL-8 was increased threefold by PGE1, suggesting that IL-8 stimulated by PGE1 may be involved in the regulation of type I collagenase activity in SDF.

These data suggest that PGE1 enhanced the activity of type I collagenase of SDF by regulating the balance of TGF-β1 and IL-8 production which may at least partly account for the beneficial effects of PGE1 in the prevention of hypertrophic scar.

References


