Inhibition of Fibrotic Contraction by C-Phycocyanin through Modulation of Connective Tissue Growth Factor and α -Smooth Muscle Actin Expression

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The effects of C-phycocyanin (C-pc), a phycobiliprotein, on the expression of pro-fibrotic mediators in hyper-tropic scarring such as connective tissue growth factor (CTGF) and α -smooth muscle actins (α -SMA) were investigated in relation to trans-differentiation of fibroblast to myo-fibroblast, an icon of scar formation. C-pc was isolated from *Spirulina Platensis* extract using sonication method and C-pc concentration was determined by Bennet and Bogorad equation. α -SMA and CTGF levels in wounded primary human dermal fibroblasts were determined by western blot analysis and immuno-fluorescence confocal microscope was employed. Fibroblast contractility was examined by three-dimensional collagen lattice contraction assay. There was an elevation of α -SMA (121%) and CTGF (143%) levels in wound cells as compared with non-wound cells. The dose-response profiles of down regulation demonstrated that the maximum inhibitions of α -SMA by 63% ($p < 0.05$) and CTGF by 50% ($p < 0.1$) were achieved by C-pc (6 nM) treated cells. In confocal assay, non-wound fibroblasts exhibited basal level of α -SMA staining, while wounded cells without C-pc treatment showed strong up-regulation of α -SMA by 147% ($p < 0.05$). C-pc (6 nM) inhibited α -SMA expression by 70% ($p < 0.05$) and reduced collagen contraction by 29% ($p < 0.05$). C-pc seemed to lessen the over expression of CTGF, α -SMA, subsequently alleviating the fibrotic contracture. This study suggests the potential application of C-pc to regulation of the expression of pro-fibrotic mediators in scarring process and its potential usage as an efficient means for anti-fibrosis therapy.

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INTRODUCTION

Wound healing is a dynamic, interactive process involving soluble mediators, blood cells and extracellular matrix. Wound healing has three phases— inflammation, tissue formation, and tissue remodeling [1].

There is a substantial evidence that inflammation is an essential prerequisite for scarring and altered inflammatory and/or cytokine profile have a significant role in scar formation [2,3]. Un-controlled inflammation has been shown to delay healing and to result in increased scarring. Hypertrophic scar formation is an aberrant form of wound healing and related to inflammation-mediated dermal fibrosis [4]. These reports suggest that inflammatory response might provide a central target to modulate the outcome of the healing response.

C-phycocyanin (C-pc) is a phycobiliprotein from *Spirulina platensis*. C-pc is composed of two α and β polypeptide subunits and their molecular weights are 18.4 kDa and 21.3 kDa, respectively [5]. C-pc has been reported to exhibit variety of pharmacological properties such as antioxidant, anti-inflammatory, neuro-protective and hepato-protective effects [6-8]. C-pc has received much attention as a potent inhibitor of cyclooxygenase-2 (COX-2) [9]. COX-2 is an inducible form that participates in inflammation, and induction of COX-2 is responsible for the production of prostaglandin at the site of inflammation. Higher prostaglandin levels have been recognized to stimulate proliferation of cells and mediate immune response [1,10]. C-pc showed comparable COX-2 inhibition with an IC_{50} value of 180 nM which was much lower than the values obtained for celecoxib (255 nM) and rofecoxib (401 nM), the known selective inhibitors of COX-2 [11].

The role of inflammation in wound healing and fibrosis, the key involvement of fibroblasts, myofibroblasts have been emphasized [12]. Fibroblasts are the predominant cell type that synthesizes and remodels the extracellular matrix and central to

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the fibrotic response. Fibroblasts display considerable variation during wound repair [13].

Fibroblastic cells acquire contractile properties during the healing of an open wound, modulating into myofibroblasts [14]. Myofibroblasts express the smooth muscle actin isoform. During normal wound repair, myofibroblasts are transiently present at the wound site causing wound contraction and restoration of tissue integrity. Once the wound has regained normal structure and function, myofibroblasts disappear by apoptosis. In pathological states, myofibroblasts persist and produce excess of extracellular matrix deposition and tissue deformation leading to scarring [15]. Even though myofibroblasts are crucial for proper wound repair, their timely disappearance is crucial for preventing undesirable scarring process [16,17]. And connective tissue growth factor (CTGF) in early wound healing stage has beneficial effects in collagen production and proper organizing and epithelialization [18]. However, sustained over-expression of CTGF induced fibrosis [19]. CTGF elevation has been implicated in number of fibrotic lesions. Recent studies have demonstrated that in addition to its direct effect on extracellular matrix turnover, it may stimulate fibroblast to myofibroblast differentiation, as it is capable of up-regulating α -smooth muscle actin (α -SMA) in fibroblast.

Prostaglandin E_2 (PGE_2) has been shown to prevent multiple aspects of fibroblast activation, including the differentiation of fibroblasts to myofibroblasts. C-pc, a COX-2 inhibitor prevented PGE_2 synthesis in a dose dependent manner and preventing fibrosis [20]. PGE_2 exerts an important anti-fibrotic effect by increasing the sensitivity of fibroblast/myofibroblast to apoptosis [21]. C-pc mediated apoptosis in AK-5 tumor cells and prevented cancer cell proliferation [22]. Conversely, increased fibroblast proliferation and enhanced wound closure with up-regulation of collagen in C-pc-treated (75 μ g/mL) group were reported [23]. The validity of the wound healing effect and anti-scarring effect of C-pc remains to be clarified.

In this study, we investigated how anti inflammatory effect of C-pc could modify the outcome of wound healing, scar disposition. The effect of C-pc on α -SMA and CTGF, a pro-fibrotic mediators involved in hyper-trophic scarring were investigated in relation with modulation of trans-differentiation of fibroblast to myofibroblast, an icon of scar formation.

MATERIALS AND METHODS

Materials

All chemicals were purchased from Sigma chemicals (MO, USA). Human dermal fibroblast was provided from dept. dermatology, Seoul National University. *Spirulina platensis* was provided from Korea Institute of Ocean Science and Technol-

ogy (KIOST). Dulbecco's Modified Eagle Medium (DMEM) culture medium and fetal bovine serum (FBS) were purchased from Cellgro (Washington DC, USA), penicillin streptomycin was purchased from Gibco (New York, NY, USA) and Cultrex® Rat Collagen I was purchased from Trevigen (Gaithersburg, MD, USA).

Extraction of C-phycocyanin from *Spirulina platensis*

The *Spirulina platensis* powder (KIOST, Ansan, Korea) was suspended in 10 mL of 0.1M buffer (sodium phosphate buffer, pH=7). C-pc was extracted by the established standard process. In brief, the suspension was processed by 1 min vortex, 1 min sonication and 1 min vortex. The suspension was stored at 4°C for 4 hr. The mixture was then centrifuged at 2500 rpm for 5 min and repeated 3 times. The C-pc containing clear blue supernatant was collected. The absorbance of phycobiliprotein containing supernatant was measured by UV Spectroscopy (SmartSpec™ 3000 Spectrophotometer, BIO-RAD Inc., Hercules, CA, USA). The C-phycocyanin concentration (C-pc) in mg/mL was calculated from the optical densities at 652 and 620 nm, using the previously reported following Bennett and Bogorad equation [24].

$$\text{C-pc concentration} = \frac{\text{OD}_{620} - 0.474 \times \text{OD}_{652}}{5.34}$$

Cell lines

Primary human foreskin fibroblasts were provided from professor KC Park, department of dermatology, Medical school, Seoul National University. Fibroblasts were cultured in a 6 well plate (5×10^5 cells/well) in DMEM medium supplemented with 10% heat inactivated FBS and 100 units/mL penicillin/streptomycin. Cells were maintained in a humidified atmosphere with 95% air and 5% CO_2 at 37°C.

In vitro wound healing study

In vitro fibroblast wound model was prepared as reported previously [25]. In brief, cells were plated in six-well plates at 1×10^5 cells/well confluence and grown for 24 hr. After scratching with a sterile 10 μ L micro tip, cells were treated with C-pc for predetermined time points (3–6 hr) in incubator. After treating with C-pc, cell morphology, cell migration and proliferation were observed and images were captured and analyzed with Olympus microscopy system composed of an inverted microscope (OLYMPUS CK40, Olympus, Tokyo, Japan) and Camera (Olympus C-3030, Olympus, Tokyo, Japan).

CTGF immunoblot analysis

After 6 hr C-pc treatment, cells were rinsed with cold phos-

phate buffer solution (PBS) and lysed in sodium dodecyl sulfate (SDS)-lysis buffer. Equal amounts of protein extracts in SDS-lysis buffer were subjected to 12% SDS-polyacryl amide gel electrophoresis (PAGE) analysis and electrophoretically transferred to nitrocellulose membrane. Anti-CTGF antibody was purchased from Santa Cruz (CA, USA). β -actin antibody was from Sigma-Aldrich (MO, USA). Enhanced chemiluminescence (Amersham Pharmacia, Buckinghamshire, UK) system was used for detection. Relative intensity of the bands was determined by an Image Analyzer Gel Logic 200 Imaging System (NY, USA).

α -SMA western blot analysis

After scratching with a sterile 10 μ L micro tip, cells were cultured with and without C-pc in the absence of FBS. The cells are washed 2–3 times with cold PBS. Each well was added 70–80 μ L lysis buffer [1 M tris (pH 6.8) 500 μ L, 10% SDS 2 mL, 0.5 M ethylenediamine tetraacetic acid (pH 8.0) 20 μ L, 1 M DDT 1 mL, protease inhibitor 1 tab, D.W 5.48 mL; total 10 mL] to prepare a supernatant. The concentration of the supernatant was measured by Bradford method using Protein Assay Reagent (Bio-Rad Inc., Hercules, CA, USA). The protein were separated by SDS-PAGE (10% polyacrylamide), transferred onto polyvinylidene difluoride membrane and probed with specific polyclonal antibodies and secondary antibodies. Protein-antibody complexes were visualized by the enhanced chemiluminescence detection system (Amersham Pharmacia, Buckinghamshire, UK).

Collagen lattice contraction

After cells were cultured with and without C-pc for 6 hr, control and C-pc-treated cells were trypsinized. Collagen gel was prepared with 1.4 mL bovine dermal collagen and 0.4 mL 5 $\times$ DMEM (Sigma, St. Louis, MO, USA) and adjust the pH to 7.2 to 7.5 with NaOH. And 0.2 mL of the cell suspension (containing 5 $\times$ 10⁵ cells) was added to the dermal collagen solution. Before making collagen mixture, six-well plate was coated with BSA for 1 hr in incubator. Place the 2 mL collagen mixture into a well of the six-well plate, then, BSA was removed. Collagen lattices were allowed to gel for 60 mins in an atmosphere with 95% air and 5% at 37°C. After 60 mins, the collagen lattices were detached from the surface of the well by rimming the lattice with a sterile spatula. 4 mL of serum-free medium (SFM) was added to each well. And the rate of contraction was determined by measuring lattice diameter at predetermined time.

Confocal immuno-fluorescence analysis

The cells were plated in 8-well-slide glass at 2.0 $\times$ 10⁴ cells/well (250 μ L) confluence and grown for 16 hr. After scratching with 0.5–10 μ L micro tips, the cells were cultured with and without C-pc for 6 hr in incubator. And then tested fibroblast samples

were fixed in 3.7% formaldehyde for 1 hr and washed twice in PBS and then placed in 1% Triton X-100 (300 μ L/well) for 15 mins and blocked with 10% FBS for 1 hr at room temperature. Following sample preparation, immunohistochemistry was performed for the detection of α -SMA using indirect immunofluorescence with the primary antibody against α -SMA, monoclonal anti- α -SMA-Cy3 conjugated (Sigma, St. Louis, MO, USA) at a dilution of 1:200 in FBS for 16 hr in incubator. Slides were washed twice in PBS. Treated samples were exposed to 4,6-diamidino-2-phenylindole (DAPI) (Sigma, St. Louis, MO, USA) staining solution at a dilution of 1:5000 for 1 min. DAPI is a blue fluorescent nuclear stain, and this step was ensured to visualize nuclei. The slides were washed twice in PBS, mounted in antifade reagent 10 μ L and fixed it with cover slips. Images were viewed with a confocal laser-scanning microscope, Zeiss Laser Confocal Microscope (ZEISS-LSM 700, Jena, Germany).

Statistical analysis

Comparisons of CTGF, α -SMA, collagen lattice contractions among control and C-pc-treated groups were performed using Student's t-test. p value <0.05 and p <0.1 was accepted as denoting statistical significance. Data are expressed as mean $\pm$ standard deviation.

RESULTS

Physico-chemical properties of C-pc

The water solubility of C-pc extracted from *Spirulina Platensis* (KIOST) was 4.06 $\pm$ 0.45 mg/mL. Partition coefficient ($P_{\text{Octanol/Water}}$) of C-pc was 0.063 ($\log P_{\text{Octanol/Water}} = -1.2$).

Stability of phycocyanin

After extraction, stability of C-pc at different temperatures was investigated. C-pc maintained its stability when stored at -10°C in nitrogen gas filled vials. During 24 hr period, 5% and 23% loss of C-pc concentration were observed at 4°C and 25°C, respectively. To maintain stability, C-pc was stored at -10°C in nitrogen gas filled vials prior to use.

Effect of C-pc on the trans-differentiation of fibroblasts to myofibroblasts

Scratch-wound model was prepared with human dermal fibroblasts and the effect of C-pc on the trans-differentiation of fibroblast to myofibroblast was investigated. Figure 1 show phase contrast Images of scratched dermal fibroblasts at 9 hr post injury time with (6 nM) or without C-pc treatment. In SFM, C-pc-untreated conditions, large squamous polygonal myofibroblasts (arrow) were observed (Fig. 1A). In C-pc-treated cells (6 nM), mostly typical biaxial spindle-shaped fibro-

Figure 1. Phase contrast images of wounded dermal fibroblasts at 9 hr post injury without C-pc serum free media (A) and C-pc treatment (B). A representative image is shown from three replicates (n=3). C-pc: C-phycocyanin.

blasts (arrow) were observed (Fig. 1B). This result clearly demonstrates the inhibitory effect of C-pc on the trans-differentiation of fibroblasts into myofibroblasts.

Effect of C-pc on the expression of cytokines

Connective tissue growth factor

Figure 2A shows western blot analysis of CTGF in fibroblasts in response to different concentrations of C-pc treatment (0 to 24 nM). Un-wounded (normal) cells express negligible CTGF. CTGF level increased 1.4-fold in wound cells and the level of CTGF was reduced to 50% in C-pc-treated cells ($p<0.1$). The extent of CTGF expression was dose dependent. Expression of CTGF relative to β -actin, normalized to non-wound is summarized in Figure 2B. The expression of CTGF was reduced in low doses of C-pc-treated cells. CTGF was observed in 6 nM C-pc-treated cells. And the extent of CTGF inhibition was leveled down as the C-pc concentration was further increased to 24 nM. The increased expression of CTGF at higher concentration (24 nM) can be explained by comparing with the previous report of significant enhancement of cell migration at higher concentration of C-pc [23]. C-pc-treated groups (75 μ g/mL) showed significant up-regulation of hydroxyprolin and collagen content and faster wound closure. High hydroxyl proline content suggests an abundance of myofibroblasts in the wound environment that facilitates collagen deposition and fibroblast migration during the proliferation stage of wound healing. In a contrary, C-pc-mediated apoptosis in Ak-5 tumor cells involves down-regulation of Bcl-2 and generation of ROS was reported [22]. To expect favorable wound healing without scarring, delicate balance between complex networks of many pro and anti-inflammatory mediators is crucial [1].

Figure 2. Expression of connective tissue growth factor (CTGF) in fibroblasts in response to C-phycocyanin (C-pc) treatment (A). Each panel represents relative CTGF expression in different concentrations of C-pc-treated (0 to 24 nM) wound fibroblast group as compared with normal, un-wounded fibroblasts (B). Statistical significances between C-pc-untreated and treated groups were evaluated using Student's t-test. Bars represent mean $\pm$ SEM of three replicates per treatment conditions. Statistically different from C-pc-untreated group (0 nM), * $p<0.05$, ** $p<0.1$.

Figure 3. Western blot analysis of α -smooth muscle actins (α -SMA) expression in wounded fibroblasts in response to different concentrations of C-phycocyanin (C-pc) treatment (A). Primary dermal fibroblasts from female human forehead were grown to 80% confluence on 100 mm tissue culture plates, and serum/growth factor starved for 4 hours. Semiconfluent cultures were treated with C-pc. Expression levels of α -SMA are represented relative to normal, non wound control (B). Bars represent mean $\pm$ SEM of three replicates per treatment conditions. Statistically different from C-pc-untreated control, * $p<0.05$, ** $p<0.1$.

Alpha smooth muscle actin

Effect of C-pc on α -SMA expression was studied with different concentration ranges (0 to 24 nM). To understand how the scratch wound condition increase the α -SMA expression, unwounded fibroblasts were studied as a comparison.

Figure 3A shows western blot analysis of α -SMA in fibroblasts in response to different concentrations of C-pc treatment. Unwounded (normal) cells express negligible α -SMA. Wounded fibroblasts up regulate α -SMA. The extent of α -SMA expression was dose dependent. Expression of α -SMA relative to β -actin, normalized to non-wound is summarized in Figure 3B. The expression of α -SMA was reduced by 36% ($p<0.05$) in 6 nM and 31% ($p<0.05$) inhibition in 8 nM C-pc-treated cells. As the concentration of C-pc further increased to 24 nM, the inhibition of α -SMA C was not significant. The less inhibition of α -SMA at higher concentration of C-pc can be explained by that pros-

taglandin has many isoforms (A to F). Among them, PGE_2 has been shown to prevent multiple aspects of fibroblast activation, including the differentiation of fibroblasts to myofibroblasts [20]. Reddy et al. [11] reported that C-pc inhibited PGE_2 synthesis in a dose dependent manner; 10 nM (36% inhibition); 30 nM (46% inhibition). C-pc at a concentration of 1 μM inhibited the release of PGE_2 to a significant extent (nearly 80% inhibition).

Collagen lattice contraction

The fibroblast-populated collagen lattice (FPCL) contraction assay was used to examine that C-pc could alter fibroblast-mediated contraction of collagen lattices. Figure 4A shows that C-pc inhibited FPCL contraction in a dose-dependent manner. C-pc treatment (6 nM) induced a decrease in contraction rate as compared to the untreated cells. Figure 4B shows the percentage of contraction of FPCL when they were treated with different concentrations of C-pc (0 to 24 nM). Without C-pc treatment, FPCL maintained its initial diameter ($50.36 \pm 0.02\%$). C-pc treatment (6 nM) maintained its initial diameter ($65.04 \pm 0.01\%$). The degree of collagen contraction is strongly correlated with α -SMA expression level.

Confocal immunofluorescence analysis

Expression of α -SMA is considered as a marker of myofibroblasts.

Confocal immune-fluorescence analysis was conducted to observe the expression of α -SMA. Figure 5A shows representative confocal sections of immune-fluorescence staining for α -SMA (red) and nuclear staining (blue) with DAPI. Normal, unscratched cells exhibit basal negligent level of α -SMA staining. In the overlaid confocal sections (staining for DAPI and α -SMA) without C-pc treatment, abundance of α -SMA were presented. In contrast C-pc-treated (6 nM) cells show negligible α -SMA staining. This result suggests optimum concentration of C-pc should be carefully selected to expect favorable effect of C-pc. In contrast, there are clear differences in α -SMA immune-reactivity between C-pc-treated and untreated fibroblasts. The untreated cells exhibited cellular hypertrophy and squamous morphology with strong staining of α -SMA and well-formed actin stress fibers. C-pc-treated fibroblasts induced a decrease of α -SMA expression compared to the control untreated fibroblasts. The quantitative comparison of α -SMA western blot analysis with different concentrations (0 to 24 nM) of C-pc treatment was performed. Calculation of the α -SMA expression ratio (number of α -SMA expressed cells/number of DAPI stained cells) was summarized in Figure 5B. The expression of α -SMA was dependent on the concentration of C-pc treatment. After scarring, α -SMA expressing cells increased to 2.4-fold and C-pc treatment (6 nM) reduced the level of α -SMA to

Figure 4. (A) Representative photographic images of fibroblast-populated collagen lattices (FPCL) after wounding and 6 hr C-phycocyanin (C-pc) treatment at 0 and 24 hr observation time: non wounded; wounded without C-pc treatment (0 nM) and wounded with C-pc (6 nM) treatment. (B) Percentage of contraction of collagen lattices 24 h after C-pc treatment. Relative percentage of collagen contraction in C-pc-treated (6, 8, and 12 nM) and without C-pc-treated groups are statistically different with 95% confidence interval ($p<0.05$). Whereas, at high concentration of C-pc-treated group (24 nM), no statistically significant differences as compared to control was observed. Bars represent mean $\pm$ SEM of three replicates per treatment conditions. Significantly different from C-pc-untreated (0 nM) wound fibroblasts, * $p<0.05$, ** $p<0.1$.

Figure 5. (A) Confocal immunofluorescence analysis [$\times 20$, confocal microscopy (ZEISS-LSM700), blue; 4,6-diamidino-2-phenylindole (DAPI), Red; Cy3]. Fibroblast differentiation to myofibroblast phenotype is characterized by the expression and assembly of smooth muscle actin into stress fibers. Immunohistochemical staining for α -smooth muscle actin (α -SMA) on day 9, using a diaminobenzidine detection system, demonstrates a reduction in the number of α -smooth muscle actin expressing cells after C-phycocyanin (C-pc) treatment (A) compared with non-wounded and no treatment (0 nM). (B) In human dermal fibroblasts cultures, C-pc induced a concentration dependent modulation of α -SMA expression. The down regulation of α -SMA expression was concentration dependent. This effect of C-pc is associated with morphological changes of cellular hypertrophy and well-formed actin stress fibers. Bars represent mean $\pm$ SEM of three replicates per treatment conditions. Significantly different from C-pc-untreated (0 nM) wound fibroblasts, * $p<0.05$, ** $p<0.1$.

70% as compared to untreated cells ($p < 0.05$). The extent of α -SMA inhibition was leveled down as the C-pc concentration was further increased to 24 nM.

DISCUSSION

To reduce the size of an open wound without scarring, delicate balance between the formation of connective tissue and degradation of excessive extracellular collagen matrix deposition is crucial [26,27]. In wound healing, the initial stage of healing is cell migration and inflammation followed by cell proliferation, matrix synthesis and resolution. In resolution phase, inflammatory cells undergo apoptosis with a resolution of inflammation and a drop in collagen synthesis so that collagen production is balanced by collagen breakdown. As this balance between collagen production and breakdown disrupted, proliferative scarring occurred [28,29]. Rinkevich et al. [3] investigated the effects of inhibiting dipeptidyl peptidase, a cell surface serine exopeptidase on scar formation during wound healing using a small molecule (diprotin A), which acts as a selective allosteric inhibitor of CD26 peptidase activity. They reported that complete healing of CD26-inhibitor-treated wounds took 5 days longer than control wounds. Most importantly, although the rate of healing was decreased in inhibitor-treated versus control wounds, treated wounds showed significantly reduced final scar size. It is strongly suggested that faster wound closure implies that there are higher extra collagen matrix disposition (scar) occurs. Down-regulation of α -SMA and CTGF expression and inhibition of myofibroblast persistence observed C-pc-treated group (6 nM) could be explained by the previous reports demonstrating phycocyanin-mediated apoptosis in Ak-5 tumor cells [22]. A recent study also lends support to this notion. Anti-inflammatory effect of C-pc can control the inflammatory signal to macrophage to produce TGF- β which functions as a pro-scarring growth factor [15]. Concentration dependency of C-pc on reduction of fibrotic contraction could be explained by that PGE₂ inhibits the differentiation of fibroblasts to myofibroblasts [20]. PGE₂ is an important antifibrotic lipid mediator. When there is a failure of induction of COX-2, thus PGE₂, fibro-proliferation and fibrosis occurs. Reduced level of PGE₂ is found in pulmonary fibrosis patients [21]. C-pc inhibits PGE₂ synthesis in a dose dependent manner; 10 nM (36% inhibition); 30 nM (46% inhibition); 1 μ M (nearly 80% inhibition) [11]. In this study, we observed that C-pc exhibits dual effects depending on the concentration applied on scratched wound dermal fibroblast model. The less inhibitory effect at high concentration of C-pc-treated group correlates with faster wound closure and high collagen production [29,30]. The role of C-pc in relation to growth factors and cell migration during

wound healing needs further investigation. The concentration dependency of C-pc in anti-scarring effect might be related to the paradoxical status of a pro-inflammatory factor with immune-suppressive activity of prostaglandin E₂ [31]. Our study gives the insight to optimize the dose of C-pc for anti scar therapy. However, further study is needed to clarify the role of C-pc in modifying wound healing process and scar prevention in relation with inhibiting PGE₂ synthesis.

Conclusion

In this study, we demonstrate that persistence of myofibroblast in wound healing could be inhibited by C-pc treatment. C-pc could modulate the over expression of CTGF, α -SMA and inhibit the fibrotic contracture in scarring process. This study suggests the potential application of C-pc for anti-fibrosis therapy and scar modulating agent.

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Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

There are no animal experiments carried out for this article.

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