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Original Research Article

Sphingosylphosphorylcholine induces α-smooth muscle actin expression in human lung fibroblasts and fibroblast-mediated gel contraction via $S1P_2$ receptor and Rho/Rho-kinase pathway

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ABSTRACT

Chronic airway diseases like COPD and asthma are usually accompanied with airway fibrosis. Myofibroblasts, which are characterized by expression of smooth muscle actin (α-SMA), play an important role in a variety of developmental and pathological processes, including fibrosis and wound healing. Sphingosylphosphorylcholine (SPC), a sphingolipid metabolite, has been implicated in many physiological and pathological conditions. The current study tested the hypothesis that SPC may modulate tissue remodeling by affecting the expression of α-SMA in human fetal lung fibroblast (HFL-1) and fibroblast mediated gel contraction. The results show that SPC stimulates α-SMA expression in HFL-1 and augments HFL-1 mediated collagen gel contraction in a time- and concentration-dependent manner. The α-SMA protein expression and fibroblast gel contraction induced by SPC was not blocked by TGF-β1 neutralizing antibody. However, it was significantly blocked by S1P2 receptor antagonist JTE-013, the Rho-specific inhibitor C3 exoenzyme, and a Rho-kinase inhibitor Y-27632. These findings suggest that SPC stimulates α-SMA protein expression and HFL-1 mediated collagen gel contraction via S1P2 receptor and Rho/Rho kinase pathway, and by which mechanism, SPC may be involved in lung tissue remodeling.

1. Introduction

Chronic airway diseases, such as asthma and chronic obstructive pulmonary disease (COPD) are characterized by fibrotic scarring of airways [1,2]. Myofibroblasts, distinguished by the expression of smooth muscle actin (α-SMA), have widely been thought to contribute to these processes [3,4]. α-SMA has already been demonstrated to be related to fibroblast contraction and tissue remodeling which are involved in normal wound healing and fibrosis [4–6].

TGF-β1 induces fibroblast transition via stimulation of α-SMA expression in fibroblasts which is thought to be mediated by Smad3 protein [5,6]. Many other reagents, like thrombin [7,8], IL-4, and IL-13 [9] also can stimulate α-SMA expression, which is mediated by a different mechanism.

Sphingosylphosphorylcholine (SPC) has been recognized as an important signal molecule and is believed to play an important role in physiological and pathophysiological conditions. It is involved in many biological processes including smooth muscle contraction, wound healing and angiogenesis [10,11]. It has also been demonstrated that SPC accelerates the healing of cutaneous wound in healing-impaired diabetic mice [12] and stimulates dermal

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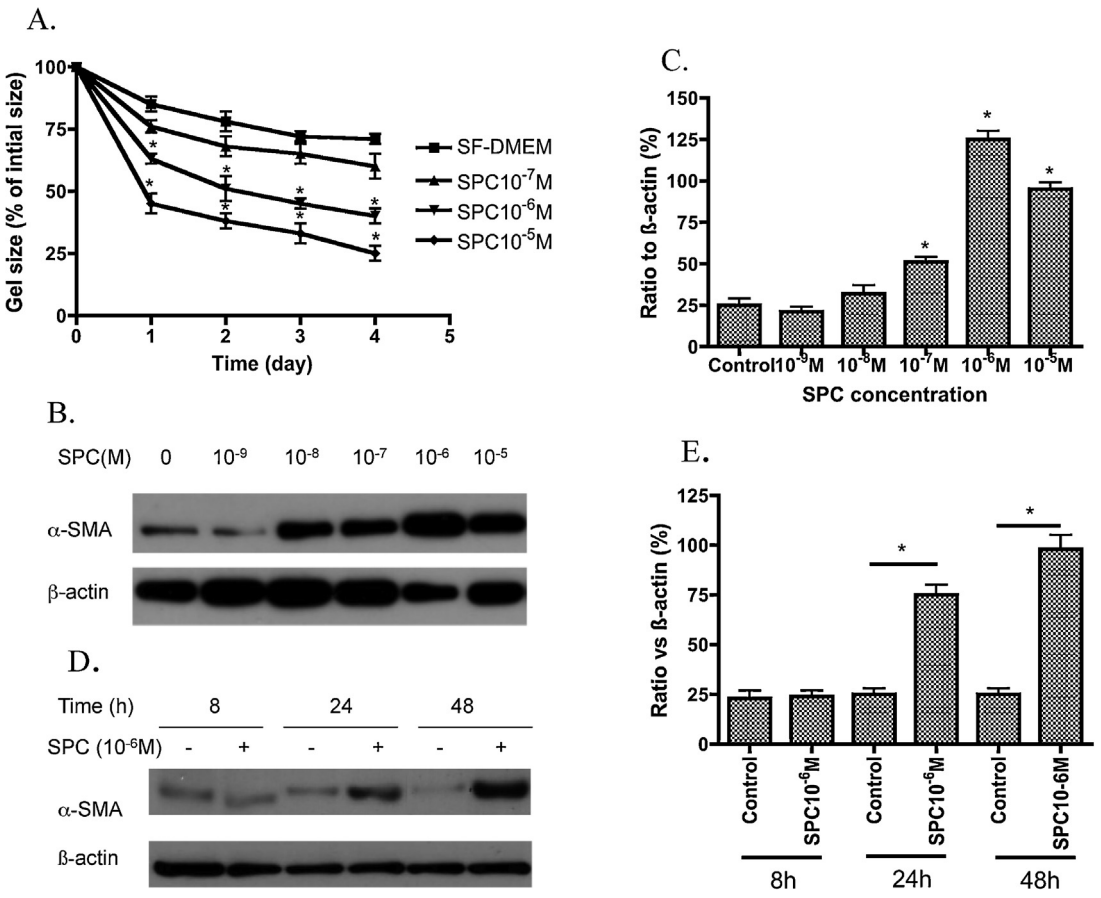

Fig. 1. Effect of pretreatment of SPC on fibroblast-mediated collagen gel contraction and α-SMA expression. Panel A: Collagen gel contraction. HFL-1 cells were pretreated with varying concentrations of SPC for 48 h. Cells were then trypsinized and cast into type I collagen gels. Gel size was measured with an image analyzer daily for 4 days. Vertical axis: gel size expressed as percent of initial size (%); horizontal axis: time (day). $^*p < 0.05$ compared to SF-DMEM (control) on each day. Panel B and C: SPC concentration-dependent effect on α-SMA expression. HFL-1 cells were serum-deprived for 24 h, and then treated with various concentrations of SPC for 48 h. Cell lysate (5 μg/lane) were separated by SDS–polyacrylamide gel electrophoresis and immunoblotted with anti-α-SMA and anti-ß-actin antibodies. Panel B: One representative of immunoblotting. Panel C: Densitometric quantification of 3 separate experiments. $^*p < 0.05$ compared to control. Panel D and E: Time-dependent effect of SPC on α-SMA expression. HFL-1 cells were serum-deprived for 24 h, and then treated with or without SPC (10^{-5} M) for 8–48 h. Cell lysate was used for immunobloting α-SMA and ß-actin. Panel D: One representative of immunoblotting. Panel E: Densitometric quantification of 3 separate experiments. $^*p < 0.05$.

fibroblast mediated gel contraction [13]. However, the mechanism is not fully defined. Thus, we hypothesized that SPC might also be involved in airway tissue remodeling by affecting the phenotype of fibroblasts through specific receptor and down stream signaling pathways. To demonstrate this hypothesis, we have conducted serial experiments to determine if SPC stimulates α-SMA expression in HFL-1 and if SPC pretreated fibroblast can augment fibroblast-mediated collagen gel contraction. Additionally, this study seeks to explore its pathway of signal transduction.

2. Materials and methods

2.1. Cell culture

Human fetal lung fibroblasts (HFL-1) were purchased from the American Type Culture Collection (Rockville, Maryland). The cells were cultured in 100 mm tissue culture dishes (FALCON, Becton-Dickinson Labware, Lincoln Park, New Jersey) in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum (FCS, Biofluid, Rockville, Maryland), 50 U/ml penicillin G sodium, 50 μg/ml streptomycin sulfate (Penicillin-streptomycin, GIBCO, Life Technologies, Grand Island, New York) and 1 μg/ml Amphotercin B (Pharma-Tek, Hutington, New York). Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2/95% air. Passages 14–19 were used in all experiments.

2.2. Materials

Sphingosylphosphorylcholine (SPC), pertussis toxin, monoclonal α-SMA-antibody and monoclonal β-actin antibody were purchased from Sigma (St. Louis, MO). Recombinant TGF-β1 and its neutralizing anti-TGFβ1 antibody were from R&D Systems Inc. (Minneapolis, MN). JTE-013 and W146 were from Cayman chemical (Ann Arbor, MI). C3 exoenzyme was provided by courtesy of Dr. Myron Toews. Y27632 were obtained from Biochem (La Jolla, CA). Anti-phospho-smad3 and anti-Smad3 antibodies were obtained from Cell signaling Technology (Beverly, MA).

2.3. Collagen gel contraction assay

Collagen gels were prepared by mixing rat tail tendon collagen (RTTC), distilled water, 4×-concentrated DMEM and cell suspension so that the final mixture resulted in 0.75 mg/ml collagen, 3×10^5 cells/ml and 1× DMEM. One-half milliliter of the collagen/cell mixture was plated into each well of a 24-well tissue culture plate. Gels were allowed to solidify at room temperature, usually within 15 min. Gels were then released into a 60-mm tissue