Figure 2. Comparison of various indicators before and after conventional and/or low-dose IL-2 treatment in DM. (A-I) Comparison of lymphocyte subsets and CD4+T cells subsets in healthy controls and DM patients before and after conventional and low-dose IL-2 treatment. Data were expressed as Median (Q25, Q75). Data were analyzed by the Independent-Samples Kruskal-Wallis test one-way ANOVA. *P<0.05; **P<0.001.

Figure 3. Comparison of serum concentrations of cytokines (including IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL-17) between PM/DM and Healthy Controls by Mann-Whitney U test. *P<0.05.

Abstract THU0332 – Figure 3

Figure 4. The correlation between multiple indicators and MYOACT. Heatmap representation of correlation of MYOACT with lymphocyte subpopulation (including the number and percentage of CD3+T, B, Th, Ts and NK cells), CD4+T subsets (including the number of Th1, Th2, Th17, Treg cells and the ratio of Th1/Th2, Th1/Treg), Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), White Blood Cell (WBC), Platelet (PLT), Lymphocyte (LYMP), Neutrophil Granulocyte (NEUT), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Creatine Kinase (CK), Lactate Dehydrogenase (LDH) and Hydroxybutyrate Dehydrogenase (HBDH) in DM patients (n=61). *P<0.05 by Spearman correlation test.

Abstract THU0332 – Figure 4

Conclusion: The decrease of Treg cells was found in PM/DM accompanied with lower serum IL-2, which should be associated with the pathogenesis of PM/DM. Meanwhile, various pro-inflammatory T cells also showed a downward trend with or without significant difference, indicating PM/DM is caused by immunodeficiency rather than hyperimmunism. Id-IL2 combined with conventional therapy could restore the reduction of CD4Treg cells and help to alleviate disease activity.

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THU0333

2-CARBA CYCLIC PHOSPHATIDIC ACID (2CCPA) SUPPRESSES PROFIBROTIC ACTIVITY IN SYSTEMIC SCLEROSIS SKIN FIBROBLASTS AND BLEOMYCIN-INDUCED SKIN FIBROSIS IN MICE

Tomoaki Higuchi^{1,1}, Kae Takagi¹, Akiko Tochimoto¹, Yuki Ichimura¹, Takanari Norose¹, Yasuhiro Katsumata¹, Ikuko Masuda¹, Hisashi Yamanaka¹, Toshihiro Moroboshi², Yasushi Kawaguchi¹. ¹Tokyo Women's Medical University, Department of Rheumatology, Tokyo, Japan; ²SANSO, Co., Ltd, Tokyo, Japan

Background: Systemic sclerosis (SSc) is a connective tissue disorder with progressive fibrosis in multiple organs including skin, lung and the gastrointestinal tract. Fibrosis is thought to be driven by activated fibroblasts. Therefore, inhibition of the profibrotic activity of activated fibroblasts may be a promising therapeutic approach in skin fibrosis in SSc. The autotaxin (ATX)/lysophosphatidic acid (LPA) axis is reportedly involved in fibrotic pathogenesis in SSc. 2-carba cyclic phosphatidic acid (2ccPA) is a naturally occurring lipid mediator and one of its pleiotropic properties is to inhibit the ATX/LPA axis.

Objectives: We investigated the anti-fibrotic effect of 2ccPA on human SSc skin fibroblasts and bleomycin-induced skin fibrosis in mice. Furthermore, we searched signaling pathways related to the anti-fibrotic effects of 2ccPA.

Methods: This study was approved by the ethics committee and the ethical review committee of animal experiments of Tokyo Women's Medical University. We informed all participants of the content of this study, and written consent was obtained. Skin fibroblasts were taken from SSc patients and adult healthy individuals were purchased. The cells were incubated with 1-10 μ M 2ccPA in the presence or absence of 10 ng/ml transforming growth factor- β 1 (TGF- β 1). Messenger RNA (mRNA) and protein expression for type I collagen, connective tissue growth factor (CTGF), α smooth muscle actin (α SMA), fibronectin (FN) and TGF- β 1 were assessed by qRT-PCR or Western blotting. Procollagen type I C-peptide, prostaglandin E₂ (PGE₂) and hepatocyte growth factor (HGF) levels in the supernatant were assessed by ELISA. Intracellular cyclic adenosine monophosphate (cAMP) levels were calculated using a commercially available EIA kit. Forskolin was used to increase intracellular cAMP levels in cultured SSc skin fibroblasts. An inhibitor of adenylate cyclase (AC), 2'-deoxyadenosine, was used to investigate whether the anti-fibrotic effect of 2ccPA was mediated via the AC/cAMP pathway. Furthermore, we used a mouse model of bleomycin-induced skin fibrosis to investigate the safety and anti-fibrotic effects of 2ccPA.

Results: Ten μ M 2ccPA significantly reduced mRNA and protein expression for type I collagen, CTGF and α SMA in SSc skin fibroblasts and adult healthy skin fibroblasts treated with TGF- β 1. PGE₂ and HGF levels in the supernatant of SSc skin fibroblasts were also reduced by 2ccPA treatment. 2ccPA increased intracellular cAMP levels as well as the AC stimulator, forskolin. In addition, forskolin decreased the mRNA expression of profibrotic markers. Reduction of COL1A1 mRNA expression by 2ccPA was blocked by treatment with 2'-deoxyadenosine in cultured SSc skin fibroblasts, suggesting that the anti-fibrotic activity of 2ccPA was partially mediated via AC stimulation. In mouse experiments, intraperitoneal injection of 10 mg/kg 2ccPA significantly reduced the development of skin thickness, collagen content and α SMA-positive cell counts.

Conclusion: 2ccPA suppressed the profibrotic activity of SSc skin fibroblasts and the development of bleomycin-induced skin fibrosis. Our experiments suggested that the anti-fibrotic property of 2ccPA was at least in part due to increased intracellular cAMP levels in skin fibroblasts. 2ccPA has been reported to be well tolerated in clinical trials of other diseases and may be expected for the treatment of fibrotic lesions in SSc.

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