A novel TGF-beta/Smad signaling inhibitor ameliorates bleomycin-induced skin fibrosis

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Introduction

Systemic sclerosis (SSc) is a chronic autoimmune disease characterized by progressive fibrosis of skin and internal organs. TGF- β /Smad signaling is well known to play a crucial role in the pathogenesis of SSc. Recently, we developed a novel compound, HPH-15, which antagonizes TGF- β /Smad signaling.

Objective

The purpose of the current study was to clarify the effects of this drug in a preclinical model of SSc and in human skin fibroblasts.

- The antifibrotic properties of HPH-15 were examined in a bleomycin-induced skin fibrosis mouse model.
- The effects of HPH-15 on migration, proliferation, expression of extracellular matrix components, and TGF- β signaling in human dermal fibroblasts were analyzed.

Materials and Methods

C57BL/6J: female, 10-12 weeks, n = 3-5

Bleomycin (BLM): subcutaneous (150 μ g/day)

HPH-15: oral (100 mg/kg daily)

Human dermal fibroblasts

Results

HPH-15 treatment ameliorated dermal thickening in the BLM-injected skin

HPH-15 treatment reduced the number of CD3⁺ T cells and F4/80⁺ macrophages in BLM-injected skin

HPH-15 treatment reduced inflammatory macrophages in the early stage and profibrotic M2 macrophages in the late stage

HPH-15 inhibited the mRNA levels of M2 macrophage markers, not M1 macrophage marker

HPH-15 recovered the mRNA levels of Fli1 and KLF5, transcription factor that repress collagen synthesis

HPH-15 inhibited fibrotic responses induced by TGF- β in cultured human dermal fibroblasts

HPH-15 inhibited fibroblasts migration and/or proliferation

HPH-15 antagonizes the over-phosphorylation of Smad3 in the mouse skin and cultured human dermal fibroblasts

Summary

Administration of HPH-15 demonstrated:

- Suppression of skin thickening and collagen accumulation in the BLM-induced skin fibrosis mouse model.
- Reduction of increased numbers of macrophages and T cells in the BLM-injected skin.
- Decreased dermal infiltration of proinflammatory macrophages (CD11b⁺Ly6C^{hi}) and M2 profibrotic macrophages (CD11b⁺CD204⁺ or CD11b⁺CD206⁺) during the early and late stages, respectively.
- Augmentation of the mRNA levels of Fli1 and KLF5, and reduced M2 macrophage markers in the BLM-injected skin.
- Inhibition of fibrotic responses induced by TGF- β in cultured human fibroblasts.
- Antagonizing Smad3 phosphorylation in the mouse skin and cultured human dermal fibroblasts.

Conclusion

Our study clearly shows that HPH-15 inhibits TGF- β /Smad signaling and fibrogenic activity of human skin fibroblasts in vitro and attenuates skin inflammation and subsequent fibrosis in a mouse model of SSc. Since the findings of this early preclinical study demonstrate several advantages of HPH-15, including the fact that it is an orally active agent with an excellent safety profile, HPH-15 shows potential as a strong candidate for SSc clinical trials.