Poly(lactic-co-glycolic acid) (PLGA) Nanoparticles Containing Pioglitazone Attenuate Bleomycin-induced Skin Fibrosis.

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Summary

We prepared controlled release matrix of PPAR γ agonist pioglitazone for local treatment of skin fibrosis. Weekly subcutaneous injections of the nanoparticles were effective for skin fibrosis.

Background and Aim

Pioglitazone and other thiazolidinedione derivatives are used for the treatment of diabetes mellitus type 2. They have been identified as high affinity ligands for peroxisome proliferator-activated receptor gamma (PPAR γ), and shown to have potent anti-inflammatory and anti-fibrotic actions (A). Poly(lactic-co-glycolic acid) (PLGA) is one of biodegradable synthetic polymers commonly used to prepare biocompatible nanoparticles (B). Water-insoluble drugs like pioglitazone can be encapsulated in PLGA nanoparticles (C). We investigated a potential of PLGA nanoparticles as the controlled release matrix of pioglitazone to treat localized skin fibrosis.

Methods

As the controlled release matrix of pioglitazone, we prepared PLGA nanoparticles containing pioglitazone (pioNP) by the conventional solvent evaporation method, followed by freeze drying. We prepared PLGA nanoparticles without pioglitazone (mockNP; as a control) and coumarin-6 containing nanoparticles (coumarinNP; as fluorescent nanoparticles) similarly. To confirm the anti-fibrotic effect of pioglitazone and pioNP *in vitro*, cultured primary murine dermal fibroblasts or human dermal fibroblasts were treated with pioglitazone or pioNP, and stimulated by 10 ng/ml of transforming growth factor beta 1 (TGFb1). The cells were then analyzed by quantitative real time PCR (qRT-PCR) and immunocytochemistry. *In vitro* scratch assay was performed by creating scratches in monolayer fibroblasts. The cellular uptake of coumarinNP by cultured fibroblasts was observed by fluorescence microscope. To confirm the anti-fibrotic effect of pioNP *in vivo*, subcutaneous injection of pioNP or mockNP to the back skin of bleomycin-induced skin fibrosis mouse model (Yamamoto T, et al. J Invest Dermatol. 1999;112:456-62.) was performed once a week. The lesional skin tissue was collected after 3 weeks and analyzed pathologically by HE staining, picrosirius red staining and immunohistochemistry of the cryosections. qRT-PCR analysis of the total RNA from the tissue was also performed. All animal experiments were performed in accordance with the guidelines of the institutional committee for the use of animals for research. The results were expressed as mean $\pm$ SD. The statistical significance was determined by Tukey-Kramer multiple comparison test, $p < 0.05$ was considered to be significant.

Results

1. PLGA nanoparticles show sustained release of pioglitazone *in vitro*

2. Pioglitazone suppress migration ability of fibroblasts and have antifibrotic effects for fibroblasts *in vitro*

3. PLGA nanoparticles are internalized by fibroblasts and have antifibrotic effects for fibroblasts *in vitro*

4. Local treatment with PLGA nanoparticles containing pioglitazone attenuates skin fibrosis *in vivo*

Female Balb / c mice had subcutaneous injections of PBS (WT) or bleomycin 4 times per week, and they concurrently had subcutaneous injections of PBS, mockNP (100 μ g) / PBS, or pioNP (100 μ g) / PBS once a week. (A) HE staining. Bars indicate the extent of the dermis. (B) PioNP treated skin showed decrease in dermal thickness. (C) Collagen deposition was investigated by fluorescent images of Picrosirius red staining. (D) Picrosirius red stained area was determined by red fluorescent area minus red and green fluorescent overlapped area. (E, F) Immunofluorescence with antibodies to α SMA (Cy3), counterstained with DAPI. PioNP treatment decreased α SMA positive mFibroblasts in immunocytochemistry. (G-I) qRT-PCR analysis. Results were normalized to GAPDH. *P < 0.05, **P < 0.01.

Conclusion

Pioglitazone and PLGA nanoparticles containing pioglitazone have antifibrotic effects for cultured dermal fibroblasts. The local treatment with PLGA nanoparticles containing pioglitazone attenuate bleomycin-induced skin fibrosis and is promising as a treatment for scleroderma and other fibrotic diseases.

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