ProGel-Tanshinone (P-Tan) Enhances Fracture Healing in a Glucocorticoid-Induced Delayed Healing Model

Shabnam Arash[1], Jie Rong[1], Yangwei Deng[1], Salma Althobaiti[1], Artem Marchenko[1], Shanshan Liu[1], Dong Wang[1]
[1]University of Nebraska Medical Center, Omaha, NE
Sarash@unmc.edu

Disclosure: NA.

ABSTRACT

Introduction

Fracture repair is a dynamic biological process that can be substantially impaired by chronic corticosteroid therapy, a treatment commonly prescribed for inflammatory/autoimmune diseases. Long-term corticosteroid exposure reduces callus formation, delays mineralization, and increases non-union risk. Tanshinone IIA (Tan) exhibits osteogenic and anti-inflammatory properties but is limited by poor aqueous solubility and rapid systemic clearance. To address these challenges, this study evaluated *ProGel-Tan*—a thermoresponsive hydrogel prodrug designed for localized, sustained Tanshinone delivery at the fracture site.

Methods

Tan was conjugated via either aromatic (terephthalate) or non-aromatic (glycine) hydrazone-containing linker chemistry to *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymers, producing ProGel-Tan prodrugs of PT2 and PT3 series, respectively. PT2 and PT3 were confirmed as optimal based on their thermoresponsive phase transition properties. A corticosteroid-induced delayed closed femoral fracture model was established on 12-week-old CD-1 female mice (n = 10/group). Prednisone (12 mg/kg/day) was administered daily through oral gavage for three weeks before fracture and continued until end of study. Six treatment groups were included: saline, free Tanshinone, Tanshinone sodium sulfonate salt, vehicle (HPMA copolymer only), aromatic ProGel-Tan (PT2), and non-aromatic ProGel-Tan (PT3). Closed femur fracture on mice without prednisone exposure was used as the healthy control. Local injections at the fracture site were performed on day 3 post-fracture surgery. For the double-dose series (PT2-2, PT3-2, salt Tan2, free Tan2) , a second injection was administered at four weeks (one month) post-fracture surgery to evaluate the impact of additional treatment on bone repair. Fracture healing was monitored via weekly *in vivo* x-ray scans; and at 3- and 10-weeks post-fracture, femurs were harvested for micro-CT analysis (BV, BV/TV, Tb.Th, Conn.Dn, and BMD), mechanical testing, and histology.

Results

At 10 weeks, both ProGel-Tan formulations demonstrated accelerated callus formation compared with prednisone and saline controls, showing early cortical bridging and enhanced mineral deposition. Micro-CT analysis revealed significant increases in bone formation metrics:

Single-dose series: Bone volume (BV) was significantly higher in PT2 and PT3 compared with the healthy baseline ($p < 0.01$–0.001). PT3 exhibited the greatest mean BV and the highest connectivity density (Conn.Dn) among prednisone-treated groups, though without statistical separation from PT2. Trabecular thickness (Tb. Th) and bone mineral density (BMD) showed upward trends in PT3 but did not reach statistical significance, suggesting partial mineral recovery rather than complete cortical restoration.

Double-dose series: A second administration further amplified the therapeutic response. PT3-2 and PT2-2 showed significantly greater BV than the saline and free Tan 2× groups ($p < 0.01$–0.001). PT3-2 achieved the highest connectivity density (Conn.Dn) among all prednisone-exposed groups ($p < 0.001$), indicating enhanced trabecular interconnectivity and advanced structural remodeling. BMD improved modestly in the salt Tan 2× group relative to saline ($p < 0.01$), while PT3-2 maintained the most balanced overall bone quality profile. Tb.Th remained similar across groups, implying that volumetric and connectivity gains predominated over changes in trabecular thickness.

Statistical analyses were conducted using unpaired two-tailed Welch's t-tests with Benjamini–Hochberg false discovery rate (FDR) correction ($\alpha = 0.05$) to adjust for multiple comparisons. Data are expressed as mean ± SD. Significance levels are indicated as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

Discussion

Localized delivery of Tan through the ProGel formulations appeared to accelerate the healing of the delayed fracture union, with effects that may exceed those of the free Tan and salt Tan. The thermoresponsive ProGel-Tan likely served as a sustained-release matrix, supporting prolonged local availability of Tan and continuous osteogenic stimulation. PT3 formulations, particularly PT3-2, showed the most notable improvements in callus volume and trabecular connectivity, suggesting that the non-aromatic linker system may offer superior therapeutic potential.

Significance

ProGel-Tanshinone offers a promising localized strategy to overcome glucocorticoid-induced fracture-healing deficits. Its injectable, self-gelling nature ensures sustained Tanshinone delivery with minimal invasiveness, providing a clinically translatable approach for enhancing bone regeneration in patients receiving long-term corticosteroid therapy.

Keywords: fracture healing, Tanshinone IIA, corticosteroids, hydrogel, prodrug, HPMA, bone regeneration

Acknowledgements: This work was supported in part by NIH R01 AR082148.

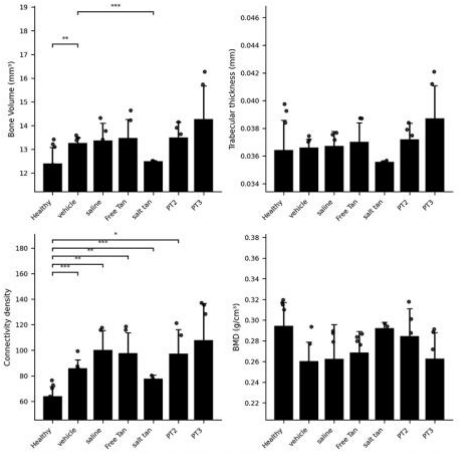

Figure 1. Single dose microCT analysis showing bone volume (BV), trabecular thickness (Tb.Th), connectivity density (Conn.Dn), and bone mineral density (BMD). PT2 and PT3 exhibited higher BV than the healthy baseline ($p < 0.01$–0.001). PT3 showed the greatest BV and Conn.Dn, while Tb.Th and BMD increased without significance. Data are mean ± SD; Welch's t-test with Benjamini–Hochberg FDR correction.

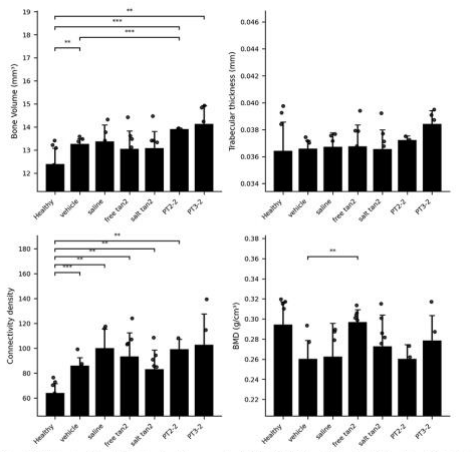

Figure 2. Double-dose microCT analysis showing enhanced bone regeneration. PT3-2 and PT2-2 had greater BV and Conn.Dn than saline and free Tan 2× groups ($p < 0.01$–0.001). PT3-2 showed the highest Conn.Dn, and BMD improved modestly in salt Tan 2× ($p < 0.01$). Data are mean ± SD; Welch's t-test with Benjamini–Hochberg FDR correction.