

Effects of Systemically Administered Mes-1022 in Rats with a Critical-Size Femoral Segmental Defect

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INTRODUCTION: Up to 5% of bone fractures exhibit delayed healing or nonunion (1) and experimental long bone critical size defects (CSD) recapitulate several aspects of nonunion (2). Mes-1022 is a novel hydroxyapatite-targeted prodrug designed to provide sustained skeletal release of a selective prostaglandin E2 receptor 4 (EP4) agonist. Mes-1022 promotes bone healing when adsorbed to mineralized bone graft material (3), and the current study assessed the bone-healing potential of systemically administered Mes-1022, in the absence of bone grafting, in a high-hurdle rodent model involving a critical size mid-diaphyseal femoral defect.

METHODS: After approval from the local Animal Care Committee, 24 ten-week-old female Sprague-Dawley rats were randomized into 3 groups (n = 8/group) that were balanced for body weight (vehicle 225 ± 17 g, low dose 227 ± 11 g, high-dose 232 ± 16 g). All animals underwent a 5 mm osteotomy of the left femoral midshaft, with stabilization provided by an external fixator (Panel A). Starting on day of surgery, all rats received 12 weekly s.c. injections of either vehicle (PBS + 25 nM EDTA) or Mes-1022 at a low dose (1.7 mg/kg) or high dose (5 mg/kg). The bone formation markers serum PINP and ALP and the bone resorption marker serum TRAcP-5b, as well as physical activity were measured at baseline and throughout the study. Rats were sacrificed at week 12 and osteotomized and unoperated femora were imaged via microCT (8 μ m voxel size), with all microCT analyses performed using Xamflow software. A threshold of 819 mg HA/cm³ was determined by Otsu in the unoperated femur. A 7 mm volume of interest (VOI) was centered within the osteotomy. MicroCT defect healing parameters included the minimum length of residual osteotomy gap (mm) and mineralized callus volume (BV, mm³). MicroCT analyses of the intact contralateral femur included trabecular analyses of the distal metaphysis (at 10% of bone length) and cortical analysis of a 7 mm segment centered at the mid-diaphysis. The unoperated femur was destructively tested in 3-point bending to evaluate biomechanical properties. Data were analyzed using one-way ANOVA followed by Tukey's post-test, with significance set at $p \leq 0.05$.

RESULTS: The high-dose Mes-1022 group had higher serum PINP (bone formation marker) at weeks 1 and 6 and lower serum TRAcP-5b (bone resorption marker) at week 10 compared with vehicle controls (all $p < 0.04$). No significant difference (NS) was observed between treatment groups for serum ALP levels. The best and worst osteotomy healing responses for each group at week 12 are depicted in the Figure. The best vehicle group response (Panel C) involved a non-bridged and possible pseudoarthrosis outcome. The best low-dose group response (Panel D) also failed to achieve bridging, whereas the best high-dose group response (Panel E) involved complete bony bridging without an obvious 'fracture' line. No other animals in any group exhibited bony bridging across the osteotomy. The worst healing responses in all 3 groups (Panels F-H) were characterized by a substantial residual osteotomy gap, with the high-dose group exhibiting new bone within the medullary spaces on both sides of the osteotomy. MicroCT analyses at week 12 showed that the minimum length of the residual osteotomy gap was 2.53 ± 1.12 mm, 2.29 ± 1.28 mm, and 1.87 ± 0.85 mm for the vehicle, low-dose, and high-dose groups, respectively (NS). Total mineralized callus volume was 23.6 ± 18.0 mm³, 28.6 ± 31.3 mm³, and 36.3 ± 24.4 mm³ for the vehicle, low-dose, and high-dose groups, respectively (NS).

The intact contralateral femur was also analyzed by micro-CT to further evaluate osteogenic responses to systemic Mes-1022 treatment. Trabecular bone volume fraction at the distal metaphysis of the intact femur increased dose-dependently with Mes-1022, with week 12 values of $27.6 \pm 9.1\%$, $32.0 \pm 16.3\%$, and $75.5 \pm 13.0\%$ for the vehicle, low-dose, and high-dose groups, respectively ($p < 0.0001$ for high-dose vs vehicle). Increased trabecular bone volume in the high-dose Mes-1022 group was driven by significantly greater trabecular thickness and trabecular number vs vehicle controls (both $p < 0.005$). At the mid-diaphyseal region of the intact femur, the high-dose Mes-1022 group had significantly greater total cortical area (7.58 ± 1.77 mm²) versus vehicle controls (5.84 ± 0.67 mm², $p < 0.05$). Cortical porosity in this intact mid-diaphyseal region was higher for the high-dose Mes-1022 group ($9.8 \pm 5.0\%$) vs vehicle controls ($3.0 \pm 4.0\%$; $p < 0.0001$). Biomechanical assessment of the intact femur indicated no significant differences in whole bone mechanical properties. Analysis of physical activity revealed no differences between Mes-1022 treated groups and vehicle for distance traveled or rearing count.

DISCUSSION: In this challenging critical size femoral defect model, Mes-1022 exhibited the dual effect of transiently increasing serum bone formation markers and reducing bone resorption markers. Osteogenic effects of high-dose Mes-1022 was also demonstrated by relative increases in trabecular and cortical bone mass parameters in the intact contralateral femur vs vehicle controls. While neither dose of Mes-1022 led to consistent osteotomy healing in the absence of bone grafting, evidence of a dose-dependent numerical reduction in minimum gap length and the achievement of complete osseous bridging in one high-dose animal suggests that Mes-1022 has the potential to exhibit more clinically meaningful bone healing effects in less extreme bone defect models. Osteotomy site histological analyses are ongoing.

SIGNIFICANCE/CLINICAL RELEVANCE: The systemic administration of Mes-1022 at 5 mg/kg/week for 12 weeks induced significant osteogenic responses, as shown by increases in serum bone formation markers and greater trabecular and cortical bone volume parameters vs vehicle controls. The osteogenic effects of Mes-1022 were not sufficient to consistently bridge these critical size defects in the absence of bone grafting. Future studies will determine whether Mes-1022 can more consistently promote bony bridging of experimental fractures with smaller osteotomy defects, or large defects treated with bone grafts.

REFERENCES: 1) Wildemann, et. al. Nat. rev. 2021. 2) Schmidt-Bleek et. al. Cyt. & GF reviews, 2016. (3) Sheikh et al. J Biomed Mater Res B 2020.

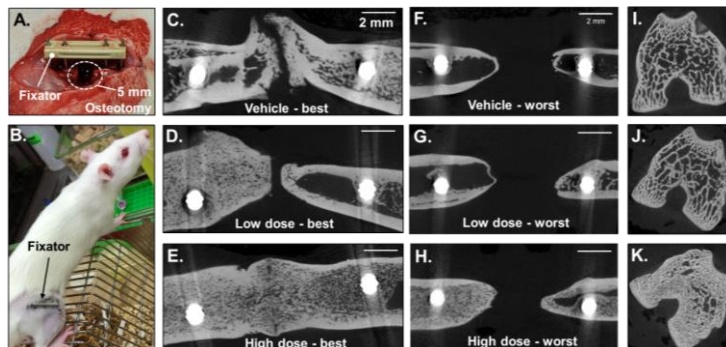


Figure. A-B: External fixator spanning the 5 mm osteotomy defect, viewed intraoperatively (A) and 6 days post-op (B). C-H: Micro-CT reconstructions of osteotomy sites. Bright spots on either side of the osteotomy gap are the inner fixation screws. The best (C-E) and worst healing responses (F-H) are shown for the vehicle, low-dose, and high-dose Mes-1022 groups, respectively. I-K: Representative micro-CT reconstructions of the distal metaphysis of the intact contralateral femur for the vehicle, low-dose, and high-dose Mes-1022 groups, respectively.