Sex as an Important Variable for cmRNA-Induced Bone Regeneration

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INTRODUCTION: Growth factors such as BMP-2 hold promise as agents of osteogenesis in clinical settings where bone healing is defective. Their clinical application is held back by problems of delivery that lead to serious side effects[1]. DNA-based gene delivery provides an alternative solution to these problems, but its clinical translation is difficult[2]. Delivery of mRNA provides an attractive alternative to circumvent these clinical translation hurdles. Our group was the first to show that chemically modified mRNA (cmRNA) is an efficient and reliable vector of BMP-2 delivery for regeneration of long bone critical size bone defects in male rats[3]. cmRNA provides improved stability and lower toxicity than native mRNA, using similar modifications to the ones used for COVID-19 vaccines[2]. In the past decade, research has shown sex to be an important, limiting variable for fracture repair and bone regeneration. The objective of the present study was to investigate whether sex is an important variable in the healing of critical sized defects by BMP-2 encoding cmRNA.

METHODS: BMP-2 cmRNA was produced from cDNA by in vitro transcription[3]. Purified BMP-2 cmRNA was complexed with a liponanoparticle and loaded onto clinically approved collagen sponges (6 x 3 x 3 mm) 15 min prior to implantation. 50 µg RNA per collagen sponge were used, the same dose as used to generate our prior results in male rats. This study was approved by IACUC. Female rats at 18 weeks of age were randomly divided into 3 groups and used for surgery (n=5/group). A 5 mm critical sized defect was created in the right femur of each rat and received a collagen sponge containing either BMP-2 cmRNA, non-coding (NC) cmRNA as a control, or 11 µg recombinant BMP-2 (rBMP-2) as a positive control. Bone healing was monitored radiologically. Animals were euthanized after 8 weeks, the femora harvested, analyzed by µCT at a 10 µm voxel size, and histology (Hematoxylin & Eosin). Statistical analysis for µCT data was performed using one-way ANOVA with Tukey post-hoc comparison. A p-value of <0.05 was considered statistically significant.

RESULTS: The rBMP-2 treated rats healed the defect consistently, presenting with radiological signs of bridging as early as 4 weeks, with a large callus extending outside the bone defect area and thin cortices, similar to historical data from our lab in male rats[2,3]. Rats treated with BMP-2 cmRNA and NC cmRNA groups consistently failed to heal the bone defect. Radiographs showed a mild bone healing response limited to the defect edges, with the defect edges capping by 8 weeks and little to no bone being formed at the defect center (Fig. 1A). The µCT analysis of BMP-2 and NC cmRNA treated rats showed minimal bone formation in the central 4 mm of the defect, evidenced as small islets surrounded by a lower density mesh tissue, suggesting persistence of the collagen sponge within the defect. No difference in bone (BV) or total volume (TV) was observed (Fig. 1B). In contrast, rBMP-2 treated defect resembled historical data from our lab with a large callus rimmed by thin cortices, and a center almost void of mineralized tissue with wispy trabeculae. BV of rBMP-2 treated defects was significantly higher than that of cmRNA treated defects. Histological analysis of the cmRNA treated femora showed little new bone tissue present within the defect, with evidence of collagen sponge remnants and fibrous tissue spanning the length of the defect (Fig. 1C). In contrast, histological analysis of rBMP-2 treated defects resembled historical data composed of a poor morphological quality bone callus, with a high quantity of adipose tissue.

DISCUSSION AND CONCLUSIONS: In this study, we set out to confirm the previous results of reliable bone regeneration by BMP-2 cmRNA in male rats[3], when using the same bone defect model in female rats. These preliminary findings, however, did not do so thus confirming the importance of considering sex as a biological variable when performing bone healing studies. Future studies to understand the underlying biological mechanisms producing this sex-dependent response to cmRNA therapy are warranted. The observation that rBMP-2 healed critical sized defects in females indicates that BMP-2 is effective in female rats, although the amounts of rBMP-2 used are high. Alternative explanations for the sex discrepancy include the need for a higher dose of cmRNA for effective healing in females and differences in the processing of RNA-liponanoparticle complexes by intra-lesional cells by males and females.

SIGNIFICANCE/CLINICAL RELEVANCE: Defective bone healing remains a pressing clinical problem. cmRNA therapies are an emerging and appealing solution in the field of regenerative medicine with a high potential for accelerated clinical translation. This study describes an important variable to consider when developing this emerging technology as a bone therapeutic.

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IMAGES:

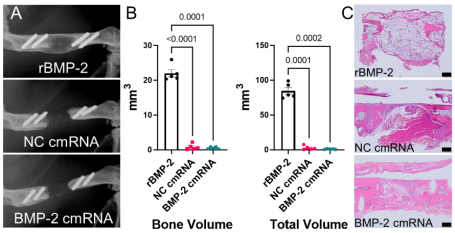

Figure 1. (A) Representative radiographs of femoral critical size bone defects 8 weeks after creation and treatment. (B) µCT analysis of the central 4 mm defect region of explanted femora, depicting measurements of bone volume and total volume. Data presented as bars depicting the mean, and SEM, along with individual data points. Values above pairwise comparison indicate p-value. (C) Representative histology images of the defect samples stained with H&E. Scale bar = 1 mm.