Zoledronic Acid Induces Muscle Regeneration After Rotator Cuff Repair in a Rodent Chronic Defect Model

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Background:

Zoledronic acid improves bone microarchitecture and biomechanical properties after rodent chronic rotator cuff repair (RCR). Besides the positive effects of zoledronic acid on bone microarchitecture, bisphosphonates have positive effects on skeletal muscle function according to the so-called "muscle-bone crosstalk".

Methods:

A total of 34 male Sprague-Dawley rats underwent unilateral supraspinatus tenotomy (timepoint 1) with transosseous RCR after three weeks (timepoint 2). Eight weeks later, all rats were sacrificed (timepoint 3). The control group obtained 1 ml subcutaneous saline solution, the intervention group was treated with a single subcutaneous dose of 100 µg/kg bodyweight zoledronic acid. All 34 study animals underwent serum micro ribonucleic acid (miRNA) analysis at all three timepoints. Furthermore, histological analyses of rotator cuff muscle tissue were conducted.

Results:

Circulating miRNAs showed significantly different expressions between both study groups. Efficacy of zoledronic acid on bone metabolism was confirmed by significantly different expressions of bone-specific miR-154-5p, miR-320-5p, and miR-410-3p in both study groups (**Fig.1**). In the control group, a significant down-regulation was observed for muscle-specific miR-1-3p ($p = 0.004$), miR-133a-3p ($p < 0.001$), and miR-133b ($p < 0.001$) (**Fig.1**). Histological analyses showed significantly higher rates of regenerating myofibers on the operated side of both study groups compared to the non-operated side ($p = 0.002$). On the non-operated side, significantly higher rates of regenerating myofibers were observed in the intervention group compared to the control group ($p = 0.031$). Muscle cross-sectional area revealed significantly smaller myofibers on both sides within the intervention group compared to both sides of the control group ($p < 0.001$) (**Fig. 2**).

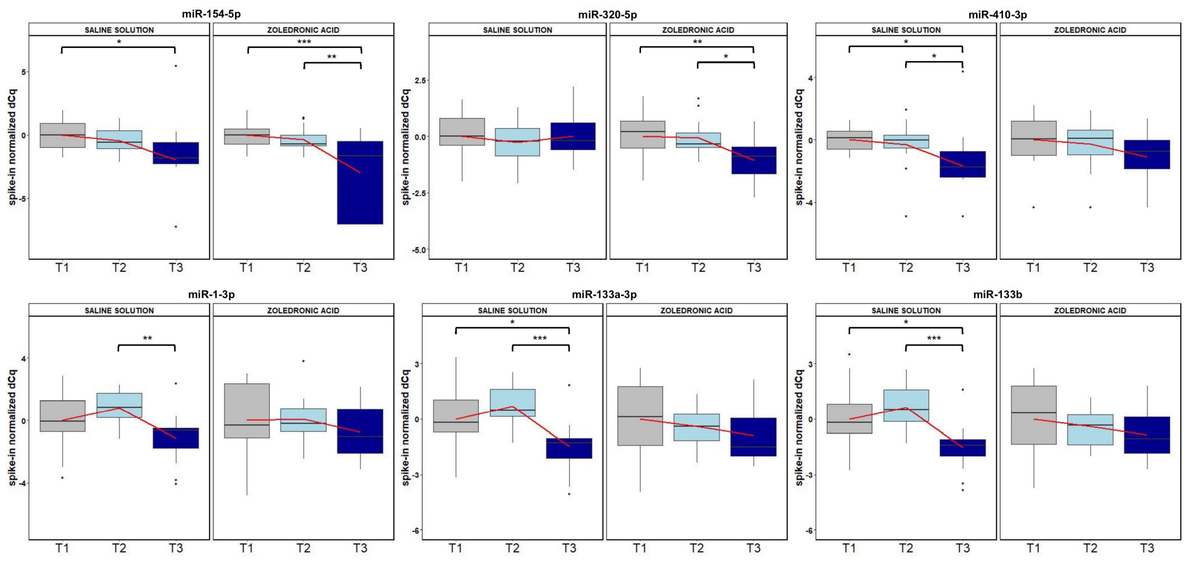

Fig. 1: Boxplot diagrams presenting the longitudinal evaluation of significantly different regulations of serum miRNAs between the control group (saline solution) and the intervention group (zoledronic acid) at supraspinatus tenotomy (timepoint 1 [T1]), RCR (timepoint 2 [T2)], and euthanasia (timepoint 3 [T3]). * <0.05, ** <0.01, *** <0.001. Values are presented as delta cycles of quantification values which are log2 scaled: A difference of one delta cycle of quantification corresponds to duplication or halving of miRNA serum levels.

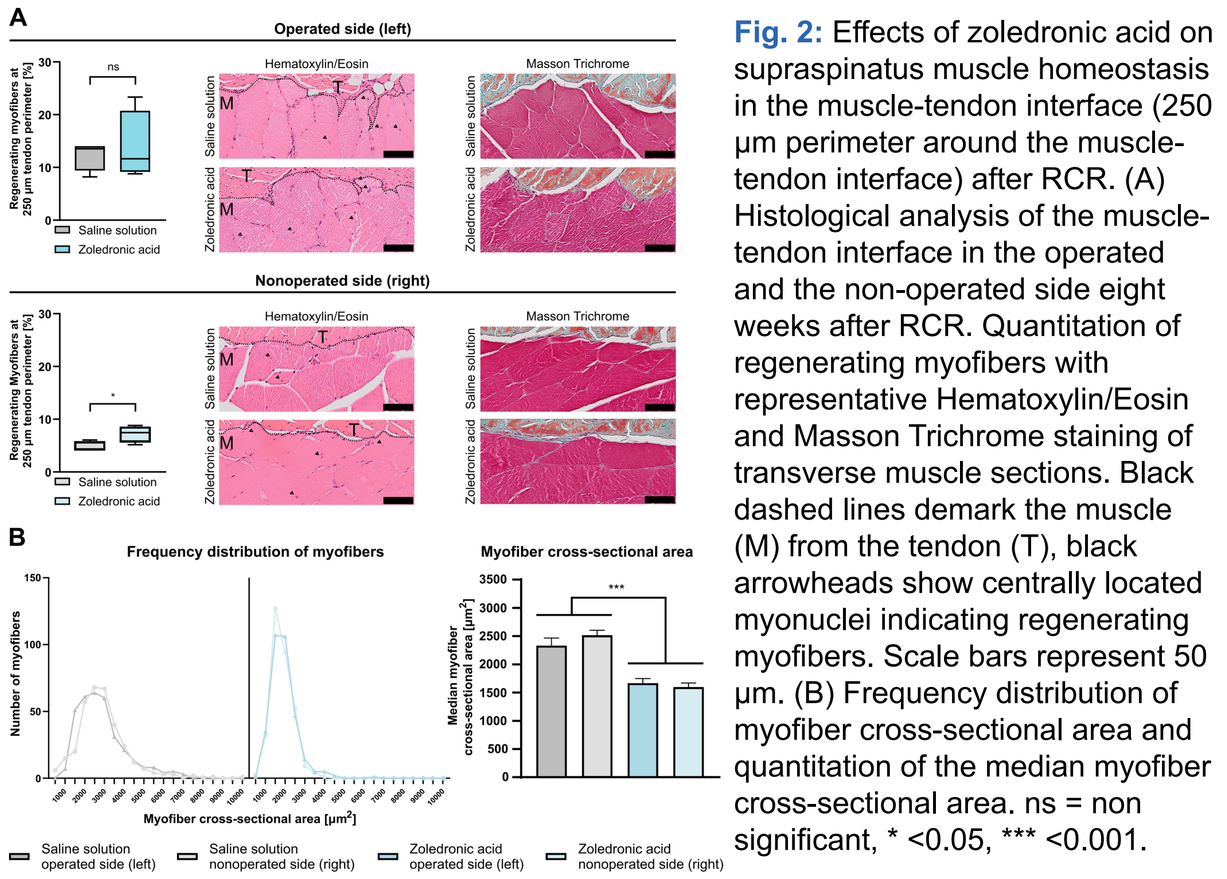

Fig. 2: Effects of zoledronic acid on supraspinatus muscle homeostasis in the muscle-tendon interface (250 µm perimeter around the muscle-tendon interface) after RCR. (A) Histological analysis of the muscle-tendon interface in the operated and the non-operated side eight weeks after RCR. Quantitation of regenerating myofibers with representative Hematoxylin/Eosin and Masson Trichrome staining of transverse muscle sections. Black dashed lines demark the muscle (M) from the tendon (T), black arrowheads show centrally located myonuclei indicating regenerating myofibers. Scale bars represent 50 µm. (B) Frequency distribution of myofiber cross-sectional area and quantitation of the median myofiber cross-sectional area. ns = non significant, * <0.05, *** <0.001.

Conclusions:

An adjuvant single-dose of zoledronic acid following RCR in a rodent chronic defect model led to significant differences in bone- and muscle-specific miRNA levels. Therefore, miR-1-3p, miR-133a-3p, and miR-133b might be used as biomarkers for muscle regeneration after RCR.

Clinical Relevance:

Adjuvant treatment with zoledronic acid may improve muscle regeneration after chronic RCR in humans, thus counteracting fatty muscle infiltration and atrophy.

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