ZOLEDRONATE PREVENTS SIMULATED WEIGHTLESSNESS-INDUCED BONE LOSS IN THE CANCELLOUS COMPARTMENT WHILE BLUNTING THE EFFICACY OF A MECHANICAL LOADING COUNTERMEASURE

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Astronauts using high-force resistance training while weightless show a high-turnover remodeling state within the skeletal system, with resorption and formation biomarkers being elevated. One countermeasure for the skeletal health of astronauts includes an anti-resorptive of the bisphosphonate (BP) drug class. We asked, does the combination of an anti-resorptive and high-force exercise during weightlessness have negative effects on bone remodeling and strength? In this study, we developed an integrated model to mimic the mechanical strain of exercise via cyclical loading (CL) in mice treated with the BP Zoledronate (ZOL) combined with hindlimb unloading (HU) to simulate weightlessness. We hypothesized that ZOL prevents structural degradation from simulated weightlessness and that CL and ZOL interact to render CL less effective.

Thirty-two C57BL/6 mice (male, 16 weeks old, n=8/group) were exposed to 3 weeks of either HU or normal ambulation (NA). Cohorts of mice received one subcutaneous injection of ZOL (45µg/kg), or saline vehicle (VEH), prior to the start of HU. The right tibia was axially compressed *in vivo* 60x/day to 9N (+1200µstrain on the periosteal surface) and repeated 3x/week during HU. Left tibiae served as a within subject, non-compressed control. *Ex vivo* µCT was performed on all subjects to determine cancellous and cortical architectural parameters. Static and dynamic histomorphometry were carried out for the left and right tibiae to determine osteoclast- and osteoblast-relevant surfaces. Further, microdamage was assessed in select groups by basic-fuchsin staining to test whether CL had an effect. For all assays, a multivariate (2x2x2) ANCOVA model was used to account for body weight changes. Additionally, for the tibiae, we incorporated a random effect for the subject (hence, a mixed model) to account for observations of both left and right tibiae within each subject. $P < 0.05$ was considered significant.

In the cancellous compartment of the proximal tibial metaphysis, we observed a main effect from each independent variable, as determined by structural and histomorphometric assays. Specifically, as expected, ZOL showed an increase in the cancellous bone volume to total volume fraction (BV/TV, +32%) and trabecular number (+18%) compared to the VEH. As expected, ZOL decreased osteoclast surface (OC/BS) by -45% compared to VEH. Surprisingly, ZOL reduced mineralizing surface (MS/BS) and bone formation rate (BFR), indicators of osteoblast activity, by -40% and -54%, respectively, compared to VEH. Altogether, ZOL-treated mice displayed a low-turnover state of remodeling in the metaphysis. In the context of skeletal aging, we speculate that ZOL prevented age-related cancellous strut loss during the experiment. As a main effect, as expected, HU decreased BV/TV by -31% via reductions in both trabecular thickness (-11%) and number (-22%) compared to NA controls. Additionally, HU decreased MS/BS by -38% and bone formation rate (BFR) by -50% compared to NA controls. Altogether, these data are consistent with structural degradation resulting from imbalanced remodeling that favors resorption. As a main effect, CL increased BV/TV by +15% via increased trabecular thickness (+12%) compared to the non-compressed limb. As expected, CL increased MS/BS (+20%) and BFR (+24%), indicating osteoblast mineralization contributed to bone gains. These data show that CL provided an anabolic stimulus to the cancellous tissue.

We observed unique interactions in ZOL*CL and HU*CL. First, ZOL prevented CL-induced increases in BV/TV and trabecular number, as compared to VEH. In the context of skeletal aging, these data suggest no added benefit from CL in the ZOL-treated mice. Interestingly, no microdamage was observed in mice that were unloaded and treated with ZOL (independent of CL). Secondly, HU prevented CL-induced increases in BFR, as compared to NA controls. These data suggest that either exercise is less effective or the kinetics of formation are slower during simulated weightlessness. Osteoclast surface was unchanged by either treatment. Thus, in contrast to exercising astronauts, these data do not suggest a high-turnover state in the metaphysis.

To assess mechanical properties as a function of HU or ZOL, we tested the left femur in three-point bending *ex vivo*. As expected, HU decreased stiffness (-30%) compared to NA, and ZOL increased stiffness compared to VEH (+28%). Interestingly, HU increased the post-yield displacement, related to collagenous tensile loading, compared to NA (+20%). ZOL increased yield force (+11%) and ultimate force (+17%), which seems to explain the significant effect of ZOL increasing total energy (work-to-fracture, +15%), while not affecting the post-yield displacement. Taken together, ZOL did not have detrimental affect on mechanical properties.

Our integrated model simulates the combination of weightlessness, exercise-induced mechanical strain, and anti-resorptive treatment that astronauts experience during space missions. We conclude that Zoledronate was an effective countermeasure against weightlessness-induced bone loss, though zoledronate, as well as weightlessness, rendered exercise-related mechanical loading less effective.

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