Bisphosphonate Treatment During an Initial Unloading Period Provides Beneficial Effects to Mechanical and Densitometric Properties of Bone for a Second Unloading

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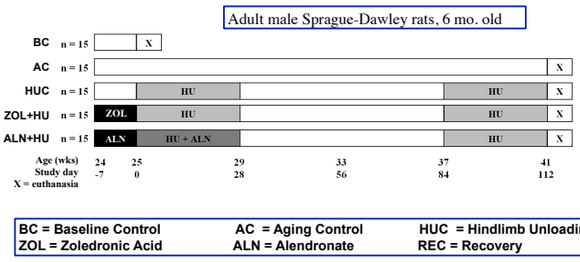
4TH Joint Meeeting of ECTS & IMBS *25-28 April 2015, Rotterdam, NL* *Poster Number P18* *hhogan@tamu.edu*

Objectives & Study Design

Bone response to multiple exposures of microgravity remains a concern for astronauts. More astronauts are making repeat flights, and some have taken bisphosphonates to prevent bone loss during flight [1]. Given the long-lasting effects of these drugs, it is possible that protection may persist for subsequent flights.

We hypothesized that 1) the beneficial effect of BP treatment given during an initial HU period would extend to a second HU, and that 2) Zoledronic Acid (ZOL) would be more effective than Alendronate (ALN) due to their differences in binding affinity.



BC = Baseline Control | AC = Aging Control | HUC = Hindlimb Unloading
ZOL = Zoledronic Acid | ALN = Alendronate | REC = Recovery

Methods & Materials

Zoledronic Acid (ZOL)
Rats were given single dose (60 µg/kg body weight) of ZOL one week prior to HU (by s.c. injection).

Alendronate (ALN)
Rats were given three doses per week (2.4 µg/kg body weight) of ALN starting one week prior to HU and continuing until the end of the first HU period (by s.c. injection); thus, 5 weeks total.

***In Vivo* Peripheral Quantitative Computed Tomography (pQCT)**
Longitudinal scans were taken at the proximal tibia metaphysis (PTM) at baseline and every 28 days using a Stratec XCT Research-M device (Norland Corp., Fort Atkinson, WI), with a voxel size of 100 µm and a scanning beam thickness of 500 µm.

Hindlimb Unloading (HU)
Using the traditional tail suspension model [2], rats were hindlimb unloaded for two periods of 4 weeks (28d) starting at day 0 and day 84.

***Ex Vivo* Femoral Neck (FN) Testing**
Femoral Neck specimens were harvested at the end of the study (Day 112). Femoral necks were placed vertically into a custom aluminum fixture and loaded until fracture with a mechanical testing system (Instron 3345, Norwood, MA). A crosshead speed of 2.54 mm/min was used.

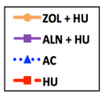
***Ex Vivo* Tibial and Femoral midshaft 3-point Bending**
Tibia and Femur specimens were harvested at the end of the study (Day 112). Tibias and Femurs were placed anterior side down with a span of 18 mm and 15 mm respectively. The samples were then loaded until fracture with a mechanical testing system (Instron 3345, Norwood, MA) at a crosshead speed of 2.54 mm/min.

***Ex Vivo* Micro Computed Tomography (µCT)**
A 1mm region of the proximal tibia metaphysis (PTM) was scanned at 12 µm resolution (SkyScan 1172; SkyScan, Kontich, Belgium) for tibia specimens at the final end point (Day 112).

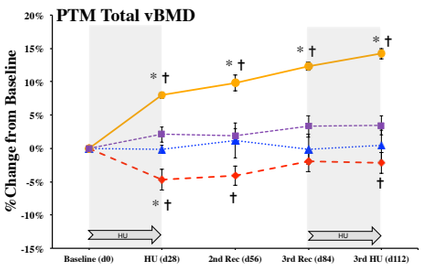
Statistical Analysis
Data were evaluated for statistical relationships using SigmaPlot 13 (Systat Software Inc., San Jose, CA). Comparisons between groups were performed using a one-way ANOVA, and pairwise comparisons of the means were evaluated with the Student-Newman-Keuls post hoc test. Repeated measure one-way ANOVA was used for longitudinal pQCT comparisons.

Results: Proximal Tibia

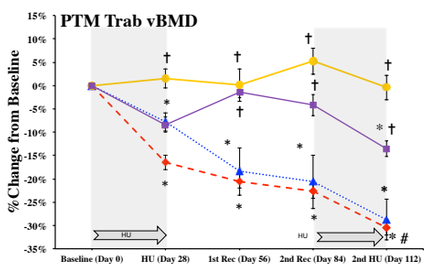
In Vivo pQCT



Data are mean ± SE
Shaded area indicates HU duration
** Different from baseline (p<0.05)*
† Different from AC at same time point (age) (p<0.05)
 # Different from d84 to d112 (p<0.05)



ALN prevents losses in Total vBMD during both HU periods, and ZOL induces higher Total vBMD throughout HU and Recovery.

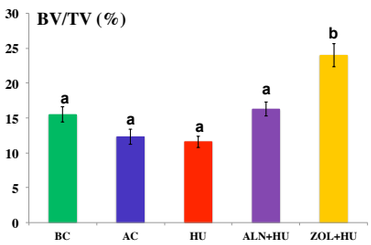


Both HU periods resulted in negative effects on untreated animals. ZOL prevented any age- or disuse-related losses in Trabecular vBMD.

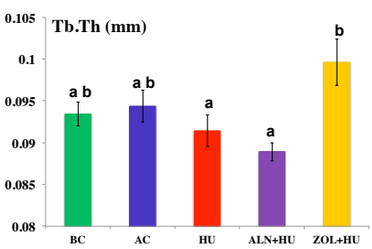
Ex Vivo µCT

BC at Day 0; All others at Day 112

Data are mean ± SE.
Those groups not sharing the same letter are significantly different from each other (p<0.05).



ZOL induces higher BV/TV compared to all other groups.



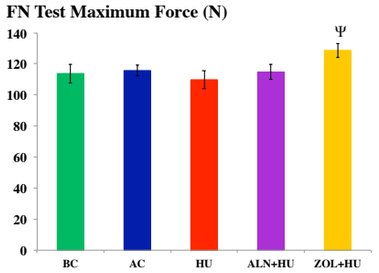
ZOL induces higher Tb.Th compared to HU and even HU+ALN.

Results: Femoral Neck

BC at Day 0; All others at Day 112

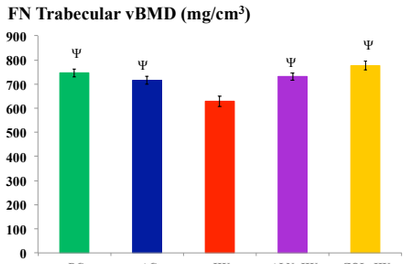
Data are mean ± SE
** Different from baseline (p<0.05)*
Ψ Different from HU (p<0.05)

Mechanical Testing



ZOL induced higher maximum breaking forces in FN mechanical tests.

Ex Vivo pQCT



All groups exceeded HU for Trab vBMD at the end point, with ZOL having the highest average value.

Conclusions

- These results clearly indicate that beneficial effects for both ZOL and ALN do extend to the second HU period, but to varying degrees.
- ZOL is much more potent than ALN in not only mitigating or preventing losses but actually enhancing skeletal parameters above controls. This is most prominent for the first HU exposure period.
- ALN is generally protective for most parameters, but is not as effective for trabecular architectural measures (BV/TV, Tb.Th).
- FN breaking strengths at the final end point also reflect the superiority of ZOL similar to pQCT and µCT results from the PTM.
- ISS crew members taking a BP for one mission may find protection for subsequent missions.

References

[1] LeBlanc et al. *Osteoporosis International* 24:7 2013.
[2] Morey-Holton et al. *Bone* 22:5 1998.

Acknowledgements: We sincerely appreciate the support provided for this work by NASA Grant NNX13AQ87G (Human Research Program Omnibus). JEB and AN are also funded through NSBRI grant NCC 9-58 for Space Life Sciences Fellowships.

Conflict of Interest: None.