Effect of Zoledronic Acid Treatment on Bone in +/G610C Mouse Model of *Osteogenesis Imperfecta*

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Introduction

Human *Osteogenesis Imperfecta* (OI) is a heterogeneous group of genetic disorders with low bone mass and occasional fractures. It can be divided into at least 17 subtypes. In most cases OI is a result of mutations in the genes encoding type I collagen.(1) The goal of the clinical management of OI is to maximize bone strength and to minimize injuries. Bisphosphonate therapy can achieve increase in bone mass of OI patients. One preclinical model for OI is 'Amish variant' mouse with a G610C mutation in the *Col1a2* gene. Heterozygous mice (+/G610C) mimic mild-to-moderate severity of OI and exhibit decreased bone mineral density (BMD) and bone strength.(2)

Aim of the study

We characterized the bone phenotype and the effects of zoledronic acid (ZOL) on bone in young adult male +/G610C mice.

Materials and Methods

Heterozygous (+/G610C) and control +/+ mice were randomized to 3 groups according to body weight: 1) Control mice receiving vehicle; 2) +/G610C mice receiving vehicle; 3) +/G610C mice receiving ZOL. Treatment was started at 2 months of age and the study was completed at 4 months. Body weight was monitored, and *in vivo* microcomputed tomography (μCT) was performed at the beginning of the study. Long bones and vertebra were collected at sacrifice for further analyses performed by *ex vivo* μCT, peripheral quantitative computed tomography (pQCT) and dual-energy X-ray absorptiometry (DXA). Biomechanical testing was performed in tibial diaphysis by 3-point bending test and in femoral neck by cantilever bending test.

SkyScan 1276 scanner (Bruker, Belgium) with 4 μm image pixel size was used for μCT. Images were reconstructed using SkyScan NRecon software, aligned using DataViewer software, and analyzed using CTAn software. Faxitron UltraFocus DXA was used for DXA measurements, and Norland Stratec XCT Research SA+ (Norland Stratec Medizintechnik) bone densitometer was used for pQCT measurements. The analyses were performed from femoral and tibial metaphysis and diaphysis, and from the vertebral body of lumbar spine (L5).

The study protocol was approved by National Animal Experiment Board, Regional State Administrative Agency for Southern Finland, Hämeenlinna, Finland. Data is presented as mean +/- SD. Statistical analysis were performed by parametric one-way ANOVA and linear contrasts of means, or non-parametric Kruskal-Wallis and Mann-Whitney *U* tests.

Beginning of the study

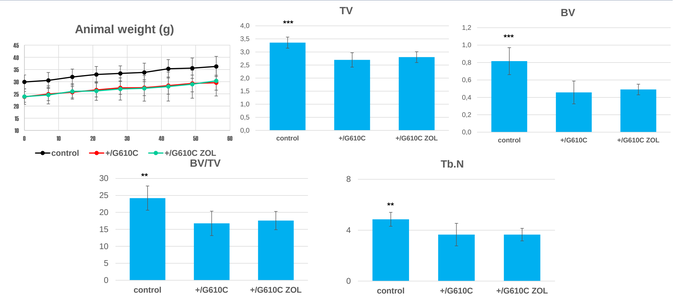

FIGURE 1. Body weight (g) follow up during the 56 days experiment, and tissue volume (TV; mm^3), trabecular bone volume (BV; mm^3), trabecular bone volume per tissue volume (BV/TV; %) and trabecular number (Tb.N; mm^{-1}) in tibial metaphysis as measured by *in vivo* μCT at the beginning of the study.

Ex vivo pQCT

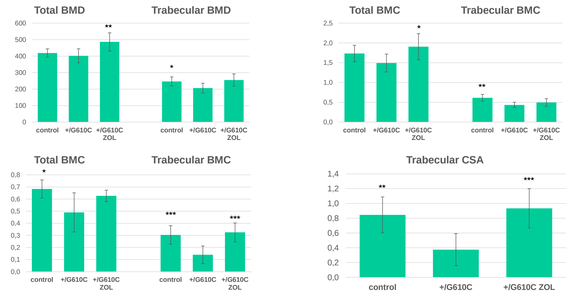

FIGURE 3. Upper row: Total and trabecular bone mineral density (BMD; mg/cm^3) and content (BMC; mg/mm) of femoral metaphysis. Lower row: Total and trabecular BMC (mg/mm), and trabecular cross-sectional area (CSA; mm^2) of lumbar vertebral body L5 at the end of the study.

Bone biomechanical testing

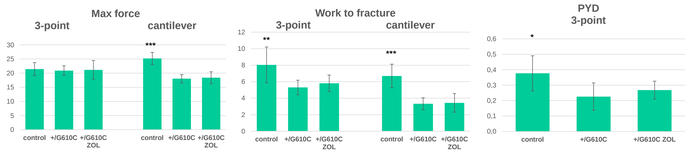

FIGURE 5. Maximal force (N), work to fracture (Nmm), and post-yield displacement (PYD; mm) as measured by 3-point bending test of tibial diaphysis and cantilever bending test of femoral neck.

Ex vivo uCT

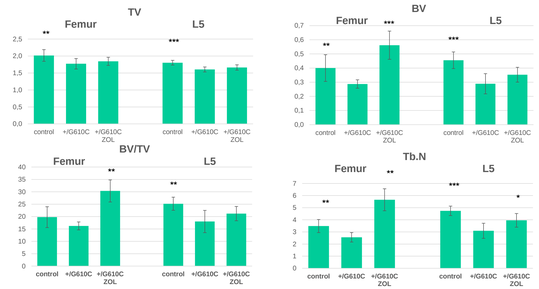

FIGURE 2. Trabecular tissue volume (TV; mm^3) , trabecular bone volume (BV; mm^3), trabecular bone volume per tissue volume (BV/TV; %), and trabecular number (Tb.N; mm^{-1}) of femoral metaphysis and lumbar vertebral body L5 at the end of the study.

Ex vivo DXA

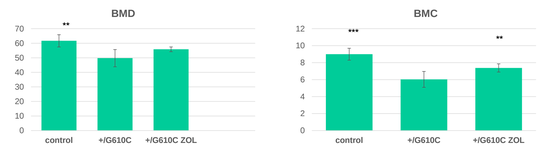

FIGURE 4. Bone mineral density (BMD; mg/cm^2) and content (BMC; mg) of lumbar vertebra L5.

Conclusions

+/G610C mice were lighter than control mice and they had reduced amount of bone tissue and trabecular bone in long bones and vertebra, and biomechanical properties of their bones were impaired.

ZOL increased the reduced amount of trabecular bone in this OI animal model, but bone biomechanical properties were not improved.

References

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2. Daley E, Streeten EA, Sorkin JD, Kuznetsova N, Shapses SA, Carleton SM, Shuldiner AR, Marini JC, Phillips CL,Goldstein SA, Leikin S and McBride DJ Jr (2010) Variable bone fragility associated with an Amish COL1A2 variant and a knock-in mouse model. *J Bone Miner Res*. 25: 247-61.

Summary

Body weight

- +/G610C mice were lighter than control mice, and ZOL had no effect on weight
- Weight of the mice of all three groups increased steadily during the study

Femur and tibia

- +/G610C mice had lower TV, BV, BV/TV and Tb.N than control mice at the beginning of the study
- At the end of the study, μCT analysis showed that TV, BV and Tb.N were lower in +/G610C mice, and ZOL increased BV, BV/TV and Tb.N values
- pQCT analysis showed that trabecular BMD and BMC were lower in +/G610C mice, and increased by ZOL
- Cantilever bending test of femoral neck showed that +/G610C mice had lower maximal force and work to fracture
- 3-point bending test of tibial diaphysis demonstrated that +/G610C mice had lower work to fracture and post-yield displacement
- ZOL had no effects on biomechanical properties in femur or tibia

Lumbar vertebral body L5

- μCT analysis showed that TV, BV, BV/TV and Tb.N were decreased in +/G610C mice, and ZOL increased lumbar vertebra Tb.N
- pQCT showed that total and trabecular BMC and CSA were lower in +/G610C mice and increased by ZOL
- DXA showed that BMD and BMC were lower in +/G610C mice, and BMC was increased by ZOL

Acknowledgements

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