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

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ASBMR 2019 SAT-924

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. 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 wild-type (+/G610C) mice exhibit mild-to-moderate severity of OI and exhibit decreased bone mineral density (BMD) and bone strength.

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 (CT) was performed at the beginning of the study. Long bones and vertebrae were collected at sacrifice for further analyses performed by ex vivo CT, 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 pixel size was used for CT. Images were reconstructed using Skyscan NRecon software, aligned using DachImport software, and analyzed using CTAn software. Fiducial UltraFocus DXA was used for DXA measurements, and Norland Slice: XCT Research SA (Norland, Stratec Medizintechnik) bone densitometer was used for QCT measurements. The analyses were performed from femoral and iliac metaphysis and diaphysis, and from the vertebral body/lumbar spine (L5).

The study protocol was approved by National Animal Experiment Board, Regional State Administrative Agency for Southern Finland, Halmeenta, Finland. Data is presented as mean ± SD. Statistical analyses 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; mm³), trabecular bone volume (BV; mm³), trabecular bone volume per tissue volume (BV/TV; %), and trabecular number (Tb.N; mm⁻¹) in iliac metaphysis as measured by in vivo uCT at the beginning of the study.](image)

Ex vivo uCT

![FIGURE 2. Trabecular tissue volume (TV; mm³), trabecular bone volume (BV; mm³), trabecular bone volume per tissue volume (BV/TV; %), and trabecular number (Tb.N; mm⁻¹) of iliac metaphysis and lumbar vertebral body L5 at the end of the study.](image)

Ex vivo pQCT

![FIGURE 3. Upper row: Total and trabecular bone mineral density (BMD; mg/cm²) and content (BMC; mg) of lumbar vertebrae L5.](image)

Ex vivo DXA

![FIGURE 4. Bone mineral density (BMD; mg/cm²) and content (BMC; mg) of lumbar vertebrae L5.](image)

Bone biomechanical testing

![FIGURE 5. Maximal force (N), work to fracture (Nm) and post-yield displacement (mm) as measured by 3-point bending test of iliac diaphysis and cantilever bending test of femoral neck.](image)

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, TV/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, TV/TV and Tb.N values.

pQCT analysis showed that trabecular BMC 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 iliac 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, TV/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.

Conclusions

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

ZOL increased the reduced amount of bone in this OI animal model, but bone biomechanical properties were unaffected.

References


Acknowledgements

We thank all Pharmatest personnel who contributed to the study.

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