FOURIER TRANSFORM INFRARED IMAGING SPECTROSCOPY (FT-IRIS) OF THE EFFECT OF ALENDRONATE ON FRACTURE HEALING IN OSTEOGENESIS IMPERFECTA AND WILD TYPE MICE

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Introduction

Alendronate, a third-generation bisphosphonate used in treating osteoporosis and more recently proposed for the treatment of osteogenesis imperfecta (OI), is known to reduce bone turnover by inhibiting osteoclast activity. Accordingly, there is a concern that fractures that occur during alendronate therapy will not heal normally. Our previous study using image analysis to evaluate bone density and callus area showed that alendronate did not significantly delay radiological remodeling of the callus in either the +/+ or the oim/oim mice, and that it may actually stabilize fractures at an earlier timepoint. In this study, we used Fourier transform infrared imaging spectroscopy (FT-IRIS) to investigate the effect of alendronate on bone quality by evaluating ultrastructural parameters of bone mineralization during fracture repair.

Methods

30 B6C3F1/e oim/oim mice and 20 B6C3F1/e +/+ wild type mice six weeks of age were used under an IACUC-approved protocol. The +/+ and oim/oim were randomly allocated into 4 and 6 groups respectively with n = 5 in each. Alendronate was administered via a subcutaneously implanted Alzet pump (Alza Corp., Palo Alto, CA) at a dosage of 26 μg alendronate/kg/day to 3 groups of oim/oim and 2 groups of +/+. The other 5 groups served as control and received an equal volume of normal saline. At 2 weeks into treatment, the right femora were fractured via a modification of the rat femoral fracture model. An intramedullary pin (25 gauge) was inserted retrograde followed by creation of an open osteotomy of the midshaft of the femur producing a transverse fracture of the femur. After fracture, the mice continued to receive alendronate or saline until they were euthanized. Oim/oim mice were euthanized at 3, 4, and 6 weeks post-fracture and +/+ mice were euthanized at 3 and 4 weeks post-fracture.

Dissected femora were fixed in 70% alcohol immediately after the sacrifice followed by embedding in PMMA. Sections 3 microns thick along the long axis of the bone were cut using a Reichart-Jung sliding microtome and tungsten carbide knife and then evaluated by FT-IRIS.

Data was acquired on a BioRad (Cambridge, MA) UMA 300A FTIR microscope with an FTS-60A step-scanning FTIR spectrometer and a 64x64 MCT FPA detector (Stingray Imaging Spectrometer) at 16 cm⁻¹ resolution under N₂ purge from a 400 x 400 μm² region of the histological section, resulting in 4096 individual infrared spectra per region. Infrared vibrations of the mineral (biological apatite) and the matrix phases were monitored. The ratio of the area of the apatite phosphate absorbance (900-1200 cm⁻¹) to the area of the protein absorbance (1590-1720 cm⁻¹) was calculated to obtain the relative amounts of mineral and matrix present (mineral/matrix), an indicator of tissue density. The ratio of the area of the carbonate absorbance (840-890 cm⁻¹) to the phosphate absorbance was calculated to obtain the relative amount of carbonate in the mineral phase (carbonate/mineral), an indicator of mineral maturity.

Results

For the +/+ mice, the mineral/matrix was about higher in the alendronate group compared to the saline group at 3 weeks and 4 weeks respectively. The carbonate/mineral in the alendronate group in +/+ mice was lower than that in the saline group at 3 weeks and 4 weeks respectively. For the oim/oim mice, the alendronate group had a higher mineral/matrix than that of the saline group at 3 weeks. At 4 weeks and 6 weeks this was reversed and the alendronate group had a lower mineral/matrix ratio. The carbonate/mineral in the alendronate oim/oim group was lower than that in the saline group at both week 3 and week 6 (p = 0.009), while there were no differences at week 4. At both 3 weeks and 4 weeks, the carbonate/mineral was lower in the oim/oim compared to the +/+ mice. At 6 weeks, the carbonate/mineral and the mineral/matrix were reduced in the alendronate oim/oim compared to the saline oim/oim mice.

Discussion

Previously, based on radiographic analysis, we reported that the fracture healing of oim/oim mice lags behind the wild type mice [1]. Interestingly, in both +/+ and oim/oim mice, alendronate treatment resulted in larger and denser calluses at earlier time points compared to their saline-treated counterparts. However, remodeling did proceed to the pre-fracture state in all groups.

The mineral/matrix is an indicator of tissue mineralization and the carbonate/mineral is an indicator of mineral maturity. Based on these ultrastructural analyses, there appears to be more mineral in the alendronate-treated +/+ mice than in the saline-treated +/+ mice. However, the carbonate/mineral ratios indicate that this mineral may be less mature, and therefore could potentially result in biomechanically less competent bone.

In the oim/oim mice, those treated with alendronate were more mineralized at week 3 than the group treated with saline. This mineral was less mature in the alendronate group than in the saline group at week 3, however. At week 6, the saline group had greater mineral than the alendronate group, and the mineral was also more mature, as shown by the difference in carbonate/mineral ratios between the two groups. These data indicate that at the early stage of fracture healing, alendronate appears to help the mineralization of bone, even though the increased mineral may be less mature. This trend disappeared as healing progressed.

Overall, this study suggests that alendronate may accelerate the mineralization of bone at an early stage of fracture healing, but the quality of bone seems less mature. In addition, the mineralization and mineral maturity of trabecular bone in fracture healing appears to be different between the oim/oim and the +/+ mice, perhaps as a result of the underlying pathology in the oim/oim mice.

Reference