Fracture Healing with Alendronate Treatment in the Brtl Model of Osteogenesis Imperfecta

INTRODUCTION

Osteogenesis Imperfecta (OI) is a genetic disease typically related to a mutation in the genes encoding type I collagen. OI patients experience a high incidence of fractures during childhood and adolescence. Bisphosphonates have been used in an effort to prevent or reduce the number of fractures. While prior studies have investigated the effect of bisphosphonate treatment on fracture healing in normal adult bone, the influence of bisphosphonates on healing in growing OI bone has not been fully examined. This may be particularly important, based on data from oim/oim mice treated with a soluble form of RANK. Similar to bisphosphonates, RANK exhibits an effect on osteoclasts and when given to oim/oim mice, leads to altered fracture healing in comparison to WT mice.[1] The purpose of this study was to investigate the influence of alendronate on fracture healing in a murine model of OI during growth. Specifically, the Brtl/+ mouse was used, since it is a knock-in model that copies the genetic mutation of an OI patient.[2]

MATERIALS & METHODS

Study Design & Animal Model: Male Brtl/+ and WT mice were enrolled into the study at 2w of age. Mice were randomly assigned to receive no treatment (A: Average untreated control), treatment until fracture (B: Before fracture), or treatment throughout the study duration (C: treated before and Continued after fracture). Treatment was administered biweekly 0.219 μg/g subcutaneous injections of alendronate. After 8 weeks of age, an intramedullary pin was inserted in one randomly chosen tibia and a fracture was created using a guillotine device under IACUC approval. A radiograph was taken and the limb was further stabilized using a tape splint. Mice were allowed to heal for 1, 2, 3, or 5 weeks.

μCT: The tibiae were scanned using a commercially available μCT system (Xplore Loris SP, GEHC PCL, London, ONT) at an 18-μm isotropic voxel size set at a voltage of 80 kVp and current of 80 μA. An aluminum filter and beam flattener were used to minimize beam hardening artifacts.[3] In the reconstructed images, the callus volume, bone volume fraction (BVF) and tissue mineral density (TMD) of bone in the callus and residual cortical bone were measured (MicroView 2.2).

Mechanical testing: A randomly assigned subset of the fractured tibiae and the contralateral (intact) controls were potted, wetted with saline and tested in torsion at 0.5% until failure. The raw data were filtered and the stiffness, angular displacement at failure, torque at failure and the energy to failure were measured.

Histomorphometric analysis: Another subset of the fractured tibia were fixed, decalcified, embedded, sectioned, and stained using Safranin-O, Fast Green and Hematoxylin. One section per bone was analyzed utilizing a color separation method and the percentage of the callus which contained cartilage was measured (MicroView 2.2).

DISCUSSION

Recently published data indicate that there was no effect of zoledronic acid on early development of callus,[5] similar to our histologic assessment. After 3w of healing, when the cartilage had essentially disappeared, more bone was present in the callus from mice with treatment C (treatment Continued during healing) that was associated with an increased stiffness. After 5w of healing, the larger callus with more bone present in treatment C was associated with an increased torque to failure and an increased stiffness in WT mice. While a study of human subjects demonstrated that pamidronate treatment led to non-union in OI patients with osteotomies,[6] the only non-unions in this study resulted from significant pin instability. The lack of non-unions in this murine study may be related to the specific animal model or the inability to reproduce the complex interaction between the fracture stability and the treatment and should be explored further. Increases in the callus size and associated structural biomechanical properties that occur with continued alendronate treatment are considered to be beneficial during healing.[7] However, while normal healing fractures continue to remodel, reducing this geometric advantage over time, the alendronate treated fractures retain the larger geometry which may have consequences that need to be explored over longer time periods. Further investigation will be required to determine the optimal clinical approach to enhance callus formation and mechanical properties while minimizing the serious consequences of non-union in OI.

RESULTS

Of the 285 mice used n this analysis, 67% had simple fractures, 11.9% had wedge fractures, 17.9% had complex fractures and 3.2 percent could not be classified based on the radiographs.

1w of healing: There was very little bony callus. A qualitative analysis of the histologic sections indicated that the tissue was primarily composed of cartilage and soft tissue.

2w of healing: A qualitative analysis of the histologic slides indicated that cartilage persisted in some regions of the callus while other portions of the callus had begun transitioning to trabeculated woven bone. There were no statistically significant changes in any of the μCT measurements, nor biomechanical properties.

3w of healing: Treatment C resulted in an increased callus BVF and higher stiffness in comparison to treatment A or B. Fractured limbs from WT mice with treatments A or B had a higher stiffness than their respective Brtl/+ counterparts.

REFERENCES