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New Treatments in Osteogenesis Imperfecta
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YOUNG INVESTIGATORS POSTER SESSION

Behavioral Characterization of Pain in a Mouse Model of Osteogenesis Imperfecta

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Osteogenesis imperfecta (OI) is a congenital disorder primarily caused by mutations in *COL1A1* and *COL1A2* genes. Clinical features vary from mild to lethal and include malformed and easily fractured bone, brittle teeth, curved spines, muscle weakness, loose joints, and blue sclera. Pain is common in OI patients which impedes their lives. Managing OI pain remains suboptimal even with available approaches. Animal models of OI provide an opportunity to better understand the sources of pain in this syndrome and to question the effectiveness of possible treatments.

Recently, an OI model was developed by a screening of N-ethyl-N-nitrosourea-induced mutagenesis resulting in T to C transition in the *COL1A1* gene. The OI model (*Col1a1Jrt/+*) was assessed for weight, skeletal events and behavioral signs of pain compared to their wild type (WT) littermates. 2-3-month-old 14 *Col1a1Jrt/+* and 15 WT males were tested for mechanical, radiant heat and cold sensitivities. Motor activity and anxiety-like behavior were evaluated using home cage running wheels for 1 hour and open field for 5 minutes, respectively.

Our data demonstrated significant differences between the 2 groups in weight progression and skeletal abnormalities including undersized long bones, joint displacement, vertebral column misalignment and small caudal vertebrae. Statistically significant differences were observed between the groups in all tests of sensory hypersensitivity and motor ability. Additionally, the effect of bisphosphonate (a current treatment for OI patients) on sensory thresholds and motor abilities was examined. Three injections of Zometa (100µg/kg) or saline were given i.p. to *Col1a1Jrt/+* (n=7/group) and WT (n=5-10/group) over 3 weeks. Preliminary results reveal insignificant effects on mechanical or cold thresholds but strong reduction in heat sensitivity.

In conclusion, increased behavioral sensitivity in *Col1a1Jrt/+* compared to WT might be due to several factors including bone dislocations, joint hyperlaxity and nerve compression. Bisphosphonate treatment may be beneficial in reducing sensitivity associated with skeletal deformities but its analgesic efficacy requires further investigation.

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Currently, I'm a PhD candidate at Faculty of Dentistry, McGill University. I obtained a Masters in oral pathology from Faculty of Dentistry, Suez Canal University, Egypt, 2009. I practiced dentistry as a resident in Faculty of Dentistry, Suez Canal University, Egypt, 2001-2003 after obtaining my DDS from the same university in 2001. My areas of research interest are pain assessment in animal models with skeletal diseases (e.g. Osteogenesis Imperfecta) and the role of bisphosphonates treatment in pain-related bone diseases. I'm also working on bone destruction associated with breast cancer metastases and the effect of mammalian target of ramaycin pathway inhibition on osteolysis and pain.

Dose response study into the effects of sclerostin antibody in Brtl/+ mouse

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Introduction

Across animal studies and human trials, sclerostin antibody has been demonstrated as a potent drug for increasing bone mass. While clinical trials and most animal studies have focused on application to diseases in older populations such as osteoporosis, sclerostin antibody (Scl-Ab) also holds great potential in reducing fracture risk in osteogenesis imperfecta (OI) patients at all ages. We previously demonstrated a significant anabolic response to Scl-Ab in Brtl/+ mice when administered for 2 week s at 25 mg/kg, twice weekly (Sinder et al, JBMR 2013 28:73). To prepare Scl-Ab for clinical applications, we investigated its potency across multiple doses to determine treatment conditions which would restore Brtl/+ bones to wild-type (WT) levels.

Methods

In this study, 87 mice (44 WT; 43 Brtl/+) were separated into 5 dose-response cohorts and compared to a control group treated with saline. Scl-Ab was administered twice per week from 3 weeks of age to 14 weeks of age at 3, 6, 12.5, 25, and 50 mg/kg (n= 6-9 per group). A group size of 7 was targeted to provide sufficient power to detect untreated genotype differences previously established at 14 weeks of age (16% cortical area, 34% bending moment of inertia; Uveges et al, JBMR 2009 24:849). Following euthanasia, femora were dissected and scanned by μCT. Mid-femoral cortical regions of interest were assessed for cross sectional geometric parameters.

Results

Brtl/+ and WT mice both showed a significant dose response in cortical area, cortical thickness, polar moment of inertia and bending moment of inertia. Untreated Brtl/+ femora had significantly less cortical area than WT. With treatment they no longer demonstrated significant reductions at therapeutic doses of ≥3 mg/kg. Bending moment of inertia and polar moment of inertia, parameters representing structural indices of bending and torsional strength respectively, were no longer different than WT at doses ≥6 mg/kg.

Conclusion

Biomechanical testing will reveal whether Brtl/+ treated femoral strength shows similar dose response effects when compared to WT. These studies will provide key data to demonstrate minimally effective dosing of sclerostin antibody in a preclinical model of the developing OI skeleton.

The authors gratefully acknowledge Amgen and UCB Pharma for providing sclerostin antibody.

David K. Barton, MS is a pre-candidate in Biomedical Engineering at the Orthopedic Research Labs at the University of Michigan. He is currently studying the bone dynamics of the Brtl/+ mouse model treated with sclerostin antibody for application to osteogenesis imperfecta, while maintaining a healthy interest low magnitude high frequency vibration, bone quality, bone architecture and ergonomics. Raised in Idaho, a graduate of the University of Utah, he also completed short summer stints at the FDA, NIH and Harvard University.

Whole Body Vibration and Resistive Exercises May Reduce Risk Factors for Fractures in Type I Osteogenesis Imperfecta

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There is a scarcity of research about whole body vibration exercise and resistance exercise as a combined program for children and adolescents with type I Osteogenesis Imperfecta (OI). Type I OI is the mildest and most common form of the disorder, characterized by mild to moderate fragility without bone deformity. The goal of treatment when working with individuals with Type I OI is reducing the risk of fractures primarily by increasing bone mineral density. Research has indicated that whole body vibrations as well as resistive exercise are effective means for increasing and preventing the loss of bone mineral density and muscle strengthening. Whole body vibration, by means of mechanical oscillation, has been shown to effectively improve bone density. Resistive exercise creates a tensile force on the bone thereby stimulating bone formation. Using the Oxford Level of Evidence, a systematic review of literature was done to determine if whole body vibration and resistive exercise training can reduce risk factors for fractures in children and adolescents with type I OI. Based on the gathered evidence, we surmise that combining whole body vibration and resistive exercises yield greater effects on bone mineral density thereby reducing risk factors for fractures in children and adolescents with type I OI.

Keywords: Osteogenesis imperfecta, whole body vibration, resistive exercise, bone density, collagen synthesis, fracture, muscle strengthening

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Elizabeth Robbins is a second year doctoral student of Physical Therapy in the College of Health Sciences at Tennessee State University. She graduated from Auburn University in 2012 with a bachelor of science degree in Exercise Science and two minors, Psychology and Sustainability.

Hindlimb Skeletal Muscle Function and Femoral Strength in *+/G610C* Osteogenesis Imperfecta Mice; Impact of Weight-bearing Treadmill Exercise

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Osteogenesis imperfecta (OI) is a heterogeneous heritable connective tissue disorder primarily due to mutations in the COL1A1 and COL1A2 genes. Although OI patients are classified into several subtypes depending on molecular basis and clinical severity, the cardinal feature of OI is skeletal fragility. Recently, 64 individuals of an Amish OI kindred were found to carry a glycine to cysteine substitution at position 610 of the 2(I) collagen chain, causing reduced bone mineral density (BMD) and increased incidence of fracture compared to non-affected family members. Heterozygous *G610C* OI model mice *(+/G610C*) mimic both the genotype and phenotype of this population with reduced BMD and femoral biomechanical strength. Bone is inherently mechanosensitive and the largest physiological loads that bone typically experience are from muscles; with bone strength directly proportional to muscle mass and strength. Physically active children accrue 10-40% more bone regionally than inactive children. Children with type I OI exhibit decreased exercise capacity and muscle strength compared to healthy peers. It is unknown whether this muscle weakness reflects decreased physical activity or a muscle pathology. In the following study, we investigated if *+/G610C* mice have a muscle pathology or altered physical activity levels prior to evaluating their ability to undergo a treadmill exercise regimen. We found that *+/G610C* mice do not exhibit muscle pathology or altered activity levels and were able to complete an 8 week treadmill regimen. Femora from exercised *+/G610C* mice demonstrated the greatest gains in whole bone and material stiffness (torsional loading to failure) with reduced collagen content (hydroxyl proline). Raman spectroscopy analysis of tibial cortical bone demonstrated that the mineral:matrix ratio were equivalent regardless of gender, genotype or exercise status. Our findings suggest muscle weakness and reduced exercise capacity in OI is potentially mutation specific (in contrast to *oim* mouse) and/or reflects potential life style preferences/limitations.

Youngjae Jeong is currently a graduate student of the University of Missouri, Columbia, Missouri. My current focus is to investigate therapeutic strategies to enhance bone quantity and quality in two mouse models of osteogenesis imperfect that exhibit different skeletal severities (*oim* and *G610C*). I am investigating whether *oim* and *G610C* mice can respond to weight bearing and non-weight bearing exercise regimens to increase their hindlimb skeletal muscle function as well as geometrical and biomechanical properties of the *oim* and *G610C* mice bones. In addition, our lab recently have designed a study introducing postnatal myostatin and acitivin deficiency in the *oim* mouse model by using fusion protein (RAP-031, Acceleron Pharma) to attempt to stimulate and enhance bone strength and muscle function. The advantage of our research strategy is to investigate both muscle and bone together where there is potential role of bone and muscle cross-talk and bone mechanosensing. In 2013, I received a travel grant from American Society of Bone and Mineral Research annual meeting by presenting poster about effect of disuse due to hindlimb unloading on mouse femora and muscle quality.

Maternal Myostatin Deficiency Reduces Femoral Strength of Osteogenesis Imperfecta Offspring

Myostatin (MSTN) is a member of the TGF-β superfamily and a negative regulator of muscle growth. Absence of functional MSTN during fetal development results in an increase in muscle mass due to both hypertrophy and hyperplasia of skeletal muscle fibers. In addition to its effects on muscle, the bones of myostatin null (*mstn/mstn*) mice have been reported to have increased bone mineral density (BMD) and strength, suggesting myostatin inhibition may be a potential therapeutic target in the treatment of bone disorders. Osteogenesis imperfecta (OI) is a heritable connective tissue disorder primarily due to mutations in type I procollagen genes, resulting in bone deformity and fragility. Osteogenesis imperfecta murine (*oim*) mice carry a *Col1a2* functional null mutation causing bone fragility and skeletal muscle impairment with heterozygous (+/*oim*) mice exhibiting a milder OI phenotype. Bone is mechanosensitive and responds to loading from the muscle by altering its geometry and biomechanical properties. In a recent study to investigate the impact of myostatin deficiency in +/*oim* mice, we bred +/*mstn* dams to +/*oim* sires. Although femoral strength in four month old male +/*oim* animals was partially rescued when +/*oim* mice were also heterozygous for myostatin deficiency (+/*mstn*), a parental effect of myostatin deficiency was also observed. Femora from +/*oim* male offspring had reduced torsional loading to failure, tensile strength, and energy to failure when the dam was +/*mstn* as compared to genetically identical offspring from +/*oim*. To investigate the mechanism responsible for reduced femoral tensile strength of +/*oim* mice from MO crosses compared to the genetically identical offspring from OO crosses, we examined the hierarchical organization of the mineral to matrix composition using Raman spectroscopy. The PO4: amide I ratio, which reflects the mineral to collagen ratio in the bone, of male +/*oim* femora from the MO cross was 26.2% greater than the PO4: amide I ratios of male +/*oim* femora from OO crosses. Taken together, these data indicate that an absence of parental myostatin influences bone mechanical properties in adult offspring, and these changes appear persistent through adulthood, implicating potential perinatal developmental programing events.

Arin Kettle Oestreich is a 4th year Life Sciences Research Fellow at the University of Missouri in the Department of Biological Sciences studying genetic and epigenetic factors contributing to bone development, maturation, and biomechanical integrity. The focus of her research is to better understand the impact of the uterine environment during development on adult bone health. Understanding the factors within the uterine environment that epigenetically contribute to offspring bone integrity will provide potential therapeutic targets for increasing bone quality of osteogenesis imperfecta patients in utero.

Sclerostin Antibody Increases Bone Mass and Strength in Rapidly Growing and Adult Brtl/+ Models of OI

BP Sinder, LE White, JD Salemi, MS Ominsky, MS Caird, JC Marini, KM Kozloff

Sclerostin is a negative regulator of the Wnt pathway, and inhibiting sclerostin activity with a monoclonal antibody (Scl-Ab) has demonstrated anabolic efficacy in other models of bone fragility. In the context of current therapies for OI, Scl-Ab may be useful for the treatment of OI patients by stimulating bone formation and reducing fracture risk.

We have pursued preclinical studies in the Brtl/+ model of Type IV OI, that carry a G349C substitution on one col1a1 allele. Specifically, 3wk and 6mo old male WT and Brtl/+ were treated for 5 weeks with Scl-Ab (25mg/kg, 2x week) or Veh. Both 3wk and 6mo Brtl/+ showed increased femoral trabecular bone after 5wks of Scl-Ab, although the effect was muted in the rapidly growing 3wk Brtl/+ animals. In cortical bone of the Brtl/+ mid-diaphyseal femur, both ages demonstrated significantly increased bone mass with treatment. This manifested in improved femoral cortical bone strength as assessed by mechanical four-point bending in both 3wk and 6mo old Brtl/+ after 5wks of Scl-Ab. Unexpectedly, we observed a decrease in bone brittleness with Scl-Ab treatment in 6mo old Brtl/+, but this was not seen in rapidly growing 3wk Brtl/+ animals.

Recent work has quantified the bone formation response in the diaphyseal femur by dynamic histomorphometry. In 6mo Brtl/+ animals, we found that Scl-Ab significantly increased Mineral Apposition Rate (MAR) and Mineralizing Surface (MS/BS). In 3wk animals, Scl-Ab did not significantly increase MAR, but was able to increase MS/BS. Detailed regional analysis of anterior and posterior sub-compartments at the femoral mid-diaphysis revealed a posterior shifting cortical drift pattern in Veh animals, although this drifting pattern was more evident in the younger 3wk animals. Scl-Ab was able to consistently increase bone formation on surfaces low in bone formation in 6mo animals (e.g., periosteal anterior), but these increases were more variable in rapidly growing 3wk Brtl/+.

Despite some differences in response to therapy with age, Scl-Ab increased bone mass and strength in a rapidly growing 3wk, and a skeletally mature 6mo, Brtl/+ model of OI. As such, Scl-Ab may be a candidate therapy for the treatment of both pediatric, and adult, OI patients.

Benjamin Sinder, MS received his undergraduate degree in Biomedical Engineering from Case Western Reserve University, and is currently a doctoral student of Ken Kozloff at the University of Michigan. He has performed the initial studies investigating Sclerostin Antibody in the Brtl/+ OI Mouse Model. Ben is attending this conference on a Young Investigator Travel Grant.