Introduction

Duchenne Muscular Dystrophy (DMD) is a fatal X-chromosome linked disease that breaks down skeletal and cardiac muscle. Boys with DMD progressively lose muscle strength, and by the ages of 12-15 are usually confined to a wheelchair. By 20-25 years, the condition is typically fatal due to respiratory or cardiac muscle failure. The limited mobility associated with DMD, along with its primary treatment – glucocorticosteroids – leads to a decrease in bone mineral content and bone mineral density in the lumbar spine, hip, and long bones of the lower limbs by 30-50%. Greater losses are observed in trabecular bone, due to increased surface area and metabolic potential. The disuse and drug-related declines in bone structural properties lead to increased fracture risk. At later stages in disease progression, after ambulatory function has been lost, 67% of boys had experienced at least one fracture. Of these fractures, 66% occurred in the long bones of the lower limbs, more often in the femur than in the tibia. 20.8% of boys with DMD sustained a fracture regardless of whether they were taking corticosteroids, compared to 0.8-3.6% fracture incidence in healthy boys of similar age.

Human recombinant parathyroid hormone is currently an FDA-approved anabolic treatment for post-menopausal osteoporosis. Black bear parathyroid hormone (bbPTH) has been explored as a treatment for osteoporosis as well. Bears spend up to half the year in hibernation, yet do not experience disuse-related osteoporosis. Serum levels of bbPTH have been correlated to bone formation markers during hibernation.

An animal model of DMD, the dystrophin-deficient mdx mouse was injected daily with bbPTH for 6 weeks. At the conclusion of the study, femurs and tibias were extracted and evaluated. We hypothesized that bbPTH would increase bone strength and volume compared to vehicle-treated animals.

Methods

Injections

4 week old male C57BL/10ScSn/DMD-mdx and wild type control mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were split into four groups (mdx PTH, mdx vehicle, wild type PTH, wild type vehicle) and injected 5 times per week for 6 weeks with either 24 nmol/kg bbPTH (1-84) or acidic saline solution. This study was approved by the Michigan Tech Animal Care and Use Committee.

Trabecular Properties

Proximal tibia metaphyses were scanned using micro-computed tomography (µCT) to determine trabecular properties. A 0.7 mm section was evaluated to determine bone volume/tissue volume (BV/TV), trabecular spacing (Tb.sp), trabecular number (Tb.n), trabecular thickness (Tb.th), and apparent bone mineral density (App.M.Dn).

Cortical Geometric Properties

A thin section at the femoral midshaft was imaged at 40x on a brightfield microscope. Using a custom macro, cross-sectional properties including medio-lateral moment of inertia (Iml), antero-posterior moment of inertia (Iap), cortical thickness, cortical area, and maximum moment of inertia (Imax) were determined.

Mechanical Properties

Femoral bending properties were determined using a 3-point bend test on an Instron test machine (Norwood MA). Test fixtures had a span of 10 mm and a radius of 1 mm. Ultimate force and energy to failure were determined using load-displacement plots, and stress-strain plots were used to determine ultimate stress and modulus of toughness.

Mineral Content

Relative mineral content was determined by ashing. Femur diaphyses were dried at 100°C for 24 hours, after which dry mass was determined. Bones were then ashed in a furnace at 600°C for 48 hours to remove all organic content, after which ash mass was determined. Ash fraction was calculated as the ratio of ash mass to dry mass.

Results

Micro-computed tomography

µCT of the proximal tibia showed that untreated mdx mice have lower BV/TV (p=0.021), Tb.n (p=0.051), Tb.th (p=0.0246), and App.M.Dn (p=0.002) than wild type mice, along with higher Tb.sp (p=0.0543). bbPTH increased trabecular properties in both mdx and wild type mice, but to a much greater degree in mdx. BV/TV increased 7-fold in PTH-treated mdx animals (p<0.0001), compared to a 2-fold increase in vehicle-treated mice (p=0.027) (Figure 1). Tb.n and Tb.th increased in PTH-treated mdx mice (p<0.0001), but not in wild type controls. Also, Tb.sp decreased in mdx mice (p<0.0001), with no change observed in wild type. App.M.Dn increased with PTH treatment in both mdx and wild type animals (p<0.0001 and p=0.0247, respectively).

Discussion

The large changes in microstructural trabecular properties suggest that bbPTH is more potently anabolic in mdx mice than wild type controls, evidenced by the 7-fold increase in BV/TV in the proximal tibia of mdx mice compared to a 2-fold change in the wild type. The increases in Tb.n, Tb.th, and App.M.Dn, and decreases in Tb.sp correspond to the increases observed in BV/TV in PTH-treated mice, but to a greater degree in mdx mice. Interestingly, PTH was shown to increase Tb.n in mdx mice, forming new trabecular struts where there were none previously. No change in Tb.n with PTH-treatment was observed in wild type mice, indicating the capability to increase trabeculae with PTH may be unique to dystrophin-deficient models. This suggests a strong interaction between dystrophin deficiency, PTH, and bone that has not been previously explored.

The change in cortical thickness in mdx mice was independent of PTH treatment, suggesting it is a factor of the disease, not the treatment. Like previously published PTH studies, black bear PTH affected cortical bone to a much lesser degree than trabecular bone. The increase in Iml in mdx mice demonstrates that PTH has an effect on cortical bone, and a longer duration treatment could significantly increase ultimate stress in bending.

Significance

An effective anabolic treatment for osteoporosis in boys with DMD could decrease fracture risk, delay the loss of ambulatory function, and improve overall quality of life.

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