Lithium Treatment Improves Vertebral Trabecular Bone Architecture in Mucopolysaccharidosis I Dogs during Postnatal Growth

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Introduction

Mucopolysaccharidosis (MPS) I is a lysosomal storage disease characterized by deficient alpha-L-iduronidase activity, which leads to abnormal accumulation of incompletely degraded dermatan and heparan sulfate glycosaminoglycans (GAGs)[1]. MPS I patients present with severe spinal deformity, due in part to impaired vertebral bone formation during postnatal growth, which decreases quality of life and increases mortality[2]. Enzyme replacement therapy (ERT), the current clinical standard of treatment for MPS I, has been shown to attenuate progression of spine disease in MPS I dogs[3,4]. However, ERT is expensive, requires frequent and regular administration throughout the duration of the patients' lifetimes, and does not fully normalize bone formation even when commenced at birth[5]. Thus, it is important to identify alternative therapies that specifically target and enhance bone formation. Our previous work in other MPS subtypes with similar bone manifestations showed that impaired bone formation is due to failed hypertrophic differentiation of vertebral epiphyseal chondrocytes during postnatal development[6], which is associated with decreased Wnt signaling[7], an important regulator of endochondral ossification during postnatal growth. Therefore, we hypothesized that stimulating the Wnt signaling pathway would result in improved bone formation in MPS I. Lithium activates the Wnt pathway by inhibiting GSK-3β and is FDA-approved for human use for the treatment of bipolar disorder[8]. Thus, the objectives of this study were to 1) establish a lithium dosing and monitoring regimen and 2) investigate whether treatment with lithium can enhance bone formation during postnatal growth in MPS I in the naturally-occurring canine model.

Methods

We used the naturally-occurring MPS I canine model, which mimics both the progression and pathological phenotype of the skeletal abnormalities found in human patients[9]. With Institutional Animal Care and Use Committee (IACUC) approval, MPS I dogs (n = 3) were treated orally with lithium carbonate daily from 14 days to 6 months-of-age. Following an initial 1 week period of low dose acclimation, the dose was gradually increased to 30-50 mg/kg in order to maintain serum lithium levels in the putative therapeutic range (0.5-1.5 mmol/L)[10]. Throughout the study, lithium doses were adjusted twice a week based on animal weight and serum analyses. At 6 months-of-age, serum was collected from the three lithium-treated MPS I dogs as well as age-matched unaffected control dogs (n = 4) and untreated MPS I dogs (n = 5) in order to measure bone-specific alkaline phosphatase (BAP) activity using a commercially available ELISA kit. All animals were euthanized following serum collection, and thoracic vertebrae were excised postmortem and analyzed using micro-computed tomography (μCT). Significant differences in BAP levels between all groups were determined using 1-way ANOVA with post-hoc Bonferroni test ($p < 0.05$). Significant differences in μCT measurements between lithium-treated and untreated MPS I animals were determined using unpaired t-tests ($p < 0.05$).

Results

After the acclimation period, lithium treated MPS I dogs generally maintained serum lithium levels within the desired therapeutic range (Figure 1). While control dogs had significantly higher serum BAP than either the untreated or treated MPS I dogs, BAP levels were significantly higher in lithium-treated than untreated MPS I dogs (Figure 2). While bone volume fraction, bone mineral density, and trabecular thickness were not significantly different in lithium-treated MPS I dogs compared to untreated MPS I dogs, connectivity density and trabecular number were significantly higher, and trabecular spacing was significantly lower (Figure 3A, B). Preliminary cortical bone analysis showed no differences between groups. Upon clinical examination, forelimb and hindlimb joints of the MPS I lithium-treated animals presented with less swelling, less fluid retention, and better mobility than untreated MPS I animals.

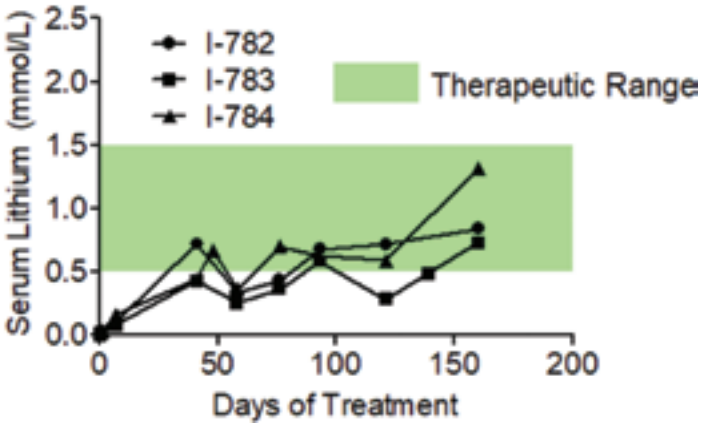

Figure 1. Lithium-treated MPS I animals generally maintained serum lithium levels in the therapeutic range throughout the duration of the study under our dosing regimen. N = 3, each line represents a lithium-treated MPS I animal.

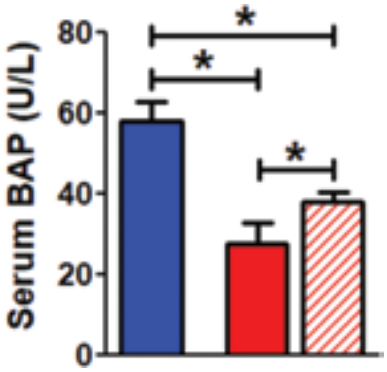

Figure 2. Lithium-treated MPS I animals exhibited higher levels of BAP activity compared to untreated animals. Control (n = 4), MPS I (n = 5), MPS I lithium treated (n = 3). $p < 0.05$.

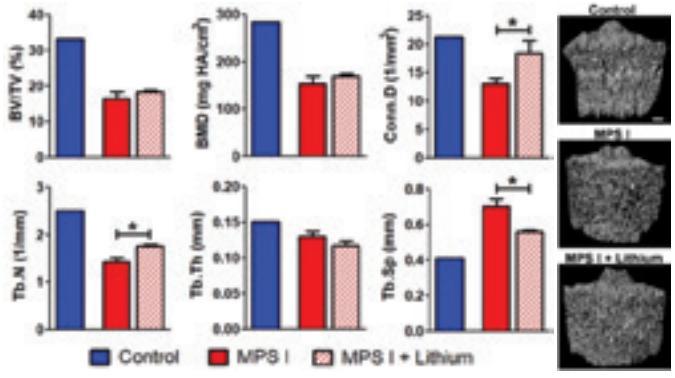

Figure 3. Micro-computed tomography (μCT) measurements of T12 vertebrae showed improvements in trabecular bone microarchitecture. (**A).** Bone volume fraction (BV/TV), bone mineral density (BMD), connectivity density (Conn.D), trabecular number (Tb.N), thickness (Tb.Th), and spacing (Tb.Sp) from control (representative baseline), MPS I (n = 3), and lithium-treated animals (= =3). *$p < 0.05$;)**B).** Representative 3D reconstructions of vertebral trabecular bone. Scale bar = 2 mm.

Discussion

Overall, these findings suggest that activating the Wnt pathway with lithium can alter bone turnover in MPS I animals. Our dosing regimen maintained serum lithium levels in the therapeutic range. Higher serum BAP levels in lithium-treated animals suggest increased bone formation, potentially due to increased osteoblast numbers or activity with treatment. Higher connectivity density and trabecular number and lower trabecular spacing in the lithium-treated animals also suggest improvements in bone microarchitecture. Importantly, these findings demonstrate that bone cells in MPS I are still able to respond to activating stimuli despite significant GAG storage and that Wnt pathway agonists may represent a potential therapeutic strategy for stimulating bone formation in MPS I. Furthermore, measuring BAP levels is a non-invasive method to detect increases in bone formation in patients as a result of therapeutic intervention. Decreased swelling and improved mobility of joints suggest that lithium treatment may be able to alleviate other related musculoskeletal symptoms of MPS I. Ongoing work will establish the underlying cellular basis of improved bone formation with lithium treatment in MPS I dogs and whether these alterations in trabecular bone architecture are associated with improved mechanical properties.

Clinical Relevance

MPS I is associated with debilitating skeletal disease stemming from impaired bone formation for which there is no effective treatment. Our results suggest that Wnt activation is a potential therapeutic strategy for stimulating bone formation in MPS I patients.

References:

1. **Scott, *et al.*** *Hum Mutat* 1995;
2. **White,** *Rheum* 2011
3. **Chiaro, *et al.*** *J Bone Min Res* 2014
4. **Dickson, *et al.*** *Mol Gen Met* 2010
5. **Yasin, *et al.*** *Spine* 2014
6. **Peck, *et al.*** *Mol Gen Met* 2015
7. **Peck, *et al.*** *ORS Trans* 2016
8. **Hedgepeth, *et al.*** *Dev Biol* 1997
9. **Haskins, *et al.*** *Pediat Res* 1984
10. **Rosenthal, *et al.*** *J Vet Pharmacol Therap* 1983