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ARTICLE

Administration of soluble activin receptor 2B increases bone and muscle mass in a mouse model of osteogenesis imperfecta

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Osteogenesis imperfecta (OI) comprises a group of heritable connective tissue disorders generally defined by recurrent fractures, low bone mass, short stature and skeletal fragility. Beyond the skeletal complications of OI, many patients also report intolerance to physical activity, fatigue and muscle weakness. Indeed, recent studies have demonstrated that skeletal muscle is also negatively affected by OI, both directly and indirectly. Given the well-established interdependence of bone and skeletal muscle in both physiology and pathophysiology and the observations of skeletal muscle pathology in patients with OI, we investigated the therapeutic potential of simultaneous anabolic targeting of both bone and skeletal muscle using a soluble activin receptor 2B (ACVR2B) in a mouse model of type III OI (*oim*). Treatment of 12-week-old *oim* mice with ACVR2B for 4 weeks resulted in significant increases in both bone and muscle that were similar to those observed in healthy, wild-type littermates. This proof of concept study provides encouraging evidence for a holistic approach to treating the deleterious consequences of OI in the musculoskeletal system.

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INTRODUCTION

Osteogenesis imperfecta (OI) comprises a group of heritable connective tissue disorders generally defined by recurrent fractures, low bone mass, short stature and skeletal fragility. The vast majority of OI cases (~90%) are the result of dominant mutations in either of the two type I collagen genes, *COL1A1* or *COL1A2*, the most abundant and primary structural proteins in the organic bone matrix.[1] Over the past 10 years, a rapidly growing list of recessive gene mutations has emerged, which account for the remaining 5%–10% of OI cases, although—with a few exceptions (e.g., *SERPINF1*, *IFITM5*, *LRP5*, *SP7*)—most of these genes are intimately involved in the trafficking or complex post-translational processing of type I collagen.[2] In the clinic, the wide spectrum of phenotypic variance in OI is classified into five types, based largely on the Sillence classification system that was originally outlined 35 years ago.[3] In order of increasing severity, the five phenotypic classifications of OI are type I (classic, non-deforming OI with blue sclerae), type IV (common variable OI with normal sclerae), type V (OI with ossification in interosseous membranes), type III (progressively deforming OI with normal sclerae) and type II (perinatally lethal OI). The majority of the rare, recessive types of OI and newly discovered non-collagen molecular defects, which had previously been assigned their own OI types, are now enfolded as subtypes of type III OI.[4]

Beyond the skeletal complications of OI, many patients also report intolerance to physical activity, fatigue, and muscle weakness, in some cases so severe that it serves as a presenting symptom for the disease.[5] While frequently reported, the impact of OI on skeletal muscle function and anatomy has only received limited investigation. A study of 20 children with type I OI demonstrated decreased resistance to fatigue and lower muscle force in the plantar flexor.[6] Another study by Takken and colleagues[7] observed decreased isometric muscle force of the shoulder abductors, hip flexors, ankle dorsiflexors, and grip strength in 17 children and adolescents with type I OI, when compared to age-/sex-matched controls. More

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recently, Veilleux and colleagues[8] studied the muscle anatomy and dynamic muscle function of 54 patients with type I OI. They observed that patients with OI had smaller muscle size, lower average peak force and lower specific peak force when compared to age-/sex-matched controls. Interestingly, the deficits in skeletal muscle observed in all of these studies were seen in the least severe form of OI, suggesting that there may be an intrinsic defect in the skeletal muscle of OI patients. Moreover, one might speculate that the deleterious effect of OI on skeletal muscle could be exacerbated in more severe types of the disease by repeated bouts of inactivity with more frequent fractures. Indeed, a recent cross-sectional study of 62 children and adolescents with varying types of OI demonstrated that moderate and severe forms of OI are associated with greater functional impairment, influenced by fracture history, which has a negative impact on ambulation.[9]

The activin signaling pathway, well known for its activity in regulating skeletal muscle mass through myostatin,[10] has recently been demonstrated to affect bone development and remodeling as well.[11–15] Systemic blockade of activin receptor signaling using a soluble activin receptor 2B (ACVR2B) has previously been demonstrated to increase muscle mass in mouse models of androgen deficiency,[16] muscular dystrophy[17] and cancer cachexia.[18] ACVR2B administration also increased bone formation rates and bone mineral density in aged mice[19] and demonstrated direct effects on osteoblast activity.[12] Given the well-established interdependence of bone and skeletal muscle in both physiology and pathophysiology[20] and the observations of skeletal muscle pathology in patients with OI, we proposed to investigate the therapeutic potential of simultaneously targeting both bone and skeletal muscle in this study, using a soluble activin receptor in a mouse model of OI. Using the *oim* mouse, which most closely resembles the severe, deforming type III OI in humans and also exhibits deficits in muscle mass and function,[21] we demonstrate that ACVR2B can increase both bone and muscle mass in OI, possibly providing a new therapeutic alternative where so few currently exist.

MATERIALS AND METHODS

Animal studies

Heterozygous *oim*/+ mice (B6C3Fe *a/a-Col1a2*^{oim}/J; Jackson Laboratory, Bar Harbor, ME, USA) were bred to produce homozygous *oim*/*oim* (*oim*) mice and wild type littermate controls (wild-type (WT)). As previous studies reported no difference in skeletal muscle phenotype between sexes, only male mice were used in the present study.[21] The soluble activin receptor (ACVR2B) is a fusion protein containing the extracellular domain of activin receptor 2B linked to a murine Fc domain. The methods for expression and purification of ACVR2B have been previously described.[22] Twelve-week-old WT and *oim* mice were treated with ACVR2B (10 mg·kg^{-1}, intraperitoneal injection) or vehicle control once per week for 4 weeks prior to euthanasia and tissue harvest (WT, n=5; WT+ACVR2B, n=5; *oim*, n=11; *oim*+ACVR2B, n=11). All animal studies were performed in accord with established protocols approved by the Animal Care and Use Committee at the Johns Hopkins University School of Medicine.

Skeletal analysis

Femora were harvested and bone volume was assessed using a desktop microtomographic imaging system (Skyscan 1172; Skyscan, Kontich, Belgium) at the Center for Musculoskeletal Research at Johns Hopkins University. Histological analyses, using a semi-automated method (Osteoplan II, Kontron), were carried out in the Department of Nephrology, Bone and Mineral Metabolism at the University of Kentucky. All analyses of bone structure—both μCT and histomorphometry—were completed in accordance with the recommendations of the American Society for Bone and Mineral Research.[23–24]

Muscle analysis

Pectoralis, triceps, quadriceps and gastrocnemius muscles were harvested to measure wet weights of various muscle groups. For measurement of muscle weights, individual muscles from both sides of the animal were dissected, and the average weight was used for the value of each muscle weight for that animal.

Statistical analysis

All values are expressed as a mean±standard error of the mean. All statistical analyses were performed using the Microsoft Excel data analysis program for ANOVA or Student's *t*-test analysis with an assigned significance level of 0.05 (α).

RESULTS

In order to assess the therapeutic efficacy of a dual anabolic (muscle and bone) treatment in OI, 12-week-old WT and *oim*/*oim* (*oim*) mice were treated with a soluble activin receptor 2B (ACVR2B) at 10 mg·kg^{-1} or vehicle via intraperitoneal injection, once per week, for 4 weeks. μCT analysis of the distal femur revealed a significant increase in trabecular bone volume, trabecular thickness and trabecular number in ACVR2B treated *oim* mice (Figure 1) similar to increases observed in WT mice receiving ACVR2B (Supplementary Figure S1).

To further analyze the anabolic effect of ACVR2B treatment in the skeleton of the *oim* mice, histomorphometric analysis was performed in the distal femur. Histomorphometry confirmed the bone volume increase

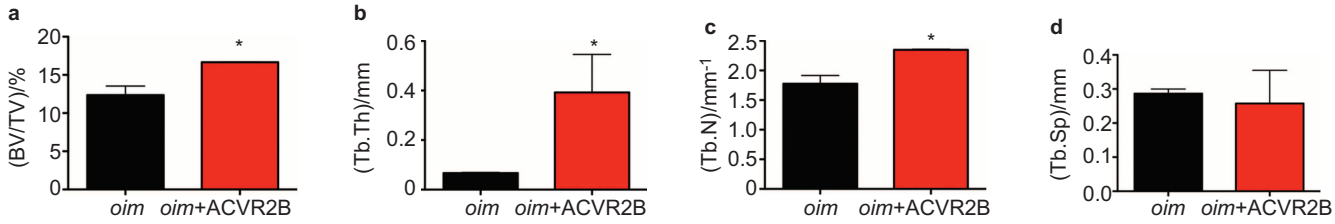

Figure 1. Administration of ACVR2B increases bone volume in *oim* mice. μCT analysis of trabecular bone in the distal femur of 16-week-old *oim* mice following 4 weeks of treatment with ACVR2B (10 mg·kg^{-1} i.p., 1×/week) or vehicle control. (**a**) Bone volume fraction; (**b**) trabecular thickness; (**c**) trabecular number; (**d**) trabecular separation.

observed in ACVR2B treated *oim* mice by μCT analysis (Figure 2a). We also observed significantly reduced osteoid volume and thickness in ACVR2B-treated *oim* mice compared to controls (Figure 2e and 2f), suggesting that the increased bone volume may result from improved osteoblast mineralization, consistent with our previous findings.[12] Osteoblast numbers tended to increase in ACVR2B-treated *oim* mice, although these values did not reach significance, as observed in their WT counterparts treated with ACVR2B (Supplementary Figure S2a–2c), likely due to variability in remodeling as a result of fractures in these severely affected OI mice.

We next examined the effect of ACVR2B administration on skeletal muscle in *oim* mice. Consistent with previous findings,[21] *oim* mice had significantly lower muscle weights than WT mice across all muscle groups examined (Figure 3a). ACVR2B treatment was able to significantly increase muscle weight in the pectoralis, triceps, and quadriceps of *oim* mice (Figure 3b–3d), but failed to show a significant effect in the gastrocnemius (Figure 3e).

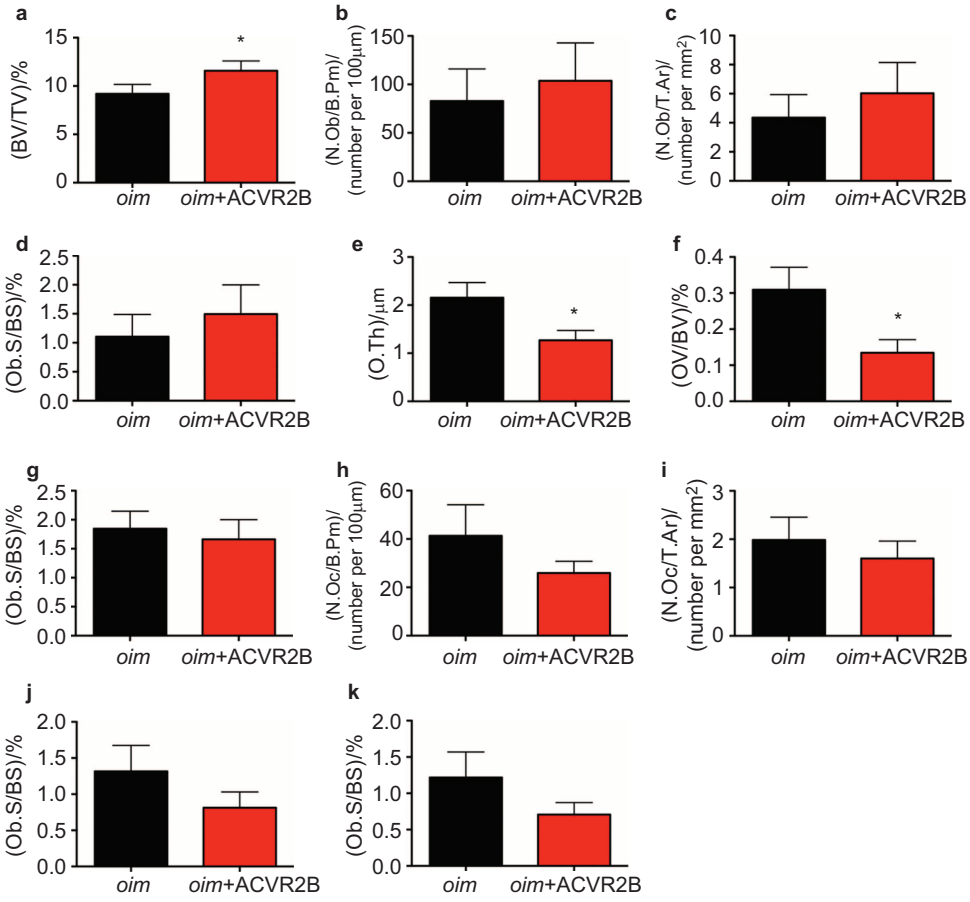

Figure 2. Administration of ACVR2B reduces osteoid volume while increasing bone volume in *oim* mice. Histomorphometric analysis of trabecular bone in the distal femur of 16-week-old *oim* mice following 4 weeks of treatment with ACVR2B (10 mg·kg^{-1} i.p., 1×/week) or vehicle control. (**a**) Bone volume; (**b**) osteoblast number per bone perimeter; (**c**) osteoblast number per tissue area; (**d**) osteoblast surface; (**e**) osteoid thickness; (**f**) osteoid volume; (**g**) osteoid surface; (**h**) osteoclast number per bone perimeter; (**i**) osteoclast number per tissue area; (**j**) eroded surface; (**k**) osteoclast surface.

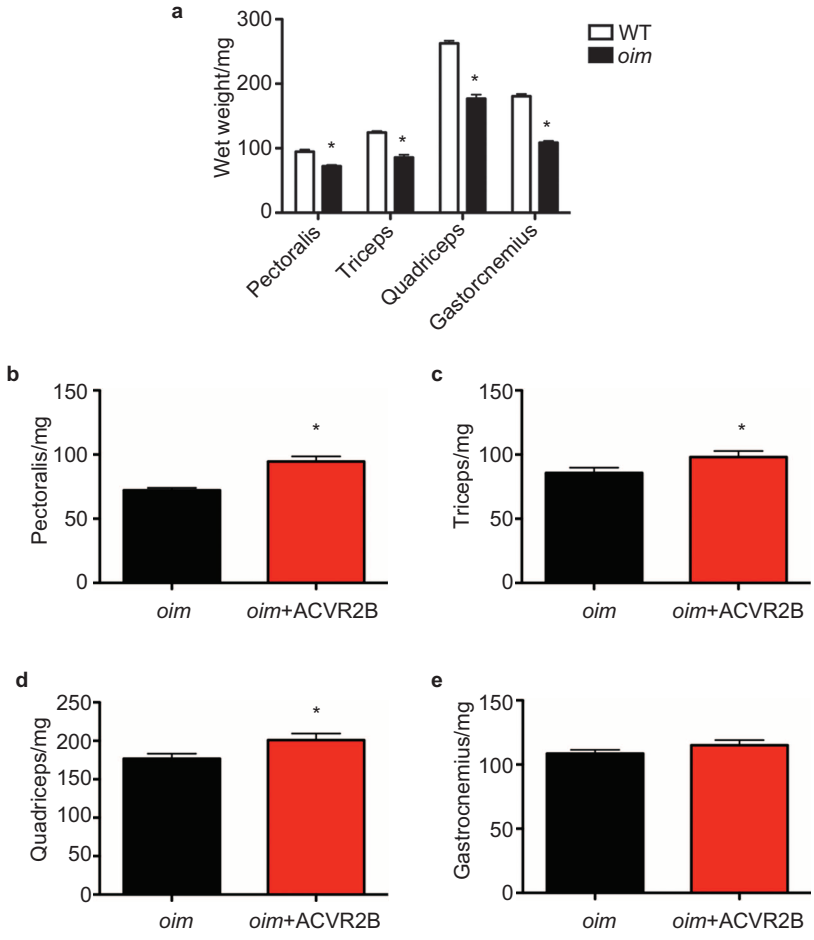

Figure 3. Administration of ACVR2B improves the reduced muscle mass observed in *oim* mice. Wet weights of skeletal muscle groups dissected from 16-week-old *oim* mice following 4 weeks of treatment with ACVR2B (10 mg·kg^{-1} i.p., 1×/week) or vehicle control. (**a**) Muscle mass is reduced in *oim* mice compared to WT in all muscle groups examined. Treatment with ACVR2B increases muscle mass in *oim* mice in the (**b**) pectoralis, (**c**) triceps, (**d**) quadriceps, but not the (**e**) gastrocnemius.

Interestingly, the magnitude of increase in pectoralis weight of ACVR2B treated *oim* mice (Figure 3a) was similar to that of WT mice treated with ACVR2B (Supplementary Figure S3a), while the anabolic effect of ACVR2B treatment in the limb muscles of *oim* mice (Figure 3b–3d) was less pronounced than that observed in ACVR2B treated WT mice (Supplementary Figure S3b–3d).

DISCUSSION

Osteogenesis imperfecta is a debilitating disease with relatively few clinical interventions. Prior to the introduction of bisphosphonate treatment over 25 years ago,[25] orthopaedic surgery and physiotherapy were the sole course of treatment for patients with OI. During this time, patients with OI suffered significantly higher rates of fracture, progressive deformity and immobility. Treatment with bisphosphonates has provided significant improvement in the quality of life of patients with OI, providing reduced bone pain, reduced rates of fracture and subsequent deformity, improved longitudinal growth, better mobility, and an improved sense of well-being in these patients.[26] Bisphosphonate treatment, despite all those positives, is not without its drawbacks. Prolonged use of bisphosphonates in children can result in impaired metaphyseal modeling, resulting in widened metaphyses and characteristic radiographic lines of unresorbed calcified cartilage from the growth plate.[27] Moreover, the junction between denser, bisphosphonate-treated bone and the less dense bone produced after discontinuation of therapy may be more prone to fracture.[28] Finally, the reduction of bone turnover by bisphosphonates can also impair bone repair in patients undergoing corrective osteotomy.[29] Thus, despite providing considerable improvement in quality of life for children with OI over surgery and physiotherapy alone, additional therapeutic options are sought.

In the present study, we examined the ability of a soluble activin receptor to improve both bone and muscle mass in a mouse model of type III OI. We believe that such a dual anabolic strategy would be ideal for the setting of OI, where low bone mass and fracture lead to

reduced physical activity, which results in muscle atrophy and further reduction in bone mass, further predisposing to fracture, and so on. Indeed, physical activity programs are highly encouraged to prevent contracture and reduce immobility-induced bone loss in patients with OI,[30] but in some patients, the risk of fracture—or progression of disease—is too great to accomplish meaningful levels of activity. It is in these patients that such a dual anabolic treatment is most attractive, as one would be able to increase bone and muscle mass to a point that would allow effective physiotherapy, improved mobility, and ultimately, improved quality of life. It is for this reason that we chose to evaluate the efficacy of ACVR2B treatment in a mouse model of the most severe (type III) OI.

Indeed, treating *oim* mice with ACVR2B was able to significantly increase both bone and muscle mass. Despite starting with a significant deficit in bone mass, the anabolic effect of ACVR2B on the skeleton of *oim* mice was equal in magnitude to that observed in WT littermates, which is especially encouraging given the severity of disease in these animals. While ACVR2B treatment only partially rescued the deficit in bone mass observed in *oim* mice, this may be further improved by increasing the duration and/or dose of treatment. Moreover, ACVR2B dramatically increased trabecular thickness in *oim* mice (Figure 1b). This is in contrast to the mechanism by which bisphosphonates increase bone mass in OI, i.e., the number of abnormally thin trabeculae is increased by inhibition of resorption of the primary trabeculae during the transition to secondary spongiosa with endochondral growth.[26] Presumably, the increase of both trabecular number and thickness should result in even greater mechanical benefit than would just the increase in trabecular number seen with bisphosphonate treatment. Unfortunately, we were unable to observe any significant difference in mechanical properties between ACVR2B-treated and vehicle-treated *oim* mice by three-point-bend testing, as the bones of the *oim* mice were almost all afflicted with at least one fracture in various states of healing (data not shown). While the precise molecular mechanism(s) by which inhibition of activin receptor signaling exerts anabolic effects in bone has yet to be defined, the efficacy of targeting this pathway to improve bone has been demonstrated in numerous settings [16,31–33]. Previous studies have demonstrated that BMP3 signaling through ACVR2B can inhibit osteoblast differentiation and mineralization.[34] More recently, however, Bialek and colleagues[31] treated *Bmp3*$^{-/-}$ mice with a soluble ACVR2B and observed further increases in bone volume in these mice, suggesting that additional TGF-β family members are also involved in regulating osteoblast function through the activin receptors.

As in the bone, *oim* mice also began treatment with ACVR2B with a significant deficit in muscle mass compared to their WT littermates. Interestingly, and by contrast to the effect of ACVR2B on the bone of *oim* mice, ACVR2B did not increase muscle mass in the *oim* mice to as great an extent as observed in healthy control animals (Figure 3b–3e *vs.* Supplementary Figure S3), with the exception of the pectoralis. One possible explanation for this difference may stem from the fact that the other three muscles examined are located in the extremities and have experienced selectively greater disuse atrophy than the pectoralis as a result of the numerous fractures observed in the long bones of the *oim* mice. Importantly for our proof of concept; however, ACVR2B was still able to improve muscle mass in these muscle groups.

While the effects of OI may be most pronounced and induce the greatest morbidity in patients through the skeletal manifestations of the disease, it is important to consider the entire musculoskeletal system in the approach to treating the disorder. Skeletal muscle is also negatively affected in OI, both by the disease directly, and secondarily, by the eventual loss of mobility due to recurrent fracture and/or surgical treatment. Moreover, improving muscle mass can have a multitude of positive secondary effects on bone via increased mechanical force, paracrine/endocrine signaling, *etc*. Thus, simultaneously improving both bone and skeletal muscle, as we have demonstrated by targeting the activin signaling pathway with ACVR2B, represents an exciting and promising new approach to treating the deleterious consequences of OI in the musculoskeletal system.

Competing interests

The authors declare no conflict of interest.

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