INTRODUCTION

Glucocorticoids are the first-line medications for Duchenne muscular dystrophy (DMD) and are widely used as immunomodulatory agents in inflammation, autoimmune diseases and cancer. However, glucocorticoid treatment is associated with significant adverse consequences, including adrenal suppression, growth retardation, increased risk of fracture due to accelerated bone loss, and muscle atrophy [1-3]. Particularly, chronic glucocorticoid treatment is one of the primary contributors to the high fracture rate in DMD patients [4-5]. Transforming growth factor-beta family members, activin A and myostatin, are potent negative regulators of musculoskeletal growth [6-9]. Inhibition of these ligands has been demonstrated, through various therapeutic modalities, to promote anabolic activities in both muscle and bone [10-11]. Moreover, chronic glucocorticoid treatment has been shown to significantly elevate the myostatin levels in skeletal muscle [12].

RKER-065 is an investigational, research form of a modified type II activin receptor (ActRII)-derived ligand trap that was designed to bind and inhibit activin A and myostatin. With the goal of reducing the adverse effect of prolonged glucocorticoid exposure while maximizing its beneficial outcomes, we evaluated if RKER-065 can prevent the negative effect of glucocorticoids on bone and muscle.

METHOD

Six-week-old male C57Bl/6 mice were randomized to three groups with baseline body weight matched. Mice in the vehicle cohort were fed with cherry syrup daily via gavage (Veh, P.O.), while mice in Pred-TBS or Pred RKER-065 cohorts received prednisolone (Pred; 5mg/kg, P.O., daily) and either tri-buffered saline (TBS) or RKER-065 (10mg/kg, i.p. weekly), for 9 weeks. All animals were assessed for lean mass, bone mineral density (BMD) and grip strength after 4 and 8 weeks of treatment. Bone volume and structure were assessed at 9 weeks.

RESULTS

1. RKER-065 preserved body weight gain in prednisolone treated mice

Figure 1. The body weight of the mice was measured 5 times a week. The Pred-TBS cohort exhibited reduced weight gain relative to the Veh cohort, while this parameter was maintained in Pred-RKER-065 treated mice throughout the study. Statistical analysis was done by two-way ANOVA and individual comparisons shown from Tukey's multiple comparison test. Data are shown as mean ±SEM, **P<0.01, ***P<0.001.

2. RKER-065 counteracted prednisolone-induced muscle atrophy and increased muscle strength

Figure 2. Lean mass was assessed at baseline, day 34, and day 54 post treatment using nuclear magnetic resonance (NMR). Forelimb grip strength was assessed at day 34 and day 55 post treatment. Mice were sacrificed 48hrs post final dose. Individual muscle weight was measured at study termination. The Pred-TBS cohort exhibited reduced lean mass gain relative to the Veh cohort, while RKER-065 treatment in prednisolone-treated mice led to a robust increase in lean mass. The observed increase in lean mass in the Pred-RKER-065 cohort was associated with a significant increase in forelimb grip strength which was apparent by day 34. Statistical analysis was done by one-way ANOVA and individual comparisons shown from Tukey's multiple comparison test. Data are shown as mean ±SEM, **P<0.01, ***P<0.001, ****P<0.0001.

3. RKER-065 attenuated bone mineral density reduction in prednisolone treated mice

Figure 3. Bone mineral density (BMD) was assessed at day 32 and day 52 post treatment using DXA scan. At day 32, right femoral (a) and whole-body BMD (b) in the Pred-TBS cohort were lower than the Veh cohort. RKER-065 treatment prevented prednisolone induced BMD reduction and there was no difference observed between the Pred-RKER-065 and Veh cohorts. Although statistical significance was not achieved, a similar trend was observed at day 52 post treatment. Statistical analysis was done by one-way ANOVA and individual comparisons shown from Turkey’s multiple comparison test. Data is shown as mean ±SEM, * P<0.05, ** P<0.01.

4. RKER-065 increased trabecular bone mass in prednisolone treated mice

Figure 4. Ex vivo CT scans of the femurs were conducted using GX2 μCT (10 mm FOV, 90 kV, 88 μA, 4 minutes, Perkin Elmer). Micro-structure of femurs was evaluated using Analyze 14.0 Bone micro-architecture Analysis software (AnalyisDirect). No obvious trabecular bone loss was observed in Pred-TBS cohort, whereas reduced cortical bone thickness was noted in this cohort of mice (g). Pred-RKER-065 treated mice showed improvements in trabecular bone parameters relative to both Veh and Pred-TBS cohorts (a-e) and a trend in preserving cortical bone in prednisolone treated mice (f-i). BV/TV: bone volume fraction, Tb.Th: trabecular thickness, Tb.N: trabecular number, Tb.Sp: trabecular separation, Ctr.Ar: cortical area bone area, Ctr.Th: cortical bone thickness, Imax: maximum moment of inertia. Polar MOI: polar moment of Inertia. Statistical analysis was done by one-way ANOVA and individual comparisons shown from Tukey’s multiple comparison test. Data is shown as mean ±SEM, * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

CONCLUSIONS

• Taken together, these data demonstrate that RKER-065 can increase muscle mass, improve muscle function and prevent bone loss in prednisolone treated mice.

• These studies support how targeting activin and myostatin could potentially be used to improve muscle and bone strength in dystrophic patients under glucocorticoid therapy.

• Future studies will assess the effect of RKER-065 on prednisolone-induced growth retardation and skeletal development impairment in young mice.

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