Edasalonexent maintains bone density and bone strength in the *mdx* mouse model of Duchenne Muscular Dystrophy

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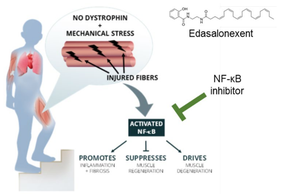
Introduction

- Duchenne muscular dystrophy (DMD) is caused by mutations in the *DMD* gene, leading to a lack of dystrophin protein. The resulting muscle disease is progressive and ultimately lethal, x-linked recessive disorder.
- Boys with DMD have multiple risk factors that make secondary bone disease all but inevitable, including muscle weakness, reduced mobility as well as glucocorticoid therapy.
- Currently, the treatment is lifelong high-dose glucocorticoids which further impact bone health, including increased risk of fractures.
- Previous studies on boys with DMD have shown that treatment with intravenous bisphosphonates improves back pain caused by vertebral fractures, increases spine bone density and promotes reshaping of fractured vertebral bodies.
- DMD mutations cause a chronic activation of nuclear factor kappa B (NF-κB) signaling in muscle tissue.
- NF-κB inhibitor edasalonexent improved the muscle phenotype in animal models of DMD.
- The purpose of this study was to understand the bone-effects of long term edasalonexent treatment in mdx mice



Methods

- Two separate reporter cell lines were used where the reporter gene, firefly luciferase, was linked to the specific consensus upstream genetic response elements (either GR or NF-kB) to drive luciferase transcription.
- *Mdx* mouse model of DMD was used for this experiment.
- Treatment: 1mg/kg/day targeted prednisolone dose by drinking water, and by 1% edasalonexent in the diet
- Left femur were used for µCT and three-point bending analysis
- Left tibia was used for histomorphometry
- Lumbar vertebra were used for µCT and histomorphometry

Results

Edasalonexent inhibits NF-κB in vitro and does not activate the Glucocorticoid Receptor

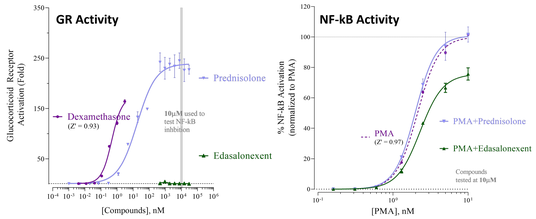


Figure 1. *Left*, GR activity in reporter cells. Concentration at which the NF-kB assay was performed is shown with a line at 10µM. *Right*, NF-κB activation normalized to PMA. All relative luminescence units were normalized between 0% (vehicle treated) to 100 (plateau response for PMA at 10nM). Z-Factor was calculated as follows: 1- [(3*[SDVehicle + SDRef Max]) / (RLURef Max- RLUVehicle)] .

Edasalonexent increases significantly P1NP at 6 months of treatment and TRAP5b increases with prednisolone

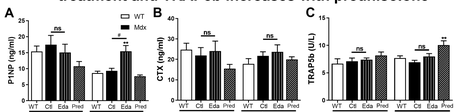


Figure 2. Serum bone resorption markers (A) CTX, and (B) TRAP5b; and bone formation marker (C) P1NP. Data represent mean +/- SEM. Differences between mdx groups were assessed by one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ns: not significant (p≥0.05). Abbreviations: WT: wild type; Ctl, control; Eda: edasalonexent; Pred: prednisolone.

Mineral apposition rate is increased at 3 months of treatment

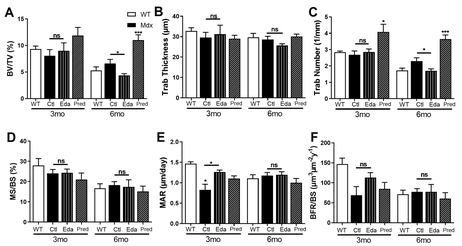


Figure 3. Histomorphometry of the left tibia (A) Trabecular volume; (B) trabecular number; (C) trabecular thickness; (D) Mineral apposition rate; (E) Bone formation rate; (F) mineralizing surface over bone surface. Data represent mean +/- SEM. Differences between mdx groups were assessed by one-way ANOVA. The level of significance for the difference to the mdx control group is indicated by symbols above each bar. In addition, the significance of the difference between edasalonexent and control are shown by symbols: *p<0.05, **p<0.01, ***p<0.001, ns: not significant (p≥0.05).

Significant increase in trabecular thickness after edasalonexent treatment, while trabecular number increased with prednisolone treatment.
Endosteal diameter, periosteal diameter, stiffness and maximum load decreased with prednisolone treatment

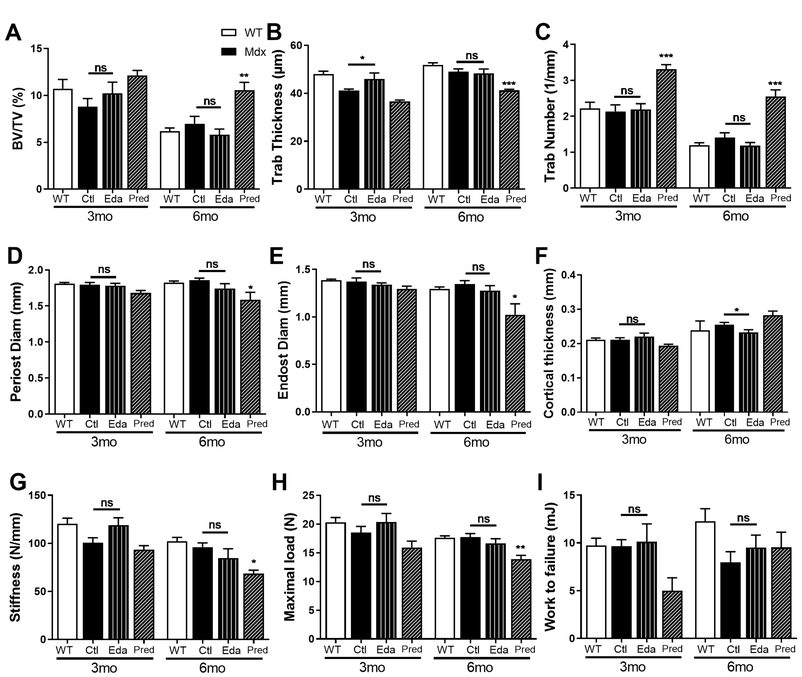


Figure 4. Femur microCT and biomechanics. (*A*) Trabecular bone volume (distal femur); (*B*) trabecular thickness (distal femur); (*C*) trabecular number (distal femur); (*D*) Periosteal diameter (midshaft femur); (*E*) Endosteal diameter (midshaft femur; corresponding to the diameter of the bone marrow cavity); (*F*)cortical thickness (midshaft femur); (G) Stiffness; (*H*) Maximal load; (*I*) Work to failure. Data represent mean +/- SEM. Differences between mdx groups were assessed by one-way ANOVA. The level of significance for the difference to the mdx control group is indicated by symbols above each bar. In addition, the significance of the difference between edasalonexent and control are shown by symbols: *p<0.05, **p<0.01, ***p<0.001, ns: not significant (p≥0.05).

Significant increase of BV/TV and trabecular number after treatment with prednisolone

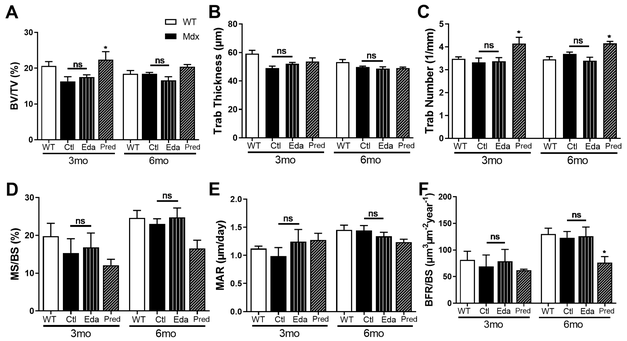


Figure 5. Trabecular bone analyses at lumbar vertebra L4. MicroCT results for (A) Trabecular bone volume; (B) trabecular thickness; (C) trabecular number. Dynamic bone formation parameters as measured by histomorphometry: (D) Mineralizing surface per bone surface; (E) Mineral apposition rate; (F) Bone formation rate per bone surface. n = 4-5 mice per group at 3 months, 9-10 mice per group at 6 months of treatment. Data represent mean +/- SEM. Differences between mdx groups were assessed by one-way ANOVA. The level of significance for the difference to the mdx control group is indicated by symbols above each bar. In addition, the significance of the difference between edasalonexent and control are shown by symbols: *p<0.05, **p<0.01, ***p<0.001, ns: not significant (p≥0.05).

Conclusions

Treatment with edasalonexent was not associated with significant changes in parameters reflecting bone mass, structure or strength at any of the investigated skeletal sites (lumbar vertebra, distal and midshaft femur, tibia). Treatment of mdx mice with prednisolone was associated with higher serum concentrations of the bone resorption marker TRAP5b and with lower stiffness and maximal load of the femur in the three-point bending test suggesting decreased bone strength.

Acknowledgments

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