The Mitochondria-targeting Peptide Elamipretide Potentiates Dystrophin Expression Induced by an Exon-skipping Morpholino in the mdx Mouse Model

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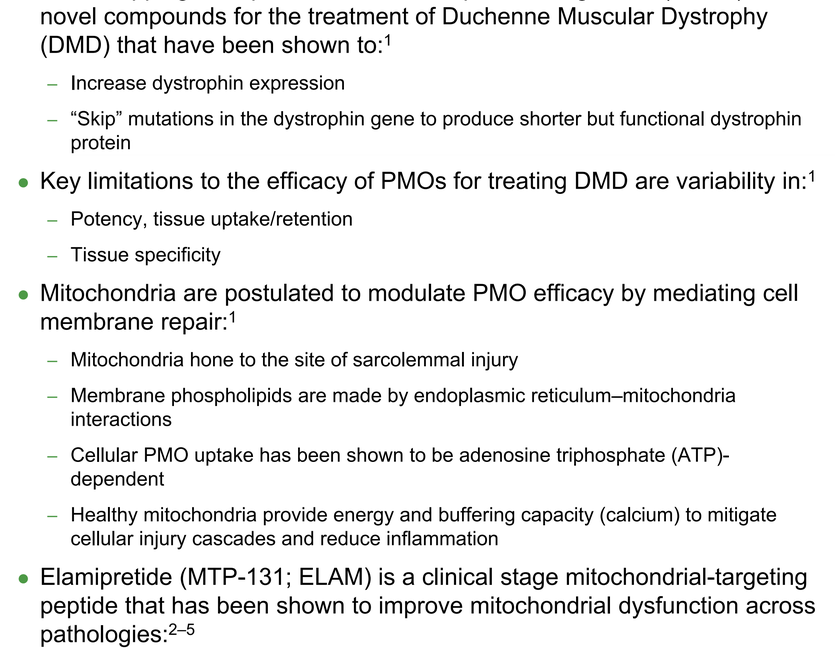
INTRODUCTION

- Exon-skipping Phosphorodiamidate Morpholino Oligomers (PMOs) are novel compounds for the treatment of Duchenne Muscular Dystrophy (DMD) that have been shown to:[1]
  - Increase dystrophin expression
  - "Skip" mutations in the dystrophin gene to produce shorter but functional dystrophin protein
- Key limitations to the efficacy of PMOs for treating DMD are variability in:[1]
  - Potency, tissue uptake/retention
  - Tissue specificity
- Mitochondria are postulated to modulate PMO efficacy by mediating cell membrane repair:[1]
  - Mitochondria hone to the site of sarcolemmal injury
  - Membrane phospholipids are made by endoplasmic reticulum–mitochondria interactions
  - Cellular PMO uptake has been shown to be adenosine triphosphate (ATP)-dependent
  - Healthy mitochondria provide energy and buffering capacity (calcium) to mitigate cellular injury cascades and reduce inflammation
- Elamipretide (MTP-131; ELAM) is a clinical stage mitochondrial-targeting peptide that has been shown to improve mitochondrial dysfunction across pathologies:[2–5]
  - Stabilizes the structure and function of the mitochondrial electron transport chain via selective interaction with cardiolipin (**Figure 1**)
  - Increases cellular ATP production in disease states
  - Reduces mitochondria-derived oxidants in affected cells

Figure 1. Elamipretide and Cardiolipin Depiction in the IMM



OBJECTIVE

- To determine if a mitochondria-protecting peptide (ELAM) would augment PMO-mediated dystrophin production in an mdx mouse model

METHODS

- Four-week-old C57BL/10ScSn-Dmdmdx/J (BL/10) mdx mice (N=60) were divided into one of four treatment groups (**Figure 2**)

Figure 2. Study Design

Can a mitochondria-protecting peptide improve the efficacy of PMOs to produce dystrophin?

Study Duration: 7 weeks

4-5 weeks of age → 11-12 weeks of age

Healthy mouse (BL/10 mouse): 1 x phosphate-buffered saline + 0.9% saline (n=15)
Saline (mdx mouse): 1 x phosphate-buffered saline + 0.9% saline (n=15)
PMO (mdx mouse): 125 mg/kg PMO[1] + 0.9% saline (n=15)
Elamipretide (mdx mouse): 1x phosphate-buffered saline + 5 mg/kg elamipretide[2] (n=15)
Combination (mdx mouse): 125 mg/kg PMO[1] + 5 mg/kg elamipretide[2] (n=15)

PMO=Phosphorodiamidate Morpholino Oligomer, ip=intraperitoneal

[1]PMO administered once weekly (retroorbital injection)
[2]Elamipretide administered once daily (ip injection)

- Variables measured included muscle force (extensor digitorum longus [EDL] max and specific force)
- At the end of the 7-week study duration:
  - Dystrophin expression in the muscle was quantified via western blot and immunofluorescdnce
  - Percent inflammation (hematoxylin and eosin [H&E] staining;TA)
  - *In-vitro* tension development from EDL muscles
- For the comparison of healthy mouse (BL/10 mouse) versus saline (mdx mouse), an unpaired t-test was performed and mean±standard error of mean (SEM) was calculated
- For the comparison of the saline and treatment groups (PMO, ELAM, and combination therapy [PMO+ELAM]), an ordinary one-way ANOVA followed by a Dunnett's Multiple Comparison test was performed and mean±SEM was calculated

RESULTS

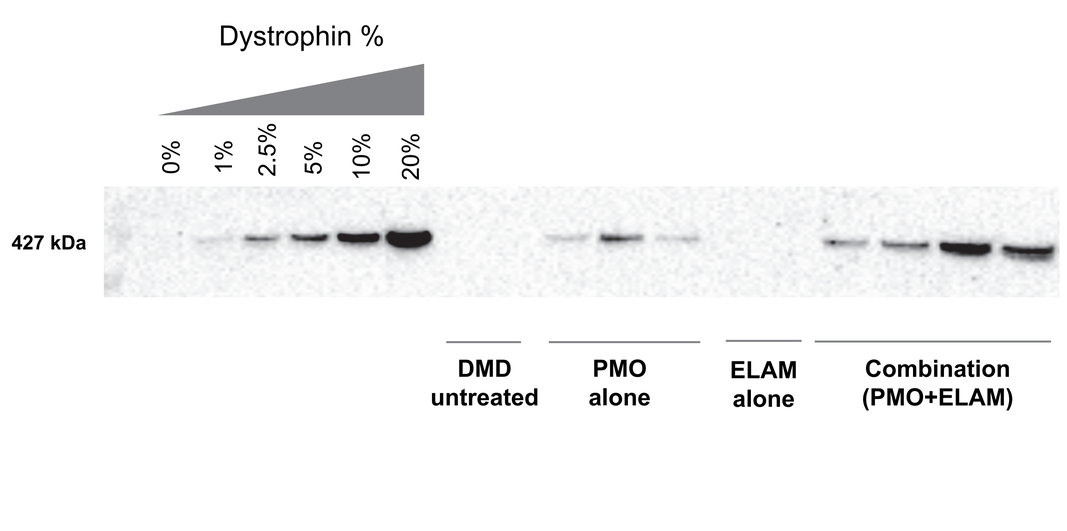
- Dystrophin protein levels were very low in the saline-treated mdx mouse (average 0.7%±0.4% standard deviation [SD] of healthy BL/10 mice) (**Table 1**)
  - ELAM treatment did not alter dystrophin expression (0.7%±0.4%)
  - Treatment with PMO increased dystrophin expression (3.7%±2.1%, *P*<0.05 versus saline-treated mdx mouse)

Table 1. Average Dystrophin Protein Levels for the Four Treatment Groups: Saline, PMO, ELAM and Combination

Treatment Group	Range of Dystrophin Protein Levels (Min, Max)	Average Dystrophin Protein Level	Standard Deviation
Saline	(0.0%, 1.2%)	0.7%	0.4%
PMO	(1.2%, 7.4%)	3.7%	2.1%
ELAM	(0.0%, 1.2%)	0.7%	0.4%
Combination (PMO+ ELAM)	(2.1%, 20.8%)	7.9%	6.0%

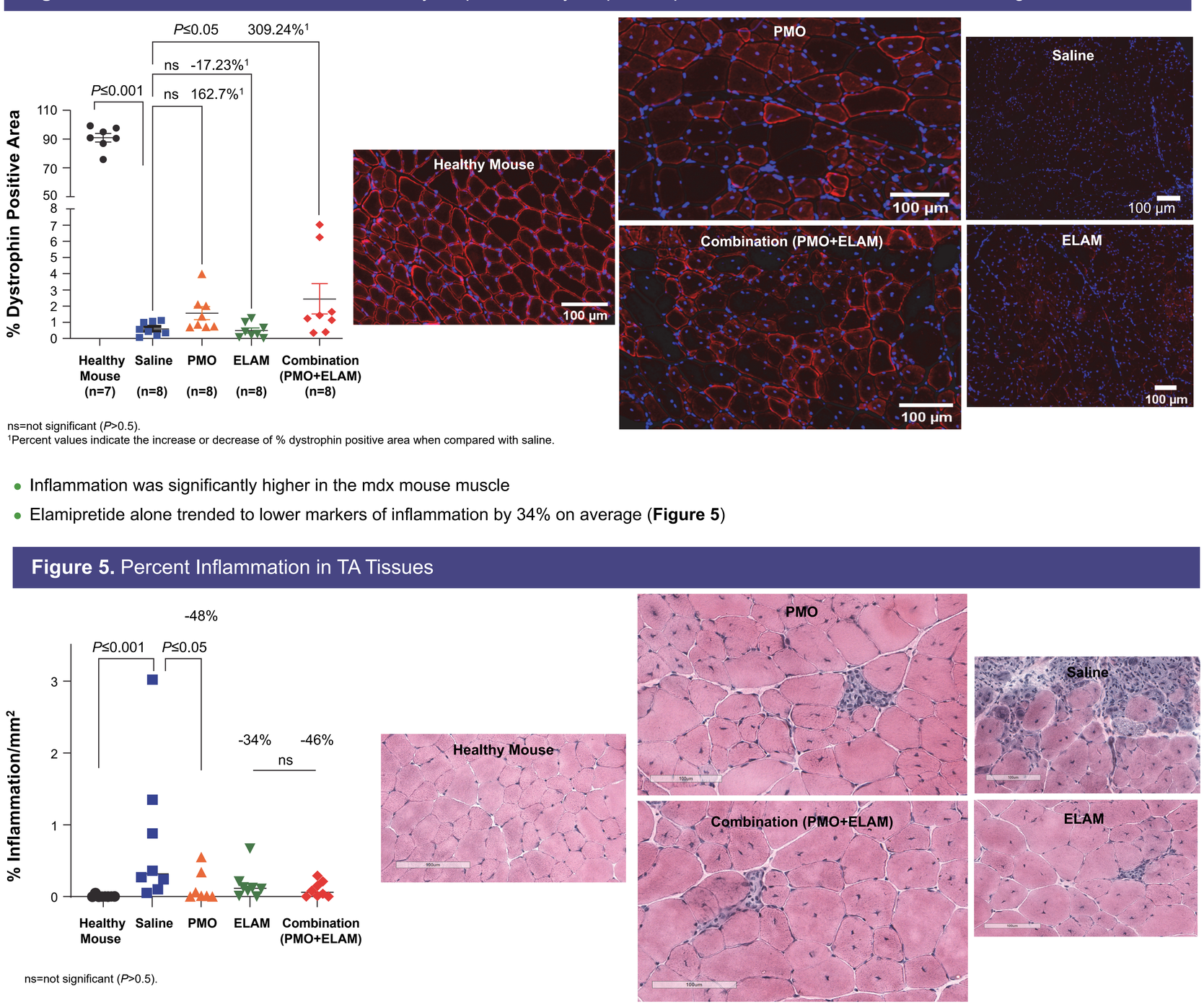
- Combination therapy (PMO+ELAM) more than doubled the mean level of dystrophin protein (7.9% ± 6.0%, *P*<0.05 versus PMO alone) (**Figure 3**)

Figure 3. Mean Levels of Dystrophin Protein for the Four Treatment Groups: Saline, PMO, ELAM and Combination



- Immunofluorescence staining with an anti-dystrophin antibody provided corroborating support for the increase in dystrophin expression (**Figure 4**)

Figure 4. Immunofluorescence TA Percent Dystrophin and Dystrophin Expression: Immunofluorescence Staining



ns=not significant (*P*>0.5).
[1]Percent values indicate the increase or decrease of % dystrophin positive area when compared with saline.

- Inflammation was significantly higher in the mdx mouse muscle
- Elamipretide alone trended to lower markers of inflammation by 34% on average (**Figure 5**)

Figure 5. Percent Inflammation in TA Tissues

ns=not significant (*P*>0.5).

- *In vitro* specific force production was significantly lower in the extensor digitorum longus (EDL) muscle from mdx mice, with no treatment effects observed

CONCLUSIONS

- This preclinical study provides compelling data that elamipretide combined with an exon-skipping PMO augments dystrophin expression in mdx mice
- Combination therapy (PMO+ELAM) more than doubled the mean level of dystrophin protein
- Elamipretide alone trended to lower markers of inflammation, consistent with effects observed in other pre-clinical disease models
- These data highlight the potential synergy between a mitochondria-targeting compound and exon-skipping PMOs for the treatment of patients with DMD

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