Edasalonexent (CAT-1004), an NF-κB inhibitor, enhances myotube formation in vitro, and increases exon-skipped sarcolemmal dystrophin in muscle of mdx mice
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Abstract
NF-κB is activated from infancy in boys with Duchenne muscular dystrophy and in mdx mice, and promotes muscle degeneration while inhibiting muscle regeneration. NF-κB driven micro-RNAs also directly impair dystrophin protein translation, further destabilizing this protein and limiting the full potential of exon-skipping therapy in dystrophic muscles. Edasalonexent is an oral NF-κB inhibitor that is currently in the MoveDMD trial in DMD boys aged 4-7, an age range where high burden of inflammation in the muscle is expected. Previously, edasalonexent has been shown to inhibit muscle inflammation and fibrosis, and to improve muscle function and exercise endurance in mdx mice and GRMD dogs. Here, we show that in primary human skeletal muscle myoblasts derived from multiple donors, treatment with edasalonexent enhances their differentiation into myotubes. In an in vitro pro-inflammatory context, simulated by the addition of IL-1β and TNFα, myobute formation was suppressed, and treatment with edasalonexent partially rescued myotube formation. In young mdx mice where muscle inflammation is prominent, edasalonexent treatment reduced inflammatory infiltration in the skeletal muscle while enhancing sarcolemmal integrity. In combination with an exon-skipping agent, edasalonexent treatment further enhanced the sarcolemmal dystrophin detected in the quadriceps of mdx mice beyond that produced by exon skipping alone. The increase in dystrophin levels with edasalonexent combination treatment extended to the heart, a tissue known to have low efficiency of dystrophin upregulation by these agents when used alone. These results demonstrate that inhibition of NF-κB by edasalonexent in a pro-inflammatory environment enhances myotube formation in vitro. Furthermore, edasalonexent treatment of dystrophic mdx mice enhances muscle fiber integrity, and in combination with an exon-skipping agent, enhances sarcolemmal dystrophin expression.

In vivo Study Design and Experimental Procedures

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment</th>
<th>Experimental Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/10 mdx</td>
<td>Saline</td>
<td>mdx mice were closed with CAT-1004 in air for 1 day and plasma and muscle drug exposure was determined. Exon skipping was performed in vivo with CAT-1004 to the day before 1 week of age and at 10 weeks of age. Microarray 23 skipping (ANI5 -1004) was administered intraperitoneally once per week.</td>
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<tr>
<td>C57BL/10 mdx</td>
<td>CAT-1004 (1%)</td>
<td>At termination, separate pieces of tissues were collected to extract RNA, DNA, and preserved for histology.</td>
</tr>
<tr>
<td>C57BL/10 mdx</td>
<td>CAT-1004 (1%)</td>
<td>Skinning of mouse-exon 23 skipping (ANI5 -1004) was administered intraperitoneally once per week.</td>
</tr>
<tr>
<td>C57BL/10 mdx</td>
<td>ANI5 -1004</td>
<td>Sarcolemmal dystrophy was detected in muscle sections by immunohistochemistry using a specific antibody to the human dystrophin epitope.</td>
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</tbody>
</table>

Pharmacokinetic and Pharmacodynamic Measurements

CAT-1004 Enhances Dystrophin Protein Expression Produced by AVI-4225 in mdx Muscle

CAT-1004 inhibits inflammation to promote in vitro primary human myotube formation.

CAT-1004 and AVI-4225 Reduce Inflammation and Fibrosis in mdx Quadriceps

Increased Dystrophin Localizes to the Sarcolemma in mdx Quadriceps

Summary and Conclusions

- Inhibition of NF-κB by edasalonexent in a pro-inflammatory context enhances myotube formation in vitro, and reduces inflammation and fibrosis in vivo.
- Inhibition of NF-κB by edasalonexent enhances protein translation and sarcolemmal expression of dystrophin produced by exon skipping with AVI-4225 in mdx mice.
- These data suggest potential for combination treatment for DMD.

Presented at: The 22nd International Annual Congress of the World Muscle Society | October 4 – 7, 2017 | St Malo, France
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