Effects of Differential Utrophin Expression on Contractile Function of Dystrophin-Deficient Mouse Muscle

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Background

Various mouse models of Duchenne muscular dystrophy (DMD) exist and include mdx, het and FIONA. These models differ in their expression of utrophin (a dystrophin homolog), which structurally replaces dystrophin. The three models investigated in this study were:
• mdx mouse – the most commonly used model for DMD. It has two functional alleles for utrophin and upregulation of this protein compensates for the lack of dystrophin and attenuates the muscle disease phenotype. Therefore, the mdx mouse is not an optimal model.
• het mouse – has only one functional allele for utrophin. Het mice have been found to have increased fibrosis and inflammation, and altered motor performance relative to mdx mice.
• Fiona mouse – transgenic mdx mice with over-expression of utrophin. The dystrophic phenotype has been shown to be greatly attenuated in ex vivo muscle and cytological testing.

These three models give a spectrum of differential utrophin expression. Using in vivo muscle testing the effect of utrophin on contractile function and susceptibility to contraction-induced injury can be investigated. This will give important insight into these dystrophic mouse models.

Does the expression level of utrophin affect dystrophic muscle’s susceptibility to contraction-induced injury?

Utrophin can rescue muscle function and mitigate torque loss in mdx mice after eccentric contraction-injury as indicated by peak isometric torque and the relationship between torque and stimulation frequency.

Study Design

Wild-type (WT)  MDX  HET  FIONA
Non-dystrophic mouse model
Most common dystrophin-deficient mouse model
MDX with utrophin (+)
MDX with over-expressed utrophin

Age: 12 to 16 weeks

Sample Size:

<table>
<thead>
<tr>
<th>Group</th>
<th>Anterior crural muscles tested</th>
<th>Posterior crural muscles tested</th>
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</thead>
<tbody>
<tr>
<td>WT</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>MDX</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>HET</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>FIONA</td>
<td>8</td>
<td>9</td>
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</tbody>
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Methods

In vivo protocol:

Peak Isometric Torque (Pre-injury)
Torque Frequency Protocol (Pre-injury)
70 Eccentric Contractions (contraction-induced injury)
Peak Isometric Torque (Post-injury)
Torque Frequency Protocol (Post-injury)

Results

Anterior Crural Muscles

Posterior Crural Muscles

Main Findings: Anterior All dystrophic models were equally susceptible to contraction-induced injury. Posterior HET mice were weak as indicated by a low pre-injury isometric torque. MDX and HET mice lost more isometric torque than WT and FIONA mice as a result of contraction-induced injury. FIONA mice were protected from torque loss. Left; there was no significant difference in torque production between groups, until after eccentric protocol where all dystrophic models produced less torque (p≤0.035). Right; HET mice produced less torque than other groups initially (p≤0.004). After injury MDX and HET mice produced less torque than both WT and FIONA mice (p<0.05). Groups with differing letters above the bar are significantly different, p<0.05.

Question/Hypothesis

Utrophin appears to play a role in the preservation of muscle function
• HET mice (one allele for utrophin) had reduced torque compared to WT mice (p<0.05), but following eccentric injury there was no difference between HET and MDX
• FIONA mice (over-expression of utrophin) were more similar to WT mice. This supports the idea that up-regulation of utrophin can rescue muscle function and protect against injury

Conclusions

The expression level of utrophin appears to affect dystrophic muscle susceptibility to contraction injury.

Acknowledgments

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References

1. FIONA mice were protected posteriorly, but not anteriorly. Upper Left; anteriorly WT mice performed better than HET and MDX mice from 7-70, and FIONA mice from 28-70 (p<0.05). FIONA mice differed from MDX at 28 (p<0.017). Upper Right; posteriorly WT mice and FIONA mice produce greater torque than HET and MDX mice from 14-70 (p<0.05), and WT mice were greater than MDX mice at all contractions, and greater than HET mice from 7-70 (p<0.05). FIONA mice were greater than MDX mice at 7-70 (p<0.05). Lower Left; in anterior muscles, MDX and FIONA mice lost a greater fraction of torque compared to WT mice (p<0.003), while HET mice did not (p>0.084). In posterior muscles HET and MDX mice lost a greater fraction of torque than WT and FIONA mice (p<0.001), while FIONA mice did not differ from WT mice (p=0.343).

Future Directions

Quantify differential expression of utrophin in MDX, HET, and FIONA mouse muscles using Western Blotting and correlate with torque loss.