Effect of Dystrophin Deficiency on Selected Intrinsic Laryngeal Muscles of the \textit{mdx} Mouse

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Intrinsic Laryngeal Muscles

- Highly specialized form of skeletal muscle
- Similar to extraocular muscle phenotype
- Areas of divergence from limb skeletal muscle
  - Morphogenesis
  - Myosin heavy chain isoforms present
  - Mitochondrial content
  - Regenerative capacity
  - Innervation patterns (motor, sensory)
  - Sensitivity to disease – DMD (2006 study)

Duchenne Muscular Dystrophy

- Most common form of MD
- 1 in every 3,500 live male births
- Motor delays, weakness by age 2
- Marked reductions in fx and muscle size by ages 6-11
- Death by end of third decade
- Some muscles preferentially spared – extraocular

Duchenne Muscular Dystrophy

- Absence of dystrophin, a protein of the cytoskeleton
- Dystrophin
  - Integral component of DGC
  - Scaffolds sarcolemma during muscle contraction
  - Possible signaling role / Control of Ca levels

Dystrophin-Glycoprotein Complex

Andrade et al., 2000; Kaminska et al., 1992; Merrache & Darris, 2001
Andrade et al., 2003; Goding et al., 1996; McLoon et al., 2004; Noden & Francis-West 2006; Porter et al., 1995; Thomas, Harrison, et al. (in press)
Ervasti et al., 1991; Lapidos, Kakkar, & McNally, 2004; Rando, 2001
Pathophysiology

In the absence of dystrophin...
- Loss of support structure leads to disruption of sarcolemmal (cell membrane) integrity
- Influx of Ca^{2+} triggers destruction of proteins
- Fiber necrosis
- Attempts at fiber regeneration (failed)
- Eventual muscle fibrosis and fatty infiltration

Menache & Darris, 2001; Petrof, 2002

Markers of Pathology

- Evidence of disrupted sarcolemma
- Fiber degeneration and regeneration
  - Inflammation, necrosis
  - Central nuclei
  - Pleomorphic fibers
- Eventual widespread fibrosis, necrosis

Davies & Nowak, 2006; Menache & Darris, 2001

The Animal Model of DMD

- *mdx* mouse – dystrophin deficient
  - Genetic and biological similarities to human DMD
  - Differences in disease severity and functional impact across species

Bulfield et al., 1984; Menache & Darris, 2001

Introduction

2006 Preliminary Study

- Examined effects of dystrophin deficiency on the thyroarytenoid (TA) and posterior cricoarytenoid muscles (PCA) of the larynx
- *mdx* mouse

Thomas, Joseph, et al. (in press)

Normal Appearing Leg Muscle (Gastrocnemius)
Introduction
2006 Preliminary Study

- Results: While deficient in dystrophin, TA and PCA did not show the effects of the disease process.
- Conclusions: TA and PCA possess special features which protect them from the effects of this disease

Thomas, Joseph, et al. (in press)

Laryngeal Muscle Specialization

- Much of research on ILM specialization conducted on 2 primary laryngeal muscles (TA, PCA)
- Are these properties true of all laryngeal muscles?
- Do all laryngeal muscles share a similar level of biological specialization?
- Implications for function and rehabilitation

Laryngeal Muscle Specialization

- Continued study in 2007 - Possible diversity among laryngeal muscles
  - Literature review showed evidence of diversity across laryngeal muscles
  - Two laryngeal muscles with features (e.g., contractile speeds, proprioceptive mechanisms, etc.) more reflective of limb muscle
    - Interarytenoid
    - Cricothyroid

Katto et al., 1987; Okamura et al., 1988; Tellis et al., 2004

Current Study

Effect of Dystrophin Deficiency on Selected Intrinsic Laryngeal Muscles of the mdx Mouse

Purpose Statement

- To further define the biological characteristics of two intrinsic laryngeal muscles (the interarytenoid and cricothyroid) and their similarity/dissimilarity to other laryngeal muscles through the use of the mdx mouse model.
**The Interarytenoid**

- Deviates from other laryngeal muscles in:
  - Motor Innervation
    - Transverse fibers are unpaired; receive bilateral innervation from RLN
    - Supplemental innervation from SLN
  - Sensory: Presence of muscle spindles
  - Contractile profile (ie, myosin isoforms) similar to limb muscle

Katto et al., 1987; Maranillo et al.; 2005; Mu et al., 1994; Tellis et al., 2004

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**The Cricothyroid**

- Deviates from other laryngeal muscles in:
  - Morphogenesis
  - Innervation
  - Contractile profile (ie, myosin isoforms) similar to limb muscle
  - Sensitivity to disease

Benninger et al., 2006; Hyodo et al., 2001; Lucas et al., 1996; Marques et al., 2004; Rhee et al., 2004; Sperber, 1981

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**Primary Investigation**

**Methods**

- Animals
  - *mdx* mouse (*n* = 8); C57BL control (*n* = 8)
  - Dissected out whole larynges and gastrocnemius muscle
  - Serial 10-um thick cryosections
- H & E Staining
- Evans blue dye tests
- Immunocytochemistry (dystrophin, utrophin)

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**Results H & E – Gastrocnemius**

**Results H & E - Laryngeal**

C57BL

**GT**

**SCA**

**PCA**
### Results – Central Nuclei

*Percentage Central Nucleation Across Muscles*

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Control</th>
<th>mdx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastroc*</td>
<td>3.93% (3.08)</td>
<td>65.93% (7.99)</td>
</tr>
<tr>
<td>PCA</td>
<td>7.13% (3.83)</td>
<td>4.88% (2.14)</td>
</tr>
<tr>
<td>SCA</td>
<td>5.33% (3.01)</td>
<td>1.60% (1.62)</td>
</tr>
<tr>
<td>CT</td>
<td>5.83% (4.3)</td>
<td>11.63% (2.76)</td>
</tr>
</tbody>
</table>

*p < .000*  
Inter Rater Reliability: ICC = .98

### Results – Evans Blue

#### Gastroc

#### Strap

### Results - Utrophin

- **Background**
  - Utrophin
    - Dystrophin homolog
    - Present only at NMJ in mature muscle fibers
  - To study
    - Mark NMJ (alpha-bungarotoxin)
    - ICC staining for utrophin
    - Expect utrophin at NMJ in mature fibers
Results – Utrophin Monoclonal

### Gastrocnemius
- Utrophin at NMJ in C57BL
- Utrophin re-localized to sarcolemma in *mdx*

### Laryngeal
- Utrophin not present at NMJ in mature C57BL fibers
- No shifts in utrophin expression in *mdx*

### Results – Utrophin Monoclonal (Laryngeal)

#### Conclusions
- SCA spared from the effects of dystrophin deficiency
- CT shows subtle changes indicative of regeneration (central nucleation)
- Utrophin upregulation not evidenced in spared muscles

#### Discussion
- SCA
  - Has response to disease similar to other laryngeal muscles
  - Has other features similar to limb muscle
  - May be “blended” form of muscle
  - Varying level of specialization within the larynx

#### Hypothesis
Muscles will show effects of disease as in limb muscle
- Neither muscle demonstrated disease effects as seen in limb muscle
  - SCA - full sparing
  - CT – subtle regenerative changes
  - Suggest SCA and CT are not comparable to limb muscle
  - Possess specialized features as other laryngeal muscles
Discussion

CT
- Two-fold increase in central nuclei (not significant)
- Similar increase shown by Marques et al
- No widespread evidence of fiber degeneration
- Comparable to results in other mildly affected muscles
  - Masseter
  - EOM (levator palpebrae superioris, retractor bulbi)
  - Previous study of CT (2007)

Marques et al. (2007)

Discussion

CT (con’t)
- Implications re: nature of the CT
  - Blended, transitional form of muscle
  - Mechanical differences
    - Different sarcolemmal management?
    - Mechanical requirements of CT?

Discussion

Mechanisms of Sparing
- Opens door for use of murine model in laryngeal study
- Organization of DGC in laryngeal muscle
- Implications for voice therapy

Discussion

Limitations
- Muscle sections
- Utrophin antibodies
- Use of the animal model

Concluding Remarks

Laryngeal muscles distinctive
- What does this mean in the clinic?
  - Study of the biology of laryngeal muscles can lay
    a foundation for physiologic therapies
  - Will require:
    Communication of clinical needs to the bench
    Translation of bench findings to clinicians

Thanks to
References


References


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References