Introduction

Duchenne muscular dystrophy (DMD) is a lethal genetic disease characterized by the loss of dystrophin, a cytoskeletal protein, leading to progressive and generalized muscle wasting and weakness. The disease process affects most muscle groups, and respiratory muscle involvement is a leading cause of mortality and morbidity. However, it is unknown whether laryngeal muscles are affected by the pathology.

DMD results in progressive degenerative changes in skeletal muscle fibers, including inflammation, myofiber degeneration (discruption of sarcoplasmic reticulum, focal contractures, sarcorm, myofiber regeneration (pleomorphic fibers, central nucleation), fibrosis, and fat infiltration.

The purpose of this study was to evaluate the consequences of dystrophin deficiency on mouse laryngeal muscle morphology. A cremaster model of DMD, the mouse estrus strain, was used to determine the effect of dystrophin deficiency on the posterior cricoarytenoid (PCA) and thyroarytenoid (TA) muscles of the larynx.

Methods

Animals: Eight-week-old male C57BL/6J (control) and mdx (Jackson Laboratories) were killed by CO2 asphyxia. Whole larynges and orbits as well as the diaphragm and gastrocnemius/soleus muscles were dissected and frozen in 2-methylbutane cooled to its freezing point in liquid nitrogen.

Histology and Immunocytochemistry: Serial 10-µm thick coronal sections of whole larynges and frozen in 2-methylbutane cooled to its freezing point in liquid nitrogen. Hematoxylin and eosin stained sections of leg muscles (top) and laryngeal muscles (bottom). Control gastrocnemius shows normal morphology with polygonal muscle fibers and peripheral nuclei. mdx gastrocnemius evidences fibrosis, pleomorphic muscle fibers and central nuclei. Control and mdx laryngeal fibers demonstrate peripheral nuclei and consistent fiber size and shape.

Results

Figure 1: Model of the dystrophin-glycoprotein complex. In DMD, the absence of dystrophin results in disruption of the entire dystrophin-glycoprotein complex and subsequent disruption of sarcoplasmic integrity.

Figure 2: Hematoxylin and eosin stained sections of leg muscles (top) and laryngeal muscles (bottom). Control muscles (left) show the presence of dystrophin in the intracellular space. Dye did not penetrate the sarcolemma of control leg muscles (middle) and mdx muscles (right). mdx muscles and controls were processed concurrently. For overall morphology, sections were done by personnel blinded to the experimental conditions.

Figure 3: Central nuclei counts for control and mdx muscles of the leg and larynx. Levels of central nuclei (a marker of fiber regeneration) were elevated in the sole gastrocnemius (sole Gs), while they remained unchanged in mdx sole laryngeal muscles (mdx TA - thyroarytenoid muscle, and mdx PCA - posterior cricoarytenoid muscle).

Conclusions

The cause of the muscle sparing is unknown; however, previous literature on spastic muscles has pointed to several possible mechanisms, including a constitutive overexpression of utrophin, reduced fiber size, improved calcium handling within the cell, and improved regenerative capacity of spastic muscles.

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Discussion

The results demonstrated the absence of typical dystrophin deficiency changes in the PCA and TA muscles of mdx mice. These findings suggest the sparing of these muscles by the disease process.

The pathologic cascade initiated by dystrophin deficiency spares few muscle groups, most notably the extracellular muscles (Andrade et al., 2001). Kaminski et al., 1992). This study adds the laryngeal muscles to this short list of spared muscles.

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References
