Dystrophin-Glycoprotein Complex and Reactive Oxygen Species

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Abstract
Duchenne’s Muscular Dystrophy (DMD) is caused by a deficiency in dystrophin protein. DMD is distinguishable through muscle degeneration and weakness. Dystrophin protein is a necessary structural link between the sarcolemma and the cytoskeleton. Studies show Neuronal Nitric Oxide Synthase (nNOS), a critical enzyme in the sarcolemma, that catalyzes nitric oxide (NO), is a molecular component of the Dystrophin-Glycoprotein Complex (DGC). To rescue cells from the effects of a dystrophin deficiency, we will expose the muscle sarcolemma to NO by using gas plasma. Three methods will be tested: 1) treatment with air through a plasma device, as our control, 2) treatment with NO through the plasma device, and 3) treatment with NO via Cold Atmospheric Pressure Source (CAPS) to generate a NO plasma. Q-RT-PCR analysis and confocal microscopy will allow quantification of DGC stability at the plasma membrane. We propose to answer mechanistic questions such as: 1) does an increase in NO levels affect the expression of muscle specific genes in the presence and absence of dystrophin, 2) will increased levels of NO stabilize the DGC within the cell, and 3) are other types of muscle cells (skeletal, cardiac, and smooth) affected by increasing NO in cells. Thus, we predict NO treatment will rescue the deficiency in absence of dystrophin.

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Abstract

Duchenne’s Muscular Dystrophy (DMD) is caused by a deficiency in dystrophin protein. DMD is distinguishable through muscle degeneration and weakness. Dystrophin protein is a necessary structural link between the sarcolemma and the cytoskeleton. Studies show Neuromuscular Nitric Oxide Synthase (nNOS), a critical enzyme in the sarcosome, that catalyzes nitric oxide (NO), is a molecular component of the Dystrophin-Glycoprotein Complex (DGC). We will expose the muscle sarcosomes to NO by using gas plasma. Three methods will be tested: 1) treatment with air through a plasma device, as our control, 2) treatment with NO through the plasma device, and 3) treatment with NO via Cold Atmospheric Pressure Source (CAPS) to generate a NO plasma. rRT-PCR analysis and confocal microscopy will allow quantification of DCG stability. We propose to answer mechanistic questions such as: 1) does increased NO levels affect the expression of muscle specific genes in the presence and absence of dystrophin, 2) will increased levels of NO stabilize the DGC within the cell, and 3) are there other types of muscle cells (skelatal, cardiac, and smooth) affected by increasing NO in cells. Thus, we predict NO treatment will rescue the deficiency in absence of dystrophin.

I. Background

Symptoms and Diagnosis of Duchenne Muscular Dystrophy
Duchenne Muscular Dystrophy (DMD) annually affects 1 in 3,500 males worldwide. DMD is an inherited, fatal disease causing muscle degeneration and weakness of all muscle types. DMD is found in young boys, between the ages of two to three, due to a X-linked recessive pattern. DMD is displayed when a frameshift mutation occurs on the dystrophin gene, Xp21, the largest gene on the human genome, preventing gene expression (refer to Figure 1). A frame shift mutation occurs when a deletion or insertion exists in a DNA sequence, which in turn, causes a shift in sequence interpretation. Abnormal behaviors are first noticed in walking, gait, and speech with the development of paraplegy/hypertrophy, or enlarged legs. Symptoms first occur in proximal muscles and progress to the distal muscles. Death occurs due to weakness of thoracic muscles and a decrease in membrane reactive oxygen species production.

II. Methods

Our first step was to establish a time course of how normal C2C12 cells differentiate into myoblasts (Figure 4). We conducted a time course in order to qualitatively determine the normal differentiation time course. We used a serial dilution in a 24 well plate to photograph each dilution (Figure 5A-C). We obtained nine days of photograph for each well (Figure 6). This was done to establish a normal baseline in order to compare it to our future experiments where gene knockdown will be conducted.

III. Future Experiments

With the use of Cold Atmospheric Plasma (CAP), we hope to induce physical and chemical changes when applying it to the biological surface, tissue, grown from C2C12 cells. CAP is an ionized gas that can be created using several different gasses such as: helium, argon, oxygen, and nitrogen. CAP can have many uses such as: 1) does increased NO levels affect the expression of muscle specific genes in the presence and absence of dystrophin, 2) will increased levels of NO stabilize the DGC within the cell, and 3) are there other types of muscle cells (skelatal, cardiac, and smooth) affected by increasing NO in cells. Thus, we predict NO treatment will rescue the deficiency in absence of dystrophin.

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