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). 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 Plasma Source (CAP) to generate a NO plasma. In RT-PCR analysis and confocal microscopy will allow quantification of DGC 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 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 protein 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 waking, gait, and speech with the development of paraplegia, 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 the respiratory system. Dilated Cardiomyopathy is the most prevalent cause of death.

The Dystrophin-Glycoprotein Complex
The Dystrophin-Glycoprotein Complex (DGC) is a multiprotein complex, functions as a structural link between the sarcolemma-cytoskeleton and the extracellular matrix (refer to Figures 2-3). It aides in blood flow regulation, and in muscle fatigue recovery. A decrease in function of this protein complex causes muscle fibers to become weakened and results in more susceptibility to muscle degeneration and tissue death.

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 determined 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 photographs for each well (Figure 4) This was done to establish a normal baseline in order to compare it to our future experiments where gene knockdown will be conducted.

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 utilizing several different gases such as helium, argon, oxygen, and nitrogen. CAP has also been used in wound healing, eradication of biofilms, oncology, and tissue regeneration. With the use of a CAP device (Figure 7) and the plasma produced with nitric oxide (NO), we intend to further our studies by applying NO plasma to muscle tissue from C2C12 cells. NO gas is our chosen gas due to the circumstantial effects of DMD where NO cannot be created. By exposure of plasma to muscle tissue, we hope to see an increase in muscle stability as NO exposure compensates for the lack of dystrophin and rescues the other components of the Dystrophin-Glycoprotein Complex.

In addition to the use of NO plasma, we will also be using a chemical compound called NONOates. Studies have shown that the use of NONOates for biological purposes have exhibited the release of NO. With this, we hope to see NO levels in our cells rise. We will be using Polymerase Chain Reaction (PCR) to conclude whether or not the levels of NO has increased in the cells. PCR is a molecular biology technique used to create amplified copies of DNA and RNA segments. PCR will determine if our experiments are successful in the increase of NO levels. If we are able to increase the levels of NO, we intend on knocking down the dystrophin gene and using the CAP device with NO gas and NONOates to help improve the functionality muscle tissue from the absence of dystrophin. Gene knockdown is a technique used to reduce the expression of one or more genes. We intend on using short hairpin RNA (shRNA) to permanently knockout the function of the dystrophin gene.

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