CORRELATION AND ROLE OF NITRIC OXIDE (NO) AND BCL-2 IN DUCHENNE MUSCULAR DYSTROPHY (DMD) PATIENTS

Thesis Submitted to Faculty of Science,
Ain Shams University
In Partial Fulfillment of Master degree of Science

BY

Fatma Sayed Mohamed Moawed
B.Sc. Biochemistry

Under Supervision Of

Prof. Dr. Fahmy Tawfik Ali
Professor of Biochemistry
Faculty of Science
Ain Shams University

Prof. Dr. Nadia Y.S. Morcos
Professor of Biochemistry
Faculty of Science
Ain Shams University

Prof. Dr. Soheir Saad Korraa
Professor of Medical Enviromental science
National Center for Radiation
Research and Technology
Atomic Energy Authority

2009

علاقة ودور اكسيد النيتريك وبى سى ال-2 فى مرضى الحثل العضلى (دوشان)

رسالة مقدمــة من
فاطمه سيد محمد معوض

كجزء متمم للحصول على درجة الماجستير فى الكيمياء الحيوية

تحت إشراف

أ.د. فهمى توفيق على استاذ الكيمياء الحيوية قسم الكيمياء الحيوية كلية العلوم – جامعة عين شمس	**أ.د. ناديه يوسف صادق مرقس** استاذ الكيمياء الحيوية قسم الكيمياء الحيوية كلية العلوم – جامعة عين شمس
أ.د.سهير سعد قراعه أستاذ العلوم البيئيه الطبيه المركز القومي لبحوث وتكنولوجيا الإشعاع هيئة الطاقة الذرية	

كلية العلوم – جامعة عين شمس
2008

CONTENTS

ACKNOWLEGEMENT

Thanks to ***Allah*** first and foremost. I feel always indebted to ***Allah*** the kindest and the most beneficent and merciful.

My great thanks and gratitude extend to include ***Professor Dr. Fahmy Tawfik Ali,*** Professor of Biochemistry, Former Head of Biochemistry Department, Faculty of Science, Ain Shams University for his continuous encouragement, valuable advice and criticism. Without his generous and valuable assistance, this work would lose its value. It is an honor working under his supervision.

My deep thanks and gratitude to Professor ***Dr.Nadia Y. S. Morcos,*** Professor of Biochemistry, Biochemistry Department, Faculty of Science, Ain Shams University for her continuous encouragement, generous supervision, valuable advice, and great support and without her great efforts this work wouldn't complete.

It is great honor for me that I take this opportunity to express my sincere appreciation and my deep respect to ***professor Dr.Soheir Saad Korraa***, Head of laboratory of toxigenomics and proteomics, National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, for her precious guidance .I owe her more than I can express for all the time, she spent in revising every detail, in spit of her busy schedule. In fact all credit goes to her in bringing this study to light.

I would like to express my profound appreciation to professor ***Dr.Ekram Abdel-Salam*** ,Professor of Pediatrics

and Genetics ,Faculty of Medicine, Cairo University ,who without her I would have never accomplished this work.

Finally, I would like to thank anyone share with advice, aid in experimental analysis and in finishing this work.

Aim of the work

The goal of this study was to evaluate some biochemical parameters in plasma of Duchenne Muscular Dystrophy patients as a diagnostic marker of the disease, such as creatine phosphokinase, nitric oxide, malondialdhyde, BCL-2, percent of apoptosis in circulating lymphocytes, catalase, total cholesterol and triacylglycerol. In addition, elucidation of the ameliorative effect of laser irradiation on disorder associated with the disease.

Introduction

Duchenne muscular dystrophy (DMD) is a form of muscular dystrophy characterized by decreasing muscle mass and progressive loss of muscle function in male children. DMD occurs in approximately 1 out of 4,000 live born males and can either be inherited or occur spontaneously. A family history of Duchenne muscular dystrophy is a significant risk factor ***(Faulkner et al., 2008).***

DMD is named after the French neurologist **Guillaume Benjamin Amand Duchenne (1806-1875),** who first described the disease in the 1860s ***(Grounds, 2008)***. The disease is usually diagnosed based on gait abnormalities at the age of 4-5 years. In some patients, neurological and cardiac symptoms may also appear by the early teens ***(Thrush et al., 2008)***. Further progression of muscle degeneration eventually leads to death in the early twenties as a result of respiratory or cardiac failure ***(Bertoni, 2008)***.

Duchenne muscular dystrophy is caused by a mutation of the dystrophin gene located at Xp21. Females can be carriers but generally do not experience the symptoms of the condition ***(Jazedje et al., 2009)***.

The dystrophin protein is located beneath the cell membrane (sarcolemma) of the muscle cell (myofiber) and serves to link the contractile machinery (sarcomeres) and associated cytoskeleton to the extracellular matrix where collagens transmit the muscle force ***(Grounds, 2008)***. Absent or defective dystrophin results in myofiber fragility leading to breakdown (necrosis) that is repeated over time until formation of new muscle (regeneration) fails and the damaged skeletal muscle is replaced by fibrous or fatty connective tissue ***(Cyrulnik and Hinton, 2008; Matsumura et al., 2009)***.

Skeletal muscle is capable of complete regeneration due to stem cells that reside in skeletal muscle and non-muscle (circulating) stem cell populations **(*Narciso et al., 2007*)**. However, in severe myopathic diseases such as DMD, this regenerative capacity is exhausted **(*Shi and Garry, 2006*)**. This exhaustion could be explained by two plausible theories: oxidative stress **(Sato *et al.*, 2008)** and replicative aging, that lead to increased rate of myofiber death **(*Abdel et al., 2007*).**

If a physician suspects DMD after examining the boy, he will use the creatine phosphokinase (CPK) test to determine

if the muscles are damaged. This test measures the amount of CPK in the blood. In DMD patients, CPK leaks out of the muscle cell into the bloodstream, so a high level (nearly 50 to 100 times more) confirms that there is muscle damage ***(Burdi et al., 2009)***.

During the 1990s and through the early years of the 21st century, many promising, sophisticated genetic techniques have been designed to ameliorate the devastating impact of muscular dystrophy on the structure and function of skeletal muscles. There is no known cure for Duchenne muscular dystrophy, although recent stem-cell research is showing promising vectors that may replace damaged muscle tissue ***(Faulkner et al., 2008)*.** Meanwhile, the use of low energy laser irradiation, is a promising means to enhance both the survival and functionality of primary myogenic cells, by promoting the cell cycle in lymphocytes and inhibiting cell apoptosis ***(Shefer et al., 2008).***

REVIEW OF LITERATURE

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is an X-linked recessive, progressive muscle-wasting disease affecting all world populations equally, with an incidence of 1 in every 3,500 live male births **(*Emery, 1993; Friedrich et al., 2008*)**. DMD was named in recognition of Dr. G. Duchenne de Boulogne from France, who was the first to attribute the signs and symptoms to a distinct familial disease entity about 150 years ago, originally describing the condition pseudo-hypertrophic muscular paralysis ***(Bogdanovich et al., 2004)***.

Clinical Progression of Duchenne and Becker Muscular Dystrophies:

Initial physical signs such as muscle weakness are not generally observed until the child reaches 2–5 years of age and are typified by a waddling gait, difficulties in running and climbing stairs. In retrospect of the diagnosis, parents often also provide a history of delays in achievement of motor milestone. Subsequent onset of pseudo-hypertrophy of the calf muscles, proximal limb muscle weakness, a positive Gowers' sign, or the child's use of arms to climb from a lying to standing position clinically suggest DMD.

Progressive muscle wasting continues throughout life, initially affecting proximal muscles, with a decrease in lower limb muscle strength resulting in loss of ambulation by about 12 years of age. In the latter stages almost all skeletal muscles are severely involved, which in turn leads to additional problems such as joint contractures and progressive kyphoscoliosis (***Blake et al., 2002; Bertoni, 2008***).

The overall clinical course is unfortunately relentless. While supportive measures such as cord lengthening, spinal stabilization, orthoses, and mechanical long-term ventilation improve the quality of life of patients, death usually occurs in the late teens or early twenties, commonly from cardiac or respiratory causes ***(Bogdanovich et al., 2004, Jazedje et al., 2009).***

Becker muscular dystrophy (BMD) is an allelic disorder that follows a similar, albeit milder DMD course distinguished by a later age at onset and slower rate of progression. A clinical continuum seems to exist between a mildly affected BMD patient and a severely affected DMD patient. Historically, the varied clinical course and milder phenotype led to reluctance in classifying these patients with

DMD, until an elegant hypothesis concerning its allelic nature of the disorder was proposed by Becker and Kiener from Germany. More than 90% of BMD patients are still alive in their 20s, with some remaining mobile until old age. Both BMD and DMD patients can present with varying degrees of cognitive impairment, indicating that brain function is abnormal in these disorders ***(Emery, 1993; Bogdanovich et al., 2004)***.

At the cellular level both disorders involve the loss of skeletal muscle fibers, with marked degeneration. Eventually the regenerative capacity of the muscles is lost, and muscle fibers are gradually replaced by adipose and fibrous connective tissue, giving rise to the clinical appearance of pseudo-hypertrophy followed by atrophy ***(Emery, 1993; Bogdanovich et al., 2004)***.

Dystrophin Protein:

Both DMD and BMD are known to be caused by mutations in the gene encoding dystrophin, resulting in the absence or expression of mutant forms of the protein. Dystrophin is the largest human gene located in chromosome Xp21 and spans up to 2.4 mega base pairs of DNA in length. The full length human dystrophin protein is composed of

3685 amino acid residues with a molecular weight of 427 kD and is a subsarcolemmal component of the cytoskeleton **(*Mital et al., 1998; Cyrulnik and Hinton, 2008; Matsumura et al., 2009).***

Dystrophin shows structural homology with spectrin and α-actinin and contains four distinct domains; amino-terminal domain, helical-rod domain, C-terminus and a cysteine-rich domain (Figure 1). Dystrophin binds F-actin filaments at its amino-terminal domain and parts of the helical-rod domain. The C-terminus and a cysteine-rich domain interact with integral membrane proteins, including sarcoglycan, dystroglycans, syntrophin, and dystrobrevin, which are assembled together to form the dystrophin- associated protein complex (DAPC) (Figure 2). The DAPC provides a crucial structural and signaling link between the extracellular matrix (ECM) and the intracellular actin cystoskeleton across the sarcolemma (***Rando, 2001; Guang-qian et al., 2006***).

Deficiency of dystrophin expression affects formation of the DAPC and causes a disruption of the molecular bridge (***Blake et al., 2002; Grounds, 2008)***.