Detailed research report for International Society for Neurochemistry - the Committee for Aid and Education in Neurochemistry (ISN-CAEN) Travel award

(Category 1: Research A. visit by the applicant to another laboratory)

Name of the fellowship recipient: Rajakumar Dhanarajan.

Country: India.

Address: PhD student,
Neurochemistry Laboratory,
Department of Neurological Sciences,
Christian Medical College,
Vellore, Tamil Nadu,
India.

Host : Prof. Gabriella Piazzei ,
Physiology Laboratory,
Department of Evolutionary Biology,
University of Florence,
Firenze,
Italy.

PhD guide: Dr. Anna Oommen,
Head, Neurochemistry Laboratory,
Christian Medical College, Vellore.

Duration: 26th May 2010 – 20th June 2010.

Title of the project:

Treatment for Muscular Dystrophies – Effect of Curcumin on muscle force generation.

Project supervisor and guide: Prof. Gabriella Piazzei .

Methods learnt:

Preparation of intact and demebranated fibers from skeletal muscle of vertebrate. Optic measurements of fiber and sarcomere parameters; mechanical measurements in intact and demembranated fibers.

Description of the work done:

Muscular Dystrophies are a major group of inherited neuromuscular disorders. Limb girdle muscular dystrophies (LGMD) a subtype of muscular dystrophies are common worldwide, and the most common adult myopathy in India with approximately 3 million people affected. LGMDs are characterized by progressive muscle wasting and weakness. LGMDs have no specific treatment, partly due to unexplored pathomechanisms.

Oxidative and nitrosative stress induced activation of NF-kB, a transcription factor of regulatory importance in muscle cell death, occurs in LGMD muscle. Curcumin (*Curcuma longa*) a spice, is a potent inhibitor of NF-kB and has anti-oxidant, anti-apoptotic and anti-inflammatory properties. In mice curcumin improves dystrophic pathology. These suggest that curcumin could be considered in treatment of LGMD.

Drugs used in treatment of LGMD should prevent muscle cell death without compromising muscle function. Although nitric oxide and intracellular calcium dysregulation underlie the pathology of LGMD, they are key regulators of muscle contraction under physiological conditions. Use of curcumin should not interfere in the normal function of these molecules and affect muscle contraction. Curcumin's effect on muscle force generation at the cellular level is not known but is important to elucidate prior to considering it for therapy.

The aim of this study was to determine the effect of curcumin on normal and oxidative stress induced single myofibre force generation .

We analyzed the effect of different concentrations (0, 0.5, 1.0, and 10.0 µM) of curcumin on single intact muscle fibers from frog (Tibialis anterior) and skinned fibers from rabbit muscle (psoas). Curcumin at lower concentrations (0.5 and 1.0 µM) did not affect isometric force (To) generation of single intact fibers. T_0 at 0µM curcumin was 167 ± 27 kPa (mean ± SD) and became 172 ± 32 kPa at 1 µM of curcumin. In the presence of 3 and 10 µM curcumin, the development of isometric force became slower and attained a lower plateau value, indicating a progressive damage of the fiber, leading to cell death. However in maximally activated skinned fibers ($[Ca^{2+}]= 3.16 * 10^{-5}$ M or pCa= 4.5) isometric force generation was not affected by curcumin at whatever concentration. In skinned fibres the relation between isometric force and Ca^{2+} concentration could be interpolated by the equation

$T_0/T_{0,4.5} = 1/(1+10^{n(pCa-pK)})$

where $T_{0,4.5}$ is the value of T_0 at pCa= 4.5, n is the Hill coefficient, that is related to the steepness of the relation, and pK is the value of pCa at which $T_0 = 0.5\ T_{0,4.5}$. The relation is not significantly affected by the addition of 10 uM curcumin (control: pK = 5.79 ± 0.10 , n = 3.60 ± 0.33; 10 uM curcumin: pk= 5.76 ± 0.07, n= 3.81 ± 0.33).

Fibers subjected to oxidative stress (5 mM H_2O_2) and treated with 1.0 µM curcumin showed fluctuations in twitch and titanic isometric forces.

This short term project at Prof.Gabriella's laboratory in Florence, Italy showed a dose dependent effect of curcumin on myocyte function. At low concentrations curcumin is nontoxic and does not alter force generation of muscle.

The evidence from the skinned fiber experiments indicate: curcumin's toxic effect on myocyte is primarily due to its effect on sarcolemmal or sarcoplasmic reticulum, and that curcumin does not alter the mechanism of force generation. Curcumin does not have any effect on the sarcomere complex, which is the functional unit of muscle force generation. It showed no interaction with contractile proteins of muscle. We did not come to a definitive conclusion on the effect of curcumin on muscle fibers subject to oxidative stress. The results of this short term project support the use curcumin at low concentrations for therapeutic purposes. Further experiments are required to study the effect of curcumin on oxidative stress induced force decline.

Other academic activities during the short term project:

Presented my research work to the laboratory.

Participated in weekly talks and discussions on muscle contraction.

Involved in active discussion and exchange of ideas with other visiting students of the laboratory.

Visited the laboratory of Dr. Thessi to observe other methods to measure force of muscle.

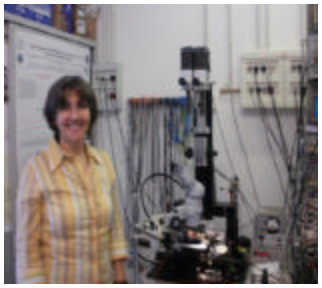
Acknolwedgments

I would like to express my gratitude to Prof. Vincenzo Lombardi, Dr.Marco Linari, Dr.Marco Caremani, Mr.Mario Dalfi and other lab members of the Physiology laboratory, University of Florence for their enthusiastic discussions, kind help and support.

I thank Prof. Thessi, and her students of the Physiology Laboratory, University of Florence, Firenze, for allowing me to observe myofibril preparations in their laboratory and for their scientific discussions.

I am extremely grateful to Prof. Gabriella Piazzei for her tireless help, from receiving me at the Florence railway station, ensuring a comfortable stay, arranging animals for my work, personally teaching me the experiments, and for arranging visits to other laboratories.

I thank The ***ISN-CAEN committee*** for providing financial support for this visit and study.



Prof. Gabriella Piazzei , at Physiology Laboratory, Department of Evolutionary Biology, University of Firenze, Florence, Italy

Rajakumar Dhanarajan, at Physiology Laboratory, Department of Evolutionary Biology, University of Firenze, Florence, Italy.