Neural Cell Adhesion Molecule (CD56) Expression in Skeletal Muscle in Older Adults with Parkinson's Disease

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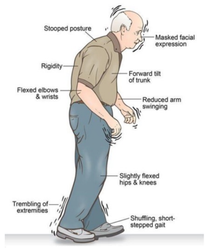
Introduction

Parkinson's disease (PD) is a condition characterized by motor dysfunction as a result of progressive neuro-degeneration. Individuals with PD experience tremors, muscle rigidity, bradykinesia, and abnormal gait among other debilitating symptoms.



T Tremor: shaking, usually starting on one side

R Rigidity: stiffness of the limbs, neck, or trunk

A Akinesia: loss or impairment in power of voluntary movement

P Posture and balance

Neural cell adhesion molecule (NCAM) is a protein expressed by muscle fibers when they are denervated, or no longer attached to a motor unit (Figure 1). NCAM is used by muscle cells to signal to other muscle fibers and motor units that reinnervation is necessary in order to avoid undergoing apoptosis[1,2]. This signal can be observed and quantified by utilizing fluorescent antibodies to tag NCAM positive fibers[3] in both Type I (TI) and Type II (TII) muscle fiber types[4]. Other fluorescent antibodies were also used to identify TI and TII muscle fibers to allow for a percentage of NCAM positive fibers in each to be determined.

The purpose of this study was to evaluate motor unit reorganization in older adults with Parkinson's Disease (PD) and healthy older adults (OLD) using immunohistochemistry to identify myofiber type and NCAM positive fibers in human skeletal muscle tissue.

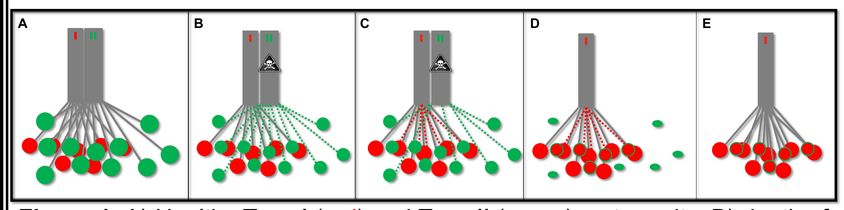


Figure 1. A) Healthy Type I (red) and Type II (green) motor units; B) death of Type II motor neuron and denervation of type II myofibers; C) axonal sprouting from Type I motor neuron to save some of the denervated Type II myofibers; D) reinnervation of some of the denervated Type II myofibers; E) reinnervated (formerly Type II) myofibers are added to Type I motor unit and begin expressing Type I myosin heavy chain.

Methods

Thirty individuals volunteered for a research study at the University of Alabama at Birmingham (UAB). Participants were recruited from the Birmingham, Alabama metropolitan area and gave written, informed consent allowing their samples and data to be used for future research as approved by the UAB Institutional Review Board. From that larger study, seven individuals with PD (65 ± 9 yrs; M=3, F=4) and two healthy older adults (65 ± 14 yrs; M=2) muscle biospecimens were available for the present study. All myofiber assessments were accomplished by utilizing available vastus lateralis muscle biopsy specimens collected from persons with PD and older adults In the same cohort. These muscle biopsy specimens were collected by percutaneous needle biopsy of The right vastus lateralis muscle under local anesthesia (1% lidocaine) using a 5-mm Bergstrom-type biopsy needle with suction (Figure 1).

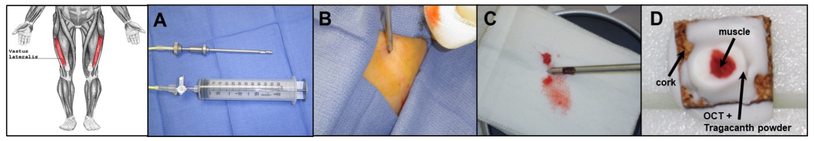


Figure 1. A) 5-mm Bergstrom-type biopsy needle with tubing and syringe for suction; B) sterile field with biopsy needle inserted into vastus lateralis; C) muscle tissue sample; C) cross-sectionally mounted tissue sample embedded in OCT + tragacanth gum for sectioning.

Figure 2. Cryostat machine that is used to slice skeletal muscle into cross-section and mount it onto glass slides.

Figure 3. KEYENCE BZ-X800 Microscope used to capture and stitch images of muscle specimen cross-sections.

Biopsies were performed in the Clinical Research Unit of the UAB Center for Clinical Translational Science and stored at -80°C. Tissue samples were shipped on dry ice to Creighton University and stored at -80°C. All immunohistochemistry was performed on fresh-frozen 6µm thick serial cryo-sections (Figure 2).

Upon completion of each stain, tissue samples were dried, mounted, cover slipped, and stored at -20°C until analysis. 10x microscopic images were captured in a grid format and stitched together using BZ-X800 microscope (KEYENCE, Inc.) software to render one seamless image of the entire cross-section of the specimen (Figure 3). ImageJ (NIH) software was used to manually analyze and count all fibers for myofiber type distribution and NCAM expression.

Independent t-tests (2-tailed, unequal variances) were used to determine differences in myofiber type distribution and NCAM expression between PD and OLD. Due o limited and unequal sample sizes, Hedges g was calculated to determine the magnitude of the effect of the differences in PD and OLD (Small ≤ 0.19; Medium = 0.20 - 0.79; Large = > 0.80).

Results

PD had higher distribution of Type II myofibers (54.7 ± 11.4%; OLD = 47.4 ± 13.6%; P > 0.05; *g* = 1.4) and lower distribution of Type I myofibers (45.3 ± 11.4%; OLD = 52.6 ± 13.6%). PD had fewer Type II/NCAM+ myofibers (8.4 ± 5.5%; OLD = 13.7 ± 10.7% P > 0.05; *g* =1.8), fewer Type I/NCAM+ myofibers (5.4 ± 4.0%; OLD = 6.2 ±3.1%; P > 0.05; *g* = 0.44), and fewer total NCAM+ myofibers (6.8% ± 4.2%; OLD = 9.2 ± 5.7%; P > 0.05; *g* = 1.2).

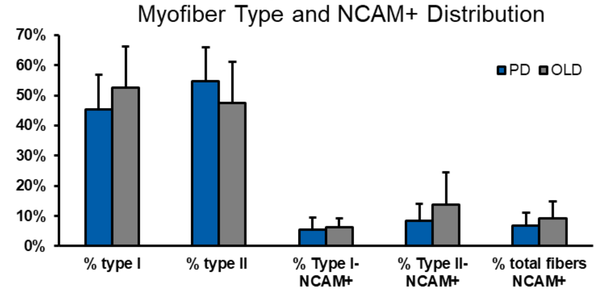


Figure 4. Myofiber type distribution Type I (slow-twitch), Type II (fast-twitch) and NCAM+ distribution in Parkinson's disease (PD) and healthy, older adults (OLD).

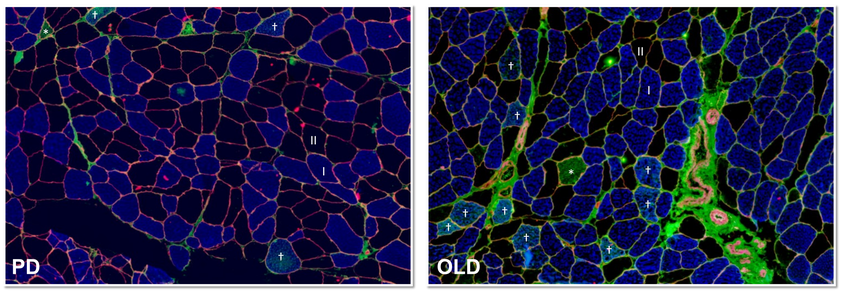


Figure 5. Representative immunohistological images are shown of both PD and OLD respectively: type I (blue); type II (black/negative); NCAM (green); laminin (red). *Type II-NCAM+; †Type I-NCAM+

Conclusions

Our data indicate: 1) there appears to be a difference in the remodeling processes of healthy old compared to PD motor units, and 2) the present study should be repeated with a larger sample size to confirm our findings.

Practical Applications

As we identify the order, rate, and incidence of motor unit remodeling in PD, we will be better equipped to optimize therapeutic exercise interventions to improve physical function, independence, and quality of life in persons with PD.

References

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