Mitochondrial Function of Subsynovial Connective Tissue in the Carpal Tunnel in Carpal Tunnel Syndrome Patients

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INTRODUCTION: In idiopathic carpal tunnel syndrome (CTS), fibrosis and thickening of the subsynovial connective tissue (SSCT) are thought to increase pressure within the carpal tunnel, resulting in median nerve entrapment [1]. In recent years, it has also been reported that mitochondrial dysfunction in tissues leads to the accumulation of senescent cells and the development of various diseases through reduced adenosine triphosphate (ATP) production and excessive production of reactive oxygen species (ROS) [2], but there have been no reports linking this to CTS. The aim of this study was to evaluate mitochondrial function in SSCT tissue from CTS patients.

METHODS: Ten SSCTs were obtained at the time of carpal tunnel release surgery in patients with CTS (CTS group) and five SSCTs obtained at the time of tendon transfer or tendon rupture surgery in non-CTS patients (control group) were included in the study. Endpoints were (i) tissue superoxide dismutase (SOD) activity, (ii) gene expression levels of the mitochondrial biosynthetic factors; peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) and mitochondrial transcription factor A (TFAM), and the ROS degrading enzymes; SOD-1 and SOD-2 by real-time PCR, (iii) mean fluorescence intensity (MFI) of mitochondrial mass, mitochondrial membrane potential and mitochondrial ROS production assessed by fluorescence staining. Images were captured using a Keyence BZ microscope and MFI was calculated using BZ-X800 analyzer software (Keyence). Differences between groups were analyzed by t-test, and $p < 0.05$ was considered a statistically significant difference.

RESULTS: (i) The SOD activity of the control group was 157 (±25.9) units/ml and that of the CTS group was 93.8 (±13.5) units/ml (Fig 1). (ii) Gene expression levels of PGC-1α, TFAM were significantly higher and SOD-2 was significantly lower in the CTS group than in the control group. SOD-1 was higher in the CTS group, but not significant (Fig 2). (iii) MFI of mitochondrial mass was higher and MFI of mitochondrial membrane potential was lower in the CTS group than in the control group. MFI of mitochondrial ROS production was significantly higher in the CTS group than in the control group (Fig 3).

DISCUSSION: Tissue SOD activity, which reflects mitochondrial function, was reduced in the CTS group. In particular, the expression of SOD-2, which is considered mitochondrial specific, was significantly reduced. The mitochondrial biosynthesis factors PGC-1α and TFAM are known to increase compensatorily in patients with impaired mitochondrial function [3], and the present study showed similar results. At the cellular level, the amount of intracellular mitochondria in aging fibroblasts was increased to compensate for mitochondrial dysfunction, while membrane potential decreased and mitochondria-derived ROS production increased [2]. In the present study, mitochondrial ROS production was upregulated and SOD-2 expression was downregulated, suggesting mitochondrial dysfunction in SSCT of CTS patients.

SIGNIFICANCE: The results suggest mitochondrial dysfunction of the SSCT in the carpal tunnel of CTS patients compared to the control group.

REFERENCES:
[1] Festen-Schrier, V.J.M.M.; Amadio, P.C. The Biomechanics of Subsynovial Connective Tissue in Health and Its Role in Carpal Tunnel Syndrome. *J. Electromyogr. Kinesiol.* **2018**, *38*, 232–239.
[2] Miwa, S.; Kashyap, S.; Chini, E.; von Zglinicki, T. Mitochondrial Dysfunction in Cell Senescence and Aging. *J. Clin. Invest.* **2022**.
[3] Brand, M.D.; Nicholls, D.G. Assessing Mitochondrial Dysfunction in Cells. *Biochem. J* **2011**, *435*, 297–312.

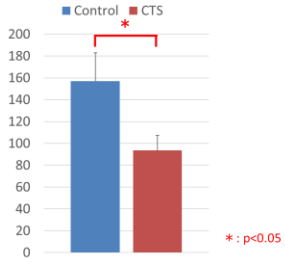

Figure 1. The SOD activity.

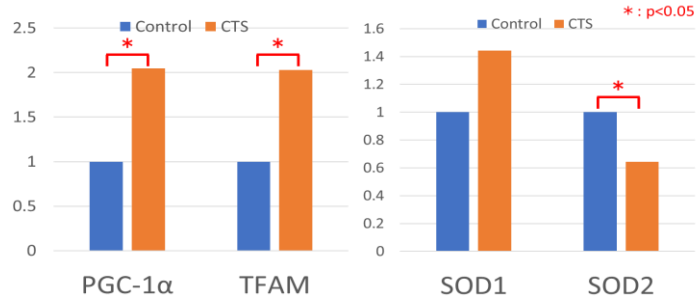

Figure 2. Gene expression levels of PGC-1α, TFAM, SOD-1 and SOD-2.

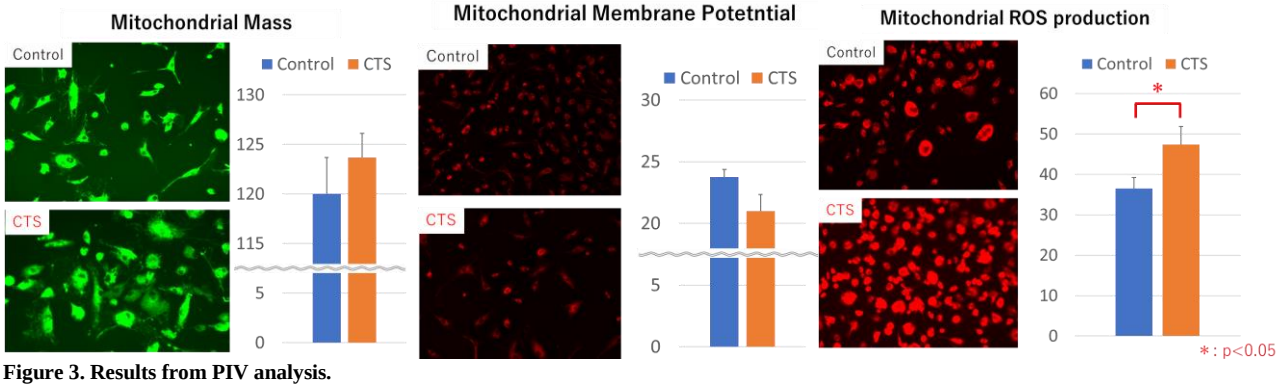

Figure 3. Results from PIV analysis.