Calcitonin Gene-Related Peptide in Charcot Foot Neuroarthropathy
Yi Guo; Lew C. Schon, MD; Sharada Paudel, PhD; Tyler Feltham; Lumanti Manandhar; Zijun Zhang

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Introduction/Purpose: Charcot neuroarthropathy (CNA) is a destructive joint condition secondary to neuropathic deficiency. For more than a century, the pathogenesis of CNA has been interpreted as unprotected injury and/or uncontrollable inflammation in a neuropathic joint, with little understanding its cellular and molecular pathology. Histologically, a hallmark of CNA is bone and cartilage fragments engulfed by hyperplastic synovium. Synovium is richly innervated. Neuropetptides, such as calcitonin gene-related peptide (CGRP), are secreted by neuronal and non-neuronal cells of the nervous, endocrine, and immune systems, and regulate inflammation. This study investigated the expression of CGRP in the synovial samples collected from CNA and non-CNA joint conditions, and the effects of CGRP on the proliferation and collagenolysis of fibroblast-like synoviocytes (FLS) isolated from CNA synovium in vitro.

Methods: For this study, six CNA and 14 non-CNA synovial samples were collected during foot and ankle surgery. The donors included 10 male and 10 female, age from 14 to 79 years (mean 48). The non-CNA conditions consisted of osteoarthritis, ankle instability, Os Trigonum and osteochondritis dissecans. Western blot and immunohistochemistry of CGRP were performed to detect and localize CGRP expression in the CNA and non-CNA synovium. FLS was isolated from CNA synovium and cultured with, or without, human CGRP (10nM) in the culture media, for comparison of cell proliferation. Additionally, FLS were seeded on the collagen-coated 24-well plate and simulated with CGRP (10nM) and recombinant human tumor necrosis factor-alpha (TNF-α). After 7 days, the residual collagen on the bottom of the culture plate were stained and imaged for area measurements. Data were analyzed with t test or one-way Analysis of Variance, followed with post hoc Tukey’s test.

Results: By western blot, there was significant CGRP expression in the CNA synovium (5/6; Fig1). Except of an intense CGRP band in one of the osteoarthritis samples, only recognizable CGRP bands were shown in the samples of other non-CNA conditions. The average density of CGRP (in greyscale) in the CNA group was about 2-fold of that in the non-CNA group (10126 ± 5346 vs. 5377±3734; p < 0.05). Immunohistochemistry demonstrated intense CGRP staining in the intimal layer of the CNA synovium (arrows) but not in the non-CNA synovium (Fig1). Treated with CGRP, the number of FLS in tissue culture increased. Cell number doubling time was 1.0 day (+-0.4) for the CGRP treated FLS and 2.2 days (+-0.5) for the control (p < 0.05). When theculture media were supplemented with CGRP and TNF-α, FLS eroded a larger area of collagen coating comparing with TNF-α alone in the media (p < 0.05).

Conclusion: A knowledge gap in understanding the molecular and cellular pathology of CNA hampers the development of disease-modifying therapies. This study showed an increased CGRP, with a concentration in the intimal layer, in the CNA synovium as compared with the synovium in the non-CNA foot conditions. The increased CGRP expression in CNA synovium may be part of the unbalanced neuropeptide signaling in the neuropathic pathology. Functionally, CGRP stimulated FLS proliferation and degrading collagen in vitro. These effects suggest that CGRP may play a role in bone and joint destruction in the Charcot foot.
CNA = Charcot neuroarthropathy
AI = ankle instability
OST = Os Trigonum
OA = osteoarthritis
OCD = osteochondritis dissecans

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