Chronic Inflammation in Painful Diabetic Neuropathy: CCR2 and other Chronic Pain Candidate Genes

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Painful diabetic neuropathy (PDN) is a diabetes complication involving pain in extremities from peripheral nerve damage. A major CCR2 chemokine receptor isoform, CCR2A or CCR2B, may be an underlying contributor to PDN. Previous experiments with normal human blood samples suggested a consistently higher CCR2B level, indicating potential functional consequences. The purpose of this study was to assess CCR2 expression among mice with neuropathic pain, with the future goal of assessing specific CCR2 isoform expression. Quantitative reverse-transcriptase PCR (RT-qPCR) was used, alongside primers designed for multiple mouse genes involved in neurological disease: CCHCR1, SNAP29, GOLGA2, APP, as well as CCR2. Mouse brain and spinal cord tissue samples were collected from neuropathic pain mouse models. A protocol was developed for extracting RNA from frozen mouse brain tissues; isolated RNA was quantified and tested for purity using fluorometric assays before undergoing TaqMan-based fluorogenic RT-qPCR with designed primers and a GAPDH internal control. Current testing has been done only on control mouse samples to streamline the RNA extraction and one-step RT-qPCR protocol for higher efficiency and to resolve inconsistencies in qPCR results. It was found that running qPCR at an annealing temperature of 58°C has produced consistently higher amplification from RNA extracted from brain tissue samples approximately 50 mg in size. In the immediate future, an optimized protocol for RNA extraction and RT-qPCR will allow for reliable results from experiments conducted with tissue from PDN mice, enabling direct assessment and comparison of expression levels of CCR2 and other neuropathic pain-related genes. Supported by NJ ACTS NIH R25TR004777 CREST Program, NIH R43NS120617, and New Jersey Commission on Science, Innovation and Technology.