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Human dorsal root ganglion pulsed radiofrequency treatment modulates cerebrospinal fluid lymphocytes and neuroinflammatory markers in chronic radicular pain

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ABSTRACT

Radicular pain is a common cause of disability. Traditionally treatment has been either epidural steroid injection providing short-term relief or surgery with associated complications. Pulsed radiofrequency (PRF) applied to the dorsal root ganglion (DRG) is a minimally invasive day-care treatment, which is gaining significant clinical acceptance in a selective group of patients with pure radicular pain. Greater insights into the immunomodulatory effects of this procedure may help to further optimise its application and find alternative treatment options. We have examined it's effect on lymphocyte frequencies and secreted inflammatory markers in the cerebrospinal fluid (CSF) and correlated this with clinical outcome to identify clinical markers of chronic radicular pain. Ten patients were recruited for the study. CSF lymphocyte frequencies and levels of cytokines, chemokines and growth factors were quantified using flow cytometry and enzyme-linked immunosorbent assay (ELISA), respectively. Clinical assessment utilised Brief Pain Inventory scores. Nine out of ten patients (90%) demonstrated significant reduction in pain severity ($p = 0.0007$) and pain interference scores ($p = 0.0015$) three months post-treatment. Our data revealed significant reductions in $CD56^+$, $CD3^-$, NK cell frequencies ($p = 0.03$) and IFN-γ levels ($p = 0.03$) in treatment responders, while $CD8^+$ T cell frequencies ($p = 0.02$) and IL-6 levels were increased ($p = 0.05$). IL-17 inversely correlated with post-treatment pain severity score ($p = 0.01$) and pre and post-treatment pain interference scores ($p = 0.03$, $p = 0.01$). These results support the concept that chronic radicular pain is a centrally mediated neuroimmune phenomenon and the mechanism of action of DRG PRF treatment is immunomodulatory.

1. Introduction

Lumbosacral radicular pain is one of the most commonly occurring forms of chronic neuropathic pain and is associated with significant disability. Conventional injection therapy relies on application of local anaesthetic and steroid to the nerve root or dorsal root ganglion (DRG). However, analgesic response to this technique is frequently short-lived and its role is often more diagnostic than therapeutic. Pulsed radiofrequency (PRF) using high-frequency current intermittently applied adjacent to the DRG has demonstrated success in treating chronic radicular pain (Van Boxem et al., 2014; Van Boxem et al., 2015). This target selective treatment, with very low complication rates, can provide long lasting analgesia extending beyond 6 months (Van Boxem et al., 2014). However, the mechanism of action of this therapy is unclear.

Growing evidence supports the concept that chronic neuropathic pain is a centrally mediated neuroimmune phenomenon (Costigan et al.,2009). Cytokines, chemokines and growth factors mediate neuroinflammation and pain propagation by acting directly and indirectly at the central nociceptors (Ramesh et al.,2013). Some of the important inflammatory mediators of central neuroinflammation, which have been identified to date include IL-2, IL-6, IL-10, IL-17, IFN-γ, brain derived neurotrophic factor (BDNF), biotinylated nerve growth factor (bNGF), vascular endothelial growth factor (VEGF), IP-10 and MCP-1(Ramesh et al., 2013; Kosek et al., 2015). Roles of cerebrospinal fluid (CSF) cytotoxic T lymphocytes, Natural Killer (NK) cells and glial cells which include microglia, astrocytes and satellite cells have been reported in chronic pain (Ramesh et al., 2013). In a healthy individual the absolute count ratio of $CD4^+$ to $CD8^+$ T cells (CD4:CD8) is higher

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in CSF than blood, with central memory $CD4^+$ T cells being more prevalent, while NK cells constitute less than 5% of lymphocytes in the CSF (Ho et al., 2013; de Graaf et al., 2011a; de Graaf et al., 2011b). Interestingly, NK cells mediate neurotoxicity and activation of NK cells has been reported following acute severe pain induced by electric stimulation on human abdominal skin (Poli et al., 2013; Greisen et al., 1999). Slight elevation of CSF NK cells and higher CD4/CD8 ratio have been found in inflammatory neurological diseases such as multiple sclerosis, bacterial and viral meningitis and neuroborreliosis (Kowarik et al., 2014). NK cells are potent producers of IFN-γ, an important mediator of neuroinflammation and may therefore be a key source of this cytokine in neurological inflammatory disease. The DRG has abundant sensory neurons and may be the core site of glial cell activation, which occurs several days after the initial insult and is long lasting (Sapunar et al., 2012). Activation of cytotoxic and NK cells around the vicinity of DRG may be responsible for establishing neuroinflammation and chronic radicular pain.

The mechanism of action of PRF is currently undergoing extensive research. Inhibition of immune function represents a major avenue for therapeutic intervention for neuropathic pain and PRF appears to have genuine immunomodulatory and biological effects on cell morphology, synaptic transmission and pain signalling (Van Boxem et al., 2015;Costigan et al., 2009). A better understanding of neuronal immune interactions and PRF treatment in patients with chronic pain conditions will help to optimise this therapy. In this study, we have examined the effect of PRF applied to the DRG in patients with lumbosacral radicular pain on CSF lymphocyte frequencies and secreted inflammatory factors and correlated this with clinical outcome to identify clinical markers of chronic radicular pain and response to PRF.

2. Methods

2.1. Subject selection

This study was approved by St James's Hospital/ Adelaide & Meath Hospital Research Ethics Committee, Dublin on 3rd of March 2015, Reference no: 2015-03, List 11(3). All investigations and procedures were conducted according to Declaration of Helsinki principles. Written consent to participate in the study was taken and the family doctor was informed. Also, written informed consent was obtained for CSF aspiration and PRF procedure. Participants were anonymised and all immunological assays were carried out in a blinded fashion.

Ten consecutive patients were enrolled for the study which was conducted at St. James's Hospital, Dublin, Ireland. All the patients included in this study were referred to the pain clinic for evaluation and management of continuous chronic lumbosacral radicular pain for duration of six months or more. Diagnosis of radicular pain was made based on a clear history and of a single dermatome being involved and clinical examination revealing hypoesthesia compared to the opposite side. Magnetic Resonance Imaging (MRI) scans were interpreted in both sagittal and axial views to confirm the exact level and extent of nerve root impingement with disc bulging but not osteophytes and correlated with clinical findings. Nerve Conduction Study excluded peripheral neuropathy with normal sensory potentials despite sensory symptoms and signs. Exclusion criteria included patient refusal, back injury or back surgery in the past, infection, pregnancy, breast feeding, psychiatric and cognitive problems and patients with other inflammatory neurological conditions or autoimmune/auto-inflammatory diseases (e.g. multiple sclerosis). Patients medicated with anticoagulants, COX inhibitors, Corticosteroid/Disease-modifying anti-rheumatic drugs (DMRDs), anti-psoriatic/methotrexate/anti-monoclonal antibody therapy were also excluded. Those who agreed to participate in the study received an information leaflet with a two-week grace period prior to enrolment to ensure that patients had time to reflect on their decision to enrol.

2.2. Pulsed radiofrequency treatment

Ten patients scheduled to have DRG PRF treatment were enrolled in the study. PRF treatment was delivered in operating suite in the Day Surgery Unit (DSU) in St. James's Hospital. All patients filled in a Brief Pain Inventory Score (BPI) Short form prior to treatment in DSU and a sample of CSF was taken prior to PRF treatment.

Patients were positioned prone and they remained unsedated during the intervention. Standard monitoring included heart rate, oxygen saturation and blood pressure. Under aseptic conditions and following skin preparation, Lignocaine 1% was used to provide local anaesthesia. A 22-gauge RF needle with 5 mm exposed tip was then inserted and advanced under fluoroscopic guidance. A Neurotherm model NT2000 PRF generator was be used for the procedure. The anatomical position of the RF needle was considered optimised when the tip of the pulsed RF needle was placed in the dorso-caudal quadrant of the foramen and lying just lateral to the DRG after contrast injection on AP fluoroscopic view, with a positive sensory stimulation (frequency 50 Hz; pulsed width 1 ms) elicited at a voltage $\leq$ 0.3 V. Motor testing was performed (frequency 2 Hz; pulsed width 1 ms: voltage 1.2 V) to ensure the RF needle was not in contact with the nerve root. Two cycles of PRF was performed after application of 1 ml of 1% lignocaine with a pulse width of 20 ms, 42 °C, at 2 Hz frequency for 2 min. On completion of the procedure patients were observed in the Day Surgical Unit (DSU) for two hours. Patients were discharged home once they had achieved standard discharge criteria for the DSU.

2.3. CSF sample collection and preparation

Two millilitres of cerebrospinal fluid (CSF) was collected from each patient before and 3 months after pulsed radio frequency (PRF) of the DRG. 1 ml was immediately stored at -20 °C in 2 ml cryovials while 1 ml was stored and stabilised in Transfix/EDTA CSF sample storage tubes (Cytomark, UK) at 4 °C for subsequent flow cytometry analysis.

2.4. Antibodies and flow cytometry

Percentages of T cells and NK cells and phenotypes were assessed in the CSF before and three months after PRF to the DRG. One patient did not consent for repeat CSF sampling after treatment. Comparison of cell populations in CSF was made pre and post treatment in the remaining eight patients who responded to the treatment and also in the treatment non-responder. CSF was returned to room temperature before 2 ml of sterile filtered phosphate buffered saline (PBS) was added to the tube and the sample was mixed gently. Following centrifugation at 540×*g* for 5 min, the supernatant was discarded and the pellet was resuspended in 300 μl of PBS. Each CSF sample was then stained with fluorochrome-conjugated monoclonal antibodies (mAbs) specific for human surface markers (CD45-APC, CD3-APC-Vio770, CD4-VioBlue, CD8-PerCP, CD56-PEVio770, CD69-PE, CD45RA-VioGreen, CD27-VioBright FITC) obtained from MiltenyiBiotec (Germany). Due to the precious nature of the CSF samples and the lower number of cells within each CSF sample, voltage and compensation settings were optimised for this lymphocyte antibody panel using peripheral blood lymphocytes. Patient-matched unstained CSF samples were used as a control for each experiment. Cells were acquired