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Multimodal assessment of nervous and immune system responses following sciatic nerve injury

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ABSTRACT

Subsequent to peripheral nerve compression and irritation, pathophysiological processes take place within nervous and immune systems. Here, we utilized a multimodal approach to comprehend peripheral and central soft tissue changes as well as alterations occurring in systemic analytes following unilateral chronic constriction injury (CCI) of the sciatic nerve in rodents. Using magnetic resonance imaging and [18F]-2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography, we demonstrated robust structural abnormalities and enhanced FDG uptake within the injured nerve and surrounding muscle, respectively. To assess whether central morphological changes were induced by nerve injury, diffusion tenor imaging was performed. A decrease in fractional anisotropy in primary motor cortex contralateral to the injury site was observed. Evaluation of a panel of circulating cytokines, chemokines, and growth factors showed decreased levels of interleukin-1β and Fractalkine in CCI animals. Area under the receiver operating curve (ROC) calculations of analyte levels, imaging, and behavioral end points ranged from 0.786 to 1, where behavioral and peripheral imaging end points (eg, FDG uptake in muscle) were observed to have the highest discriminatory capabilities (maximum area under ROC = 1) between nerve injury and sham conditions. Lastly, performance of correlation analysis involving all analyte, behavioral, and imaging data provided an understanding of the overall association amongst these end points, and importantly, a distinction in correlation patterns was observed between CCI and sham conditions. These findings demonstrate the multidimensional pathophysiology of sciatic nerve injury and how a combined analyte, behavioral, and imaging assessment can be implemented to probe this complexity.

1. Introduction

In the presence of sciatic nerve compression or irritation, symptoms of pain, numbness, weakness, and loss of normal motor control can be experienced. Additionally, aberrant inflammatory and immune responses at systemic or local levels can coexist with nerve trauma, and also contribute to the experienced symptoms. The existence of pain and proper sensorimotor function along with the above-mentioned pathologies can be indicative of sciatica, a nervous system condition influenced by occupational, routine physical activity and genetic factors [29]. In some patients, sciatica and the associated symptoms are resolved over time following a conservative treatment regimen [35]. However, in cases where the disease state and symptoms, particularly pain, become chronic, resolving the condition over a long-term period can at times be difficult, despite implementation of invasive procedures such as epidural steroid injections or surgery [19].

Today, magnetic resonance imaging (MRI) of the periphery is commonly used to assist in diagnosing chronic sciatica [37], as well as in preclinical settings where models of sciatica are evaluated [3,4,15,38]. From clinical MRI investigation specifically, previous work suggests discordance between MRI observations and symptoms experienced (or lack thereof) by patients. Jensen et al. and el Barzouhi et al. have reported that anatomical abnormalities (eg, scar tissue, protrusions, and extrusions) observed in repeat lumbar spine MRI were not predictive of chronic symptomatic vs asymptomatic sciatica in patients, nor was it indicative of

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treatment outcome [14,21]. Thus, a discrepancy exists between MRI-based detection of peripheral pathology, such as qualitative proof of neuropathy and symptoms of sciatica (eg, chronic pain).

The objective of the current study was to implement a multimodal approach in order to better comprehend the disease processes and symptoms of sciatic nerve injury. In the rodent chronic constriction nerve injury (CCI) model, pain was assessed behaviorally, after which, blood samples were collected in order to quantify a panel of 26 analytes associated with pain and inflammation. To measure structural and regional metabolic demand along the distribution of the sciatic nerve, T2-weighted MRI and [18F]-2-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET)/computed tomography (CT), respectively, were used. Lastly, diffusion tensor imaging (DTI) of the brain was performed to determine if structural plasticity was induced by nerve injury.

Nervous and immune system functionality deviate from their respective baseline states in response to peripheral nerve injury [34]. Thus, we tested the hypothesis that structural and metabolic properties quantified along the distribution of an injured sciatic nerve as well as within the central nervous system parallel the activity of immune system regulators relevant to pain and inflammation. Structural properties of the injured nerve, structural and metabolic properties within leg muscle, systemic levels of interleukin (IL)-1β, mechanical allodynia, and fractional anisotropy (FA) within the primary motor cortex were observed to best differentiate CCI rats from sham controls. Correlation analysis performed amongst analyte, behavioral, and imaging data demonstrated associations and clustering amongst these distinct end points. By calculating the area under the receiver operating curve (ROC) and performing least absolute shrinkage and selection operator regression analysis, between-group classifier assessment and predictive modeling of end points were respectively enabled.

2. Method

2.1. Animals

All studies were conducted in accordance with Institutional Animal Care and Use Committee guidelines and the National Institutes of Health Guide for Care and Use of Laboratory Animals. AbbVie facilities are accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Adult male Sprague-Dawley rats (Charles River, Portage, MI, USA) were used (n = 16; 250-325 g at start of imaging). The current study contained one sham cohort (n = 8) and one CCI model cohort (n = 8). Data from one sham animal were not collected in this study.

2.2. CCI Surgery

Initially, rats were anesthetized with isoflurane (4% to induce and 2%-3% to maintain). The incision site was sterilized using ethanol and 10% povidone-iodine solution prior to and after surgeries. CCI of the sciatic nerve in rats was produced by following the method of Bennett and Xie [6]. The right common sciatic nerve was isolated at mid-thigh level, and loosely ligated by 4 chromic gut (4-0) ties separated by an interval of ~1 mm; for sham rats, the right common sciatic nerve was isolated but not ligated. All animals were allowed to rest and then placed in a cage with soft bedding for 2 weeks prior to further experimental procedures.

2.3. Behavioral testing

Sensitivity to mechanical stimulation was blindly tested 2 and 3 weeks post surgery with tactile allodynia using calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA), as previously described [10]. The maximum force applied was 15 g, a force that normally does not evoke a response in a naive rat. Rats with scores ⩽5 g were considered allodynic, confirming successful model creation.

2.4. Blood collection

Blood was collected (0.5 mL) 2 weeks following surgery and prior to FDG injection. Following room temperature exposure for ~45 minutes, each sample was spun at 4°C and 1000 g for 10 minutes. The serum layer was siphoned off and frozen at −80°C. Serum samples were run through a panel of 26 analytes (AssayGate, Ijamsville, MD, USA).

2.5. Peripheral MRI data acquisition

Animals were fasted for a minimum of 6 hours prior to imaging data acquisition. Each subject was anesthetized with 2%-3% isoflurane. A 4.7T Bruker Pharmascan (Karlsruhe, Germany) was used for data acquisition. Each animal was secured headfirst to the MRI bed with hind paws slightly extended and away from the body. A heating pad was placed beneath the animal to maintain 37°C body temperature and a pressure respiration pad (SA Instruments, Stony Brook, NY, USA) was used to monitor animal health for the entire duration of the scan. A 16-cm (inner diameter) volume coil (Bruker) was used to image the hip and lower limbs, covering the distribution of the sciatic nerve. The incision site was used as an initial localization point for positioning within the coil. Final positioning was adjusted using a 3-dimensional reference scan. A fat-suppressed, T2-weighted rapid acquisition with relaxation enhancement (RARE) scan was used for structural imaging of the periphery. T2-weighted RARE scan parameters: RARE factor = 3, # of dummy scans = 2, # of averages = 8, flip angle = 180°, # of slices = 20, effective echo time (TE) = 28 ms, temporal resolution (TR) = 4000 ms, spatial resolution = 0.205 mm × 0.205 mm × 1.5 mm.

2.6. Peripheral PET/CT data acquisition

Approximately 500 micro-Curie (μCi) of FDG (purchased from IBA Molecular, Dulles, VA, USA) in 100-200 μL were injected via tail vein 50-80 minutes before PET/CT imaging and just prior to MRI data acquisition. Once MRI data acquisition was completed, animals were kept secured on the MRI bed and transferred to a PET/CT station. Animals were imaged on a docked PET/CT station (Siemens Inveon microPET/CT, Knoxville, TN, USA). Positioning of each animal was carried out such that the field of view encompassed the hips and lower limbs. CT images were acquired at 80 kV and 500 uA, with an exposure time of 210 ms, and 200 steps. CT images were reconstructed using filtered back projection with a Shepp-Logan filter. PET was a 7-minute scan and reconstructed using an ordered subsets expectations maximization 2D algorithm.

2.7. Peripheral MRI and PET/CT data analyses

All peripheral MRI and FDG-PET data analysis was performed using VivoQuant 1.20 (inviCRO, LLC, Boston, MA, USA). Initially, each subject-specific PET/CT dataset was co-registered to the respective MRI dataset using a semi-automated, rigid body registration process. Subsequent to quality assurance/quality control of co-registration output, regions of interests (ROI) were masked and positions using the MRI datasets given spatial resolution as well as soft and hard tissue specificity (Fig. 1A).