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Laboratory studies

Diagnostic accuracy of evoked potentials for functional impairment after contusive spinal cord injury in adult rats

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ABSTRACT

Iatrogenic spinal cord injury (SCI) is a cause of potentially debilitating post-operative neurologic complications. Currently, intra-operative neurophysiological monitoring (IONM) via somatosensory evoked potentials and motor-evoked potentials is used to detect and prevent impending SCI. However, no empirically validated interventions exist to halt the progression of iatrogenic SCI once it is detected. This is in part due to the lack of a suitable translational model that mimics the circumstances surrounding iatrogenic SCI detected via IONM. Here, we evaluate a model of simulated contusive iatrogenic SCI detected via IONM in adult female Sprague-Dawley rats. We show that transient losses of somatosensory evoked potentials responses are 88.24% sensitive (95% confidence interval [CI] 63.53–98.20) and 80% specific (95% CI 51.91–95.43) for significant functional impairment following simulated iatrogenic SCI. Similarly, we show that transient losses in motor-evoked potentials responses are 70.83% sensitive (95% CI 48.91–87.33) and 100% specific (95% CI 62.91–100.00) for significant functional impairment following simulated iatrogenic SCI. These results indicate that our model is a suitable replica of the circumstances surrounding clinical iatrogenic SCI.

1. Introduction

Many processes contribute to spinal cord injury (SCI) during corrective spine surgery, including vascular compromise, the placement of spinal column instrumentation, and compressive injury from bony elements during deformity correction [1]. The SCI that result from these processes can ultimately manifest as post-operative paraplegia or paraparesis [2]. The incidence of paraplegia after corrective surgery varies between 1–3.8% depending on the age of the patient and the type of spinal deformity being corrected [2,3]. Intra-operative neurophysiological monitoring (IONM) with somatosensory evoked potentials (SSEP) and motor-evoked potentials (MEP) has been shown to reduce the risk of motor deficits due to SCI during corrective surgery [4,5].

To our knowledge at the time of writing, pharmacological therapies for SCI developed in the laboratory have been unsuccessful in the clinic [6]. One possible limitation has been the lack of a laboratory animal model of SCI that accurately replicates the conditions surrounding clinical iatrogenic SCI. Previous animal models of SCI have incorporated IONM [7–9], but have not evaluated the diagnostic accuracy of IONM changes in a real-time fashion similar to an actual procedure. The development of a preclinical model of SCI that combines the parameters of IONM and functional recovery in a way that mimics actual conditions in the operating room has the potential to aid development of more effective interventions targeting iatrogenic SCI. Here, we present a model that assesses the diagnostic accuracy of SSEP and MEP as biomarkers of iatrogenic SCI using a clinically relevant model of spinal cord contusion.

2. Methods

2.1. Experimental groups

In four experimental groups, adult female Sprague-Dawley rats received a contusive SCI of variable severity via the Infinite Horizon impactor (Precision Systems and Instrumentation, KY, USA), ranging from 100 (n = 7), 125 (n = 6), 150 (n = 7), or 200 (n = 6) kilodyn

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(Kdyn). These levels of impact were chosen to reflect mild, light moderate, severe moderate, and severe spinal cord contusions, respectively. In a fifth group, rats underwent an identical surgical procedure, but did not receive a spinal cord contusion (control group; n = 6). We chose to mimic iatrogenic SCI using this forced contusive model because we believe that this most closely replicates the type of injury inflicted during spinal column instrumentation – the most common mechanism of intra-operative SCI during scoliosis correction. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

2.2. Electrode placement

All laboratory animals were anesthetized prior to surgery using a combination of ketamine (60 mg/kg) and dexmedetomidine (0.5 mg/kg) administered via intraperitoneal injection. Following pre-operative anesthesia, the scalp was shaved and aseptically treated using iodine in preparation for surgery. A midline incision was made along the scalp to reveal the skull. A dental drill was used to create three holes in the skull to accommodate screw electrodes (E363/20, PlasticsOne, Roanoke, VA, USA) for subsequent neurophysiological monitoring. One electrode was placed 2 mm posterior to bregma and 3 mm to the left of the longitudinal fissure, a location corresponding to the left primary motor cortex (P3). A second electrode was placed 2 mm posterior to bregma and 3 mm to the right of the longitudinal fissure, a location corresponding to the right primary motor cortex (P4). A third electrode was placed 2 mm anterior to bregma in the midline of the skull for use as a reference (FZ). Electrodes were secured in place using superglue and covered by the scalp, which was closed using 4-0 sutures, allowing the wire leads of the electrodes to exit from the back of the neck.

2.3. SCI procedures

Immediately following cranial electrode implantation, the back region of the rat was shaved and aseptically treated using iodine in preparation for surgery. A midline incision was made along the thoracic vertebrae and the paravertebral muscles traversing T6–T12 were retracted. A laminectomy was performed at T9 to expose the dorsal surface of the T8 spinal cord which was subsequently contused using the Infinite Horizon impactor. During the contusion, the T6 and T12 spinous processes were secured in stabilization clamps to reduce the motion of the vertebral column during impact. After the injury, the muscles were sutured in layers using absorbable 4-0 sutures. The skin was then closed using wound clips. All rats were allowed to recover in a cage with food and water kept easily accessible. Gentamicin (5 mg/kg, intramuscular) was administered daily for 7 days starting immediately after surgery. The analgesic rimadyl (5 mg/kg, subcutaneous) was given daily for 3 days starting immediately after surgery. Bladders were manually emptied twice daily until control of urination was regained.

2.4. Neurophysiological testing

2.4.1. SSEP acquisition

Subdermal needle electrode pairs (Rhythmlink International, Columbia, SC, USA) were used to stimulate the median and tibial nerves in the fore and hind limbs, respectively. Recordings were performed from the skull electrodes. A 32 channel device (XLTEK Protektor, Natus Medical, San Carlos, CA, USA) was used to generate and record SSEP. Stimulation was performed one limb at a time using repetitive square electrical pulses of 6 mA in magnitude, 0.02 ms in duration at a frequency of 3.43 Hz. Responses were recorded using two channels – Fz-P4 and Fz-P3 – for both the fore and hind limbs. We averaged 256 SSEP trials to improve the signal-to-noise ratio. SSEP signals were filtered using a bandpass filter of 10 Hz to 250 Hz. A sensitivity of 20 μV/div and time base of 5 ms/div were used to display the SSEP responses.

2.4.2. MEP acquisition

Constant current stimulation was used for the generation of MEP. The skull electrodes were used for anodal stimulation. MEP were recorded using sub-dermal needle electrode pairs from the extensor digitorum communis in the forelimb and tibialis anterior in the hindlimb. Single trial MEP were obtained with a current intensity of up to 16 mA and a pulse width of 50 μs at a frequency of 350 Hz for 1 minute duration.

2.5. Neurophysiological assessment

Onset latency and peak-to-peak amplitude of the responses were measured at baseline, after laminectomy, after SCI, 1 hour post-injury, and 1 day post-injury. The onset latency was measured from the delivery of the stimulus to the first positive or negative deflection from baseline. Peak-to-peak amplitude was defined as the maximum amplitude between the largest positive and negative peak. To maintain consistency in measurements, the sensitivities of the waveforms were maintained throughout the analysis. The mean latency and amplitude of the SSEP response from the contralateral somatosensory cortex were calculated at every response obtained. The mean latency, amplitude, and number of MEP phases were calculated for each instance in which MEP were obtained. The SSEP/MEP responses post-injury were classified into three neurophysiological categories: [1] no change in responses; [2] significant change in responses; and [3] loss of responses. Significant changes in SSEP were defined as a decrease of 50% in the amplitude of the response and/or a 10% increase in the latency of the response when compared to baseline values. Significant changes in MEP responses were defined as an increase in latency of 10% of the onset latency and/or a decrease in amplitude by 50%. Loss of response was defined as a complete loss of SSEP/MEP amplitude after SCI. Loss of response was further classified as transient or persistent loss. Persistent loss was defined as a loss of response that did not improve on the first day of recording. Day 1 was used for this measure so that neurophysiological status could be directly correlated to neurobehavioral assessment. SSEP and MEP responses recorded from each limb were classified separately. For the final analysis, the lowest classification grade of SSEP or MEP changes among the four limbs was utilized as the definitive data point.

2.6. Post-operative behavioral assessment

Hind limb motor function was assessed using the open-field Basso, Beattie, and Bresnahan (BBB) locomotor test [10]. We used a high-definition camera to record each session, which was then analyzed by two individuals blinded to the injury groups. A separate score was assigned to each hind limb. Based on their BBB scores, rats were grouped into one of two functional groups at each time point. Rats with BBB scores 0–7 were identified as ‘‘severely impaired”. Rats with BBB scores 8–21 were identified as ‘‘not severely impaired”. These cutoffs were chosen because they reflect the inherent demarcations between different levels of recovery as specified by the BBB scoring system. This grouping of rats allowed us to calculate the predictive values of SSEP and MEP monitoring during a subsequent phase of the study. The 4 day and 7 day BBB scores were not part of this analysis.