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QUANTITATIVE ANALYSIS OF HINDLIMBS LOCOMOTION KINEMATICS IN SPINALIZED RATS TREATED WITH TAMOXIFEN PLUS TREADMILL EXERCISE

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Abstract—Locomotion recovery after a spinal cord injury (SCI) includes axon regeneration, myelin preservation and increased plasticity in propriospinal and descending spinal circuitries. The combined effects of tamoxifen and exercise after a SCI were analyzed in this study to determine whether the combination of both treatments induces the best outcome in locomotion recovery. In this study, the penetrating injury was provoked by a sharp projectile that penetrates through right dorsal and ventral portions of the T13-L1 spinal segments, affecting propriospinal and descending/ascending tracts. Intraperitoneal application of Tamoxifen and a treadmill exercise protocol, as rehabilitation therapies, separately or combined, were used. To evaluate the functional recovery, angular patterns of the hip, knee and ankle joints as well as the leg pendulum-like movement (PLM) were measured during the unrestricted gait of treated and untreated (UT) animals, previously and after the traumatic injury (15 and 30 days post-injury (dpi)). A pattern (curve) comparison analysis was made by using a locally designed Matlab script that determines the Frechet dissimilarity. The SCI magnitude was assessed by qualitative and quantitative histological analysis of the injury site 30 days after SCI. Our results showed that all treated groups had an improvement in hindlimbs kinematics compared to the UT group, which showed a poor gait locomotion recovery throughout the rehabilitation period. The group with the combined treatment (tamoxifen + exercise (TE)) presented the best outcome. In conclusion, tamoxifen and treadmill exercise treatments are complementary therapies for the functional recovery of gait locomotion in hemi-spinalized rats.

Key words: tamoxifen, treadmill exercise, kinematics analysis, Frechet distance.

INTRODUCTION

Significant spinal neuronal dysfunction is characterized by co-activation of antagonistic muscles, exhaustion of locomotive muscle activity, and gait pattern deterioration (Beauparlant et al., 2013). For motor function repair, diverse therapeutic interventions are focusing on the promotion of axon regeneration of severed neural pathways. The recovery of sensory-motor capacities also depends on the integrity of the neuronal spinal networks located caudal to the injury (Beauparlant et al., 2013). Two processes occur after a spinal cord injury (SCI): first, an acute primary mechanical trauma and then a progressive secondary phase of neuronal damage produced by inflammation and excitotoxicity that lead to apoptosis and demyelination. Therefore, an early and sustained rehabilitation therapy has a good locomotive outcome due to an increased plasticity in spared neuronal networks (Edgerton et al., 2008). Complex motor tasks such as gait locomotion could occur in spite of supraspinal neural pathways disruption. Spared spinal pathways undergo an extensive reorganization to achieve locomotive recovery (Bareyre et al., 2004). Thus, useful treatments to repair damaged axons and reconnect them are under design. Axonal regeneration and functional recovery occurred after blocking axonal growth inhibitors. However, most of the regenerated axons do not connect with the correct targets (Zhang et al., 2014). Spinal networks can be retrained to reestablish movement of the hindlimbs (Edgerton et al., 2004). Exercise training promotes effective rewiring after SCI (Wernig and Muller, 1992) and in addition, produces systemic changes, such as increased cardiovascular fitness, improved blood circulation and neuroendocrine changes that have a great impact on brain function and plasticity (Graziano et al., 2013).

Neuronal circuits (central pattern generators (CPGs)) for stepping are located in the lumbar spinal cord and even a few descending tracts fibers can activate them

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Abbreviations: CPG, central pattern generator; dpi, days post-injury; Fd, Fréchet distance; NTFs, neurotrophic factors; PBS, phosphate-buffered saline; PLM, pendulum-like movement; SCI, spinal cord injury; TE, Tamoxifen plus exercise; TMX, Tamoxifen treated; UT, untreated.

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(Ziegler et al., 2011). The motor output also depends on the sensory feedback information provided by propriospinal neurons. However, mammal CPGs can produce rhythmic alternating activity on motoneurons in the absence of sensorial and proprioceptive feedback (Rybak et al., 2006). Local spinal cord circuitry is capable of coordinating gait in the absence of supraspinal inputs (Chi, 2009).

Tamoxifen is a selective estrogen receptor modulator that exerts favorable effects on cellular and regional specificity in the nervous system (Kimelberg et al., 2000; Silva et al., 2000; Franco Rodriguez et al., 2013). In spinal-injured rats, Tamoxifen administration (after 30 min post-injury) showed a decrease in IL-1β production, reduced edema and apoptotic cells. Furthermore, at 3 days post-injury (dpi), Tamoxifen caused a significant attenuation in myelin loss and associated axonal myelin inhibitors (Tian et al., 2009). Tamoxifen evaluation on IL-1β y TNF-α expression levels supports its neuroprotective and neuroregenerative roles, preserves oligodendrocytes, increased myelin levels and decreased reactive astrocytes (Ismailoglu et al., 2010). However, practically there are no studies about the benefits of exercise on the kinematics of gait locomotion in spinal-injured animals.

In this study, the combined effects of exercise and tamoxifen on rats hindlimbs gait locomotion were analyzed after a SCI produced at the spinal T13-L1 level.

EXPERIMENTAL PROCEDURES

All procedures were performed in accordance with the ethical considerations and guidelines of the Mexican Official Norm (NOM-062-ZOO-1999) and the National Institute of Health Guide NIH Publication No. 8023 (1996) for the Care and Use of Laboratory Animals. In addition, the experimental protocols were approved by the Institutional Bioethics Committee of the Institutional Animal Care and Use Committee (IACUC).

Experimental subjects

Young adult female Sprague–Dawley rats (250–300 g) submitted to a penetrating thoracic-lumbar injury (T13-L1) were divided in four groups ($n = 6$ each): Untreated (UT); Tamoxifen treated (TMX; 1 mg/kg per day for 3 days, the first dose 30 min post-surgery); Exercise (EX; treadmill walk); and Tamoxifen plus exercise (TE). The animals were kept with food and water *ad libitum*.

SCI surgical procedure

Experimental animals were anesthetized with isoflurane gas (2–3%) and surgeries were performed under aseptic conditions. To expose the vertebral apophyses and laminae an incision was made in the back skin and muscles at the thoracic-lumbar level and subsequently the animals placed in a stereotactic device that holds and immobilizes the spine. As an impact model injury, the device was placed in the T13 and L1 right hemicord to discharge a projectile ($1 \times 1 \times 10$ mm), the vertebrae laminae break and the projectile penetrates into the spinal cord through dorsal and ventral fasciculus (Fig. 1A, B) and is removed almost immediately. Subsequently, back muscles, aponeurosis and skin were sutured with polysorb 3-0 and nylon 3-0 threads and the animals were treated for 3 days with postoperative antibiotics (Enroxil 2 mg/kg). In the corresponding treated groups of animals, Tamoxifen (1 mg/kg) was injected intraperitoneally immediately after, 24 and 48 h after the penetrating injury [17] and housed in individual cages at room temperature after surgery.

Locomotive training

Previous to the injury, rats were trained to walk on a treadmill (5 min for 5 days) and through a passageway acrylic tunnel (100 cm length, 10 cm high and 9 cm width) where the animals were video-recorded. Rats in the exercise groups began the treadmill training protocol at 3 dpi, 5 days per week, increasing the session duration every week (10–20 min, for 4 weeks) and with a variable treadmill velocity (8–17 cm/s). At the first days after injury, the animals received manual assistance to perform quadrupedal locomotion by holding their tale with an elastic thread.

Kinematic analysis

In order to evaluate treatment effectiveness, hindlimbs kinematic analysis was performed. The animals gait was video recorded (Digital CANON 120 fps) in three stages: previousl to the injury (control), 15 and 30 dpi. Hindlimbs were marked in the iliac crest, hip (greater trochanter), knee, ankle (lateral malleolus), and the fifth metatarsal phalangeal joints (Fig. 1C) and the steps of both ipsilateral and contralateral hindlimbs were obtained in the same recording time. Subsequently the Cartesian coordinates of each joint were obtained from all video frames using the Image J software. Line drawings were constructed between each joint point and three to four consecutive strides of the animal gait were reconstructed and a personalized MatLab script calculated the joint angular values (Fig. 1C, D) of the hip, knee, ankle and leg pendulum-like movement (PLM).

The angular control curves (previous to SCI) of strides executed during the gait of all the animals were aligned with respect to the time axis to obtain a robust control mean curve $\hat{C}(t)$. Then, each of the experimental stride angular curves (after SCI) was aligned with $\hat{C}(t)$ to determine the degree of similarity with respect to the control strides. The alignment consisted on finding the time shifting that maximizes the cross-correlation of the first derivative (computed using finite differences method) of the given curves. A quantitative similarity score was determined, which corresponds to each experimental stride curve with respect to the control curve by computing its corresponding discrete Fréchet distance (Fd) (Alt and Godau, 1995; Martinez et al., 2013), which is a metric extensively studied in computational geometry that allows to quantitatively evaluate the similarity between two curves taking into account the location, and ordering of the points that define those