INTRODUCTION Compartment Syndrome (CS) can occur when the circulation within a closed space is compromised, resulting in muscle necrosis, contractures, sensory deficits, and rhabdomyolysis (1-5). Reliable small animal models emulating CS injury in humans are lacking; we therefore developed a new rat animal model using a tourniquet placed over the trochanter and wrapping the limb in an external compression device (ECD). This group has investigated CS associated muscle damage by histological and molecular markers, and has used muscle-derived stem cells to enhance repair of the CS damaged tibialis anterior (TA) in this rat model. In order to investigate the functional improvement in muscle strength and the amelioration of damage to the skeletal muscle, nerves, and other soft tissues of the hind limb, gait and balance of the animals were tested. This study reports on the utility of gait analysis using the DigiGait™ system and balance testing using the RotoRod™ system to objectively quantify the differences in injuries caused by tourniquet alone and tourniquet plus the ECD.

METHODS All procedures described here were performed at the Rodent Behavior Analysis Core, University of Pittsburgh Health Sciences, following IACUC approvals. We used 6 Sprague-Dawley (SD) rats (male, 11 weeks old, Jackson Laboratories, ME). We trained the rats on the systems described below, to allow the rats to gain proficiency with the instruments and minimize noise in the data. Baseline gait dynamics were recorded prior to CS injury with the treadmill speed at 25 cm/s, and baseline RotoRod™ measurements with the axel speed at 0-40 rpms. Gait analysis: Gait analysis was quantified with the DigiGait™ system (Mouse Specifics Inc., Boston, MA) which has been used by other groups studying muscle defects (10-12). Briefly, this system utilizes a transparent treadmill designed for rodents and fitted with a high definition video camera mounted underneath to image the animal’s ventrum (Fig. 1). Digital images of the rodent paw prints are captured. The images are analyzed by a proprietary software package provided by Mouse Specifics, Inc. to define the area of each paw. The automated analysis of pixels in each paw print allows kinematic parameters to be measured.

Balance testing: These data were collected using a RotoRod™, a device that measures an animal’s latency to fall from a rotating axel. The axel’s rotational speed and the latency to fall are recorded. These are indicators of balance and motor function, and can provide data on the consistency of the injury, variability among individuals, and, of course, the functional outcome of the therapeutic strategy.

RESULTS To evaluate the effect of the ECD used in our CS model, we compared the use of the tourniquet plus ECD to the use of the tourniquet alone (Figures 2 and 3). Our gait analysis data show a decrease in % time the rodent dedicates to propulsion and an increase in % braking when injured with the ECD (TD7). A compensatory adjustment in the actions of the right forelimb are seen in these rats as they use this forelimb relatively more for propulsion than for braking after injury. When injured without the ECD (T7), the gait is not as affected: the swinging, braking, and propulsion phases of injured rats are comparable to those of uninjured controls. Looking at the RotoRod data, rats injured with tourniquet and the ECD (TD) were more affected than rats injured with the tourniquet alone (T), as indicated by TD group’s significantly (p=0.049) lower ratio. These findings are in agreement with our histological analyses, which show more TA damage when the tourniquet plus the ECD were used. We hypothesize that the use of the ECD prevents the facia from expanding and allows for sustained pressure throughout the duration of the injury. The use of the tourniquet and the ECD emulates a CS injury better than an ischemia reperfusion.

DISCUSSION: A reproducible gait signature in the CS-injured limb was noted; however, due to constraints of the analysis software, only certain segments of the captured video can be analyzed with an accepted degree of accuracy. As a result, the n for this experiment currently is only 1 per group, and Day-14 data were not able to be included. Nevertheless, the DigiGait™ system enables application of an objective and reliable method for quantification of early behavioral changes in lower limb injuries. The balance testing is less stringent and can also be less reliable, because animals can compensate for the injury and still perform the test as well as pre-injury. However, like gait analysis testing, the RotoRod™ is a non-terminal, non-invasive procedure that allows for longitudinal studies of rodents and requires less technical skill to perform compared to current functional physiological tests. All of these factors make both assays valuable tools for quantifying the consistency of the injury and the efficacy of treatment.


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