A new reliable murine model for Compartment Syndrome

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Introduction

Compartment Syndrome (CS) can occur when the circulation and function of tissues within a closed space are compromised and, ultimately, causes tissue necrosis (leading to contractures, muscle weakness and sensory deficits), rhabdomyolysis, renal failure, and even death (Matsen 1975, Finkelstein 1996, McQueen 1996, Matsuoka 2002, Boody 2005). The average annual incidence of compartment syndrome is 7.3 per 100 000 for men and 0.7 per 100 000 for women (McQueen 2000). The exact mechanisms are poorly understood; thus, effective treatments are lacking. The management of CS has changed little in the 70 years since fasciotomy was first described (Wang 2005). Therapies aimed at repairing deficits are only just emerging and new therapies could improve patient’s quality of life and outcomes. For this to be accomplished, reliable animal models must be developed. To date, most studies have been performed in large animal models (dogs, goats, pigs) hindering faster increase in knowledge because of the high costs and high maintenance needed for this large animals. Here we describe a new murine animal model for CS and list preliminary data on tissue damage associated with CS.

Methods

All procedures described here were approved by our University’s Institutional Animal Care and Use Committee. 25 Sprague-Dawley rats (male, 7/8 weeks old, Jackson Laboratories, ME) were used.

Injury model: initially a tourniquet is placed around the hindlimb just above the trochanter major (Walters 2008, Schoen 2007). Blood-flow occlusion is confirmed with the visual assessment of color changes of the sole of the foot and by palpation of femoral pulse. Following this, an external compression device (ECD; modified after Coutinho 2002) is placed over the hindlimb to impede swelling. This device is made of steel mesh (number 6), cotton cloth and adhesive tape and is easily fitted and fixed to the animal; the upper part of the device is similar to a T-shirt made of cotton cloth to better fix the device to the animal (Fig. 1). Before fixing the device to the hindlimb, a small cotton pad is placed around the hip and knee joints to prevent possible skin ulceration. The device’s weight is 12g. Both the tourniquet and the ECD are placed on the animal for 3 hours while the animal is under anesthesia (isoflurane 2%).

Analysis: The tibialis anterior (TA) muscle was evaluated at 5 different time points (5 animals at each time point): 1, 3, 5, 7 and 14 days post injury. TAs were harvested and placed in liquid nitrogen pre-cooled 2-methyl butane, and stored for further analysis. Tissue samples were cryosectioned and analyzed for tissue damage, fibrosis, necrosis, and immunohistochemistry. Hematoxylin and Eosin stain for general tissue morphology and Masson’s Trichrome stain for muscle and fibrotic tissue. Immunohistochemical methods for tissue characterization included staining for CD31 (BD Biosciences, NJ), a marker associated with endothelial cells in vascular tissue, CD68 (Abcam, MA), a marker associated with macrophages, and dystrophin, a sarcolemmal protein responsible for maintaining muscle membrane stability. Evaluations were made with a fluorescence microscope (Nikon E800) and analyzed with Northern Eclipse and Image J (NIH) software.

Results:

The animals presented, after injury, with difficulty in ambulation and apparent soreness. After 5 days the rats presented with an alteration in toe spread that could indicate nerve injury. The macroscopic evaluation of the TA showed increase degeneration and adipose tissue infiltration that increased with time (Fig. 2).

Hematoxylin and Eosin staining of TA muscles displayed intact muscle fibers at the periphery that were lost at the core of the TA muscle. These figures also reveal a buildup of mononuclear cells along the periphery of injured muscles. The Masson’s Trichrome mediated histological analysis revealed immature collagen deposition and fibrosis along the front of these infiltrating mononuclear cells.

Examining the injured muscle fibers in the TA central core reveals the loss of muscle fibers and nuclei. The progressive loss of nuclei from the TA core over a fourteen day time period was apparent in the injured TA after observing nuclear staining with Dapi. CD31 and dystrophin showed some preserved muscle fiber structure and an increased presence of microvasculature when compared to the enucleated TA center. The immunohistochemical staining with anti CD68 revealed the presence of macrophages in the injured TA. Similar to the CD31 staining, damaged muscle peripheries showed an increased presence of macrophages when compared to the enucleated TA core. These non-phagocytic macrophages are distributed in higher densities near newly regenerated muscle fibers.

Discussion

To better understand CS, we created a rat model by increasing the compartment pressure in the hindlimb. The use of a tourniquet and ECD over a period of three hours proved to be effective and consistent method of inducing CS injury after qualitative differences between the control and injured TA were observed up to 14 days using immunohistochemical staining methods. In our CS model, the muscle affected by CS has an ischemic, necrotic core. This decellularized compartment devoid of capillaries and populated with macrophages should be the target of future therapies.

References


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