Quantitative MRI analysis of skeletal muscle injury and regeneration in a rat hindlimb model

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Introduction:

Contusion injuries to muscle, resulting from high energy impacts that rapidly compress local tissues, are common in the athletic population. Given the frequency of these injuries, it is important to develop a clearer understanding of the injury process and skeletal muscle regeneration. Although qualitative human MRI studies have been performed [1,2], quantitative reports are sparse. Animal models of injury, whereby contusions are induced under controlled experimental conditions, are useful for characterizing normal healing [3]. We have designed a contusion device using a standard weighted free falling mass as a method of inducing injury, and assessed the use of volumetric and relaxation-time MRI methods to follow the dynamics of injury and regeneration.

Subjects & Methods:

Male Wistar rats (240-290 g) were anesthetized via inhaled anesthetic (isoflurane/oxygen), the hindlimbs were shaved, cleansed with alcohol and the mid-belly of the gastrocnemius muscle were determined and marked via palpations. Animals were then placed prone in the injury device with hind limb extended to ensure direct impact over the gastrocnemius muscle. Animals served as their own control with only the left hind limb being injured. Force output, displacement, and energy were recorded. A free falling mass with a 97.1g weight attached was dropped from 70, 60, 50, or 40 cm. At specific time points after the injury (6, 12, 24, 48, 72, and 96 hours) the rats were anaesthetized via inhaled anesthetic (isoflurane/oxygen), and MRI imaging was conducted on a Varian 7 tesla horizontal system with a quadrature birdcage. Five spin echo experiments (56 slices) with a resolution of (215 x 194 x 500) μm^3 and increasing echo times (11, 20, 30, 40, and 60 ms) were acquired to calculate T2 maps of the muscle in the hind limb. Segmentation using AMIRA of 30 slices starting at the mid-joint line of the knee of each animal (using the images acquired with TE=11 ms) was used to calculate the three-dimensional volume of the hind limb, as well as the three-dimensional T2-map. In order to estimate the effects of injury and post-injury recovery from the T2 maps, the average and standard deviation of the T2 value of the control limb was calculated, and the number of pixels with a T2 value larger than the mean by two standard deviations or more was calculated: the number of pixels was then converted into an absolute volume.

Results:

Figure 1 A shows representative axial slices through one rat at 6 hours and 96 hours. The “anatomical” image at 6 hours shows a substantial increase in volume of the injured left leg compared to the control right leg: large localized increases in the T2 value are also apparent. At 96 hours the volumes of the two legs are identical, but there is still a substantially elevated T2 value in many areas of the tissue, although the fluid present at 6 hours has been reabsorbed. Figures B and C shows volume and relaxation time changes of the 30 slices as a function of time and drop-weight.

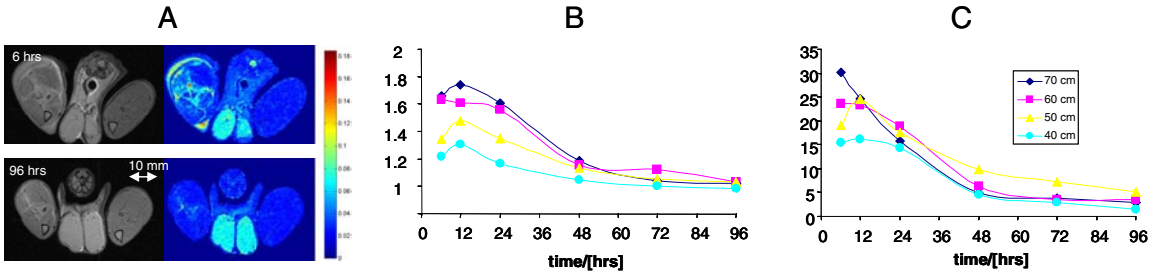

Figure 1. A. Anatomical images (left) and T2 map (right) of respresentative slices acquired at 6 (upper row) and 96 hours after contusion of the left hindleg: the impact of the injury can be seen by the enlarged size and the elevated T2 values (colourbar). B. The ratio of the volume of the injured leg vs control leg as a function of time. C. Ratio of the volume of tissue of the injured vs. control leg with a T2 value more than two standard deviations greater than the mean of the control leg.

Discussion:

Figure 1B shows that the volume of the hind limb muscle returns to normal, for all of the injury conditions, at approximately 96 hours. However, as shown in Figure 1C, the T2 data show that there are still measurable effects of injury for all of the conditions, as evidenced by the presence of a significant region with elevated T2 values over the control leg: thus T2 measurements add valuable information on the healing process. For the lower impact injuries (40 and 50 cm drop depths) the greatest muscle injury was recorded at 12 hours (by both measures), whereas maximum injury occurred earlier for the higher impact cases. For all injuries, the period of greatest recovery was between 24 and 48 hours. Having established a quantitative measure for injury and regeneration, current experiments aim to: (i) evaluate the effects of analgesics such as buprenorphine, (ii) correlate MRI measures with histological stains for neutrophils, macrophages and satellite cells, and (iii) investigate the effects of therapeutic modalities (i.e. ice, ultrasound, laser) on the dynamics of muscle regeneration.

References:

[1] Verrall, G., J. Slavotinek, P. Barnes, and G. Fon. Diagnostic and prognostic value of clinical findings in 83 athletes with posterior thigh injury. Amer J Sports Med. 31:969-973, 2003. [2] Smet, A, Best, T. MR Imaging of the distribution and location of acute hamstring injuries in athletes. Amer J Rad 174:393-399, 2000. [3] Crisco, J., P. Jokl, G. Heinen, M. Connell, and M. Panjobi. A muscle contusion injury model. Amer J Sports Med. 22:702-710, 1994.