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Impaired muscle function in a mouse surgical model of post-traumatic osteoarthritis

C. van der Poel †, P. Levinger ‡, B.A. Tonkin §, I. Levinger ‡, N.C. Walsh § ‖ * [a]

† Department of Physiology, Anatomy and Microbiology, La Trobe University, Melbourne, Australia
‡ Institute of Sport, Exercise & Active Living (ISEAL), College of Sport and Exercise Science, Victoria University, Melbourne, Australia
§ St Vincent's Institute of Medical Research, Melbourne, Australia
‖ Department of Medicine, St Vincent's Hospital, University of Melbourne, Melbourne, Australia

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SUMMARY

Objectives: Using a mouse surgical model of post-traumatic osteoarthritis (OA), we sought to determine if muscle function is altered following acute joint injury and whether this relates to OA progression.
Design: Male C57BL/6 mice underwent surgical transection of the medio-meniscal tibial ligament destabilisation of the medial meniscus (DMM) or sham surgery on one knee. Tibialis anterior (TA) muscle function was assessed *in situ* at 1, 4 and 8 weeks post-surgery. Cartilage damage and joint inflammation were assessed by histologic scoring. Muscle mRNA expression was quantified by qRT-PCR.
Results: Tetanic and twitch force production between DMM and sham muscle did not differ at 1 week post-surgery. Muscle function improved in both groups with time, but specific force production in DMM muscles was 18% and 22% lower than sham muscles at 4 and 8 weeks post-surgery respectively. At 8 weeks post-surgery, DMM muscles had a 40% slower relaxation rate and reduced expression of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (Serca) pump mRNA compared to sham muscles; both observations indicate likely alterations in muscle Ca^{2+} handling. There were no histologic signs of muscle atrophy or inflammation in DMM TA muscles. Specific force production in both sham and DMM mice showed a negative correlation with the severity of joint inflammation.
Conclusions: Acute knee injury in the DMM model of post-traumatic OA leads to a persistent deficit in TA muscle function that occurs in the absence of muscle atrophy. This study highlights that the impact of acute knee injury is unlikely to be limited to the muscles controlling knee movement.

Introduction

Osteoarthritis (OA) comprises a heterogenous group of degenerative diseases that irrespective of the suspected cause result in low level joint inflammation associated with cartilage damage and loss, thickening of the subchondral bone, and formation of osteophytes on the outer bone surfaces[1]. Muscle weakness is also common in OA patients[2]. However unlike cartilage and bone pathology in OA, the aetiology of muscle weakness and how it relates to OA onset and progression is unclear, and few studies have focused on this pathology in pre-clinical animal models of OA.

Healthy muscles are integral to efficient joint function as they not only aid joint movement and stability but also absorb and transduce limb loads. In patients with early or established OA, quadriceps strength was reduced by 20–40% compared to healthy controls[2–4]. This pathology has been attributed to limb disuse due to reduced mobility and pain within the affected limb, however, several studies have shown that muscle weakness[3,4] and muscle atrophy[5] are evident in patients with radiographic OA without persistent joint pain. This suggests that other factors may initiate and/or contribute to loss of muscle strength in OA patients.

Individuals with acute joint injuries are at high risk of developing post-traumatic OA with approximately 50% of patients presenting with OA 10–20 years post-injury[6]. Patients with acute joint injury, such as ligament or meniscal tears, also show muscle weakness following injury, which persists following surgical repair

* Address correspondence and reprint requests to: N.C. Walsh, Level East 5th floor, MCRI, Royal Children's Hospital, Flemington Road, Parkville, VIC 3052, Australia. Tel: 61-3-8341-6467.
E-mail address: nicole.walsh@mcri.edu.au (N.C. Walsh).
[a] Present address: Now Located at Murdoch Childrens Research Institute, Melbourne, Australia.

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and rehabilitation[2,7,8]. With many of these post-traumatic OA patients being young, it is important that the aetiology of OA development post-injury, including factors contributing to muscle weakness are defined.

In this study, we use a mouse surgical model of post-traumatic OA to investigate how muscle function is affected following joint injury and how this relates to the onset and early progression of post-traumatic OA. The destabilisation of the medial meniscus (DMM) model of post-traumatic OA is a widely accepted preclinical model of OA[9,10] which relies on the transection of the ligament attaching the medial meniscus to the tibia[11]. This destabilizes the knee joint and results in increased loading within the medial compartment of the knee leading to focal cartilage damage similar to human OA[9,11]. Muscle function has not been assessed in any rodent model of post-traumatic OA. In this study, we have assessed muscle function *in situ* in mice following DMM surgery to identify how joint injury alters muscle function and whether this is associated with onset or progression of the early pathologic events associated with post-traumatic OA. Our results demonstrate that muscle function is impaired following acute joint injury and occurs in the absence of changes in muscle morphology.

Methods

Mice

All procedures were approved by the Animal Ethics Committees of St. Vincent's Health and La Trobe University and conformed to the Australian Code of Practice for the Care and Use of Animals for Scientific purposes as stipulated by the National Health and Medical Research Council (Australia). 10 week old male C57BL/6Arc mice were purchased from the Animal Resource Centre (Canning Vale, Australia) and housed in groups of three/cage in microisolator cages with a 12 h light and dark cycle with food and water provided *ad libitum*. The C57Bl/6 mouse strain was chosen for these studies since it is commonly used for the study of the effect of deletion, mutation or overexpression of single genes *in vivo*. Mice were randomly allocated to surgical groups and time-points.

Induction of post-traumatic OA

12 week old male C57Bl/6Arc mice (six mice per time point, 3 time points, total 18 mice) had surgical transection of the mediomeniscal tibial ligament (MMTL) in the right limb as per Glasson *et al.*[11] with the exception that the fat pad was not removed from the joint. Sham surgery on the right limb was also done in separate groups of mice (six mice per time point, 3 time points, total 18 mice). In both DMM and sham mice, the surgical wound was closed with continuous suture from the distal quadriceps muscle, through the patellar ligament finishing in the proximal gastrocnemius muscle using tapered Vicryl 8-0. The skin wound was closed by discontinuous suturing of the subcutaneous layer with reverse cutting Vicryl 7-0 and application of Vetbond tissue adhesive. All mice received carprofen analgesia (5 mg/kg) at the time of surgery. Six mice per surgical group were analysed at 1, 4 and 8 weeks post-surgery.

In situ analysis of muscle function

Tibialis anterior (TA) muscle function was assessed *in situ* at each time point in all mice (6 DMM and 6 Sham mice per time point) as previously described[12]. The TA muscle is not injured directly by the surgical procedure and therefore analysis of this muscle is not confounded directly by wound repair from surgical intervention. Absolute maximal isometric tetanic force (Po) was determined from the plateau of the frequency-force relationship and normalized to the cross-sectional area of the muscle to calculate maximum specific force (sPo). Twitch force parameters including time to peak tension (TPT) and time taken to relax to 50% of peak twitch tension (1/2RT) were also recorded. Once *in situ* muscle function analyses were completed the mice were euthanased by cervical dislocation and tissues dissected as follows. The TA muscles were dissected and weights recorded before transecting them midbelly and embedding half in Tissue-Tek OCT or freezing in liquid nitrogen for RNA isolation for further analyses. Knee joints were dissected and fixed in 4% paraformaldehyde overnight before decalcification and embedding in paraffin for histologic analyses.

Histologic analyses

Muscle

Serial transverse sections (8 μm) were cut from the midbelly of each TA muscle using a cryostat microtome and stained with hematoxylin-eosin for visualization of muscle morphology using a Leica DMRBE microscope. Muscle fibre cross sectional area (CSA) was measured from tissue sections using Leica Qwin software (Leica Microsystems, Wetzlar, Germany) and the mean muscle fibre CSA for each mouse TA muscle was calculated. Four TA muscles from individual mice per surgical group per time point were assessed for mean fibre CSA (the remaining muscles were not suitable for analysis due to poor quality of sections).

Knee joints

Knee joints were sectioned coronally (5 μm) and three sections from the mid-posterior region of the joint separated by 100 μm were stained with Safranin-O Fast green for histologic scoring. Cartilage damage was assessed according to the OARSI histologic guidelines; scores for the medial tibial and medial femoral compartments were summed for each tissue section and a total score for an individual joint obtained by summing the score for all three section[13]. Joint inflammation was assessed according to the following scheme which was guided by scoring schema published by Lewis *et al.*[14]: 0, normal; 0.5, thickening of synovial lining layer (>3–4 cell layers thick); 1, thickened synovium adjacent bone and/or cartilage; 2, significant inflammation within the synovium with increased cellularity and protrusion of synovial villi into the joint space; 3, significant inflammation of the synovium, increased cellularity and protrusion of synovial villi into the joint space and inflammation within the surrounding joint structures. Sum of the scores for all three sections gave a total score for inflammation for each joint. Several knee joints did not decalcify fully and the quality of sections was not sufficient for analysis. Therefore the group sizes assessed were as follows: 1 week DMM and sham, $n = 6$ per group; 4 week DMM and sham $n = 5$ per group; 8 week sham, 4 per group; 8 week DMM 6 per group. The scorer (NCW) was blinded to sample identification for analysis.

RNA preparation and quantitative (q)RT-PCR analysis of gene expression

qRT-PCR was done to determine if expression of genes encoding proteins that contribute to muscle function were altered in DMM TA muscle biopsies compared to sham TA muscles. Muscle biopsies were homogenized individually, and mRNA was extracted using an aurum fatty and fibrous tissue RNA extraction kit (Bio-Rad) according to the manufacturer's instructions. mRNA was transcribed into cDNA using the Iscript cDNA synthesis kit (Bio-Rad) according to the manufacturer's instructions. qRT-PCR was performed using an iCycler Thermal Cycler (Bio-Rad) with EVA Green (Bio-Rad). The expression levels of all genes ($2^{-(\text{deltaCT})}$) was determined relative to