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Case report

Muscle biopsies off-set normal cellular signaling in surrounding musculature

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Abstract

Studies of muscle physiology and muscular disorders often require muscle biopsies to answer questions about muscle biology. In this context, we have often wondered if muscle biopsies, especially if performed repeatedly, would affect interpretation of muscle morphology and cellular signaling. We hypothesized that muscle morphology and cellular signaling involved in myogenesis/regeneration and protein turnover can be changed by a previous muscle biopsy in close proximity to the area under investigation. Here we report a case where a past biopsy or biopsies affect cellular signaling of the surrounding muscle tissue for at least 3 weeks after the biopsy was performed and magnetic resonance imaging suggests that an effect of a biopsy may persist for at least 5 months. Cellular signaling after a biopsy resembles what is seen in severe limb-girdle muscular dystrophy type 2I with respect to protein synthesis and myogenesis despite normal histologic appearance.

Keywords: Muscle biopsy; Muscle regeneration; Wound healing; Cellular signaling; Limb girdle muscular dystrophy type 2I

1. Introduction

Muscle biopsies are performed for diagnostic and research purposes on a routine basis, and it is not uncommon that multiple biopsies from the same muscle are required. Many studies rely on the assumption that a biopsy has little effect surrounding musculature and that long-term effects are minimal. Performing a biopsy will result in a flurry of cellular events, i.e. immediate wound healing, pro-inflammatory response and muscle regeneration. Hence, significant changes in mRNA and protein-content should be expected soon after the biopsy has been performed in the immediate surroundings of the biopsy-site. However, little is known about the effect of such biopsies on muscle morphology and cellular signaling in the surrounding musculature. Only a few short-term exercise studies described cellular signaling after multiple biopsies but have focused on the effect of exercise [1–5]. However, acute changes in cellular signaling may be irrelevant in a long-term study. Recently, we had the opportunity to investigate a man, who had participated in various unrelated experiments on muscle physiology involving performance of multiple biopsies in each vastus lateralis (VL) muscle in the past years. With a biopsy performed in close proximity to another biopsy that had been taken 3 weeks earlier, we investigated any differences in myogenesis/regeneration and protein turnover (Fig. 1a) between this healthy, multi-biopsied subject and controls. The study demonstrates that multiple biopsies may lead to long-term alterations in important cellular signaling

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pathways concealed by a normal histologic appearance of the muscle.

2. Case report

2.1. Case

We report the case of a 67 year-old healthy male who had approximately 20 muscle biopsies performed in the vastus lateralis of both legs over the course of 5 years. The subject did not have any evidence of muscle disease and had not experienced any systemic diseases or muscle trauma other than the performed muscle biopsies. The most recent biopsy had been performed only 3 weeks before the biopsy we took. We performed a biopsy within 3–4 cm from the most recent biopsy. As controls we used healthy male subjects (age 42 ± 15 years), who never had a biopsy performed before, and a man (age 37 years) severely affected by limb-girdle muscular dystrophy type 2I (LGMD2I), who had not been biopsied before in the same limb. The Ethical Committee of the Capital Region had approved the study (Approval # H-1–2010–021), and all participants had provided written consent to the participation in the study.

2.2. MRI

We performed a short tau inversion recovery (STIR) MRI of the thigh muscles of the subject who had multiple biopsies performed to visualize potential edema in the muscle, using a Siemens 3T MRI system (Siemens, Erlangen, Germany).

2.3. Histology and immunoblots

Muscle biopsies were obtained using a Bergstrøm needle, and were frozen in isopentane cooled by liquid nitrogen. Biopsies were sampled from vastus lateralis (VL) and tibialis anterior (TA). TA acted as an internal control, since no other biopsies had been taken from the tibialis muscle in the multi-biopsied subject. Cryosections, 10 μm thick, were fixed and stained with hematoxyline and eosine (H & E) for histopathologic evaluation. Sections for immunohistology were blocked in buffer (3% fetal calf serum in PBS) prior to staining. Primary antibodies were diluted 1:100 and incubated overnight. The level of active regeneration was determined by staining for embryonic myosin heavy chain (eMHC, F1.652, DSHB, Iowa City, IA) and neural cell adhesion molecule (NCAM/CD56, Becton–Dickinson, UK). Fibers showing an intermediate eMHC or NCAM stain and nuclear clumps (with positive staining) were excluded. Differentiating satellite cells were visualized by staining for myogenin (clone F5D, DSHB, Iowa City, IA), DAPI nuclear stain (Invitrogen, Carlsbad, CA) and laminin (L9393; Sigma, St. Louis, MO). Alexa 488 and 594 (Invitrogen, Carlsbad, CA) secondary anti-mouse and anti-rabbit antibodies were used at 1:500 dilution in PBS buffer and observed under a Nikon Eclipse 80i epi-fluorescence microscope. We used Western blotting to determine if important myogenesis (MyoD-Myogenin-p38) [6,7] and protein turnover (Akt-mTOR-p70S6K and MAFBx-MuRF1-Myostatin) [8–10] as well as mitogen activated kinase (MAP) signaling pathways were differentially activated. Briefly, muscle was homogenized in ice-cold lysis buffer using a bead-mill at 4 °C. Supernatants were collected and protein concentrations were determined using a Bradford assay. Equal amounts of extracted muscle proteins were separated on 10% TGX polyacrylamide gels (Bio-Rad, Hercules, CA) at 200 V for 30 min. Proteins were transferred to PVDF membranes and post-transfer membranes were stained with Sypro Ruby (Sigma–Aldrich, St. Louis, MO) to facilitate equal protein transfer. Membranes incubated overnight with primary antibodies (phospho-Akt_{S473}, phospho-$JNK_{T183/Y185}$, phospho-$p38_{T180/Y182}$, phospho-$ERK1/2_{T202/Y204}$, phospho-$p70S6K_{T389}$, phospho-$p90RSK_{S380}$, phospho-$PDK1_{S241}$, phospho-$PI3K_{Y199}$, phospho-$p4E\text{-}BP1_{T37/46}$, phospho-$SMAD2_{S245/250/255}$, Cell Signaling Technologies, Danvers, MA; MyoD, Vectorlabs, Burlington, CA; Myostatin, Millipore, Billerica, MA; MAFBx, MuRF1, Sigma–Aldrich; α-tubulin clone 12G10 and myogenin clone F5D, Developmental Studies Hybridoma Bank, University of Iowa). Secondary antibodies coupled with horseradish peroxidase diluted 1:10,000 were used to detect primary antibodies (DAKO, Denmark). Immuno-reactive bands were detected using SuperSignal West Dura kit (Thermo Scientific, Waltham, MA) and visualized using a Gbox XT16 darkroom (Syngene, UK). Immuno-reactive band intensities were normalized to the intensity of the α-tubulin bands for each subject to correct for differences in total muscle protein loaded on gel.

3. Results

3.1. MRI

Due to the discrepancy between histology and cellular signaling we decided to scan the multi-biopsied subject by STIR MRI. Prior to this there had been no reason to perform an MRI scan of the subject as he was considered perfectly healthy. The vastus lateralis (VL) was scanned five months following the last biopsy, and this showed a focal edema around a biopsy site spanning approximately 5 cm in the VL muscle (Fig. 1b). Due to location and focal pattern of the edema, and lack of a history of trauma or neurologic deficits, the edema is likely a reaction to one of the biopsies or both, performed 5–6 months before the MRI scan.

3.2. Histology

The morphology of VL muscle biopsy from the multi-biopsied person was normal with no signs of