Experimental Gerontology 80 (2016) 1–5

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Short communication

Association between myocyte quality control signaling and sarcopenia in old hip-fractured patients: Results from the Sarcopenia in HIp FracTure (SHIFT) exploratory study

Emanuele Marzetti [a,1], Riccardo Calvani [a,*,1], Maria Lorenzi [a], Fabiana Tanganelli [a], Anna Picca [a], Maurizio Bossola [b], Amerigo Menghi [a], Roberto Bernabei [a], Francesco Landi [a]

[a] Department of Geriatrics, Neurosciences and Orthopedics, Catholic University of the Sacred Heart School of Medicine, Teaching Hospital "Agostino Gemelli", Rome, Italy
[b] Department of Surgery, Catholic University of the Sacred Heart School of Medicine, Teaching Hospital "Agostino Gemelli", Rome, Italy

ARTICLE INFO

Article history:
Received 18 February 2016
Received in revised form 21 March 2016
Accepted 4 April 2016
Available online 7 April 2016

Section Editor: Christiaan Leeuwenburgh

Keywords:
Fusion
Fission
Mitochondrial biogenesis
Muscle atrophy
Muscle biopsy
Orthogeriatrics

ABSTRACT

Background: Sarcopenia has been proposed as a potentially amenable factor impacting the clinical outcomes of hip-fractured elderly. The identification of specific biological targets is therefore crucial to developing pharmacological interventions against age-related muscle wasting. The present work reports promising preliminary data on the association between alterations of myocyte quality control (MQC) signaling and sarcopenia in old patients with hip fracture.
Methods: Twenty-five elderly hip-fractured patients (20 women and 5 men; mean age 84.9 ± 1.65 years) were enrolled as part of the Sarcopenia in HIp FracTure (SHIFT) study. Intraoperative biopsies of the vastus lateralis muscle were obtained and assayed for the expression of a set of MQC signaling proteins. The presence of sarcopenia was established according to the European Working Group on Sarcopenia in Older People (EWGSOP) criteria, with bioelectrical impedance analysis used for fat-free mass estimation.
Results: Sarcopenia was identified in 10 patients (40%). Protein expression of the mitochondrial fusion factor mitofusin (Mfn) 2 and the autophagy mediator microtubule-associated protein 1 light chain 3B (LC3B) was significantly lower in patients with sarcopenia compared with non-sarcopenic controls. No differences between groups were observed for Mfn1, optic atrophy protein 1 (OPA1), fission protein 1 (Fis1), and the master regulator of mitochondrial biogenesis peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α).
Conclusion: Data from this exploratory study show that a reduced expression of the mitochondrial fusion factor Mfn2 and the autophagy mediator LC3B is associated with sarcopenia in old hip-fractured patients. Future larger-scale studies are needed to corroborate these preliminary findings and determine whether MQC pathways may be targeted to improve muscle health and promote functional recovery in old patients with hip fracture.

1. Introduction

More than 1.6 million older adults worldwide sustain a hip fracture annually, of whom 20–30% die within one year of the event (Hung et al., 2012). Among survivors, approximately 50% show incomplete functional recovery and develop various degrees of disability (Maggi et al., 2010). The hip fracture "epidemic" has a considerable socio-economic impact, because of the burden of direct and indirect costs linked to fracture treatment and the frequent occurrence of disability and the need for long-term care. Hence, the development of new strategies to improve survival and functional recovery in hip-fractured patients is perceived as a major public health priority.

Among the factors that may impact the clinical outcomes of hip-fractured elderly, sarcopenia has been indicated as a condition potentially amenable to interventions (Visser et al., 2000; D'Adamo et al., 2014; Marzetti et al., 2014). However, except for physical exercise combined with nutritional supplementation, no treatments are currently available against sarcopenia. This situation is primarily attributable to incomplete knowledge of the signaling pathways underlying age-related muscle wasting and the consequent lack of meaningful biological targets for drug development.

Alterations of myocyte quality control (MQC) processes may play a role in sarcopenia of aging and could therefore represent a novel target for interventions (Marzetti et al., 2013; Fan et al., 2015). Indeed, a reduced expression of various autophagy signaling molecules, suggestive of decreased autophagic flux, has been documented in the

* Corresponding author at: Center for Geriatric Medicine (CeMI), Institute of Internal Medicine and Geriatrics, Catholic University of Sacred Heart, L.go Agostino Gemelli 8, 00168 Rome, Italy.
E-mail address: riccardo.calvani@gmail.com (R. Calvani).
[1] These two authors contributed equally to the present work.

http://dx.doi.org/10.1016/j.exger.2016.04.003
0531-5565/

muscles of older adults (Wohlgemuth et al., 2011; Drummond et al., 2014). This, in conjunction with altered mitochondrial dynamics and turnover, may result in the inefficient clearance of damaged mitochondria, the accumulation of dysmorphic and dysfunctional organelles and, ultimately, muscle degeneration (Brunk and Terman, 2002; Leduc-Gaudet et al., 2015). Despite the fact that MQC may be critically involved in the origin of sarcopenia, only a handful of studies have attempted to characterize the alterations of autophagy and mitochondrial dynamics that accompany human muscle aging. The invasive nature of the muscle sampling procedure (i.e., needle biopsy) is a major obstacle to investigating these signaling pathways. Accessing muscle tissue during hip fracture surgery may therefore represent a valuable way in which to avoid patient discomfort and increase participation.

The Sarcopenia in HIp FracTure (SHIFT) study was designed to gather preliminary data on the possible involvement of MQC dysfunction in sarcopenia among old patients with hip fractures. In particular, in this initial investigation, we explored the hypothesis that sarcopenic patients would show altered expression of signaling molecules suggestive of reduced autophagy and impaired mitochondrial homeostasis relative to their non-sarcopenic counterparts.

2. Materials and methods

2.1. Study sample

The study was performed in 25 older persons (mean age: 84.9 ± 1.65 years; 80% women) admitted for hip fractures due to accidental falls to the Emergency Department of the Teaching Hospital "Agostino Gemelli", Catholic University of the Sacred Heart School of Medicine (Rome, Italy). Exclusion criteria were age <65 years, unwillingness to take part in the investigation, presence of peripheral edema, pathologic fractures, cognitive impairment (Cognitive Performance Scale, CPS < 3), presence of a pacemaker or implantable cardioverter defibrillator, and being wheel-chaired or bedridden before the hip fracture. The study was approved by the Institutional Review Board of the Catholic University of the Sacred Heart, and all participants signed a written consent before enrollment.

2.2. Identification of sarcopenia

The presence of sarcopenia was established according to the European Working Group on Sarcopenia in Older People (EWGSOP) criteria (Cruz-Jentoft et al., 2010). Whole-body fat-free mass was measured by bioelectrical impedance analysis (BIA) using a Quantum/S Bioelectrical Body Composition Analyzer (Akern Srl, Florence, Italy), as previously described (Marzetti et al., 2014). Measurements were taken under standard conditions, with the patient in a supine position and surface electrodes placed on the wrist and ankle contralateral to the side of the fracture. Muscle mass was estimated using the equation developed by Janssen et al. (2000). The skeletal muscle index [SMI, (kg/m^2)] was obtained by dividing absolute muscle mass by squared height. According to the EWGSOP indications, low SMI was defined based on the following cut-offs: <8.87 kg/m^2 in men and <6.42 kg/m^2 in women (Cruz-Jentoft et al., 2010).

Muscle strength was assessed by means of a North Coast handheld dynamometer (North Coast Medical, Gilroy, CA), with the patient lying supine, as described elsewhere (Marzetti et al., 2014). The maximal value of three consecutive measurements in the dominant arm was used for the analysis. Low grip strength was defined as values <30 kg and <20 kg, in men and women, respectively (Cruz-Jentoft et al., 2010).

2.3. Collection of muscle biopsies

Muscle samples (250–350 mg) were obtained intraoperatively from the vastus lateralis. Muscle specimens were cleaned of any visible blood and fat, snap-frozen in liquid nitrogen, and stored at −80 °C until analysis.

2.4. Preparation of mitochondrial fractions and whole cell homogenates

For cellular fractionation, muscle samples (~150 mg) were minced in 2 mL of phosphate buffered saline (PBS) and incubated on ice for 3 min in PBS with 0.3 mg/mL trypsin. Samples were subsequently centrifuged at 700 ×*g* for 1 min at 4 °C.

To obtain the mitochondrial fraction, the pellet was resuspended in 800 μL of PBS containing 4 mg/mL bovine serum albumin (BSA), homogenized with a Dounce homogenizer and centrifuged at 1000 ×*g* for 3 min at 4 °C. The pellet was then resuspended in 800 μL of isotonic buffer containing 20 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) and 4 mg/mL BSA, vortexed at medium speed for 5 s and incubated on ice for 2 min. Cell lysis was achieved by adding 10 μL of lysis buffer containing 20 mM HEPES and 8% 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), pH 7.4. The sample was then vortexed at maximum speed for 5 s and incubated on ice for 5 min. During incubation, the sample was further vortexed at maximum speed every minute. Subsequently, 800 μL of sample buffer containing 20 mM HEPES, 2 mM ethylene glycol tetraacetic acid (EGTA), 420 mM mannitol, and 140 mM sucrose (pH 7.4), were added. The sample was then shaken by inversion and centrifuged at 700 ×*g* for 10 min at 4 °C to remove nuclei and cellular debris. The supernatant was finally collected and centrifuged at 3000 ×*g* for 15 min at 4 °C to obtain the mitochondrial pellet.

Mitochondrial proteins were solubilized by resuspending the pellet in 40 μL of solubilization buffer containing 50 mM tris(hydroxymethyl)aminomethane hydrochloride (TRIS/HCl), 150 mM NaCl, 1% Nonidet P-40 (NP-40), and 0.5% sodium dodecyl sulphate (SDS), pH 8.0, and incubated on ice for 30 min. The suspension was subsequently centrifuged for 2 min at 12,000 ×*g* and the supernatant, containing solubilized mitochondrial proteins, collected and stored at −80 °C until analysis.

For the preparation of whole-tissue extracts, the original pellet was resuspended in 400 μL ice-cold lysis buffer containing 50 mM TRIS/HCl, 2 mM EGTA, 1% SDS, and 0.01% protease inhibitor cocktail (EMD Millipore, Billerica, MA), pH 7.5. The sample was then homogenized using a Dounce homogenizer and centrifuged at 12,000 ×*g* for 10 min at 4 °C. The supernatant, corresponding to the whole-tissue protein extract, was aliquoted and stored at −80 °C until analysis. Protein concentration in the mitochondrial fraction and whole-tissue extracts was determined by the Bradford method.

2.5. Western immunoblotting

Mitochondrial and total proteins (50 μg) were separated by gel electrophoresis on 10–15% polyacrilamide gels and wet-transferred onto nitrocellulose membranes at 4 °C. Membranes were subsequently blocked at room temperature in TBS with 0.1% Tween (TBS-t) containing 20 mM TRIS/HCl, 150 mM NaCl, and 5% BSA (pH 7.5) for 1 h, and incubated overnight with primary antibodies at 4 °C (Table 1). Membranes were then washed in TBS-t and incubated with appropriate horseradish peroxidase-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA), 1:5000, for 1 h at room temperature. Finally, the Immun-Star™ WesternC™ Chemiluminescent Solution Kit (Bio-Rad, Hercules, CA) was applied, and the chemiluminescent signal captured with a Chemidoc XRS imager (Bio-Rad). Digital images were analyzed using the Image Lab software (Bio-Rad). For whole-tissue extracts, spot density of target bands was normalized to glyceraldehyde 3-phosphate dehydrogenase (GADPH) loading controls. For the mitochondrial fraction, the total amount of protein loaded in each lane, as determined by densitometric analysis of the corresponding Ponceau S-stained membranes, was used as the loading control, as previously described