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Upregulation of MuRF1 and MAFbx participates to muscle wasting upon gentamicin-induced acute kidney injury

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ABSTRACT

Acute Kidney Injury (AKI) is frequently encountered in hospitalized patients where it is associated with increased mortality and morbidity notably affecting muscle wasting. Increased protein degradation has been shown to be the main actor of AKI-induced muscle atrophy, but the proteolytic pathways involved are poorly known. The Ubiquitin Proteasome System (UPS) is almost systematically activated in various catabolic situations, and the E3 ligases MuRF1 and MAFbx are generally up regulated in atrophying muscles. We hypothesized that the UPS may be one of the main actors in catabolic skeletal muscles from AKI animals. We used gentamicin-induced acute kidney disease (G-AKI) in rats fed a high protein diet to promote acidosis. We first addressed the impact of G-AKI in the development of mild catabolic conditions. We found that both muscle atrophy and UPS activation were induced with the development of G-AKI. In addition, the phasic muscles were more sensitive to 7-days G-AKI (−11 to −17%, $P < 0.05$) than the antigravity soleus muscle (−11%, NS), indicating a differential impact of AKI in the musculature. We observed an increased expression of the muscle-specific E3 ligases MuRF1 and MAFbx in phasic muscles that was highly correlated to the G-AKI severity ($R^2 = 0.64$, $P < 0.01$ and $R^2 = 0.71$, $P < 0.005$ respectively). Conversely, we observed no variation in the expression of three other E3 ligases (Nedd4, Trim32 and Fbxo30/MUSA1). Altogether, our data indicate that MuRF1 and MAFbx are sensitive markers and potential targets to prevent muscle atrophy during G-AKI.

1. Introduction

Mortality remains high in acute kidney injury failure (AKI) despite different support strategies. Even a moderate acute renal failure, which is the majority of medical cases, is associated with increased mortality (Chertow et al., 2005). This has recently led to the revised definition of AKI (Acute Kidney Injury Work Group, 2012). AKI patients exhibit negative nitrogen balance linked to an increased protein catabolism. Different factors are responsible for the development of a hyper catabolism in AKI patients, like insulin resistance, circulation of inflammatory cytokines, acidosis, increased secretion of catabolic hormones, and inadequate delivery of nutritional substrates. Nitrogen loss induces a marked decrease in lean body mass in AKI patients and is associated with impaired protein metabolism, gluconeogenesis and changes in amino acid metabolism, which complicates nutritional intervention (Lee et al., 2004; Druml, 2005).

In uremic rats, increased protein and branched chain amino acids degradation and little or no variation of protein synthesis have been observed (Clark and Mitch, 1983; Flugel-Link et al., 1983 and Price et al., 1998). However, these studies were carried out using nephrectomized rats, animals were used 30–48 h after surgery with both food and water being removed at least 4 h before experiments. Surgery-linked stress may have impacted the

Abbreviations: UPS, ubiquitin proteasome system; AKI, acute kidney injury; ATN, acute tubular necrosis; CKD, chronic kidney disease; MuRF1, muscle ring finger protein-1; MAFbx, muscle atrophy F-box protein; MUSA1, muscle ubiquitin ligase of the SCF complex in atrophy-1; Nedd4, neuronally expressed developmentally down-regulated 4 protein; Trim32, tripartite motif-containing protein 32; Ub, ubiquitin; EDL, extensor digitorum longus; TGF, transforming growth factor.

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conclusions, as protein degradation is sensitive to both stress and trauma (Baliga and Shah, 1991 and Lecker et al., 2006). By contrast, few studies addressed the mechanisms of muscle protein wasting upon AKI from acute tubular necrosis (ATN). In human, ATN from toxic exposure, notably of aminoglycosides such as gentamicin, is one of the most frequent causes of AKI. A single study used a treatment of gentamicin (7 days) to induce G-AKI in rats and showed that muscle protein degradation was increased upon G-AKI in incubated epitrochlearis muscle with no significant variation in protein synthesis (Baliga and Shah, 1991).

Upon 7/8 nephrectomy, the caspases and ubiquitin proteasome system (UPS) are the main proteolytic systems activated in rat skeletal muscles (Du et al., 2004; Workeneh et al., 2006). The relative importance of each pathway in renal failure-linked muscle wasting is not fully understood, but the UPS is known for being the main actor of skeletal muscle catabolism in most catabolic situations (Temparis et al., 1994; Taillandier et al., 1996a,b; Bodine et al., 2001; Clarke et al., 2007; Fielitz et al., 2007; Heng et al., 2008; Polge et al., 2011). The UPS is a highly sophisticated pathway involving hundreds of proteins that controls most cellular pathway [see (Ciechanover and Stanhill, 2014) for a recent review]. The target proteins are labeled with a ubiquitin chain by an enzymatic cascade. The small protein ubiquitin is activated by an E1, transferred onto one of the 35 ubiquitin-conjugating enzymes (E2s) and covalently linked to the substrate recognized by one of the numerous E3 ligases (>600). The E2 and E3 enzymes define the specificity and allow the UPS to potentially target any cellular protein (Ravid and Hochstrasser, 2008 and Polge et al., 2015). Indeed, the muscle-specific E3 ligases MuRF1 and MAFbx were reported to be upregulated in tens multiple catabolic models (see Bodine and Baehr, 2014 for a review) but other E3 ligases like Nedd4 and fbxo30/MUSA1 are also important regulators of muscle atrophy in denervated muscles (Nagpal et al., 2012; Sartori et al., 2013). However, the ubiquitous upregulation of the latters in other catabolic situations still needs to be investigated. Amongst these E3 ligases, MuRF1 is the only one that targets the main contractile proteins actin, myosin and troponin I for subsequent degradation by the UPS (Kedar et al., 2004; Clarke et al., 2007; Fielitz et al., 2007; Polge et al., 2011). We previously demonstrated that telethonin, a small sarcomeric protein implicated in sarcomere stability, is also an UPS substrate during severe atrophying situations and others found that it may interact with MuRF1 (Heng et al., 2008; Witt et al., 2008). This suggests that the UPS may be implicated in the degradation of most, if not all, sarcomeric proteins, with MuRF1 as a key player.

By contrast with renal failure caused by partial nephrectomy, the mechanism of skeletal muscle protein wasting has not been evaluated in an acute tubular necrosis model despite its high negative impact in patients. Thus, the goal of our study was to determine whether the UPS is activated during toxic G-AKI-linked muscle wasting. Using a rodent model of toxic acute tubular necrosis (G-AKI), we obtained a moderate catabolic situation in which we mainly focused on the potential activation of muscle atrophy-linked E3 ligases as they may represent early and sensitive markers of UPS activation.

2. Materials and methods

2.1. Materials

Antibody recognizing polyubiquitin (polyUb) chains was from Millipore (clone FK1, Millipore, Darmstadt, Germany). Anti-telethonin and anti-MuRF1 antibodies were from SantaCruz (SantaCruz Biotechnology, CA, USA) and anti-MAFbx was from ECMBiosciences (Versailles, KY, USA), anti-phospho AKT, anti-AKT, anti-phospho p70 S6 kinase, anti p70 S6 kinase, anti-phospho S6 and anti-S6 were from Cell Signaling (Cell Signaling technology, MA, USA). Fluorescent secondary antibodies were from ScienceTec (ScienceTec, Courtaboeuf, France) and detection was performed using a Li-COR Odissey Imager (LI-COR Biosciences, Lincoln, NE, USA). Membrane loading was determined using Blot-FastStain (G-Biosciences). Densitometric analysis of images was performed using ImageJ v. 1.47 software. Oligonucleotides for amplifying cDNAs encoding for MAFbx, MuRF1, Nedd4, Fbxo30/MUSA1 and Trim32, YWHAZ, HPRT1 and 36B4 were designed using Primer3 software and purchased from Sigma-Aldrich (St Louis, MO, USA). (see Suppl. Table 1 for primers in the online version at DOI: 10.1016/j.biocel.2016.04.006).

Table 1
Muscle weight and biochemical data from control (CT) and gentamicin-induced kidney injury (G-AKI) animals.

	CT	G-AKI
Urea (mmol/l)	7.1 ± 3	58 ± 30*
Creatinin (μmol/l)	23 ± 3.4	135 ± 89*
Bicarbonate (mmol/l)	27 ± 4	20 ± 3*
Weight increase, D7-14 (g)	24 ± 10	6.5 ± 5*
Soleus		
Muscle mass, mg	87 ± 9	78 ± 11
Muscle wt per whole body wt, mg/100 g	43 ± 1.3	41 ± 1.6
EDL		
Muscle mass, mg	104 ± 8	89 ± 4*
Muscle wt per whole body wt, mg/100 g	48 ± 3	45 ± 1*
Tibialis anterior		
Muscle mass, mg	404 ± 35	364 ± 15*
Muscle wt per whole body wt, mg/100 g	188 ± 11	183 ± 10
Gastrocnemius		
Muscle mass, mg	1280 ± 94	1142 ± 57*
Muscle wt per whole body wt, mg/100 g	597 ± 26	574 ± 22

Weight increase of animals was determined between day 7 and 14 (D7-14), i.e. when saline (CT) or gentamicin (120 mg kg^{-1} d^{-1}) were injected; EDL, extensor digitorum longus; Values are means ± SE, n = 4-6; *p < 0.05 vs. control (CT).

2.2. Animals

The experiments were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals. All animals were maintained in a temperature-controlled room (22 ± 1C) with a 12:12 h light:dark cycle. Acute tubular necrosis (ATN) was induced in rats by subcutaneous injection of gentamicin (aminoglycoside) at a dose of 120 mg kg^{-1} d^{-1} (Baliga and Shah, 1991).

Six-week-old rats were acclimatized with a normal protein diet (NP diet) ad libitum for 7 days, and then with a high protein diet (HP diet) containing 46% protein for 7 more days. Animals were randomly separated into 2 groups (n = 6 per group). One group was injected subcutaneously daily with gentamicin for 7 days (concomitant to HP diet) to induce acute kidney injury (G-AKI group), while the control group (CT) received the same amount of 0.9% sodium chloride (normal saline). G-AKI animals were fed ad libitum and controls were pair-fed accordingly.

2.3. Muscle and sampling extraction

Rats were fasted the night before sacrifice to avoid the variability in protein metabolism that could end up with differential food absorption. Animals were anesthetized and venous blood was collected to determine serum urea, creatinine and bicarbonate. Skeletal muscles (Tibialis Anterior, Extensor Digitorum Longus [EDL], Gastrocnemius and Soleus) were removed and immediately frozen in liquid nitrogen and rats were sacrificed by exsanguination and cutting the diaphragm.

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