Toxicology and Applied Pharmacology 278 (2014) 190–199

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Mechanisms of cisplatin-induced muscle atrophy

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ARTICLE INFO

Article history:
Received 4 February 2014
Revised 23 April 2014
Accepted 1 May 2014
Available online 10 May 2014

Keyword:
Cisplatin
Muscle atrophy
Fatigue
Muscle atrophy F-box
Muscle RING finger-1

ABSTRACT

Fatigue is the most common side effect of chemotherapy. However, the mechanisms of "muscle fatigue" induced by anti-cancer drugs are not fully understood. We therefore investigated the muscle-atrophic effect of cisplatin, a platinum-based anti-cancer drug, in mice. C57BL/6J mice were treated with cisplatin (3 mg/kg, i.p.) or saline for 4 consecutive days. On Day 5, hindlimb and quadriceps muscles were isolated from mice. The loss of body weight and food intake under the administration of cisplatin was the same as those in a dietary restriction (DR) group. Under the present conditions, the administration of cisplatin significantly decreased not only the muscle mass of the hindlimb and quadriceps but also the myofiber diameter, compared to those in the DR group. The mRNA expression levels of muscle atrophy F-box (MAFbx), muscle RING finger-1 (MuRF1) and forkhead box O3 (FOXO3) were significantly and further increased by cisplatin treated group, compared to DR. Furthermore, the mRNA levels of myostatin and p21 were significantly upregulated by the administration of cisplatin, compared to DR. On the other hand, the phosphorylation of Akt and FOXO3a, which leads to the blockade of the upregulation of MuRF1 and MAFbx, was significantly and dramatically decreased by cisplatin. These findings suggest that the administration of cisplatin increases atrophic gene expression, and may lead to an imbalance between protein synthesis and protein degradation pathways, which would lead to muscle atrophy. This phenomenon could, at least in part, explain the mechanism of cisplatin-induced muscle fatigue.

Introduction

Chemotherapy compromises the patient's quality of life (QOL), an important indicator of patient outcome and survival in numerous cases (Montazeri, 2009). A component of QOL is the patient's perceived fatigue, which is one of the most common symptoms of cancer (Patrick et al., 2004). This type of fatigue is reported by cancer patients through QOL self-assessments and increases with the aggressiveness of chemotherapy (Cella et al., 2002; Prue et al., 2010; Stromgren et al., 2002; Wu and McSweeney, 2004). Over half of patients undergoing chemotherapy exhibit cognitive impairment (Jansen et al., 2011), which is associated with the patient's perceived fatigue (Bender et al., 2006). Although the documentation of perceived fatigue is useful clinically, it is difficult to discriminate this from physiological fatigue. Physiological fatigue can be divided into two components, central and peripheral. Central fatigue involves the central nervous system and the inhibition of neurological reflexes, whereas peripheral fatigue is more likely to be muscle-specific. Muscle fatigue is defined as a loss of force that can be reversed with rest, while muscle weakness is an impaired ability to generate force and is not relieved by rest (Wanner et al., 1990).

Muscle mass is maintained through an interplay between anabolic and catabolic pathways. The insulin-like growth factor-1 (IGF-1)/PI3K/Akt/ mammalian target of rapamycin (mTOR) pathway along with the ubiquitin–proteasome pathway and myostatin (Mstn) pathway maintain this homeostasis with the aid of various transcriptional and genetic factors (Banerjee and Guttridge, 2012). The activation of IGF-1 and Mstn signaling causes muscle hypertrophy and atrophy, respectively. Skeletal muscle atrophy is a debilitating consequence of multiple chronic diseases and conditions that involve starvation. It reduces treatment options and positive clinical outcomes as well as compromising QOL and increasing morbidity and mortality (Lynch, 2001; Zinna and Yarasheski, 2003). While considerable research has been undertaken to identify the molecular mechanisms that control skeletal muscle

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http://dx.doi.org/10.1016/j.taap.2014.05.001
0041-008X/

atrophy, the signaling pathways involved and effective treatments that are currently available to prevent, attenuate or reverse muscle atrophy are not clearly defined.

ActRIIB is a high affinity activin type 2 receptor and mediates the signaling by a subset of TGF-β family ligands including Mstn, activin, and others (Lee, 2008). Increased signaling by the ActRIIB pathway has been implicated in many cancers (Costelli et al., 2008; Seder et al., 2009; Wildi et al., 2001). Zhou et al. (2010) has been demonstrated that in cancer cachexia models, ActRIIB pathway blockade abolished the activation of the ubiquitin–proteasome system and the induction of muscle-specific E3 ubiquitin ligases in muscles.

The discovery of two muscle-specific E3 ubiquitin ligases, Muscle atrophy F-Box (MAFbx; also known as atrogin-1) and Muscle RING Finger-1 (MuRF1), prompted renewed interest in identifying muscle-specific targets for therapeutic manipulation. MAFbx and MuRF1 are parts of the ubiquitin–proteasome pathway, which is the primary pathway involved in intracellular protein degradation in skeletal muscle (Lecker et al., 1999). Consistent increases in their gene expression have been observed in a wide range of in vivo models of skeletal muscle atrophy including diabetes, cancer, renal failure, denervation, unweighting and glucocorticoid or cytokine treatment (Bodine et al., 2001; Gomes et al., 2001). The expression of MAFbx and MuRF-1 mRNAs increases dramatically in catabolic states and mice deficient in either of them are partially resistant to muscle atrophy. Therefore, it is most likely that MAFbx and MuRF-1 play crucial roles in the loss of muscle proteins and their mRNAs are now considered to be specific markers of atrophy.

Chemotherapy generally consists of the administration of anti-cancer drugs in a standardized treatment regimen, which is specific for the type of cancer. Cisplatin, a platinum-based anti-cancer drug, is one of the most effective broad-spectrum anti-cancer drugs used against cervical, head and neck, prostrate, breast, lung, testicular and ovarian cancers. However, fatigue, including muscle fatigue, is the most common side effect of cisplatin after/during treatment. Damrauer et al. (2008) previously has been reported that the effect of cisplatin on muscle wasting was mediated through activation of the NF-κB signaling pathway. However, few reports have addressed the mechanisms that underlie cisplatin-induced muscle atrophy. In the present study, we investigated the mechanisms of cisplatin-induced muscle fatigue.

Methods

Animals. Male C57BL/6J mice (8–9 weeks of age, 23–27 g) were used. All experiments were approved by the Animal Care Committee at Hoshi University (Tokyo, Japan).

Treatment protocol. Mice were given a single intraperitoneal injection of cisplatin (1 or 3 mg/kg) daily for 4 days, with saline (vehicle) used as a control (Fig. 1A). As the body weight of mice was decreased by the administration of cisplatin, we prepared dietary restriction (DR) as another vehicle control by carrying out pair feeding. Only 2, 2, 2 and 1.5 g meal and 4, 4, 3 and 3 mL water were given to the DR mouse at Days 0–1, 1–2, 2–3 and 3–4. Twenty-four hours after the final injection of cisplatin (Day 3), animals were killed under deep anesthesia with isoflurane, and the femoral quadriceps and hind limb muscles were removed, and their wet weight were measured. In RT-quantitative PCR (qPCR), quadriceps muscle and liver were removed, washed with cold saline, and stored in TRI Reagent™ (Sigma-Aldrich) at −80 °C. Referred to previous reports (Fujimoto, 2006; Khasabova et al., 2012), we injected cisplatin (1, 3 and 10 mg/kg) to the mice daily for 4 days in our preliminary study. As the mouse injected with cisplatin (10 mg/kg), 3 mice in 5 mice died. We thus determined the given dose as 3 mg/kg, i.p.

Histology. Standard hematoxylin and eosin (HE) staining was performed on quadriceps muscle from cisplatin or its vehicle treated mice. Briefly, the quadriceps muscle was removed from anesthetized mice, and then fixates with 10% formalin for paraffin embedding and sectioning followed by HE staining. To analyze the length of the longest minor axis (the perpendicular bisector of the major axis) as diameters of the myofibers, the sections were observed under a light microscope in quadriceps muscles using the ImagePro Plus (Media Cybernetics, Silver Spring, MD) as shown Fig. 2A. For each animal at least 100 fibers were measured.

RT-qPCR. mRNA levels of cytokines and AQPs were examined by real-time RT-PCR as described previously (Sakai et al., 2013). Briefly, total RNA was extracted from various tissues with a one-step guanidium–phenol–chloroform extraction procedure using TRI Reagent™ (Sigma-Aldrich). cDNAs were prepared from total RNA (1.0 μg) by using QuantiTect Reverse Transcriptase (Qiagen, Germany) after incubation with gDNA wipeout buffer at 42 °C for 3 min to remove contaminating genomic DNA. The reaction mixture (2 μL) was subjected to PCR (50 nM forward and reverse primers, Fast SYBR Green Mastermix; Applied Biosystems) in a final volume of 10 μL. The PCR primer sets used are shown in Table 1. The thermal cycle profile used was 1) denaturing for 30 s at 95 °C, and 2) annealing for 30 s at 60 °C. PCR amplification was performed for 40 cycles. Data are expressed as the expression relative to GAPDH mRNA as a housekeeping gene using the $2^{-\mathrm{deltadeltaCT}}$ method (Sakai et al., 2013). In the case of tissue distribution of gene expression, data are expressed as the expression relative to 18 s rRNA as a housekeeping gene using the $2^{-\mathrm{deltadeltaCT}}$ method.

Immunoblotting. Homogenates of quadriceps muscle were prepared as follows. In brief, the muscle tissues were removed and immediately soaked in ice-cold, saline. The tissue was then homogenized in ice-cold T-PER™ Tissue Protein Extraction Reagent (Pierce). The tissue homogenate was centrifuged (1000 ×*g*, 4 °C for 15 min), and supernatants were stored at −80 °C until use. To quantify the phosphorylation of Akt and forkhead box 3a (FOXO3a), Western blotting was performed. In brief, the samples (10 μg of total protein per lane) were subjected to 10–20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were then electrophoretically transferred to a PVDF (polyvinylidene difluoride) membrane. After being blocked with 3% bovine serum albumin, transferred PVDF membranes were incubated with the primary antibodies. Rabbit anti-MuRF1 (1:1000 dilution; ECM Biosciences), rabbit anti-MAFbx (1:1000 dilution; ECM Biosciences), rabbit anti-FOXO3a (1:1000 dilution; Cell Signaling), rabbit anti-Akt (1:1000; Cell Signaling), rabbit anti-phospho-Akt (1:1000; Cell Signaling), rabbit anti-phospho-FOXO3a (1:1000; Cell Signaling), rabbit anti-Smad2 (1:1000; Cell Signaling) and rabbit anti-phosphor-Smad (1:1000; Cell Signaling) were used as a primary antibody. The membranes were then incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (Ig) G (1:5000 dilution; GE Healthcare), and detection was performed with an ECL system. The housekeeping gene was detected on the same membrane by using monoclonal mouse anti-GAPDH (1:5000 dilution; Chemicon International) and horseradish peroxidase-conjugated sheep anti-mouse IgG (1:5000 dilution; GE Healthcare) to confirm that the same amounts of proteins were loaded. To normalize the phosphorylated protein contents to the total protein, the ratio of the corresponding phosphorylated Akt or FOXO3a to total Akt or FOXO3a were calculated as an index of phospho-Akt or phospho-FOXO3a. In the level of protein expression, to normalize the Akt or FOXO3a contents to GAPDH, the ratio of the corresponding Akt or FOXO3a to GAPDH was calculated as an index of Akt or FOXO3a.

Statistical analysis. The statistical significance of differences was determined by an unpaired Student t-test or one-way analysis of