Reduction of Skeletal Muscle Atrophy by a Proteasome Inhibitor in a Rat Model of Denervation

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The ubiquitin-proteasome system is the primary proteolytic pathway implicated in skeletal muscle atrophy under catabolic conditions. Although several studies showed that proteasome inhibitors reduced proteolysis under catabolic conditions, few studies have demonstrated the ability of these inhibitors to preserve skeletal muscle mass and architecture *in vivo*. To explore this, we studied the effect of the proteasome inhibitor Velcade (also known as PS-341 and bortezomib) in denervated skeletal muscle in rats. Rats were given vehicle or Velcade (3 mg/kg po) daily for 7 days beginning immediately after induction of muscle atrophy by crushing the sciatic nerve. At the end of the study, the rats were euthanized and the soleus and extensor digitorum longus (EDL) muscles were harvested. In vehicle-treated rats, denervation caused a 33.5 ± 2.8% and 16.2 ± 2.7% decrease in the soleus and EDL muscle wet weights (% atrophy), respectively, compared to muscles from the contralateral (innervated) limb. Velcade significantly reduced denervation-induced atrophy to 17.1 ± 3.3% in the soleus ($P < 0.01$), a 51.6% reduction in atrophy associated with denervation, with little effect on the EDL (9.8 ± 3.2% atrophy). Histology showed a preservation of muscle mass and preservation of normal cellular architecture after Velcade treatment. Ubiquitin mRNA levels in denervated soleus muscle at the end of the study were significantly elevated 120 ± 25% above sham control levels and were reduced to control levels by Velcade. In contrast, testosterone proprionate (3 mg/kg sc) did not alleviate denervation-induced skeletal muscle atrophy but did prevent castration-induced levator ani atrophy, while Velcade was without effect. These results show that proteasome inhibition attenuates denervation-induced muscle atrophy *in vivo* in soleus muscles. However, this mechanism may not be operative in all types of atrophy. Exp Biol Med 231:335–341, 2006

Key words: ubiquitin-proteasome pathway; Velcade; PS-341; skeletal muscle atrophy; sarcopenia; muscle denervation

Introduction

Skeletal muscle mass is a balance between protein synthesis and degradation. An imbalance such that proteolysis prevails over synthesis is associated with skeletal muscle atrophy. For instance, a decrease in circulating growth hormone or androgen levels as a consequence of aging causes a decreased rate of protein synthesis that leads to skeletal muscle wasting or sarcopenia (1, 2). When the rate of protein degradation exceeds that of protein synthesis as a consequence of disease states, such as cancer cachexia, renal failure, sepsis, and skeletal muscle denervation, skeletal muscle atrophy ensues (3–7). Although sarcopenia of aging and other forms of muscle wasting are important clinically, there are few therapeutic options for the treatment of any form of skeletal muscle atrophy.

Although there is evidence for the involvement of several regulatory mechanisms in the breakdown of myofibrillar proteins, it is thought that protein degradation in skeletal muscle occurs primarily through the ATP-dependent ubiquitin-proteasome pathway (9, 10, 15). In this pathway, proteins are marked for degradation by the attachment of several moieties of the 76-amino acid polypeptide, ubiquitin. Ubiquitination is mediated by a series of enzymes: E1, or ubiquitin-activating enzyme; E2, or ubiquitin-conjugating enzyme; and E3, or ubiquitin ligase. The polyubiquitinated proteins are degraded by the proteasome, a large multi-subunit protease complex, into short oligopeptides that are rapidly degraded to amino acids by cytosolic peptidases.

There are several chemical classes of compounds that inhibit proteasomal activity, including peptide analogs of substrates with different C-terminal groups, such as aldehydes, epoxyketones, boronic acids, and vinyl sulfones (11, 12). A selective boronic acid proteasome inhibitor, Velcade (also known as PS-341 and bortezomib), directly inhibits the proteasome complex without direct effects on ubiquitination. This potent compound is orally active and is presently approved for treating multiple myeloma (13, 14).

In a variety of muscle-wasting conditions, the ubiquitin-proteasome pathway is largely responsible for the

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degradation of skeletal muscle proteins and is implicated as a therapeutic target for skeletal muscle wasting (9, 10, 15). Although there are numerous reports of inhibitors of proteasomal activity reducing skeletal muscle proteolysis *ex vivo* under conditions of muscle wasting (15–19), few studies measuring the effect of these inhibitors on muscle mass and architecture *in vivo* have been done. It is unclear if the changes in proteolysis observed *ex vivo* can predict changes in muscle mass. To explore the effect of proteasome inhibitors on skeletal muscle mass and histopathology during skeletal muscle wasting, we determined the effect of the proteasome inhibitor Velcade in an *in vivo* model of skeletal muscle wasting induced by denervation of the rat hind limb (6, 20). In this denervated muscle model, the soleus muscle (type I, slow-twitch) and extensor digitorum longus (EDL) muscle (type II, fast twitch) undergo rapid atrophy and increased protein turnover after cutting or crushing the sciatic nerve (6, 20). While the proteasome pathway appears to be the primary mediator of this muscle atrophy, calcium-dependent or lysosomal pathways are implicated, although to a lesser degree (6).

For comparison, we determined the effect of testosterone on denervated hind limbs, although testosterone is not particularly active in rodent skeletal muscle, except in the levator ani muscle (21–23). To make sure we were using an efficacious dose of testosterone, we determined its effects on the levator ani muscle in castrated rats. The levator ani muscle (type II, fast twitch) undergoes atrophy and apoptosis with depleted testosterone (21, 24). We also tested the effect of Velcade on the levator ani muscle in castrated animals.

The results from the hind limb–denervation study show that proteasome inhibition attenuated atrophy in the denervated soleus muscle with less effect on the EDL. Testosterone had no effect in denervated soleus or EDL muscles in doses sufficient to rescue levator ani muscle in castrated rats, whereas Velcade had no effect on the levator ani.

Methods

All animal test methods used in these studies were approved by the Institutional Animal Care and Use Committee. The animals were cared for according to American Association for the Accreditation of Laboratory Care guidelines. Sprague-Dawley rats were obtained from Harlan Teklad (Madison, WI). Testosterone proprionate was acquired from Sigma-Aldrich (St. Louis, MO), and Velcade (Pyz-Phe-boroLeu; Pyz, 2,5-pyrazinecarboxylic acid) was synthesized at Bristol-Myers Squibb. Statistical analyses of the data were performed using ANOVA (Newman-Keuls post-hoc test).

Sciatic Denervation. Male Sprague-Dawley rats (200–250 g) underwent sciatic denervation surgery under anesthesia (ketamine 60–80 mg/kg plus xylazine 8–10 mg/kg ip) using aseptic techniques. The right hind limb was denervated with a hemostat by crushing the sciatic nerve immediately proximal to the division of the peroneal and tibial branches. The contralateral leg was left intact. Denervation was known to be complete by observing post-operative disuse of the lower limb and loss of a withdrawal response to a pinch in the sciatic distribution area. No dysfunction of the contralateral leg was observed, and no behavioral aberrations were observed. Crushing the nerve effectively eliminated autotomy (self-mutilation) observed with nerve transection (25). For the purpose of comparison, one group of rats received a sham operation in which the sciatic nerve was exposed but not crushed and was not exposed to drug treatment.

The rats were divided into treatment groups ($n = 5$ per group) and administered vehicle (0.5% DMSO in 0.9% saline po), Velcade (3 mg/kg po), or testosterone proprionate (3 mg/kg sc in a 10% ethanol/90% peanut oil vehicle) immediately after surgery and then once daily for the duration of the 7-day study. At the end of the study, the rats were euthanized by CO_2 asphyxiation, and the soleus and EDL muscles were harvested from both denervated and innervated (contralateral) hind limbs, weighed, and cut into two equal pieces. One piece was immediately frozen in liquid nitrogen for mRNA purification and the other was fixed in 10% neutral buffered formalin. As a means for comparison, the wet weight of the muscles from the denervated limb was measured against the muscles from the nonsurgical, contralateral limb. To normalize muscle wet weight to body weight, the data were standardized as mg/100 g of body weight. For histological evaluation, the fixed muscles were paraffin embedded, sectioned 6- to 8-μm thick, and stained with hematoxylin-eosin. The slides were evaluated by a pathologist who was blinded to the treatment.

The Levator Ani Muscle Assay in the Rat. Male Sprague-Dawley rats (200–250 g) were surgically castrated under anesthesia (ketamine 60–80 mg/kg plus xylazine 8–10 mg/kg ip) using aseptic techniques to deplete the rat of androgens and consequently cause atrophy of the levator ani muscle. Five animals were used for each test group. Intact (sham surgery, $n = 5$) rats were included for the purpose of comparison. To determine the effect of exogenous addition of androgen or the proteasome inhibitor Velcade on skeletal muscle wet weight, testosterone proprionate (3 mg/kg sc in a 10% ethanol/90% peanut oil vehicle) or Velcade (3 mg/kg po in a 0.5% DMSO/0.9% saline vehicle) was administered immediately after surgical castration and once daily thereafter for 10 days. At the end of the 10-day treatment period, the rats were euthanized by CO_2 asphyxiation, and the levator ani muscle was harvested, weighed, and flash frozen in liquid nitrogen for mRNA purification. To normalize muscle wet weight to body weight, the data were standardized as mg of levator ani muscle/100 g of body weight.

Proteasomal Activity Assay. Rat blood was drawn from the tail vein of denervated rats just described 1 hr after administration of vehicle or Velcade on Day 1 and at the end of the study on Day 7. The *ex vivo* 20S proteasome activity assay was performed as previously described (26).

In brief, 20 μl of heparinized rat blood was washed with 200 μl of phosphate-buffered saline and centrifuged at 6600 *g* for 10 min at 4°C. The resulting pellet was resuspended in 200 μl phosphate-buffered saline and centrifuged again. EDTA (200 μl of 5 m*M*; pH 8.0) was added to the pellet for cell lysis, left on ice for 15 min, and then centrifuged at 6600 *g* for 10 min at 4°C. The resulting supernatant was used for protein determination and the 20S proteasome assay. Protein content was determined using a Bio-Rad DC protein assay. For measurement of proteolytic activity, sample (80 μg protein per sample well) and buffer (20 m*M* HEPES, 0.5 m*M* EDTA, 1% DMSO; pH 8.0) containing fluorogenic peptide substrate (40 μ*M* Chymotrypsin Suc-Leu-Leu-Val-Tyr_AMC; Bachem, Bubendorf, Switzerland) are mixed, incubated 4 hrs at 37°C, and hydrolysis measured using a Millipore Cytoflor plate reader (excitation: 360, emission: 480).

Ubiquitin mRNA Expression. Soleus, EDL, and levator ani muscles were weighed, harvested, and placed into liquid nitrogen. The muscles were individually pulverized in Trizol Reagent (Life Technologies Inc., Carlsbad, CA) using Lysing Matrix D pulverizing beads (Q-BIOgene Inc., Irvine, CA) and then processed according to the Trizol method to isolate total RNA. Each sample was subsequently DNase treated to rid the sample of contaminating genomic DNA and then further purified by LiCl precipitation. cDNA was synthesized from the purified RNA by reverse transcription of 2.5 μg of total RNA using random hexamers and Superscript II reverse transcriptase according to the manufacturer's recommendations (Life Technologies). Real-time quantitative polymerase chain reaction (PCR) was performed on the Applied Biosystems 7700 Sequence Detection System using AmpliTaq Gold PCR Master Mix and a 1-μl aliquot of the resultant cDNA with the following primers (300 n*M* final concentration for each) and probe (200 n*M* final concentration): Ubiquitin-F 5′-cggcaagaccatcacccta-3′, Ubiquitin-R 5′-ggatcttggccttcacgttc–3′, Ubiquitin-Probe VIC-tggagcccagtgacaccatcg-TAMRA (Applied Biosystems, Foster City, CA). Rat GAPDH primers/probe were used for normalization and acquired as a prepackaged and validated kit from Applied Biosystems. Thermocycling conditions were 50°C/2 min, denaturation of template and activation of AmpliTaq Gold® at 95°C/10 min, and amplification (95°C/15 secs, 60°C/60 secs) for 40 cycles. Quantities of mRNA for both ubiquitin and GAPDH were determined using the relative standard curve method. Amplification efficiencies for the ubiquitin and GAPDH primers/probe set were nearly identical based on slopes of respective standard curves (–3.45 for ubiquitin and –3.42 for GAPDH).

Results

Effects of Velcade on Denervated Soleus and EDL. The *ex vivo* 20S proteasome activity assay in whole blood was used as a surrogate measure to determine the effectiveness of Velcade at inhibiting proteasomal activity after oral administration in rats without having to euthanize the animals. The results showed that oral administration of Velcade inhibits blood proteasomal activity by 81 ± 3% (mean ± SE) at 1 hr after the first dosing and is maintained at 70 ± 7% inhibition 1 hr after dosing on Day 7. These data show that Velcade is effectively absorbed into the blood. This dose has been previously shown to inhibit tissue proteasomal activity in tumor-bearing rodents (26).

The effects of Velcade and testosterone on denervated rat skeletal muscle are listed in Table 1 and illustrated in Figure 1. In the vehicle-treated rats, denervation caused a 33.5 ± 2.8% decrease in soleus wet weight and a 16.2 ± 2.7% decrease in EDL wet weight after 7 days when compared with muscles from the contralateral (innervated)

Table 1. Effects of Velcade on Denervated Soleus and EDL Muscle[a]

Treatment	Soleus		
	Denervated wet weight (mg/100 g body weight)[b]	Contralateral wet weight (mg/100 g body weight)[b]	% atrophy[c]
Sham	38.8 ± 1.5	40.0 ± 1.6	2.8 ± 3.0**
Vehicle control	26.3 ± 1.5	39.4 ± 1.1	33.5 ± 2.8
Velcade	33.0 ± 1.4	39.8 ± 1.3	17.1 ± 3.3**
Testosterone	22.6 ± 1.9	37.6 ± 0.8	39.8 ± 4.7
	EDL		
	Denervated wet weight (mg/100 g body weight)[b]	Contralateral wet weight (mg/100 g body weight)[b]	% atrophy[c]
Sham	38.7 ± 1.2	41.2 ± 1.8	5.7 ± 2.9*
Vehicle control	33.7 ± 1.2	40.2 ± 0.6	16.2 ± 2.7
Velcade	34.5 ± 1.3	38.4 ± 1.2	9.8 ± 3.2*
Testosterone	30.8 ± 1.3	38.4 ± 2.3	19.4 ± 2.2

[a] Data are mean ± SE ($n = 5$).
[b] Body weights were within the range of 200 to 250 g.
[c] % atrophy represents change in wet weight of the denervated muscle compared with muscle from the contralateral (innervated limb).
** $P < 0.01$ and * $P < 0.05$ (compared with vehicle control) as determined by ANOVA (Newman-Keuls test).

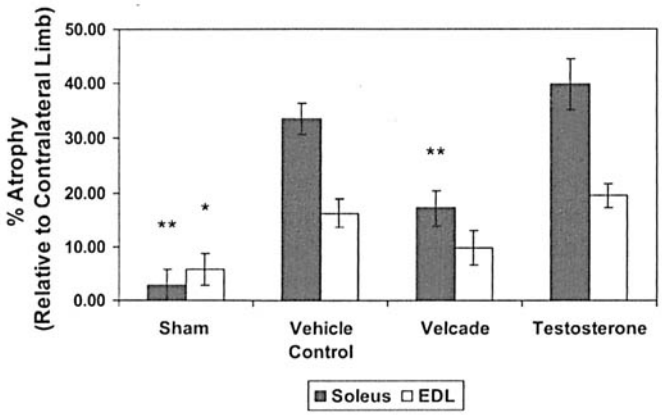

Figure 1. Inhibition of muscle wasting by proteasome inhibitor in the denervated rat skeletal muscle model. Data are presented as percent atrophy for each group from its respective contralateral, innervated hindlimb such that each animal serves as its own control. Little effect was seen for sham animals, and denervation reduced both soleus and EDL mass. Velcade significantly attenuated loss of muscle mass due to denervation in the soleus but not the EDL muscle. Values are means ± SE. ** = $P < 0.01$ and * = $P < 0.05$ (compared with vehicle denervated, denoted as vehicle control in graph) as determined by ANOVA (Newman-Keuls test).

limb. No significant change in muscle wet weights was noted in rats that received sham surgery when compared with their contralateral limb. Velcade significantly reduced denervation-induced atrophy to 17.1 ± 3.3% in the soleus ($P < 0.01$), a 51.6% reduction in atrophy associated with denervation, while it had little effect on the EDL (9.8 ± 3.2% atrophy). The attenuation of atrophy by Velcade in soleus muscles was significant, although the weights were not completely returned to those seen in sham animals. Not surprisingly, treatment with the anabolic steroid testosterone proprionate caused no net change on the wet weight of the denervated muscles when compared with the vehicle-treated controls (Table 1 and Fig. 1). On average, the wet weight of nerve-intact soleus and EDL muscles was 96.7 ± 3.8 mg (mean ± SE) and 99.7 ± 4.3 mg, respectively.

Histologically, Velcade significantly ($P < 0.01$) attenuated the reduction in size of individual muscle fibers in the soleus after denervation in a similar manner to which it attenuated wet weight (Fig. 2). In sham control rats, soleus muscle fiber diameter was 93.0 ± 2.4 μm (mean ± SE) compared with 59.7 ± 2.2 μm in the vehicle-treated denervated rats and 78.0 ± 2.4 μm in Velcade-treated denervated rats. In addition, histological analysis revealed no significant compound-related adverse effects in skeletal muscle from Velcade-treated rats (Fig. 2). Both soleus and EDL muscle from vehicle-treated rats showed atrophy of individual muscle fibers and a moderate, diffuse increase in nuclear size and numbers of satellite cells suggestive of a regenerative or compensatory response. Short-term denervation stimulates satellite cells to enter the mitotic cycle (27). Activated satellite cells form new myotubes on muscle fibers reactivating myogenesis between 10 and 21 days after experimental nerve transection in rats (28). Atrophic fibers were less frequent in skeletal muscle from Velcade-treated rats, but the satellite cell proliferative changes were unaltered by treatment. There were no other histological changes observed in skeletal muscle from Velcade-treated rats compared with non-denervated muscle.

Ubiquitin mRNA Expression in Denervated Rat Soleus. The effect of denervation alone or in combination with Velcade treatment on the mRNA expression of ubiquitin in soleus muscle is illustrated in Figure 3. After 7 days of denervation, polyubiquitin mRNA expression from vehicle-treated denervated soleus muscle was elevated 120 ± 25% above sham surgery control levels and was significantly reduced by Velcade to 23 ± 4% above sham

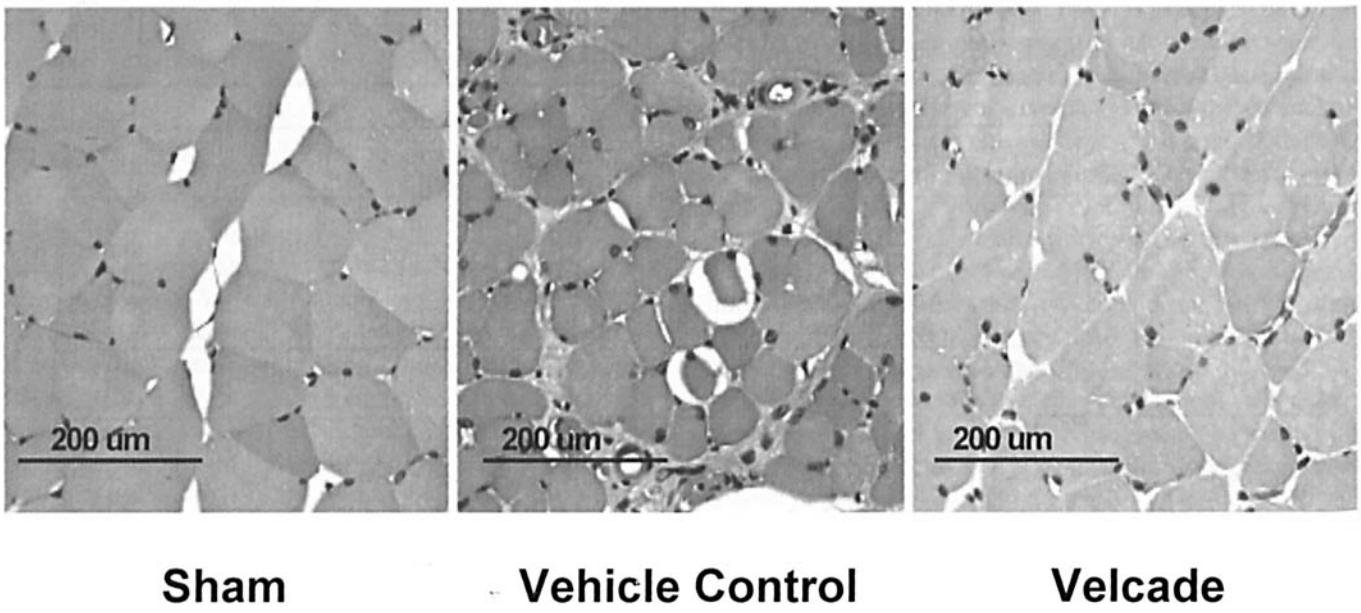

Figure 2. Histology of rat denervated skeletal muscle with and without proteasome inhibitor treatment with Velcade relative to sham (nerve intact) control. Sections were stained with hemâtoxylin-eosin.

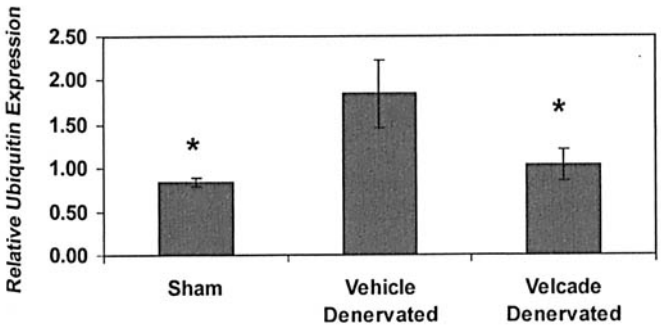

Figure 3. Induction of polyubiquitin mRNA expression (normalized to GAPDH) in rat denervated soleus muscle and suppression by the proteasome inhibitor Velcade. Values are means ± SE. * = $P < 0.05$ (compared with vehicle denervated) as determined by ANOVA (Newman-Keuls test).

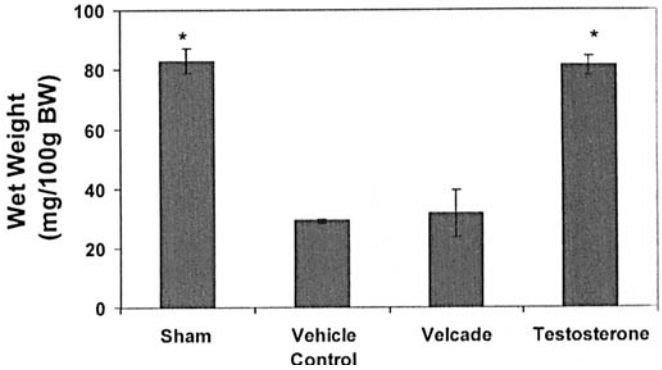

Figure 4. Prevention of castration-induced levator ani muscle atrophy by testosterone. Velcade had no effect in this model of muscle atrophy. Values are means ± SE. * = $P < 0.05$ (compared with vehicle castrated) as determined by ANOVA (Newman-Keuls test).

control levels ($P < 0.05$ compared with vehicle-treated denervated soleus). Therefore, polyubiquitin mRNA was returned nearly to baseline levels.

Effects of Testosterone or Velcade on the Levator Ani Muscle in Castrated Rats. Because testosterone did not attenuate muscle wasting after denervation, we tested the same dose in levator ani muscles to ensure an efficacious dose. Castration (vehicle treatment) caused a 65% reduction in the wet weight of the levator ani muscle 10 days after castration surgery (82.9 ± 4.2 and 29.3 ± 0.6 mg/100 g body wt for sham and vehicle-treated, castrated rats, respectively [Fig. 4]). Treatment with Velcade caused no change in levator ani wet weight (31.8 ± 7.9 mg/100 g body wt) compared with the vehicle-treated, castrated group. These data are consistent with the absence of an elevation in ubiquitin mRNA expression in the levator after castration (data not shown). As expected, testosterone treatment (3 mg/kg) prevented levator ani muscle atrophy (81.1 ± 3.1 mg/100 g body wt) as a consequence of castration.

Discussion

Skeletal muscle atrophy is associated with many disease states, including cancer, AIDS, sepsis, diabetes, burn injury, inactivity, aging (sarcopenia), and nerve injury (2–6, 9, 29). Much needs to be learned about the mechanisms attributing to skeletal muscle atrophy associated with each of these disease states. The development of therapies designed to prevent or attenuate skeletal muscle atrophy may fill an important, unmet medical need.

The mechanisms that maintain skeletal muscle protein homeostasis involve a balance between protein synthetic mechanisms and protein breakdown. For most conditions in humans, this is primarily reflective of an increased breakdown of skeletal muscle proteins (8–10). Although there are multiple mechanisms that can contribute to muscle catabolism, the ubiquitin-proteasome pathway is now recognized as the major proteolytic pathway involved, although calcium-dependent or lysosomal pathways can induce modest levels of myofibrillar proteolysis (8–10). Therefore, pharmacological inhibition of the ubiquitin-proteasome pathway is one approach to attenuate muscle atrophy. Velcade is a dipeptidyl boronic acid that is a direct inhibitor of the proteasome (11–13, 26). It is well distributed in the body and does not cross the blood-brain barrier. A high percentage of proteasome inhibition by Velcade is well tolerated (14, 26). Other proteasome inhibitors are known, such as peptide aldehydes, epoxomicin, pentoxifylline, and lovastatin, although many are not ideal tools for *in vivo* studies due to multiple actions and poor pharmacokinetic activity (11, 17, 18).

Previous well-executed studies showed reduced rates of skeletal muscle proteolysis *ex vivo* using proteasome inhibitors (15–19), setting the stage for our studies that sought to determine whether the reduction in proteolysis translates into improved muscle mass *in vivo* after denervation. The rat denervation model causes rapid and marked skeletal muscle atrophy, especially in the soleus muscle. Consistent with the atrophy was an activation of satellite cells most likely seen as a compensatory response (27, 28). Treatment with the proteasome inhibitor Velcade significantly reduced the denervation-induced atrophy in the soleus muscle with an insignificant effect on the EDL muscle. Of course, other proteolytic mechanisms, such as calcium-dependent or lysosomal pathways, may also be important in denervation atrophy (8–10). This may explain why the soleus muscle was only partially rescued by Velcade and perhaps why there was minimal effect on the EDL. This could also be an issue related to dose. We gave what we thought was a maximum tolerated dose of Velcade, which may not be the same as the maximum inhibitory dose.

Ubiquitin mRNA increased after denervation in our studies as in previous reports (20). Interestingly, Velcade attenuated this response in soleus muscle. It is not clear why Velcade caused a reduction in ubiquitin mRNA, because it does not directly affect the ubiquitination process. This suggests a feedback mechanism by which proteasomal activity at some level activates the ubiquitin gene. Previous studies are conflicting, with one study showing reduced ubiquitin message with proteasome inhibition (18) and

another showing increased ubiquitin message after proteasome inhibitor treatment after burn injury (16). These conflicting data highlight how little we know about the regulatory pathways involved in regulation of ubiquitin-proteasomal activity.

Testosterone did not attenuate denervation-induced muscle atrophy, although testosterone prevented atrophy of the rat levator ani muscle in castrated rats as previously shown (21–23) while Velcade was ineffective. Testosterone is not effective in preventing muscle atrophy after disuse in the rat, possibly because of low expression of androgen receptors in rat skeletal muscle or because androgens work best in combination with exercise (30, 31). Atrophy of the levator ani after loss of anabolic signaling is mediated through different mechanisms, such as apoptosis (24), which may account for the lack of effect of Velcade in this model.

Although the data presented here show some promise for the use of proteasome inhibitors in skeletal muscle–wasting syndromes, an obstacle facing proteasome inhibition is the ubiquitous expression of proteasomes and the possibility of unacceptable toxicity. Although the ubiquitin-proteasome pathway has a general function in intracellular protein turnover, it also plays a key role in many biological processes, such as MHC class 1 antigen presentation, cell division, and NF-κB activation (7, 9, 11, 12). Selectivity and a lack of toxicity are particularly critical for many elderly patients who may have some degree of muscle atrophy but are otherwise healthy. One potential strategy to accomplish this is to develop compounds inhibiting protein ubiquitination (32). The large number of isoforms of E2 and E3 enzymes identified may allow tissue-specific inhibition of ubiquitination (32, 33). Recent studies have shown skeletal muscle–selective E3 ligases that may be targets for drug development (34, 35). Muscle RING Finger 1 (MuRF1) and Muscle Atrophy F-box (MAFbx) are E3 ligases discovered by gene profiling and are expressed exclusively in striated muscle. MuRF1 and MAFbx genes are upregulated in models of skeletal muscle atrophy, and knockout mice lacking these genes show reduced muscle atrophy after denervation (34, 35).

In conclusion, we showed the protective effects of a proteasome inhibitor in an animal model with increased skeletal muscle proteolysis. Although a reduction in skeletal muscle atrophy was observed in denervated rats with Velcade treatment, it is uncertain if this translates into a preservation of muscle strength. It is also unknown how useful a proteasome inhibitor may be for muscles that are already atrophied. Clearly, more studies are needed to fully understand the utility of a proteasome inhibitor for conditions of skeletal muscle wasting. Nonetheless, our studies demonstrating a preservation in skeletal muscle mass with Velcade in an *in vivo* model of skeletal muscle atrophy complement studies that have shown reduced proteolysis measured *ex vivo* after proteasomal inhibition. These findings show promise for the use of proteasome inhibitors in skeletal muscle–wasting syndromes.

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