Chemotherapy Inhibits Skeletal Muscle Ubiquitin-Proteasome-dependent Proteolysis

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ABSTRACT

Chemotherapy has catabolic effects, but it is unknown whether cytostatic agents alter skeletal muscle proteolysis. We hypothesized that chemotherapy-induced alterations in protein synthesis should result in the increased incidence of abnormal proteins, which in turn should stimulate ubiquitin-proteasome-dependent proteolysis. The effects of the nitrosourea Cys were investigated in skeletal muscles from both healthy and colon 26 adenocarcinoma-bearing mice, an appropriate model for testing the impact of cytostatic agents. Muscle wasting was seen in both groups of mice 4 days after a single Cys injection, and the drug further increased the loss of muscle proteins already apparent in tumor-bearing animals. Cys cured the tumor-bearing mice with 100% efficacy. Surprisingly, within 11 days of treatment, rates of muscle proteolysis progressively decreased below basal levels observed in healthy control mice and contributed to the cessation of muscle wasting. Proteasome-dependent proteolysis was inhibited by mechanisms that include reduced mRNA levels for 20S and 26S proteasome subunits, decreased protein levels of 20S proteasome subunits and the S14 non-ATPase subunit of the 26S proteasome, and impaired chymotrypsin- and trypsin-like activities of the enzyme. A combination of Cisplatin and ifosfamide, two drugs that are widely used in the treatment of cancer patients, also depressed the expression of proteasomal subunits in muscles from rats bearing the MatB adenocarcinoma below basal levels. Thus, a down-regulation of ubiquitin-proteasome-dependent proteolysis is observed with various cytostatic agents and contributes to reverse the chemotherapy-induced muscle wasting.

INTRODUCTION

The syndrome of cancer cachexia, which includes progressive weight loss, anorexia, weakness, and anemia, is an important complicating factor in the management of cancer patients. Wasting is particularly evident in adipose tissue and skeletal muscle (1) and has a negative impact on overall survival (2). Chemotherapy itself increases the wasting seen in cancer patients (3). However and surprisingly, the effects of cytostatic agents on skeletal muscle protein turnover are extremely poorly documented. We have reported recently that in vivo protein synthesis decreased transiently in muscles from healthy and tumor-bearing mice treated with Cyst (4). This was not unexpected because Cyst is a chlolethyo-nitrosourea that induces DNA damage and more particularly interstrand DNA cross-linking (5, 6). By contrast, we also reported in these experiments that the drug may have inhibited skeletal muscle proteolysis (4). This finding was suspect for two reasons: (a) protein breakdown rates were estimated indirectly in vivo as the difference between protein synthesis and protein deposition, and thus were questionable (7); and (b) it is now well established that the major proteolytic process in skeletal muscle is Ub and proteasome dependent (8–11). Well-known substrates of this pathway are abnormal proteins, e.g., misfolded, and thus were questionable (7); and (b) it is now well established that the major proteolytic process in skeletal muscle is Ub and proteasome dependent (8–11). Well-known substrates of this pathway are abnormal proteins, e.g., misfolded, folded, and mislocalized proteins (12). Chemotherapy-induced DNA damages should have resulted in the increased incidence of such proteins, which in turn should stimulate Ub-proteasome-dependent proteolysis.

The present experiments were undertaken to specifically study the effects of Cyst on skeletal muscle proteolysis in healthy and colon 26 adenocarcinoma-bearing mice and to identify molecular mechanisms by which proteolysis is affected. There are very few rodent models that are suitable to study cancer cachexia and cytostatic agents (13, 14). Among these, the murine colon 26 adenocarcinoma identified by Tanaka et al. (13) fulfills several criteria. The tumor elicits extensive weight loss, hypoglycemia, hypercorticism, and several disorders of hepatic function, including increased synthesis of acute phase proteins, decreased number of hepatic glucocorticoid- cytosol receptors, and decreased activities of hepatic catalase and drug-metabolizing enzymes. Whole-body weight loss mainly reflects adipose tissue and muscle wasting, is not associated with anorexia, and occurs with relatively small tumor burdens (13).

Cyst is a plasma metabolite identified in animals after oral administration of 2-chloroethyl nitrosocarbamoylcystamine (15, 16), which possesses excellent cytostatic activity against a range of murine tumors, including the colon 26 adenocarcinoma (4, 5, 17). This agent has limited toxicity, shows good efficacy on melanoma, induces redifferentiation of primary tumors, and confers protection against secondary tumor growth in a melanoma murine model (18). Cyst is therefore appropriate to study the effects of chemotherapy on skeletal muscle proteolysis.

MATERIALS AND METHODS

Animals, Tumor Implantation, and Chemotherapy. Young male Balb/c mice, with an initial weight of 19–20 g, were purchased from Ifa Credo (L’Arbresle, France) and maintained in a temperature-controlled room (22 ± 1°C) on a 12-h light-dark cycle. They had free access to a standard diet (A03; UAR, Epinay sur Orge, France) and water. Animals were randomly divided into two groups. On day 0, the tumor-bearing group was inoculated s.c. with a homogenate of colon 26 adenocarcinoma (50 mg of solid tumor tissue in 0.1 ml of sterile 0.9% NaCl). The control group was injected with 0.1 ml of sterile saline solution. In each group, on day 15, animals received a single i.p. injection of either Cyst (20 mg/kg body weight) or saline (17). This time point was chosen to ensure significant weight loss and muscle wasting (4, 13) before initiation of chemotherapy. The average tumor mass was 0.80 ± 0.02 g at that time. Animals were weighed daily (from days 0 to 44, and from days 8 to 44 in the tumor-bearing and control groups, respectively). According to the changes in body weight, animals were killed by cervical dislocation on days 16, 19, 26, and 44. Days 16 and 19 were used to determine the acute effects of chemotherapy. By days 26 and 44, the tumor had fully regressed and therefore
served as a good indicator of short- and long-term recovery, respectively. EDL, tibialis anterior, and gastrocnemius muscles were dissected, weighed, and either incubated (EDL) or frozen at −80°C until analysis. We did not use pair-fed animals because: (a) colon 26 adenocarcinoma does not significantly affect food intake (13, 17); and (b) Cyst administration has very little and transient (2 days) effect on food intake, both in healthy and in tumor-bearing mice (17).

Eighteen female Fischer 344 rats with an initial body weight of 183 ± 7 g were also used. Six animals served as controls, and 12 rats were implanted s.c. with 200 µl of a suspension of MatB 13762 tumor cells. Six days after tumor implantation, six tumor-bearing rats received a single i.p. dose of ifosfamide (100 mg/kg body weight) and cisplatin (3 mg/kg body weight; Refs. 19 and 20). Untreated and treated rats bearing this mammary carcinoma were killed 14 days after tumor implantation, along with the group of control animals. Gastrocnemius muscles were dissected and frozen at −80°C until analysis.

**Rates of Protein Breakdown.** EDL muscles were carefully dissected from mice killed at days 16, 19, and 26 and attached at approximated resting length by pinning their tendons on inert plastic supports. Muscles were preincubated at 37°C in Krebs-Henseleit buffer equilibrated with 95% O2 and 5% CO2, containing 5 mM glucose, 0.1 units/ml insulin, 0.17 mM leucine, 0.10 mM isoleucine, and 0.20 mM valine to improve protein balance and energy status (21). After 1 h of preincubation, muscles were transferred into fresh medium of identical composition containing 0.5 mM cycloheximide and further incubated for 2 h. At the end of the incubation, muscles were blotted and homogenized in 10% TCA. TCA-insoluble material was washed three times with 10% TCA and solubilized in 1 N NaOH at 37°C for determination of protein content. Tissue protein mass was determined using the bicinchoninic acid procedure (22).

Total rates of protein breakdown were measured by following the rates of tyrosine release into the medium (21). When proteasome-dependent proteolysis was measured, one EDL was incubated in a calcium-free medium containing 10 mM methylamine and calcium-dependent proteinases, and the contralateral EDL was incubated in this medium also containing 40 µM of the proteasome inhibitor MG132 (Affiniti, Marnhead, Exeter, United Kingdom). Proteasome-dependent proteolysis was calculated by the difference between nonsolubilized, calcium-independent proteolysis (measured in the medium containing methylamine and leupeptin) and nonsolubilized, calcium-independent and proteasome-independent proteolysis (measured in the medium containing methylamine, leupeptin, and MG132). Tyrosine release was assayed by the fluorometric method of Waalkes and Udenfriend (23).

**Northern Blot Analysis.** Total RNA was extracted from tibialis anterior muscles (from mice killed at day 26) as described by Chomczynski and Sacchi (24). Twenty µg of total RNA were electrophoresed on agarose gels and then electrophoretically transferred and covalently bound to nylon membranes as described previously (25, 26). The membranes were hybridized with cDNA probes encoding polyUb: 14-kDa E2; the C2, C5, and X subunits of the 20S proteasome; and the S4, S5a, S7, and S14 subunits of the 19S regulatory complex of the 26S proteasome. After stripping of the different probes, the filters were reprobed with a cDNA fragment encoding the 18S rRNA. The complexes of the 26S proteasome. After stripping of the different probes, the filters were autoradiographed, and signals were quantified as described (25, 26). Autoradiographic signals were normalized using the corresponding 18S signals to correct for variations in RNA loading. Northern blots were also performed as described above in the gastrocnemius muscles from control rats and animals bearing the MatB tumor treated or not with ifosfamide and cisplatin.

**Western Blot Analysis.** When Ub-protein conjugates were conjugated, gastrocnemius muscles (from mice killed at day 26) were homogenized as described previously (27) to obtain myofibrillar and soluble proteins. Twenty-five µg of myofibrillar and soluble proteins were electrophoresed on 7.5% polyacrylamide gels and transferred onto nitrocellulose membrane (0.45 µm; Schleicher & Schuell France, Ecquevilly, France). An ECL kit and Hyperfilm ECL (Amersham Pharmacia Biotech Europe GmbH) were used as the detection system with antibodies against iota and zeta subunits of the 20S proteasome (kindly provided by Dr. H-P. Schmid, University of Clermont-Ferrand, Clermont-Ferrand, France) and S14 and S5a subunits of the 19S complex (kindly provided by Dr. H. Sawada, University of Hokkaido, Sapporo, Japan, and purchased from Affiniti, respectively).

Membranes were stained with amido black, and autoradiographic signals were quantified in arbitrary units using digital image processing and analysis (NIH Image 1.54; Apple Computer, Cupertino, CA). The data were normalized using the corresponding amido black staining signals to correct for variations in protein loading.

**Proteasome Activities.** Proteins from pooled gastrocnemius muscles of animals killed at day 26 were extracted in a 10% glyceral, 1 mM Tris ice-cold buffer containing protease inhibitors (0.2 mM phenylmethylsulfonyl fluoride, 0.5 mM Nα-p-tosyl-l-lysine chloromethyl ketone, 0.5 mM Nα-p-tosyl-l-phenylalanine chloromethyl ketone, 10 mM N-ethylmaleimide, 10 µg/ml leupeptin, 10 µg/ml pepstatin A, and 10 µg/ml soybean trypsin inhibitor), 10 mM ATP, and 10 mM MgCl2. Samples were then centrifuged for 1 h (100,000 × g, 4°C). Supernatant was removed and submitted to a 10.2-ml 10% TCA, 10 mM MgCl2, and 10 mM MgCl2 buffer. Samples were centrifuged for 22 h (100,000 × g, 4°C). Fractions (0.45-ml) were then collected from the bottom of the tubes. Each fraction was tested for the chymotrypsin-like activity of proteasome using the following procedure. Fractions (20 µl) were incubated for 45 min with 100 µM Suc-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin (Affiniti) at 37°C in a 1 ml Tris, 10 mM ATP, and 10 mM MgCl2 buffer: ± 40 µM proteasome inhibitor MG132 (Affiniti) in a final 200-µl volume. The reaction was stopped with the addition of 800 µl of ice-cold 100 mM monochloroacetate, 40 mM sodium acetate, and samples were placed on ice for 10 min. The chymotrypsin-like activity was measured as the 7-amido-4-methylcoumarin release using a Perkin-Elmer fluorimeter (excitation, 365 nm; emission, 430 nm). The difference between the fluorescence units recorded ± MG132 in the reaction medium was calculated, and final data were corrected by the amount of protein loaded onto the gradient. The same procedure was used to determine the trypsin-like activity, by using Boc-Leu-Arg-Arg-7-amido-4-methylcoumarin (Affiniti) as a substrate.

**Statistics.** All data are expressed as means ± SE and are representative of at least two different experiments. Statistical analyses were performed using ANOVA or an unpaired Student’s t test when appropriate. Significance was defined at the 0.05 level.

**RESULTS**

**Cyst Injection First Results in Muscle Wasting and Then Allows Recovery in Tumor-bearing Mice.** A single i.p. injection of Cyst at day 15 resulted in a rapid body weight loss in healthy animals within 5 days (Fig. 1A). Animals lost 5.0 ± 0.5 g, i.e., 19% of their initial body weight. Changes in both EDL and tibialis anterior muscle mass paralleled body weight loss; muscle mass was not affected significantly by 1 day of Cyst treatment (i.e., at day 16), but both were reduced (P < 0.005) at day 19 (Fig. 2A). By contrast, mice were recovering in terms of body weight by day 26 (Fig. 1A), but muscle mass was still reduced with respect to the control animals (Fig. 2A). An improvement in muscle mass was only seen at day 44 (Fig. 2A).

Colon 26 adenocarcinoma induced weight loss starting on day 10 and reached a maximum at day 17 (Fig. 1B). The body weights of the tumor-bearing mice then gradually increased, but this was almost totally accounted for by the development of the tumor (Fig. 1B, inset). Indeed, muscle wasting occurred very progressively (Fig. 2B). The Cyst injection at day 15 did not induce further body weight loss compared with the tumor group (Fig. 1B). By contrast, at day 19, muscle wasting was more pronounced in the Cyst-treated tumor-bearing mice than in the untreated tumor-bearing animals (Fig. 2B). Thus, in this model, the cytostatic agent had an additive effect with tumor-induced muscle wasting. However, body weight loss after day
Cyst Inhibits Skeletal Muscle Ub-Proteasome-dependent Proteolysis. Variations in muscle mass reflected changes in protein pools, because protein concentration was not affected by Cyst, the presence of the tumor, or both (data not shown). Total rates of proteolysis progressively decreased after Cyst administration in both healthy and tumor-bearing mice (Fig. 3A). These variations, however, were only statistically significant at day 26 when protein breakdown was inhibited by 35–37% \((P < 0.001)\) in healthy and tumor-bearing mice.

We next investigated whether Cyst inhibited proteasome-dependent proteolysis in vitro. As shown in Fig. 3B, Cyst depressed proteasome-dependent proteolysis at day 26 by 65 and 61% in healthy and tumor-bearing mice, respectively. Interestingly, Cyst depressed proteasome-dependent proteolysis in tumor-bearing mice below values observed in healthy untreated animals. Thus, Cyst not only reversed a trend for enhanced proteasome-dependent proteolysis in tumor-bearing animals but also inhibited the system below basal values. It is not clear whether the colon 26 adenocarcinoma significantly stimulates the Ub-proteasome pathway in skeletal muscle. Although early experiments support this interpretation (28), more recent studies show no significant changes in lactacystin-sensitive proteolysis, proteasome activity, amounts of Ub-conjugates, and expression of the 14-kDa E2 in the muscles from mice bearing the colon 26 adenocarcinoma compared with controls (29). Our data directly support the latter observations because although we noticed a trend for an activation of proteasome-dependent proteolysis in tumor-bearing animals (Fig. 3B), this difference was small and not statistically significant \((P > 0.05)\). Similarly, we also observed no increase in Ub conjugates or in the expression of many components of the Ub-proteasome pathway, including the 14-kDa E2, in the muscles of our tumor-bearing animals compared with healthy controls (see below). Thus, it seems that depressed protein synthesis is the major determinant of muscle wasting in colon 26 adenocarcinoma-bearing mice, in accordance with recent in vivo studies (4).

Cyst Administration Inhibits Multiple Steps in the Ub Pathway. We next examined the mechanisms responsible for the inhibition of proteasome-dependent proteolysis at day 26 in Cyst-treated animals. There are two main steps in the Ub-proteasome pathway: (a) Ub

![Diagram](image_url)
chemotherapy was given on day 15. Data are expressed as a percentage of

The Inhibition of Skeletal Muscle Proteasomes Occurs with Other Cytostatic Agents in Rats. To demonstrate whether the inhibition of muscle Ub-proteasome-dependent proteolysis was seen with other drugs, we next measured the expression of subunits C2 and S5a of the proteasome in the muscles from tumor-bearing rats treated with a combination of cisplatin and ifosfamide for 8 days. These animals treated tumor-bearing mice, suggesting a reduced muscle proteasome content. In addition, the chymotrypsin-like activity of muscle proteasomes decreased after Cyst treatment (Fig. 6), as well as the trypsin-like activity (data not shown). In contrast, the muscle content of the non-ATPase subunit S5a remained unaffected (Fig. 5D), although S5a mRNA levels decreased (Fig. 5A). S5a is, thus far, the only subunit identified within the 19S complex that recognizes polyUb chains (34). However, Mcb1 (the yeast equivalent of S5a) is not the unique receptor of the polyUb chain degradation signal and exists as a free entity, because this 19S subunit is not entirely incorporated into 26S proteasomes (35).

conjugation to protein substrates (reviewed in Ref. 30) that involves in skeletal muscle at least the 14-kDa E2 and the Ub-protein ligase E3α (31); and (b) the subsequent breakdown of Ub conjugates by the 26S proteasome (reviewed in Ref. 32). Fig. 4A shows that the mRNA levels for both Ub and the 14-kDa E2 decreased in the muscles from tumor-bearing mice treated by Cyst. Similar observations were obtained in the muscles from healthy Cyst-treated mice (data not shown). However, subsequent Western blot experiments showed that neither the muscle levels of the 14-kDa E2 protein (Fig. 4B) nor the amount of Ub conjugates (Fig. 4C) were depressed in the muscles from the Cyst-treated tumor-bearing mice. Because proteasome activities also decreased (see below), the latter observation suggests that the rate of ubiquitination should also be decreased.

We next investigated whether modifications occurred at the level of the proteasome. mRNA levels for subunits of the 20S proteasome (8–11, 25, 26) and some subunits of the 19S complex (9, 27, 33) are elevated when Ub-proteasome-dependent proteolysis is activated in muscle wasting. Conversely, Fig. 5 shows that mRNA levels for subunits X, C2, and C5 of the 20S proteasome and for ATPase (S7, S4) and non-ATPase (S5a, S14) subunits of the 19 complex decreased in muscles from Cyst-treated tumor-bearing (Fig. 5A) and healthy (Fig. 5B) mice. The lack of concomitant variation in mRNA levels for glyceraldehyde phosphate dehydrogenase and other genes (data not shown) clearly rules out the possibility that chemotherapy had a nonselective effect on all mRNA levels within muscle cells. Protein levels for subunits iota and zeta of the 20S proteasome (Fig. 5C) and for the non-ATPase subunit S14 of the 19S complex (Fig. 5D) also decreased by 24, 50, and 51%, respectively, in muscles from Cyst-

Fig. 3. A, effect of Cyst injection on total muscle proteolysis in healthy and tumor-bearing mice at days 16, 19, and 26, expressed as a percentage of respective saline-injected controls. Values are means for n = 10 mice; bars, SE. *, P < 0.05 versus saline-injected controls. B, effect of Cyst at day 26 on proteasome-dependent proteolysis in muscles from healthy and tumor-bearing mice treated with Cyst (Cyst+) or saline (Cyst−). Chemotherapy was given on day 15. Data are expressed as a percentage of healthy saline-injected mice. Values are means for n = 10 mice; bars, SE. *, P < 0.05 versus saline-injected healthy mice.

Fig. 4. Effect of Cyst on Ub, 14-kDa E2, and Ub conjugates at day 26. A, Northern blots for Ub and 14-kDa E2. B, Western blot for 14-kDa E2 in muscles from tumor-bearing mice, saline (TB) or Cyst (TC) injected. Data are expressed as a percentage of TB mice and are corrected for 18S rRNA abundance (A). Values are means for n = 10 mice (bars, SE), and representative Northern blots or Western blots are shown. *, P < 0.05 versus TB. Note that in A, values for Ub are the average of the 6.4- and 1.2-kb polyUb transcripts, and values for the 14-kDa E2 only refer to the smallest 1.2-kb transcript. Note also that in A, the upper polyUb transcript migrates as a 6.4-kb band (instead of a 2.6-kb band) in tissues from Balb/c mice (this report and Ref. 17), and that in B, the 14-kDa E2 protein migrates as a 17-kDa band. C, Ub-protein conjugates in myofibrillar and soluble fractions of gastrocnemius muscle extracts. Tumor-bearing mice, saline (TB) or Cyst (TC) injected, and healthy mice, saline (H) or Cyst (HC) injected, are shown. Note that there is no obvious difference in the profile of high molecular weight conjugates (HMWC) in tumor-bearing and healthy mice treated or not with Cyst.
also started to recover muscle mass and body weight after chemotherapy (data not shown). In agreement with data in Cyst-treated mice (Fig. 5, A and B), mRNA levels for both C2 and S5a in chemotherapy-treated rats bearing the MatB tumor decreased below levels observed in control healthy animals (P < 0.05; Fig. 7). These data support the concept that at least several alkylating agents (ifosfamide belongs to the same class of drugs as Cyst) down-regulate the proteasome pathway in skeletal muscle.

**DISCUSSION**

Chemotherapy results in negative nitrogen balance (36); however, the precise mechanisms responsible for this catabolic effect remain unknown. We provide here direct and strong evidence for a surprising inhibition of Ub-proteasome-dependent proteolysis in skeletal muscle from both healthy and tumor-bearing mice. These findings were totally unexpected, because we hypothesized that chemotherapy should result in the synthesis of abnormal proteins that are good substrates of the proteasome. Chemotherapy clearly down-regulated muscle proteasome-dependent proteolysis by mechanisms that include reduced mRNA levels for 20S and 26S proteasome subunits associated with decreased protein levels of proteasome subunits and impaired chymotrypsin-like and trypsin-like activities of the enzyme.

The Ub-proteasome-dependent pathway has been identified as the critical system responsible for the bulk of muscle proteolysis (8–11, 25–27, 31, 33) including the major contractile components actin and myosins (8–11, 37, 38). However, the demonstration that the proteasome plays a major role in muscle is mostly on the basis of indirect measurements, such as elevated rates of proteolysis that are blocked...
by proteasome inhibitors in vitro and/or enhanced mRNA levels for proteasome subunits in catabolic states. In the present experiments, we demonstrated that reductions in mRNA levels for proteasome subunits correlated with: (a) decreased protein levels of 20S proteasome subunits and of at least subunit S14 of the 19S complex; (b) decreased rates of proteasome-dependent proteolysis; and (c) decreased proteasome activities. Taken together, our analysis has provided comprehensive data that clearly and unequivocally support a role for proteasomes in skeletal muscle proteolysis. Moreover, our study is the first to characterize muscle proteolysis at this level of detail. This approach is essential in determining the rate-limiting step(s) in the proteolytic process.

The reasons why skeletal muscle proteolysis is depressed by chemotherapy are unclear. Proteolysis may have been depressed directly by chemotherapy. A number of drugs used in chemotherapy have been reported recently to impair proteasome function, e.g., the incubation of human proteasomes in the presence of vinblastine resulted in an inhibition of their peptidase activities (39). When purified muscle proteasomes were incubated in the presence of various concentrations of Cyst, we were, however, unable to demonstrate any inhibitory effect of the drug on their peptidase activities (data not shown). In related experiments, we also investigated whether chemotherapy may result in apoptosis. However, again we failed to detect any DNA fragmentation in muscles after Cyst treatment (data not shown).

There are other possibilities to explain reduced proteolysis. A simple possibility could be that the inhibition of muscle proteolysis reflected catch-up growth, a general mechanism by which muscle recovery might occur (40). For example, muscle protein breakdown is reduced during catch-up growth after Escherichia coli infection in weanling rats. However, under such circumstances, the inhibition of proteolysis occurred immediately and briefly after the cessation of the stress (40), whereas in the present study, the inhibition of muscle protein breakdown occurred very progressively and was delayed (Fig. 3A). If decreased proteolysis in our study was merely catch-up growth, it should have occurred immediately. Alternatively, chemotherapy is often associated with malnutrition and/or malabsorption (41, 42), and Cyst induces rapid intestinal damage (17). Malnutrition is well known to result in an inhibition of skeletal muscle protein breakdown (7, 43). For example, dietary protein deficiency reduced ATP (and presumably Ub)-dependent proteolysis (44) and the proteasome content (45) in rat skeletal muscles. Whether the Cyst-induced depressed proteasome-dependent proteolysis reflects impaired dietary protein utilization requires further investigation. However, it is noteworthy that the reduction in ATP-dependent proteolysis in muscles from rats fed a very protein-deficient diet (1% lactalbumin) occurred slowly and was clearly apparent after 7 days of treatment (44). This effect is consistent with the delayed inhibition of muscle proteolysis observed here (Fig. 3A). Furthermore, no significant changes in mRNA levels for components of the Ub-proteasome system were seen 1 or 4 days after Cyst administration (data not shown). Finally, and more simply, depressed proteolysis may be the only alternative for animals to recover muscle mass. Indeed, in related experiments we have observed that rates of protein synthesis were still depressed at day 26 in Cyst-treated animals compared with healthy mice (46). Whether impaired muscle proteolysis occurs with cytotoxic agents other than Cyst was unknown until now. The observation that cisplatin and ifosfamide also depressed mRNA levels for proteasome subunits below basal levels in muscle from adult rats (Fig. 7) supported this hypothesis. In addition, this is consistent with a reduction in whole-body protein breakdown observed in adult cancer patients after administration of vinblastine, cisplatin, and bleomycin (47). Thus, reduced muscle proteolysis may represent a generalized response to chemotherapy.

In summary, the present studies clearly demonstrated that a single injection of Cyst and other cytotoxic drugs resulted in an inhibition of Ub-proteasome-dependent proteolysis, which significantly contributed to the reversal of muscle wasting in both healthy and cured tumor-bearing mice. Our data demonstrate a robust capacity for muscle recovery in chemotherapy-treated animals, which includes down-regulation of muscle proteolysis. This recovery period may be a window of opportunity for nutritional and metabolic approaches to stimulate growth after chemotherapy. Our studies were performed in sexually mature, growing rodents, and our data clearly showed that a long-lasting impairment in growth prevailed in the Cyst-treated mice. This is very similar to the growth defect observed in chemotherapy-treated children and/or adolescents, which is assumed to result mainly from growth hormone and other hormone-related deficiencies, as well as from reduced long bone growth (48). Our experimental model may therefore be very useful to further define the mechanisms responsible for growth defects after chemotherapy. Finally, it would be extremely valuable to investigate whether muscle protein breakdown may be down-regulated after repeated cycles of chemotherapy, as is the more usual clinical practice.

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REFERENCES


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