Anti-TNF treatment reduces rat skeletal muscle wasting in monocrotaline-induced cardiac cachexia

Brian T. Steffen, Simon J. Lees, and Frank W. Booth

Departments of Medical Pharmacology and Physiology and of Biomedical Sciences, Dalton Cardiovascular Institute, University of Missouri, Columbia, Missouri

Submitted 8 July 2008; accepted in final form 16 September 2008

Steffen BT, Lees SJ, Booth FW. Anti-TNF treatment reduces rat skeletal muscle wasting in monocrotaline-induced cardiac cachexia. J Appl Physiol 105: 1950–1958, 2008. First published September 18, 2008; doi:10.1152/japplphysiol.90884.2008.—The aim was to explore efficacy of tumor necrosis factor (TNF) inhibitors in attenuating increases in anorexia and ubiquitin proteasome pathway transcripts in cardiac cachexia, a potentially lethal condition that responds poorly to current treatments. Cardiac cachexia was rapidly induced with monocrotaline in Sprague-Dawley rats. Either soluble TNF receptor-1 or the general inhibitor of TNF production, pentoxifylline, was given to diminish TNF action on the first indication of cachexia. Animals were anesthetized with a ketamine-xylazine-acepromazine cocktail, and then skeletal muscles were removed for subsequent measurements including ubiquitin proteasome pathway transcripts and Western blots. Both soluble TNF receptor-1 and pentoxifylline attenuated losses in both body and skeletal muscle masses and also reduced increases in selected ubiquitin proteasome pathway transcripts. The action of soluble TNF receptor-1 was partly through reversal of reduced food consumption, while the effects of pentoxifylline were independent of food intake. Here we demonstrate, for the first time, that attenuation of anorexia by soluble TNF receptor-1 treatment in monocrotaline-induced cardiac cachexia is responsible for attenuating increases in some ubiquitin proteasome pathway transcripts as well as preserving body mass and attenuating loss of skeletal muscle mass.

Cachexia is a highly complex metabolic disorder characterized by inflammation, anorexia, and severe muscle loss in the presence of an underlying illness. Both inflammation and anorexia can independently contribute to skeletal muscle wasting. Cachexia associated with congestive heart failure (CHF), or cardiac cachexia, has a 50% mortality rate at 18 mo for the 10–16% of CHF patients diagnosed as cachectic (2).

Increased protein degradation by the ubiquitin-proteasome pathway (UPP) is implicated in skeletal muscle wasting in cachectic conditions (15, 29). In those cachetic states investigated to date, UPP activity is upregulated in skeletal muscle; transcript levels for UPP members are also upregulated 8-, 2-, 4-, and 8-fold for ubiquitin, E2 ubiquitin conjugating enzymes, E3 ubiquitin ligases, and subunits of the 26S proteasome, respectively (4, 6, 15). Collectively, these mRNAs in skeletal muscle form a subpopulation of ∼120 genes coordinately induced or suppressed in different catabolic states and are termed “atrogenes” (25). However, insufficient information exists on whether atrogene mRNA levels in skeletal muscle increase in cardiac cachexia and on whether inflammation and anorexia each play some role. One purpose of the present study is to test the hypothesis that monocrotaline (MCT)-induced cachexia increases atrogene mRNAs.

Although there is no intervention that exhibits 100% therapeutic efficacy in attenuating muscle loss associated with cardiac cachexia (34), pharmacological intervention against the proinflammatory cytokine TNF-α has had partial success in attenuating the pathological effects in other cachectic conditions. The general inhibitors of TNF production, torbafylline (HWA 448) and pentoxifylline (PTX), have been observed to attenuate losses in skeletal muscle mass and body weight, as well as to attenuate upregulation of UPP transcripts in skeletal muscle of animals cachectic from cancer or sepsis (9–11). Any treatment option that maintains body weight and muscle mass in cardiac cachetic patients has been predicted to improve overall outcome (3). We hypothesized that TNF inhibitors soluble TNF receptor-1 (sTNFR1) and PTX would attenuate increases in all MCT-induced UPP mRNAs.

Apart from their putative effects on muscle wasting and UPP transcripts, TNF inhibitors sTNFR1 and PTX have been shown to attenuate anorexia in sepsis and cancer models of cachexia, respectively (24, 30). Thus a further aim of this study is to determine whether any of the hypothesized attenuation of MCT-induced cachexia by sTNFR1 or PTX could be due to antianorectic effects on atrogene expression and muscle mass. In the present study, we found that not only does sTNFR1 treatment attenuate anorexia but that its antianorectic effect is unexpectedly responsible for partially attenuating the induction of UPP transcripts as well as completely preserving body weight and some skeletal muscle mass in cardiac cachexia.

MATERIALS AND METHODS

Animals. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). All procedures were approved by the Institutional Animal Care and Use Committee at the University of Missouri-Columbia. Male Sprague-Dawley rats were housed at 21°C, maintained on a 12:12-h light-dark cycle, and allowed free access to water and food unless otherwise specified. Once rats reached 85-100 g in body weight (point A in Fig. 1), they were intraperitoneally injected with 30 mg/kg MCT (Sigma), an alkanoid that produces severe pulmonary hypertension and subsequent right ventricular (RV) failure, following the procedure described by Vescovo et al. (31). The MCT model was selected for the rapidity of the onset of cachexia, thus allowing an enhanced sensitivity of detection due to larger magnitude of change due to the short time frame.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
MCT has been shown to reduce food intake; to control for this effect, a healthy control was paired with an animal administered MCT. The healthy control was then pair-fed with the MCT animal from the time of MCT injection. The amount of food consumed by the MCT animal was given to the healthy control the following day. In addition, a healthy, ad libitum, continuously fed control group was included, thus controlling for any observed effects of MCT on food intake. When MCT-injected animals had lost ≥8.5% of their peak body weight or had not eaten in the previous 24 h, animals were given an intraperitoneal injection of ketamine (80 mg/kg), xylazine (10 mg/kg), and acepromazine (4 mg/kg) to harvest muscle when the animal was killed (point C in Fig. 1); extensor digitorum longus (EDL) was excised, weighed, frozen in liquid nitrogen, and stored at −80°C.

**TNF inhibitor treatments.** On the first signs of cardiac cachexia, either a loss or a plateau in body weight in these growing rats, a TNF inhibitor treatment was given on successive days (point B in Fig. 2). One subcutaneous injection of sTNFR1 [15 mg/kg body wt (22)] diluted in PBS or daily injections of PTX [10 mg/100 g body wt (9)] were administered. Each TNF inhibitor-treated rat, as well as respective controls, was exposed to the same number of days in a cachectic state as rats given MCT alone. In addition, each TNF inhibitor treatment group was further subdivided into two groups, 1) ad libitum fed (AL), and 2) pair-fed to MCT animals (PF), to address whether TNF inhibitors were affecting body weight and muscle masses as well as atrogene transcript levels through any antianorectic effects of the respective treatment.

**RNA isolation and real-time PCR.** Total RNA was extracted from preweighed frozen muscle samples using Trizol reagent (Invitrogen Technologies, San Diego, CA) following the Chomczynski-Sacchi method (8). Qiagen RNeasy (no. 74104) and RNase-Free DNase Set (no. 79254) were used for RNA purification.

RNA was reverse transcribed with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). For all genes examined (Table 1), cDNA was amplified by real-time PCR in a reaction consisting of 25 ng of each cDNA sample (run in duplicate) in a mix of 250 nM primers and SybrGreen PCR mix (Applied Biosystems). Values were normalized to 18S (Applied Biosystems), data were analyzed using comparative ΔCt method, where Ct is threshold cycle, and statistics were run on ΔCt values.
observations within subjects for different days were correlated, and using an autoregressive model of order 1, analysis was carried out in SAS; comparison of means was done using least-squares means. Data from Western blots were analyzed with SigmaStat software (Chicago, IL) using one-way ANOVA; all post hoc comparisons were made with Fisher’s LSD test with significance designated at $P \leq 0.05$.

All remaining data were analyzed using SAS (mixed procedure); treatment groups were treated as fixed effects, and the blocks of similarly fed animals were treated as random effects. The heterogeneous variances were accounted for by using the group option in the repeated statement. If a convergence failure occurred using this design, remaining groups were analyzed using two-sample $t$-tests (using the Satterthwaite method to allow for unequal variances).

RESULTS

**MCT treatment induces cardiac hypertrophy and cachexia.**

Figure 1 depicts the timeline for the MCT-induced cardiac cachexia model. Our preliminary findings verified previous published reports (12–14, 31–33). sTNFR1 treatment resulted in increased masses of the right atria and ventricles and reduced whole body and muscle weights (Table 2; see Supplemental Fig. 3S, available with the online version of this article), indicating that these animals were cachectic. MCT administration (at point A in Fig. 1) led to a significant reduction in food intake 2 days before and through the day that animals were killed (between points B and C, Fig. 2; $P \leq 0.05$), indicating animals were anorectic. Notably, body and EDL weights in MCT-administered animals were less ($P \leq 0.05$) than PF animals, indicating that reduced food consumption (anorexia) accounts for a proportion of the changes observed (Table 2).

**sTNFR1 attenuates anorexia in rats with cardiac cachexia.**

The above observations led to a modified approach (point B in Fig. 2) in which pharmacological attenuation of the inflammatory molecule TNF-$\alpha$ was tested to determine if its action played a role in anorexia. The anorectic state induced by MCT administration was attenuated by sTNFR1 treatment ($P \leq 0.05$), and food intake in these animals was not different from control (no treatments) on both days before death (Fig. 2). On the day animals were killed, sTNFR1-treated animals consumed less than control animals, but still consumed threefold more than animals given MCT alone (Fig. 2, $P \leq 0.05$). Taken together, the data show that sTNFR1 has an antianorectic effect in MCT-administered animals.

While sTNFR1 is a specific inhibitor of TNF-$\alpha$, a second less specific inhibitor of TNF-$\alpha$, PTX, was also tested to determine if its outcome would be identical. MCT animals treated with PTX did not have a greater food intake than those given MCT alone on any day (Fig. 2, $P \leq 0.05$). Notably, 1 day before animals were killed, food intake for PTX animals was not statistically different from control; thus the possibility that PTX has a modest antianorectic effect the day before death cannot be excluded.

**sTNFR1 attenuation of body weight loss in cardiac cachexia is largely associated with rescue of food intake.** Since sTNFR1 had attenuated anorexia and the loss of muscle and body weights, we next pair-fed animals given sTNFR1 to determine whether the improved outcomes associated with anti-TNF-$\alpha$ treatment are solely due to improved appetite. Importantly, whereas sTNFR1 prevented all of the body weight loss in treated rats that were fed ad libitum, sTNFR1-treated animals that were pair-fed lost more body weight than sTNFR1-treated animals fed ad libitum, indicating that the majority of sTNFR1’s rescue of body mass was due to its maintenance of food intake (Fig. 3A, $P \leq 0.05$). PTX-treated rats that were pair-fed to MCT rats also showed less body weight loss (~60%) compared with those given MCT alone (Fig. 3A, $P \leq 0.05$), but there was no difference between the pair- and ad libitum-fed PTX subgroups. Vehicles used for sTNFR1 and PTX administration had no effect on body and EDL masses produced by MCT, suggesting the rescue effect was due to the TNF inhibitors (data not shown). Together, these data indicate that improvement in body weight for sTNFR1 treatment is largely dependent on food intake, whereas PTX treatment improves body weight independent of food intake.

sTNFR1 attenuation of skeletal muscle mass loss in cardiac cachexia is partly dependent on its rescue of food intake. Since sTNFR1 attenuated body weight mass loss with MCT, EDL muscle mass was examined to determine if fast-twitch muscle mass contributed to the body mass effect. MCT administration results in lower EDL muscle masses compared with control and pair-fed animals (Fig. 3B, $P \leq 0.05$), consistent with published reports (12–14, 31–33). sTNFR1 treatment resulted in partial sparing of EDL muscle mass in sTNFR1 animals fed ad libitum compared with animals given MCT alone (Fig. 3B,

---

**Table 1. Primer sequences: all atrogene and control targets for real-time PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5’-3’)</th>
<th>Reverse (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E214K</td>
<td>TCCAAACGCTGCACGCAATA</td>
<td>ATGGCCGAACCCTGCTTTC</td>
</tr>
<tr>
<td>UBC7</td>
<td>CATTGAATTGTTTGAAGAGTGATATGGG</td>
<td>ATGCCAGTGGCTGCAAGAT</td>
</tr>
<tr>
<td>PSM3</td>
<td>TCAGGACCTGCGGCTGAGAGA</td>
<td>TTTGACCTGACGGGACGTG</td>
</tr>
<tr>
<td>PSM1</td>
<td>GCCGACTGTAGGCTGATTCT</td>
<td>CATTATTCTACACAGGAGTTGCATATCT</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>CGGGACCTGATGGCTGATTCT</td>
<td>TGGGATGAAGCAAGGATCAG</td>
</tr>
<tr>
<td>PSMC2</td>
<td>CACCCAGGAGCAGAGGACAC</td>
<td>CAATCTGTGCTCTGGCTTCAA</td>
</tr>
<tr>
<td>PSMC6</td>
<td>AAAACGACAGACGGCAAGATAC</td>
<td>CATTACCAGTGGTGATGCAAGATG</td>
</tr>
<tr>
<td>MaFbx</td>
<td>AACACAAAGACCTGGCAGACTAA</td>
<td>CATTTCTGGAAATGCTTTG</td>
</tr>
<tr>
<td>18S</td>
<td>GCCGCTAGAGTGTAAGATCTT</td>
<td>TGGGACCTGACGGGACGTG</td>
</tr>
</tbody>
</table>

**Table 2. Final body, EDL, right atrial, and right ventricular weights of control, MCT, and pair-fed rats**

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>EDL, mg</th>
<th>Right Atrium, mg</th>
<th>Right Ventricle, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>216.4 ± 8.4</td>
<td>87.6 ± 3.6</td>
<td>36.4 ± 3.6</td>
<td>171.9 ± 6.6</td>
</tr>
<tr>
<td>MCT (n = 7)</td>
<td>154.3 ± 2.2*</td>
<td>63.4 ± 1.4*</td>
<td>66.3 ± 7.4*</td>
<td>283.5 ± 12*</td>
</tr>
<tr>
<td>Pair fed (n = 6)</td>
<td>168.1 ± 3.8†</td>
<td>73.6 ± 1.8†</td>
<td>22.7 ± 2.6†</td>
<td>131.5 ± 6.8†</td>
</tr>
</tbody>
</table>

Values are mean ± SE. EDL, extensor digitorum longus; MCT, monocrotaline. *Significantly different from control. †Significantly different from control and MCT.
The losses in EDL muscle mass were attenuated with PTX treatment in ad libitum- and pair-fed groups compared with animals given MCT alone (Fig. 3B, \(P \leq 0.05\)). No differences were observed between AL or PF groups treated with PTX. Together, the data indicate that PTX treatment partially spares muscle mass in the cachectic state independent of food intake.

**sTNFR1 and PTX attenuate MCT-induced increases in select atrogene transcript levels.** Our next aim was to determine the effect of anti-TNF-\(\alpha\) and pair-fed treatments on the up-regulation of UPP transcripts in skeletal muscle as other cachetic models have shown increased UPP mRNAs (19). Transcript levels for ubiquitin and the E3 ligase muscle atrophy F-box (MaFbx) were first examined as these have been shown to be consistently upregulated in muscle atrophy and wasting conditions. Moreover, MaFbx knockout mice retain 56% more muscle mass than wild types when placed under atrophy conditions (5). Consistent with other models of cachexia, MCT administration increased ubiquitin and MaFbx transcripts over control animals fed ad libitum (Fig. 4, A and B, \(P \leq 0.05\)). Both transcripts in MCT-treated only animals were elevated over pair-fed animals without other treatments; the vast majority of their increases were not due to anorexia.

sTNFR1 treatment attenuated MaFbx and ubiquitin transcript levels but only in those animals allowed to feed ad libitum (Fig. 4, A and B, \(P \leq 0.05\)); the attenuation of the transcripts was not observed in sTNFR1-treated animals that were pair-fed to MCT animals, indicating that the antianorectic effect of sTNFR1 is responsible for the attenuation of these transcript levels. In contrast, both feeding groups of PTX-treated animals showed attenuated ubiquitin transcript levels (Fig. 4B, \(P \leq 0.05\)); MaFbx transcript levels were attenuated in pair-fed PTX-treated animals (Fig. 4A, \(P \leq 0.05\)) while PTX-treated animals fed ad libitum approached significance in MaFbx transcript levels (Fig. 4A, \(P = 0.053\)). Together, the data suggest that PTX attenuates ubiquitin and MaFbx transcripts independent of food intake.

As E3 ligases, such as MaFbx, catalyze ubiquitination of specific proteins, which targets these proteins to the proteasome, we next sought to learn whether upregulation of structural components of the proteasome were also affected by TNF-\(\alpha\) inhibitors and/or anorexia. First, essential ATPase subunits of the 20S and 19S proteasome were examined. 20S proteasome subunits PSMA1 and PSMA3 as well as 19S proteasome subunits PSMC2 and PSMC6 were upregulated in MCT-administered animals over control animals fed ad libitum (Fig. 4, C and D, \(P \leq 0.05\)). Neither sTNFR1 nor PTX treatment reduced PSMA1, PSMA3, and PSMC6 compared with MCT-treated animals; however, PSMA1 and PSMC6 were not different compared with control in PTX-treated animals and PSMA3 was not different from control for sTNFR1 and PTX (Fig. 4, C and D). In contrast, the 19S proteasome subunit PSMC2 was attenuated in sTNFR1-treated animals that were fed ad libitum, and, like ubiquitin and MaFbx transcripts, attenuation was not observed in sTNFR1-treated pair-fed animals, indicating the attenuation was dependent on food intake. PTX-treated animals that were pair-fed to animals given MCT alone showed an attenuation of PSMC2 (Fig. 4D, \(P \leq 0.05\), indicating its reduction was independent of food intake. Taken together, mRNAs for structural subunits of the proteasome show a complex variation in pattern, suggesting that regulation of the UPP pathway to sTNFR1 or PTX does not employ common regulatory responses.
E2 conjugating enzymes were next examined in MCT-administered animals since these enzymes are essential in carrying activated ubiquitin to a targeted protein substrate and its corresponding E3 ligase; E2 enzyme transcripts have been shown to be increased in muscle atrophy and wasting conditions (1, 17, 18, 20). As reported in these other models of cachexia, MCT administration upregulates the E2 conjugating enzymes E214K and UBC7 (Fig. 5, \( P < 0.05 \)). As with 20S proteasome subunit transcripts (Fig. 4), neither PTX nor sTNFR1 treatment attenuated transcript levels for E214K or UBC7 (Fig. 5).

When the increases in UPP transcripts are attenuated, this is reflected in sparing of muscle mass. Linear regression analysis including animals from all experimental groups revealed that increased transcripts of MaFbx, ubiquitin, PSMC2, and UBC7 were correlated with a lower EDL muscle mass (Fig. 6, A–D, \( P \leq 0.001 \)). These data suggest that increases in particular transcripts for the UPP may be important in the increased muscle loss observed, potentially by increased protein degradation. In addition, these data show that pharmacological treatments that attenuate expression of these transcripts may result in a sparing of muscle mass in fast-twitch muscles such as the EDL.

Regulation of MaFbx mRNA levels. The upregulation of MaFbx mRNA expression in MCT-administered animals was next examined to determine the possible mechanism of its increased transcript expression. Since ad libitum feeding, but not pair feeding, with sTNFR1 treatment attenuated the upregulation of MaFbx mRNA (Fig. 4A), we chose to examine signaling pathways putatively involved in MaFbx expression in ad libitum groups only. Divergent from previous studies in models of cell culture and in vivo injection of TNF-\( \alpha \) (18, 20), an increase in phosphorylation of p38 MAPK was not observed in animals with increased MaFbx expression in any experimental group nor was it attenuated in PTX or sTNFR1-treated animals fed ad libitum (Fig. 7). These data suggest that p38 MAPK may not be involved in the upregulation of MaFbx mRNA in the MCT model of cardiac cachexia.

Given the abundant evidence that Akt and FoxO3a are involved in the regulation of MaFbx (26, 28), it was then hypothesized that the phosphorylation of both Akt and FoxO3a would be decreased in MCT-administered animals compared with ad-libitum fed controls. Expectedly, Western analysis revealed a decrease in the ratio of phospho-Akt/total Akt and a concomitant decrease in phospho-FoxO3a/total FoxO3a levels of MCT-administered animals (Fig. 8, A and B, \( P \leq 0.05 \)); together, the data suggest an inactivation of Akt results in dephosphorylation and activation of FoxO3a and thus increased MaFbx transcript levels. The ratios of phospho-Akt/total Akt and phospho-FoxO3a/total FoxO3a were not in-
creased in response to sTNFR1 or PTX treatment animals fed ad libitum compared with MCT treatment alone (Fig. 8, A and B), indicating that this pathway in skeletal muscle does not account for the attenuated expression of MaFbx in presence of the inhibitors in these animals.

Soleus muscle is resistant to MCT-induced changes in muscle mass and atrogene transcript levels. Soleus muscle mass was not different between MCT and pair-fed animals, indicating that loss of soleus muscle mass was dependent on reduced...
Heart failure, muscle and body mass losses, anorexia, and reduced food intake in these animals. MCT-administered only animals depended solely on the re-expression of atrogin-1 transcripts, and attenuate whole body and muscle wasting. We show for the first time that 1) sTNFR1’s action of reversing low food consumption in MCT-administered animals is wholly responsible for body mass loss, partly responsible for EDL mass loss, and increases in some atrogin-1 transcripts; and 2) MCT administration increases mRNAs for MaFbx, some 26S proteasome subunits, and an E2 conjugating enzyme.

Treatment with sTNFR1 was shown to have a clear antianorectic effect in MCT-administered, ad libitum-fed animals in the present study. Although our study does not allow for determining a mechanism, it is noteworthy that TNF-α has been well documented to induce an anorectic state at the level of the hypothalamus (23). Thus sTNFR1 treatment may be preventing the cytokine from interacting with hypothalamic TNF receptors in the present model. In addition, our finding that sTNFR1 attenuates anorexia in MCT-administered animals supports a previous study in which a similar specific TNF receptor, TNF-binding protein, was shown to attenuate anorexia in a cancer cachexia model (30). Importantly, the antianorectic effect of sTNFR1 proved to be an essential component for attenuating muscle and whole body wasting in MCT-administered animals.

Both sTNFR1 and PTX reduced the amount of body weight loss in animals administered MCT; however, sTNFR1 pair-fed animals showed no difference in body weight from MCT-administered animals. Expressed another way, the sparing of body weight by sTNFR1 was completely abolished when food was restricted. The finding shows that the antianorectic effect of sTNFR1 was responsible for improvements in body weight. In contrast, both feeding groups of PTX-treated animals showed attenuated body weight loss, thus indicating that the effect of PTX in MCT-administered animals is independent of food intake.

In accordance with their effects on body weight, both sTNFR1 and PTX were shown to reduce the degree of fast-twitch hindlimb skeletal muscle wasting in MCT-administered animals. In contrast to their effects on body weight, however, both feeding groups of sTNFR1-treated animals attenuated the loss of EDL muscle mass. However, the effects were not equal in both feeding groups. Ad libitum-fed sTNFR1-treated animals attenuated EDL muscle loss by 45% compared with those given MCT alone. When food was restricted, sTNFR1 only attenuated EDL loss by 20% compared with those given MCT alone. Thus roughly one-half of EDL sparing in ad libitum-fed sTNFR1-treated animals occurred through a mechanism independent of food intake. In contrast to sTNFR1, PTX treatment spared 26% of EDL muscle mass in MCT-administered animals, regardless of feeding group. To begin to explain the sparing of EDL mass in sTNFR1 and PTX treatment groups, transcriptional activation of the UPP was examined.

Given the well defined upregulation of UPP transcripts in muscle wasting and cachexia, any treatment such as sTNFR1 or PTX that spares muscle mass would be expected to be effects on additional atrogin-1 transcripts are unknown. In addition, the efficacy of anti-TNF pharmacotherapy has not been explored in any model of cardiac cachexia. Therefore, it was the goal of the present study to determine whether employing TNF-inhibitor treatments sTNFR1 and PTX in MCT-administered animals would result in better outcomes of cardiac cachexia, specifically, prevent anorexia, reduce the expression of atrogin-1 transcripts, and attenuate whole body and muscle wasting. We show for the first time that 1) sTNFR1’s action of reversing low food consumption in MCT-administered animals is wholly responsible for body mass loss, partly responsible for EDL mass loss, and increases in some atrogin-1 transcripts; and 2) MCT administration increases mRNAs for MaFbx, some 26S proteasome subunits, and an E2 conjugating enzyme.

Treatment with sTNFR1 was shown to have a clear antianorectic effect in MCT-administered, ad libitum-fed animals in the present study. Although our study does not allow for determining a mechanism, it is noteworthy that TNF-α has been well documented to induce an anorectic state at the level of the hypothalamus (23). Thus sTNFR1 treatment may be preventing the cytokine from interacting with hypothalamic TNF receptors in the present model. In addition, our finding that sTNFR1 attenuates anorexia in MCT-administered animals supports a previous study in which a similar specific TNF receptor, TNF-binding protein, was shown to attenuate anorexia in a cancer cachexia model (30). Importantly, the antianorectic effect of sTNFR1 proved to be an essential component for attenuating muscle and whole body wasting in MCT-administered animals.

Both sTNFR1 and PTX reduced the amount of body weight loss in animals administered MCT; however, sTNFR1 pair-fed animals showed no difference in body weight from MCT-administered animals. Expressed another way, the sparing of body weight by sTNFR1 was completely abolished when food was restricted. The finding shows that the antianorectic effect of sTNFR1 was responsible for improvements in body weight. In contrast, both feeding groups of PTX-treated animals showed attenuated body weight loss, thus indicating that the effect of PTX in MCT-administered animals is independent of food intake.

In accordance with their effects on body weight, both sTNFR1 and PTX were shown to reduce the degree of fast-twitch hindlimb skeletal muscle wasting in MCT-administered animals. In contrast to their effects on body weight, however, both feeding groups of sTNFR1-treated animals attenuated the loss of EDL muscle mass. However, the effects were not equal in both feeding groups. Ad libitum-fed sTNFR1-treated animals attenuated EDL muscle loss by 45% compared with those given MCT alone. When food was restricted, sTNFR1 only attenuated EDL loss by 20% compared with those given MCT alone. Thus roughly one-half of EDL sparing in ad libitum-fed sTNFR1-treated animals occurred through a mechanism independent of food intake. In contrast to sTNFR1, PTX treatment spared 26% of EDL muscle mass in MCT-administered animals, regardless of feeding group. To begin to explain the sparing of EDL mass in sTNFR1 and PTX treatment groups, transcriptional activation of the UPP was examined.

Given the well defined upregulation of UPP transcripts in muscle wasting and cachexia, any treatment such as sTNFR1 or PTX that spares muscle mass would be expected to be
mirrored by attenuation in UPP transcripts. Indeed, sTNFR1 treatment coupled with ad libitum feeding led to a significant reduction in MaFbx, ubiquitin, and PSMC2 transcript levels in MCT-administered animals; however, the attenuation was not observed in the remaining transcripts measured. Furthermore, the attenuations observed in MaFbx, ubiquitin, and PSMC2 transcript levels were uniquely completely abolished in sTNFR1 pair-fed animals; these data suggest that sTNFR1 treatment must be coupled with a normal food intake for the full benefit of sTNFR1 in terms of both suppression of UPP transcripts and improved muscle sparing.

Contrary to our hypothesis, neither sTNFR1 nor PTX had a significant effect on transcript levels for the majority of 26S proteasome subunits. However, it is putatively the availability of substrates, not the structural components of the 26S proteasome, that may limit the activity of the UPP. Thus, modest changes in mRNA expression of 26S proteasome subunits observed in the present study may be less important than expression of UPP components that affect substrate availability, i.e., expression of the E3 ligase MaFbx. Intriguingly, increased transcript levels of MaFbx, ubiquitin, and UBC7 were associated with lower EDL masses. If this finding holds for human patients, treatments such as sTNFR1 with increased nutritional support should attenuate increases in these transcript levels as well as resulting in greater muscle mass in patients with cardiac cachexia, potentially improving mortality.

The mechanism by which sTNFR1 or PTX treatment leads to the decrease in MaFbx was investigated in ad libitum-fed animals by targeting pathways that have previously been shown to regulate MaFbx, specifically the Akt/FoxO3a pathway (25, 26) and p38 MAPK (18, 20). Unexpectedly, none of our data suggests that reduced MaFbx transcript levels in sTNFR1 or PTX treatment groups were attenuated through either the Akt/FoxO3a pathway or through a mechanism involving p38 MAPK. Previous studies have demonstrated that fast decreasing phosphorylation of Akt and FoxO3a, resulting in increased MaFbx expression. In the present study, we observed that decreased food intake is associated with decreased phosphorylation of Akt and FoxO3a, as well as increased MaFbx expression. Interestingly, we observed a significant increase in MaFbx expression above that observed in pair feeding, which cannot be attributed to by changes in Akt/FoxO3a signaling. Therefore, our data indicate that there is an alternative, parallel signaling pathway that regulates MaFbx expression due to cardiac cachexia, in addition to the Akt/FoxO3a signaling pathway, that is responsive to food intake.

Although we have shown the potential for PTX to attenuate the loss of body and muscle weights independent of food intake, the mechanism behind this phenomenon remains unknown. Unlike sTNFR1, PTX is not a specific inhibitor of TNF and is documented to have additional effects including increasing limb blood flow (27) as well as gas exchange in the lung (16), which could contribute to the improved status of these animals.

In summary, the antianorectic effect of sTNFR1 in sparing EDL and body weights of MCT-administered animals, as well as preferentially attenuating increases in some UPP transcripts, opens further awareness to pursue anorexic mechanisms of cardiac cachexia in a search for potential therapies. We reported that anti-TNF treatment reduced EDL wasting in cardiac cachexia, and we observed a further reduction in EDL wasting, by improving appetite in sTNFR1-treated, ad libitum-fed animals. These findings emphasize the need for multiple, simultaneous treatments, which not only target inflammatory molecules, but other important clinical outcomes like anorexia, to counter muscle wasting from cardiac cachexia.

ACKNOWLEDGMENTS

Anti-TNF-α sTNFR1 was provided by Amgen. This work was done in partial fulfillment of requirements for the Ph.D degree for B. T. Steffen, who was supported by the Heartland Affiliate of the American Heart Association.

GRANTS

This study was support by American Heart Association Grant 610006Z to B. T. Steffen.

REFERENCES


