Mechanisms of Cachexia in Chronic Disease States

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Abstract: Sarcopenia and cachexia are muscle wasting syndromes associated with aging and with many chronic diseases, such as congestive heart failure (CHF), diabetes, cancer, chronic obstructive pulmonary disease and chronic kidney disease (CKD). While mechanisms are complex, these conditions are often accompanied by elevated angiotensin II (Ang II). Patients with advanced CHF or CKD often have increased Ang II levels and cachexia, and angiotensin-converting enzyme inhibitor treatment improves weight loss. It was found that Ang II infusion in rodents leads to skeletal muscle wasting. Ang II increases cytokines and circulating hormones, such as tumor necrosis factor-α, interleukin-6, serum amyloid-A and glucocorticoids, which regulate muscle protein synthesis and degradation. Ang II–induced muscle wasting is caused by alterations in insulin-like growth factor-1 signaling, enhanced muscle protein breakdown via the ubiquitin-proteasome system and decreased appetite resulting from the downregulation of hypothalamic orexigenic neuropeptides, such as Npy and orexin. Ang II also inhibits 5′ adenosine monophosphate–activated protein kinase activity and disrupts normal energy balance via the activation of 5′ adenosine monophosphate–activated protein kinase phosphatase PP2Cα. Furthermore, Ang II inhibits skeletal muscle stem (satellite) cell proliferation, leading to lowered muscle regenerative capacity. Distinct satellite cell angiotensin receptor subtypes have different effects on different stages of differentiation and are critical for the regulation of muscle regeneration. These data suggest that the renin-angiotensin system plays a critical role in mechanisms underlying cachexia in chronic disease states, and it is a promising target for the treatment of muscle atrophy in patients with diseases such as CHF and CKD.

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Patients with cachexia, or wasting syndrome, develop weight loss, muscle atrophy, fatigue, weakness and often loss of appetite without actively trying to lose weight. Patients with cachexia are defined as those who lose more than 5% of body weight over 12 months or less in the presence of a chronic disease such as congestive heart failure (CHF), chronic kidney disease (CKD), chronic obstructive pulmonary disease and cancer. However, 10% to 30% of the patients with these diseases develop cachexia, and it affects more than 5 million people in the United States.[1] Cachexia is a multifactorial disease and, importantly, nutritional support cannot fully reverse the syndrome. In cachexia conditions, the degradation of myofibrillar proteins is increased and protein synthesis is decreased, leading to the rapid loss of muscle mass. Weight loss and reduced muscle mass are associated with a reduction in the quality of life and increased mortality. Thus, cachexia is a major public health issue, and the development of interventions to block or attenuate this process would have significant therapeutic benefits in a wide array of chronic diseases.

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MECHANISMS AND POTENTIAL THERAPIES FOR CACHEXIA

Among the candidate mediators of cachexia that have been investigated, proinflammatory cytokine tumor necrosis factor-α (TNF-α) is the most prominent and well-characterized factor. TNF-α has been shown to induce cachexia in mice[2] and cause myotube atrophy *in vitro* via the activation of E3 ubiquitin ligases.[3] Although many rodent tumor models of cancer cachexia showed an increased TNF-α level,[4] the relevance of TNF-α to human cancer cachexia is unclear. Maltoni et al[5] found that circulating levels of TNF-α in patients with cancer cachexia had no correlation with weight loss and anorexia. Furthermore, a clinical trial designed to block TNF-α signaling using anti-TNF-α antibody (infliximab) in patients with cancer cachexia closed early because the treatment prevent or palliate cancer-associated weight loss, and patients developed greater fatigue and worse global quality of life scores.[6]

Another candidate mediator of cachexia is interleukin-6 (IL-6). It has been shown in some,[7,8] but not all,[5] studies that different kinds of cancer cells secrete IL-6 and that circulating levels of IL-6 correlate with weight loss in patients with cancer. Strassmann et al[9] showed that increasing levels of IL-6 in tumor-bearing mice correlated with the development of cachexia and that an antibody against IL-6, but not against TNF-α, suppressed cachexia development. However, a clinical trial of IL-6 antibody in weight-losing patients with lung cancer did not show a significant effect on the loss of lean body mass, although anorexia, fatigue and anemia were prevented.[10]

Myostatin and activin A are the most recent and promising target molecules related to cancer cachexia. Myostatin and activin A are members of the transforming growth factor-β (TGF-β) family, and both are upregulated in patients with various kinds of wasting diseases.[11] Animals and humans with null mutations of myostatin show dramatic muscle hypertrophy[12,13] and blockade of activin A–restored regenerative capacity of human myoblasts in the presence of high cytokines (TNF-α or IL-1β).[14] Myostatin and activin A signal through the common receptor, activin type II receptor B (ActRIIB). To inhibit both myostatin and activin A signaling at the same time, soluble ActRIIB-Fc decoy protein was developed. Treatment of tumor-bearing mice with soluble ActRIIB-Fc decoy protein prevented cachexia development without affecting the tumor growth and prolonged survival.[15] Thus, blockade of ActRIIB signaling seems to be a very promising treatment of cancer cachexia, and clinical trials are ongoing to treat patients with sarcopenia and cachexia.[16]

Cachectic patients with CHF showed increased growth hormone levels with lower insulin-like growth factor-1 (IGF-1), suggesting growth hormone resistance.[17] Also, it has been shown that patients with CHF had higher glucose and insulin levels, an indication of insulin resistance.[18] Because patients

with CHF have higher Ang II levels and Ang II causes insulin resistance in skeletal muscle by inhibiting insulin-stimulated GLUT4 translocation,[19,20] it was postulated that blockade of Ang II could benefit skeletal muscle function in chronic diseases with high levels of Ang II. There are 2 main pharmacological approaches to target the effects of Ang II: (1) inhibiting the formation of Ang II by angiotensin-converting enzyme (ACE) inhibitor (ACEi) and (2) blocking the AT1R by angiotensin receptor blocker (ARB). Because alternative ACE-independent pathways of Ang II formation exist, the use of ARB could be more specific in targeting Ang II–mediated muscle wasting. The ACEi enalapril was shown to reduce the risk of weight loss in patients with CHF,[21] and ACEi helped maintain body weight but not muscle strength in patients with CHF or hypertension.[22] Elderly patients without heart failure on antihypertensive treatment with ACEi had higher muscle mass than those receiving other antihypertensive therapy.[23] In addition, insulin sensitivity was improved by losartan and lisinopril in patients with hypertension.[24] There have been a few clinical trials designed to protect muscle wasting by blocking Ang II. Ark Therapeutics has completed a phase III clinical trial treating patients with cancer cachexia with the ACEi imidapril. Imidapril prevented weight loss in non–small cell lung cancer and colorectal cancer but not in pancreatic cancer, but its effect to prevent weight loss did not reach the statistical significance when the data were combined. The company discontinued development of the product after the failed clinical trial, although it remains convinced of the value of this approach.[25,26] Blockade of AT1R signaling could be another approach to prevent weight loss in patients with cachexia, but Merck & Co, Inc, which marketed losartan as Cozaar, has no plans to develop it for new indications in the United States because it went off patent.[26]

One of the potential reasons why clinical trials of Ang II blockade have so far been unsuccessful is that the underlying mechanisms of renin-angiotensin system–mediated muscle wasting is not fully understood. For instance, Ang II and other angiotensins act on different subtypes of receptors, such as AT1R, AT2R, Mas and IRAP.[27] Because ACEi blocks the conversion of Ang I to Ang II, ACEi treatment results in a decrease in Ang II level, whereas Ang I level is increased. Conversely, blockade of AT1R by ARB results in a compensatory increase in Ang II, which may activate AT2R-mediated signaling. Thus, it is critical to understand the multiple signaling pathways that mediate the effect of the renin-angiotensin system (RAS). Current progress in understanding the role of the RAS in muscle wasting is summarized and discussed below.

ANG II AND MUSCLE WASTING

It was first demonstrated that Ang II infusion in the rat caused a significant loss of body weight through 2 independent mechanisms, a reduction in food intake and increased proteolysis in skeletal muscle.[28] Both of these effects were completely prevented by losartan but not by the vasodilator hydralazine, showing that Ang II causes wasting through the AT1R and that its effect is independent of blood pressure regulation. It was found that Ang II caused an increase in muscle protein breakdown via the ubiquitin-proteasome system (UPS). Accelerated proteolysis via the UPS plays a major role in muscle atrophy in several different types of cachexia.[29] The genes of muscle-specific E3 ubiquitin ligases atrogin-1 and muscle RING finger-1 (MuRF-1) were identified to be strongly upregulated in different muscle atrophy conditions, and knockout mice for either of these genes partially prevented muscle wasting.[30] In Ang II–induced muscle wasting, expression of atrogin-1 and MuRF-1, levels of ubiquitin-conjugated proteins and 20S proteasome activity were robustly increased.[31–33]

In the Ang II–induced wasting condition, it was also found that there was a decrease in skeletal muscle IGF-1 signaling, which is the main anabolic pathway in skeletal muscle.[28,34] IGF-1 modulates muscle size via autocrine and paracrine signals, by directly stimulating protein anabolism in myofibers and by the activation of satellite cell proliferation.[35] IGF-1 signals through PI3K/Akt and induces muscle hypertrophy by stimulating GSK and mTOR kinases, which regulate protein translation.[36] Multiple studies have shown the involvement of IGF-1/PI3K/Akt signaling in muscle cell size regulation and atrophy. For instance, inhibition of PI3K and expression of dominant-negative Akt reduce the size of myotubes *in vitro*,[37] mice deficient for Akt1 and Akt2 have smaller muscle size[38] and activation of Akt in rat muscle prevents denervation-induced atrophy.[36,39] The authors used a transgenic mouse strain in which IGF-1 was overexpressed under the control of a skeletal muscle–specific promoter[40] and showed that the local increase in IGF-1 could prevent Ang II–induced muscle wasting.[31] Interestingly, although Ang II rapidly increased both atrogin-1 and MuRF-1 expression, IGF-1 prevented only the increase in atrogin-1.[32] These data may be consistent with the current evidence suggesting the distinct roles of atrogin-1 and MuRF-1 in muscle wasting.[41] Myofibrillar proteins have been identified as the target of MuRF-1,[42,43] and it is suggested that MuRF-1 is likely involved in skeletal muscle proteolysis. However, atrogin-1 has been shown to target MyoD, the regulator of myogenesis, and eIF-e, the eukaryotic initiation factor of protein synthesis, suggesting that its main role is the regulation of protein synthesis.[44,45] Although precise signaling pathways whereby Ang II and IGF-1 regulate atrogin-1 and MuRF-1 remain to be elucidated, it is of note that atrogin-1 and MuRF-1 are regulated by distinct mechanisms.[46,47] In summary, Ang II and IGF-1 have opposing roles in regulating muscle protein synthesis and degradation. Disruption of IGF-1 signaling by Ang II plays a critical role in Ang II–induced atrophy, and local activation of IGF-1 signaling can prevent Ang II–induced muscle wasting.

There have been studies reporting that the effect of Ang II to cause muscle wasting is via the direct action of Ang II on skeletal muscle cells and is indirectly mediated by other circulating factors. It has been shown that Ang II directly acts on cultured muscle cells and induces proteolysis via the UPS pathway.[48,49] However, it has been demonstrated that multiple circulating hormones and cytokines mediate Ang II's action on skeletal muscle. Glucocorticoids are required for the activation of the UPS in acidosis and diabetes, and glucocorticoid inhibition restored Ang II–induced loss of muscle mass.[31] After Ang II infusion, there is an increase in circulating IL-6 and serum amyloid-A, and blockade of IL-6/serum amyloid-A prevented Ang II–induced wasting.[50] These studies suggest that the catabolic effect of Ang II on skeletal muscle *in vivo* is, at least in part, mediated via intermediate molecules activated by Ang II.

ANG II AND OXIDATIVE STRESS

Reactive oxygen species (ROS) play an important role in Ang II–induced signaling in different cell types, contributing to cardiac myocyte and vascular smooth muscle cell hypertrophy, endothelial dysfunction, hypertension and insulin resistance.[51,52] Ang II has been shown to induce ROS generation in skeletal muscle,[53,54] and ROS contributes to disuse muscle atrophy.[55] NAPDH oxidase and mitochondria are major sources