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Review

Animal models of cardiac cachexia

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ABSTRACT

Cachexia is the loss of body weight associated with several chronic diseases including chronic heart failure (CHF). The cachectic condition is mainly due to loss of skeletal muscle mass and adipose tissue depletion. The majority of experimental in vivo studies on cachexia rely on animal models of cancer cachexia while a reliable and appropriate model for cardiac cachexia has not yet been established.

A critical issue in generating a cardiac cachexia model is that genetic modifications or pharmacological treatments impairing the heart functionality and used to obtain the heart failure model might likely impair the skeletal muscle, this also being a striated muscle and sharing with the myocardium several molecular and physiological mechanisms. On the other hand, often, the induction of heart damage in the several existing models of heart failure does not necessarily lead to skeletal muscle loss and cachexia.

Here we describe the main features of cardiac cachexia and illustrate some animal models proposed for cardiac cachexia studies; they include the genetic calsequestrin and Dahl salt-sensitive models, the monocrotaline model and the surgical models obtained by left anterior descending (LAD) ligation, transverse aortic constriction (TAC) and ascending aortic banding.

The availability of a specific animal model for cardiac cachexia is a crucial issue since, besides the common aspects of cachexia in the different syndromes, each disease has some peculiarities in its etiology and pathophysiology leading to cachexia. Such peculiarities need to be unraveled in order to find new targets for effective therapies.

1. Cardiac cachexia

Cachexia is the loss of more than 5% of body weight over 12 months occurring in the presence of a chronic illness such as cancer, sepsis, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis, acquired immunodeficiency syndrome (AIDS) and chronic heart failure (CHF). Cachexia is a complex syndrome whose diagnosis requires the presence of other symptoms e.g. fatigue or reduced muscle strength, loss of lean body mass and biochemical abnormalities (e.g. anemia, inflammation or low albumin) [1]. CHF is the final outcome of several cardiac dysfunctions such as ischemic heart disease, idiopathic dilated cardiomyopaty and valvular dysfunctions [2]. Breathlessness, muscle fatigue and exercise intolerance are typical features while cachexia occurs in 5–15% of end-stage CHF patients. When cachexia is associated with CHF (hereinafter referred to as cardiac cachexia; CC), the efficacy of treatments decreases and patients have a devastating prognosis independently of the left ventricular (LV) ejection fraction [3].

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2. Pathophysiology of CC

To date attempts to unveil the pathogenetic mechanisms leading to CC have failed; etiology and pathophysiology of cachexia might vary according to the underlying disease although the final outcome of cachexia is similar from one disease to another. The weight loss characterizing CC is mainly due to the progressive loss of skeletal muscle mass, but also to fat and bone mass loss which are affected later in the disease [3].

Several players have been proposed as possibly having a role in causing CC. However, their exact role and the correlation between them remain unknown. Lean mass loss occurring in CC results from an imbalance between myofibril anabolism and catabolism with an excess of catabolism and myocyte apoptosis over anabolism, triggered by an increased systemic inflammatory response. TNF-α is considered one of the main players involved in the pathogenesis of CC. This pro-inflammatory cytokine is activated in all forms of cachexia and its increased levels are associated with poor survival. Elevated circulating levels of other pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and interferon-γ, along with the C-reactive protein, have also been involved in the pathogenesis of CHF and CC [4] and represent a strong predictor for pathological weight loss [5].

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Some data suggest that pro-inflammatory cytokines, as well growth and differentiation factor 15 (GDF-15) not only act peripherally on lipolysis and proteolysis, but also act centrally on hypothalamic appetite regulation. They might also trigger an imbalance between the leptin and the ghrelin systems, thus generating food intake alterations [6]. In fact, lack of appetite and malabsorption occur in CC, while leptin and ghrelin are among the most influent hormones regulating energy balance [3]. Ghrelin is mainly produced by the stomach; it stimulates the secretion of growth hormone (GH) and blocks the leptin-mediated decrease of food intake. Notably, CC patients show paradoxically higher levels of plasma ghrelin and GH, compared to controls and non-cachectic patients [7,8]. As regards leptin, it is secreted by adipocytes and its plasma concentration directly correlates with total fat mass [9]. Leptin inhibits food intake; however, its levels normalized for fat tissue amount are not higher in cachectic compared to non-cachectic CHF patients; this suggests, once again, that appetite and food intake might not be particularly relevant for CC's development [10,11].

A critical role in cachexia is played by myostatin (also known as growth and differentiation factor 8; GDF-8), a member of the transforming growth factor beta (TGF-β) family. Myostatin is a negative regulator of muscle growth. Accordingly, it was found that the administration of myostatin-blocking antibodies reversed muscle mass loss in the myostatin wild-type mice [12–14].

The stimulation of the renin-angiotensin system and increased levels of angiotensin-II and glucocorticoids (aldosterone and cortisol) are features of CC patients. It has been clearly shown that injection of systemic angiotensin-II triggers skeletal muscle atrophy in rodents [15]. Moreover, angiotensin converting enzyme (ACE) inhibitors blocking angiotensin-II reduce weight loss and skeletal muscle mass loss occurring in CC, thus increasing muscle strength [16].

It has also been hypothesized that molecular mechanisms initially evoked to protect the heart from damage might lead to neuroendocrine dysregulation which finally produce profound immune and metabolic alterations leading to cachexia [3,4,17]. In the early phase of the acute illness, the body initiates several processes to mobilize energy stores in order to allow the acute phase response; in this phase, the catabolism of skeletal muscle provides fundamental energy substrates. The hypothalamus-pituitary gland axis regulates glucocorticoid release [18] which promotes catabolism in peripheral tissues [19] among which skeletal muscle. When the inflammation persists, this mobilization of protein from skeletal muscle leads to atrophy [20]. Moreover, in the context of subacute and chronic disease, the persistent inflammation determines also the onset of hypothalamic inflammation (responsible for the regulation of appetite, body mass and energy homeostasis) and the transition from sickness to cachexia [21].

Finally, it has been found that CC patients show high plasma levels of noradrenaline and brain and atrial natriuretic peptides (BNP and ANP) and that TNF-α is strongly associated with BNP and ANP secretion [22,23]. As stated above, along with skeletal muscle mass wasting, there is also a decrease in fat mass in CC. Loss of adipose tissue may be mediated by increased lipolysis (typically occurring in CC) and, for example, induced by catecholamines or reduced lipogenesis [24]. Also the bone density is reduced in CHF patients with lower calcium and vitamin D levels [3]. Skeletal muscle wasting has also been associated with high free-radical production [48] and to reduced regeneration capacity of the impaired skeletal muscle [25].

3. Animal models of CC

Unraveling the pathophysiological mechanisms leading to CHF-associated cachexia is crucial in order to highlight new targets for efficient therapies, as yet still lacking. Animal models mimicking human diseases represent important tools for understanding the pathogenetic pathways associated with many diseases.

Most studies on cachexia use animal models of cancer cachexia. Since some mechanisms leading to cachexia are independent of the etiology of the disease, the therapies identified for cancer cachexia might also be potentially used for CC patients. However, some differences must exist between different diseases and a specific animal model for CC is essential in order to focus studies on this syndrome. It is plausible that most of the differences occur early in the development of the syndrome; therefore identifying the stage of pre-cachexia will allow early intervention and the delay or avoidance of the onset of severe wasting.

Genetic, pharmacological, surgical, models have been proposed for CC, although as yet there is no appropriate animal model. Here we illustrate some suggested animal models of CC.

3.1. Calsequestrin (CSQ)-overexpressing mice: a genetic model of CC

Transgenic mice overexpressing cardiac CSQ in atrium and ventricle under control of the α-myosin heavy chain (MyHC) promoter, are a model for studying skeletal muscle atrophy following non-ischemic heart failure , i.e. CC [26]. CSQ-overexpressing mice develop a severe cardiac hypertrophy at 8 weeks of age, with a significant increase in the size of cardiac myocytes, this leading to CHF. Although the exact molecular mechanism is unknown, this phenotype has been associated with the impairment of both Ca^{2+} level modulation and β-adrenergic receptor signaling both of which are triggered by CSQ-overexpression [27–29].

Defective Ca^{2+} homeostasis plays a critical role in human heart failure [30]. Ca^{2+} plays an active role in the excitation-contraction coupling; when released into the cytosol, Ca^{2+} mainly targets myofilaments and generates the contractile force of the heart, whereas the removal of Ca^{2+} from the cytosol induces heart relaxation. Ca^{2+} levels and flux in and out of the sarcoplasmatic reticulum (SR) are tightly controlled by specific proteins, CSQ being one of them. In cardiomyocytes, intracellular Ca^{2+} is mostly sequestered into the SR where it is bound to CSQ located within the lumen of the SR. CSQ is associated with the ryanodine receptors through the proteins junctin and triadin, thus forming a quaternary complex [31]. CSQ-transgenic mice show 10-fold higher levels of CSQ compared to WT mice, this impairing Ca^{2+} flux modulation [27]. It has been suggested that CSQ might play both a storage and a dynamic regulatory role in Ca^{2+} homeostasis and cycling and that the chronic alteration of Ca^{2+} release caused by overexpression of CSQ may initiate a cascade of molecular events activating the hypertrophic program possibly as a consequence of a chronic reduction in force-generating capacity. Moreover, the transgenic myocardium adapts to CSQ overexpression by downregulating other proteins involved in the Ca^{2+}-release cascade and binding to CSQ (such as ryanodine receptor, junctin and triadin); this strongly correlates with the functional impairment of Ca^{2+} release typical of transgenic hearts overexpressing CSQ.

In addition to Ca^{2+} level modulation, it has also been found that CSQ-transgenic mice show abnormal G protein-coupled β-adrenergic receptor signaling. This can also be associated with cellular contractile failure typical of this mouse model. Indeed, severely impaired β-adrenergic receptor function appears to be a critical event in the failing heart [28] and the use of β-blockers increases the survival of the transgenic animals [26,32].

CSQ-transgenic mice have no growth retardation [33] but die prematurely by the age of 10–16 weeks [28]. They display features of human dilated cardiomyopathy by echocardiography, with a twofold cardiac hypertrophy and increased wall thickness and, later on, LV enlargement, induction of fetal expression and increased ANP, depressed contractile function and severe cardiac dysfunction [27,32,33]. At 8 weeks of age, CSQ-overexpressing mice develop severe cachexia with a significant decrease in body weight and reduced exercise tolerance in performing a treadmill running test [33]. These mice develop cardiac impairment very rapidly (3 to 4 weeks) compared to CHF patients which usually develop the disease in a space of years. Consistently, also skeletal muscle impairment occurs very quickly and, soon after, mice die [29]. In CHF, the molecular signaling leading to muscle