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Role of Granulocyte-Colony Stimulating Factor on Skeletal Muscle in Animal Model of Chronic Kidney Disease

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Abstract

Loss of muscle proteins is a deleterious consequence of chronic kidney disease (CKD) that causes a decrease in muscle strength and function, and can lead to a reduction in quality of life and increased risk of morbidity and mortality. The effectiveness of current treatment strategies in preventing or reversing muscle protein losses is limited. The limitations largely stem from the systemic nature of diseases such as CKD, which stimulate skeletal muscle protein degradation pathways while simultaneously activating mechanisms that impair muscle protein synthesis and repair. Stimuli that initiate muscle protein loss include metabolic acidosis, insulin and IGF1 resistance, changes in hormones, cytokines, inflammatory processes and decreased appetite. A growing body of evidence suggests that signaling molecules secreted from muscle can enter the circulation and subsequently interact with recipient organs, including the kidneys, while conversely, pathological events in the kidney can adversely influence protein metabolism in skeletal muscle, demonstrating the existence of crosstalk between kidney and muscle. Together, these signals, whether direct or indirect, induce changes in the levels of regulatory and effector proteins via alterations in mRNAs, microRNAs and chromatin epigenetic responses. Advances in our understanding of the signals and processes that mediate muscle loss in CKD and other muscle wasting conditions will support future development of therapeutic strategies to reduce muscle loss. Administration of granulocyte colony stimulating factor has an ameliorating role in regeneration of CKD induced skeletal muscle damage.

Keywords: Granulocyte-Colony Stimulating Factor, Skeletal Muscle, Chronic Kidney Disease.

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1. Introduction

There are different animal models available for induction of CKD. These models are important to understand the pathology of renal fibrosis and for the evaluation of new treatments. They include surgical, chemical and physical, genetic and in vitro models [1]. Whenever surgical models are considered, we cannot ignore the most popular model, the 5/6 nephrectomy that mimics CKD in humans, by the removal of one whole kidney and two-thirds of other kidney. In this model, glomerulosclerosis phenomena appear in the first phase, followed by tubule interstitial fibrosis and tubular atrophy [2]. Another popular model is induction of interstitial fibrosis phenomena by unilateral ureteral obstruction. In this case there is advantage of using contralateral kidney as a control [3] However, these surgical techniques are often accompanied by high mortalities and require well trained surgeons [4-5]. CKD induction medication may sometimes be preferred because they are not as invasive as models that require certain surgical techniques and may simulate CKD as well. They include Cisplatin, folic acid exerting toxicity in renal tubule, streptozocin is a good marker for the simulation of diabetic nephropathy by tubular and glomerular impairment in type 1 diabetes and adriamycin [6]. Adenine-induced CKD is also used by some researchers. Major mechanisms include inflammation, oxidative stress, programmed cell death and metabolic disorders. However, this usually requires at least 16 weeks of adenine administration [7].

Also, male patients with CKD may develop gonadal abnormalities that lead to impotence, reduced libido, decreased testicular size, impaired spermatogenesis and gynaecomastia [8-9]. Folic acid (FA) also known as vitamin B9 is a vital component of a healthy diet, being essential for numerous bodily functions and has a well-known role in prevention of neural tube defects. As some vitamins, FA could not be synthesized in mammalian cells and is delivered from exogenous sources including foods and intestinal micro biota. Animal liver and kidney, mushrooms, spinach, yeast, green leaves, and grasses are richest in folates according to the National Institute of Health in 2021 [10]. Deficiency of folate is common, with studies suggesting prevalence of deficiency as high as 85.5% as was shown in women between the ages of 16 and 49. Causes of folate deficiency range from diet and lifestyle, to pathological & pharmacological causes. So, numerous countries have implemented strategies to increase folate intake with programs such as mandatory grain

fortification. The US Institute of Medicine and European Food Safety Authority suggest tolerable upper limit of folic acid to be 1000 µg. Higher levels of intake may cause or exacerbate vitamin B12 deficiency related neurological damage [11]. High prolonged folic acid intakes have been linked to increased cancer risk and progression within certain patient groups and also to insulin resistance in children, interaction with epilepsy medications, masking vitamin B12 deficiency & hepatotoxicity at high concentrations.

The Proposed mechanisms for this include folic acid enhancing DNA synthesis and replication within cells, while reducing the natural killer cell response to carcinogenic cells [12]. While a low dose of FA (usually less than 10 mg/day) is nutritionally beneficial, a high dose of FA (250 mg) is very toxic to the kidneys and causes damage mainly to the proximal tubular epithelial cells. Therefore, folic acid (FA) may be used as animal model of CKD [13]. FA is a vitamin and is not environmentally toxic. So, routine handling in laboratories does not have any hazards. Second, unlike ischemic surgery of kidney injury, of FA is administered as a simple intraperitoneal injection, which does not require surgery and animal friendly. Third, unlike the cadmium and cisplatin toxicity models, which induce multiple organ injury, the FA model mainly injures the kidney can simulate the clinical symptoms of human kidney disease without deleterious effects on other organs [14-15]. Folic acid can accumulate in larger amounts in the kidney than in other tissues because of the high content of folate receptors causing tubular obstruction by FA crystals and so acute kidney injury within 72 h of FA administration. If left untreated, acute damage occurs within 2 weeks with tubulointerstitial fibrosis becoming apparent between 3 and 4 weeks. Biochemical markers of renal impairment occur in parallel with renal fibrosis making this a particularly useful model for studying the consequences of CKD [16-19].

2. Granulocyte-colony-stimulating factor

Granulocyte colony-stimulating factor (G-CSF) is a cytokine most well-known for maturation and mobilization of bone marrow neutrophils. So, it is used therapeutically to treat chemotherapy induced neutropenia, [20]. G-CSF acts by binding to its cognate cell surface receptor on target cells, causing the activation of intracellular signaling pathways mediating the proliferation, differentiation, function, and survival of cells in the neutrophil lineage. Studies in humans and mice demonstrate that GCSF also contributes to protecting the host against infection [21]. Human G-CSF is a glycoprotein located on chromosome 17 and encoded by CSF3 gene. However, this gene encodes two different products: G-CSFa contains 177 amino acids and G-CSFb contains 174 amino acids. The difference between the two types is that G-CSFa contains additional three residues after Leucine 35 (Valine-Serine-Glycine). The G-CSFb contains a glycosylation site on the oxygen known as O- linked glycosylation and this form is expressed in mammalian cells. It has been reported that G-CSFb obtains more biological activity (about 20 times more than G-CSFa), which makes it the source of commercial pharmaceutical products for G-CSF [22-23].

Granulocyte colony-stimulating factor acts as a powerful mobilizer of peripheral blood stem cells. Recombinant human G-CSF has been used to treat granulocytopenia and neutropenia after chemotherapy for cancer patients [23]. Food and Drug Administration (FDA) has approved G-CSF as a drug for its antiapoptotic and immune modulatory properties [24]. Granulocyte colony-stimulating factor has also several effects outside the hematopoietic system [25]. The hematopoietic stem cells (HSCs) reside in the microenvironment of the bone marrow in a highly organized manner. It is composed of at least three different niches: endosteal, peri-sinusoidal and peri-arteriolar. The endothelial and stromal cells occupy the perisinusoidal and peri-arteriolar niches, while macrophages and megakaryocytes occupy the perisinusoidal niche [26]. Bone marrow-derived mesenchymal stem cells are multi potent and capable of self-renewal. Also, they can move from the bone marrow into the circulation to support different tissues [27]. Hira et al. [28] reported that HSCs express specific protein called integrin α4β1 that mediate their adherence to bone marrow microenvironment via interaction with another protein expressed in bone marrow stromal cells called vascular cell adhesion molecule (VCAM1).

Following treatment of G-CSF, bone marrow neutrophils release specific proteases, such as matrix metalloproteinase 9 (MMP 9), neutrophil elastase and cathepsin G. These proteases cleave the interactions between C-X-C Motif Chemokine Ligand 12 (CXCL12) and chemokine receptor type 4 (CXCR4) and other factors that allow cells mobilization and release into circulation [29]. Cluster of differentiation 34 (CD34) is a Trans membrane glycoprotein first identified as a biomarker for hematopoietic stem cell progenitors. CD34 expression of these stem cells has been exploited for therapeutic purposes in various hematological disorders. It has been also found expressed on early hematopoietic stem cells, progenitor cells, endothelial cells, embryonic fibroblasts and some cells in fetal and adult nervous tissue [30]. In last few decades, studies have revealed the presence of CD34 expression on other types of cells with non-hematopoietic origins, such as interstitial cells & muscle satellite cells. This transmembrane phosphorglycoprotein present at the cell surface in humans and various animal species functioning as an adhesion factor between cells [30]. CD34 can mediate the attachment of different stem cells to the extracellular matrix in the bone marrow or straight to the tissue by binding to L-selectin expressed on endothelial cells and involved in the recruitment of leukocytes to sites of inflammation and infection.

From a medical perspective, this protein is involved in the process of extracting and enriching hematopoietic stem cells in order to perform bone marrow transplantation [31]. The mobilization of HSCs from the BM involves the interaction between stromal cell derived factor-1 (SDF-1) – CXCL 12- and CXCR4. SDF-1 is a chemokine protein that activates CXCR 4 that regulates stem cells migration and chemotaxis [32]. Many body cells can produce G-CSF after appropriate stimulation; cells from the monocyte and macrophage series are the most significant G-CSF source; however, normal cells of mesodermal origin can also produce G-CSF. G-CSF is present at low levels in healthy individuals and at increased levels in infections and inflammations. Normally, levels of circulating G-CSF are very low (<100 pg/mL). Nevertheless, G-CSF levels can increase to 20 times baseline levels in conditions of stress resulting in a rapid increase of circulating neutrophils [33]. Some pre-clinical studies have highlighted the role of G-CSF in different organs. Pourtaji et al., [34] mentioned that G-CSF exerts its

beneficial effects on cardiac cell repair through either stem cell mobilization or direct angiogenesis promotion, so that their regeneration might serve as a novel target in cardiac remodeling prevention and congestive heart failure treatment.

Moreover, neuroprotective effects of G-CSF were observed in other studies triggered by inhibition of apoptosis, inflammation and stimulation of neurogenesis [35]. Multiple courses of G-CSF, with or without stem or progenitor cell or growth factors (erythropoietin or growth hormone) infusion, might be associated with accelerated hepatic regeneration, improved liver function and survival [36]. The identification of the G-CSF receptor (G-CSFR) being expressed outside the hematopoietic system has revealed wider roles for G-CSF, particularly in tissue repair and regeneration. Skeletal muscle damage, including that following strenuous exercise, induces an elevation in plasma G-CSF, implicating it as a potential mediator of skeletal muscle repair [37]. The recombinant G-CSF named filgrastim was first approved in 1991, and its value has evolving since 2015. It has been included on the World Health Organization (WHO) Model List of Essential Medicines which is defined as a list of medicines needed for a basic healthcare system & & includes most efficacious, safe & cost–effective medicines for priority conditions [38]. Some in vitro studies found that administration of G-CSF had anabolic effect on muscle, so that G-CSF can be a potential agent for treating patients with muscle loss and sarcopenia. Also, there is growing evidence for G-CSF treatment of skeletal muscle myopathies [37-39].

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