Hepcidin: an important iron metabolism regulator in chronic kidney disease

Hepcidina: um importante regulador do metabolismo de ferro na doença renal crônica

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ABSTRACT

Anemia is a common complication and its impact on morbimortality in patients with chronic kidney disease (CKD) is well known. The discovery of hepcidin and its functions has contributed to a better understanding of iron metabolism disorders in CKD anemia. Hepcidin is a peptide mainly produced by hepatocytes and, through a connection with ferroportin, it regulates iron absorption in the duodenum and its release of stock cells. High hepcidin concentrations described in patients with CKD, especially in more advanced stages are attributed to decreased renal excretion and increased production. The elevation of hepcidin has been associated with infection, inflammation, atherosclerosis, insulin resistance and oxidative stress. Some strategies were tested to reduce the effects of hepcidin in patients with CKD, however more studies are necessary to assess the impact of its modulation in the management of anemia in this population.

Keywords: anemia; chronic; inflammation; peptides; renal dialysis; renal insufficiency.

RESUMO

Anemia é uma complicação frequente e seu impacto na morbimortalidade é bem conhecido em pacientes com doença renal crônica (DRC). A descoberta da hepcidina e de suas funções contribuíram para melhor compreensão dos distúrbios do metabolismo de ferro na anemia da DRC. Hepcidina é um peptídeo produzido principalmente pelos hepatócitos, e através de sua ligação com a ferroportina, regula a absorção de ferro no duodeno e sua liberação das células de estoque. Altas concentrações de hepcidina descritas em pacientes com DRC, principalmente em estádios mais avançados, são atribuídas à diminuição da excreção renal e ao aumento de sua produção. Elevação de hepcidina tem sido associada à ocorrência de infecção, inflamação, aterosclerose, resistência à insulina e estresse oxidativo. Algumas estratégias foram testadas para diminuir os efeitos da hepcidina em pacientes com DRC, entretanto, serão necessários mais estudos para avaliar o impacto de sua modulação no manejo da anemia nessa população.

Palavras-chave: anemia; doença renal crônica; diálise renal; inflamação; peptídeos.

INTRODUCTION

Anemia is a frequent complication in patients with chronic kidney disease (CKD), and its impact on morbidity and mortality is well known in this population.[1-3] Anemia starts manifesting in the initial stages, and increases its prevalence with the CKD progression.[4] Data from the Brazilian Society of Nephrology from 2012 showed that anemia, defined by hemoglobin < 11 g/dL, was present in 34% of the patients in dialysis, despite the free access to erythropoiesis stimulating agents and iron supplementation in our country.[5]

Erythropoietin deficiency is the main cause of anemia in this population[1] However, factors such as iron deficiency and chronic inflammation contribute greatly for its occurrence.[6]

Iron is a vital mineral for cell homeostasis. Its absolute or functional deficiency is associated with impaired hemoglobin synthesis; causing anemia and reduced tissue oxygenation.[7] The iron used by the body is derived from two main sources: food and the recycling of senescent red blood cells. Iron homeostasis is governed by two mechanisms: one

intracellular, according to the amount of iron present in the cell; and one systemic, wherein hepcidin plays a major role.[8] The discovery of hepcidin and its role in controlling iron availability to the tissues has contributed to improving the understanding the iron deficiency pathophysiology, including CKD-related anemia.[9]

Hepcidin, a peptide which gene is located on chromosome 19, is synthesized and secreted by various cells, and hepatocytes are its primary production site. This protein has four disulfide bridges among eight cysteine residues, which gives it a molecular configuration similar to that of drosomycin, a potent antifungal present in the Drosophila insect.[10]

Initially, a precursor protein with 84 amino acids is synthesized (pre-pro-hepcidin), which is cleaved to form pre-hepcidin with 60 amino acids and, thereafter the active form, a 25-aminoacid peptide.[1,11,12] Forms with lower numbers of amino acids are less abundant and probably represent degradation products.[11]

It is estimated that 11% of the plasma hepcidin is free in the circulation and the remainder circulate associated with α2 macroglobulin, a plasma protein with high affinity for hepcidin[12], and albumin.[13] Hepcidin-25 serum levels, established by using ELISA assay in normal volunteers, range between 2-56 ng/mL.[14] The clearance of hepcidin occurs through cell degradation after its binding to ferroportin and renal excretion.[13]

MECHANISM OF HEPCIDIN ACTION

Although hepcidin was initially isolated in human urine in 2001,[10] its role in iron homeostasis was established later using *in vitro* studies with mice.[15] The main action of this protein is to control serum iron, wherein a high hepcidin expression decreases iron, while its low expression increases serum iron concentration.

Hepcidin exerts its function by binding to ferroportin, a protein present in the cell membrane of macrophages, enterocytes, hepatocytes and placental syncytiotrophoblasts, preventing iron from leaving the cells. After the hepcidin-ferroportin complex is formed, it is internalized and subsequently degraded into lisossomes.[11,16,17] Note that ferroportin is the only pore from where iron leaves the cell.[16] Although ferroportin is a carrier of other metals such as manganese, zinc and cobalt, its affinity is greater towards iron.[18,19]

ADJUSTING THE CONCENTRATION OF HEPCIDIN

Hepcidin synthesis is mediated by physiological and pathological processes.[12] Physiologically, it is regulated by serum iron, and when in sufficient amounts, iron binds to the transferrin 1 receptor, shifting the hemochromatosis protein. This complex binds to the transferrin 2 receptor, increasing hepcidin transcription directly or by activating the morphogenetic 6-hemojuvelin bone protein complex.

In addition, hepcidin synthesis can be suppressed by endocrine signals (testosterone, estrogen and growth factors) and by the erythropoietic activity. However, this mechanism is not yet fully understood.[20] Erythroferrone, a recently found hormone produced by erythroblasts in response to erythropoietin, seems to mediate the suppression of hepcidin during erithropoieses.[21]

In disease conditions such as iron deficiency and hypoxia, there is a reduction in the liver synthesis of hepcidin. Infection, inflamation[12] and physical activity[22] induce an increased liver synthesis of hepcidin mediated by interleukin-6. High hepcidin concentrations have been described in association with elevated levels of inflammatory markers (such as interleukin-6 and C reactive protein), anemia (such as hemoglobin and endogenous erythropoietin) and iron parameters (such as ferritin), and the latter may reflect the inhibition of iron leaving its stocks.[23]

CLINICAL IMPLICATIONS

In studies carried out in mice, it was found that in the absence of hepcidin gene expression, the animals developed hemochromatosis framework similar to those developed by humans,[24] while the excessive production of this protein entailed anemia because of a decreased availability of iron for erithrogenesis.[25]

It is noteworthy that hepcidin is produced by various cell types in addition to hepatocytes, such as macrophages,[26] adipocytes,[27] cardiomyocites[28] and kidney cells (mainly distal tubule cells),[29] having autocrine effects in these tissues, in addition to the effect on iron metabolism.[12]

In vitro studies suggest an antimicrobial effect associated with hepcidin.[10] Notwithstanding, this effect requires much higher concentrations than those found in the circulation, even in the presence of infection.[10] This suggests that these high concentrations seem to be achieved locally on phagosomes of infected macrophages.[30] Hepcidin's ability to cause iron

deficiency, i.e., a decrease in extracellular iron, would also have a protective effect on the host, since low iron levels appear to be bacteriostactic[12] and some species of bacteria have iron as an important factor of increased virulence.[31]

In fact, some studies have shown that increased hepcidin can promote increases in the intracellular growth of some specific pathogens; among them we stress Salmonella, Chlamydia psittaci, and Legionella pneumophila,[32] exactly by increasing the concentration of iron within infected cells.

More recently, the expression of hepcidin was detected in multiple epithelial barriers,[33,34] often encountering pathogens, including renal tubule cells.[29] An experimental study in mice demonstrated that the decrease in hepcidin expression was involved in the decrease in the epithelial barrier resistance to infection by E. coli. In this study the authors suggest the possibility of new therapeutic strategies with hepcidin, to increase host defense or prevent maladaptive inflammatory responses that cause kidney damage in a variety of infectious and non-infectious conditions.[35]

On the other hand, a high level of hepcidin has been considered an independent risk factor for atherosclerosis in different populations[36,37], including patients in hemodialysis.[9,36] Some studies have shown an iron buildup in endothelial cells and macrophages in the atheroma plaque.[38] Furthermore, recent studies have shown a positive association between the concentration of hepcidin and mean intimal thickness,[36] just like the pulse wave velocity in hemodialysis patients.[39]

High serum concentration of hepcidin have been associated with higher levels of systolic blood pressure in healthy male individuals.[40] The role of hepcidin in ischemic disease is not fully understood, but it has been suggested an association between an increase in its concentration and insulin resistance,[41] inflamation[36] and increased oxidative stress.[42]

Hepcidin in CKD

Since the kidney is responsible for hepcidin excretion, many studies have shown increased concentrations of this protein in CKD patients, especially among those with more advanced stages of the disease and those in dialysis.[12,14,20,43] Coyne[43] describes an inverse correlation between the glomerular filtration rate and the plasma concentration of hepcidin in CKD patients not on dialysis. Some authors, however, did not report such association.[44] Different methods used to measure hepcidin, studies with small samples and different characteristics of the populations studied are the factors mentioned to explain these differences.[45]

In patients undergoing hemodialysis there are elevated concentrations of hepcidin.[14,46,47]. In fact, Ashby et al.[14] reported that the median active form of hepcidin was 26.5 and 58.5 ng/mL in patients with CKD and under hemodialysis, respectively. Zaritzky et al.[46], also using the ELISA method, showed an even higher median of 270 ng/ml in patients with CKD and 652 ng/ml in patients receiving dialysis.[47]

Increasing the concentration of hepcidin in CKD has been attributed not only to decreased renal excretion, but also by increased synthesis in response to inflammation,[9] hepcidin is the link between inflammation and iron metabolism disorders in this population.[3]

Hepcidin measurement methods

Initially, the measurement of serum and urinary levels of hepcidin was carried out by mRNA detection in laboratory animals and cell cultures; and only rarely it was performed in humans due to technical difficulties.[15] After the ELISA method was developed, which measured pro-hepcidin and could not distinguish between its different isoforms, studies with serum levels of this protein started to be carried out.

However, the relevance of such data is questionable, since the pro-hepcidin levels did not correlate with the levels of serum and urinary hepcidin-25.[15,48] Thereafter, ELISA assays for human hepcidin-25 were made commercially available, and more recently two new trials using mass spectrometry were published.[20,48]

The latter technique allows for a reliable quantitative and reproducible measurement of serum and urinary hepcidin, but it still has a high cost, which limits its use in clinical practice.[48] There is a strong correlation between the tests used; however, we found a difference in the absolute concentrations in different studies.[46,47,49] The reason for these differences is not clear; however, it clearly complicates the direct comparison of hepcidin values between studies and the establishment of reference serum levels.[49]

New horizons

Although there has been a little more than a decade since the first publication on hepcidin, a large number of studies involving treatments are under development, which was expected, considering the important role of this protein in iron metabolism and its various clinical implications.

Some studies have tried to suppress the expression of hepcidin, directly modifying the medical condition that caused its elevation. Wang et al.[50] used anti-BMP with the aim of reducing the expression of hepcidin and increasing serum iron levels in laboratory animals with colitis. In this study they found a reduction of inflammatory cytokines, but modest improvement in anemia. In a study of patients undergoing hemodialysis, Antunes et al.[51] found no effect concerning pentoxifylline used as an anti-inflammatory agent, on the serum levels of hepcidin.

The use of stabilizing agents, such as HIF (hypoxia-induced factor) seems to reduce hepcidin expression by acting directly through the reduction of inflammatory cytokines,[52-54] or increased erythropoiesis, providing an improvement in the iron profile.[55] Knowing that this factor stimulates the production of erythropoietin and restores the physiological rhythm of its secretion, HIF stabilizers, which are given orally, have been studied as a new therapeutic approach to chronic inflammation anemia in patients with CKD.[53,55]

In a recent study, Brigandi et al.[56] showed improvements in anemia using an HIF inhibitor in CKD patients, for those under conservative treatment as well as for those under dialysis.

In a recent review, Fung and Nemeth[57] described several strategies that have been studied to decrease the effects of elevated hepcidin. Among them, the decrease in hepcidin production through actions in regulatory pathways (pathway related to iron, inflammation or erythropoietic path); hepcidin neutralization; interference in the binding between hepcidin and ferroportin (preventing its degradation); and stimulation of ferroportin production.

Despite major advances in the knowledge of this protein over the past decade, we still need further studies to assess the real impact of hepcidin modulation in the management of anemia in CKD patients.

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