Editorial Comments

Hepcidin: a molecular link between inflammation and anaemia

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Introduction

Hepcidin is a small, cysteine-rich cationic peptide that was purified only recently from human urine and plasma ultrafiltrate [1,2]. It forms a short hairpin with the two arms linked by four disulfide bridges in a ladder-like fashion. One of the disulfide bridges lies between two adjacent cysteine residues near the turn of the hairpin. The peptide appears to be conserved among species, and, due to its unusual disulfide motifs, it may represent a new class of antimicrobial peptides [3]. In mice, hepcidin mRNA was found to be induced by iron overload as well as by treatment with lipopolysaccharide [4]. Hence, a role in iron homeostasis and acute phase response was suggested. Nicolas et al. [5] submitted the idea that hepcidin constitutes a humoral factor regulating intestinal iron absorption and iron storage in macrophages. Because loss of hepatic hepcidin expression in a murine knockout model led to visceral iron overload and a decrease of splenic iron load [3], Fleming and Sly [6] proposed that an elevated expression of hepcidin should result in features commonly encountered during the anaemia of inflammation, namely a decrease of circulating iron, an increase of iron within cells of the reticuloendothelial system (RES) and a reduced intestinal iron absorption. Nicolas et al. [7] found that the gene encoding hepcidin is regulated in response to anaemia, hypoxia and inflammation: Acute haemolysis induced by phenylhydrazine and blood loss following repeated phlebotomies dramatically decreased hepatic hepcidin mRNA expression, even after increasing hepatic hepcidin mRNA expression to high levels by iron loading [4,7]. Hypoxia (2% oxygen) specifically downregulated hepcidin transcription in hepatoma cells, and the same response was observed in mice that were kept in hypobaric hypoxia chambers [7]. Induction of an acute inflammatory response by a single turpentine injection resulted in a 2-fold reduction of serum iron in wild-type mice but, importantly, not in hepcidin-deficient mice [7]. In a murine model of chronic inflammation (three repeated turpentine injections within 4 weeks), hepatic hepcidin mRNA increased 6-fold in comparison with saline-treated animals, and red blood cell parameters and serum iron levels decreased [7]. These results strongly suggest that hepcidin exerts an inhibitory effect on iron absorption by duodenal enterocytes and, possibly, on iron release from the RES [7].

Hepcidin: targeting duodenal enterocytes

Enterocytes take up dietary iron by the membrane transporter divalent metal transporter 1 (DMT1) after reduction of ferric iron to the ferrous form by a duodenal brush border ferrireductase (duodenal cytochrome b, Dcytb). In rats, changes in hepcidin expression show a close temporal relationship with changes in duodenal iron transporter expression [8]. Duodenal iron absorption increases with decreasing liver hepcidin expression, and vice versa [9]. The lack of a significant lag time between iron withdrawal and the increased, uniform immunolocalization of Dcytb and DMT1 on rat duodenal villus enterocytes advocates the notion that basal crypt cells as well as mature villus enterocytes are susceptible to hepcidin signalling [8]. Since the mucosal absorption of iron is significantly decreased in haemodialysis patients on maintenance erythropoietin therapy, and absorption is even lower with increased levels of C-reactive protein (CRP) [10], it is interesting to speculate that hepcidin expression may play a role in these anomalies.

From the nephrologist’s perspective, iron deficiency and chronic inflammation are two major, commonly encountered causes of hyporesponsiveness to exogenous erythropoietin. It may be speculated that appropriately high levels of circulating hepcidin impede
adequate iron absorption in chronic kidney disease patients, particularly since the majority of these patients have elevated levels of CRP [11]. Early studies reported that inflamed mice failed to show an increase in iron absorption in response to hypoxia [12]. The inflammatory signal for enhanced hepcidin expression may outcompete that of anaemia and/or hypoxia for the downregulation of hepcidin transcription, and serum and liver iron content may be even less relevant for the modulation of hepcidin expression [7].

Hepcidin: targeting monocytic cells?

Loss of hepatic hepcidin expression led to a decrease of splenic iron load [5]. Conversely, inflammation-induced hepcidin expression might account for iron sequestration within cells of the RES. Direct evidence, however, is lacking. Monocytes express DMT1 and IREG1 (an iron exporter molecule, also termed ferroportin or MTP1). Expression of IREG1 was downregulated in a lipopolysaccharide model of acute inflammation in RES cells of the spleen, liver and bone marrow, and in a Leishmania donovani model of chronic infection [13]. Transcription and surface expression of the transferrin receptor (TfR) and iron uptake were reduced after combined treatment of human monocytes with interferon-γ and lipopolysaccharide, DMT1 expression increased in conjunction with increased uptake of non-transferrin bound iron, and IREG1 mRNA and iron release declined [14]. Interleukin (IL)-4 and IL-13 have been reported to increase iron uptake and storage by activated murine macrophages via stimulation of ferritin translation and/or TfR transcription [15]. Cytokines thus exert a pivotal role in the control of iron sequestration within cells of the RES. A direct inhibitory role for hepcidin on iron release by cells of the RES has been proposed but not proven, as hepcidin-deficient mice did not show reduced plasma iron concentrations in response to an inflammatory stimulus within 16 h, in contrast to wild-type mice [7]. A potential NF-κB-binding site has been identified in the promoter region of mouse hepcidin [4]. In humans with anaemia of inflammation, urinary hepcidin excretion correlated with serum levels of the acute-phase protein ferritin [16]. Plasma ferritin is secreted from cells of the RES depending on the iron concentration within the cell. High ferritin levels in chronic inflammatory conditions result from the reduced uptake of iron into erythroid precursors, and may serve as an indicator of how much iron is being deposited within the RES [17]. Are high plasma levels of hepcidin indicative of an increased iron storage within cells of the RES? IL-6, the main stimulator of the production of most acute-phase proteins [18], but not IL-1 or tumour necrosis factor-α, induced hepcidin mRNA in cultured hepatocytes [16]. In the rat, acute inflammation-induced changes in hepatic hepcidin expression preceded the decline of plasma transferrin saturation and occurred more rapidly than decreases of intestinal iron transporter expression [19]. Collectively, these results suggest that iron sequestration in response to inflammatory stimuli is a coordinated event of iron uptake and storage within monocyctic cells, that is induced by cytokines and probably modified by hepcidin and a hepcidin-dependent reduction of duodenal iron absorption.

Hepcidin: a target for erythropoietin?

The expression of hepcidin appears to be sensitive to the activity of the erythroid bone marrow. Hepatic hepcidin mRNA expression was lower in iron-deficient, anaemic mutant mice in comparison with wild-type mice, even in the setting of replete liver iron stores [20]. It decreased in rats after the switch from a normal diet to an iron-deficient diet before any changes in hepatic iron stores [8]. In a recently suggested model for the regulation of hepatic hepcidin expression [9], hepatocyte surface HFE (the haemochromatosis protein) competes with diferric transferrin for the binding on surface TfR. Unbound surface HFE was proposed to somehow increase hepatic hepcidin expression and release. According to the model, iron deficiency would lead to a decrease of circulating diferric transferrin, and the number of free surface TfRs would increase, resulting in a decreasing fraction of free surface HFE. Hepatic HFE, however, has been localized predominantly to cells of the RES; hence, the signalling cascade may involve further steps between RES cells and hepatocytes [21]. Importantly, the degree of transferrin saturation may serve as a main switch for the modulation of circulating hepcidin concentration. Erythropoietin increases the number of erythroid precursors, and transferrin saturation declines due to increased iron uptake.

Chronic kidney disease patients on maintenance erythropoietin therapy frequently present with functional iron deficiency, i.e. with a low transferrin saturation but normal or high serum ferritin levels [11]. According to the above model, hepcidin expression should be minimized unless other more potent stimuli upregulate hepcidin expression, such as a chronic elevation of circulating proinflammatory cytokine levels. Vitamin C has been proposed to improve intestinal iron uptake and iron utilization by the erythron, and erythropoietin requirements decreased as long as patients received adequate vitamin C supplements [22]. It may be speculated that vitamin C antagonizes the action of hepcidin regarding the regulation of iron trafficking. Vitamin C could even affect hepatic hepcidin expression. If hepcidin indeed represents the key modulator of intestinal iron uptake, iron absorption should improve to a large extent with hepatic antagonistic drugs.

Hepcidin: a possible target for nephrologists?

To date, the relevance of hepcidin for the effectiveness of erythropoiesis in chronic kidney disease patients
remains unclear. Absolute iron deficiency rapidly develops during therapy with exogenous erythropoietin, and persistently high levels of hepatic hepcidin expression might explain why duodenal iron absorption remains inadequately low. A large proportion of patients with functional iron deficiency exhibit elevated levels of CRP. In the rat, inflammation-induced changes of hepatic hepcidin expression precede the decline of plasma transferrin saturation [18]. If future studies confirm a primary role for hepcidin in controlling iron metabolism in response to inflammatory stimuli, hepcidin could evolve as a potent marker of functional iron deficiency in chronic kidney disease patients. The observation that serum ferritin levels correlate with urinary hepcidin excretion in patients with anaemia of inflammation [16] supports this notion. Finally, hepcidin could accumulate with declining residual renal function. The preserved renal function of peritoneal dialysis patients could be of benefit with regards to hepcidin removal in comparison with haemodialysis patients. Hepcidin clearance could also be dependent on the dialysis procedure itself. Pharmacokinetic data, however, are lacking.

Conclusion

Hepcidin is a small defensin-like peptide whose production by hepatocytes is modulated in response to anaemia, hypoxia or inflammation. Iron uptake by duodenal enterocytes appears to be largely regulated by hepcidin. Anaemia and/or hypoxia suppress the expression of hepatic hepcidin, but inflammatory stimuli that are strong enough to induce acute-phase responses induce its release even in the setting of anaemia. Both the anaemia of renal disease and the anaemia of chronic inflammation result from an inappropriate production of erythropoietin, a blunted bone marrow response to erythropoietin due to various cytokines, an excessive loss of blood, a reduced red cell life span and reduced iron availability. Hepcidin has been implicated in the latter by reducing intestinal iron absorption and, possibly, by favouring iron sequestration within cells of the RES. Since the majority of pre-dialysis, haemodialysis and peritoneal dialysis patients have serological evidence for an upregulation of acute-phase proteins, an inappropriate hepcidin expression appears conceivable for a large proportion of these patients. Moreover, hepcidin could act as an indicator of functional iron deficiency in chronic kidney disease patients. It remains to be seen to what extent uraemic-specific factors such as residual renal function also determine hepcidin clearance. To date, serological data are lacking due to the restricted availability of specific antibodies, and possible changes of hepcidin expression in experimental models of renal failure have not yet been reported.

Conflict of interest statement. None declared.

References

New European guidelines for management of hypertension: what is relevant for the nephrologist

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It is well established among nephrologists that advanced renal failure is associated with an increased prevalence of cardiovascular (CV) disease including myocardial infarction, stroke and heart failure [1]. Recently, a great amount of information has become available, which demonstrates that the finding of minor abnormalities of renal function also predicts more CV risk in the general population, as well as in hypertensive patients [2].

Recently, the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) have published their guidelines for the management of arterial hypertension [3]. This came only a short time after the JNC 7 report had been published [4]. Some of the relevant changes refer to the introduction of new risk factors that have come to complete the list of major CV risk factors. In this sense, both guidelines recognize the relevance of minor abnormalities of renal function, easily detectable by practicing physicians, for the stratification of patients with arterial hypertension. The Seventh Report of the Joint National Committee [4] considers the findings of microalbuminuria or diminished estimated level of glomerular filtration rate (eGFR) (<60 ml/min) as major CV risk factors. These also include hypertension, cigarette smoking, obesity, physical inactivity, dyslipidaemia, diabetes mellitus, age >55 years in men and 65 years in women, and a family history of premature CV disease. Similarly the ESH/ESC guidelines [3] contemplate among the factors influencing prognosis in hypertensive patients the finding of a slight elevation in serum creatinine (>1.3 mg/dl in men and 1.2 mg/dl in women) and/or microalbuminuria. The presence of chronic kidney disease, defined in ESH/ESC guidelines as serum creatinine values >1.5 mg/dl in men and 1.4 mg/dl in women or by the presence of proteinuria (>300 mg/day), is also considered as a CV risk factor. The ESH/ESC guidelines also recommend to estimate either the creatinine clearance (using the Cockcroft–Gault formula) or the glomerular filtration rate [eGFR—using the modified Modification of Diet in Renal Disease (MDRD) formula] [3].

Detection and prevalence of minor abnormalities of renal function in clinical practice

The finding of subtle changes in renal function, by non-nephrologists, is usually based on the determination of serum creatinine, creatinine clearance and/or urinary albumin excretion. The Hypertension Detection and Follow-up Program trial [5] showed for the first time that the presence of elevated serum creatinine values (>1.7 mg/dl) at baseline was a very potent predictor for 5- and 8-year all-cause mortality. The data were later confirmed and extended by the Hypertension Optimal Treatment (HOT) trial [6], and many other trials performed in hypertensive patients. Serum creatinine was an excellent predictor, as good as or better than any of the well established major CV risk factors such as diabetes or a history of myocardial infarction [7]. Importantly, in hypertensive subjects, creatinine concentrations that are still within the normal range may already predict outcome [8].

In hypertensive patients the prevalence of an elevated serum creatinine has been shown to be progressively