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Journal of Hepatology 49 (2008) 680–685

Journal of Hepatology

www.elsevier.com/locate/jhep

Editorial

Hepcidin in the management of patients with mild non-hemochromatotic iron overload: Fact or fiction? ☆

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Several non-hereditary liver diseases are associated with mild hepatic iron overload such as viral hepatitis, excessive alcohol consumption, non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) [1–7]. In fact, the term insulin resistance or dysmetabolic iron overload syndrome was introduced to describe the association of body iron overload and NAFLD [8]. The contribution of HFE-mutations to the development of iron overload in these diseases remains debated [9,10] (NAFLD/NASH [8,11,12]) (HCV [13–15]) (alcohol [16]), but is probably slight.

The underlying molecular mechanism centers on dysregulation of the iron regulatory hormone hepcidin. Hepcidin is primarily produced by hepatocytes, but there is also evidence for production by macrophages [17] and fat cells [18]. Hepcidin internalizes and subsequently degrades the cellular iron exporter ferroportin, present on the cell surface of macrophages and enterocytes, resulting in hypoferremia. Hepcidin is upregulated by inflammation, by the presence of increased body iron levels and inhibited by increased erythropoietic activity. These respective regulatory pathways are thought to protect the body from (i) extracellular proliferating pathogens, (ii) the harmful effects of iron overload and to (iii) ensure that body iron availability matches iron needs.

Recent evidence has demonstrated that perturbation of hepcidin underlies non-hereditary (or non-hemochromatotic or acquired) mild iron overloading hepatic diseases (NHIOD). Hepcidin expression is affected by the upstream stimulatory factor 1 (USF1), a well established susceptibility gene for familial combined hyperlipidemia, a complex disorder of lipid metabolism [19]. USF1 together with USF2 controls *cis* and *trans* regulation of hepcidin expression [20]. Other circumstantial evidence of a link between hepcidin, glucose and lipid metabolism comes from hepcidin producing hepatic adenomas in severe anemic type 1a glycogen storage disease (GSD1a) patients [21], and hepcidin production by fat cells [18]. Interestingly, many of the diseases that are part of the metabolic syndrome have a disrupted iron homeostasis [3,4] (e.g. diabetes [1,2,8], hypertension [22] and elevated body mass index [23]). Thus, we are only starting to discover the pleiotropic role of hepcidin.

Alcoholic and hepatic viral infections are the most typical examples of acquired mild forms of iron overload. Liver biopsies in these disorders consistently show iron deposits in hepatocytes and Kupffer cells [5,24]. Elevated liver iron deposits are also commonly observed in NAFLD [5,24].

Iron can induce oxidative stress, which can cause tissue injury. Indeed, hepatic iron deposits have been associated with more advanced forms of NHIOD, including liver fibrosis, cirrhosis and hepatocellular cancer in some reports (NAFLD/NASH [9,11,25,26] , HCV [27–29] , alcohol [30,31]), but this was not confirmed in others (NAFLD/NASH [12,32,33] , HCV [16,30]).

In NAFLD, there is increased urinary hepcidin excretion [34] and elevated liver hepcidin expression [35] but hepcidin levels are relatively low for the level

Associate Editor: Y.M. Deugnier

☆ The authors declare that they do not have anything to disclose regarding funding from industries or conflict of interest with respect to the manuscript.

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doi:10.1016/j.jhep.2008.08.004

of iron overload [34], suggesting ineffective liver iron sensing.

In this issue of the *Journal of Hepatology*, Fujita et al. [36] confirm that in chronic hepatitis C virus (HCV), hepatic hepcidin expression is inadequate for the body iron stores, but can be restored upon viral eradication. In addition, they found that hepatic hepcidin expression reflects circulating hepcidin levels. This finding suggests that serum hepcidin measurements might become a proxy parameter for hepatic hepcidin and aid in the management of patients with acquired forms of mild hepatic iron overload such as in HCV.

1. Hepcidin peptide

Human hepcidin is mainly produced by hepatocytes as an 84 amino-acid (aa) pre-prohepcidin [37]. Subsequent posttranslational processing results in the biologically active 25 aa form (hepcidin-25, molecular mass is 2789 Da) that is secreted in the plasma. Additional amino-acid degradation results in the production of two smaller isoforms (hepcidin-20 and -22) [38,39], that have unknown biological significance.

2. Hepcidin assay

Progress in this field has been hampered by the difficulties many researchers face by measuring hepcidin. Until recently, only few tools were available to measure hepcidin. Largely because antibodies are hard to raise. Hepcidin has a small and compact structure and antigenic epitopes are scarce. In addition, hepcidin is conserved among a wide range of species which complicates the induction of antibodies in hosts such as rabbit. As a consequence, immunochemical methods based on specific anti-hepcidin antibodies are largely unavailable. Lastly, quantitation of hepcidin is complicated by its tendency to aggregate [40] and stick to laboratory plastics, necessitating implementation of robust laboratory procedures.

Currently, many studies use the serum-based commercial immunoassay that measures the hepcidin precursor prohepcidin rather than the bioactive peptide. The relevance of these data is controversial because prohepcidin levels fail to correlate with urinary and serum hepcidin [41,42]. Furthermore, when measured in healthy controls, serum prohepcidin levels are around 40–50 times higher than those observed for bioactive hepcidin (≈180 ng/ml versus ≈4 ng/ml) (unpublished observations). This suggests that prohepcidin and hepcidin have different biological profiles [41–44].

Ganz and Nemeth et al. have developed an immunodotblot assay using non-commercially available antibodies for the semi-quantitative measurement of the sum of all urinary hepcidin isoforms [45]. The results of studies that employ this method have greatly improved our understanding of the regulation of hepcidin in patients with distorted iron homeostasis [46].

More recently, new assays for the various hepcidin isoforms: hepcidin-25, -22 and -20 have been developed on a platform of novel technologies such as surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) and liquid chromatography tandem MS (LC/MS–MS) [38,47–49]. With the use of internal standards these MS techniques enable reliable quantitative hepcidin analysis in urine and serum (Fig. 1) [50,51]. In fact, MS optimally uses an internal standard to correct for extraction, variable ionization and suppression of the analyte. Internal standards are spiked to the clinical samples and co-analysed. The ratio between the synthetic internal standard and endogenous peptide is determined by MS, allowing calculation of the absolute amount of hepcidin.

In the current issue Fujita et al. exploit SELDI-TOF MS to (semi)quantify circulating hepcidin-25 levels [36,47]. This method shares the drawback of most currently available hepcidin analysis since it lacks the use of an internal standard. This precludes value assignment, necessitating expression in arbitrary units, and resulting in somewhat imprecise measurements. Nevertheless, their results provide important proof of principle insights in biologically relevant circulating hepcidin levels in HCV before and after treatment.

3. Clinical utility of the hepcidin assay

Before measurement of hepcidin in urine and serum can be introduced into routine clinical practice, several issues need to be addressed. This starts with establishment of reference values among a wide variation of controls, and the assessment of a diurnal variation or fluctuation in relationship to meals.

Proof of principle findings of a circadian (either intrinsic or diet related) variation with generally lower values in the fasted morning samples than in the afternoon [38], may necessitate standardized sampling in the morning after an overnight fast (as was nicely done by Fujita et al.).

Another relevant question is whether urine is a reliable alternative for serum to assess biologically relevant hepcidin levels. Urine hepcidin levels correlate with hepcidin expression in liver biopsies [52], but discrepancies have been reported [34]. Urine levels also correspond to circulating levels [38] and nicely fit in the concept of hepcidin as the iron regulatory peptide [46,53]. Still, we caution against the uncritical use of urine concentrations as a universal surrogate for circulating levels since urinary secretion of hepcidin will depend on glomerular filtration and tubular re-absorption. In addition, hepcidin mRNA has been detected in the kidney, which suggests the potential for local production and release into the urine [54].