Low hepcidin triggers hepatic iron accumulation in patients with hepatitis C

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ABSTRACT

Persistent hepatitis C virus (HCV) infection is a major cause of chronic liver disease including fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Chronic hepatitis C (CHC) is also a problem in patients with chronic kidney disease (CKD), particularly in those on haemodialysis. Excessive iron in the liver of CHC patients contributes to hepatic fibrosis, cirrhosis and finally HCC, while iron depletions are beneficial. In CHC patients without CKD, in HCV-infected experimental animals and in cell culture studies, serum hepcidin levels and/or cellular hepcidin expression are low and directly suppressed by HCV, radical oxygen species, growth factors and/or transcription factors. In contrast, antiviral therapy (e.g. with pegylated interferon-alpha combined with ribavirin) raises hepcidin levels and reduces iron overload in patients with CHC. Hepcidin directly inhibits HCV replication mediated by STAT3 activation. HCV circumvents hepatic innate antiviral defence by lowering hepcidin. If hepcidin is also low in CKD patients with CHC, iron supplementation should be avoided even in CKD patients with CHC treated with erythropoiesis-stimulating agents.

Keywords: hepcidin, hepatitis C, iron, liver

Persistent hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide [1, 2]. Approximately 20% of patients with chronic hepatitis C (CHC) will progress to liver cirrhosis within 20 years, resulting in an annual risk of 3–7% for hepatocellular carcinoma (HCC) [3]. HCV infection is also common among chronic haemodialysis patients with a prevalence ranging between 5 and 40% [4–6]. Iron is responsible for the formation of toxic hydroxyl radicals and favours fibrosis as well as tumouriogenesis. Liver iron content correlates with fibrosis in patients with hepatitis B virus (HBV) and HCV. In 38 patients with HCV infection, fibrosis was minimal in 77% of patients if hepatic iron was absent versus 24% with minimal fibrosis if hepatic iron was present. In contrast, marked fibrosis was present in 56% with iron but only in 15% without iron [7]. In CHC patients, hepatic iron concentration predicts the response to antiviral therapy [10]. Iron removal by phlebotomy improves liver function tests [11, 12] and liver histology [13]. Iron depletion also increases the probability of sustained HCV eradication with antiviral therapy [14–16] and decreases the development of HCC in patients with CHC [17].

HEPCIDIN LEVELS IN HAEMODIALYSIS

Circulating hepcidin was found to be bound to α2-macroglobulin with high affinity and based on theoretical calculations, 11% of hepcidin has been estimated to be freely circulating. Hepcidin clearance occurs via cellular co-degradation with ferroportin at its sites of action, and via excretion by the kidneys. Because of its low-molecular weight and the small radius of the molecule, unbound hepcidin is likely to pass freely into the glomerular filtrate. In human studies, the fractional excretion has been calculated to be as low as 0–5%.

Hepcidin, prohepcidin and metabolites in the blood increase in CKD and are very high in dialysis patients, but intradividual levels of hepcidin are highly variable [18]. CKD is an inflammatory state (malnutrition-inflammation complex syndrome: MICS) and this and other factors, like iron supplementation, frequent bacterial infections and removal of hepcidin by haemodialysis membranes might contribute to this fact. The situation is even more complex in patients on haemodialysis with CHC: hepcidin production seems to be suppressed by the viral infection and this suppression may even contribute to lower erythropoietin and iron demand in these patients [19]. But, clear data are still missing.
MECHANISMS OF HEPcidIN SUPPRESSION IN CHc

The hepatic peptide hormone hepcidin is the major regulator of iron metabolism [20]. Animal and cellular models have suggested that HCV infection may directly modulate hepcidin expression. HCV-induced reactive oxygen species (ROS) have been shown to raise iron level by reducing hepcidin transcription in animals [21]. In hepatoma cell lines expressing HCV core and non-structural proteins, HCV-induced oxidative stress suppressed hepcidin production through increased histone deacetylase activity [22]. Girelli et al. [23] measured serum hepcidin levels in 81 untreated CHc patients and 57 control subjects with rigorous definition of normal iron status (serum ferritin levels). All CHc patients underwent liver biopsy. In CHc patients, serum hepcidin significantly correlated with serum ferritin and histological iron score in the liver. After stratification for ferritin quartiles, serum hepcidin increased significantly across quartiles in both controls and CHc patients, but hepcidin was significantly lower in CHc patients than in controls for each corresponding quartile. The investigators concluded that suppression of hepcidin by HCV is likely an important factor in liver iron accumulation in this condition [23]. Tsouchatzis et al. [24] confirmed that serum hepcidin concentration is significantly lower in Greek patients with chronic HCV infection than in healthy subjects.

Fujita et al. [25] determined the hepatic expression levels in patients with various liver diseases. Hepcidin expression levels were strongly correlated with serum ferritin (P < 0.0001), serum iron (P = 0.0012), transferrin saturation (TSAT) (P < 0.0001), haemoglobin (P = 0.0073), transaminase levels (P = 0.0013) and the degree of the iron deposit in liver tissues (P = 0.0001) of these patients. The hepcidin/ferritin ratio was significantly lower in HCV-positive patients than in HBV-positive patients (P = 0.0129) or in control subjects (P = 0.0080). After adjustments for either serum ferritin or hepatic iron scores, hepcidin indices were significantly lower in HCV-positive patients than in HBV-positive patients, suggesting that hepcidin may play a pivotal role in the pathogenesis of iron overload in CHc patients [25]. Stainable iron in hepatocytes and portal tract cells predicts progression and clinical and histological outcomes in advanced CHc [26].

Effect of antiviral therapy

The current therapy of CHc involves pegylated interferon alpha (PEG-IFN-alpha) in combination with the nucleoside analogue ribavirin. Administration of PEG-IFN-alpha causes an immediate decline in HCV load within 24–48 h [27]. Ryan et al. [28] have recently shown that in HCV patients a single dose of PEG-IFN-alpha/ribavirin resulted in a significant increase in serum hepcidin, peaking at 12 h, coinciding with a 50% reduction in serum iron and TSAT within 24 h. The 24-h serum iron levels were an independent predictor of the immediate HCV decline. In cell culture, the STAT3 transcription factor controlled the induction of hepcidin by PEG-IFN-alpha [27]. Thus, iron depletion may be part of a successful antiviral therapy in patients with CHc.

Effect of iron depletion by phlebotomy

Sigumoto et al. [29] evaluated the appropriateness of hepcidin expression relative to iron overload in patient with chronic HCV infection using the hepcidin/ferritin ratio. This ratio was significantly lower in patients with chronic HCV infection than in healthy controls (0.33 ± 0.41 versus 0.73 ± 0.36; P = 0.0068). Removal of a mean blood volume of 2910 ± 920 mL by 9.0 ± 2.89 venesection times performed over a period of 12.0 ± 2.8 months significantly decreased red blood cell counts, haemoglobin concentrations, serum iron, ferritin, transaminase and hepcidin levels, but did not normalize the low hepcidin/ferritin ratio (0.33 ± 0.14). The authors concluded that hepcidin expression is impaired in chronic HCV-infected patients when adjusted for serum concentrations of ferritin, and that this impairment is not improved when the iron overload condition is cured [29].

Mitsyoshi and coworkers [30] examined the significance of iron deposition in hepatocytes and reticuloendothelial cells in CHc patients. Serum transaminase levels and hepatic scores of stage, grade and steatosis were higher in CHc patients with reticuloendothelial cell iron staining than in those without. Additionally, patients with iron overload had decreased ratios of hepcidin mRNA to serum ferritin when compared with those without stainable iron. The efficacy of phlebotomy was greater in CHc patients with reticuloendothelial cell iron than in those without.

Effects of growth and transcription factors

Hepatocyte growth factor (HGF) and epidermal growth factor (EGF) are important mediators of liver repair and regeneration after liver injury. HGF, EGF and possibly other growth factors contribute to hepcidin suppression in chronic liver disease, including alcoholic hepatitis and viral hepatitis, promote iron accumulation in the liver and exacerbate the destructive disease process.

The suppression of hepcidin by these growth factors in primary mouse hepatocytes as well as EGF administration in mice is transcriptional and is mediated by a direct effect of HGF and EGF on the bone morphogenetic protein (BMP) pathway [31], essential for iron and hepcidin regulation [32]. Additionally, growth factors interfered with nuclear localization of activated sons of mothers against decapentaplegic (Smad) and increased the nuclear pool of the BMP transcriptional corepressor TG-interacting factor (TGF). Suppression of hepcidin by HGF is prevented by inhibitors in the phosphoinositide-kinase pathway and in the mitogen-activated ERK kinase/extracellular signal-regulated kinase (MEK/ERK) pathway [31].

In transgenic mice expressing the HCV polyprotein, even modest iron supplementation induced HCC [33]. In contrast, phlebotomy and low iron diet normalized elevated hepatic 8-hydroxy-2'-deoxyguanosine levels in patients with chronic HCV infection and thus the progression to HCC [17]. Transgenic mice expressing the HCV polyprotein have increased hepatic iron concentration, decreased hepatic hepcidin expression and increased ferroportin expression in the duodenum and liver when compared with control animals. Hepcidin promoter activity and the DNA-binding activity of CCAAT/
These patients [43]. The transcription factor C/EBP alpha regulates hepatic hepcidin transcription [34]. The HCV protein induces ROS production raising hepatic iron levels by the reduction of DNA-binding activity of C/EBP alpha, which results in inhibition of hepcidin transcription [21]. Increased ROS levels in these transgenic mice may cause the up-regulation of the nuclear protein C/EBP homology protein (CHOP), an inhibitor of C/EBP alpha DNA-binding activity. Thus, a model for HCV-induced iron loading was proposed, where HCV protein increases ROS production, up-regulation of CHOP, which prevents C/EBP alpha binding to the hepcidin promoter and reduces hepcidin expression. This, in turn, up-regulates ferroportin expression, increasing iron export from the duodenum and macrophages, raising serum iron levels and TSAT resulting in hepatic iron overload [35]. In contrast, recent data of Kotta-Loizou et al. [36] suggest that hepcidin is down-regulated via AP1-mediated transcription by the HCV core + 1/ARF protein using hepatoma cells, while the previously described C/EBP and STAT sites are probably not essential.

As discussed before, the increased deposition of iron in the liver may trigger oxidative stress, inflammation, liver cell damage, liver cirrhosis and progression to HCC [37, 38]. Iron is required by cancer cells to proliferate [39]. Alcohol consumption, a risk factor for HCC, can decrease hepcidin transcription and cause hepatic iron overload [40]. Downregulation of hepcidin may stimulate tumour progression in patients with chronic HCV infection. In HCV-positive cells and in HCC, hepcidin expression is reduced by histone acetylation but not by DNA methylation [41]. In contrast, in cell lines with established hepcidin expression, hepcidin directly inhibits HCV replication mediated by STAT3 activation, indicating that the iron regulator hepcidin exhibits antiviral activity against HCV. The investigators proposed a novel mechanism by which HCV circumvents hepatic innate antiviral defence [41].

**CONCLUSIONS**

CHC is frequently associated with hepatic iron overload. Iron-induced oxidative stress may cause liver injury resulting in fibrosis, cirrhosis and finally HCC, while iron depletion is beneficial for CHC patients. Low serum hepcidin and low hepcidin expression in the liver caused by ROS, growth factors and transcription factors alleviates hepatic iron accumulation in CHC. In contrast, antiviral therapy immediately upregulates hepcidin and reduces hepatic iron content. Chronic kidney disease (CKD) patients with CHC may probably also have lower hepcidin levels than CKD patients without CHD. If so, anaemia should not be supplemented with iron, even if treated with erythropoiesis-stimulating agents.

**AREAS OF CONTROVERSY**

Phlebotomy with iron depletion was shown to be beneficial in patients with hepatitis C, and iron depletion was reported to support antiviral therapies.

It seems to be a logical approach to withhold iron i.v. substitution in this patient group. Nonetheless, this approach might not be risk free. Iron depletion in turn can further decrease hepcidin levels. As hepcidin exerts bactericide and virucide properties, this could theoretically increase the susceptibility to infections in this high-risk group. Iron depletion might deteriorate anaemia, but could also aggravate other manifestations of iron deficiency, like restless leg and Plummer Vinson syndromes.

Up to now, there are no trials supporting the benefit of this hypothesis. Hence, a potential benefit of such an approach has to be proved in prospective trials in haemodialysis patients with CHC—in those with peginterferon/ribavirin therapy as well as those without—using histological evaluation of the liver and clearly defined goal levels of iron metabolism.

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This was the very last manuscript Prof. Hörl worked on when he passed away on the 25 June 2013. We, the Department of Nephrology of the General Hospital/Medical University of Vienna, dedicate this paper in memory of Prof Dr Walter Hermann Hörl, the head of our department, a passionate scientist and empathic physician.

**CONFLICT OF INTEREST STATEMENT**

None declared.

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**POTENTIAL ROLE OF PROHEPCIDIN**

Prohepcidin is an easily measurable precursor of hepcidin but the results on serum prohepcidin levels in CHC patients are controversial. In both patients with chronic hepatitis and those with liver cirrhosis, serum prohepcidin levels were significantly lower in HCV-positive patients than in HBV-positive patients. Additionally, serum prohepcidin levels were significantly lower in patients with liver cirrhosis than in patients with chronic hepatitis [42]. Haemodialysis patients with chronic HCV infection have lower serum prohepcidin levels associated with lower iron and epoetin requirements when compared with HCV-negative haemodialysis patients [43, 44]. Serum interleukin (IL)-6 levels of HCV-positive haemodialysis patients were also significantly lower than those of HCV-negative haemodialysis patients (P = 0.016). Thus, low levels of serum prohepcidin and IL-6 might account for iron accumulation together with lower iron and epoetin requirements in these patients [43].
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