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Pathogenesis and clinical consequences of iron overload in chronic hepatitis C – impact of host and viral factors related to iron metabolism

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Abstract

Chronic hepatitis C virus infection is a leading cause of progressive liver fibrosis, liver cirrhosis and hepatocellular carcinoma. Iron overload is frequently observed in cases of chronic hepatitis C and has been suggested as a negative prognostic factor for this disease. Although the mechanisms leading to iron accumulation are not fully explained yet, both host and viral factors seem to contribute towards the development of this pathology. Better understanding of the interplay between hepatitis C virus replication and expression of iron regulatory molecules may elucidate new and interesting targets for the effective treatment of chronic hepatitis C.

Key words: hepatitis C, pathogenesis, iron

Introduction

Chronic hepatitis C virus infection, affecting more than 170 million people worldwide, is a leading cause of progressive liver fibrosis, liver cirrhosis and hepatocellular carcinoma (HCC). Hepatitis C virus (HCV)-associated end-stage liver disease is the major indication for liver transplantation in the United States and Europe (Lavanchy, 2007). The current standard of care, i.e., antiviral therapy with interferon and ribavirin, is burdened with the risk of complications and only effective in half of the treated patients (Manns et al., 2006). Iron overload in the liver induces oxidative stress leading to cell membrane damage, DNA instability and mutagenesis (Isom et al., 2009). Due to these effects, iron can be considered a proinflammatory, profibrogenic factor and a potential carcinogen. Since the implementation of serological diagnostic tests for HCV identification, elevated serum iron-overload indices or appearance of iron deposits in liver cells have been observed in 10-40% of patients with chronic hepatitis C (CHC) and 50% of patients suffering both from CHC and HCC (Di Bisceglie et al., 1992; Arber et al., 1994; Piperno et al., 1995; Ludwig et al., 1997). In addition, feeding HCV-infected chimpanzees a diet with excess iron increased the level of alanine aminotransferase activity (ALT). Histological changes that were observed provided further evidence of iron-related exacerbation of liver injury (Bassett et al., 1999).

Based on the above-mentioned observations, iron overload has been suggested as a negative prognostic factor of CHC, with possibly influences on the increase in aminotransferase activity, exacerbation of inflammation, progression of liver fibrosis and decrease in antiviral therapy effectiveness (Bonkovsky et al.., 1997; Metwally et al., 2004). However, detailed molecular mechanisms of liver iron overload in CHC have not yet been fully elucidated.

The use of phlebotomy to remove excess iron from tissues, although controversial, has been proposed as an adjuvant form of therapy. It has been suggested that such a therapy might reduce the serum activity of aminotransferases, improve histopathological indices of liver injury in CHC and improve sustained virological response (SVR) after the treatment with interferon or with interferon plus ribavirin (Di Bisceglie et al., 2000; Fontana et al., 2000; Yano et al., 2002; Fargion et al., 2002; Desai et al., 2008; Sartori et al., 2010).

Iron overload related liver injury

The liver serves as an important reservoir of iron, where it is mostly bound to ferritin or hemosiderin.

About a third of the body's total iron is stored in hepatocytes and liver macrophages. However, the liver's physiological iron storage capacity is limited, and excessive iron accumulation can lead to liver damage. Pathological accumulation of iron exacerbates oxidative stress resulting in increased lipid peroxidation. This leads to destruction of organelle membranes and, in turn, cell death via hepatocyte necrosis or/and apoptosis. Products of oxidative stress induce a focal inflammatory reaction that plays a role in the stimulation of liver macrophages and release of profibrogenic cytokines (Pietrangelo, 1996; Videla et al., 2003). Those mechanisms trigger the activation of hepatic stellate cells (HSC), which are major sources of collagen and other extracellular matrix elements that gradually accumulate in the perisinusoidal spaces of liver parenchyma (Cassiman et al., 2002; Hübscher, 2003). Due to the presence of high-affinity receptors for transferrin on activated HSC, iron has also been proposed as a direct activator of HSC (Bridle et al., 2003). Longer hepatocyte exposure to excess iron is associated with a greater risk of progressive fibrosis and the development of liver cirrhosis with irreversible, nodular reconstruction of the organ, replacement of the functional liver parenchyma with connective tissue, and a loss of function (Niederau et al., 1996).

Increased hepatic iron stores are associated with a higher risk of the HCC development due to pathological nodular regeneration of cirrhotic liver and DNA damage, genome instability and mutagenesis caused by products of iron induced-oxidative stress (Fracanzani et al., 2001; Kowdley, 2004). Immune surveillance for malignant transformation may be decreased by free iron-induced immunological abnormalities (Deugnier et al., 1998).

Hepatitis C virus – general characteristics

Hepatitis C virus, a member of family *Flaviviridae*, was discovered in 1989. The HCV genome consists of a positive-polarity single-stranded RNA encoding a large polyprotein. The polyprotein is cleaved by host peptidases and virus encoded proteases to generate 3 structural (S; core-C; envelope-E1, E2) and 7 nonstructural (NS) proteins (Fig. 1). Nonstructural proteins take part in the synthesis of viral particles (Lindenbach et al., 2005).

Initiation of HCV RNA translation is mediated by the unique mechanism of internal ribosomal entry which differs from what is observed in most eukaryotes. Translation proceeds under the control of a highly structured internal ribosomal entry site (IRES). IRES constitutes 341-nucleotide 5′ untranslated region (5′UTR) of the HCV genome. IRES contains binding sites for ribosomal subunits and eukaryotic initiation factor 3 (eIF3), both of which are necessary for the commencement of translation from HCV RNA (Hellen et al., 1999; Otto et al., 2004). Although the 5′UTR is a highly conserved region of HCV genome, subtypes 1b, 2b, 6a of the virus differ in the IRES location and the conformation of eIF3 binding sites. Thus, the translation of different HCV genotypes is not equally efficient (Collier et al., 1998).

The HCV is characterized by a high replicative potential and a high mutation rate. Due to the diversity of HCV genomes, the virus is classified into 6 genotypes, with several subtypes within each genotype. Chronic hepatitis C develops in approximately 70-80% of HCV-infected patients. HCV replicates mainly in hepatocytes, but its nucleic acids have also been found in peripheral blood mononuclear cells and in central nervous system cells. The pathogenesis of chronic liver disease is complex, including the multifactorial mechanisms of cellular and humoral immune response with the induction of oxidative stress (Isom et al., 2009; Juszczyk, 2009; Levent et al., 2006).

Iron overload in pathogenesis of chronic hepatitis C

The pathogenesis of iron accumulation in CHC is not well understood, but both host and viral factors seem to play roles. Two different interpretations of iron overload in CHC have been proposed. Guyader et al. negated the role of iron as a fibrogenic factor per se and demonstrated it as a surrogate marker for disease severity (Guyader et al., 2007). All factors known to influence iron overload and fibrosis (age, sex, duration of HCV infection, alcohol consumption, features of metabolic syndrome including hyperglycemia, hyperlipidemia, obesity, non-alcoholic fatty liver disease) were analyzed in 586 patients with CHC. After the analysis of the data, it was concluded that iron accompanied more severe forms of necroinflammatory disease and fibrosis as a mere bystander. However, other authors have demonstrated a significant association of iron overload with the promotion of liver necroinflammation and fibrosis (Bonkovsky et al., 1997; Metwally et al., 2004; Fujita et al., 2007a). Independent of the hypotheses of iron-enhanced fibrosis progression or iron as a surrogate marker of severe fibrosis,

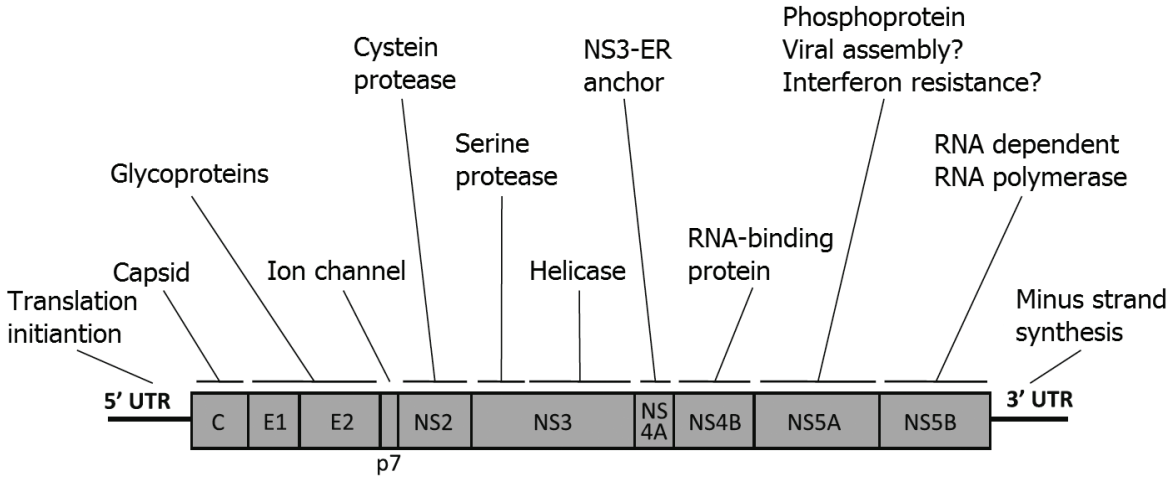

Fig. 1. HCV genome – its structure and function (adapted from Sakamoto N., Watanabe M. (2009) *New therapeutic approaches to hepatitis C virus*. J. Gastroenterol. 44: 643-649): UTR – untranslated region; ER – endoplasmic retikulum

there are evidences that iron overload leads to an increased risk of HCC, failure of antiviral treatment and significantly worsens clinical outcomes in patients suffering from chronic HCV infection (Fujita et al., 2007a; Ko et al., 2007; Sikorska et al., 2010).

Genetic host factors

Iron homeostasis in the human body is maintained by mechanisms controlling iron absorption from the intestinal tract, iron recycling from macrophages and mobilization of hepatic iron stores (Crichton, 2009). Multiple genes regulating iron homeostasis have been discovered in the past few years. Based on clinical observations, mutations in at least five genes may lead to an iron overload syndrome (Pietrangelo, 2010). In humans, carefully regulated system of iron absorption control is accompanied by a lack of effective physiological mechanisms for the excretion of excess iron accumulating in different tissues. This is the main reason for progressive iron loading and, ultimately, multiple organ damage determined by genetic defects. Hepcidin and its interaction with the transmembrane iron transporter ferroportin (FPN) play crucial roles in the systemic iron balance through down-regulation of iron release from enterocytes and phagocytes (Nemeth et al., 2004). The expression of hepcidin is a complex process, strongly inhibited by hypoxia, anemia and iron deficiency while being activated by inflammation and iron overload. Molecular mechanisms of hepcidin regulation involve stimulation of hepcidin mRNA transcription through the interleukin-6 (IL-6)/STAT3 pathway, which is responsible for signaling in inflammation. The iron-induced production of bone morphogenetic proteins (BMPs; one of the multifunctional growth factors) with its co-receptor hemojuvelin likely constitutes a key, endogenous signaling pathway for hepcidin activation also through its interaction with IL-6/STAT3 (Pietrangelo, 2010). Disturbances of hepatic BMP signaling (e.g., mutations of the hemojuvelin gene) can cause inhibition of hepcidin expression and iron overload (Corradini et al., 2009).

Primary iron overload disorder (hereditary hemochromatosis; HH) is one of the most frequently inherited metabolic diseases, occurring at rates as high as 3 to 8 cases per 1000 among Caucasians (Phatak et al., 1998). Classification of HH takes into account the loss of functional proteins encoded by different genes including mutations that alter hepcidin synthesis (HFE, hemojuvelin, hepcidin, transferrin receptor 2 genes) or its interaction with FPN (ferroportin disease). However, the most frequent form of HH observed in Caucasians is related to *HFE* gene mutations (>80% of HH cases). Juvenile hemochromatosis (with rapid progression and serious organ damage in early adulthood, caused by mutations of hepcidin (HAMP) or hemojuvelin (HJV) genes) transferrin receptor 2 (TfR2) mutation-related forms of HH and ferroportin disease are rarely diagnosed (EASL, 2010).

HFE gene mutations are responsible for HFE protein conformation changes leading to down-regulation of hepcidin synthesis through a postulated impaired signaling response of the TfR1-HFE/TfR2 complex to BMP (Pietrangelo, 2010). The low circulating levels of hepcidin lead to high *FPN* expression, with an increase of iron

absorption in the gut and release from phagocytes. The clinical presentation of the defined genotype for type 1 HH, homozygotic missense mutation C282Y or compound heterozygosity for C282Y and H63D, is limited. This frequent genotype predisposition results in an iron overload-related disease in 38-50% of C282Y homozygotes (EASL, 2010). Evidence for iron overload has been also reported for other HFE genotypes (Ellervik et al., 2007).

Patients suffering from both CHC and type 1 HH have a significant risk of severe iron overload syndrome and show a rapid development of progressive liver disease leading to an unfavorable outcome (Sikorska et al., 2010; Diwakaran et al., 2002).

The role of non-HH genotypes (heterozygotic C282Y, H63D, S65C mutations) in the development of iron overload and acceleration of liver fibrosis in CHC has yet to be explicitly determined (Smith et al., 1998; Thorburn et al., 2002; Tung et al., 2003; Gehrke et al., 2003; Geier et al., 2004). The association varies due to ethnic differences in the frequency of *HFE* mutations and the influence of other environmental and genetic factors modulating the phenotypic expression of those mutations (gender, genetic polymorphism in antioxidant enzymes, alcohol consumption, metabolic syndrome) (Wood et al., 2008). Some authors have found that *HFE* gene mutations can function as prognostic factors and are associated with the progressive course of HCV-related chronic liver disease (Smith et al., 1998; Tung et al., 2003; Gehrke et al., 2003; Geier et al., 2004; Bonkovsky et al., 2002; Erhardt et al., 2003).

Analyses of the possible immunological function of HFE protein, which could shape response to antiviral treatment, have brought disparate results. Based on the observations of better interferon and ribavirin antiviral treatment efficacies in carriers of *HFE* gene mutations, some researchers have proposed that HFE protein may function as a possible immunomodulatory non-classical major histocompatibility complex class I (MHC-I) molecule (Lebray et al., 2004; Bonkovsky et al., 2006). Researchers have examined the impact of *HFE* gene mutations on the alteration of iron metabolism and modulation of the immune response and the accompanying effect on SVR. Bonkovsky et al. showed improved results for antiviral therapies in carriers of *HFE* mutations (especially H63D), despite the positive correlations between the presence of *HFE* mutations and serum measures of iron status. However, the presence of iron deposits in hepatocytes did not affect the rate of SVR. The effect of *HFE* mutations in the studied populations could be race dependent; the Caucasians, who are the main carriers of *HFE* mutations, show higher rates of SVR. Moreover, the above-mentioned authors have shown the improved results of antiviral therapy only in patients without confirmed hereditary hemochromatosis or intensified iron overload based on a liver biopsy. Their extensive study provided an insight into the potential of mutated HFE protein, a non-classical MHC-I molecule, as a beneficiary modulator of the immune response against the virus (Bonkovsky et al., 2006). Earlier, Lebray et al. also observed a positive influence of the *H63D* mutation on responses to interferon treatment (Lebray et al., 2004). Since a later Polish study did not confirm the earlier results, additional studies are required to fully confirm the role of HFE in modulating SVR (Sikorska et al., 2010).

Impact of the HFE protein malfunction on cellular iron availability in lymphocytes and phagocytes appears complex and may be associated with an immune dysfunction and attenuation of host response against HCV infection (Drakesmith et al., 2008). The immune cells are involved in the modulation of iron homeostasis through the expression of iron-related genes and proteins. Perturbations of iron levels may inhibit the efficient stimulation of macrophages, differentiation and proliferation of specific clones of lymphocytes, and increase the susceptibility of lymphocytes to oxidative stress with an accompanying higher risk of DNA damage (Pietrangelo, 2003; Kruszewski et al., 2008). Decreased intraphagocytic iron, as observed in hepcidin deficiency and ferroportin up-regulation, inhibits the translation of the pro-inflammatory cytokines TNF-alpha and IL-6 (Wang et al., 2008). The differentiation of monocyte-derived dendritic cells into active antigen presenting cells as well as the dendritic cell stimulation of lymphocytes are both dependent on the decreased expression of TfR1 and the down-regulation of ferroportin (Porto et al., 2007). Low $CD4^+$ and NK T lymphocyte populations, major increases in IL-4 and IL-10 synthesis, diminished $CD8^+$ T lymphocytes cytotoxic activity and changes in the expression of T cell receptors have been described in C282Y homozygous patients (De Almeida et al., 2005). The severity of iron load, rather than the type of *HFE* mutation, appears to influence the lymphocyte populations. A disturbance of iron homeostasis has been shown to be

associated with altered susceptibility to some bacterial infections (Wang et al., 2008), but the underlying molecular mechanisms are still poorly understood. The impact of iron disorders on the innate or adaptive immunity in viral infections is not yet fully described (Ward et al., 2010).

The roles of other iron metabolism-related genes in the pathogenesis of iron overload in cases of chronic hepatitis C have not been examined extensively. It is likely that this lack of information is due to the rare frequency of *TfR2*, *HAMP*, *HJV* and *FPN* mutations. There is no accurate data on iron metabolism-related gene polymorphisms in different world populations. It seems to be an interesting task to examine the impact of *FPN* mutations that cause hepcidin resistance or HAMP and HJV mutations especially in the context of the interesting recent discoveries that indicate both HCV-dependant hepcidin inhibition and hepcidin-dependant HCV replication (Nishina et al., 2008; Miura et al., 2008; Tai et al., 2009). In one Italian study, no mutations of *HJV*, *FPN* or *TfR2* genes were detected in 143 CHC patients. Four patients were carriers of hepcidin mutations, with two of them presenting iron overload and liver cirrhosis (Valenti et al., 2007).

Currently, in the transplantation era, problems of genetic predisposition to pathogenic iron accumulation in HCV infection and genetic screening of donors are arising. HCV-associated end-stage liver disease is one of the main reasons for liver transplantation in the United States and Europe. Liver transplant recipients who also suffer from iron overload are more susceptible to other diseases like serious fungal, bacterial and viral infections (aspergillosis, cryptococcosis, cytomegaly, infections with *Staphylococcus aureus*, *Yersinia* species), relapse of hepatocellular carcinoma and nonhepatic cancers, which significantly decrease the post-transplantation survival rate (Kowdley et al., 2005; Alexander et al., 2006; Singh et al., 2008; Dar et al., 2009). Moreover, hemochromatosis phenotypes as a post-transplantation complication were also observed in HFE-wild-type recipients of livers from donors with *HFE* mutations (Wigg et al., 2003; Ismail et al., 2009).

Modulation of iron homeostasis in chronic hepatitis C

Iron availability and its safe storage in cells are achieved by maintaining appropriate iron concentrations in both the blood and intracellular iron pools. The balance of iron plasma levels is controlled by hepcidin/ferroportin interactions. The amount of iron in intracellular compartments is regulated by the modulation of the posttranscriptional expression of ferritin and transferrin receptor 1. The expression of these iron-related proteins depends on the binding of iron regulatory proteins -1, -2 (IRP1, IRP2) to iron responsive elements (IREs) that are present in untranslated regions of the proteins' mRNA. Iron deficiency activates the binding of IRP to IREs, which leads to the stabilization of TfR1 mRNA (IRE in 3′untranslated region) and inhibition of ferritin mRNA (IRE in 5′untranslated region) (Lipiński et al., 2006). Extracellular oxidative stress activates IRP1 in livers (Mueller et al., 2001). Chronic liver inflammation, as is observed in CHC, leads to an activation of immune effector cell responses and the release of reactive oxygen species. Such inflammation could result in increased intracellular iron. Additional studies are needed to fully elucidate the function of the IRP/IRE regulatory network and its possible impact on hepcidin/FPN system in HCV-induced inflammation.

More is known about the influence of HCV on the modulation of hepcidin. Experiments employing cell lines and transgenic mice expressing HCV proteins have shown that HCV-induced oxidative stress leads to a decreased hepcidin expression through the reduced DNA binding activity of the transcription factor C/EBPα (Nishina et al., 2008; Miura et al., 2008).

These results suggest that the HCV polyprotein may enhance iron overload and disease progression (Fig. 2). The lower levels of hepcidin resulting in up-regulation of ferroportin and increased iron export from enterocytes and macrophages might explain elevated biochemical iron markers and hepatic iron observed in CHC patients. However, increases in hepatic iron may be part of a host antiviral strategy in which iron is used to inhibit HCV replication. The above-mentioned preliminary models are not ideal because they disregard the HCV infection related inflammatory activity. Inflammation and increased body iron are strong hepcidin stimulators. The interaction of the factors regulating hepcidin levels is more complex in chronically HCV-infected patients. Clinical observations of CHC patients have shown some discrepancies in the assessment of the role of hepcidin. Regulatory failure of serum prohepcidin (prohormone) and low hepcidin mRNA accumulation in HCV infection have been observed (Nagashima et al., 2006; Fujita et al., 2007b).

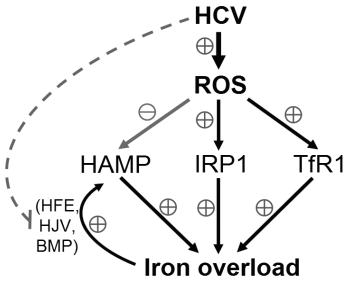

Fig. 2. Iron regulation in HCV infection. ROS are generated in the hepatic tissue in chronic hepatitis C. Iron overload may be a result of oxidative stress leading to hepcidin suppression, IRP1 activation and TfR1 activation (Andriopoulos et al., 2007). Iron overload and inflammation cause the induction of HAMP but this effect may be changed by the possible interactions between HCV and HFE, HJV or BMP, which at present, are unknown. ROS – reactive oxygen species; IRP1 – iron regulatory protein 1; HAMP – hepcidin; TfR1 – transferrin receptor 1; HJV – hemojuvelin; BMP – bone morphogenetic protein

HCV-dependent hepcidin production impairment in the liver appears to be reversible after successful HCV eradication (Fujita et al., 2008). In addition, hepcidin gene expression has been shown to be positively correlated with hepatic iron concentration (HIC) and the concentrations of reactive oxygen species (Fujita et al., 2007c; Aoki et al., 2005). However, contrary to the expected inflammatory response, hepcidin levels were independent of other markers of inflammation and were positively associated with HIC. Due to these factors it was concluded that liver iron stores regulate hepcidin expression (Aoki et al., 2005). In a Polish study, prohepcidin serum concentration was correlated neither with HCV viral load nor serum iron indices and decreased in patients who responded to antiviral therapy. Interestingly, serum prohepcidin was higher in patients infected with HCV genotype 3a, that is characterized by less resistance to interferon therapy (Jaroszewicz et al., 2010). The association of hepcidin with the efficacy of interferon therapy and the relation between serum and tissue hepcidin are also interesting in the context of hepcidin significant inhibition in hepatocellular carcinoma, probably due to the impairment of the tumor suppressor gene *p 53* (Kijima et al., 2008).

Regulation of other iron transporters in relation to HCV infection has been the subject of a few clinical studies. In two studies of Japanese patients, the impact of HCV infection on the up-regulation of TfR2, ferroportin and subsequent iron accumulation in the liver was shown (Takeo et al., 2005; Mifuji et al., 2006).

Iron as a modulator of hepatitis C virus replication

Studies on HCV replication and iron impact on the pathogenesis of CHC have yielded divergent results. Any analysis of the correlation between iron and HCV should take into account not only the possible disorders of iron metabolism induced by HCV infection and chronic inflammation but also the alterations of HCV replication caused by iron.

Molecular mechanisms of the probable modulation of the HCV life cycle by iron are not obvious. Results of previous research are divergent but confirm the iron interference with virus replication and viral protein expression. Using a semiquantitative reverse transcription-PCR assay, Kakizaki et al. found that supplementation of an HCV-infected non-neoplastic human hepatocyte cell line with different concentrations of iron salts enhanced HCV replication (Kakizaki et al., 2000). In other studies, it has been proposed that iron promotes HCV translation. Theurl et al. showed an increased production of eIF3 mRNA and eIF3 protein in HepG2 cells (human hepatocellular liver carcinoma cell line characterized by a high degree of morphological and functional differentiation) treated with ferric chloride. After transfection of the cells with vectors containing the parts of the 5′ untranslated region of HCV genome, the authors observed strong activation of IRES-dependent HCV 1b mRNA translation in cells treated with iron. An association of the increased eIF3 mRNA synthesis with liver iron concentration in liver biopsy-samples derived from HCV 1b-infected patients was also found. Based on these observations, it was suggested that iron promoted the translation of HCV by stimulating the expression of eIF3 (Theurl et al., 2004). Cho et al. also reported a positive correlation of increased intracellular iron with the efficiency of HCV IRES-dependent translation in Chang's liver cells derived from non-malignant tissue. The authors demonstrated that two common proteins bind to both HCV RNA 5′UTR and IRE in an iron-dependent manner (Cho et al., 2008).

Completely different results, showing a link between iron metabolism and HCV life cycle, have been presented by Fillebeen et al., who utilized Huh7 hepatoma cells transfected with a genotype 1b HCV replicon. Exposure to increasing concentrations of hemin or Fe-SIH (Ferric-salicylaldehyde isonicotinoylhydrazone) as iron donors inhibited the expression of NS5A and core proteins of the subgenomic HCV replicon. Fillebeen et al. also found an iron-induced decrease in the replicon RNA expression

without alterations of HCV translation and confirmed iron-mediated inhibition of RNA-dependent RNA polymerase (NS5B) (Fillebeen et al., 2005). In other experiments, the same authors observed alterations of iron homeostasis induced by the presence of the subgenomic HCV replicon in Huh7 cells. It was shown that the modulation of the expression of iron-related genes and iron metabolism proteins associated with subgenomic HCV replication leads the host cells to reduce iron uptake and increase iron release. Reduction in TfR1 accumulation and high increase in ferroportin production accompanied paradoxically by the increase both in total IRE-binding activity and IRP2 level were suggested to indicate iron deficiency in HCV replicon expressing cells. The authors hypothesized that the reduction of host intracellular iron may be a kind of virus adaptive strategy enabling its growth, especially during an early stage of infection (Fillebeen et al., 2010). Recently, Fillebeen (2010) presented an evidence for iron-induced inhibition of HCV replication in Huh7cells transfected in vitro with a consensus clone of HCV genotype 2a (JFH-1). JFH-1 was derived from a Japanese patient who developed fulminant hepatitis, an extremely rare clinical presentation of HCV infection (Fillebeen et al., 2007).

Due to the different outcomes of studies in this area and various factors that could influence the study outcome, continued research is necessary. Genetic polymorphisms in different cell lines and the variable effectiveness of the multiplication mechanisms of different HCV genotypes may be influencing experimental results. Further studies are warranted to explain the observed correlations between HCV replication and iron.

The divergent results of studies analyzing the impact of iron on HCV replication make it difficult to fully understand the role of hepcidin in CHC (Kakizaki et al., 2000; Theurl et al., 2004; Cho et al., 2008; Fillebeen et al., 2005; Fillebeen et al., 2007; Fillebeen et al., 2010). Does low hepcidin in CHC followed by an increase in liver iron content enhancing virus replication signal inefficiency in human innate immune system? Or is low hepcidin a part of an antiviral defense system targeting HCV's evolutionary strategy to bypass iron-dependent inhibition of viral replication? Both of these hypotheses require thorough study (Fillebeen et al., 2007). A clear explanation of the mechanisms behind hepcidin dysregulation may lead to new possibilities in using the modulation of iron metabolism as therapies for difficult-to-treat HCV patients.

The role of iron overload in antiviral treatment

The most important known predictive factors of positive response to treatment with interferon and ribavirin include infection with HCV genotype 2 and 3, pretreatment low viral load, rapid response and early virological response. Age, race, body mass index, liver fibrosis stage, insulin resistance and mutations in the interferon sensitivity-determining (ISDR) region of the HCV genome may also serve as predictors of treatment failure (Juszczyk, 2009). It is important to note that effectiveness of IFN-α therapy depends on the regulation of HCV translation factors, the expression of which is proposed to be up-regulated by iron (Theurl et al., 2004).

In the past, when interferon-α was used only in monotherapy, high liver iron concentration was proposed as a negative predictor of antiviral therapy in different ethnic populations. There were no consensus on whether iron accumulation in hepatocytes or the sinusoidal cells and portal track macrophages was more significant for poor response to treatment (Olynyk et al., 1995; Barton et al., 1995; Ikura et al., 1996).

Use of combination therapy with interferon followed by pegylated interferon with ribavirin has substantially improved the rates of SVR. Observations on the efficacy of the current standard therapy in relation to biochemical iron parameters such as serum iron, ferritin, transferrin saturation or hepatic iron content appear to be incompatible. Some research confirmed an association between elevated serum iron indices or high hepatic iron concentration and the lack of SVR (Fujita et al., 2007a; Sikorska et al., 2010; Bonkovsky et al., 2006; Distante et al., 2002), whereas others showed no positive correlation between HIC and decreased frequency of SVR (Pianko et al., 2002; Hofer et al., 2004). In a large study, Bonkovsky et al. found the presence of iron in endothelial cells with triad iron score (not global iron score) as a predictor of decreased SVR (Bonkovsky et al., 2006). These contradictory results from different parts of the world may possibly have their source in ethnic differences and the variable polymorphisms of iron metabolism-related genes found in different populations. To fully answer this question further, larger prospective studies are needed.

According to clinical observations, it is probable that in many cases, increased biochemical iron parameters and hepatic iron storage accompany a more aggressive

course of CHC characterized by intense necroinflammatory activity and much advanced fibrosis (Guyader et al., 2007). Such an unfavorable course of CHC may be the result of specific HCV virulence changes that breed resistance to antiviral treatment.

At present, there is no consensus opinion on the influence of iron overload or iron reduction on the efficacy of antiviral therapy. Current recommendations for iron reduction therapy by phlebotomy generally concern patients with CHC linked to proven hepatic iron storage disease, especially in cases with coexistence of hereditary hemochromatosis (Bassett, 2007). Patients diagnosed with HH should be treated with bloodletting, even if they achieve sustained viral response after treatment with interferon and ribavirin. Phlebotomies have appeared to be effective in Japanese patients free from *HFE* mutations and have been shown to cause a decline in serum aminotransferase activities and an improvement of liver inflammation with the suppression of the liver fibrosis development confirmed by histological examination (Yano et al., 2002). As iron depletion itself does not decrease HCV viremia in the treated patients, it has been speculated that the beneficial effect of phlebotomy results from the protection of hepatocytes from an injury caused by iron-generated free radicals (Fong et al., 1998; Herrera, 1999). Controlled, randomized, prospective studies evaluating the effect and safety of phlebotomies, also in comparison with orally-active iron chelators in patients treated with interferon and ribavirin, are needed. If the advantageous impact of iron deficiency on CHC progression can be confirmed, such results might lead to interesting long-term management alternatives for nearly half of the patients who do not currently respond to standard antiviral drugs.

Despite the results of some research indicating iron as an inhibitor of HCV replication (Fillebeen et al., 2005; Fillebeen et al., 2007; Fillebeen et al., 2010), there is no convincing, clinical evidence supporting iron substitution as a desirable factor favoring CHC remission. Hemodialized HCV-infected patients who received iron intravenously, presented significant elevation in both alanine and aspartate aminotransferase levels (Kahraman et al., 2005). In addition, the observations of patients with thalassemia, the carriers of hemoglobin gene mutations who develop hemosiderosis as a result of hematological disease (independent of HCV infection), do not prove any beneficial effects from iron excess. On the contrary, Italian HCV-infected patients who were the carriers of β-globin mutations presented higher hepatic iron concentrations and more advanced liver fibrosis in multivariate analysis (Sartori et al., 2007).

Conclusions

Twenty years of observations of chronic hepatitis C in relation to iron confirms its role as a non-overrated factor in pathogenesis and clinical course of disease. There are still many questions about molecular mechanisms underlying the accumulation of iron in CHC. It is also unclear as to how such mechanisms depend on host responses against virus, HCV replication and HCV-induced modulation of iron homeostasis. A better understanding of the interplay between HCV and iron may help create novel effective strategies of CHC treatment.

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