

Relationship between Hepatitis C Virus Infection and Iron Overload

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Abstract

Objective: The aim of this study was to summarize the interactions between hepatitis C virus (HCV) infection and iron overload, and to understand the mechanisms of iron overload in chronic hepatitis C (CHC) and the role iron plays in HCV life cycle.

Data Sources: This review was based on data in articles published in the PubMed databases up to January 28, 2017, with the keywords “hepatitis C virus”, “iron overload”, “iron metabolism”, “hepcidin”, “translation”, and “replication”.

Study Selection: Articles related to iron metabolism, iron overload in patients with CHC, or the effects of iron on HCV life cycle were selected for the review.

Results: Iron overload is common in patients with CHC. The mechanisms involve decreased hepcidin levels caused by HCV through signal transducer and activator of transcription 3, mitogen-activated protein kinase, or bone morphogenetic protein/SMAD signaling pathways, and the altered expression of other iron-metabolism-related genes. Some studies found that iron increases HCV replication, while other studies found the opposite result. Most of the studies suggest the positive role of iron on HCV translation, the mechanisms of which involve increased expression levels of factors associated with HCV internal ribosome entry site-dependent translation, such as eukaryotic initiation factor 3 and La protein.

Conclusion: The growing literature demonstrates that CHC leads to iron overload, and iron affects the HCV life cycle in turn. Further research should be conducted to clarify the mechanism involved in the complicated interaction between iron and HCV.

Key words: Hepatitis C Virus; Hepcidin; Iron Overload; Replication; Translation

INTRODUCTION

Iron is an important trace element in the human body. The absorption of iron is strictly controlled through hepcidin, while there are no efficient physiologic mechanisms to excrete iron from the body. Under conditions where iron metabolism is disturbed, such as in transfusion-dependent patients with thalassemia major, myelodysplastic syndromes, aplastic anemia, or sickle cell disease, excessive iron deposition occurs in the liver, heart, and endocrine organs, resulting in reactive oxygen species (ROS) through the Fenton and Haber–Weiss reaction.^[1] ROS can attack components of cells^[2,3] and further induce organ dysfunction, such as liver fibrosis, cirrhosis, hepatic carcinoma,^[2] myocarditis, pericarditis,^[4] and other diseases.^[4-8]

In the liver of chronic hepatitis C (CHC) patients, iron deposition has been found in both hepatocytes and reticuloendothelial cells^[9-13] although the mechanism is not fully clarified. Like other viruses, hepatitis C virus (HCV)

needs constituents of host cells to proliferate, and iron is one of the most important constituents. Many studies have explored the connection between iron overload and HCV life cycle with differing results. Some studies have found that iron promotes HCV replication,^[14,15] while others have shown that iron suppresses HCV replication.^[16-19] Most of the studies suggest the positive role of iron on HCV translation.^[20-23] To summarize the interactions between HCV and iron, and understand the mechanisms of iron overload in CHC, we searched articles published in the PubMed databases up to January 28, 2017, to gain an in-depth understanding and knowledge of relevant areas.

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Received: 01-12-2016 **Edited by:** Qiang Shi
How to cite this article: Zou DM, Sun WL. Relationship between Hepatitis C Virus Infection and Iron Overload. *Chin Med J* 2017;130:866-71.

Access this article online

Quick Response Code:



Website:
www.cmj.org

DOI:
10.4103/0366-6999.202737

IRON METABOLISM AND HOMEOSTASIS

In the human body, iron is critical for maintaining the fundamental function of many proteins.^[24-28] Iron in the diet is absorbed through divalent metal transporter 1 (DMT1),^[29] a multi-transmembrane protein, or heme carrier protein 1^[29] on duodenal and jejunal enterocytes.^[30] It is then exported by the ferroportin (FPN) to bind to transferrin in the bloodstream,^[31,32] and taken to the erythroblasts for erythropoiesis. Iron that is not utilized can also be stored as ferritin or hemosiderin^[29] in enterocytes, macrophages, and hepatocytes. There are no efficient physiologic mechanisms to excrete iron, a little iron (about 2 mg per day) is lost by sloughing of intestinal epithelial cells, desquamation of skin and urinary cells, blood loss, and sweat.^[33]

Iron homeostasis in the human body is mainly regulated by the hepcidin/FPN and iron-regulatory protein/iron-responsive element (IRP/IRE) systems. Hepcidin is a peptide hormone mainly secreted by the liver.^[29] When iron levels increase, hepcidin negatively regulates iron levels by binding to the FPN and promoting the internalization and degradation of FPN.^[34] This reduces the amount of iron absorbed by enterocytes, released by hepatocytes, and recycled from macrophages, and finally reduces transferrin levels in the bloodstream. In contrast, when iron levels decrease, hepcidin is downregulated to improve transferrin levels. Transferrin receptor 1 (TfR1)^[35] and bone morphogenetic protein 6 receptor (BMP-6R)^[29,36] can sense transferrin saturation (TS) and tissue iron content, respectively, further regulating hepcidin concentration. Hepcidin can also be affected by other factors, including inflammation, erythropoietic activity of the bone marrow, and the oxygen tension within hepatocytes.^[29] While hepcidin regulates systemic iron metabolism transcriptionally and posttranslationally, cellular iron metabolism is regulated by the IRP/IRE system posttranscriptionally. The IRP/IRE system alters the expression of iron-metabolism-related proteins, such as ferritin, FPN, hypoxia-inducible factor 2 α (HIF2 α /EPAS1), DMT1, TfR1, and other proteins.^[37]

IRON OVERLOAD IN CHRONIC HEPATITIS C PATIENTS

About 170 million people worldwide are infected with HCV.^[38] It is widely accepted that CHC is associated with iron overload. Many studies have found iron deposition in the liver,^[9-13] and in both hepatocytes and reticuloendothelial cells.^[10-12] Di Bisceglie *et al.*^[11] were the first to find elevated serum ferritin and iron levels in patients with chronic hepatitis (both chronic hepatitis B and CHC). Subsequently, in 1999, a study of 209 CHC patients found liver iron accumulation detected by liver biopsy in 42.1% of patients, the majority of which was mild liver iron accumulation (35.4% of the 209 CHC patients). Those with liver iron accumulation had significantly higher levels of serum iron (SI), ferritin, and TS; liver iron accumulation was also found to have a significant relationship with the severity of histological activity based on METAVIR

classification and cirrhosis.^[10] In a study of 100 consecutive patients with HCV infection who underwent liver biopsy, 19 patients were found to be hepatic iron stain positive, which is associated with Stage III or IV fibrosis. Fifty-five patients had at least one abnormal value of SI, ferritin, or TS. In multivariate analysis, the only independent predictive factor of severe hepatic fibrosis was serum ferritin. The serum ferritin value and tissue iron stain had a significantly positive correlation.^[39] These two studies^[10,39] demonstrate that elevated serum ferritin is associated with iron overload status. However, elevated serum ferritin can also be caused by hepatic inflammation.^[40] In 1994, a study of 123 chronic hepatitis patients (including 63 CHC patients) found increased SI, iron saturation, and ferritin in CHC patients, while no evidence of hepatic iron accumulation could be found in any of these patients. In this study, serum ferritin was elevated in the absence of liver iron overload, which indicates that elevated serum ferritin may also be caused by inflammation itself. Thus, elevated ferritin reflects hepatic iron accumulation, as well as hepatic inflammation, and also predicts severe hepatic fibrosis. In other words, hepatic iron accumulation can lead to elevated ferritin, while elevated ferritin is not only induced by hepatic iron accumulation but also caused by hepatic inflammation. Thus, serum ferritin levels only serve as a reference to evaluate liver iron status in CHC patients, and liver biopsy is the gold standard for diagnosis of iron overload.

THE EFFECT OF HEPATITIS C VIRUS INFECTION ON IRON METABOLISM

So far, plenty of studies have been devoted to exploring the mechanism as to how HCV leads to iron overload. It is generally accepted that HCV alters iron metabolism by reducing the level of hepcidin.^[13,41-48] Fujita *et al.*^[43] measured hepcidin messenger RNA (mRNA) levels in liver samples from 56 patients with HCV infection, and revealed that the expression of hepcidin is strongly correlated with serum ferritin and the degree of iron deposition in liver tissues. The hepcidin-to-ferritin ratio was significantly lower in HCV(+) patients than HBV(+) patients or controls.^[43] Girelli *et al.*^[44] detected s-hepcidin (the 25-amino acid bioactive peptide in serum) levels in 81 untreated CHC patients and 57 controls with a rigorous definition of normal iron status and found that s-hepcidin was significantly lower in CHC patients than in controls. In CHC patients, s-hepcidin levels are significantly correlated with serum ferritin and histological total iron score. Tsochatzis *et al.*^[45] examined the determinants of serum hepcidin and liver hepcidin mRNA levels and their association with histological lesions in 96 patients with CHC and 30 controls; they concluded that serum hepcidin was significantly lower in patients with CHC compared to healthy controls, and that liver hepcidin mRNA levels did not differ between patients and controls. A study using FL-N/35 transgenic mice harboring the HCV polyprotein also found decreased hepcidin expression in the liver, accompanied by an increase in FPN expression in the duodenum, spleen, and

liver. This suggests that HCV proteins may directly lead to increased duodenal iron absorption, macrophage iron release, and hepatic iron accumulation.^[42] Elevated duodenal FPN levels^[47] and a significant relationship between HCV and hepcidin^[48] in CHC patients were also found by other studies. All of these studies suggest that hepcidin may play a pivotal role in the pathogenesis of iron overload in patients with CHC.

There are many studies examining the mechanism by which CHC suppresses hepcidin expression. Kohjima *et al.*^[49] analyzed iron-metabolism-related gene expression profiles in 100 patients with CHC (genotype 1b, $n = 50$; genotype 2, $n = 50$) and 18 living donors of liver transplantation. They found that expression of genes related to iron absorption (transferrin, and DMT1), iron export (FPN), cellular iron metabolism (IRP1 and IRP2), and hepcidin-regulation (BMPRI1, BMPRI2, and hemojuvelin) were significantly higher, indicating that HCV affects the expression of iron-metabolism-related genes, leading to iron accumulation in hepatocytes. Besides, patients with a sustained virological response (SVR) had significantly lower transcription and protein expression levels of hepcidin, FPN, BMPRI2, and hemojuvelin before therapy, indicating the importance of hepatocytic iron retention for viral response during pegylated interferon plus ribavirin treatment. Almost all HCV proteins, including core, E1, E2, NS3, NS4A, NS4B, and NS5A, have been shown to regulate hepcidin expression through signal transducer and activator of transcription 3, mitogen-activated protein kinase, or BMP/SMAD signaling pathways,^[50] and increased histone deacetylase activity,^[41] which further clarifies the mechanism of decreased levels of hepcidin caused by HCV.

THE EFFECT OF IRON ON HEPATITIS C VIRUS REPLICATION AND TRANSLATION

Like other viruses, HCV needs constituents of host cells to replicate and translate, and iron is one of the most important constituents. It is indispensable for several basic metabolic processes in viruses, as well as in mammalian cells, as described in many studies.

Kakizaki *et al.*^[14] were the first to analyze the effect of iron on HCV replication *in vitro*. In 2000, they cultured a nonneoplastic HCV-infected human hepatocyte line (PH5CH8) treated with FeSO_4 and proved that iron can promote HCV replication in liver cells. In 2016, Foka *et al.*^[15] found that iron-overloaded macrophages was infected with HCV when co-cultured with HCV-infected human hepatoma cell line (Huh7.5). These iron overloaded macrophages also enhanced HCV replication in co-cultured HCV-infected Huh7.5 cells through reversed ferritin “flow” from macrophages to Huh7.5 cells. Macrophages without overloaded iron was also infected with HCV and enhance HCV replication in Huh7.5 cells, but the rate of infection was slower and the effect on HCV replication was weaker.

On the other hand, several studies have found that iron suppresses HCV replication. A study using Huh7 cells in

2005 concluded that iron could inhibit HCV replication by inactivating the RNA polymerase NS5B, without significant effect on translation.^[16] Fillebeen *et al.*^[17] concluded that iron could decrease HCV replication by reducing the activity of NS5B in Huh7.5.1 cells, similar to the result of the study in 2005. A later study, in 2011, again found that increased iron status and down-regulated hepcidin inhibited HCV replication.^[18] Another study also found that iron administration could suppress HCV replication *in vitro*.^[19]

The studies described above hold different opinions toward the effect of iron on HCV replication. This may be due to different cell types. Besides, in clinical practice, CHC with primary or secondary iron overload have different prognoses. CHC patients with hereditary iron overload due to hemochromatosis gene mutations have increased SVRs to antiviral therapy,^[51] when excess iron deposits in parenchymal cells and macrophages become iron deficient.^[52] In contrast, iron overload secondary to CHC or other chronic liver diseases significantly aggravates the disease,^[1] when excess iron deposits in macrophages.^[53] It seems that the iron content of macrophages is associated with the clinical outcome of disease, because iron overload may impair the immune function of macrophages through oxidative stress.^[54]

Although the role of iron in promoting or suppressing HCV replication is debatable, there are few controversies about the effect of iron on HCV translation. Apart from a group in the USA that reported that iron suppresses HCV translation through increasing heme oxygenase-1 *in vitro*^[55] and another group^[16] that found no effect of iron on HCV translation, all other studies confirm the positive role of iron on HCV translation,^[20-23] but the mechanisms are not fully understood. Expression of HCV is predominantly controlled at the translational level, which involves the interaction between cellular translation initiation factors and a specific mRNA stem-loop structure – the internal ribosome entry site (IRES) – within the 5' untranslated region.^[56] Iron is reported to stimulate the expression of eukaryotic initiation factor 3 (eIF3), thus enhancing HCV IRES-dependent translation both in HepG2 cells and HCV-infected patients, whereas iron chelation reverses it *in vitro*.^[20] Another group also found that hepatic iron load promotes HCV translation initiation *in vitro* with the mechanism involving iron-dependent increased expression of eIF3. Expression of another element, La protein, is also significantly increased by hepatic iron load which can promote HCV translation. Expression of both eIF3 and La protein can be partially inhibited by the iron chelator deferoxamine.^[21,22] Cho *et al.*^[23] also found that iron changes the affinities of common cellular factors to HCV IRES which modulates HCV IRES-dependent translation. In summary, iron can regulate HCV IRES-dependent translation by increasing expression levels of associated factors.

The outcome of iron perturbation varies according to HCV genotype. Translation of both HCV 1b and HCV 2b is significantly increased after iron treatment, while translation

of HCV 6a shows little difference. Compared with subtype 1b, HCV 6a has a modified eIF3 binding site within hairpin III.^[20] It is reported that hepatic iron levels are higher in patients infected with subtype 1b than those in patients infected with subtypes 2a or 2b, which may explain the different responses of HCV genotypes to iron overload.^[57]

Nowadays, liver biopsy is the gold standard for diagnosis of hepatic iron overload, and there are no uniform diagnostic criteria about extracellular iron concentration. Most of the studies described in the paper used cell models incubated in ferric or ferrous iron solutions with maximum concentrations ranging from 50 to 300 $\mu\text{mol/L}$, whether they reach the standard of iron overload is difficult to judge. Therefore, whether these results from *in vitro* research could be suggestive to understand the effect of iron on HCV is still debatable.

CONCLUSIONS

Each part of iron metabolism, including absorption, restoration, recycling, and utilization, is regulated by hepcidin and the IRE/IRP system. There is no efficient way to eliminate iron from the body, so any abnormality in the process of iron metabolism may lead to excessive iron burden. The relationship between HCV and iron overload is complicated [Figure 1]. Many studies have

observed iron overload in patients with CHC, and most suggest that decreased hepcidin expression, as well as increased HCV protein-mediated expression of other iron metabolism-related genes, play a role. As to the detailed mechanism of HCV proteins inducing iron overload, there is still much to discover. In turn, as an essential element for all living bodies, iron can also affect the life cycle of HCV. Nearly all studies so far confirm the positive role of iron on HCV translation, while the role of iron in promoting or suppressing HCV replication is still unclear. More work is needed to understand this, and the mechanisms by which iron affects HCV life cycle. Excessive iron can produce hydroxyl radicals and other ROS, further inducing organ dysfunction. Iron chelation therapy can reverse the negative effect of iron overload, which offers important information for clinical application.

Financial support and sponsorship

This work was supported by grants from the 215 Project of Beijing Municipal Commission of Health and Family Planning (No. 303-01-005-0068) and the Basic-clinic Cooperative Foundation of Capital Medical University (No. 15JL-L07).

Conflicts of interest

There are no conflicts of interest.

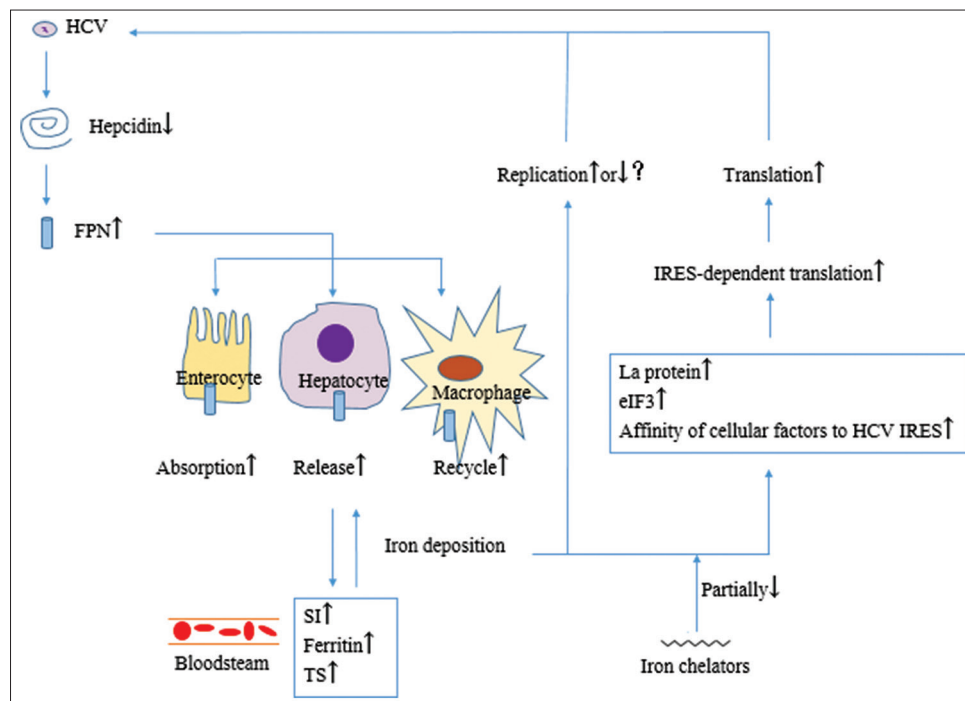


Figure 1: Relationship between HCV infection and iron overload. HCV reduces the levels of hepcidin, and elevates the levels of FPN, resulting in elevated iron absorbed by enterocytes and iron deposition in hepatocytes and macrophages. Although the role of iron in HCV replication is disputable, it is strongly suggested the positive effect of iron on HCV translation by increasing expression of both eIF3 and La protein, and changing the affinities of common cellular factors to HCV IRES. This positive effect can be partially inhibited by iron chelators. HCV: Hepatitis C virus; FPN: Ferroportin; SI: Serum iron; TS: Transferrin saturation; IRES: Internal ribosome entry site; eIF3: Eukaryotic initiation factor 3; ↑: Upregulated; ↓: Downregulated.

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