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REVIEW ARTICLE

The effect of iron depletion on chronic hepatitis C virus infection

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Abstract Increasing evidence exists that iron overload, a common finding in chronic hepatitis C virus (HCV) infection, plays an important role in the pathophysiology of this disease. The mechanisms by which iron excess induces liver damage along with the benefit of iron depletion via phlebotomy on biochemical and histological outcomes in patients with chronic HCV infection have been discussed in this review. Finally, we focus on the effect of iron reduction on the rate of response to interferon antiviral therapy.

Keywords Iron · Phlebotomy · HCV · IFN · Therapy

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Introduction

Hepatitis C virus (HCV) infection, which affects nearly 2% of the human population, is a major cause of liver disease worldwide. Following acute HCV infection, a chronic state is established in as many as 80% of infected individuals. Although many subjects carrying the virus remain asymptomatic, chronicity is often accompanied by altered liver function and progressive liver disease and culminates in cirrhosis or hepatocellular carcinoma in up to 20% of infected individuals [1].

Mild-to-moderate iron overload is a common finding among patients with chronic HCV infection; indeed, up to 30–40% of them may show increased serum transferrin-iron saturation and serum ferritin or increased hepatic iron concentration [2, 3]. On the other hand, elevated iron indices have been correlated with a progression of the liver disease and a decreased response to antiviral therapy [4–9].

The association between iron overload and chronic HCV infection, along with the effect of iron depletion on the course of chronic HCV infection and the response to antiviral therapy, has been addressed in this review.

Sources

We first performed an electronic search on MEDLINE, EMBASE, SCOPUS, and OVID databases without temporal limits using different combinations of the following keywords: "phlebotomy," "iron depletion," "iron overload," "iron reduction," "hepatitis C virus infection," "HCV," "chronic hepatitis C," "antiviral therapy," "interferon," and "ribavirin." In addition, the bibliographic references of all retrieved studies and reviews were assessed for additional reports of clinical trials. Only full-

text articles published in English were considered in this review.

Iron overload and chronic HCV infection

The mechanism of hepatotoxicity of iron accumulation in chronic HCV infection is still unclear. The deposition of iron predominantly within Kupffer cells and portal macrophages, the correlation between hepatic necroinflammatory activity and iron accumulation, and the reduction of hepatic iron content following response to interferon (IFN) therapy suggest that hepatic iron overload is the result of hepatocyte necrosis, which leads to release of ferritin from hepatocytes and subsequent uptake by macrophages [10–12]. However, another possible mechanism to explain the elevated iron stores could be the increased intestinal iron absorption. Indeed, recent investigations have found decreased levels of hepcidin (a peptide hormone produced in the liver that has an inhibitory effect on iron absorption) and increased levels of transferrin receptor 2 (which is located on the hepatocyte membrane and is involved in the uptake of iron by hepatocytes) in chronic HCV infection [13]. Thus, the resulting effect of these abnormalities could be an increased delivery of iron to hepatocytes from macrophage iron stores and intestinal mucosa.

Excess iron increases the formation of reactive oxygen species leading to lipid peroxidation, damage to protein and DNA, and thereby to cell membranes and genomic damage. Reactive oxygen species, which include hydroxyl radicals, may cause hepatic stellate cell activation and proliferation and upregulate synthesis of smooth muscle actin and collagen, thus contributing to hepatic fibrogenesis [11, 14, 15]. In vitro studies also suggest that iron deposition in hepatocytes enhances HCV replication, thus facilitating the viral infection in the liver [16]. Moreover, these hydroxyl radicals are known to generate promutagenic bases, such as 8-hydroxy-2′-deoxyguanosine (8-OHdG), which have been implicated in spontaneous DNA mutagenesis and carcinogenesis [12].

Finally, hemochromatosis gene mutations could play a role in the pathogenesis of iron overload among patients with chronic HCV infection [17]. Indeed, after the initial observation by Smith and colleagues [18] that HCV-infected patients who were carriers of C282Y mutation had higher serum ferritin levels than patients homozygous for the wild type of the *HFE* gene, several other reports have found that heterozygous HFE mutations are more frequent and are associated with increased iron storage among patients with chronic HCV infection [19–24]. However, studies examining the relationship between HFE mutations and disease progression in chronic HCV infection have given conflicting results. Indeed, while some studies have found a positive correlation between HFE mutations and the severity of liver disease [19, 22, 25–30], others failed to found such association [31–36]. Methodological- or population-based differences among studies could account for these discrepant findings [37]. For instance, Tung and colleagues [19] studied the liver histology in 316 patients with chronic HCV infection at various stages and found that the presence of HFE mutations was independently associated with iron loading and advanced fibrosis in patients with compensated liver disease. In contrast, Thorburn and colleagues [31] performed liver biopsies in 164 chronically HCV-infected patients and observed that the carriage of HFE mutation did not have a role in the iron accumulation or the progression of liver disease.

The effect of iron depletion on liver status in chronic HCV infection

Several groups have evaluated the effects of iron reduction on chronic HCV infection [38–46]. The majority of the studies performed phlebotomies of 400–500 ml of whole blood every 1 or 2 weeks until the development of an iron-deficient anemia. Hayashi and colleagues [39] first reported that iron reduction performed by repeated venesection led to normalization of serum alanine aminotransferase (ALT) levels in 5 of the 10 patients. However, serum ALT levels significantly decreased in all patients (from 152 ± 49 to 55 ± 32 U/L, $P < 0.001$). According to a report by Piperno and colleagues [41], serum ALT levels significantly improved in 32 iron-depleted patients with chronic HCV infection. Similar results were subsequently reported by other groups with phlebotomy alone [42–45]. However, no significant reduction in serum HCV RNA levels was observed [42, 46]. The long-term effect of phlebotomy on biochemical and histological parameters of chronic HCV infection was addressed by Yano and colleagues in 25 patients undergoing a 5-year maintenance phlebotomy program [47]. Interestingly, the authors found that the mean serum ALT levels decreased significantly during the initial phlebotomy program (from 117 to 75 U/l, $P < 0.05$) and this improvement persisted during the study period. Furthermore, phlebotomies were able to prevent the progression of liver histology because the severity of liver fibrosis (staging score) decreased from 2.3 to 1.7 ($P < 0.05$) in the iron-reduction group, whereas the mean values increased from 1.7 at baseline to 2.0 at the end of follow-up in controls ($P =$ NS). Likewise, the severity of inflammation (grading score) remained unchanged in the study group (1.8 vs. 2.0, $P =$ NS) but progressed in the control group (2.0 vs. 2.9, $P < 0.005$). Thus, the authors concluded that long-term maintenance of iron depletion is a safe and effective alternative to IFN treatment and could be

particularly indicated for those patients who do not respond to antiviral therapy or cannot tolerate such drugs.

Recently, Alexander and colleagues [48] found that iron depletion was associated with a biochemical response in 22% of patients who did not respond to IFN monotherapy and that, among patients with serum ALT normalization, there was a significant reduction of serum markers of liver fibrosis (procollagen III peptide). Kaito and colleagues [49] found that iron-reduction therapy by phlebotomy significantly reduced lipid peroxidation and oxidative stress, which mediate the deleterious effect of iron overload on the liver.

Finally, other groups have demonstrated that the association of a low-iron diet to phlebotomy has an additional effect in removing iron-induced oxidative stress [50, 51]. Indeed, in a study conducted by Kato and colleagues [51], 34 patients with chronic HCV infection unresponsive to IFN therapy were maintained in an iron-depleted state with phlebotomy and a low-iron diet for 6 years. This therapy was associated with a high rate of biochemical response (65%), improvement in liver histology, and reduction in hepatic levels of 8-OHdG, a marker of oxidant stress. In a recent cohort study, the same authors demonstrated that long-term phlebotomy with a low-iron diet therapy reduced the risk of progression of chronic HCV infection to hepatocellular carcinoma [52].

Table 1 summarizes the results of the most important studies on the effect of iron depletion by phlebotomy on chronic HCV infection.

The effect of iron depletion on response to antiviral therapy

Based on the findings that iron overload has been associated with more advanced liver disease and that increased hepatic iron concentration is predictive of a poor response to IFN monotherapy [4, 9], some investigators have evaluated the impact of phlebotomy on the response to IFN therapy in chronic HCV infection [38, 41, 46, 53–60]. In a study on 81 patients with chronic HCV infection, Piperno and colleagues [41] found that iron depletion by phlebotomy did not improve the response to IFN-α in both naïve and nonresponder patients. Similarly, Herrera [46] and Guyader and colleagues [53] concluded that iron depletion was not effective in inducing a virologic response in patients with chronic HCV infection who failed to respond to IFN therapy. In a multicenter, prospective, randomized controlled trial on 96 patients with chronic HCV infection who were nonresponders to IFN, Di Bisceglie and colleagues [55] compared iron reduction by phlebotomy with iron reduction plus retreatment with IFN and concluded that although prior phlebotomy therapy did not improve the rate of sustained virologic response to IFN retreatment, it resulted both in a decrease of serum ALT levels and in a slight improvement in liver histopathology. In contrast, Tsai and colleagues [54] observed that IFN retreatment preceded by iron depletion rescued 15% of previously nonresponder patients. In a multicenter study, Fargion and colleagues [56] randomized 114 previously untreated patients with chronic HCV infection to receive IFN alone or phlebotomy followed by IFN therapy and found that iron removal improved the rate of response to IFN. In fact, patients in the combination group (phlebotomy followed by IFN) had a 2.32 odds ratio (95% confidence interval = 0.96–6.24) of obtaining a sustained virologic response in comparison with patients in the IFN-alone treatment group. Similarly, the reduction of necroinflammatory activity and the improvement of the response to IFN was observed by Carlo et al. [57] in the group of patients who underwent phlebotomy before antiviral therapy.

Three randomized controlled studies compared the combination of iron depletion plus IFN with IFN alone [57–59]. Van Thiel and colleagues [58] showed that the combination treatment was effective in previous IFN nonresponders (60% of sustained virologic response in the combination group compared with 13% in the IFN-alone group). However, the intensified IFN regimen used in this

Table 1 Summary of the most important studies on the effect of iron depletion by phlebotomy on chronic hepatitis C virus infection

Author [ref]	Number of patients	Characteristics	Results
Hayashi et al. [40]	40	40 naïve	Phlebotomy significantly reduced mean ALT levels
Sartori et al. [42]	24	12 IFN NR, 12 naïve	Reduction of ALT levels and inflammatory grading score, suppression of the progression of staging score for fibrosis
Yano et al. [43]	33	NI	Phlebotomy significantly reduced ALT
Tanaka et al. [44]	22	NI	Phlebotomy significantly reduced ALT, AST, and α-fetoprotein levels
Yano et al. [47]	25	22 IFN NR, 3 naïve	Reduction of ALT levels, improvement of liver inflammation, and suppression of the progression of liver fibrosis
Alexander et al. [48]	18	18 NR	Biochemical response was accompanied by a reduction of markers of fibrogenesis

Abbreviations: IFN, interferon; NR, nonresponders; NI, not indicated; ALT, alanine aminotransferase; AST, aspartate aminotransferase

Table 2 Summary of the most important studies on the effect of iron depletion by phlebotomy on the response to IFN therapy in chronic hepatitis C virus infection

Author [ref]	Number of patients	Characteristics	Results
(a) Iron depletion before IFN therapy			
Piperno et al. [41]	16	8 IFN NR, 8 naïve	Iron depletion decreased ALT levels but did not improve SVR to IFN
Herrera [46]	28	28 IFN NR	No patient achieved an SVR
Guyader et al. [53]	16	16 IFN NR	Despite a significant effect on serum ALT levels, no effect on viremia was observed
Tsai et al. [54]	20	20 IFN NR	IFN retreatment preceded by iron depletion produced a 15% of SBR and SVR
Di Bisceglie et al. [55]	96	96 IFN NR	No patient achieved an SVR
Fargion et al. [56]	114	114 naïve	Iron removal by phlebotomy improved the rate of SVR to IFN
Carlo et al. [57]	83	83 IFN naïve	Iron removal by phlebotomy improved serum ALT levels and the rate of SVR to IFN
(b) Iron depletion plus IFN			
Van Thiel et al. [58]	30	30 IFN NR	Combination therapy increased SVR (60% vs. 13% in the IFN-alone group)
Fong et al. [59]	38	38 IFN naïve	Combination therapy increased SVR (29% vs. 5% in the IFN-alone group)
Fontana et al. [60]	82	82 IFN naïve	Combination therapy improved virologic and histological response to IFN

Abbreviations: IFN, interferon; NR, nonresponders; ALT, alanine aminotransferase; SBR, sustained biochemical response; SVR, sustained viral response

study (5 million units daily) could account for the high rate of virologic response observed. Fong and colleagues [59] observed a higher rate of sustained virologic responses in naïve patients treated with phlebotomy before and during IFN treatment than those receiving IFN alone (29% vs. 5%). In a similar trial, Fontana and colleagues [60] showed that iron reduction via therapeutic phlebotomy improved the virologic and histologic response to IFN therapy. Recently, Desai et al. [61] performed a meta-analysis of six prospective randomized controlled trials and concluded that phlebotomy improves the response to IFN in patients with chronic HCV infection. Table 2 summarizes the most important studies analyzing the effect of iron depletion and IFN in the treatment of chronic HCV infection.

Conclusions

On the whole, the literature data suggest that iron depletion via phlebotomy improves biochemical and histological outcomes in patients with chronic HCV infection. In addition, a number of studies have documented that combining iron depletion with IFN monotherapy may improve the rate of virologic responses in previously untreated and nonresponder patients.

Of note, recent investigations have found that hepatic iron concentration does not influence the response to antiviral therapy with IFN plus ribavirin [62, 63]. In addition, an iron-deficiency anemia due to repeated phlebotomies could be a risk factor for unsuccessful outcome of the antiviral treatment because of dose reduction of ribavirin. Thus, it will be interesting to see the results of future randomized controlled trials assessing the efficacy of combining iron-depletion therapy with pegylated IFN and ribavirin in chronic HCV infection.

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