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Is there any association between HCV multiplication and iron induced liver injury in chronic hepatitis C?

To the Editor:

We were very interested in the excellent paper by Fillebeen *et al.* published in a recent issue of the Journal of Hepatology [1]. The paper presents unexpected results on the role of iron in hepatitis C virus (HCV) replication. The authors confirm the results of a previous publication and show, in *in vitro* experiments, the iron-mediated inhibition of HCV replication.

These results are surprising and are not consistent with those of earlier studies in which iron was implicated in the upregulation of HCV translation [2,3] or the enhancement of HCV replication [4]. These results are also intriguing in the context of clinical observations of patients with chronic hepatitis C (CHC). It is rather difficult to determine a simple correlation between the iron-induced inhibition of HCV replication and the less favourable prognosis of CHC patients with iron overload [5].

The attempt to apply the results of the experimental models to clinical situations, according to some selected reports, is quite risky. Fillebeen *et al.* cited three previous reports showing the association of *HFE* gene mutations, an etiologic agent of HFE hemochromatosis, with efficacy of antiviral treatment. Perhaps, they selected these papers because of the possible associations between iron concentration, intensity of HCV multiplication, and viral load, which is a known strong predictor of SVR.

Distante *et al.* found that C282Y mutations (2 C282Y homozygotes and 31 C282Y heterozygotes) were independent predictors of SVR but this effect was probably caused by a lack of HFE phenotypic expression and by factors that were associated with the virus. In their study, carriers of C282Y mutations did not show any significant increase in serum concentrations of ferritin nor increased transferrin saturation compared to patients with wild type *HFE*. Moreover, the authors stated that serum ferritin concentration was predictive of non-response to interferon and ribavirin therapy [6]. Based on our own observations, two out of 152 CHC patients with iron overload were homozygous for the C282Y mutation and died at the age of 40, with rapidly progressive liver failure due to liver cirrhosis and hepatocellular carcinoma, after unsuccessful antiviral therapies. Lebray *et al.* did not confirm that the H63D mutation was a significant predictor of SVR ($p = 0.089$) [7]. In a large study by Bonkovsky *et al.*, iron and *HFE* gene mutations were analysed in relation to PEG-IFN therapy in advanced CHC patients. A possible positive influence of *HFE* mutations on the effectiveness of antiviral therapy was found, especially with the H63D mutation, but only in patients without confirmed hereditary hemochromatosis or iron overload based on a liver biopsy. Moreover, the presence of iron deposits in hepatocytes did not affect the rate of SVR [8].

In contrast, in our study, we found that treatment failure was positively correlated with elevated iron serum concentration and the presence of *HFE* gene mutations, in 61 out of 152 CHC patients treated with PEG-IFN plus ribavirin. However, *HFE* gene mutations did not appear to be the main factor responsible for iron overload in CHC [9]. In conclusion, additional experiments on cellular models and clinical studies are required to elucidate the impact of iron on HCV replication in terms of the pathogenesis of acute and chronic HCV infection and to explain the differences between clinical and experimental observations.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Reply to: Is there any association between HCV multiplication and iron induced liver injury in chronic hepatitis C?

This is a reply to the letter to the Editor by Sikorska et al.:
We appreciate the interest of Sikorska *et al.* in our recent study [1] and would like to comment on the issues raised in their letter. We fully agree that, in light of clinical literature and earlier biochemical evidence, the iron-mediated inhibition of HCV replication is surprising. This matter has been extensively discussed in a previous related publication [2], which established that iron attenuates the expression of subgenomic HCV replicons in culture and blocks the enzymatic activity of recombinant HCV polymerase NS5B *in vitro*. Our recent study validated the antiviral activity of iron in a more physiologically relevant setting of infectious HCV in permissive Huh7.5.1 cells. Certainly, HCV replicon models do not recapitulate the entire range of molecular responses triggered in the host during acute infection with HCV and progression to CHC; nevertheless, they provide a valuable framework to elucidate important aspects of HCV biology in a reductionist approach. The employment of such models uncovered that iron inhibits HCV replication. We speculated that this surprising finding may account for some unexpected clinical observations, where HCV-infected patients with mutations in the *HFE* gene responded better to antiviral therapy [3–5].

Sikorska *et al.* challenge this idea by arguing that it is merely supported by selected reports. We believe that published clinical literature qualifies to accommodate hypotheses arising from biochemical data. Moreover, we wish to emphasise that an increased frequency of *HFE* gene mutations in HCV patients who are responders to antiviral therapy has been documented in major clinical studies (reviewed in [6]). Thus, in a cohort of 256 CHC patients, the presence of *HFE* C282Y mutation positively correlated with sustained response in multivariate analysis ($p = 0.012$) [3]. Likewise, CHC patients with *HFE* H63D mutation showed a significant improvement in both their primary response and sustained response to interferon, although statistical significance for the latter did not reach the level of 0.05 [5]. These data suggested that *HFE* may constitute part of a battery of host genes that affect responses to antiviral therapy. Similarly, in the largest study thus far, on the effects of *HFE*-related iron overload in anti-HCV therapy (HALT-C trial) that included 1051 patients, Bonkovsky *et al.* found that subjects harboring *HFE* mutations, particularly H63D, had significantly higher likelihood of both end-of-treatment virological response ($p = 0.0078$) and sustained virological response ($p = 0.009$) to re-treatment with pegylated interferon alpha-2α plus ribavirin [4]. Again, both the *HFE* mutation and/or associated genetic variants were considered as possible causes of the improved response to therapy.

The iron-dependent inhibition of HCV replication documented in our studies is consistent with the above clinical findings and it is tempting to speculate that even minimal *HFE*-related hepatic iron overload may contribute to viral clearance in HCV-infected patients subjected to antiviral treatment. There is no doubt that this hypothesis requires further validation in animal models of HCV infection. It should also be noted that the adverse effects of iron overload in the liver and in the immune system preclude any exploitation of the iron-dependent inhibition of HCV replication for therapeutic purposes.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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