Dual chronic hepatitis B virus and hepatitis C virus infection

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Abstract Dual hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are common in HBV or HCV endemic areas. However, several clinical and pathogenetic issues remain unresolved. First, clinical and in vitro studies suggest the interactions between two viruses. The dynamics of the interaction in untreated setting versus treated setting and its influence on the long-term outcomes await further studies. A key issue regarding viral interactions is whether modulation of infection occurs in the same dually infected individual hepatocyte of the liver. Clarifying this issue may help to understand the reciprocal interference between HCV and HBV and provide clues for future immunopathogenetic studies. Second, the prevalence and clinical significance of coexisting occult HBV infection in patients with chronic HCV infection need further investigations. Third, combination therapy of peginterferon alfa-2a and ribavirin appears to be just as effective and safe for the treatment of hepatitis B surface antigen (HBsAg)-positive patients chronically infected with active chronic hepatitis C as it is in patients with HCV mono-infection. Nevertheless, one-third of dually infected patients with nondetectable serum HBV DNA-level pretreatment developed HBV reactivation posttreatment. How to prevent and treat this reactivation should be clarified. Furthermore,

about 10% of the dually infected patients lost HBsAg. Underlying mechanisms await further investigations. Finally, the optimal treatment strategies for dually infected patients with hepatitis B e antigen-positive chronic hepatitis B should be identified in future clinical trials.

Keywords Dual infection · Hepatitis B virus · Hepatitis C virus · Interferon · Pegylated interferon · Ribavirin · Sustained virologic response · HBsAg clearance

Introduction

Around the world, most hepatitis C patients suffer from a chronic hepatitis C virus (HCV) mono-infection. For example, in northern Europe where the hepatitis B surface antigen (HBsAg) carriage rate is low (<1%), patients dually infected with hepatitis B virus (HBV) and HCV are few [1]. However, in areas where HBV infection is endemic such as Southeast Asia, the Far East, and southern Europe, a substantial number of patients are infected with both HBV and HCV (Table 1) [1–16]. Worldwide, if the prevalence of anti-HCV positivity is around 1–2% in the general population, then the number of individuals with HCV–HBV dual infection will be at least 3.5–7 million among the 350 million HBV carriers. However, the worldwide prevalence of dual HBV–HCV infection is very likely underestimated because occult HBV infection is often not taken into account.

In patients with dual chronic hepatitis B and C infection, the clinical presentations and disease outcomes are usually more severe than in patients with single hepatotropic virus infection [1, 2, 17, 18]. For example, a study from Taiwan revealed that the prevalence of anti-HCV was 8% in patients with chronic hepatitis B infection, 10% in patients

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Table 1 Epidemiology of hepatitis B and C dual infection in different countries

	HBsAg positive rate	Estimated prevalence of HBV–HCV dual infection	Comments
<i>HBV high endemic area</i>			
Taiwan [2, 3]	10–20%	0.26–2.4%	2.6–12% of HBsAg carriers also positive for anti-HCV
China [4–6]	5.3–12%	0.6–1.74%	11.4–14.5% of HBsAg carriers also positive for anti-HCV
Italy [7, 8]	0.9–13.6%	0.04–0.95%	4.3–7% of HBsAg carriers also positive for anti-HCV
Thailand [4, 9]	4.6–8%	0.12–0.22%	2.7% of HBsAg carriers also positive for anti-HCV
<i>HBV intermediate endemic area</i>			
Japan [10]	1–5%	Unclear	1–2% of patients with chronic hepatitis positive for both anti-HCV and HBsAg
Spain [4, 11]	1.2%	0.16%	13% of HBsAg carriers also positive for anti-HCV
Brazil [12]	0.1–5.2%	0.1% (donor population)	0.1% of blood donors positive for both HBsAg and anti-HCV
India [13]	1–5%	0.03–0.15%	3% of patients with HBV-related chronic liver disease also positive for anti-HCV
<i>HBV low endemic area</i>			
United States [14]	0.7–2.1%	0.09–0.29%	14% of HBsAg carriers also positive for anti-HCV
	Anti-HCV positive rate	Estimated prevalence of HBV–HCV dual infection	Comments
<i>HCV high endemic area</i>			
Egypt [15, 16]	15%	Unclear	5% of community-based population positive for both anti-HCV and HBsAg and/or anti-HBc

with HBV-related liver cirrhosis, and increased to 17% in patients with HBV-related hepatocellular carcinoma (HCC) [2]. A meta-analysis suggested a synergistic effect of HBV and HCV infection in causing HCC [19]. Supplementing these cross-sectional data, long-term follow-up studies demonstrated the combined effect of HCV and HBV infection in the progression of chronic liver disease [18]. Taken together, most studies have provided substantial evidence to support that dual HBV–HCV chronic infection increases the risk of fulminant hepatic failure, the progression of liver disease, and the development of HCC. Therefore, patients dually infected with hepatitis C and B need attention from the medical profession and require effective treatment. Data from recent clinical trials suggest that pegylated interferon (Peg-IFN) alfa plus ribavirin is effective in the clearance of HCV in dually infected patients with active hepatitis C [20, 21], though optimal treatment strategy for dually infected patients with active hepatitis B is still unknown.

From the virologic point of view, clinical observations and in vitro studies have shown that both HBV and HCV could interact with each other and possibly affect the host immune responses. Then, a key issue in dual infection would be whether the modulation of infection occurs in the same dually infected individual hepatocyte of the liver. If dual infection does not naturally occur in individual hepatocyte, then the influence of replication of either virus is more likely a result of indirect modulation of distinct HBV- or HCV-infected cell populations in proximity to each other [22].

This review article summarizes current status and points out these unresolved issues.

Viral interactions between HBV and HCV and their impact on the natural and treatment outcomes

Hepatitis B virus and hepatitis C virus may interact with each other [23]. Clinical observations in HBV endemic

areas showed that most of the HBV–HCV dually infected cases have similar HCV RNA levels as in patients with HCV mono-infection but relatively low levels of serum HBV DNA compared with patients with chronic HBV mono-infection [24–28]. In patients with chronic hepatitis B along with HCV superinfection, hepatitis B e antigen (HBeAg) seroconversion and even HBsAg clearance have been documented [29, 30]. Acute HCV superinfection in chimpanzees with chronic HBV infection was found to result in a significant reduction of serum HBsAg. These data suggest that the interference between the two viruses is more frequently characterized by an inhibition of HBV exerted by HCV. These observations may also explain the phenomenon why occult HBV infection is frequently found in patients with chronic hepatitis B superimposed by acute HCV infection in HBV endemic areas. In vitro studies consistently revealed that HCV is capable of suppressing HBV replication, and this inhibitory effect is possibly mediated by HCV core protein acting on HBV enhancer activity [31–34]. Nevertheless, conflicting results with respect to the effects of HCV core and NS5A proteins on the level of HBV replication have been reported [34–37].

Clinical studies do not universally demonstrate a dominant role of HCV in patients with dual HBV–HCV infection [25, 38–40]. HCV RNA levels were interestingly found to be significantly decreased in some HBV–HCV dually infected patients with detectable serum HBV DNA as compared with HBV DNA-negative cases [38]. Another study showed that dually infected patients had a significantly higher rate of HCV RNA clearance than those with HCV mono-infection [41]. Thus, a dominant effect of HBV can indeed be found in a proportion of the dually infected patients.

Using a novel in vitro system, a recent study well demonstrated the interaction between HBV and HCV [42]. In this model, stable Huh-7 cell lines inducibly replicating HBV were transfected with selectable HCV replicons or infected with cell culture-derived HCV. Both viruses were found to replicate in the same cell without overt interference. Specific inhibition of one virus did not affect the replication and gene expression of the other. Furthermore, cells harboring replicating HBV could be infected with cell culture-derived HCV, arguing against superinfection exclusion. Finally, cells harboring replicating HBV supported efficient production of infectious HCV. On the basis of these findings, the viral interference observed in dually infected patients is probably due to indirect mechanisms mediated by innate and/or adaptive host immune responses. These findings provide new insights into the pathogenesis of HBV–HCV dual infection and suggest that future researches should be focused on immunologic aspects.

Patients with dual HBV–HCV infection may exhibit a wide spectrum of virologic profiles. Although most dually

infected patients appear to have both active HCV and inactive HBV replication, some patients indeed have high HBV DNA levels and undetectable HCV RNA. Because most available data are based on cross-sectional observations, the possibility cannot be ruled out that HBV and HCV can alternate their dominance during different periods of coinfection. A multicenter longitudinal follow-up study in Italy indeed revealed that patterns of virologic dominance in these cases vary and change over time [43]. This study enrolled 133 untreated HBV–HCV dually infected patients. Active infection with both HBV and HCV was revealed in 24 cases, inactive infection by both the viruses was seen in 15 cases, active HBV/inactive HCV was seen in 15 cases, and inactive HBV/active HCV was seen in 49 cases. During 1-year prospective follow up, 32 subjects (31%) presented dynamic virologic profiles characterized by the fluctuation of HBV and/or HCV viremia levels at different time points. In summary, this study showed that the virologic patterns in HBV–HCV dual infection are widely divergent and have dynamic profiles. A careful longitudinal evaluation of the viremia levels of both viruses is essential for making a correct diagnosis. This phenomenon should be taken into consideration before determining the dominance of viral infection and designing therapeutic strategies in dually infected patients.

Hepatitis B and hepatitis C viruses are hepatotropic viruses whose primary replication occurs in the liver; however, significant differences exist between these two viruses regarding the immune response after acute infection and the underlying mechanism that precipitates liver injury. In the chimpanzee model of acute infection, it was found that HBV is a stealth virus that establishes the infection very efficiently without altering the innate immune system to its presence, although it is readily controlled when the adaptive immune response is induced [44]. In contrast, HCV strongly induces yet cunningly evades the innate response and also defeats the adaptive immune response by mutation and functional inactivation. When two viruses exist in the same liver, the situation may be even more complex. A key issue regarding viral interactions is whether the influence of infection occurs in the same dually infected individual hepatocyte of the liver [22]. If HBV and HCV infect the same hepatocyte, then HCV structure or functional proteins may directly influence the replication of HBV and the expression of HBsAg, or vice versa. Alternatively, if dual infection does not naturally occur in the same individual hepatocyte, then the modification of the replication of either virus is more likely a result of paracrine and autocrine modulation of distinct HBV- or HCV-infected cell populations in proximity to each other. Clarifying this issue may help to understand the reciprocal interference between HCV and HBV and provide clues for future immunopathogenetic studies.

Prevalence and clinical significance of occult HBV infection in patients with chronic HCV infection

Occult HBV infection can be defined by the presence of HBV DNA in the liver and/or serum of individuals who are tested negative for HBsAg by currently available assays [45]. It is not common in general population even in HBV endemic areas. A recent study from Taiwan suggested that the prevalence of occult HBV infection was about 0.11% among healthy blood donors with a clean, automatic, and most sensitive nucleic acid amplification assay [46]. In contrast, occult HBV infection has been more frequently identified in patients with chronic HCV infection. The prevalence of occult HBV infection among HCV-infected persons has been detected in 11–50% of patients in previous series [47–49].

Current evidence suggested that occult HBV infection might negatively influence the clinical outcome of chronic HCV infection and contribute to the development of liver cirrhosis and HCC [50–53]. Regarding this aspect, occult HBV infection retains several of the oncogenic mechanisms of HBV such as the capacity to be integrated into the host's genome and the production of transforming proteins. However, the detailed underlying molecular pathogenesis remains to be clarified. Furthermore, prospective studies using standardized assay for occult HBV and well-defined selection criteria for patients with occult HBV infection are needed to address this issue.

Occult HBV infection might also affect the response to anti-viral therapy in patients with chronic HCV infection. However, currently available data do not allow any conclusion regarding this aspect, partly because of the heterogeneity of the patients enrolled and the lack of data regarding the current standard-of-care treatment, i.e., Peg-IFN alfa-2a or -2b plus ribavirin combination therapy [45].

Treatment of HBV–HCV dual infection

Ideally, effective anti-viral therapy for dually infected patients should eradicate both the viruses. Alternatively, the treatment may be against the virus that is likely responsible for the liver injury in the majority of these patients or the one most likely responsive to anti-viral therapy. A previous study revealed that active hepatitis C can be found in around 70% of these patients [43]. Besides, HCV can be successfully eradicated in about 70% of the patients with chronic HCV monoinfection using current standard-of-care combination therapy [54–56]. Accordingly, HCV infection seems to be the priority target for treatment in dually infected patients with active hepatitis C.

Small studies showed that IFN alone was not effective in clearing HCV in dually infected patients [1, 57]. Combining IFN with ribavirin improved the response rates in patients monoinfected with HCV as well as in dually infected patients [58–61]. Nevertheless, sustained virologic response (SVR) rates were significantly lower in those dually infected with HCV genotype 1 than in those with genotype 2/3 infection (44 vs. 85%, $P < 0.05$).

In the treatment of chronic hepatitis C monoinfection, Peg-IFN was shown to be superior to conventional IFN [54] and combination with ribavirin became the standard of care due to further improvement of efficacy over conventional IFN plus ribavirin [55, 56]. We hypothesized that the efficacy of treatment of dually infected patients may also be enhanced through Peg-IFN plus ribavirin [61] and therefore conducted a large multicenter clinical trial in Taiwan [21]. A total of 321 eligible patients with active hepatitis C were enrolled. The study group comprised 161 patients with coexisting HBsAg, and 160 without HBsAg served as controls. Patients with HCV genotype 1 infection received 48-week Peg-IFN alfa-2a 180 µg weekly plus 1,000–1,200 mg of ribavirin daily. Patients with HCV genotype 2/3 received 24-week Peg-IFN alfa-2a weekly plus 800 mg of ribavirin daily. We found that for genotype 1 infection, HCV SVR was 72.2% in dually infected patients versus 77.3% in monoinfected patients. For genotype 2/3 infections, SVR was 82.8 and 84.0%, respectively.

The results proved our hypothesis that replacing conventional IFN with Peg-IFN in the combination therapy and extending the treatment from 24 to 48 weeks significantly improved the SVR rate of HCV genotype 1 from 44 to 72% in the dually infected patients. Contrary to previous reports [47], we found that a coexisting occult HBV infection did not influence the HCV SVR rate in short-term follow-up. In addition to our multicenter trial, a satisfactory HCV SVR rate was also documented in a series of 19 German patients with HBV–HCV dual infection by using Peg-IFN alfa-2b plus ribavirin [20].

These data indicated that in dually infected patients with predominant hepatitis C, the same genotype-dependent treatment recommendations for single chronic hepatitis C still hold true [62]. Nevertheless, the long-term hepatitis C SVR remains to be carefully followed. Besides, in retrospective analyses of randomized trials on chronic hepatitis C, the assessment of HCV RNA at 4 weeks (rapid virologic response, RVR) and 12 weeks (early virologic response, EVR) of treatment has been identified as a useful early predictor of SVR [56, 63]. The values of RVR and EVR in predicting response to anti-HCV therapy in dually infected patients also remain to be studied.

Virologic response of HBV to Peg-IFN and the potential risk for the reappearance of HBV DNA during treatment of coexisting chronic hepatitis C are 2 major clinical concerns

Table 2 Interferon-based therapy for HCV–HBV dually infected patients

Author	Subjects	Case no.	Treatment regimen	HCV SVR	HBV DNA disappearance	HBsAg loss	HBeAg loss	Biochemical response
<i>IFN monotherapy</i>								
Gehenot et al. [65]	Anti-HCV+	16	IFN alfa 3 MU TIW for 6 mo	NA	NA	13%	NA	19%
	HCV RNA+							
	HBsAg+							
	HBV DNA–							
Liaw et al. [66]	Anti-HCV+	15	IFN alfa 9 MU TIW for 14 wk or 4–6 MU TIW for 12 wk	0%	7% ^a	7%	7%	7%
	HBsAg+							
	HBeAg+							
	HBV DNA+							
Guptan et al. [67]	Anti-HCV+	7	IFN alfa 6 MU TIW for 6 mo	29%	86% ^a	29%	100%	0%
	HCV RNA+							
	HBsAg+							
	HBV DNA+							
Utili et al. [68]	Anti-HCV+	16	IFN alfa 5 MU TIW for 12 mo	44%	NA	NA	15%	50%
	HBsAg+							
Villa et al. [57]	Anti-HCV+	16	IFN alfa 9 MU TIW for 6 mo	31%	100% ^b	3%	NA	38%
	HCV RNA+							
	HBsAg+							
	HBeAg–							
<i>IFN plus RBV</i>								
Liu et al. [58]	Anti-HCV+	24	IFN alfa 6 MU TIW for 3 mo then 3 MU TIW for 3 mo + RBV for 6 mo	43%	35% ^c	0%	100%	43%
	HCV RNA+							
	HBsAg+							
	(HBeAg+ in 3)							
Hung et al. [60]	Anti-HCV+	36	IFN alfa 3–5 MU TIW for 6 mo + RBV for 6 mo	69%	11% ^c	0	0%	56%
	HCV RNA+							
	HBsAg+							
	(HBeAg+ in 1)							
Chuang et al. [59]	Anti-HCV+	42	IFN alfa 6 MU TIW for 6 mo + RBV for 6 mo	69%	31% ^c	14%	50%	55%
	HCV RNA+							
	HBsAg+							
	(HBeAg+ in 2)							

Table 2 continued

Author	Subjects	Case no.	Treatment regimen	HCV SVR	HBV DNA disappearance	HBsAg loss	HBeAg loss	Biochemical response
<i>Peg-IFN plus RBV</i>								
Liu et al. [21]	HCV genotype 1 Anti-HCV+ HCV RNA+ HBsAg+	97	Peg-IFN alfa-2a 180 mcg/wk for 12 mo + RBV for 12 mo	72%	58% ^d	12%	NA	62%
	HCV genotype 2/ 3 Anti-HCV+ HCV RNA+ HBsAg+	64	Peg-IFN alfa-2a 180 mcg/wk for 6 mo + RBV for 6 mo	83%	53% ^d	9%	NA	67%
Pothoff et al. [20]	Anti-HCV+ HCV RNA+ HBsAg+ (HBeAg + in 1)	19	Peg-IFN alfa-2b 1.5 mcg/kg/wk for 12 mo + RBV for 12 mo	74%	33% ^e	0%	0%	63%
<i>IFN plus lamivudine</i>								
Marrone et al. [64]	Anti-HCV+ HBsAg+ HBeAg+ HBV DNA+	8	IFN alfa 5 MU TIW for 12 mo + lamivudine for 18 mo	50%	38% ^c	0%	38%	50%

IFN interferon, RBV ribavirin, mo month, wk week, TIW thrice weekly, SVR sustained virologic response, HBsAg hepatitis B s antigen, HBeAg hepatitis B e antigen, NA not available

^a Commercial hybridization-based quantitative assay

^b Dot blot assay

^c Commercial quantitative assay (Cobas Amplicor HBV Monitor, Roche Diagnostics)

^d In-house real-time polymerase chain reaction (PCR) assay

^e Commercial quantitative real-time PCR assay (Cobas Amplicor, Roche Diagnostics)

that needed to be addressed in dually infected patients receiving effective anti-HCV therapy. We found that HBV virologic response was obtained in 56% of the dually infected patients with pretreatment hepatitis B viremia. Posttreatment HBsAg clearance was found in 18 (11.2%) of the 161 dually infected patients. It would be interesting to study the mechanism of HBsAg clearance in this unique cohort. On the other hand, 28 (36%) of the 77 dually infected patients whose pretreatment serum HBV DNA levels were undetectable experienced a reappearance of HBV DNA. Nevertheless, the reappearance of HBV did not result in clinically evident hepatitis, even if ALT levels were elevated in some patients. However, further follow up is needed to clarify the clinical significance of HBV DNA reappearance in patients with dual chronic HCV–HBV infection. In such cases, anti-HBV therapy can be implemented if hepatitis flares up.

Although our study shows that Peg-IFN alfa-2a plus ribavirin can be used for the treatment of active hepatitis C in patients coinfecting with HBV, it remains to be determined whether this approach is also suitable for dually infected patients with a dominant HBV infection who are HBeAg positive and are likely to have a higher hepatitis B viral load. In our pilot study, 3 of the 24 dually patients were positive for HBeAg [58]. All 3 patients lost HBeAg after treatment with 6-month conventional IFN plus ribavirin, and 1 even seroconverted to anti-HBe. Marrone et al. [64] treated 8 patients with dual chronic hepatitis C and HBeAg-positive chronic hepatitis B with 5 MU of IFN and lamivudine 100 mg/day for 12 months followed by lamivudine alone for 6 months. Three patients (37.5%) had loss of HBeAg, 3 (37.5%) had undetectable HBV DNA, and posttreatment alanine aminotransferase (ALT) levels normalized in 4 (50%) of the 8 treated patients. Four patients (50%) also had sustained clearance of HCV RNA at 12 months posttreatment. This case series suggests that the addition of lamivudine to IFN might be effective in dually infected patients with chronic hepatitis C and active HBV replication. However, the case number is too small to draw any definite conclusion. Alternatively, we may use Peg-IFN alfa, ribavirin, and nucleos(t)ide analog all together to treat both viruses, or use nucleos(t)ide analog alone to treat active hepatitis B if HCV RNA is undetectable. Nevertheless, these strategies should be tested in further large clinical trials.

In summary, combination therapy of Peg-IFN alfa and ribavirin appears to be just as effective for the treatment of HBsAg-positive patients chronically infected with active hepatitis C as it is in patients with HCV mono-infection (Table 2). The treatment recommendations regarding therapy duration according to genotype in HCV mono-infection appear to be applicable also for this patient group.

Summary and future directions

HBV–HCV dual infection is common in endemic areas and among subjects at risk of parenterally transmissible infections. Sensitive assays for both HBsAg and HBV DNA are needed to elucidate the prevalence and clinical significance of occult HBV infection in patients with HCV infection. How two viruses interact with each other in the same liver awaits further *in situ* and *in vitro* studies. Apart from academic interests, these data will help to develop more effective anti-viral therapy. Before the administration of anti-viral therapy, thorough serologic and virologic examinations are required to determine both the viral dominance and the optimal anti-viral regimen. For dually infected patients with predominant hepatitis C, the same genotype-dependent treatment recommendations for single chronic hepatitis C still hold true. However, for patients with active hepatitis B, more studies are needed to determine the optimal regimen to treat both viruses.

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