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Abstract

To evaluate viral interference between hepatitis B and C, we studied coinfected patients serologically and molecular biologically. Twenty-seven patients positive for hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus (HCV) antibody, were classified into Groups BC-L and BC-H according to DNA-polymerase activity (less or greater than 100 cpm, respectively). Patients with hepatitis B or C alone were also enrolled as controls. HCV-RNA was detected more often in Group BC-L than in Group BC-H. Genotype 1b of HCV was determined in 75% of Group BC-H, 87.5% of Group BC-L, and 70.7% of hepatitis C-only patients. Activity of DNA-polymerase in coinfected patients was lower in patients positive for HCV-RNA as compared with those negative. HBsAg titers tended to be lower in coinfected patients than in patients with hepatitis B virus (HBV) alone. In conclusion, in coinfection, HBV may suppress the replication of HCV and HCV appears to reduce the expression of HBsAg and probably suppresses HBV replication.

KEYWORDS: hepatitis B virus, hepatitis C virus, double infection, hepatitis B surface antigen, hepatitis C virusRNA

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To evaluate viral interference between hepatitis B and C, we studied coinfected patients serologically and molecular biologically. Twenty-seven patients positive for hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus (HCV) antibody, were classified into Groups BC-L and BC-H according to DNA-polymerase activity (less or greater than 100 cpm, respectively). Patients with hepatitis B or C alone were also enrolled as controls. HCV-RNA was detected more often in Group BC-L than in Group BC-H. Genotype 1b of HCV was determined in 75% of Group BC-H, 87.5% of Group BC-L, and 70.7% of hepatitis C-only patients. Activity of DNA-polymerase in coinfected patients was lower in patients positive for HCV-RNA as compared with those negative. HBsAg titers tended to be lower in coinfected patients than in patients with hepatitis B virus (HBV) alone. In conclusion, in coinfection, HBV may suppress the replication of HCV and HCV appears to reduce the expression of HBsAg and probably suppresses HBV replication.

Key words: hepatitis B virus, hepatitis C virus, double infection, hepatitis B surface antigen, hepatitis C virus-RNA

In areas where viral hepatitis is endemic, individuals may be infected by multiple hepatitis viruses. The suppressive influence of non-A, non-B hepatitis viruses on the replication of hepatitis A virus (HAV) and hepatitis B virus (HBV) has been suggested in transmission experiments in chimpanzees (1, 2). Since the diagnosis of hepatitis C virus (HCV) became possible (3, 4), double infection or superinfection of HBV and HCV has been reported by several groups (5-9) and by our group in Japan (10, 11). It has been suggested that HCV infection suppresses HBV-associated DNA polymerase (DNA-p) activity on HBV carriers who are positive for hepatitis B e antigen (HBeAg), leading to severe liver disease and removal of the hepatitis B surface antigen (HBsAg) (12-16). On the other hand, positivity and titer of anti-c100-3 antibody (Ab) in patients with coinfection of HBV and HCV, were higher in anti-HBe positive patients than in HBeAg positive patients (10). These results may indicate that there is interference in the replication of HBV and HCV. To clarify the relationship between HBV and HCV in double infection and whether superinfected HCV represses the replication of HBV to terminate progress of hepatitis B, we quantitatively examined viral markers in coinfected patients.

Patients and Methods

Patients. We studied 27 patients with chronic liver disease (CLD) positive for both HBsAg and anti-HCV Ab. All of them were negative for anti- hepatitis D virus (HDV) Ab (Table 1). Six patients with DNA-p activity greater than 100 cpm, were categorized as Group BC-H (high), and the remainder as Group BC-L (low). All patients of Group BC-H except one with pre-C mutant virus had HBeAg and all patients of Group BC-L had anti-HBe Ab. Twenty-one patients who were positive for HBsAg and anti-HBe Ab and who were negative for anti-HCV Ab, were randomly selected as Group B, matching the age and gender with Group BC-L. All patients in Group B were shown to be free of liver cirrhosis (LC) by ultrasonography and showed normal alanine aminotransferase (ALT) levels (< 40 IU/l) at

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Table 1 Patients with double infection of hepatitis B virus (HBV) and hapatitis C virus (HCV)

Patient	Age (Years)	Sex	AST/ALT (IU/l)	HBeAg	Anti-HBe	DNA-p (cpm)	HCV-RNA	Genotype	Diagnosis
Group BC-H									
1	40	M	418/673	+	−	1738	−	1b#	CAH2B
2	75	M	80/71	+	−	1019	−		CH*
3	82	M	42/35	+	−	678	+	2b	CH*
4	45	M	46/89	+	−	132	+	1b	CPH
5	70	F	55/17	+	−	118	−		LC*
6	33	M	47/74	−	+	1530	−	1b#	CAH2B
Group BC-L									
7	66	M	48/45	−	+	39	+	1b	HCC
8	34	M	28/45	−	+	6	+	2a	ASC*
9	38	F	41/39	−	+	2	+	1b	CAH2B
10	53	M	38/39	−	+	0	+	1b	LC*
11	67	F	33/31	−	+	0	+	2a	LC*
12	60	M	67/21	−	+	0	−		HCC
13	43	F	130/64	−	+	0	+	1b	HCC
14	69	F	72/46	−	+	0	+	1b	HCC
15	46	M	24/28	−	+	0	−		ASC*
16	62	F	145/103	−	+	0	−		HCC
17	45	F	11/7	−	+	0	−		ASC*
18	40	M	54/74	−	+	11	+	1b	LC
19	43	M	31/52	−	+	0	+	1b	CAH2B
20	61	M	196/205	−	+	0	+	1b	LC
21	59	M	27/28	−	+	0	+	1b	CAH2B
22	56	M	76/57	−	+	13	+	1b	HCC
23	55	M	64/88	−	+	5	+	1b	CAH2B
24	44	M	104/85	−	+	0	+	1b	LC*
25	46	M	69/97	−	+	31	+	1b	CH*
26	49	M	51/72	−	+	13	+	1b	CAH2B
27	55	M	77/74	−	+	12	+	1b	LC

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; HBeAg: Hepatitis B e antigen; DNA-p: DNA polymerase.
Group BC-H: Six patients with DNA-p activity greater than 100 cpm; Group BC-L: Twenty-one patients with DNA-p activity lower than 100 cpm.
#: Genotype was determined in samples positive for HCV-RNA taken at different times; *: Diagnosed clinically not histologically.
ASC: Asymptomatic carrier; CH: Chronic hepatitis; CPH: Chronic persistent hepatitis; CAH2B: Chronic active hepatitis with severe activity; LC: Liver cirrhosis; HCC: Hepatocellular carcinoma.

least for six months. One hundred sixteen patients with CLD who were positive for anti-HCV Ab and negative for HBsAg were also enrolled as Group C. None of the patients had serological markers that would indicate recent infection of HAV, cytomegalovirus, or Epstein-Barr virus, or a history of alcoholism or drug abuse. Eleven patients with chronic hepatitis were histologically classified (17). Six patients with hepatocellular carcinoma (HCC) were diagnosed by angiography, computed tomography, and magnetic resonance imaging, or specimens obtained by ultrasound-guided biopsy.

Methods. Sera from patients were stored at − 20 °C until analyzed. Commercially available radioimmunoassays (RIA) were used for the detection of HBsAg, anti-HBs Ab, HBeAg and anti-HBe Ab. HBsAg was semiquantified by ELISA (Hepanostica, Organon Teknika KK, Tokyo, Japan) and its optical density was measured by spectrophotometer (Photo-ELISA 1, Organon Teknika KK) at a wavelength of 492 nm (arbitrary unit, cutoff 0.04). DNA-p activity was determined by Kaplan's method (18). HBV-DNA were detected by the nested polymerase chain reaction (PCR) method (Otsuka Assay Laboratory, Tokushima, Japan).

Abs against HCV antigens, including 5-1-1, c100-3, c33c and c22-3, were determined by recombinant immunoblot assay (RIBA; Chiron RIBA-II Test, Ortho

Diagnostic Systems, Tokyo, Japan). Reverse transcriptase-PCR (RT-PCR) using primers to the 5' non-coding region of the HCV genome was carried out to detect HCV-RNA as described previously (19). Quantitation of serum HCV-RNA was performed using competitive RT-PCR as previously reported (20). RT-PCR with mixed type-specific primers against NS5 region was used to determine HCV genotype as previously reported (21). In this paper, the nomenclature for HCV genotype was that proposed by Simmonds *et al.* (22).

Statistical analyses. Values are described as (mean ± standard deviation) or (median, range). Chi-square test, two-tailed unpaired t-test, and Wilcoxon rank sum test were used for statistical analysis and $P < 0.05$ was considered to be statistically significant.

Results

HCV in coinfected patients. In this study, 13 (48 %) of 27 patients coinfected with HBV and HCV had advanced liver diseases such as LC and HCC (Table 1). The detection rate of HCV-RNA was lower in Group BC-H (33 %) than in Group BC-L (81 %) ($P < 0.05$ by chi-square test). The quantity of HCV-RNA tended to be lower in Group BC-L (6.9 ± 0.7, log copies/0.5 ml) than in Group C (7.4 ± 0.8, log copies/0.5 ml), but the difference was not statistically significant (Fig. 1). Distribution of genotypes in coinfected patients examined was as follows: type 1b, 86 %; type 2a, 10 %; and type 2b, 4 % (Table 1). Genotype 1b of HCV was determined in 3/4 (75 %) of Group BC-H, 14/16 (87.5 %) of Group BC-L, and 82/116 (70.7 %) of Group C. Regarding disease pathology, genotype 1b was detected in 8 (89 %) out of 9 patients with LC or HCC, and in 9 (82 %) out of 11 patients with chronic hepatitis or asymptomatic carriers.

Abs against several viral peptides of HCV were assayed utilizing RIBA. Anti-c100-3 Ab was detected in 2 members (33 %) of Group BC-H, and in 13 (62 %) of Group BC-L (the difference was not significant). Three patients (60 %) of Group BC-H and 12 (57 %) of Group BC-L were positive for anti-5-1-1 Ab. All patients (100 %) of Group BC-H and 19 patients (90 %) of Group BC-L were positive for anti-c33c Ab. All patients in both Groups had anti-c22-3 Ab.

HBV in coinfected patients. To determine whether the coinfection of HCV could influence the status of HBV, several viral markers of HBV were assayed. DNA-p activity more than 100 cpm were detected in 2 (11 %) of 19 patients seropositive for HCV-RNA and 4 (50

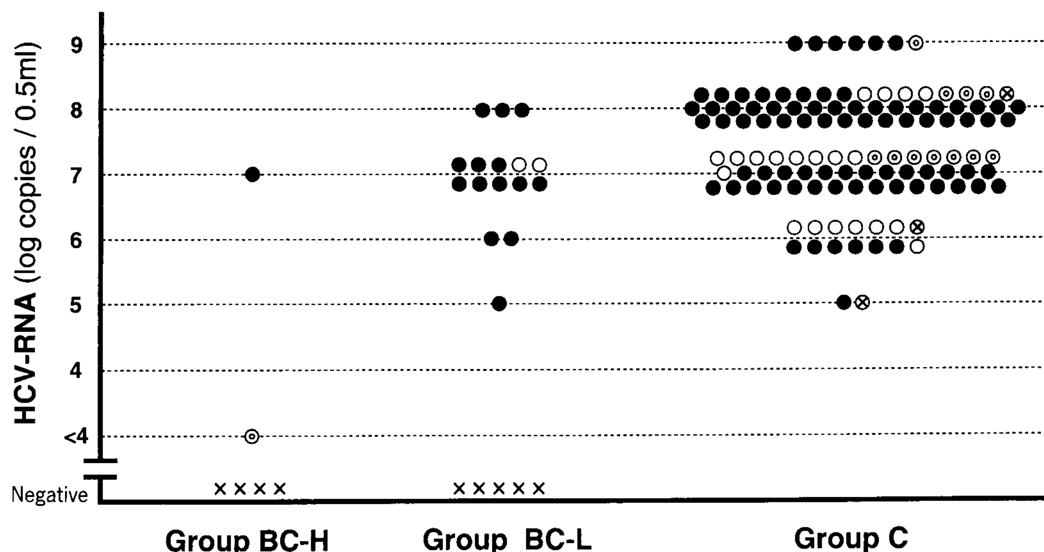

Fig. 1 Quantification and genotype of hepatitis C virus (HCV)-RNA in patients infected with HCV only and in patients coinfected with hepatitis B virus (HBV) and HCV. HCV-RNA was quantified by competitive reverse transcriptase polymerase chain reaction (RT-PCR) and genotype was determined by RT-PCR with mixed primer. Closed circle, type 1b; open circle, type 2a; double circle, type 2b; open circle with cross, mixed type. Cross indicates undetermined type.
Group BC-H; Group BC-L: See legend to Table 1.
Group C: One hundred sixteen patients with chronic liver disease who were positive for anti-HCV antibody and negative for HBsAg.

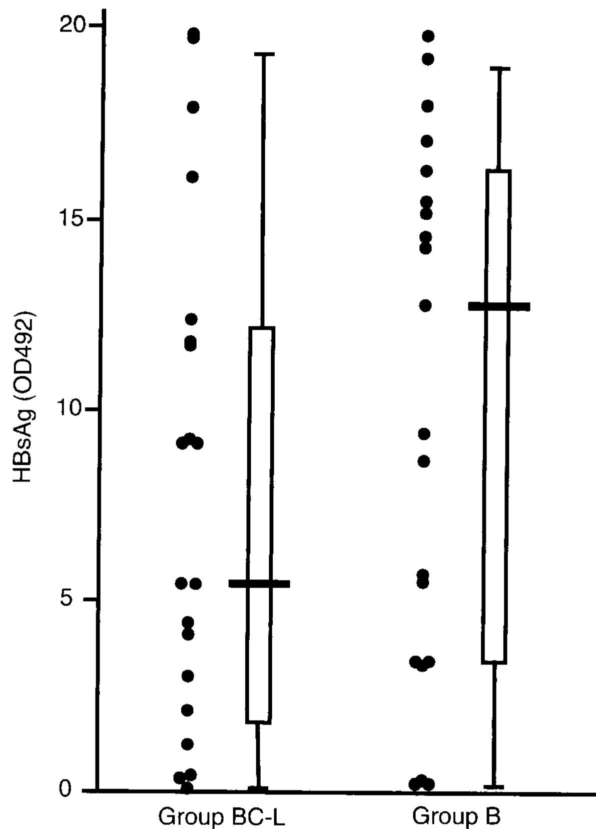

Fig. 2 HBsAg titer in Groups BC-L and B. HBsAg was semi-quantified by ELISA. Distribution of values in each group is described by box plot (thick bar, median; lower and upper side of box, 25 and 75 percentile; lower and upper bar, 10 and 90 percentile)
Group BC-L: See legend to Table 1.
Group B: Randomly-selected 21 patients who were positive for HBsAg and anti-HBe antibody and negative for anti-HCV antibody.

%) of 8 patients seronegative for HCV-RNA among all members of Groups BC-H and BC-L (Table 1; $P < 0.05$ by chi-square test). HBV-DNA was detected in 9 members (43 %) of Group BC-L and 11 (52 %) of Group B. Because of low titers of DNA-p and HBV-DNA in Groups BC-L and B, HBsAg was semiquantified to assess HBV replicative activity. HBsAg in Group BC-L tended to be lower than in Group B (median, range; 5.37, 0.15–19.81; 12.84, 0.19–19.8, respectively; Fig. 2), although the difference was not statistically significant.

Discussion

Since the diagnosis of HCV infection became possible, it has been reported that some HBV patients carry HCV. In Japan, the prevalence of anti-HCV Ab is about 0.9–1.0 % in blood donors, and 7 to 15 % in HBsAg carriers (10, 23). Viral interference among multiple hepatotropic viruses has been documented. Evidence suggests that HAV, HDV, or HCV can suppress replication of HBV (8, 9, 14, 24–26). In this study, to semiquantify the viral genomes and gene products, we found that patients coinfected with HBV and HCV had lower titers of HBsAg than those with HBV alone, and that they also had lower titers of HCV-RNA than those with HCV alone.

In HBV and HCV coinfection, HCV-RNA in serum was detected more often in patients with infrequently replicating HBV (Group BC-L) than in patients with highly replicating HBV (Group BC-H) (Table 1). As all patients but one in Group BC-H were positive for HBeAg and all patients in Group BC-L were seroconverted to be positive for anti-HBe Ab (Table 1), patients in Group BC-L seemed to be in a clinical stage with lower potential of HBV replication. HCV-RNA titers in Group BC-L tended to be lower than those in patients with anti-HCV Ab and without HBsAg (Group C) (Fig. 1). This result suggests that active replication of HBV may suppress replication of HCV or release of HCV particles from hepatocytes. Furthermore, even minimal HBV replication in the liver may be enough to suppress HCV replication. In patients without HCV-RNA, HCV infection must be confirmed in addition to anti-HCV Ab. We performed RIBA as confirmatory test and showed that most patients' sera reacted with more than two antigens of HCV. We, therefore, considered anti-HCV Ab in this group to be truly related to HCV infection. Pontisso *et al.* reported that HBsAg carriers positive for anti-HCV Ab but negative for HCV-RNA in sera often had HCV-RNA in the liver (15).

The HCV genotypes observed in coinfected patients were not different from those in blood donors with HCV as we reported previously (21). The correlation of HCV genotype distributions between double infected patients and blood donors supports the assertion that the subjects of the present study were not deviated from the general population. However, genotype 1b tended to be more common in coinfected patients than hepatitis C alone and this suggests that HCV of genotype 1b persists despite of interference by HBV as compared with type 2a or 2b.

Next we examined the influence of HCV on HBV replication. The activity of DNA-p in patients negative for serum HCV-RNA was higher than in those positive for serum HCV-RNA (Table 1). Also, HBV-DNA tend-

ed to be detected in more patients without anti-HCV Ab than with anti-HCV Ab. In semiquantitative assays, HBsAg levels tended to be lower in coinfected patients (Group BC-L) than in HBsAg carriers (Group B). These results were consistent with the spontaneous HBV clearance with HCV infection as reported by Sheen (16).

The mechanisms of viral interference responsible for virus absorption, penetration, and/or replication have been put forth (1). Liaw suggested that HCV-induced liver injury and accompanying cell renewal would increase the expression of HBV epitopes on the hepatocyte surface and that this may lead cytotoxic T cells to effect the elimination of HBV-infected hepatocytes (7). A recent *in vitro* study revealed that core antigens of HCV may directly suppress the expression of HBsAg in HuH-7 cells (26).

The high percentage of severe liver diseases like LC and HCC in Groups BC-H and BC-L argues against the existence of viral suppression. The clinical course of patients who were examined retrospectively using stored sera (patients 1 and 6) indicated that the dominance in viral replication alternated between HBV and HCV (data not shown). The progression of liver damage may be brought by continuous inflammation alternating between HBV and HCV. Crespo *et al.* and our group have found that double infection causes more severe pathological and clinical liver damage than single infection (11, 27). On the other hand, another researcher showed neither exacerbation nor amelioration of histological findings (28). Another reported that superinfection of HCV on HBV improved the clinical outcome of HBV patients undergoing liver transplantation (29). The clinical outcome of coinfection of HBV and HCV still remains to be clarified by the long-term observation.

In conclusion, our study of coinfected patients with HBV and HCV suggests that HCV replication may be suppressed by active HBV replication. It also suggests that HCV suppresses HBsAg expression and probably HBV replication.

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