

Entecavir Treatment of Chronic Hepatitis D

Gökhan Kabaçam,¹ F. Oğuz Önder,¹ Mustafa Yakut,¹ Gülseren Seven,¹ Senem C. Karataylı,³ Ersin Karataylı,³ Berna Savaş,² Ramazan Idilman,¹ A. Mithat Bozdayı,^{1,3} and Cihan Yurdaydin^{1,3}

Departments of ¹Gastroenterology and ²Pathology, University of Ankara School of Medicine, and ³Hepatology Institute, University of Ankara, Turkey

Background. Hepatitis D virus (HDV) requires hepatitis B surface antigen (HBsAg) to propagate infection and cause disease. Entecavir is a nucleoside analog with potent antiviral efficacy, and in the woodchuck animal model it also decreased hepatitis B virus (HBV) cccDNA and woodchuck surface antigen. The aim of this study was to investigate the efficacy of entecavir in chronic hepatitis D (CHD).

Methods. This single-center study was conducted in patients with compensated liver disease. All patients had to have detectable hepatitis HDV RNA and elevated levels of alanine aminotransferase (ALT). Entecavir was given at a dosage of 1 mg/d for 1 year. The primary end point was achievement of undetectable HDV RNA at the end of treatment.

Results. Thirteen consecutive patients were assessed. All patients had detectable HDV RNA, and 8 had detectable HBV DNA at baseline. At the end of treatment, HBV DNA became undetectable in all patients ($P = .001$). No significant decline in HDV RNA, ALT, or quantitative HBsAg levels was observed. The primary end point of undetectable HDV RNA at the end of treatment was achieved in 3 patients who had significantly lower baseline HDV RNA levels than nonresponders ($2.99 \log_{10}$ copies/mL $\pm .70$ vs $4.68 \pm .97$; $P = .0185$). In all 3 patients, ALT levels were also normal at the end of treatment.

Conclusions. One year of entecavir treatment is ineffective in CHD. Any generalized beneficial effect of nucleoside/nucleotide analog treatment may necessitate prolonged treatment. Patients with CHD with HBV dominance, which is likely to occur in the later phases of CHD, may be a reasonable patient cohort in which to target nucleoside/nucleotide analog therapy.

Chronic hepatitis D (CHD) develops mostly as a result of superinfection of a hepatitis B carrier with the hepatitis D virus (HDV) and represents the most severe form of chronic viral hepatitis [1]. As such, treatment needs in CHD are high. However, the major advances seen in the management of chronic hepatitis B (CHB) and C chronic hepatitis C (CHC) were not witnessed for this form of chronic viral hepatitis. One hope was that CHD could benefit from the success of new treatments for CHB, because HDV leads to disease only in the presence of hepatitis B virus (HBV). In several studies, different nucleoside/nucleotide analogs (NAs)

have been tested for CHD treatment, such as famciclovir, lamivudine, and adefovir, both as monotherapy and also in combination with interferon or pegylated interferon for the latter 2 NAs [2–5]. None of them proved to be effective either as monotherapy or as combination treatment with interferons, although a combination of pegylated interferon and adefovir was associated with a significant decrease in quantitative hepatitis B surface antigen (HBsAg) levels [5]. The only beneficial effect so far with NAs was reported in patients with HDV–human immunodeficiency virus (HIV) coinfection; in 13 of 16 patients, a significant decline in HDV RNA levels was observed with tenofovir treatment [6].

The only helper function needed by HDV from HBV to cause disease in humans is the production of HBsAg [1, 7, 8]. In this context, an NA affecting HBsAg levels should be beneficial in CHD. Entecavir is not only an NA with potent antiviral efficacy but has also been reported to substantially decrease woodchuck hepatitis virus cccDNA in liver samples as well

Received 20 January 2012; accepted 10 April 2012; electronically published 9 May 2012.

Correspondence: Cihan Yurdaydin, MD, Department of Gastroenterology, University of Ankara Medical School, Dikimevi 06100, Ankara, Turkey (cihan.yurdaydin@medicine.ankara.edu.tr).

Clinical Infectious Diseases 2012;55(5):645–50

© The Author 2012. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com.

DOI: 10.1093/cid/cis459

as woodchuck surface antigen in serum [9]. In the current retrospective study, the effect of entecavir use in CHD was investigated.

PATIENTS AND METHODS

Patients selected for the study were consecutive patients who received entecavir at the Department of Gastroenterology of the University of Ankara Medical School in Ankara for CHD for 1 year and met the following inclusion criteria: documented HBV and HDV infection of ≥ 6 months duration, age between 18 and 70 years, detectable HDV RNA levels, and compensated liver disease. Other inclusion criteria included alanine aminotransferase (ALT) concentration above the upper limit of normal but ≤ 10 times that limit, negative serologic findings for hepatitis C virus and HIV, and no treatment for CHD in the previous 6 months. Patients had to have a white blood cell count >3000 cells/mm³, a neutrophil count >1500 cells/mm³, and a platelet count $>75\,000$ cells/mm³. Patients were excluded if they had a serum albumin level <3.5 g/L or a serum bilirubin level >3 mg/dL. Patients were also excluded from the study if they had evidence of other genetic, autoimmune, or metabolic liver diseases or alcohol and/or drug abuse within 1 year of study entry or had evidence of ascites or hepatocellular carcinoma on an imaging study up to 6 months before commencement of entecavir treatment. The study was approved by the ethics committee of the university hospital.

Study Design

This was an open-label retrospective study to assess the effect of 1-year entecavir treatment in patients with CHD. Patients were followed up without treatment for ≥ 6 months after discontinuation of entecavir. Entecavir was given at a dosage of 1 mg/d, before breakfast. The primary end point was achievement of undetectable HDV RNA levels at the end of treatment. Secondary end points were normal ALT levels at the end of the study and undetectable HDV RNA at the end of follow-up. Patients were followed up monthly for the first 3 months and then every 6 weeks until the end of the study. Treatment-free follow-up consisted of assessments 3 and 6 months after treatment discontinuation. At each visit, blood samples were obtained for hematologic and biochemical work-up. In addition, serum samples were collected to determine HBV DNA and HDV RNA levels at baseline, at months 6 and 12, and at follow-up visits. Quantitative HBsAg levels were determined only at baseline and the end of treatment. All patients underwent liver biopsy before treatment commencement because this is required by the state insurance body to start antiviral treatment in CHB or CHD. After treatment discontinuation, liver biopsy was offered to patients and was performed in those patients who gave consent. Liver biopsy

samples were assessed by a single pathologist (B. S.) after all data were collected. The pathologist was blinded to the treatment patients were receiving. Histologic findings were assessed according to Ishak et al [10]. A change in fibrosis was defined as a change of ≥ 1 in the fibrosis score, and a change in necroinflammation was defined as a change of ≥ 2 in the necroinflammatory score.

Methods

For HDV RNA measurement, viral RNA was extracted from 200 μ L of serum by a commercial kit (Viral RNA Extraction Kit; Roche Diagnostics) according to the manufacturers' instructions. Complete genome sequences belonging to different HDV genotypes retrieved from GenBank, or genotype I from our own database, were compared to determine the conserved regions of HDV genome for the design of primers and probes used in real-time polymerase chain reaction (PCR) settings. A plasmid containing the hepatitis D antigen region of genotype I HDV genome was constructed to use as the standard for measuring viral load. Among several primer and probe systems, 2 primers targeting the hepatitis D antigen region and a TaqMan probe labeled with Fam and Tamra by their 5' and 3' ends, respectively, had the best performance with the 1-step EZ RT PCR kit. This kit uses rTth enzyme, allowing reverse transcription and polymerization in the same closed tube and uracil-N-glycosylase (UNG) enzyme treatment to prevent PCR contamination with ABI 7300 (Applied Biosystems) and LightCycler 480 Real-Time (Roche) PCR systems. The primers used for PCR amplification and the TaqMan probe were as follows: DF1, 5'- CTC GTT CCA CCT TCG AGG G -3'; DR1, 5'- CGA GGA AGA CGA GAG AAG GGA -3'; and DPR1, 5'-FAM- ACC TGC GGG CCG GCT ATT CTT CT - TAMRA- 3'. Reverse transcription and amplification were performed using a 1-step EZ RT PCR kit in 25 μ L of reaction mixture containing 3 mmol/L Manganese II-acetate (Mn [OAc]₂); 0.3 mmol/L deoxy adenosine triphosphate (dATP), deoxy cytosine triphosphate (dCTP), and deoxy guanosine triphosphate (dGTP); 0.6 mmol/L deoxy uracil triphosphate (dUTP); 400 nmol/L forward and 400 nmol/L reverse primers; 200 nmol/L TaqMan probe; 0.01 U/ μ L UNG; 0.1 U/ μ L rTth DNA polymerase; and 5 μ L of template DNA. Briefly, UNG is activated at 50°C for 2 minutes, and RNA is reverse transcribed at 60°C for 60 minutes, with UNG inactivation at 95°C for 5 minutes and 40 cycles of denaturation at 95°C for 20 seconds followed by elongation at 60°C for 1 minute. The sensitivity of the assay to detect HDV RNA was 100 copies/mL.

HBV DNA levels were measured with a fully automated system which combines automated extraction of DNA on the COBAS AmpliPrep Instrument, coupled with a real-time PCR on the COBAS TaqMan Analyzer using COBAS TaqMan HBV 48 test kit with a detection limit of 20 IU/mL (Roche Diagnostics).

Table 1. Baseline Characteristics of Patients

Patient Characteristic	Data
Age, median (range), years	51.5 (26–62)
Sex, No.	5 male, 8 female
ALT (IU/L, $\bar{x} \pm \text{SD}$)	120 $\pm$ 110
AST (IU/L, $\bar{x} \pm \text{SD}$)	80 $\pm$ 64
Alkaline phosphatase (IU/L, $\bar{x} \pm \text{SD}$)	87 $\pm$ 34
GGT (IU/L, $\bar{x} \pm \text{SD}$)	82 $\pm$ 53
HBeAg positive at serology, No. (%)	4 (38.5)
HBV DNA, log ₁₀ IU/mL	3.80 $\pm$ 2.58
HDV RNA, log ₁₀ copies/mL	4.22 $\pm$ 1.47
HBsAg level, log ₁₀ IU/mL (n:11)	3.49 $\pm$.79
Fibrosis grade (n:10)	2.6 $\pm$ 1.5
HAI (n:10)	8.1 $\pm$ 2.1
Previous interferon treatment, No.	9
Previous NA treatment, No.	8

Fibrosis grade and HAI assessed according to Ishak et al (10; n = 13 if not otherwise indicated).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transpeptidase; HAI, histologic activity index; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HDV, hepatitis D virus; NA, nucleoside/nucleotide analog; SD, standard deviation.

HBsAg was quantified by the Architect HBsAg assay (Abbot Diagnostics), according to the manufacturer's instructions. HDV genotypes, and HBV genotypes in patients with detectable HBV DNA, were determined as described elsewhere [11].

Statistics

All data are presented as mean and/or median values, as specified. Comparisons were made using unpaired and paired Student *t* test, Mann–Whitney *U* test for continuous variables, where appropriate, and Fisher's exact test for categorical variables. Patients with undetectable HBV DNA or HDV RNA levels were given the values of 20 (1.3 log₁₀) IU/mL and 100 (2 log₁₀) copies/mL, respectively, during statistical assessment. Differences were considered significant at *P* < .05.

RESULTS

Baseline Characteristics

The main baseline demographic, clinical, and virologic characteristics of the patient cohort are given in Table 1. The cohort had a female predominance. Four patients had hepatitis B e antigen (HBeAg)-positive CHD, of whom 1 was also positive for anti HBe. HBV DNA levels at baseline were undetectable in 4 patients; all were serologically negative for HBeAg and positive for anti-HBe antibody. All patients had detectable HDV RNA at baseline. The HBV genotype was D in all 7 patients in whom this assessment was possible, and the HDV

genotype was 1 in all patients. Nine patients had received interferon α or pegylated interferon α in the past, and 8 had received NAs (lamivudine in 7, adefovir in 1). The baseline liver biopsy specimen was considered inadequate in 2 patients, in 1 of whom ultrasound demonstrated a cirrhotic liver. In the other 11 patients, liver tissue samples displaying 6–32 portal tracts were obtained, with mild to moderate fibrosis revealed in 8 of them. However, 1 patient (patient number 2) had grade 0 fibrosis in the baseline sample, which included 10 portal tracts, and grade 4 fibrosis in the sample obtained at the end of treatment; this difference was probably due to a sampling error in the baseline liver biopsy, which is frequently observed in patients with advanced liver disease.

Response to Treatment

After 48 weeks of treatment with entecavir, HBV DNA levels became undetectable in all patients (Tables 2 and 3). However, no significant change compared with baseline was observed in ALT or HDV RNA levels, although mean ALT levels ($\pm$ standard deviation [SD]) decreased from 126 $\pm$ 108 to 61 $\pm$ 34 (*P* = .07; Table 3). Quantitative HBsAg levels showed a mild increase (Table 3). Six months after treatment-free follow-up, HBV DNA levels became detectable in 5 patients (*P* < .05 for comparison with end-of-treatment values), 5 of the 9 with detectable HBV DNA levels at baseline. Two patients with detectable baseline HBV DNA levels refused to stop treatment and continued to have undetectable HBV DNA levels. Excluding the patients who continued to take entecavir after 1 year, ALT levels at the end of follow-up were similar to those at the end of treatment (mean $\pm$ SD, 57 IU/L $\pm$ 15 vs 69 $\pm$ 32, respectively; *P* = .18). HDV RNA levels at the end of follow-up were also similar to end-of-treatment levels (4.55 $\pm$ 1.12 vs 4.45 $\pm$ 1.09 log₁₀ copies/mL, respectively; *P* = .6). No patient had a biochemical or virologic flare after treatment discontinuation, and no adverse effects were noted during treatment or after treatment discontinuation.

Paired liver biopsies with satisfactory specimen ($\geq$ 8 portal tracts) were available in 9 patients. In 7 patients, histologic findings showed deterioration, with histologic improvement seen in only 1 patient; no change was observed in 1 patient. (Table 2).

The 4 patients with HBeAg-positive serologic findings had baseline HBV DNA levels between 4.96 and 7.08 log₁₀ IU/mL. Their baseline HBV DNA levels were higher than those in the 8 patients with HBeAg-negative CHD (5.65 $\pm$.99 vs 3.17 $\pm$ 1.87 log₁₀ IU/mL; *P* = .032). In these 4 patients baseline HDV RNA levels ranged from 4.20 to 6.32 log₁₀ copies/mL and were similar to those in HBeAg-negative CHD. No change in HDV RNA levels occurred in HBeAg-positive patients at the end of treatment (mean $\pm$ SD for baseline vs end of treatment, 5.01 $\pm$.37 vs 5.13 $\pm$.39 log₁₀ copies/mL, respectively;

Table 2. Effects of Entecavir Treatment on Biochemical and Virologic Variables

Patient	HBeAg	HBeAb	ALT (IU/L)		HDV RNA, log ₁₀ Copies/ mL		HBV DNA, log ₁₀ IU/mL		HBsAg		Fibrosis Grade ^a		HAI ^a	
			BL	EOT	BL	EOT	BL	EOT	BL	EOT	BL	EOT	BL	EOT
1	Positive	Negative	79	51	5.1	5.24	4.23	UD	4.16	4.13	1	3	7	9
2	Negative	Positive	63	68	4.16	4.53	3.12	UD	4.29	4.26	0	4	10	16
3	Negative	Positive	61	66	4.16	4.63	UD	UD	4.11	4.27	4	4	9	9
4	Negative	Positive	94	103	3.82	4.16	UD	UD	3.37	4.29	5	5	10	12
5	Positive	Negative	54	39	5.49	5.33	4.79	UD	3.6	4.6	3	3	6	10
6	Positive	Negative	197	128	4.63	4.56	6.32	UD	2.17	2.18	3	NA	10	NA
7	Negative	Positive	69	42	5.72	5.53	UD	UD	4.18	4.14	1	3	6	10
8	Negative	Positive	250	54	2.85	2.98	4.84	UD	3.3	4.23	3	4	10	11
9	Negative	Positive	105	98	6.09	5.32	UD	UD	4.23	4.4	3	4	11	14
10	Positive	Positive	44	85	4.82	5.4	4.2	UD	3.55	4.26	4	3	11	10
11	Negative	Positive	32	23	2.23	UD	3.38	UD	2.05	1.95	NA	NA	NA	NA
12	Negative	Positive	400	23	3.62	UD	4.79	UD	NA	NA	NA	NA	NA	NA
13	Negative	Positive	201	18	3.12	UD	4.18	UD	NA	3.14	5	NA	11	NA

Abbreviations: ALT, alanine aminotransferase; BL, baseline; EOT, end of treatment; HAI, histologic activity index; HBeAb, hepatitis B e antibody; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HDV, hepatitis D virus; NA, not available; UD, undetectable.

^a Fibrosis grade and HAI scored according to Ishak et al [10].

$P = .5115$). Similarly, ALT levels did not change (mean $\pm$ SD, 94 ± 71 vs 76 ± 40 IU/L; $P = .49$).

The primary end point of undetectable HDV RNA at the end of treatment was achieved in 3 patients (patients 11, 12 and 13). In all 3 patients, ALT levels were also normal at the end of treatment (Table 2; Figure 1). In these 3 patients with virologic and biochemical responses, baseline HDV RNA levels were generally lower (2.23 , 3.62 , and 3.11 log₁₀ copies/mL) than in the other 10 patients (2.65 ± 1.26 vs 4.58 ± 1.21 copies/mL; $P = 0.028$). In the latter group, only 1 patient, whose HDV RNA level was 2.85 log₁₀ copies/mL, had lower HDV RNA levels than 1 of the virologic responders. In this non-responder patient, HDV RNA levels did not decline, but

ALT levels decreased substantially from 250 IU/L at baseline to 64 at the end of treatment. None of the other patients had normalized ALT levels at the end of treatment (Figure 2). Among the baseline characteristics, platelet counts seemed lower in the responders than in the nonresponders (82 ± 6.1 vs $166 \pm 66 \times 10^9/L$ respectively; $P = .0554$).

DISCUSSION

The main finding of this study is that 1-year treatment of CHD with entecavir is without effect. This may have been expected because treatment of CHD with other NAs, such as famciclovir [2], lamivudine [3, 4], and adefovir [5], has also

Table 3. Overall Assessment of Treatment Response to Entecavir in Patients With Chronic Hepatitis D

Measure	Baseline	End of Treatment	P^a	End of Follow-up ^b	P^b
HDV RNA, copies/mL	4.29 ± 1.16	4.13 ± 1.39 4.52 ± 1.12^b	.38	4.55 ± 1.09^b	.60
HBV DNA, IU/mL	3.41 ± 1.75	1.3 ± 0	.001	2.52 ± 1.50^b	.022
ALT	121 ± 108	61 ± 34 69 ± 32^b	.0734	57 ± 15^b	.20
HBsAg, IU/mL	$3.55 \pm .80$	$3.88 \pm .91$	.03	NA	NA

Data are given as means $\pm$ standard deviations.

Abbreviations: ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HDV, hepatitis D virus; NA, not available; RNA, ribonucleic acid.

^a End of treatment versus baseline.

^b End of follow-up versus end of treatment. The 2 patients who did not stop treatment at the end of the official treatment period were excluded from the analysis.

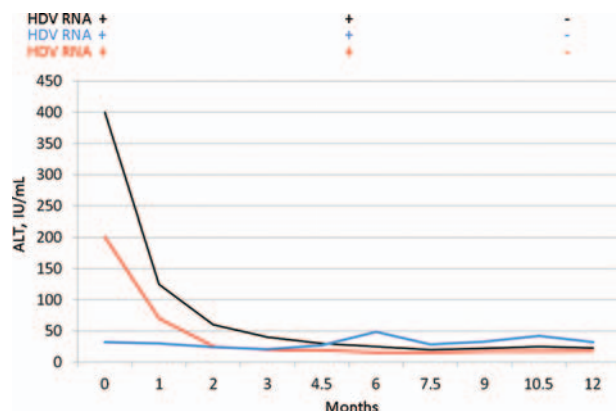


Figure 1. Alanine aminotransferase levels in the 3 patients who cleared hepatitis D virus RNA at the end of entecavir treatment. Abbreviations: ALT, alanine aminotransferase; HDV, hepatitis D virus; RNA, ribonucleic acid.

been reported to not be efficacious. However, entecavir and tenofovir represent the most potent NAs for the treatment of CHB [12] and if NAs were to be considered for preventing or decreasing the helper HBV function in CHD, it seems reasonable to consider these 2 NAs. This rationale is supported by the recent demonstration of the beneficial effect of long-term tenofovir use in patients with HIV-HDV coinfection [6]. In this latter study, tenofovir was used for a median of 6.1 years, compared with only 1 year of entecavir use in the current study. It must be remembered that HDV needs HBV not for its replication but for its assembly, and here HBsAg is needed. Because NAs have no direct effect on HBsAg production, which occurs through HBV cccDNA or alternatively from HBV DNA integrated into the host genome [13], any potential effect of an NA could only be indirect. This indirect path

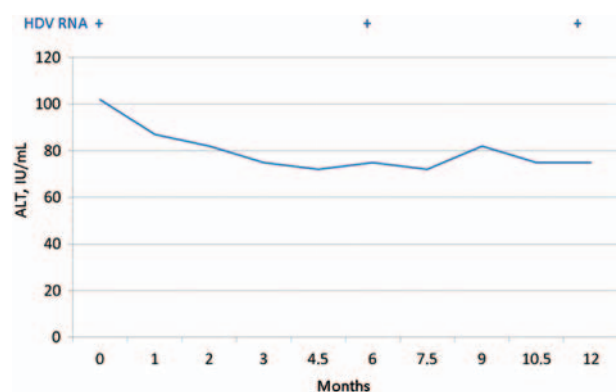


Figure 2. Mean alanine aminotransferase levels in the 10 patients who did not clear hepatitis D virus RNA at the end of entecavir treatment. Abbreviations: ALT, alanine aminotransferase; HDV, hepatitis D virus; RNA, ribonucleic acid.

would involve decreased recycling of viral nucleocapsids to the nucleus because of strong inhibition of viral DNA synthesis and less incoming virus from the blood [14]. Although such a mechanism may have accounted for the beneficial effects observed in patients with HIV-HDV coinfection receiving long-term tenofovir treatment, a treatment duration of 1 year is apparently too short to see such an effect, as has been recently shown in patients with HBeAg-negative CHB, in whom HBsAg levels did not change after 1 year of entecavir treatment [15].

In CHD, HDV classically suppresses HBV, so treatment has to be tailored toward HDV. However, a dynamic shift of the dominant virus with time may also be possible [16], and this has been reported in CHD [17, 18]. It is reasonable to consider that NA therapy may be effective in cases where HBV, not HDV, is the dominant virus. Patients with HBeAg-positive CHD are one CHD cohort in which HBV may be the dominant virus. Although HBV DNA levels in such patients have been found to be higher compared with HBeAg-negative CHD, HDV RNA levels were similar [19], suggesting that HBV is not suppressing HDV in HBeAg-positive CHD. This hypothesis is also supported in the current study, in which 4 patients with HBeAg-positive CHD had higher HBV DNA levels than patients with HBeAg-negative CHD, but none of them showed a virologic or biochemical response.

However, 3 patients (patients 11, 12, and 13) had undetectable HDV RNA levels at the end of treatment. These patients had low HDV RNA and higher HBV DNA levels. Two of them (patients 12 and 13) refused to stop treatment and continued to have undetectable HBV DNA and HDV RNA levels along with normal ALT levels. The other patient had a cirrhotic liver at ultrasound, associated with splenomegaly. Furthermore, baseline platelet levels in these 3 patients were lower than in the nonresponders. This may have some relevance, because the late phase of CHD infection has been reported to be associated with reactivation of HBV [20]. However, no recommendation can be made based on these 3 patients, and a placebo effect cannot be ruled out. In a randomized study of the use of lamivudine in 31 patients for 1–2 years, 2 patients who had taken lamivudine for 2 years had a sustained virologic response, and both of them had undetectable HBV DNA at baseline with a sensitive PCR-based assay, contradicting the observations made in our study [3]. It is important to note that 2 of the 3 patients who cleared HDV RNA had high ALT levels to start with (400 and 201 IU/mL), and it is possible that clearance of HDV RNA in these 2 patients may have simply been immune mediated. The limitations of the current study should also be considered; the study was retrospective and based on a rather small number of patients with heterogeneous baseline properties; some were treatment naive, and others had been treated in the past with interferons or other NAs.

In this pilot study, 1 year of entecavir treatment proved ineffective for the treatment of CHD. Any generalized beneficial effect of NA treatment may necessitate prolonged treatment, because NAs do not interfere directly with HBsAg synthesis, the only product of HBV needed by HDV. The occasional beneficial effect of NAs in CHD also observed in this study may require further clarification. Patients with CHD and HBV dominance, which is probably more likely to occur later in the natural history of CHD, seem to be a reasonable patient cohort in which to target NA therapy. It is also important to note that in the current study, in 7 of 9 patients with available paired liver biopsy samples, histologic deterioration was observed. This finding suggests that NA therapy should be considered only in patients in whom interferon therapy cannot be used or who show no change in viral load during interferon therapy.

Note

Potential conflicts of interest. C. Y. has been on the advisory board of Merck and Gilead Pharma, is on the speaker's bureaus of Roche, Gilead, BMS, and Merck Pharma, and has received research grant from BMS. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Yurdaydin C, Idilman R, Bozkaya H, Bozdayi AM. Natural history and treatment of chronic delta hepatitis. *J Viral Hepat* **2010**; 17:749–56.
2. Yurdaydin C, Bozkaya H, Gürel S, et al. Famciclovir treatment of chronic delta hepatitis. *J Hepatol* **2002**; 37:266–71.
3. Niro GA, Ciancio A, Tillman HL, et al. Lamivudine therapy in chronic delta hepatitis: a multicentre randomized-controlled pilot study. *Aliment Pharmacol Ther* **2005**; 22:227–32.
4. Yurdaydin C, Bozkaya H, Onder FO, et al. Treatment of chronic delta hepatitis with lamivudine vs lamivudine + interferon vs interferon. *J Viral Hepat* **2008**; 15:314–21.
5. Wedemeyer H, Yurdaydin C, Dalekos G, et al. Pegyinterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med* **2011**; 364:322–31.
6. Sheldon J, Ramos B, Toro C, et al. Does treatment of hepatitis B virus infection reduce hepatitis delta virus replication in HIV-HBV-HDV-coinfected patients? *Antiviral Ther* **2008**; 13:97–102.
7. Wedemeyer H, Manns MP. Epidemiology, pathogenesis and management of hepatitis D: update and challenges ahead. *Nat Rev Gastroenterol Hepatol* **2010**; 7:31–40.
8. Rizzetto M. Hepatitis D: thirty years after. *J Hepatol* **2009**; 50:1043–50.
9. Yurdaydin C. Entecavir: a step forward in combating hepatitis B disease. *Expert Opin Pharmacother* **2008**; 9:3095–107.
10. Ishak K, Baptista A, Bianchi I, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* **1995**; 22:696–9.
11. Bozdayi AM, Aslan N, Bozdayi G, et al. Molecular epidemiology of hepatitis B, C and D viruses in Turkish patients. *Arch Virol* **2004**; 149:2115–29.
12. Woo G, Tomlinson G, Nishikawa Y, et al. Tenofovir and entecavir are the most effective antiviral agents for chronic hepatitis B: a systematic review and Bayesian meta-analyses. *Gastroenterology* **2010**; 139:1218–29.
13. Nguyen T, Thompson AJV, Bowden S, et al. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. *J Hepatol* **2010**; 52:508–13.
14. Wursthorn K, Lutgehetmann M, Dandri M, et al. Peginterferon alpha 2b plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. *Hepatology* **2006**; 44:675–84.
15. Reijnders JGP, Rickborst V, Sonneveld MJ, et al. Kinetics of hepatitis B surface antigen differ between treatment with peginterferon with and entecavir. *J Hepatol* **2011**; 54:449–54.
16. Boyd A, Lacombe K, Mialhes P, et al. Longitudinal evaluation of viral interactions in treated HIV-hepatitis B co-infected patients with additional hepatitis C and D virus. *J Viral Hepat* **2010**; 17:65–76.
17. Schaper M, Rodriguez-Frias F, Jardi R, et al. Quantitative longitudinal evaluations of hepatitis delta virus RNA and hepatitis B virus DNA shows a dynamic, complex replicative profile in chronic hepatitis B and D. *J Hepatol* **2010**; 52:658–64.
18. Yalcin K, Degertekin H, Yurdaydin C, Bozdayi M, Bozkaya H. The role of HBeAg seroconversion in acute exacerbation of liver disease with termination of hepatitis B and D virus infection in a chronic hepatitis D patient during alpha interferon therapy. *Eur J Gastroenterol Hepatol* **2003**; 15:819–23.
19. Heidrich B, Wedemeyer H, Bektaş M, et al. HBeAg-positive delta hepatitis: virological, biochemical and clinical features in a large European multicenter database [abstr]. *Hepatology* **2008**; 48:1184A.
20. Wu JC, Chen TZ, Huang YS, et al. Natural history of hepatitis D virus superinfection: significance of viremia detected by polymerase chain reaction. *Gastroenterology* **1995**; 108:796–802.