Safety and Efficacy of Oral Entecavir Given for 28 Days in Patients With Chronic Hepatitis B Virus Infection

Robert A. de Man,[1] Leonieke M. M. Wolters,[1] Frederik Nevens,[2] David Chua,[3] Morris Sherman,[4] Cing L. Lai,[5] Adrian Gadano,[6] Youngmee Lee,[7] Fransesco Mazzotta,[8] Neil Thomas,[9] and Deborah DeHertogh[9]

Entecavir is an oral antiviral drug with selective activity against hepatitis B virus (HBV). We conducted a randomized, placebo-controlled, dose-escalating study in patients with chronic hepatitis B infection in which we evaluated the efficacy and safety of entecavir given for 28 days. Follow-up was 24 weeks. All doses of entecavir (0.05 mg, 0.1 mg, 0.5 mg, and 1.0 mg) showed a pronounced suppression of replication of the HBV with a 2.21, 2.29, 2.81, and 2.55 mean log_{10} reduction of viral load, respectively. Approximately 25% of patients on entecavir showed a decline of HBV DNA below the limit of detection of the Chiron HBV-DNA assay (<0.7 MEq/mL). In the postdosing follow-up period patients who were treated with 0.5 and 1.0 mg of entecavir showed a considerably slower return in their HBV DNA levels to baseline compared with those patients treated with lower dosages ($P < .05$). All doses of entecavir were well tolerated with no significant difference between treated patients and those receiving placebo. No significant changes in alanine transaminase (ALT) levels within the dose groups and the placebo group between baseline and the end of treatment were observed. Three patients (9%) (1 each in the 0.05-, 0.1-, and 0.5-mg groups) experienced asymptomatic hepatitis flares 16 weeks (2 patients) and 24 weeks (1 patient) after withdrawal of entecavir. In conclusion, in this 28-day study of entecavir a pronounced decrease of HBV DNA was observed and there were no significant side effects in entecavir patients in comparison with placebo-treated patients. (Hepatology 2001;34:578-582.)

Although the introduction of an effective vaccine against hepatitis B has drastically reduced the incidence of new infections, more than three hundred million people are affected by chronic hepatitis B infection worldwide. The infection may eventually lead to a substantial percentage of deaths caused by cirrhosis with complications of liver failure and hepatocellular carcinoma.[1] Interferon alfa has been the only registered therapy during recent years but it is effective in only one third of patients,[2] requires parenteral administration, and causes many side effects especially in cases of cirrhosis.[3] Lamivudine, a cytosine nucleoside analogue, is an orally administered antiviral agent with few side effects. It has recently been registered for the treatment of chronic hepatitis B infection.[4-6] However, the development of mutations with decreased sensitivity of the virus for lamivudine,[7-9] and the rebound of viral replication after withdrawal of the drug[10,11] leave room for further improvement of nucleoside analogue therapy. The increase in viral replication after withdrawal of the drug is based on residual covalently closed circular DNA (cccDNA) inside the nucleus of the hepatocyte, which is not affected by lamivudine.[12,13]

Entecavir, a new deoxyguanine nucleoside analogue, is a selective inhibitor of the replication of the hepatitis B virus (HBV).[14-16] In HepG2.2.15 cell lines, this compound has proved to be 30 times more potent than lamivudine in suppressing viral replication with an EC_{50} of 4 nmol/L compared with 116 nmol/L for lamivudine.[14] The *in vitro* therapeutic index (a marker indicating the range of doses that can be applied safely without causing toxicity) is 8,000.[15] In addition, incorporation of entecavir into cellular polymerases appears to be very inefficient thus bypassing an important cause of *in vivo* toxicity of nucleoside analogues. In chronically infected woodchucks, up to 8 log_{10} reductions in viral DNA have been observed after a mean of 32 weeks of therapy. Ten woodchucks that were treated for at least 14 months were both negative for cccDNA and hepatitis B core antigen in the liver biopsy.[17,18] Five animals that were kept on maintenance therapy for up to 3 years showed sustained drops in woodchuck hepatitis virus DNA with no evidence of resistance. In addition, woodchuck hepatitis virus DNA remained undetectable for 21 months after withdrawal of the drug.[19] This may indicate that entecavir is not only capable of interfering with viral replication, but that it also has a direct effect on cccDNA. In untreated historical controls, all chronically infected woodchucks died of hepatocellular carcinoma within 4 years. In contrast, entecavir reduces the incidence of liver cancer resulting in prolonged survival of the animals.

To be able to explore the initial antiviral effect and safety of entecavir in chronic hepatitis B patients, a placebo-controlled, dose-escalating study was performed.

Abbreviations: cccDNA, covalently closed circular DNA; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; bDNA, branched DNA; ULN, upper limit of normal; HBeAg, hepatitis B e antigen; ALT, alanine transaminase; HIV, human immunodeficiency virus; PCR, polymerase chain reaction.

From the [1]Erasmus University Hospital, Rotterdam, The Netherlands; [2]University Hospital Gasthuisberg, Leuven, Belgium; [3]Research Concepts, Chicago, IL; [4]Toronto General Hospital, Toronto, Canada; [5]Queen Mary Hospital, Hong-Kong, Hong Kong special; administrative region of China; [6]Hospital Italiano, Buenos Aires, Argentina; [7]NE Medical Center, Boston, MA; [8]S. M. Annunziata Hospital, Florence, Italy; and [9]Bristol-Myers Squibb, Wallingford, CT.

Received March 13, 2001; accepted June 11, 2001.

Supported by a research grant from Bristol-Myers Squibb (BMS). M.S. and C.L.L. are members of the BMS hepatitis advisory board. N.T. and D.D.H. are employees of BMS. C.L.L. has been awarded the unrestricted BMS research grant 2001-2005.

Address reprint requests to: Robert A. de Man, M.D., Ph.D., Department of Gastroenterology and Hepatology, University Hospital Rotterdam, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands. E-mail: deman@mdl.azr.nl; fax: (31) 10 436 5916.

0270-9139/01/3403-0019$35.00/0
doi:10.1053/jhep.2001.26815

PATIENTS AND METHODS

Study Design. We conducted a double-blind, placebo-controlled dose-escalating study of 4 dosages of entecavir (0.05 mg, 0.1 mg, 0.5 mg, and 1.0 mg) once daily. If proved eligible during the 2 screening visits, patients were treated for 4 weeks and followed-up for 24 weeks after discontinuation of the drug. Each cohort started when the evaluation of the previous cohort treated with a lower dosage had proved to be safe. In each cohort, patients were randomly assigned to a dose of entecavir or placebo (8:2). Blinded randomization was performed using a predetermined schedule maintained by the sponsor; bottle assignments were communicated by phone to the individual investigator. HBV DNA was assessed at 2 screening visits, day 1 (baseline); weeks 1, 2, 3, and 4 during dosing; and weeks 2 and 4 postdosing. HBV serology was obtained during initial screening, day 1, week 4, and final postdosing visit. Safety analysis, based on laboratory results, clinical evaluation of adverse events, and physical examination, was assessed on a weekly basis during therapy and monthly during follow-up. The protocol was approved by the Medical Ethics Committee of each participating center. All patients had to give written informed consent.

Selection of Patients. Eligible patients included men and women older than 16 years. Chronic hepatitis B infection was documented by hepatitis B surface antigen (HBsAg) positivity in the serum for over 24 weeks before start of therapy. Each individual had to have an HBV DNA greater than 20 MEq/mL by the branched DNA (bDNA) assay on 2 determinations at least 2 weeks apart. Patients had to have a compensated liver disease as documented by serum transaminase activity below 5 times the upper limit of normal (ULN), serum albumin greater than 30 g/L, serum bilirubin less than 51.3 μmol/L, a prothrombin time that was not elongated for more than 3 seconds, and the absence of significant ascites, hepatic encephalopathy, or variceal bleeding. Both hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients were eligible. Previous antiviral therapy, such as interferon alfa and other nucleoside analogues, was permitted but had to be withdrawn 6 months before the start of therapy in this trial. Patients were excluded if they were coinfected with the hepatitis C virus, the hepatitis D virus, or the human immunodeficiency virus (HIV); had another concomitant liver disease; had any signs or a history of pancreatitis; or had received immunosuppressive therapy within 6 months before start of therapy. Both male and female patients had to practice a reliable method of contraception.

Assays. HBsAg, antibodies to HBsAg, HBeAg, antibodies to HBeAg, anti-HCV, anti-HDV and anti-HIV were analyzed by an enzyme immunoassay (Abbott Diagnostics, Abbott Chicago, IL). If the anti-HIV assay proved to be positive, an HIV Western blot (Biorad, Hercules, CA) was performed for confirmation. HBV DNA was detected with the Chiron bDNA signal amplification assay (Chiron, Emeryville, CA;) lower limit of detection of 0.7×10^6 genome equivalents/mL (geq/mL). HBV DNA was assessed with a sensitive polymerase chain reaction (PCR) assay (Roche Monitor, limit of detection 400 geq/mL; Roche, Indianapolis, IN) at baseline and on day 28.

Statistics. The primary end point of this study was the proportion of subjects who had either a ≥ 2 $\log_{10}$ reduction from baseline in their day-28 bDNA assay or who achieved undetectable levels of HBV DNA by the bDNA assay and a ≥ 2 $\log_{10}$ reduction from baseline in their day-28 Roche HBV DNA PCR assay. The secondary efficacy summaries were the proportion undetectable by the bDNA assay, and the mean $\log_{10}$ reduction from baseline by the bDNA assay regarded as a continuous parameter at days 7, 14, 21, and 28 and postdosing days 14 and 28.

For comparison between entecavir and placebo for end points represented by binary variables we used Fisher's exact test. Comparisons of HBV DNA mean reductions were based on *t* tests.

One subject receiving entecavir 0.1 mg had a large spontaneous reduction in HBV DNA levels between the screening and baseline visits and was excluded from all of the efficacy analyses. The placebo subjects from each cohort in the sequential designs were pooled for comparison with each entecavir dose group. For binary end points, subjects who discontinued before day 28 because of an adverse event were assigned the failure value. Estimates and comparisons of mean differences were based on all available measurements at each time point, with measurements made after a subject who prematurely discontinued dosing was excluded.

RESULTS

A total of 42 patients were randomized into this study. Initially, the protocol was scheduled to include doses of 0.1 mg (cohort 1), 0.5 mg (cohort 2), 1.0 mg (cohort 3), and 2.5 mg (cohort 4) of entecavir. However, after virologic evaluation of the 0.1 mg and 0.5 mg dosages of entecavir, it was decided to amend the protocol to exclude the highest dose of 2.5 mg and instead to include a lower dose of 0.05 mg (cohort 5). Eight, 9, 9, and 8 patients were treated with 0.05 mg, 0.1 mg, 0.5 mg, and 1.0 mg, respectively. The evaluation was completed with a placebo group of 8 patients in which all data of placebo-treated patients per cohort were pooled. Baseline characteristics are shown in Table 1. All entecavir groups and the placebo group were comparable with regard to sex and age. Fifty-two percent of patients were of Asian origin (n =

TABLE 1. Baseline Demographics/Characteristics, Virology, and Chemistry

	Entecavir 0.05 mg N = 8	Entecavir 0.1 mg N = 9	Entecavir 0.5 mg N = 9	Entecavir 1.0 mg N = 8	Placebo N = 8
Sex (M:F)	6:2 (75%M)	8:1 (89%M)	7:2 (78%M)	8:0 (100%M)	7:1 (88%M)
Age (Mean years)	33.8	45.1	35.2	41.1	42.1
Race					
Asian	5 (63%)	6 (67%)	3 (33%)	2 (25%)	6 (75%)
White	2 (25%)	3 (33%)	4 (44%)	3 (38%)	2 (25%)
Black	0	0	1 (11%)	0	0
Hispanic/Latino	0	0	0	3 (38%)	0
Other	1 (13%)	0	1 (11%)	0	0
Prior HBV therapy	4 (50%)	5 (56%)	4 (44%)	3 (38%)	2 (25%)
IFN only	0	1 (11%)	4 (44%)	1 (13%)	1 (13%)
Lamivudine only	0	1 (11%)	0	0	0
Lamivudine + IFN	4 (50%)	2 (22%)	0	2 (25%)	1 (13%)
Lamivudine + polyclonal antibodies	0	1 (11%)	0	0	0
HBeAg positivity	7 (88%)	8 (89%)	7 (78%)	8 (100%)	8 (100%)
Mean ($\log_{10}$ HBV bDNA)	2.7	3.0	3.2	3.1	3.0
ALT (IU/L, SD)	77 (52)	78 (54)	106 (74)	124 (79)	86 (65)

TABLE 2. Response During One Month of Entecavir

	Entecavir 0.05 mg N = 8	Entecavir 0.1 mg N = 8*	Entecavir 0.5 mg N = 9	Entecavir 1.0 mg N = 8*	Placebo N = 8
Mean log_{10} change in HBV DNA	−2.21 (0.10)	−2.29 (0.33)	−2.81 (0.21)	−2.55 (0.17)	+0.01 (0.13)
Patients reaching the primary end point†	7 (88%)	4 (50%)	8 (89%)	6 (75%)	0
P value for comparison to placebo	.0001	.077	.0004	.007	
Number undetectable by the bDNA assay (%)	2 (25%)	2 (25%)	3 (33%)	1 (13%)	0

*Subjects discontinuing dosing before week 4 are excluded from the mean log_{10} change and are assigned failure for the analysis of the primary end point.
†≥2 log_{10} reduction in HBV DNA by bDNA or undetectable HBV DNA by bDNA and ≥log_{10} reduction by Roche PCR. Level of detection of bDNA was 0.7 × 10^6 Eq/mL.

22). Approximately 50% of patients were treatment-naive at start of entecavir therapy. In the 2 lower dosage entecavir groups, half of the patients were previously treated with lamivudine. The majority of patients were HBeAg positive (n = 38, 90%). The baseline level of HBV DNA was comparable in all cohorts. Mean alanine transaminase (ALT) level was above the ULN in all dosage groups.

Virologic Response. All entecavir-treated patients showed a pronounced decline of serum HBV DNA after 4 weeks of therapy. Treatment with 0.05 mg, 0.1 mg, 0.5 mg, and 1.0 mg of entecavir resulted in a 2.21, 2.29, 2.81, and 2.55 mean log_{10} decline, respectively, versus a 0.012 log_{10} increase in the placebo group (Table 2 and Fig. 1). Reduction in HBV DNA was similar between pretreated patients and treatment-naive patients. HBV DNA below the limit of quantification of the bDNA assay was observed in 25% of patients in the 2 lowest dosage groups (0.05 and 0.1 mg), in 33% of the patients treated with 0.5 mg of entecavir, and in 13% of patients treated with 1.0 mg of entecavir. HBV-DNA levels in the first 4 weeks postdosing remained significantly lower for the 2 higher dosages of entecavir (0.5 and 1.0 mg) than for the 0.05 or 0.1 mg dosages ($P = .005$). Three entecavir-treated patients had undetectable HBV DNA by the bDNA assay 6 months postdosing (2 at 0.5 mg and 1 at 0.1 mg). Two patients, 1 treated with 0.1 mg and 1 with 0.5 mg, lost HBeAg transiently. In these patients HBeAg was undetectable at day 28 and 4 weeks postdosing, but was again detectable at 6 months postdosing. A third subject in the 0.05-mg group had lost HBeAg at 6 months postdosing.

Liver Transaminase Levels. Mean transaminase levels were elevated in all dosage groups at the start of therapy (Table 1). Both at baseline and at the end of therapy, no significant difference was observed between the 4 dosage groups and the placebo group. Moreover, no significant difference between baseline and end of therapy levels of ALT were observed within the treatment groups.

In 2 patients, both in the 0.1-mg group, ALT levels rose during the dosing period. Both patients were clinically asymptomatic while bilirubin stayed normal. Patient 1 entered the study with an ALT level of 73 IU/L, and at the end of the

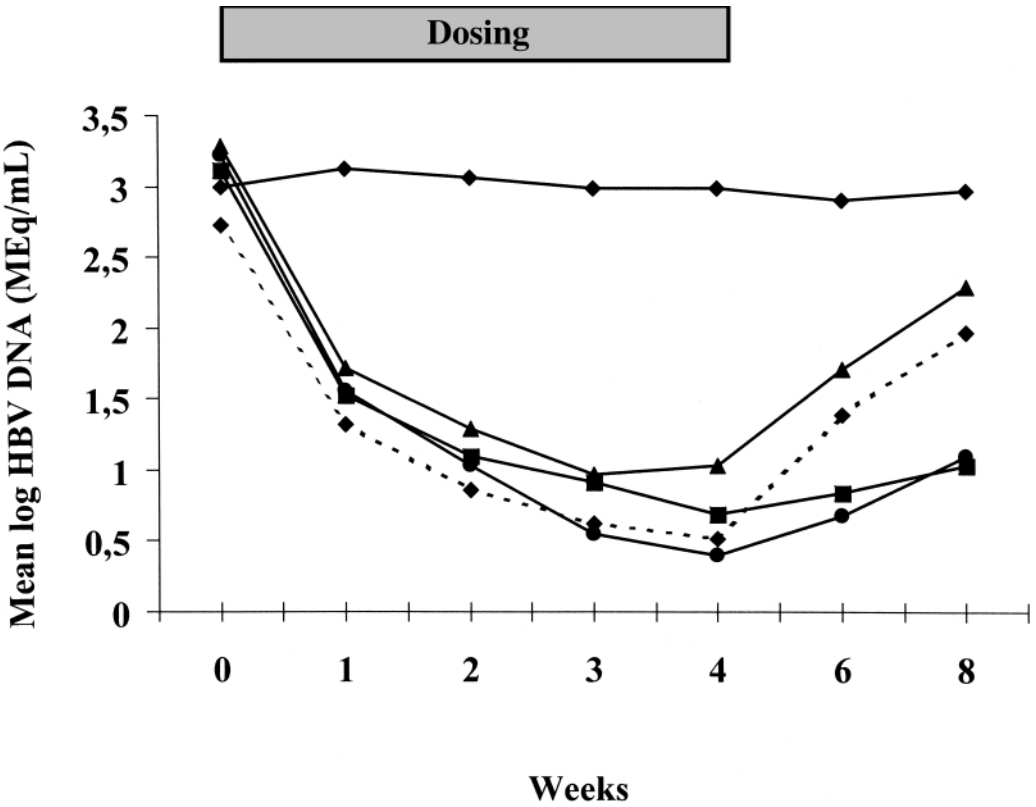

FIG. 1. Mean HBV DNA during therapy and 1 month follow-up. (—◆—) Placebo, (- -◆- -) 0.05 mg, (—▲—) 0.1 mg, (—●—) 0.5 mg, (—■—) 1.0 mg.

treatment period ALT was 165 IU/L. Patient 2 entered the study with an ALT level of 173 IU/L, which increased to 452 IU/L; entecavir was discontinued with a subsequent drop in ALT. Patient 1 had a 2.8 log reduction in HBV DNA and patient 2 had 3.3 log reduction in HBV DNA at study drug discontinuation mandated by the protocol.

Elevation of serum transaminases during the post-treatment follow-up are presented in Table 3. During follow-up, 3 patients experienced a hepatitis flare with ALT levels that were more than 2 times baseline and greater than 10 times the ULN. None of these were associated with increases in bilirubin. In 2 patients (1 patient treated with 0.05 mg entecavir and 1 patient treated with 0.1 mg) the flare was observed at 16 weeks postdosing. Serum ALT levels had declined to 5.7 times the ULN and 2.2 times the ULN, respectively, at the last follow-up visit (24 weeks postdosing). In the third patient, who had been treated with 0.5 mg of entecavir, the flare was observed at 24 weeks postdosing. No further follow-up data on this patient are available.

Safety. Overall, entecavir was well tolerated and no dose-related or dose-limiting toxicity occurred. The majority of patients reported adverse events that were of mild to moderate severity but not significantly different from placebo-treated patients. Fatigue and headache were reported most frequently (Table 4).One subject in the 0.5-mg entecavir group experienced asymptomatic grade 1 elevation of amylase and a grade 3 elevation of serum lipase on day 8 of therapy. This patient was known to have had elevation of lipase in the past. The study drug was withdrawn for several days during which time both enzymes returned to normal and the study drug was reintroduced and completed without further problems.

During the dosing and postdosing period, a total of 5 serious adverse events were reported, most of which could be related to progression of liver disease or external interference. One occurred in the first cohort (0.1 mg). This patient was diagnosed with hepatocellular carcinoma and pulmonary fibrosis 6 months postdosing. In the second cohort (0.5 mg), 1 patient was hospitalized for a cholecystectomy for biliary stones 3 months after withdrawal of therapy and another patient experienced septic shock after a heroin overdose 1 month postdosing. In the third cohort (1.0 mg), 1 patient was withdrawn from therapy after 6 days as specified in the protocol because of persistent headache lasting longer than 12 hours. Extensive neurologic evaluation did not show any abnormalities. The other patient was involved in a car accident, which required short-term hospitalization 2 months after withdrawal of therapy. No adverse events have been reported in the fifth cohort (the lowest dosage applied).

TABLE 3. Elevation of Serum Transaminases in the Post-Treatment Period of the Study

	Entecavir 0.05 mg N = 8	Entecavir 0.1 mg N = 9	Entecavir 0.5 mg N = 9	Entecavir 1.0 mg N = 8	Placebo N = 8
ALT					
>2× baseline value	3 (38%)	0	3 (33%)	2 (25%)	1 (13%)
>3× baseline value	3 (38%)	1 (11%)	2 (22%)	1 (13%)	0
>10× ULN	1 (13%)	1 (13%)	1 (13%)	0	0
AST					
>2× baseline value	2 (25%)	1 (11%)	3 (33%)	1 (13%)	1 (13%)
>3× baseline value	2 (25%)	1 (11%)	2 (22%)	1 (13%)	0
>10× ULN	1 (13%)	0	0	0	0

TABLE 4. Most Common Nonserious Adverse Events Reported on Study

	Entecavir 0.05 mg N = 8	Entecavir 0.1 mg N = 9	Entecavir 0.5 mg N = 9	Entecavir 1.0 mg N = 8	Placebo N = 8
Fatigue	2 (25%)	4 (44%)	2 (22%)	4 (50%)	3 (38%)
Headache	2 (25%)	3 (33%)	4 (44%)	3 (38%)	3 (38%)
Infection	2 (25%)	0	1 (11%)	3 (38%)	0
Abdominal pain	1 (13%)	3 (33%)	1 (11%)	3 (38%)	2 (25%)
Flu syndrome	1 (13%)	0	2 (22%)	4 (50%)	3 (38%)
Pharyngitis	1 (13%)	3 (33%)	1 (11%)	0	2 (25%)
Nausea	0	2 (22%)	1 (11%)	3 (38%)	0

NOTE. Includes events reported during 4-week dosing period plus 24-week postdosing follow-up.

DISCUSSION

The present study reports experience with the new nucleoside analogue, entecavir, in patients with chronic hepatitis B. All dosages of entecavir showed a pronounced decline of HBV DNA of more than 2 log after 4 weeks. In some patients, HBV DNA declined below the limit of detection (0.7×10^6 Eq/mL) of the bDNA assay. Both interferon and lamivudine pretreated patients and treatment-naive patients responded well to therapy. A transient loss of HBeAg occurred in 2 patients. One month of treatment is limited and not capable of inducing a pronounced viral suppression coinciding with HBeAg seroconversion. After withdrawal of entecavir in the 0.5- and 1.0-mg dosage groups, HBV DNA remained significantly below baseline levels for 4 weeks. These data are in agreement with results from *in vitro* studies of entecavir and data on inhibition of viral replication in woodchucks.[16,17] Rebound of virus after withdrawal of lamivudine occurs quickly. Return of HBV DNA to baseline was observed within 1 month of cessation of lamivudine in a phase-II 24-week dosing study for the treatment of chronic hepatitis B patients.[5] A comparison of studies in which patients were treated with lamivudine for 3, 6, and 12 months[4-6] showed that 12 months of therapy resulted in a more gradual rebound of viral load after discontinuation of therapy. However, the latter study did not report on the extent of decline of HBV DNA with more sensitive assays. A larger percentage of patients with undetectable HBV DNA by PCR, may cause a slower return to baseline. In our study of entecavir, most of the patients in all dosage groups still had detectable HBV DNA by the bDNA assay at the end of 4 weeks of therapy although the higher dosage groups showed a more gradual return to baseline in the postdosing period.

As has been observed after withdrawal of lamivudine therapy,[20] withdrawal of entecavir with a subsequent return of viral replication may induce a flare of serum transaminases. Spontaneous hepatitis flares occur at an annual rate of 27% in HBeAg-positive patients,[21] and larger patient populations should be evaluated to explore the relationship between entecavir therapy and the occurrence of hepatitis flares. None of the patients in the placebo group in our study population experienced a flare of serum transaminases, whereas 9% of patients who were treated with entecavir experienced transient hepatitis flare 16 to 24 weeks after withdrawal of entecavir. More pronounced suppression of HBV DNA may lead to

a delay in recurrence in viral replication as well as a delay of the hepatitis flare.

Nucleoside analogues are capable of interfering with the replication of HBV because they have the same characteristics as natural nucleosides. However, this may also lead to interference with cellular polymerases. As has been seen with some other nucleoside analogues, inhibition of cellular enzymes may cause significant clinical events and even deaths.[22-25] Entecavir has proved to have very low affinity for cellular DNA. In particular, mitochondrial DNA does not use entecavir at all. In this human study, no major adverse events were detected that could have been related to this characteristic of nucleoside analogues, but long-term studies will be needed to clarify this further.

In conclusion, entecavir can be given safely for a short period of time and causes pronounced reduction in HBV DNA levels with a slower rebound after stopping therapy than has been reported for lamivudine. Entecavir should be studied in longer-term dosing trials to be able to evaluate more definitively its effect on viral replication and cccDNA, and ultimately cure of chronic hepatitis B infection.

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