

RESEARCH ARTICLE

Results of Entecavir treatment in patients with chronic hepatitis B

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ABSTRACT

Objective: This study was designed to determine the efficacy and safety of Entecavir (ETV) after 96 weeks treatment in patients with chronic viral hepatitis B (CHB).

Methods: Thirty-eight patients were included into the study. The criteria for starting ETV treatment were as follows: elevated ALT levels >upper limit of normal (ULN) two times, with HBV-DNA levels $\geq 5 \log_{10}$ copies/ml (≥ 20000 IU/mL), in HBe Ag positive patients, $\geq 4 \log_{10}$ copies/ml (≥ 2000 IU/mL) in HBe Ag negative patients and liver damage was confirmed by histopathology (Knodell HAI ≥ 4 or fibrosis ≥ 1). Patients were followed up every 12 weeks by virological and biochemical tests.

Results: Twenty-four of 38 patients (63.2%) were male. Mean age of patients were 38.6 years, 14 of them were HBeAg positive (36.8%). At baseline, median ALT level was detected as 106.7 IU/ml, median HBV DNA levels were 4.8×10^7 copy/ml, and mean Knodell HAI score was nine.

Eleven of 14 HBe Ag positive patients (78.6%) were treatment-naïve. No resistance mutation was determined during treatment. Biochemical responses (BR) at 48th and 96th week were 100% and virologic response (VR) were 57.1%, and 50%, respectively. Serological response (SR) at 48th and 96th weeks were 35.7% and 42.8% respectively.

Fifteen (62.5%) of 24 HBe Ag negative patients were treatment-naïve; two patients were detected to have lamivudine resistance mutation. At 48th and 96th week, BR was 95.8%, and 100%, respectively; and VR were 83.3% both.

Conclusion: In our study, virologic response was significantly high after two years of therapy with Entecavir in HBe Ag negative patients. *J Microbiol Infect Dis 2013;3(4): 176-180*

Key words: Chronic hepatitis B, Entecavir, therapy

Kronik hepatit B hastalarında Entecavir tedavi sonuçları

ÖZET

Amaç: Bu çalışmada kronik hepatit B hastalarında 96 haftalık Entekavir (ETV) tedavi etkinliğinin ve güvenliğinin değerlendirilmesi amaçlandı.

Yöntemler: Kronik Hepatit B tanısıyla 38 hasta çalışmaya alındı. Entekavir tedavisine başlama kriterleri: yükselmiş ALT düzeyleri, (>normalin iki katında fazla), HBe Ag pozitif hastalarda HBV DNA $\geq 5 \log_{10}$ kopya/ml (≥ 20000 IU/ml), HBe Ag negatif hastalarda $\geq 4 \log_{10}$ kopya/ml (≥ 2000 IU/ml) karaciğer hasarının histopatolojik olarak gösterilmesi (Knodell HAI ≥ 4 , fibrozis ≥ 1). Hastalar 12 haftada bir biyokimyasal ve virolojik testlerle takip edildi.

Bulgular: Hastaların 24'ü (%63,2) erkekti. Yaş ortalaması 38,6 (17-67) yılı. Tüm hastaların 14'ünde (%36,8) HBe Ag pozitif. Başlangıç ortalama ALT düzeyleri 106,7 IU/ml, HBV DNA düzeyleri $4,8 \times 10^7$ kopya/ml ve Knodell HAI skoru dokuz idi. HBe Ag pozitif hastaların 11'i (%78,6) naivdi. Hiçbirinde direnç mutasyonu yoktu. Biyokimyasal yanıt (BY) 48. ve 96. haftalarda %100, virolojik yanıt (VY) sırasıyla %57,1 ve %50 idi. Serolojik yanıt (SY) sırasıyla %35,7 ve %42,8 olarak saptandı. HBeAg negatif hastaların 15'i (%62,5) naivdi; iki hastada lamuvidin direnç mutasyonu saptandı. Kırksekizinci ve 96. haftalarda sırasıyla BY %95,8 ve %100, VY %83,3 saptandı.

Sonuç: Çalışmamızda iki yıllık entekavir tedavisi sonucunda, özellikle HBe Ag negatif hastalarda tedaviye yüksek oranda yanıt sağlanmıştır.

Anahtar kelimeler: Kronik hepatit B, entekavir, tedavi

INTRODUCTION

Infection of HBV virus remains an important public health problem. Approximately 350 million people worldwide are chronically infected and each year is responsible for more than one million deaths from HBV associated complications.^{1,2} Therefore the principal goal of CHB therapy is to suppression of HBV replication in order to preventing progression to decompensated cirrhosis and hepatocellular carcinoma.³⁻⁵

During the last two decades much progress has been made in treatment CHB, oral nucleos(t)ide analogs have been used as a new antiviral agents. Currently available antiviral treatment for CHB includes two immunomodulatory agents (interferon alpha-IFN- α , pegylated interferon (peg-IFN), and five nucleos(t)ide analogs (lamivudine-LAM, adefovir dipivoksil-ADV, telbivudine, Entecavir (ETV), and tenofovir disoproksil fumarat (TDF). Among the nukleos(t)ide analogs, ETV and TDF are the most effective agents since these two drugs have shown superior virologic, biochemical, and histologic response and lower rate of developing drug resistant mutans.^{6,7} Entecavir was introduced in 2007 as a new antiviral agent in Turkey.

Entecavir is a potent and selective guanosine analogue with significant activity against hepatitis B virus (HBV) which blocs HBV replication at three essential steps: priming of the HBV polymerase, elongating of the DNA strand via reverse transcription, and DNA dependent plus- strand DNA synthesis and polymerization.^{8,9} ETV has been shown to have a high genetic barrier to resistance through five years of treatment has been reported to be 1.2% and has been known to suppress serum HBV-DNA to undetectable levels in 67%, 80% and 82% of patients after^{1,2} and 3 years of therapy respectively.^{10,11}

The current study was designed to determine the efficacy and safety of ETV after 96 weeks of treatment in patients CHB.

Patients and Methods

Thirty-eight patients with diagnosis of CHB who followed in our clinic between 2007-2010 (who were positive for hepatitis B surface antigen for at least six months) was included into the study. Patients who had history of alcohol ingestion, co-infection with chronic hepatitis C or human immunodeficiency virus, primary biliary cirrhosis, autoimmune diseases, cirrhosis, hepatocellular carcinoma were excluded.

The criteria for starting ETV treatment were as follows: elevated ALT levels > upper limit of normal (ULN) two times, with HBV-DNA levels $\geq 5 \log_{10}$ copies/ml (≥ 20000 IU/mL), in HBeAg positive patients, $\geq 4 \log_{10}$ copies/ml (≥ 2000 IU/mL) in HBeAg negative patients and liver damage was confirmed by histopathological liver examination (Knodell HAI ≥ 4 or fibrosis ≥ 1).

While 36 patients with no LAM/ADV resistance were given Entecavir at a dose of 0.5 mg/day for 96 week, two patients with LAM resistance received the drug as 1 mg/day for the same period.

According to Association for the Fight Against Viral Hepatitis 2009 criteria, biochemical response (BR) was defined as a decrease in serum ALT levels to within the normal range; virological response (VR) was defined as a negative in serum HBV-DNA levels at 48th and 96th week, and a serological response (SR) was defined as a loss of serum HBeAg.

Patients were followed up every 12 weeks. Liver biochemistry and AFP were measured at every follow-up. HBV-DNA levels and mutational analysis of the viral polymerase gene were determined at baseline and every 12 weeks.

Serum alanine transaminases (ALT) and aspartate transaminases (AST) were measured using an automated biochemistry analyzer. HBs Ag, anti HBs, HBe Ag, anti HBe, anti HBc, anti HIV, anti HCV, anti HDV were measured with ELISA (Liason, Diasory, Italy), HBV-DNA was quantified by real-time polymerase chain reaction (PCR) assay (COBAS TaqMan 48, Roche, Branchburg, NJ) which had a lower limit of quantification of 20 copies/ml. The viral mutational analysis was performed by an reverse hybridization (Inno-LIPA HBV DR v2, Innogenetics, Belgium) Necro-inflammation and fibrosis were assessed with the Knodell histology activity index (HAI) scoring system.

Statistical Analysis

Data are expressed as mean \pm standard deviation of the mean. Proportions were compared by chi-square analysis. Mean values of two groups were compared by the Student's t-test or by a nonparametric test if the data were not normally distributed. $P < 0.05$ was accepted as statistically significant.

RESULTS

A total of 38 patients treated with ETV included in the study. Twenty-four of 38 (63.2%) patients were male. Mean age of patients were 38.6 (range: 17-

67). Twenty six of patients (68.4%) have not received previous nucleos(t)ide analogue (NA) treatment (naïve); whereas 12 patients (31.6%) were previously received a NA. HBeAg positive patients constituted 36.8% of the study population. Before treatment only one person have normal aminotransferase level. At baseline, median ALT level was detected to be 106.7 IU/ml, median HBV DNA levels were 4.8×10^7 copy/ml, and mean Knodell HAI score was nine. ALT/AST ratio and HBV DNA levels of patients were evaluated at 48th and 96th week.

Eleven of HBe Ag positive patients (78.6%) were treatment-naïve, and 3 of them (21.4%) had received previously NA treatment. No resistance mutation was determined during treatment. Biochemical responses (BR) (ALT normalization) at 48th and 96th week were 100% and virological response (VR) (undetectable lowering of HBV DNA levels) were 57.1%, and 50%, respectively. Viro-

logical breakthrough occurred in one patient after treatment of 48th week. Serological responses (SR) (HBeAg seroconversion) at 48th and 96th week were 35.7% and 42.8% respectively.

Fifteen (62.5%) of HBeAg negative patients were treatment-naïve and nine of them (37.5%) were given NA treatment previously; two patients were detected to have lamivudine resistance mutation. At 48th and 96th week, BR was 95.8%, and 100%, respectively; and VR were 83.3% both (Table 1). Between two groups; virologic response at 96th week was significantly high in HBeAg negative group. Comparison of demographic characteristics, baseline laboratory analysis and ETV treatment results at 96th week in virologic response and non-response group has been shown at Table 2. At 96th week of the treatment serologic response of the group in which virological response has been sustained was significantly higher than the non-responsive group.

Table 1. Entecavir treatment results of HBeAg positive and negative groups

Variables	HBeAg (+) n (%)	HBeAg (-) n (%)	P value
Total	14 (36.8)	24 (63.2)	
Mean age (years)	30±13	43±8	0.001
Female	6 (42.9)	8 (33.3)	ns
Naïve	11 (78.6)	15 (62.5)	ns
LAM/ADV-resistant patient	0	2	
ALT (IU/mL)	86 ± 33	118 ± 70	ns
HBVDNA (log ₁₀ copies/ml)	$9 \times 10^7 \pm 5.1 \times 10^7$	$3 \times 10^7 \pm 3 \times 10^7$	<0.001
HAI (Knodell)	7.8±4.3	9.6±3.9	ns
Week 48 BR (%)	14 (100.0)	23 (95.8)	ns
Week 96 BR (%)	14 (100.0)	24 (100.0)	ns
Week 48 VR (%)	8 (57.1)	20 (83.3)	0.07
Week 96 VR (%)	7 (50.0)	20 (83.3)	0.03
Week 48 SR (%)	5 (35.7)	-	-
Week 96 SR (%)	6 (42.8)	-	-

Ns=not significant

DISCUSSION

The goal of CHB treatment is to improve survival by preventing disease progression to decomposed cirrhosis, and hepatocellular carcinoma and liver related death.

Major problems in use of increasingly available antiviral treatment options in chronic hepatitis B are rebound replication upon discontinuation of treat-

Table 2. Comparison of virological response and nonresponse group at 96th week

	Response, n (%)	Non-response, n (%)	P value
Total	27 (71.1)	11 (28.9)	
Mean age(years)	40 ± 10	34 ± 15	ns
Female	12 (44.4)	2 (18.2)	ns
Naïve	18 (72.7)	8 (66.7)	ns
ALT(IU/mL)	115 ± 65	84 ± 42	ns
HBVDNA (log ₁₀ copies/ml)	$4 \times 10^7 \pm 5 \times 10^7$	$6 \times 10^7 \pm 4 \times 10^7$	ns
HAI (Knodell)	8.9 ± 4.0	9.0 ± 4.5	ns
Hbe Ag (+)	7 (25.9)	7 (63.6)	0.03
Week 48 VR (%)	27 (100)	1 (9.1)	-
Week 96 BR (%)	27 (100.0)	-	-
Week 96 SR (%)	5 (71.4)	1 (14.3)	0.03

ns=not significant

ment and resistance issues. Previous studies have shown that Entecavir had lowest level of antiviral resistance compared to LAM, ADV, and telbivudine. Yet, since resistance to LAM and ETV may develop through similar mechanisms, use of Entecavir in LAM-resistant CHB was found to be suspicious.^{12,13}

Long-term treatment is required in HBeAg negative CHB patients in order to provide remission

of liver disease and viral suppression. Though pegylated interferon alpha offers efficacy, it has limited use due to its unwanted side effect profile.

In studies performed in naïve CHB patients, Hadziyannis et al., after a year of Entecavir treatment, found virological response as 90% and biochemical response as 78% in HBe Ag negative patients and 67% and 68% in HBeAg positive patients, respectively.¹⁴ In a study performed by Chang et al. comparing Entecavir and LAM treatment in naïve HBe Ag positive patients, virological response was detected as 67% and 36% in Entecavir and LAM arms, respectively; and biochemical response was found as 68% and 60% in Entecavir and LAM groups, respectively. Furthermore, HBe seroconversion rate was 21% in Entecavir, which was 18% in LAM patients.¹⁵ The study by Lai et al. where Entecavir and LAM were compared in naïve HBe negative CHB patients revealed that virological response were 90% and 72% in Entecavir and LAM groups; whereas biochemical response were 78% and 71% in these groups, respectively.¹⁶ In our study comprising of CHB cases treated with Entecavir for two years, virological response rates were detected to be higher in HBe Ag negative patients, compared to HBe Ag positive patients. We found the VR rates at HBe Ag positive and negative patients at week 48th 57%, 83.3%, at week 96th 50%, 83%, respectively. Biochemical response rates were determined to be high in both groups. All patients included into the study, serological response rates at the end of 96 week was 42.8%, most of which consisted of naïve HBe Ag positive patients. In contrast to previous studies, SR rates of our study were found to be higher.

Moreover, ETV studies have shown that possibility of a successful outcome after long-term treatment of Entecavir was low in LAM-resistant cases. In the study of Hadziyannis et al, while 3-year success of Entecavir was 94% in naïve patients, it was detected to be 40% in LAM-resistant cases. Same study revealed that resistance to Entecavir after 3 years was <1% in naïve patients although being 6% at the end of year 1 and 30% at the end of year 3 in LAM-resistant cases.¹⁴ Nagasaki et al. reported that 3 of 4 LAM-resistant cases developed resistance at 52nd to 130th weeks after Entecavir treatment.¹³ A German study by Tillmann et al reported that nine percent of LAM-resistant cases developed resistance to Entecavir after 96 week.¹⁷ In a multicenter study consisting of numerous LAM-resistant HBeAg positive CHB cases, virological and biochemical response rate was 4% (6/145) in the group continued to be treated with LAM for 52 weeks whereas

it was detected to be 55% in those subjects who received Entecavir at a dose of 1 mg/day. While genotypic resistance against Entecavir was detected in ten of 141 patients, 2 of them exhibited virological rebound.¹⁸ In our study, Entecavir treatment of 1 mg was given to 2 patients who were confirmed to have genotypic LAM-resistance, where virological response after two years of treatment was achieved in only one patient. In our study, the treatment with Entecavir resulted in increased rates of virologic suppression, especially in HBeAg negative patients. This result was found to be consistent with previous studies. In addition, HBeAg seroconversion rate was 42.8% in HBeAg positive patients, which is higher than previous studies.¹⁹⁻²¹ In conclusion, further studies with longer duration and larger numbers of subjects are needed to evaluate longer term efficacy, development of resistance, and adverse effects of Entecavir.

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