The role of entecavir in the treatment of chronic hepatitis B

Evangelini Dimou
Vasilios Papadimitropoulos
Stephanos J Hadziyannis

Department of Medicine and Liver Unit, Henry Dunant Hospital, Athens, Greece

Abstract: Entecavir (ETV) is a potent and selective inhibitor of hepatitis B virus replication. In HBeAg-positive and HBeAg-negative lamivudine-naïve patients with chronic hepatitis B (CHB), treatment with ETV at a dose of 0.5 mg daily is associated with a more potent viral suppression, a higher rate of biochemical remission and a greater improvement of liver histology compared to Lamivudine (LAM). After 3 years of ETV treatment, the majority of patients (94%) may achieve serum HBV DNA levels undetectable by sensitive PCR assays. ETV treatment of patients with LAM-resistant HBV mutants requires a higher daily dose of 1 mg yet, potent HBV suppression at 3 years is achieved only in 40% of them while the cumulative rate of genotypic HBV resistance increases from 6% in the first year to >30% in year 3. ETV resistance of HBV is rare in lamivudine-naïve patients with a reported rate of <1% after three years of treatment. In conclusion, ETV is a very potent anti-HBV drug with a high genetic barrier to resistance, highly effective in lamivudine-naïve CHB patients and most promising for their long-term treatment but not very suitable for CHB patients harboring LAM–resistant HBV mutants.

Keywords: entecavir, chronic hepatitis B, nucleoside analogue, HBV resistance

Introduction

Chronic hepatitis B virus (HBV) infection is a major health problem, affecting hundreds of millions of people worldwide. It may progress to cirrhosis and hepatocellular carcinoma (HCC) and is responsible for almost 1 million deaths annually (Lee 1997; Maddrey 2000). Eradication of HBV infection with currently available therapies is not really possible. Resolution of the infection, as indicated by HBsAg loss and development of anti-HBs, is also extremely rare. Thus, the most realistic goal of hitherto approved therapies for chronic hepatitis B (CHB) is potent and durable suppression of viral replication aiming at stop of progression and remission/regression of the underlying liver disease (Di Marco et al 2004; Liaw et al 2004). More specifically, in patients with HBeAg-positive CHB the end point and goal of treatment is HBeAg seroconversion, while in patients with HBeAg-negative CHB is durable suppression of HBV to HBV DNA levels undetectable by real time PCR assays (Lok et al 2001).

Six drugs have been approved for the treatment of chronic hepatitis B, standard interferon-α (IFN-α), the Pegylated form (PEG)-IFN-α-2a, and the nucleos(t)ide analogues Lamivudine (LAM), Adefovir Dipivoxil (ADV), Entecavir (ETV) and most recently Telbivudine (LdT).

Interferon-alpha

Interferon-alpha is the first drug approved for the treatment of CHB. In HBeAg-positive CHB finite course of treatment with standard IFN-α has achieved HBeAg loss in 33% of treated patients vs 12% of placebo-treated controls, p = 0.0001 (Wong et al 1993), with responses being durable after stopping treatment (Niederaou et al 1996). In HBeAg-negative CHB, long-term biochemical remission

Correspondence: Stephanos J Hadziyannis
Department of Medicine and Liver Unit, Henry Dunant Hospital, 107 Messogion Ave, 11526 Athens, Greece
Tel +30 210 697 2937
Fax +30 210 697 2974
Email hadziyannis@ath.forthnet.gr

has been reported in 15%–25% of patients treated for 6 or more months with a significant percentage of them (31.6%) also loosing HBsAg (Hadziyannis et al 1990; Manesis and Hadziyannis 2001; Papatheodoridis et al 2001). On the other hand PEG-IFN-α-2a either alone or in combination with LAM in HBeAg-positive CHB, achieved similar rates of HBeAg seroconversion in 32% and 27% of patients, respectively and both regimens were superior to LAM monotherapy (32% vs 19%, $p < 0.001$ and 27% vs 19%, $p = 0.02$) (Lau et al 2005) (see also Figure 1). In HBeAg-negative CHB, PEG-IFN-α-2a alone or in combination with LAM achieved similar rates of virologic remission (HBV-DNA < 20.000 cp/mL) in 43% and 44% of patients, respectively and again the two regimens were clearly superior to LAM monotherapy (29%, $p = 0.007$ and $p = 0.003$, respectively) (Marcellin et al 2004). Furthermore, follow-up of patients, 2 years after discontinuation of treatment has shown that the response appears to be durable (Marcellin et al 2006). Treatment with PEG-IFN-α-2a is probably superior to standard IFN-α, but there is no comparative study between the two forms of interferon-α applied in courses of the same duration. Although treatment with IFN-αs has many adverse events and the disadvantage of subcutaneous administration, these are the only available drugs that can achieve HBsAg seroconversion in finite courses of treatment of less than one year duration (Wong et al 1993; Marcellin et al 2004; Lau et al 2005).

In view of the above data it is clear that both in HBeAg-positive and HBeAg-negative patients with chronic hepatitis B pegylated IFN-α2a monotherapy in finite courses of 48 weeks duration, achieves higher rates of responses sustained after their discontinuation compared to LAM monotherapy.

Lamivudine

Lamivudine, the first licensed nucleoside analogue (Lai et al 1998; Dienstang et al 1999), may achieve HBeAg seroconversion, in 18% of HBeAg-positive CHB patients at the first year and in 27% and 40% at the second and third year respectively (Lai et al 1998; Dienstang et al 1999; Liaw et al 2000; Leung et al 2001). In HBeAg-negative CHB, LAM therapy for one year resulted in undetectable serum HBV DNA in 65% of patients (Tassopoulos et al 1999). The high rate of virologic response at one year is not sustainable after discontinuation of therapy, while prolongation of treatment is associated with decreasing rates of virologic response due to the emergence of LAM-resistant HBV mutants in progressively increasing rates reaching levels above 60% in 4 to 5 years time (Lai et al 1998; Dienstang et al 1999; Liaw et al 2000; Leung et al 2001; Papatheodoridis et al 2002; Lok et al 2003). The development of viral resistance may be associated with worsening of liver histology, exacerbation of liver disease and even with liver failure and deaths especially in patients with cirrhosis (Dienstag et al 2003; Lok et al 2003; Di Marco et al 2004; Liaw et al 2004; Papatheodoridis et al 2005).

Adefovir dipivoxil

Adefovir dipivoxil, a nucleotide analogue, the second licensed oral anti-HBV agent, is effective in the treatment of both wild type and LAM-resistant HBV (Hadziyannis et al 2003; Marcellin et al 2003; Peters et al 2004).

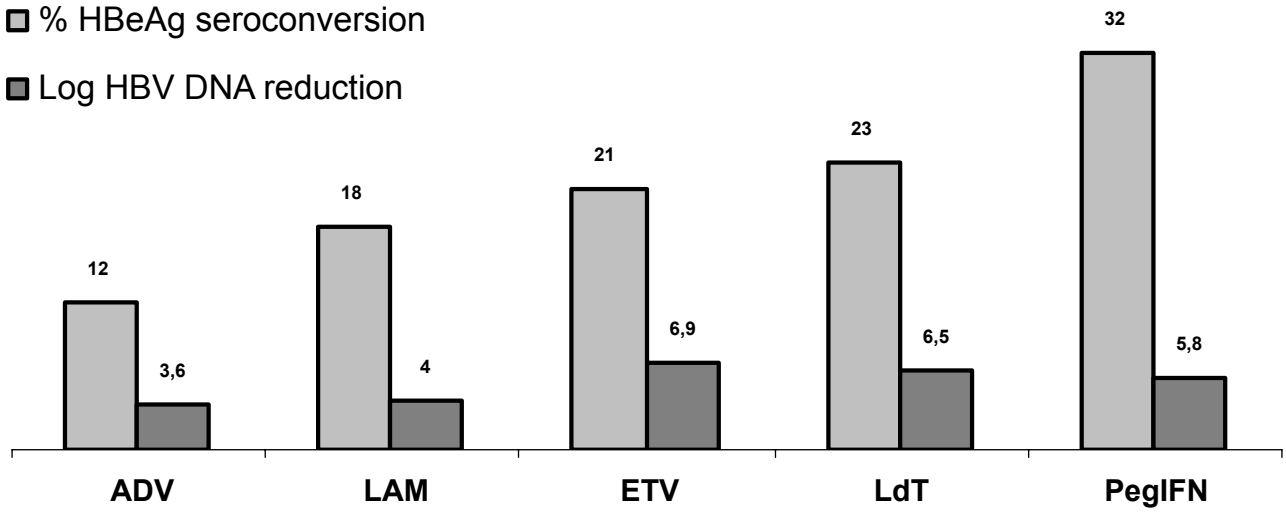

Figure 1 Comparison of responses to treatment (HBeAg seroconversion and log reduction of serum HBV DNA) of HBeAg positive chronic hepatitis B.
Lau et al. 2005. *N Engl J Med*, 352:2682–95. Dienstag et al. 1999. *N Engl J Med*, 341:1256–63. Marcellin et al. 2005. *EASL*, Abstract 73. Lai et al. 2005. *AASLD*, Abstract 72404.(LB1) Chang et al. 2004 *AASLD*, Abstract 70. Telbivudine package insert. Marcellin et al. 2003. *N Engl J Med*. 348:808–16. Chang et al. 2006. *N Engl J Med*. 354:1001–10.

In HBeAg-positive CHB, HBeAg seroconversion was achieved in 12% of patients at the first year, increasing to 29% and 43% at the second and third year of therapy respectively (Marcellin et al 2003, 2005). In HBeAg-negative CHB, long-term ADV therapy is associated with undetectable serum HBV DNA in 71%, 79%, 65% and 67% at year 2, 3, 4 and 5, respectively (Hadziyannis et al 2003, 2005a, 2005b, 2006). ADV resistance is delayed and infrequent developing after the first year of treatment. Genotypic HBV resistance may emerge in 3%, 11%, 18% and 29% of patients at years 2, 3, 4 and 5 of treatment, respectively (Hadziyannis et al 2003, 2005a, 2005b, 2006). However, virologic and biochemical breakthroughs at year 5 are restricted only to 16% and 11% respectively (Hadziyannis et al 2006). In patients with LAM-resistant HBV strains, treatment with ADV alone or in combination with LAM has similar rate of virological (26% vs 35%) and biochemical response (47% vs 53%) at one year (Peters et al 2004). However, longer duration of treatment has shown that adding adefovir on lamivudine rather than switching from lamivudine to adefovir represents the treatment of choice in patients developing LAM-resistant HBV mutants (Fung et al 2005, 2006; Lampertico et al 2006; Rapti et al 2007).

Telbivudine

Telbivudine (LdT, β-L-2'-deoxythimidine, Idenix, Cambridge, MA) is an L nucleoside analogue of thymidine, approved by FDA on late 2006 for the treatment of chronic hepatitis B under the trade name of Tyzeka at a daily dose of 600 mg. It is a reverse transcriptase inhibitor and acts by competing with the natural substrate thymidine 5' triphosphate and its incorporation into viral DNA causes chain termination, resulting in inhibition of HBV replication. It is a potent anti-HBV agent inhibiting the synthesis both of the first and the second DNA strand of the virus and in phase II studies it has been found to be more effective than lamivudine. In HBeAg-positive patients it achieved a greater reduction in HBV DNA levels (6.01 log10 vs 4.57 log10, $p < 0.05$) as well as higher rates of HBV DNA undetectability (61% vs 32%, $p < 0.05$) and normalization of ALT levels (86% vs 63%, $p < 0.05$) (Lai et al 2004; Lai et al 2005a). The year 1 and 2 results of a very large registration trial in 1367 individuals with HBeAg-positive and -negative CHB, the GLOBE trial, have been reported in the 2005 AASLD (Lai et al 2005b) and the 2006 EASL (Thongsawat et al 2006) and AASLD (Lai et al 2006a) Meetings. The following important observations came out from the analyses of the results at weeks 52 and 104 of this pivotal LdT study:

1. LdT is equally safe but more effective than lamivudine in terms of absolute HBV DNA reduction from baseline (HBeAg-positive: 6.5 log10 vs 5.5 log10 at week 52, $p < 0.05$ and 5.7 log10 vs 4.4 log10, $p < 0.05$, at week 104, respectively, HBeAg-negative: 5.2 log10 vs 4.4 log10, $p < 0.05$ at week 52 and 5 log10 vs 4.2 log10, $p < 0.05$ at week 104, respectively) and time of undetectability. However there is no significant difference from LAM in HBeAg loss and seroconversion to anti-HBe (26% vs 23% at week 52, 35% vs 29% at week 104 and 22% vs 21% at week 52, 30% vs 25% at week 104, respectively) (Lai et al 2006b).
2. Treatment failures and HBV resistance are less frequent with LdT compared to LAM (treatment failure in HBeAg-negative patients 0.9% vs 7.6%, $p < 0.001$ and in HBeAg-positive 6.8% vs 18.8%, $p < 0.001$, respectively) (Lai et al 2006b).
3. Both in LdT and LAM treated HBeAg-positive patients, the rates of HBeAg seroconversion are significantly increased if a profound and rapid HBV suppression to undetectability of serum HBV DNA at week 24 is achieved, this effect being also associated with enhanced T-cell reactivity to HBc protein (Cooksley et al 2006).

Entecavir

Entecavir, a novel carbocyclic analog of 2' deoxyguanosine (Figure 2), has been approved in 2005 in USA and in 2006 in Europe for naïve and lamivudine-resistant chronic hepatitis B treatment. Data on its safety, side effects, short- and long-term efficacy and HBV resistance in the various subsets of HBV patients are critically reviewed in this article.

In Figure 1 the HBeAg response rates achieved with one year of therapy by ETV, the other three approved nucleos(t)ides and pegylated interferon-alfa2a together with their potency in terms of log reduction in HBV DNA levels are shown in a comparative way.

Entecavir in chronic hepatitis B

Entecavir therapy in treatment-naïve patients with chronic hepatitis B

The safety and efficacy of ETV were initially evaluated in a randomized, placebo-controlled, dose-escalating study (De Man et al 2001). In patients with CHB, ETV was administered at doses of 0.05 mg, 0.1 mg, 0.5 mg and 1 mg and compared to placebo for 28 days (De Man et al 2001). All patients were followed-up 24 weeks off therapy. All doses of ETV were well tolerated and were associated with a significant decline in serum HBV DNA levels. However, a slower

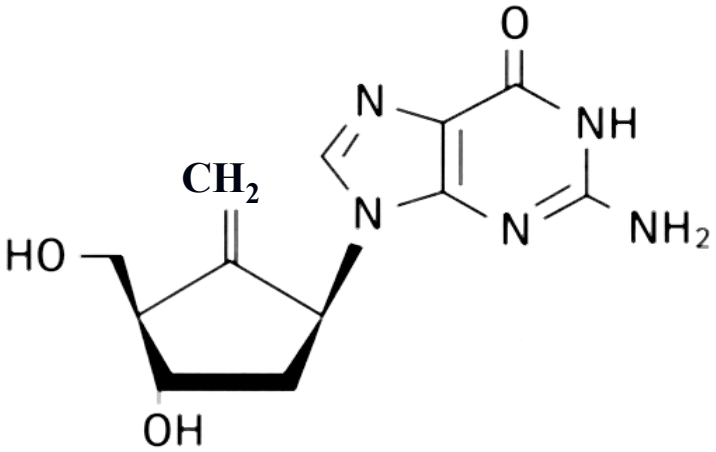

Figure 2 The chemical structure of entecavir.

rebound of viremia in the post treatment follow-up period was observed with the 0.5 and 1 mg of ETV compared with the other two lower doses ($p < 0.05$) (De Man et al 2001). During the post-treatment period, 9% of ETV treated patients experienced hepatic flares defined as elevation in ALT greater than twice the baseline level and more than 10 times the upper limit of normal. None of these flares were associated with elevated bilirubin levels and were not clinically significant. This study has showed that ETV is a potent inhibitor of HBV in humans, but longer duration dosing trials should be performed before definite conclusions about the safety and the role of ETV in the treatment of chronic hepatitis B infection are made.

In another double-blind randomized study, the safety and efficacy of 3 doses of ETV were evaluated and compared to LAM in patients with CHB (Lai et al 2002). Entecavir doses of 0.01 mg, 0.1 mg, 0.5 mg and LAM 100 mg daily were administered for 24 weeks. Complete response was defined as undetectable serum HBV DNA by bDNA (cut-off 0.7 Meq/mL) at week 22, with normal ALT and undetectable HBeAg for HBeAg-positive patient at baseline. Treatment was discontinuated in individuals with complete response and they were followed-up for 12 weeks. Partial response was defined as undetectable serum HBV DNA by bDNA assay at week 22, but without loss of HBeAg for HBeAg-positive patients or with elevated ALT for HBeAg-negative patients at baseline. In these patients, LAM 100 mg were given for 48 weeks. The nonresponders (detectable HBV DNA by bDNA) were managed after week 24 by their physician and followed-up for 12 weeks. One hundred sixty-nine patients (81% HBeAg-positive) completed 24 weeks of treatment period. At the end of therapy a significantly higher proportion (83.7%) of patients receiving 0.5 mg of ETV had undetectable HBV DNA levels (bDNA < 0.7Meq/mL) compared to LAM 100 mg (57.5%, $p = 0.008$), while the response to the ETV 0.1 mg dose was similar with LAM (61.8% vs 57.5%). Serum HBV DNA undetectable by the b-DNA assay and ALT normalization were observed in 23.1% and 50%, 61.8% and 83.3%, 83.7% and 69%, 57.5% and 59.1% at the 0.01, 0.1, 0.5 mg ETV and the 100 mg LAM, doses respectively (Lai et al 2002). Complete or partial response occurred in 23%, 62%, 84% and 57% of patients in the 0.01 mg, 0.1 mg and 0.5 mg ETV and LAM group, respectively. After discontinuation of treatment, ALT elevation $>$ 3xULN was observed in 21% of patients in the 0.01 mg ETV group compared to 10.5% in the LAM group and in 3% and 4.5% in the 0.1 and 0.5 mg ETV groups respectively. On the basis of the results of this study it was considered that the optimal dose of ETV for the treatment of naïve patients should be 0.5 mg daily (Lai et al 2002).

Efficacy in HBe-positive patients

In a randomized phase III double-blind trial, 715 treatment-naïve, HBeAg-positive (+) CHB patients were assigned to receive either 0.5 mg of ETV or 100 mg of LAM once daily for a minimum period of 52 weeks (Chang et al 2006a). According to the study protocol clinical-management decisions were made at week 52 on the basis of the results of HBV DNA levels (b-DNA) and HBeAg assays on serum samples obtained at week 48. Patients who had a complete response defined as undetectable serum HBV DNA levels (< 0.7 Meq/mL) and HBeAg loss and the nonresponders defined by serum HBV DNA levels ≥ 0.7 Meq/mL discontinued treatment at week 52. Patients who achieved only virologic response [undetectable serum HBV DNA levels (<0.7 Meq/mL), without HBeAg loss] were continued on therapy up to 96 weeks

(Chang et al 2006a). At week 48, histologic improvement (necroinflammation score) occurred in significantly higher proportion of patients treated with ETV compared to LAM (72% vs 62%, p = 0.009). Serum HBV DNA levels became undetectable by PCR methods in 67% and 36% ($p < 0.001$) of the patients treated with ETV and LAM, respectively. Moreover, the mean reduction from baseline in the serum HBV DNA levels at week 48 was 6.9 log and 5.4 log in the ETV and LAM group respectively ($p < 0.001$). Although ETV achieved a significantly greater suppression of HBV DNA levels than LAM, the rate of HBeAg loss and HBeAg seroconversion did not differ significantly between the two treatment groups being 22% and 21% in the ETV vs 20% and 18% in the LAM arm (Chang et al 2006a, Table 1). At week 48, 21% of the patient in the ETV group and 19% of those in the LAM group had achieved a complete response, 70% and 46% respectively had only virologic response and 5% and 26% respectively had no response. Among patients with response at week 48, 82% in the ETV and 73% in the LAM group sustained their response 24 weeks after discontinuation of treatment. There was no evidence of emergence of ETV resistant variants among 339 evaluated patients and although 6 (2%) of ETV treated individuals experienced a virologic rebound during the first year of therapy, samples obtained from these patients retained full phenotypic susceptibility to ETV. The adverse events were similar in the two groups. In the ETV group 3% of patients experienced alanine aminotransferase flares (ALT levels more than twice the baseline level and more than 10 times the ULN) during treatment while such flares were observed in 6% of the patients in the LAM group. All flares in the ETV group were associated with a decline of HBV DNA levels by $\geq$ 2 log10 and all but one were self-limited with continuation of treatment, without any evidence of hepatic decompensation. On the other hand half of the flares in the LAM group were associated with increasing HBV DNA levels and in one patient hepatic decompensation developed. Post-treatment ALT flares were observed in 1% in the ETV group and in 7% of patients in the LAM group (Chang et al 2006a).

The efficacy of extension of ETV and LAM treatment to week 96 in those HBeAg-positive patients who had only a virological response at week 48 without HBeAg seroconversion, have also been evaluated (Gish et al 2005). During this period return of ALT to normal was achieved in 50% of the ETV and 42% of the LAM-treated patients. The cumulative virologic response rate at week 96 (Table 1), defined by HBV DNA <300copies/mL, was 80% in the ETV and 39% in the LAM group ($p < 0.001$). Despite the significantly greater suppression of HBV DNA in the ETV group, the cumulative HBeAg seroconversion rate did not differ significantly between the two treatment groups (Table 1) Furthermore, patients achieving only virologic response during the second year of therapy could receive double dose of ETV (1 mg) for at least one additional year. Thus 122 from 151 eligible HBeAg-positive patients were enrolled and evaluated at week 144. Serum HBV DNA <300copies/mL and ALT normalization were observed in 87% and 85% of patients respectively, while HBeAg loss and HBeAg seroconversion were achieved in 31% and 16% of them (Chang et al 2006b).

Efficacy in HBeAg-negative patients

In a randomized double-blind trial, treatment with ETV 0.5 mg was compared to LAM 100 mg once daily for at least 52 weeks (Lai et al 2006b). According to the study protocol, similarly with the trial in HBeAg-positive patients, clinical-management decisions were made at week 52 on the basis of the results of HBV DNA levels (b-DNA) and ALT levels on serum samples obtained at week 48. Response was defined as

Table 1 Comparison of entecavir vs lamivudine in HBeAg positive and HBeAg-negative treatment-naïve patients with chronic hepatitis B (CHB)

	HBeAg (+) CHB				**HBeAg (–) CHB**			
	LAM		**ETV**		**LAM**		**ETV**	
	Year 1	**Year 2**	**Year 1**	**Year 2**	**Year 1**	**Year 2**	**Year 1**	**Year 2**
Undetectable HBV DNA	36%	39%	67%	80%	72%	77%	90%	94%
ALT normal	60%	77%	68%	84%	71%	84%	78%	89%
HBeAg loss	20%	28%	22%	32%	NA	NA	NA	NA
HBeAg seroconversion	18%	25%	21%	31%	NA	NA	NA	NA
Histologic improvement	62%	NA	72%	NA	61%	NA	70%	NA
HBV resistance	13%	NA	0%	0%	6%	NA	0%	0%

Abbreviations: CHB, chronic hepatitis B; ETV, entecavir; LAM, lamivudine; ALT, alanine aminotransferase; HBV, hepatitis B virus; NA, non applicable; vs, versus.
(Gish et al 2005; Chang et al 2006a; Lai et al 2006b; Shouval et al 2006).

undetectable serum HBV DNA (<0.7Meq/mL by the b-DNA assay) and ALT levels below 1.25 times the upper limit of normal (ULN) and non response as a serum HBV DNA levels ≥0.7 Meq/mL. Treatment was discontinued at week 52 both in responders and non-responders. Patients with only virologic response (= undetectable serum HBV DNA levels <0.7 Meq/mL) and ALT levels ≥1.25xULN were offered continued therapy for up to 96 weeks. Histologic improvement (reduction of necroinflammation) occurred at week 48 in significantly more patients in the ETV than in the LAM group (70% vs 61%, p = 0.01). Undetectable serum HBV DNA levels by PCR and ALT normalization were observed in 90% and 78% and in 72% and 71% of patients treated with ETV and LAM, respectively (Lai et al 2006b). Eighty-five percent of patients in the ETV group and 78%, (p = 0.04) in the LAM group had responded at week 48 while 10% and 11% of each group had only virologic response. At the end of 24 weeks follow-up the response was sustained in 48% and 35% of the patients in the LAM and ETV group, respectively. There was no evidence of ETV resistance at week 48, in paired samples from 211 randomly selected patients in the ETV group but 5 (2%) of these patients experienced virologic rebound. Genotypic analysis from these patients revealed no emerging substitutions that confer resistance to ETV. Twenty-five patients (8%) in the LAM group had a virologic rebound during the treatment period (Lai et al 2006b). The adverse events between the two treatment groups were similar. Three patients in the ETV group experienced ALT flares, associated with a reduction of HBV DNA levels by at least 2 log10, that resolved spontaneously, while such flares occurred in 5 patients in the LAM group. In two of these 5 patients alanine aminotransferase flares were associated with a reduction of HBV DNA levels by at least 2 log10 and in the other 3 with increasing HBV DNA levels. One of the last experienced the development of ascites. Post-treatment ALT flares occurred in 8% of the patients in the ETV and in 11% in the LAM group (Lai et al 2006b).

Twenty-six patients in the ETV and 28 in the LAM group continued therapy up to week 96 (Shouval et al 2006). The cumulative virologic response through week 96 was 94% and 77%, ($p < 0.0001$) among ETV and LAM treated patients respectively, while biochemical response occurred in 89% and 84% of them (Shouval et al 2006).

In Table 1 the response rates at year 1 and 2 in HBeAg-positive and HBeAg-negative patients treated by ETV or LAM are depicted. It is obvious that ETV is superior to LAM in terms of liver histology improvement, HBV DNA suppression, ALT normalization and the viral resistant strains development rate. Moreover despite the higher rate of HBV DNA suppression in ETV group the rate of HBeAg seroconversion was similar between the two groups for the treatment period.

Data from the above studies also indicated that response rate to ETV did not differ between patients with and without cirrhosis on liver biopsy (Schiff et al 2005). Although the number of cirrhotic patients was small in the two studies, histologic improvement occurred in a similar rate between patients with or without cirrhosis on liver biopsy at baseline, 76% vs 72% in HBeAg-positive and 74% vs 70% in HBeAg-negative patients, respectively (Schiff et al 2005).

Efficacy of entecavir in patients with lamivudine- and adefovir-resistant HBV mutants

The efficacy and safety of ETV in LAM-resistance patients were initially evaluated in a double-blind randomized dose-ranging trial. In this study, 182 patients with HBeAg-positive and -negative CHB and LAM-resistant HBV were enrolled and treated with 3 different daily doses of ETV (0.1 mg, 0.5 mg and 1 mg) in comparison to LAM 100 mg daily (Chang et al 2005). A significant higher proportion of patients in the ETV 1 mg (79%) and 0.5 mg (51%) groups achieved serum HBV DNA levels < 0.7 Meq/mL (by bDNA assay) compared to the LAM group (13%, $p < 0.0001$), after 24 weeks of treatment (Chang et al 2005). Moreover the proportion of patients with undetectable serum HBV DNA by PCR assay was higher in ETV 1 mg (26%) and 0.5 mg (26%) groups compared to LAM (4%, $p < 0.01$) at 48 weeks of treatment. HBeAg seroconversion was achieved in a minority of HBeAg-positive patients in all treatment groups (Chang et al 2005). The rate of biochemical remission at week 48 was superior in ETV 1 mg (68%) and 0.5 mg (59%) than in LAM (6%, $p < 0.001$). In this study superiority of ETV at dose of 1 mg for the treatment of LAM-resistant patients was revealed. At baseline, 87% of the patients had lamivudine resistance-associated substitutions and at week 48, 80% of them retained this substitution, regardless of treatment group. Entecavir-associated resistance substitutions (rtT184, rtS202 and rtM250) were detected in 6 patients at baseline and emerged in 2 entecavir-treated patients (1 receiving 0.5 mg and 1 receiving 0.1 mg) during treatment period, but only one patient experienced viral rebound at week 48 (Chang et al 2005). Viral rebound was also observed in 5 ETV treated patients, but genotypic analysis could not reveal any entecavir-resistance mutations (Chang et al 2005).

In a randomized phase III trial, in HBeAg-positive CHB patients with LAM-resistant HBV, ETV 1 mg daily was compared with LAM 100 mg daily for at least 52 weeks (Sherman et al 2006). In this study clinical management decisions were again made at week 52, based on week 48 results. Responders defined by serum HBV DNA levels < 0.7 Meq/mL by bDNA assay and HBeAg loss at week 48 and nonresponders (HBV DNA levels > 0.7 Meq/mL) discontinued treatment and followed-up for 24 weeks period. Patients with only virologic response at week 48 as defined by serum HBV DNA levels < 0.7 Meq/mL by bDNA assay, without HBeAg loss, continued study medication until week 96 (Sherman et al 2006). At week 48, histologic improvement observed in a higher proportion of patient in the ETV than in LAM group (55% vs 28%, $p < 0.001$) (Table 2). Moreover the rates of virological response (by PCR assay) and return of ALT to normal were higher in the ETV than in the LAM treated patients, 19% vs 1%, ($p < 0.0001$) and 61% vs 15%, ($p < 0.0001$), respectively. The rate of HBeAg seroconversion did not differ between the two groups (ETV group 8% and LAM group 3%, $p = 0.06$). At baseline 18 (6%) of lamivudine-refractory patients had substitutions that confer resistance to lamivudine and entecavir. Genotypic analysis of paired baseline and week 48 samples were performed for 134 of the 141 ETV-treated patients and reveled 7 patients with mutations associated with ETV resistance at baseline. None of these patients exhibited virologic rebound during the first year of therapy. Two other patients (1.4%) experienced a virologic rebound during ETV-treatment and genotypic analysis revealed mutations that confer resistance to ETV (Sherman et al 2006).

A number of patients in the ETV and the LAM groups continued treatment for a second year (Yurdaydin et al 2006). The cumulative confirmed virologic, serologic (HBeAg seroconversion) and biochemical response at week 96 in the ETV and LAM groups were 30% and 1%, ($p < 0.0001$), 16% and 4%, ($p = 0.0011$) and 85% and 29%, ($p < 0.0001$), respectively. Nine percent of the ETV treated patients exhibited viral rebound due to ETV resistant mutations (Yurdaydin et al 2006).

After several years of use of adefovir dipivoxil, HBV resistance to this compound has been recognized among patients under long-term ADV monotherapy particularly after the second year of treatment with genotypic ADV resistance reaching 29% at year five. In vitro studies have shown that ETV inhibits effectively the replication of ADV resistant HBV mutants (Brunelle et al 2005). However, clinical studies on the efficacy of ETV in patients with ADV-resistant HBV mutants have not been performed yet, but in one recent report ETV treatment in two patients with ADV resistance showed a ≥3 log10 decline of HBV DNA levels after 6 months of therapy (Fung et al 2005).

Table 2 Comparison of entecavir vs lamivudine in HBeAg positive lamivudine-resistance patients with chronic hepatitis B (CHB)

	Week 48		Week 96	
	ETV	LAM	ETV	LAM
Undetectable HBV DNA	19%	1%	30%	1%
ALT Normal	61%	15%	85%	29%
HBeAg seroconversion	8%	3%	16%	4%
Histologic improvement	55%	28%	NA	NA
ETV resistance	6%	NA	9%	NA

Abbreviations: ETV, entecavir; LAM, lamivudine; ALT, alanine aminotransferase; NA, non applicable; vs, versus.
(Sherman et al 2006; Yurdaydin et al 2006)

Entecavir resistance

Entecavir resistance was first been identified in two patients with LAM-resistant strains, who experienced virologic breakthrough after more than 1 year of ETV therapy (Tenney et al 2004). Genotypic analysis of the polymerase region before the initiation of ETV treatment, had shown substitutions conferring HBV resistance to LAM therapy: rtL180M, rtM204V, rtV173L in both patients. Genotypic analysis of the polymerase region after virologic breakthrough under ETV treatment showed that in addition to the LAM-resistance substitutions, the unique substitutions rtI169T (domain B) and rtM250V (domain E) in the first patient and the rtS184G (domain B) and rtS202I (domain C) in the second one emerged (Tenney et al 2004). Phenotypic analysis of recombinant HBV genomes, containing patients' RT domains or specific mutations, was performed. Substitution rtI169T alone or in combination with LAM-resistance substitutions did not decrease the susceptibility to ETV. Substitution rtM250V alone conferred a low level of ETV resistance, while in combination with LAM-resistance substitutions, a >1,000-fold reduction in ETV susceptibility was observed. Substitutions rtS184G and rtS202I alone did not confer resistance to ETV, while either substitution in combination with LAM-resistance substitutions slightly reduced the susceptibility to ETV. HBV containing all 4 substitutions rtL180M, rtM204V, rtS184G and rtS202I exhibited the highest level

of resistance (>1,000-fold reduction in ETV susceptibility) (Tenney et al 2004). It is obvious that the pattern of ETV-resistance is more complicated than LAM or ADV resistance because 3 or 4 substitutions in the polymerase region are required. Moreover, LAM-resistance substitutions are necessary for the development of ETV resistant mutant. Until now three patterns of ETV resistance have been detected in LAM resistant strains: (1) substitutions rtI169T and rtM250V, (2) substitutions rtS184G and rtS202I and (3) substitutions rtS202G (Villet et al 2005).

In vitro studies have shown that ETV-resistant strains have no effect on the susceptibility to ADV. In 2 patients with ETV-resistance, ADV was administered in a dose of 10 mg daily with marked reduction in the viral load (Tenney et al 2004). Thus, on clinical grounds ADV seems quite effective in patients with HBV mutants resistant to ETV and is probably the treatment of choice.

No evidence of resistance up to week 96 has been reported among ETV-treated nucleoside naïve patients (Gish et al 2005; Chang et al 2006a; Colonno et al 2006a; Lai et al 2006b; Shouval et al 2006). However, data from a recent study of 3 years of ETV treatment have shown that the previous concept of no-resistance in naive patients on ETV monotherapy is under question (Colonno et al 2006b). In this study, 3 HBeAg-positive patients experienced a virologic breakthrough with genotypically confirmed resistance in the third year of treatment. Although this data, obtained under a complex study design, are compatible with a rate of resistance lower than 1% at the third year of treatment, this has to be confirmed following a wide use of the drug in clinical practice (Figure 3). Moreover, while ETV genotypic resistance in LAM resistant mutants has been reported to be very low (6%) during the first year of therapy increasing to approximately 14% at year two, new calculations on genotypic resistance have disclosed a cumulative rate of more than 30% at year 3 (Figure 3) (Colonno et al 2006b). Though a number of these patients may remain in low serum HBV-DNA levels, viral rebound increases from 1% at the first to 10% at the second and 25% at the third year of treatment. For the time being the rate of biochemical resistance remains unknown because the definition of biochemical breakthrough (ALT > 2x baseline or >10xULN) applied in this study (Colonno et al 2006b) actually refers to biochemical flares and not to biochemical breakthroughs which represent increase of normal ALT values to >1.25xULN.

As in the case of ETV therapy, patients with LAM-resistant HBV mutants treated with Adefovir monotherapy

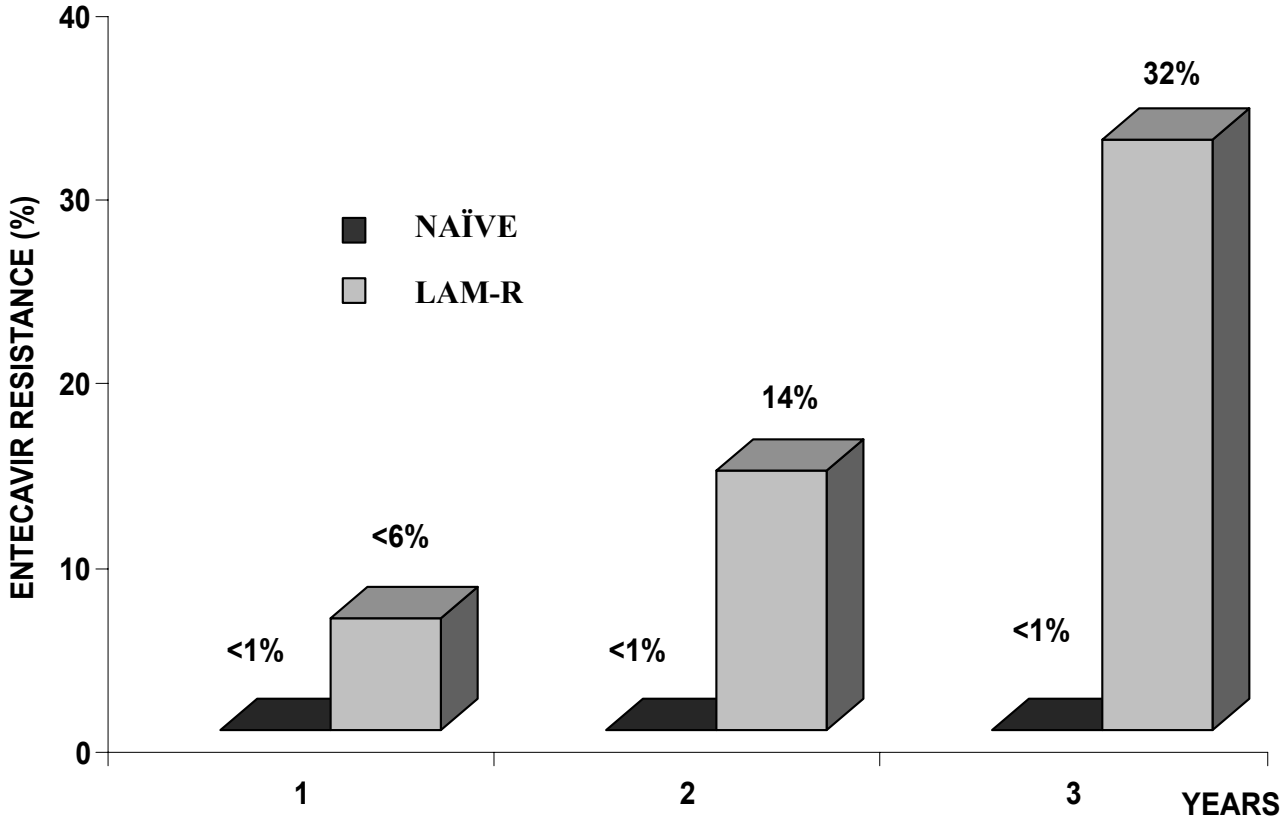

Figure 3 The rate of Entecavir resistance in Naïve and Lamivudine-resistance chronic hepatitis B patients during 3 years of treatment.
Colonno RJ. AASLD 2006. Abstract 110.

are also at significantly higher risk to develop ADV resistant HBV mutants than LAM-naïve patients (Fung et al 2005, 2006; Villet et al 2005; Yim et al 2006). On the other hand no patient with LAM-resistant strains treated by the combination of ADV with LAM for up to 4 years, has hitherto developed ADV resistant HBV mutants (Lampertico et al 2005; Rapti et al 2007). It is therefore reasonable to suggest that in patients with LAM-resistance HBV mutants sequential nucleos(t)ide monotherapy should be avoided and combination therapy be applied by 2 potent antiviral agents with different resistant profiles as is the case of combination of ETV with adefovir and preferable with tenofovir.

Conclusions

The high anti-HBV potency of entecavir, its impressive efficacy in terms of rapid HBV suppression to undetectability of HBV DNA by most sensitive PCR assays, combined with its high genetic barrier to HBV resistance, make ETV monotherapy a very attractive option as first line treatment in lamivudine-naïve CHB patients both HBeAg-positive and HBeAg-negative. However, the hitherto duration of ETV phase III trials is short, their extension design complex and appropriate long-term studies are needed before reaching definite conclusions on its very long-term safety and resistance.

In the setting of cirrhosis and liver transplantation, existing data are promising but limited.

In CHB patients with LAM-resistant HBV mutants, particularly those with advanced chronic liver disease, long term ETV monotherapy should be avoided, however this being true not only for ETV but for any sequential nucleos(t)ide analog monotherapy because of the increased risk for emergence of additional HBV resistance and multi-drug resistant HBV strains. In such setting the addition of adefovir or preferably tenofovir while continuing lamivudine, currently represents the treatment of choice but in the near future combination of ETV with ADV or tenofovir may turn out as the most preferable long term treatment strategy.

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