

ENTECAVIR, FTC, LFMAU, LDT AND OTHERS

Maria Buti, Rafael Esteban, Liver Unit, Vall d'Hebron General Hospital, Barcelona, Spain.



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In chronic hepatitis B therapy there are new and exciting developments in antivirals such as nucleoside analogs. One of them, lamivudine, is currently widely used as a first treatment for patients with chronic hepatitis B. Another, adefovir has finished registration studies and is currently used for patients with lamivudine resistant mutants. Both have the advantages of oral administration and excellent safety profiles. However, the therapy of chronic hepatitis B is still an open issue. The sustained response rate to these new therapies are still low, drug resistance to lamivudine limits its efficacy and new drugs are necessary for therapy of patients with chronic hepatitis B in different situations: immunocompromised and decompensated patients, patients with normal ALT levels, those resistant to lamivudine and non-responders to lamivudine or interferon (1).

This review focuses on new antiviral agents such as entecavir, emtricitabine, clevudine, β -L nucleosides. Some of them are still in phase I/II clinical studies; therefore the information available is limited. Only entecavir recently has started phase III clinical trials.

ENTECAVIR

Entecavir, a cyclopentyl guanosine analogue, is a potent inhibitor of HBV DNA polymerase, inhibiting both the priming and elongation steps of viral DNA replication (1-2). Entecavir is phosphorylated to its triphosphate, the active compound, by cellular kinases. It is a selective inhibitor of HBV DNA because it has little or no inhibitory effect on the replication of other DNA viruses such as herpes simplex, cytomegalovirus and RNA viruses such as HIV. Entecavir is also effective against lamivudine-resistant mutants, but less effective than against wild-type HBV (2-3).

In an "in vitro" assay using Hep G2.2.15 human liver cells, the EC₅₀ for entecavir was 0.00375 μ M compared with 0.116 μ M for lamivudine, therefore it is 30 times more potent than lamivudine in suppressing viral replication. Oral administration of entecavir also proved efficacious against woodchuck hepatitis virus (WHV) and duck hepatitis virus (DHBV). The antiviral effect is dose-dependent. In woodchucks with chronic woodchuck infection, doses of 0.1 mg/kg of entecavir reduced WHV titers by 7 logs (4).

Long-term maintenance therapy with weekly dosing has also shown to decrease the incidence of hepatocellular carcinoma and to increase survival in woodchucks with chronic hepatitis B infection compared with untreated woodchucks. In addition, after 14 months of entecavir therapy, viral core antigen, WHV and cccDNA were undetectable in liver biopsy samples of the 9 animals tested. This long-term study with entecavir in woodchucks indicated that viral replication could be suppressed for years with a weekly maintenance therapy (5).

Entecavir has been evaluated in Phase I/II clinical studies. In a randomized, double blind, escalating-dose, placebo-controlled Phase II trial, four doses of entecavir (0.05, 0.1, 0.5 and 1.0 mg once daily for 28 days) were evaluated. Serum HBV DNA levels decreased by 2-3 logs by day 28 and approximately 25% of patients showed a decline in HBV-DNA below the limit of detection of the Chiron HBV DNA assay (<0.7 ME/ml). After stopping therapy, all patients showed a rebound in ALT levels and HBV DNA. Entecavir was well tolerated at all dose levels with no significant differences in adverse events experienced by patients receiving entecavir versus placebo (6).

Based on the results of the above-mentioned trial, a phase II dose-ranging study was designed to further evaluate the safety and antiviral activity of entecavir in comparison with lamivudine. In a 24-week, double blind, randomized, multicenter, clinical trial, the safety and efficacy of three different doses of entecavir (0.01 mg/day, 0.1 mg/day, or 0.5mg/day orally) were compared to lamivudine (100 mg orally daily). One hundred and sixty-nine patients chronically infected with Hepatitis B Virus (HBeAg-positive and HBeAg-negative) were treated for 24 weeks (7). Both the 0.1 mg/day and 0.5 mg/day doses of entecavir were superior to lamivudine in viral load reduction, as measured by the Amplicor® polymerase chain reaction assay. Compared to lamivudine, entecavir therapy reduced HBV DNA by an additional 0.97 logs₁₀ with the 0.1-mg/day dose and an additional 1.28 logs₁₀ with the 0.5 mg/day dose (p<0.0001 for both comparisons). A clear dose-response relationship was observed for entecavir with both the 0.1 mg/day and 0.5 mg/day doses demonstrating significantly greater viral suppression of HBV DNA than the 0.01 mg/day dose (p<0.0001 for each). The 0.5 mg/day dose of entecavir demonstrated superiority to the 0.1 mg/day dose (p=0.018). In patients treated with entecavir 0.5 mg/day, 83.7% had an HBV DNA level below the lower limit of detection of the Quantiplex® branched chain DNA (bDNA) assay, compared to 57.5% treated with 100 mg/day of lamivudine and compared to 62% treated with 0.1 mg/day of entecavir. Only a few patients achieved HBeAg loss and/or seroconversion by Week 22, with no significant differences between treatment arms. Entecavir was well tolerated at all dose levels; most adverse events were mild-to-moderate and transient with no significant differences observed between any the different doses of entecavir and lamivudine. This study demonstrates that entecavir has a potent antiviral activity against HBV. The 0.1 mg/day and the 0.5 mg/day entecavir doses were superior to lamivudine in chronically infected HBV patients. Following the results of this study, 0.5 mg daily of entecavir can be recommended as the optimal dose for previously untreated patients.

The effect of antiviral therapy on the reduction of HBV DNA typically follows a bi- or tri-phasic kinetic pattern, with a rapid fall in HBV DNA levels observed during the first four weeks of therapy (8-9). During this time it is believed that free virions in the plasma are rapidly cleared due to the direct effect of the drug, followed by a slowing in the rate of HBV decline as infected hepatocytes are attacked by cytotoxic T-lymphocytes eliminating the infected cells which are then replaced by uninfected cells (8-9). The viral dynamics during and after entecavir therapy were studied in a small number of patients with chronic hepatitis B receiving different doses of entecavir ranging from 0.05 to 1.0 mg of entecavir daily. The median effectiveness in blocking viral production was 96%. The median half-life of viral turnover was 16 hours and the median half-life of infected hepatocytes was 257 hours (=10.7 days). Rebound of viral replication also followed a bi-phasic return to baseline levels (10). In the future, data on the amount of cccDNA in the liver in these entecavir treated-patients could be helpful in supporting the outcome of the parameters estimates by the mathematical model.

During short-term therapy, entecavir seems to show stronger antiviral activity than lamivudine, but this assumption should be validated by head-to-head studies. In addition, entecavir shows continuous activity in patients with detectable lamivudine-induced mutant virus. Results from a recent trial of entecavir against YMDD-variant HBV demonstrate its activity in this setting (11). Three doses (0.1, 0.5 and 1 mg daily) of entecavir were tested and compared with lamivudine in 181 patients who failed to respond to lamivudine therapy and had YMDD mutants. At week 24, the percentage of patients with undetectable HBV DNA by bDNA (Quantiplex assay) was 19% with 0.1 mg of entecavir, 53% with 0.5 mg and 79% with 1 mg daily of entecavir while with lamivudine (100 mg/day), only 13% had undetectable HBV DNA. The 0.5 and 1 mg doses were superior to lamivudine (p<0.0001). The mean log₁₀ decrease in HBV DNA levels by PCR assay with entecavir was 1.95 with 0.1 mg, 3.85 with 0.5 mg and 4.36 with 1 mg in contrast to 0.92 with lamivudine. Therefore, in patients failing lamivudine therapy because of YMDD mutants, entecavir significantly decreased Hepatitis B viremia and in this setting, 1 mg of entecavir daily seems to be the optimal dose in contrast to the 0.5 dose recommended for untreated patients.

The safety and efficacy of entecavir was also evaluated in 9 liver transplant patients failing lamivudine therapy. These patients receive 1 mg of entecavir daily for 42 weeks (12). A median 4.2 log reduction in HBV DNA levels was observed and in 5 patients HBV DNA became undetectable. No clinically important adverse events were observed.

Different on-going multicenter phase III studies are currently evaluating the efficacy and safety of entecavir in HBeAg positive patients, HBeAg negative patients and in patients resistant to lamivudine. These studies are

comparing entecavir vs. lamivudine for 48 weeks.

EMTRICITABINE

Emtricitabine (FTC) is a cytosine nucleoside analogue with antiviral activity against both HBV and HIV. It differs from lamivudine in having a fluorine at the 5-position of the nucleic acid. In a pilot study, 49 patients with HBeAg positive chronic hepatitis B received five different doses of emtricitabine: 25, 50, 100, 200, or 300 mg daily for 8 weeks. At the end of treatment, serum HBV DNA levels decreased by 2-3 logs in patients receiving the higher doses (1-13).

In a second randomized, double blind study, three doses of emtricitabine were compared for 48 weeks (14). Ninety-eight Asian patients (77 HBeAg positive and 21 HBeAg negative) were randomized to receive 25, 100, or 200 mg of emtricitabine daily. At week 48, HBeAg loss was observed in 40% of the 77 HBeAg positive patients (ranging from 32% to 50% depending on the dose group). For all patients, the median decrease in viral load was 2.59 log₁₀ copies/ml for the 25 mg dose, 3.12 log₁₀ copies/ml for the 100 mg dose and 2.92 log₁₀ copies/ml for the 200-mg dose, with a range up to 5.5 log₁₀ copies/ml in patients given 100 or 200 mg emtricitabine daily. The proportion of patients with undetectable HBV DNA at week 48 was 38%, 42% and 61% for the 25, 100, and 200 mg dose groups respectively. Genotypic analysis performed at week 48 showed that 12% of patients treated with 100 mg of emtricitabine and 6% of those treated with 200 mg had detectable viremia with phenotypic changes associated with HBV drug-resistance. The results of this study suggest that the optimal FTC dose is 200 mg once daily. This dose is well tolerated, produces the highest rate of Hepatitis B Virus suppression and is associated with the lowest incidence of HBV drug resistant mutants.

In the anti-HBe positive study that included 21 patients, HBV DNA loss occurred in a higher proportion (79%) than in those HBeAg positive. However, when HBV DNA results were adjusted for baseline viral load there was no differences between patients who were HBeAg positive and HBeAg negative in the proportion of patients with undetectable HBV DNA at week 48. Overall, ALT levels became normal in 95% of patients at week 48. These results suggest that emtricitabine has a potent antiviral activity in HBeAg-negative patients with detectable HBV DNA and it is an active therapeutic agent in this setting (15).

Phase III clinical trials are under way to determine the long-term safety and efficacy of emtricitabine. However, the role of emtricitabine in the treatment of chronic hepatitis B may be limited by its structural similarity to lamivudine and hence, the potential for cross-resistance and the development of mutations.

CLEVUDINE

Clevudine (L-FMAU;1-(2-fluoro-5methyl-β-L-arabinosyl uracil) is a pyrimidine analogue with marked “in vitro” activity against HBV but not HIV (1, 13). The active triphosphate inhibits HBV DNA polymerase but is not an obligate chain terminator. “In vitro”, clevidine has an EC50 value ranging from 0.02 to 0.15 μM with a mean of 0.08 μM. “In vitro” studies suggest that it may also be effective against lamivudine-resistant HBV mutants. “In vivo” studies of the infected woodchuck model have been demonstrated that a once daily dose of 10 mg/kg of clevidine resulted in as much as a 9 log₁₀ decrease in viral load. Clevidine also delayed the time to viral recrudescence in a dose-dependent manner (16). Treatment was associated with a decrease in intrahepatic WHV replicative intermediates and woodchuck hepatitis surface and core antigens. No evidence of drug-related toxicity was observed in treated animals.

An open labeled phase I/II, non-randomized, dose-escalation study was performed in patients with chronic hepatitis B. Twenty-five patients were enrolled: 5 received 10 mg daily of clevidine for 28 days, 10 received 50 mg of clevidine daily and 10 were treated with 100 mg of clevidine daily for the same period of time and were followed by a 24 week posttreatment period (17). The majority of the patients were Asian. All of them were HBV DNA positive (more than 3 x 10⁶ copies/ml). At the end of the dosing period, the median reduction in serum HBV DNA was 2.48 log₁₀, 2.74 log₁₀ and 2.95 log₁₀ in the 10 mg, 50 mg and 100 mg daily cohorts respectively. At the end of follow-up (20 weeks post-treatment), the median decrease in serum HBV DNA levels was 1.84 log₁₀ and 2.38 log₁₀ in the 10 mg and the 50 mg daily cohorts respectively. No data was available for the 100 mg dose cohort. Clevidine was well tolerated without associated adverse

events. These preliminary results show that clevudine has potent antiviral activity in all of the three doses tested and maintains a sustained post-treatment antiviral effect for at least 6 months after the 28-day treatment period. More studies in patients with chronic hepatitis B are planned.

β -L-NUCLEOSIDES

The natural nucleosides in the β -L-configuration (β -L-thymidine (LdT), β -L-2-deoxycytidine (L-dC) and β -L-2-deoxyadenosine (L-dA)) represent a newly discovered class of compounds with potent, selective and specific activity against hepadnavirus. "In vitro" studies have shown that these compounds are not active against other viruses such as herpes viruses or HIV, but these compounds have marked effects on HBV replication. It is not yet clear if these compounds are active against lamivudine-resistant HBV mutants (1, 18-21).

LdT is at the most developed stage of clinical investigation. It has a remarkably clean pre-clinical toxicology profile. So far, it doesn't have mitochondria toxicity and it appears not to be mutagenic. A phase I/II, 4-week dose-escalation trial has been completed; with 35 adults with chronic hepatitis B enrolled. All of them were HBeAg positive and HBV DNA positive. Subjects were randomized to receive five different oral doses of LdT: 25, 50, 100, 200, or 400 mg daily. HBV DNA level reductions were dose proportional and were observed in all the three doses tested. The dose-dependent antiviral effects of LdT was especially evident after the first week of treatment. The median HBV DNA reduction for the 400 mg cohort, assessed by Roche PCR assay, was 3.6–4.0 log₁₀ by week 4. This reduction seems greater than those reported for previous HBV antivirals (lamivudine, adefovir, entecavir). The safety profile of LdT appeared similar to placebo (22). A viral dynamic study was performed during LdT treatment to identify HBV viral dynamics during LdT therapy and to define the relationship between LdT dose and virologic response. This study was based on the data from the phase I/II trial of LdT. A biphasic HBV DNA response pattern was observed with LdT treatment over 4 weeks. The viral clearance during week 1 of therapy, about 2 log₁₀ or 99%, was independent of LdT dose probably because all doses tested were active. Second-phase clearance was dose-dependent with the dose of 400 mg daily achieving the highest reduction by week 12 (23-24).

A phase II study comparing five different therapeutic strategies for 1 year is currently ongoing: The strategies being analyzed are as follows: LdT 400 mg daily, LdT 600 mg daily, LdT 400 mg and lamivudine 100 mg daily, LdT 600 mg and lamivudine 100 mg daily and lamivudine 100 mg daily. Up to now, this multicenter study has enrolled 106 patients and some of them have treated for as long as 3-4 months. Initial HBV DNA data has confirmed the active antiviral effect of LdT, with most patients achieving more than 4 log₁₀ HBV DNA reductions. On the basis of this satisfactory clinical data, phase III studies with LdT are going to start soon.

Another promising β -L-nucleoside compound is val-LdC. It is in the middle of phase I/II testing and preliminary results indicate substantial antiviral activity with a good safety profile (1).

Combinations of β -L-nucleosides appear to have additive or synergistic effect against the Hepatitis B Virus. "In vitro" studies and animal testing showed that there is no evidence of cellular or mitochondrial toxicity. The combination of LdT and Val-LdC was analyzed in a woodchuck study. Over a 12-week treatment period, the combination of LdT and val-LdC cleared PCR detectable WHV DNA in 5 of the 5 animals tested with no safety issues noted. If similar profound antiviral effects are achievable in humans, a relatively rapid and profound clearance of HBV viremia may be a realistic goal in many patients.

To summarize, the future of chronic hepatitis B therapy seems to be in the combination of different drugs. Ideally, the optimal drugs to combine would meet the following criteria: they should be orally administered, they should have an excellent safety profile and the duration of therapy should be limited. Currently, the drugs most likely to fulfill these criteria are the nucleoside analogues.

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