New Nucleoside Analogs for the Treatment of Chronic Hepatitis B

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Three agents have been approved for the treatment of chronic hepatitis B virus (HBV): interferon-alpha, lamivudine and adefovir. Each agent has certain limitations and none has an excellent efficacy. Lamivudine and adefovir have the advantages of oral administration and excellent safety profiles. However, optimal treatment of chronic hepatitis B is still under debate. The sustained response rates to these new therapies are still low, drug resistance to lamivudine limits its efficacy and new drugs are necessary for the treatment of chronic hepatitis B in different situations: immunocompromised and decompensated patients, patients with normal ALT levels, lamivudine-resistant patients and non-responders to lamivudine or interferon [1].

This review focuses on new antiviral agents such as entecavir, emtricitabine, clevudine and beta-L nucleosides. Some of them are still in phase II clinical studies; therefore available information remains limited.

ENTECAVIR

Entecavir, a cyclopentyl guanosine analog, 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. Although entecavir is

also effective against lamivudine-resistant mutants, it is less effective than against wild-type HBV [2, 3].

In a randomized, double blind, escalating-dose, placebo-controlled phase II trial, four doses of entecavir (0.05, 0.1, 0.5 and 1.0mg once daily for 28 days) were evaluated. Serum HBV DNA levels decreased by 2-3 logs by day 28 and approx 25% of patients showed a decline in HBV DNA below the limit of detection of the Chiron HBV DNA assay (<0.7ME/mL). After stopping therapy, all patients showed a rebound of ALT levels and HBV DNA [3].

In a 24 week, double blind, randomized, multicenter clinical trial, the safety and efficacy of three different oral doses of entecavir (0.01, 0.1, or 0.5mg/day) were compared to lamivudine (100mg/day). One hundred and sixty-nine patients chronically infected with HBV (HBeAg-positive and HBeAg-negative) were treated for 24 weeks [4]. Both the 0.1 and 0.5mg/day doses of entecavir were more effective than lamivudine for viral load reduction, as measured by the Amplicor® polymerase chain reaction (PCR) assay. Compared to lamivudine, entecavir therapy reduced HBV DNA by an additional 0.97 $\log_{10}$ with the 0.1mg/day dose and an additional 1.28 $\log_{10}$ with the 0.5mg/day dose (P<0.0001 for both comparisons). A clear dose-response relationship was observed for entecavir with both the 0.1 and 0.5mg/day doses demonstrating significantly stronger viral suppression of HBV DNA than the 0.01mg/day dose (P<0.0001 for each). The 0.5mg/day dose of entecavir was more effective than the 0.1mg/day dose (P=0.018). 83.7% of the patients treated with entecavir 0.5mg/day had a decrease in HBV DNA levels to below the lower limit of detection of the Quantiplex® branched chain DNA (bDNA) assay, compared to 57.5% treated with 100mg/day of lamivudine and 62% treated with 0.1mg/day of entecavir. Entecavir was well tolerated at all dose levels; most adverse events were mild to moderate and transient with no significant differences observed between any of the different doses of entecavir and lamivudine. This study shows that entecavir has potent antiviral activity against HBV. The 0.1 and the 0.5mg/day entecavir doses were more effective than lamivudine in chronically HBV infected patients. Based on the results of this study, 0.5mg/day of entecavir can be recommended as the optimal dose for previously untreated patients. In addition, entecavir has antiviral activity in patients with lamivudine-resistant mutants. Results from a recent trial of entecavir against YMDD-variant HBV confirm that it is active in this setting [5]. Three doses of entecavir

(0.1, 0.5 and 1mg/day) 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 0.1mg, 53% with 0.5mg and 79% with 1mg entecavir daily while with lamivudine (100mg/day), only 13% had undetectable HBV DNA. The 0.5 and 1mg doses were more effective than lamivudine (P<0.0001). The mean $\log_{10}$ decrease in HBV DNA levels by PCR assay with entecavir was 1.95 with 0.1mg, 3.85 with 0.5mg and 4.36 with 1mg in contrast to 0.92 with lamivudine [5]. Therefore, in patients with lamivudine resistant YMDD mutants, entecavir significantly decreased hepatitis B viremia and 1mg of entecavir daily seems to be the optimal dose in these patients in contrast to the 0.5mg dose recommended for untreated patients.

Preliminary results do not show any emergence of entecavir resistance in lamivudine refractory patients treated for at least one year with entecavir. One hundred thirty two patients with lamivudine resistance were treated with entecavir for 48 weeks without the emergence of other reverse transcriptase sequences.

Different ongoing multicenter phase III studies are currently evaluating the efficacy and safety of entecavir in HBeAg positive and, HBeAg negative patients as well as in patients resistant to lamivudine. These studies are comparing entecavir vs. lamivudine for 48 weeks.

EMTRICITABINE

Emtricitabine (FTC) is a cytosine nucleoside analog with antiviral activity against both HBV and HIV. Unlike lamivudine, it has 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 300mg/day for 8 weeks. At the end of treatment, serum HBV DNA levels decreased by 2-3 logs in patients receiving the higher doses [1].

In a second randomized, double blind study, three doses (25, 100, or 200mg/day) of emtricitabine were compared for 48 weeks in 98 Asian patients (77 HBeAg positive and 21 HBeAg negative) [6]. 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 $\log_{10}$ copies/mL for the 25mg dose, 3.12 $\log_{10}$ copies/mL for the 100mg

dose and 2.92 $\log_{10}$ copies/mL for the 200mg dose, with a range of up to 5.5 $\log_{10}$ copies/mL in patients receiving 100 or 200mg emtribitabine/day. The proportion of patients with undetectable HBV DNA at week 48 was 38%, 42% and 61% for the 25, 100, and 200mg dose groups, respectively. Genotypic analysis performed at week 48 showed that 12% of patients treated with 100mg of emtricitabine and 6% of those treated with 200mg had detectable viremia with phenotypic changes associated with HBV drug-resistance. The results of this study suggest that the optimal FTC dose is 200mg once daily. This dose is well tolerated, produces the highest rate of HBV suppression and is associated with the lowest incidence of drug resistant mutants.

HBV DNA loss occurred in a higher proportion (79%) of the 21 anti-HBe positive patients, than of the HBeAg positive patients. However, when HBV DNA results were adjusted for baseline viral load there was no difference between patients who were HBeAg positive and HBeAg negative in the proportion of patients with undetectable HBV DNA at week 48. Overall, ALT levels normalized in 95% of patients at week 48. These results suggest that emtricitabine has potent antiviral activity in HBeAg-negative, HBV DNA positive patients and it is an active therapeutic agent in this setting [7].

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. For this reason some clinical trials comparing emtricitabine alone or in combination with adefovir are ongoing.

CLEVUDINE

Clevudine (L-FMAU;1-[2-fluoro-5methyl-β-L-arabinosyl uracil]) is a pyrimidine analog with marked “in vitro” activity against HBV but not HIV [1, 2]. The active triphosphate inhibits HBV DNA polymerase but is not an obligate chain terminator. “In vitro”, clevudine has an EC_{50} value ranging from 0.02 to 0.15μM with a mean of 0.08μM. “In vitro” studies suggest that it may also be effective against lamivudine-resistant HBV mutants. “In vivo” studies of the infected woodchuck model have demonstrated that a once daily dose of 10mg of clevudine resulted in as much as a 9 $\log_{10}$ decrease in viral load. 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 10mg daily of clevudine for 28 days, 10 received 50mg of clevudine/day and 10 were treated with 100mg of clevudine/day for the same period of time and were followed by a 24 week postreatment period [8]. All patients were HBV DNA positive (more that 3 x 10^6 copies/mL). At the end of the dosing period, the median reduction in serum HBV DNA was 2.48 $\log_{10}$, 2.74 $\log_{10}$ and 2.95 $\log_{10}$ in the 10mg, 50mg and 100mg/day cohorts, respectively. At the end of follow-up (20 weeks post-treatment), the median decrease in serum HBV DNA levels was 1.84 $\log_{10}$ and 2.38 $\log_{10}$ in the 10mg and the 50mg/day cohorts, respectively. No data was available for the 100mg dose cohort. Clevudine was well tolerated without associated adverse events. These preliminary results show that clevudine has potent antiviral activity at all 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.

BETA-L-NUCLEOSIDES

The natural nucleosides in the beta-L-configuration (beta-L-thymidine [LdT], beta-L-2-deoxycytidine [L-dC] and beta-L-2-deoxyadenosine [L-dA]) represent a new class of compounds with potent, selective and specific activity against hepadnaviruses. “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 whether these compounds are active against lamivudine-resistant HBV mutants [1, 9, 10].

LdT is at the most developed stage of clinical investigation. A phase I/II, 4-week dose-escalation trial has been completed with 35 adults with chronic hepatitis B. 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 400mg/day. HBV DNA level reductions were dose dependent and were observed at all five doses tested. The dose-dependent antiviral effects of LdT were especially evident after the first week of treatment. The median HBV DNA reduction for the 400mg cohort, assessed by Roche polymerase chain reaction (PCR) assay, was 3.6–4.0 $\log_{10}$ by week 4. This reduction seems greater than those previously reported for other

antiviral drugs (lamivudine, adefovir, entecavir). The safety profile of LdT appeared similar to placebo [11]. A phase IIb study comparing five different therapeutic strategies for 1 year was recently completed. One hundred and four patients were randomized to receive LdT 400mg/day, LdT 600mg/day, LdT 400mg and lamivudine 100mg/day, LdT 600mg and lamivudine 100mg/day and lamivudine 100mg/day for one year. Median serum HBV DNA reductions at week 52 in $\log_{10}$ copies/mL for the five treatment groups were 4.66 for standard lamivudine therapy, 6.43 for LdT 400mg <7day, 6.09 for LdT 600mg/day, 6.40 for combination 400mg and 6.05 for combination 600mg/day. HbeAg loss was observed in 28% of the patients treated with lamivudine, 33% of those treated with LdT and 17% of those treated with Ldt plus lamivudine [12]. Therefore after one year of treatment, viral suppression, PCR non-delectability of serum HBV DNA and ALT normalization were significantly greater for LdT than lamivudine.

Another promising beta-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 beta-L-nucleosides appear to have additive or synergistic effects against HBV. “In vitro” studies and animal tests have shown that there is no evidence of cellular or mitochondrial toxicity. The combination of LdT and Val-LdC was analyzed in woodchucks. Over a 12-week treatment period, the combination of LdT and val-LdC cleared PCR detectable woodchuck hepatitis virus (WHV) DNA in 5 of the 5 animals tested with no safety problems noted. If similar antiviral activities are observed in humans, a relatively rapid clearance of HBV viremia could be a realistic goal in many patients.

SUMMARY AND CONCLUSIONS

Although many of the new antiviral agents discussed above are promising, it is unlikely that any of these compounds will result in a definitive answer to the treatment of chronic hepatitis B and the future of chronic hepatitis B therapy seems to be the combination of different drugs with two aims: to improve response to therapy and to avoid or reduce viral resistance.

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