New nucleoside analogues for chronic hepatitis B

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For chronic hepatitis B,interferon-alpha and lamivudine are now established drugs ; however, the impact of these monotherapies is still limited. Combination therapy has the potential to be more effective with two modalities : 1. An antiviral in combination with an immunomodulatory drug, or 2. A combination of two antivirals.

For combination antiviral strategy, the therapeutic goal should be to optimise antiviral activity while minimising the emergence of viral resistance. This may be accomplished by avoiding sequential antiviral therapy and using antiviral combinations that avoid the emergence of multi-drug resistance. Anno the year 2000 lamivudine is likely to be the basic antiviral compound.

Lamivudine, a cytosine analogue lacking a 3'-hydroxy group, inhibits the synthesis of negative strand HBV DNA from pre-genomic viral RNA by chain termination. The compound is well absorbed from the gastrointestinal tract and has excellent bioavailability.

Lamivudine monotherapy results in suppression of serum HBV DNA, normalisation of serum transaminase ALT (1,2) and improvement in inflammatory activity in the liver biopsy in 60-70% of patients (2). Importantly, lamivudine has been very well tolerated. After withdrawal of lamivudine, HBV DNA and hepatic inflammation return to pre-treatment levels within 1-3 months, except in 15-20% of patients with HBeAg seroconversion (2-4). A clinically significant correlation between the degree of elevation of baseline ALT levels and the HBeAg response rate has been observed ; patients with baseline ALT levels above 2× ULN seemed to benefit most from larnivudine therapy (5).

Prolonged lamivudine therapy is hampered by the emergence of drug resistant mutant strains, reported in 14-50% of patients at 1-3 years of treatment (2,3,6,7). Lamivudine resistance is usually conferred by mutations within the HBV polymerase gene ("YMDD" mutation). The emergence of resistant mutants has been associated with elevation of serum ALT and HBV DNA ; both of these variables usually remain below pre-treatment baseline levels with continued therapy. In patients with recurrent hepatitis B after liver transplantation, drug-resistant mutants have been associated with a rapidly accelerating, severe hepatitis that has resulted in allograft failure and death (8). However, drug withdrawal has resulted in a 'lamivudine-withdrawal' hepatitis, decompensation of liver disease and death in patients with cirrhosis (Nevens, personal communication). As liver disease remains stable in most patients who develop resistant viruses, continued antiviral therapy is a reasonable option.

In the last three years famciclovir has been tested in large phase III studies ; they have shown minimal anti-HBV activity of famciclovir and interest in the drug is now rapidly fading.

At least 3 new anti-HBV nucleoside analogues have been undergoing clinical testing. Two drugs (lobucavir and entecavir) are guanidine derivatives, and the third is a competitive inhibitor of deoxyadenosine (adefovir). The drugs have been identified on the basis of their *in vitro* activity in HBV transfected hepatocyte cultures, followed by *in vitro* cytotoxicity studies (9,10). The results of these studies are summarised in the table.

	Lobucavir	Entecavir	Adefovir
Antiviral EC50 (μM)	2.5	0.004	0.7
Cytotox CC50 (μM)	30	30	150
Therapeutic Index	15	8000	214

The mechanism of action of nucleoside analogues is basically competitive inhibition of cellular nucleotides for the HBV DNA polymerase, and preferential incorporation of the drugnucleotide into viral DNA leading to chain termination. Since deoxyguanine triphosphate usually is the initiating deoxynucleotide which interacts with the acceptor tyrosine residue of the polymerase, the guanine analogues lobucavir and entecavir have an additional effect and inhibit the priming of the HBV DNA polymerase (10). Adefovir is exceptional since the active moiety is the diphosphate which inhibits deoxyadenosine triphosphate binding to the HBV polymerase and leads to chain termination. For all three drugs the site of interaction with the viral enzyme is different from the YMDD locus.

The clinical applicability of these drugs is based on their antiviral properties but decided by the toxicology profile. In 1999 lobucavir was withdrawn because of carcinogenicity in mice and rats with lifelong exposure. Carcinogenicity studies (6-mo, 1-yr, 2-yr, and lifelong) are ongoing for entecavir and adefovir. No evidence has yet emerged on teratogenicity and mutagenicity. Adverse effects of adefovir in human studies are pre-

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dominantly of renal nature : dysuria due to high concentration of drug in the urine ; elevation of creatinine, proteinuria and glycosuria due to drug interaction in the renal tubulars (11) ; in addition long- term administration can lead to carnitine deficiency due to interaction of pivalic acid of the dipivoxil moiety with carnitine. Adverse effects of entecavir point to the neurologic system with headache, dizziness and photophobia ; in dogs but not in other animals, inflammatory changes were noted in the cerebrospinal fluid.

Adefovir and entecavir are being tested in phase II studies ; results from the adefovir studies have been reported.

In one double-blind study (12) 53 HBeAg positive chronic HBV patients with elevated serum ALT were randomised to receive 5 mg, 30 mg, 60 mg or placebo daily for 12 weeks. Serum HBV DNA levels were monitored by a quantitative PCR-based assay with a lower limit of detection of 400 copies/ml. At week 12 the median decrease in HBV DNA was 4.0 log10 ; 20% of patients had HBeAg seroconversion at week 36.

In this study the kinetics of viral decline was analysed, first by the model of Nowak. In that model the assumptions are that the viral production rate during nucleoside-analogue therapy is zero and that the infection rate of uninfected cells is also zero. The data obtained during adefovir therapy fitted the model well only for the first two weeks of therapy. When the data were analysed by a new model that assumes incomplete inhibition of viral production with an efficiency between 0 and 1, a good fit could be obtained for the whole period of 12 weeks. As for other viruses (HIV, HCV), the viral decline follows a biphasic pattern : the first phase reflects clearance of free virus, and the second phase loss of infected cells. With a daily dose of 30 mg the clearance of free virus had a T1/2 of 1,1 days and the loss of infected cells a T1/2 of 18,2 days. The average inhibition of viral production was 0.983 (i.e. 1.7% persistent replication). Comparison of the viral kinetics in patients receiving different dosages (5, 30 and 60 mg/d) showed varying efficacy of 0,80 to 0,99 ; the varying efficacy to inhibit viral production appeared to have no effect on the rate of virus clearance or on the rate of infected cell loss, but only on the extent or duration of the initial rapid phase (13).

Daily dose mg/d	Efficacy of inhibition	Magnitude of initial phase (log 10)	Duration of initial phase (days)	Estimated time to eliminate HBV (days)
5	0,80	0,65	4,4	892
30	0,98	1,97	10,8	502
60	0,99	2,15	10,0	484

In the phase II studies resistance to adefovir has not yet been detected. In addition, several independent groups have described maintenance of the antiviral activity of adefovir for lamivudine resistant strains of HBV (14, 15). Therefore, adefovir is currently a major candidate for combination with lamivudine in antiviral combination therapy.

In addition to lamivudine, adefovir and entecavir, several other nucleoside analogues are in clinical development including dideoxyfluorothiacytidine (FTC), L-fluromethylarabinosyl uracil (L-FMAU) and D-diaminopurindixolane (DAPD). Upon phosphorylation, FTC and LFMAU are potent inhibitors of HBV reverse transcriptase (polymerase) and this results in marked reductions in HBV levels in woodchucks. DAPD is first deaminated to an intermediate metabolise (DXG), which upon phosphorylation actively inhibits HBV polymerase. In contrast to D-FIAU, which was associated with multi-organ failure in human clinical trials, mitochondrial damage has not been observed with L-FMAU. Preliminary preclinical toxicity profiles of these compounds are excellent ; safety studies are ongoing.

The use of targeted nucleotides is another evolving approach to treat chronic viral hepatitis. This approach relies on identifying essential and accessible viral targets and on specifically delivering antiviral elements to hepatocytes, the primary replication site of hepatitis viruses. Several potential classes of compounds have been identified that can be used to disrupt viruses including antisense oligonucleotides. Developing efficient hepatocyte-specific delivery systems has been a more difficult task. Although a number of delivery systems have been developed including adenoviruses, adeno-associated viruses, liposomes, retroviruses and asialoglycoprotein receptor-mediated endocytosis, none are ideal (16).

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