Seminar

Viral hepatitis B

Ching Lung Lai, Vlad Ratziu, Man-Fung Yuen, Thierry Poynard

More than 400 million people worldwide are chronically infected by the hepatitis B virus. The virus is responsible for more than 300 000 cases of liver cancer every year and for similar numbers of gastrointestinal haemorrhage and ascites. Major breakthroughs have been achieved in diagnosis and treatment of this virus. Hepatitis B vaccine reduces incidence of liver cancer. As with hepatitis C, advances have been made in molecular virology, especially for naturally occurring and treatment-induced mutant viruses. The clinical significance of low viral load and genotypes are also under investigation. Currently available monotherapies—interferon, lamivudine, and adefovir dipivoxil—very rarely eradicate the virus, but greatly reduce its replication, necroinflammatory histological activity, and progression of fibrosis. Lamivudine, and presumably other nucleoside analogues, can reverse cirrhosis of the liver.

Introduction

More than 400 million people worldwide are chronically infected by the hepatitis B virus (HBV).[1] 82% of the world's 530 000 cases of liver cancer per year are caused by viral hepatitis infection, with 316 000 cases associated with hepatitis B and 118 000 with hepatitis C[2] (see also seminar on hepatitis C[3]).

HBV infection is one of the commonest infections in the world. According to WHO, a third of the world's population (2 billion people) has been infected with HBV,[2] and about 5% are chronically infected. Fortunately, there are very effective vaccines against the virus, which are about 95% effective. One hepatitis B vaccine (plasma-derived HBV vaccine) has been shown to be effective against hepatocellular carcinoma—an important human cancer—in children in Taiwan.[4] More than 90 countries are now implementing universal vaccination of newborns against HBV. The vaccine is very safe and there is no convincing evidence of any long-term undesirable sequelae. Eradication of HBV infection worldwide is a distinct possibility.

Epidemiology

Like hepatitis C virus infection, chronic hepatitis B can cause cirrhosis and liver cancer. More than a quarter of people with chronic hepatitis B will die of liver disease,[5] and more than 1 million people with this infection are estimated to die every year.

Transmission of HBV, like hepatitis C virus, is parenteral. One of the distinctive features of HBV infection is that the risk of chronicity varies greatly with the age at which the infection is acquired.[6] For neonates and children younger than 1 year who acquire the infection, the risk of the infection becoming chronic is 90%. For children aged 1–5 years, the risk is about 30%, and for children older than 5 years and for adults, the risk from pooled data decreases to around 2%.[6]

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Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong, People's Republic of China (C L Lai MD, M-F Yuen MD)**; Service d'Hepato-Gastroenterologie, Groupe Hospitalier Pitié-Salpêtriére, Université Paris, France** (T Poynard MD, V Ratziu MD)

Correspondence to: Dr Ching-Lung Lai, Department of Medicine, University of Hong Kong, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong, People's Republic of China
(e-mail: hrmelcl@hkucc.hku.hk)

The reason for the high risk of chronicity in neonates and in children younger than 1 year is still uncertain. Although transplacental passage of the hepatitis B e antigen (HBeAg) from an infected mother to the fetus might induce immunological tolerance to the virus,[7] results of a study[8] in transgenic mice show that the placenta is an efficient barrier for HBeAg transfer.

Because of the age-related risk for chronicity, the main sources of transmission for people who become chronically infected are at birth or in the postnatal period from infected mothers, and less commonly, through close contact with infected fathers, siblings, and relatives during early childhood. In regions such as subSaharan Africa, tribal rituals, like scarification, might also be a potent source of infection during early childhood.

Sexual contact, intravenous drug use, and acupuncture can also transmit the disease. In 1997, an outbreak of hepatitis B involving 57 patients was caused by autohaemotherapy, a technique involving injection of the patient's own blood into acupuncture points.[9] Transfusion-related hepatitis B is rare since screening for hepatitis B has been routine in most transfusion centres for at least two decades. However, risk of chronicity is low for transmission through sexual contact, intravenous drug use, acupuncture, and transfusion.

Three-quarters of patients with chronic hepatitis B in the world are Chinese, and subSaharan Africa also has a high endemicity. Prevalence of chronic hepatitis B in these

Search strategy and selection criteria

We searched MEDLINE and PubMed, from 2000 to 2003, using the search terms HCV or HBV, both spelt out and abbreviated, and, epidemiology, clinical manifestation, biological manifestation, virological test, biopsy, or treatment. We selected publications mostly from the past 5 years but did not exclude commonly referenced and highly regarded older publications. We also searched the reference list of articles identified by the search strategy and selected those that were relevant. Selected review articles and meta-analyses or book chapters were included because they provide comprehensive overviews that would be beyond the scope of this seminar. The reference list was subsequently modified during the peer review process on the basis of comments from the reviewers and editors. Because one of the authors (TP) has a financial interest in the success of a non-invasive diagnostic marker of fibrosis, mention of this marker has been excluded.

regions is 10–20%, with most infections occurring in the neonatal period or during early childhood. By contrast, prevalence is low (0·2–0·5%) in North America, northern, western, and central Europe, and in Australia, where most infections are transmitted during adolescence or adulthood through sexual contact or intravenous drug use.

Natural history

Patients with chronic HBV infection seldom have extrahepatic manifestations. They often remain without symptoms until they present with cirrhosis-related complications, hepatocellular carcinoma (peak age of presentation at the sixth decade of life), or both. Occasionally, patients present earlier with symptoms of acute exacerbation.

The three phases of chronic hepatitis B disease have been well described: a long immune tolerance phase in children and adolescents with near normal histology, high concentrations of HBV DNA, and HBeAg positivity; the immune clearance phase with seroconversion from HBeAg to antibody against HBeAg accompanied by active inflammation and fibrosis and fluctuating serum alanine transaminase concentrations; and the residual phase with low concentrations of HBV DNA and normal concentrations of alanine transaminase.

However the age of acquiring the infection affects the course of the disease. For those who acquire the disease during adolescence or adulthood (this applies for most of the white population), there is no immune tolerance phase. Instead, the disease progresses directly to the immune clearance phase and is of short duration, which probably accounts for the better response to immunomodulatory therapy in white patients than in those of other ethnic origins. It also explains why white patients with HBeAg seroconversion and low concentrations of HBV DNA do not have progressive disease, becoming what Hoofnagle and colleagues[10] described as healthy carriers.

By contrast, for those who acquire the disease during the neonatal period and early childhood (this applies for most of the Asian and African population), the response to immunotherapy is worse. The disease continues to progress in many patients after HBeAg seroconversion. Although Asian patients who are HBeAg negative have lower concentrations of HBV DNA than do those who are HBeAg positive, they continue to have exacerbations. There is no single cutoff HBV DNA value to differentiate whether patients with antibodies against HBeAg will have inactive disease or continue to have exacerbations.[11] Severe exacerbations occur with equal frequency in patients who are HBeAg positive and in those with antibodies against HBeAg.[12] The picture is further complicated by the fact that precore mutations, previously thought to emerge as a result of immune pressure on HBeAg-producing HBV during the process of HBeAg seroconversion and to be responsible for viral replication and disease activity after HBeAg seroconversion, can already be detected in up to 44% of patients who are HBeAg positive.[13] Furthermore, disease activity after HBeAg seroconversion has no relation with the presence or otherwise of precore and core promoter mutations.[14] Finally, two-thirds of cirrhosis-related complications and hepatocellular carcinomas in Asian patients with hepatitis B occur after HBeAg seroconversion.[15]

Hepatitis B genotypes

There are seven major HBV genotypes (A to G) prevailing in different parts of the world. The distribution of various genotypes are as follows: A is pandemic, B and C are found in Asia, D in southern Europe, E in Africa, F in the USA, and G in the USA and France. Another newly discovered HBV, genotype H, has been found in central America. Genotype B can be further classified into two subtypes, Bj and Ba, representing the HBV that originates from Japan and the rest of Asia, respectively. Ba is probably derived from the recombination of Bj with the precore and core region of genotype C.[16] Unlike the role of hepatitis C virus genotypes, the role of HBV genotypes in treatment response is not as clear cut. However, HBV genotypes might affect disease profiles. White patients with genotype A have a higher chance of clearance of HBV DNA and HBeAg and sustained remission after HBeAg seroconversion compared with patients with genotype D; they also have a better histology activity index in liver biopsy samples.[17] Compared with patients with genotype C, Asian patients with genotype B have HBeAg seroconversion at an earlier age, less serious liver disease, and better response to interferon.[18–20] However, whether patients with genotype B differ from those with genotype C in development of hepatocellular carcinoma is still controversial.[21–23]

Pathophysiology

One of the most important discoveries in HBV is elimination of the virus during acute HBV infection in chimpanzees. Previously thought to be the result of lysis of infected hepatocytes by CD8 cells, elimination of the virus is now shown to occur mainly through a curative non-cytolytic mechanism mediated through cytokines such as interferon γ and tumour necrosis factor α, released from CD8 cells.[24,25] However this mechanism has yet to be proven in people. In chronic HBV infection, the damage to the liver starts in the immune clearance phase. This phase is probably triggered by a decrease in host tolerance to HBV antigens with a concomitant increased expression of class I HLA molecules, resulting in a decrease in HBV DNA and an increase in alanine transaminase concentrations. After a long period of repeated exacerbations, HBeAg seroconversion is finally achieved but cirrhosis might have been established in some patients. The process of cirrhosis sometimes continues with persistent low-grade viraemia even after HBeAg seroconversion. However, the notion of non-cytolytic CD8 T-cell response occurring in acute infection might also be true for chronic infection. Functionally active CD8 cells in chronic infection need not cause damage to infected hepatocytes, thus explaining the non-active carrier state.[26,27]

Diagnostic tests

Hepatitis B surface antigen, HBeAg and antibodies against HBeAg are tested by ELISA. The presence of hepatitis B surface antigen in the serum for 6 months or longer is indicative of chronic hepatitis B infection. The estimated yearly frequency for clearance of hepatitis B surface antigen in chronically infected patients is low (0·1–0·8%). Seroclearance of the surface antigen is usually caused by a decrease in viraemia rather than emergence of surface antigen mutants.[28]

Measurement of HBV DNA in serum

In-house assays for measurement of HBV DNA are notoriously variable in standardisation and sensitivity. The commonly used commercial assays for HBV DNA concentrations are a branched DNA assay and a hybrid capture test.[29] The lower limits of detection for these two assays are 700 000 copies/mL and 140 000 copies/mL, respectively.

One commercially available PCR assay allows for detection of 200 copies/mL. Attention is now being drawn to lower concentrations of HBV DNA. Although a concentration of fewer than 10^5 copies/mL has been associated with HBeAg seroconversion and inactive

disease,[30] Chu and colleagues[11] could not identify a single cutoff HBV DNA concentration for inactive disease. Furthermore, after 6 months' lamivudine, patients with HBV DNA concentrations of more than 10^3 copies/mL have a 63·2% chance of subsequently developing lamivudine resistance, whereas those with concentrations of 10^3 copies/mL or fewer have only a 13·0% chance.[31] Thus, quantitative PCR should ideally be used for monitoring the progression of disease and the effectiveness of treatment.

The supercoiled or covalently closed circular HBV DNA is receiving renewed interest. Because it does not directly replicate, it is resistant to nucleoside analogue treatment and is the probable source for DNA rebound once the therapy is stopped. Monitoring the concentrations of covalently closed circular HBV DNA in liver biopsy samples after long-term treatment could gain importance in the future.[32]

Liver biopsy

As with hepatitis C, a biopsy sample is recommended for initial assessment of the severity of the disease in chronic hepatitis B. Biopsy is generally not regarded essential for determining whether treatment is needed, even though it is preferable to have a baseline histological assessment. The decision to treat is usually based on alanine transaminase concentrations of more than 1·5 times the upper limit of normal and HBV DNA concentrations detectable by branched DNA or hybrid capture assays.

Treatment

The arrival of nucleoside analogue treatment marks a new era in treatment of chronic hepatitis B. In most clinical trials, the standard therapeutic endpoints have been loss of HBeAg (with or without antibodies against HBeAg), together with undetectable HBV DNA, as measured by branched DNA or hybrid capture assays. The endpoint for treatment of patients with antibodies against HBeAg is still controversial. For patients with high concentrations of alanine transaminase, long-term suppression of HBV DNA is probably indicated.[33] From a regulatory perspective, the US Food and Drug Administration (FDA) requires and uses histological analysis as the main endpoint for patients with HBeAg and those with antibodies against HBeAg. Total eradication of the virus is almost never achieved and is rarely used as a clinical trial or therapeutic endpoint.

However, with the knowledge that disease activity can continue with low viral titres, and with the availability of more potent nucleoside analogues for clinical trials, HBV DNA concentrations as measured by PCR assays are already being used as primary endpoints. The effect of the newer agents on covalently closed circular HBV DNA concentrations in liver biopsy samples are also being investigated.

The two modes of treatment for hepatitis B are immunomodulation and viral suppression—or interferon α and nucleoside analogues, respectively. Liver transplantation is effective and should be considered in patients with end-stage disease not responding to medical therapy.

Interferon alfa

According to a meta-analysis[34] of 498 patients receiving 3–6 months' interferon alfa compared with 339 untreated patients, being followed up for 6–12 months, interferon alfa induced loss of HBeAg, undetectable HBV DNA, and loss of hepatitis B surface antigens in 33%, 37%, and 8% of patients, respectively. The corresponding figures for patients receiving no treatment were 12%, 17%, and 2% (p=0·0001, p=0·0001, and p=0·001), respectively. The long-term follow-up data for interferon alfa treatment (16-week course of either 5 mU daily or 10 mU three times weekly) have been analysed. For white patients with chronic hepatitis B, up to 65% of patients who have HBeAg seroconversion will eventually also lose hepatitis B surface antigen. HBV DNA is usually still detectable after HBeAg seroconversion if the patients remain positive for hepatitis B surface antigen, but will become undetectable by PCR in 60–100% of those who lose the surface antigen. However, irrespective of whether or not they remain positive for hepatitis B surface antigen, patients who have HBeAg seroconversion have a lower risk of developing hepatitis B cirrhosis-related complications than do those who remain positive for HBeAg. They also have longer survival rates and longer intervals free from clinical complications.[35] This trend applies for patients who have received interferon alfa and for those who are untreated, but the clearance rate for HBeAg (and hepatitis B surface antigen) remains significantly higher in patients given interferon alfa during long-term follow-up.

Two long-term follow-up studies[36,37] have been done for interferon alfa in the Asian population. In the Taiwan study[36] of 101 patients whose median alanine transaminase on entry into the study was greater than 200 U/L, cumulative HBeAg seroconversion was higher in those given interferon alfa, especially in those who had steroid priming before receiving the drug, than in those who were not treated (p=0·049). However, development of new cirrhosis and of cirrhosis with complications did not differ between treated and untreated patients. In view of this finding, that hepatocellular carcinoma was more frequent in untreated patients than in treated patients is surprising.

In the second study[37] consisting of 411 patients from Hong Kong, the cumulative HBeAg seroconversion did not differ between those given interferon alfa and those who were not treated, irrespective of the concentration of alanine transaminase. HBV DNA was positive by PCR in 88·6% of treated patients and in 91·1% of untreated patients who had HBeAg seroconversion. Complications of cirrhosis and hepatocellular carcinoma occurred with equal frequency in both groups (nine of 208 treated patients and two of 203 untreated patients). Some of the differences in the two studies are probably related to the fact that the patients in the Taiwan study had very active disease with high concentrations of alanine transaminase on entry into the trial. Asian patients with chronic hepatitis B often have near normal concentrations of alanine transaminase throughout the course of their disease, or mild to moderate increases in alanine transaminase during acute exacerbation. The Taiwan study[36] therefore represents only a specific subgroup of Chinese patients.

Lamivudine

Lamivudine, the (negative) enantiomer of the deoxycytidine analogue 2′-deoxy-3′-thiacytidine, is the first nucleoside analogue to be licensed for treatment of chronic hepatitis B. The table summarises the 1-year results from three large scale trials[39–41] in Asian and white people who were HBeAg-positive and in those with precore HBV mutants. There are few side-effects. Results of a histological study[42] showed that 3 years' lamivudine treatment not only reduces necroinflammatory activity but also reverses fibrosis (including cirrhosis) in most patients. For patients who are HBeAg positive, therapy can be stopped 6–9 months after HBeAg seroconversion. The time for stopping treatment for patients with antibodies against HBeAg is difficult to determine and is still controversial.

The major drawback of lamivudine monotherapy is emergence of resistant HBV with mutation of the tyrosine-methionine-aspartate-aspartate (YMDD) motif at the

	Lai[39]*			Dienstag[40] †			Tassopoulos[41]‡		
	Patients (n=143)	Placebo (n=72)	p	Patients (n=66)	Placebo (n=71)	p	Patients (n=60)	Placebo (n=64)	p
Histological improvement§	56%	25%	<0·001	52%	23%	0·001	60%	..	..
Worsening fibrosis	0	15%	0·01	5%	20%	0·01	25	..	..
HBeAg seroconversion	16%	4%	0·02	17%	4%	0·04	NA	NA	..
Reduction of HBV DNA	98%	23%	<0·001	95–99%	40%	..	..	..	..
Sustained suppression of HBV DNA	..	..	..	44%	16%	<0·001	65%	..	..
Normalisation of alanine aminotransferase	72%	24%	<0·001	41%	7%	<0·001	..	..	..
Complete biochemical and HBV DNA response	..	..	..	..	..	..	63%¶	32%	<0·01
YMDD variant	15%	..	..	32%	..	..	27%	..	..

Values are proportion of patients with variable. *All patients were Chinese. †59% of patients were white. ‡Patients had precore mutant HBV. §Defined as two or more points reduction in Knodell necroinflammatory score. ¶Assessed at 24 weeks. Table adapted from CL Lai and colleagues.[38]

Effect of 1 year of 100 mg lamivudine daily in three placebo controlled trials

catalytic domain (C domain) of the viral reverse transcriptase/DNA polymerase. Incidence of YMDD mutants rises from 15–32% in the first year to 67–69% by the fifth year of treatment. Patients with the YMDD mutants tend to have alanine transaminase and HBV DNA concentrations that are lower than concentrations before treatment,[43] probably because the YMDD mutants have less replication competence.[44] However, replication competence can be partly restored when the B domain of the polymerase gene has a concomitant mutation (rtL180M), or when specific sites of the overlapping envelope S gene have rtL180M or concomitant mutations.[45] HBeAg seroconversion can still occur after the emergence of YMDD mutants.[43,46] Histological results worsen in similar proportions in patients with and without YMDD mutants,[42] but patients without YMDD mutants are more likely to improve and less likely to deteriorate. However in some patients with YMDD mutants, the HBV DNA concentrations can become higher than they were before treatment, which might result in varying degrees of hepatic decompensation.

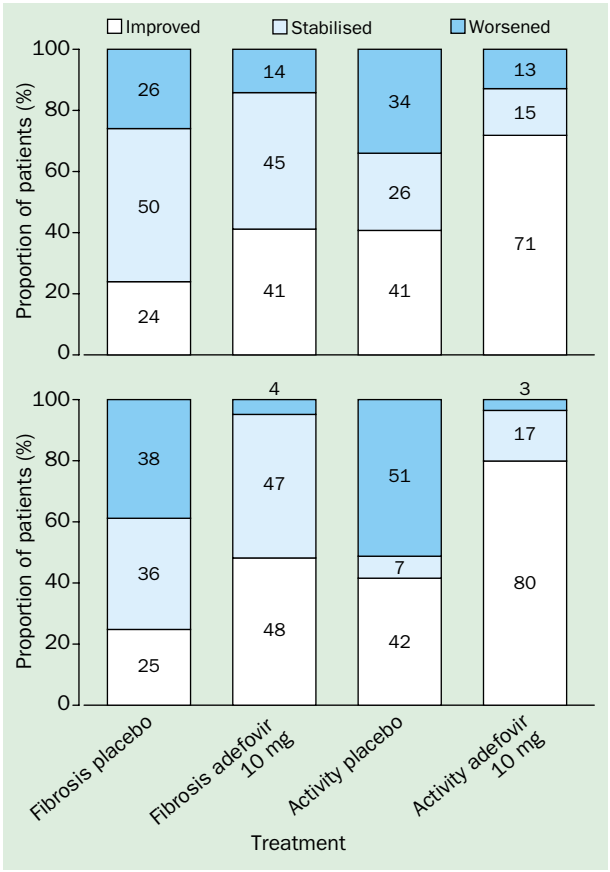
Lamivudine, unlike interferon alfa, is safe and effective in patients with decompensated liver disease, significantly improving liver function and survival in many patients.[47,48]

Adefovir dipivoxil

Adefovir dipivoxil is the second nucleoside analogue to be approved for use in chronic hepatitis B in the USA and in Europe. Adefovir, an acyclic analogue of deoxyadenosine monophosphate, inhibits amplification but not de-novo formation of covalently closed circular DNA in HBV-infected hepatocytes in ducks. In a phase III placebo-controlled trial,[49] 10 mg adefovir given daily for 48 weeks in 171 patients was associated with a significantly better improvement in the histological results, a higher rate of HBeAg seroconversion, a three logarithmic reduction of HBV DNA concentrations, and a higher chance of normalisation of alanine transaminase concentrations when compared with 167 patients receiving placebo. The same effectiveness on HBV DNA and alanine transaminase has been recorded in another randomised trial[50] of 10 mg adefovir given daily for 48 weeks in patients with chronic hepatitis B who are HBeAg negative. In these two trials, the histological results improved greatly with adefovir compared with placebo (figure).

The chance for development of adefovir resistance is low. A new adefovir-resistant mutant, rtN236T, has been identified in 1·6% of patients positive for HBeAg at 96 weeks' follow-up.[51] The low resistance to adefovir might be related to the close resemblance of adefovir to its natural substrate or the flexible acyclic structure of the adefovir molecule, which allows for several binding modes. The steric hindrance between the mutant aminoacid chain and drugs such as lamivudine that induce drug resistance is therefore subverted.[52] Adefovir dipivoxil is also active against lamivudine-resistant YMDD mutants.[53,54]

In the two large trials,[49,50] the overall adverse-event profile of a 10-mg dose of adefovir dipivoxil was similar to that of placebo. No renal toxic effects have been recorded with the 10 mg dose recommended for treatment of chronic hepatitis B, but renal function must be monitored closely. Mild changes were seen with the 30 mg dose. Adefovir dipivoxil causes proximal renal tubular dysfunction at higher doses of 60–120 mg used in earlier trials for patients with HIV. The changes were mild to moderate and reversible. The renal toxic effects are mediated through the human renal organic anion transporter 1.



Effect of 10 mg adefovir on fibrosis stage and necroinflammatory activity grade in patients with chronic hepatitis B

(Upper) antigen HBe positive: 168 patients with baseline biopsy given adefovir, 161 given placebo. (Lower) antigen HBe negative: 121 patients with baseline biopsy given adefovir, 57 given placebo. There was a significant difference (p<0·001) both for fibrosis and necro-inflammatory (activity) scores.

New nucleoside analogues

Tenofovir disoproxil fumarate, structurally similar to adefovir and approved for treatment of HIV, is also effective in suppressing replication of YMDD mutants.[55,56] However, no studies have been done in patients with the HBV infection alone.

Emtricitabine is similar to lamivudine in structure and potency. Clevudine, a pyrimidine analogue, is distinguished by a very slow rebound in HBV DNA concentrations after treatment is stopped.[57] Neither drug is effective against the YMDD mutants.

Entecavir, a guanosine analogue, has a strong inhibitory effect on the priming of HBV polymerase by guanosine triphosphate.[58] No entecavir-resistant mutants were detected in woodchucks after 3 years of treatment. A phase 2 trial[59] shows that at 6 months, both 0·1 mg and 0·5 mg daily doses of entecavir are more effective than 100 mg lamivudine in viral suppression. Entecavir, at a higher dose of 1 mg daily, is also effective against YMDD mutants.

Telbuvidine is one of three L-nucleosides with specific HBV inhibitory activity.[60] Like lamivudine, which is also an L-nucleoside analogue, telbuvidine has almost no side-effects. A phase 2 trial[60] shows that at 12 months, telbuvidine, either alone or in combination with lamivudine, causes significantly better viral suppression than lamivudine monotherapy. Telbuvidine 400 mg daily and 600 mg daily both cause an unprecedented reduction in median HBV DNA concentrations of more than 6 $\log_{10}$ at 12 months' treatment.

HBV and HIV coinfection

Lamivudine, at a dose of 300 mg daily, is used in patients coinfected with HIV and HBV for control of both infections. However such patients can develop YMDD HBV that is resistant to lamivudine. Adefovir dipivoxil has both anti-HBV and anti-HIV activities, but only the 10 mg dose effective for HBV should be used[54] and not the potentially renal toxic higher dose that is needed for HIV infection. As mentioned above, tenofovir disoproxil fumarate is active against HIV and HBV (YMDD wild-type and mutants). It should become a useful drug for patients coinfected with HBV and HIV.[61,62]

Combination treatment

The future probably lies with combination therapy, although with potent agents such as adefovir, entecavir, and telbuvidine, resistance would probably take a long time to develop even with monotherapy. Combination treatment can have additive and synergistic effects if the sites of action of the drugs are different, reduce any side-effects by reducing the dose of each drug used, and keep the risk of resistant mutants to a minimum through maximum viral suppression.[63] Immunomodulation can be combined with viral suppression or two or more viral suppressive agents can be combined together. For combination with viral suppression, initial trials of 16 weeks of interferon alfa combined with lamivudine show little additional benefit.[64] But the results of the combination of a longer duration of pegylated interferon (eg, for 1 year) and lamivudine are eagerly awaited.

Although steroid priming followed by lamivudine has shown encouraging results (60% HBeAg seroconversion for patients with alanine transaminase rebound after steroid withdrawal),[65] such a combination is not recommended for routine use because of the potential danger of severe, even fatal, HBV reactivation after steroid withdrawal.

The other option, of combining two (or more) nucleoside analogues, as in HIV therapy, is theoretically sound. It keeps viral suppression to a maximum, thereby reducing the chance that resistance will develop.[60] Moreover, the nucleoside analogues that are licensed or under trial have few side-effects. Preliminary results of the combination of telbuvidine and lamivudine in a phase 2 trial show no additional benefit over telbuvidine monotherapy.[66] A similar trial of the combination of adefovir and lamivudine also showed no additional benefit over lamivudine monotherapy.[67] Phase 3 trials of other combinations of nucleoside analogues have yet to be started and might show exciting results.

Conflict of interest statement

C L Lai has taken part in trials of lamivudine for GlaxoSmithKline, adefovir for Gilead, L-deoxythymidine for Idenix, and entecavir for Bristol-Myers Squibb. He received a Bristol-Myers Squibb unrestricted biomedical research grant for infectious disease. V Ratziu has taken part in trials of telbivudine for Idenix. M-F Yuen has taken part in trials of lamivudine for GlaxoSmithKline, adefovir for Gilead, L-deoxythymidine for Idenix, and entecavir for Bristol-Myers Squibb. T Poynard is a consultant for and owns 15% of Biopredictive, which markets FibroTest-ActiTest. The patent for this test belongs to Assistance Publique Hopitaux de Paris, a public organisation (reference US Patent Application Serial No 09/68,459. T Poynard has taken part in trials of pegylated interferon and ribavirin for Schering and Roche, lamivudine for GlaxoSmithKline, adefovir for Gilead, telbivudine for Idenix, and entecavir for Bristol-Myers Squibb. His department has participated in the trial of BILN2061 for Boehringer.

References

1 Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997; **337:** 1733–45.
2 WHO. World Health Report 1996: fighting disease, fostering development. Geneva: World Health Organization, 1996.
3 Poynard T, Yuen M-F, Ratziu V, Lai CL. Viral hepatitis C. *Lancet* 2003; **362:** 2095–100.
4 Chang MH, Chen CJ, Lai MS, et al, for The Taiwan Childhood Hepatoma Study Group. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. *N Engl J Med* 1997; **336:** 1855–59.
5 Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer* 1988; **61:** 1942–56.
6 Hyams KC. Risks of chronicity following acute hepatitis B virus infection: a review. *Clin Infect Dis* 1995; **20:** 992–1000.
7 Milich DR, Jones JE, Hughes JL, Price J, Raney AK, McLachlan A. Is the function of the secreted hepatitis Be antigen to induce immunologic tolerance in utero? *Proc Natl Acad Sci USA* 1990; **81:** 6599–63.
8 Reifenberg K, Deutschle T, Wild J, et al. The hepatitis B virus e antigen cannot pass the murine placenta efficiently and does not induce CTL immune tolerance in H-2B mice in utero. *Virology* 1998; **243:** 45–53.
9 Webster GJ, Hallett R, Whalley SA, et al. Molecular epidemiology of a large outbreak of hepatitis B linked to autohaemotherapy. *Lancet* 2000; **356:** 379–84.
10 Hoofnagle JH, Shafritz DA, Popper H. Chronic type B hepatitis and the "healthy" HBsAg carrier state. *Hepatology* 1987; **7:** 758–63.
11 Chu CJ, Hussain M, Lok ASF. Quantitative serum HBV DNA levels during different stages of chronic hepatitis B infection. *Hepatology* 2002; **36:** 1408–15.
12 Yuen MF, Sablon E, Hui CK, et al. Prognostic factors in severe exacerbation of chronic hepatitis B. *Clin Infect Dis* 2003; **36:** 979–84.
13 Yuen MF, Sablon E, Yuan HJ, et al. Relationship between the development of precore and core promoter mutations and hepatitis B e antigen seroconversion in patients with chronic hepatitis B virus. *J Infect Dis* 2002; **186:** 1335–38.
14 Chan HL, Leung NW, Hussain M, Wong ML, Lok ASK. Hepatitis B e antigen-negative chronic hepatitis B in Hong Kong. *Hepatology* 2000; **31:** 763–68.
15 Yuen MF, Lai CL. Natural history of chronic hepatitis B virus infection. *J Gastroenterol Hepatol* 2000; **15** (suppl)**:** E20–24.
16 Sugauchi F, Orito E, Ichida T, et al. Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. *J Virol* 2002; **76:** 5985–92.
17 Sanchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodes M. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. *Gastroenterology* 2002; **123:** 1848–56.
18 Chu CJ, Hussain M, Lok ASF. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. *Gastroenterology* 2002; **122:** 1756–62.
19 Kao JH, Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000; **33:** 998–1002.
20 Wai CY, Chu CJ, Hussain M, Lok AS. HBV genotype B is associated

with a higher response rate to interferon therapy than genotype C. *Hepatology* 2002; **36:** 1425–30.
21 Yuen MF, Sablon E, Yuan HJ, Wong DKH, Hui CK, Wong BCY, Chan AOO, Lai CL. Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications and hepatocellular carcinoma. *Hepatology* 2003; **37:** 562–67.
22 Orito E, Ichida T, Sakugawa H, et al. Geographic distribution of hepatitis B (HBV) genotype in patients with chronic HBV infection in Japan. *Hepatology* 2001; **34:** 590–94.
23 Sumi H, Yokosuka O, Seki N, et al. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* 2003; **37:** 19–26.
24 Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. *Science* 1999; **284:** 825–29.
25 Webster GJ, Reignat S, Maini MK, et al. Incubation phase of acute hepatitis B in man: dynamic of cellular immune mechanisms. *Hepatology* 2000; **32:** 1117–24.
26 Maini M, Boni C, Lee CK. The role of virus-specific CD8+ cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* 2000; **191:** 1269–80.
27 Rapicetta M, Ferrari C, Levrero M. Viral determinants and host immune responses in the pathogenesis of HBV infection. *J Med Virol* 2002; **67:** 454–57.
28 Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? *Hepatology* 2001; **34:** 194–203.
29 Yuen MF, Lai CL. Debates in hepatitis: How to assess HBV DNA reductions in association with therapy. *Viral Hep Reviews* 1999; **5:** 159–75.
30 Martinot-Peignoux M, Boyer N, Colombat M, et al. Serum hepatitis B virus DNA levels and liver histology in inactive HBsAg carriers. *J Hepatol* 2002; **36:** 543–46.
31 Yuen MF, Sablon E, Hui CK, Yuan HJ, Decraemer H, Lai CL. Factors associated with hepatitis B virus DNA breakthrough in patients receiving prolonged lamivudine therapy. *Hepatology* 2001; **34:** 785–91.
32 Yuen MF, Lai CL. Specific considerations in the design of hepatitis B virus clinical studies in the Far East. In Lau JYN, ed. Hepatitis B protocol (in press).
33 Yuen M-F, Lai C-L. Treatment of chronic hepatitis B. *Lancet Infect Dis* 2001; **1:** 232–41.
34 Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. *Ann Intern Med* 1993; **119:** 312–23.
35 Niederau C, Heintges T, Lange S, et al. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. *N Engl J Med* 1996; **334:** 1422–27.
36 Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *Hepatology* 1999; **29:** 979–75.
37 Yuen MF, Hui CK, Cheng CC, Wu CH, Lai YP, Lai CL. Long-term follow-up of interferon-alpha treatment in Chinese patients with chronic hepatitis B infection: the effect on HBeAg seroconversion and the development of cirrhosis-related complications. *Hepatology* 2001; **34:** 785–91.
38 Lai CL, Yuen MF, Locarnini S. Treatment of chronic hepatitis B infection. In Lai CL, Locarnini S ed. A guide to hepatitis B virus. International Medical Press, 2003: 193–214.
39 Lai CL, Chien RN, Leung NWY, et al, for the Asia Hepatitis Lamivudine study Group. A one-year trial of lamivudine for chronic hepatitis B. *N Engl J Med* 1998; **339:** 61–68.
40 Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 1999; **341:** 1256–63.
41 Tassopoulos NC, Volpes R, Pastore G, et al, for the lamivudine precore mutant study group. Efficacy of lamivudine in patients with hepatitis B e antigen-negative/hepatitis B virus DNA-positive (precore mutant) chronic hepatitis B. *Hepatology* 1999; **29:** 889–96.
42 Dienstag JL, Goldin RD, Healthcote EJ, et al. Histological outcome during long-term lamivudine therapy. *Gastroenterology* 2003; **124:** 105–17.
43 Lai CL, Dienstag JL, Schiff E, et al. Prevalence and clinical correlates of YMDD variants during lamivudine therapy of patients with chronic hepatitis B. *Clin Infect Dis* 2003; **36:** 687–96.
44 Allen MI, Deslauriers M, Andrew CW, et al. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. *Hepatology* 1998; **27:** 1670–77.
45 Torresi J. The virological and clinical significance of mutations in the overlapping envelope and polymerase genes of hepatitis B virus. *J Clin Virol* 2002; **25:** 97–106.
46 Yuen MF, Sablon E, Hui CK, Yuan HJ, Decraemer H, Lai CL. Factors associated with hepatitis B virus DNA breakthrough in patients receiving prolonged lamivudine therapy. *Hepatology* 2001; **34:** 785–91.
47 Kapoor D, Guptan RC, Wakil SM, et al. Beneficial effects of lamivudine in hepatitis B virus-related decompensated cirrhosis. *J Hepatol* 2000; **33:** 308–12.
48 Yao FY, Terrault NA, Freise C, Maslow L, Bass NM. Lamivudine treatment is beneficial in patients with severely decompensated cirrhosis and actively replicating hepatitis B infection awaiting liver transplantation: a comparative study using a matched, untreated cohort. *Hepatology* 2001; **34:** 411–16.
49 Marcellin P, Chang TT, Lim SG, et al, for the Adefovir Dipivoxil 437 Study Group. Adefovir dipivoxil for the treatment of HBeAg-positive chronic hepatitis B. *N Engl J Med* 2003; **348:** 808–16.
50 Hadziyannis SJ, Tassopoulos NC, Heathcote J, et al, for the Adefovir Dipivoxil 438 Study Group. Adefovir dipivoxil for the treatment of patients with Hepatitis B e antigen-negative chronic hepatitis B. *N Engl J Med* 2003; **348:** 800–07.
51 Angus P, Vaughan R, Xiong S, et al. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. *Gastroenterology* 2003; **125:** 292–97.
52 Das K, Xiong X, Yang H, et al. Molecular modeling and biochemical characterization reveal the mechanism of hepatitis B virus polymerase resistance to lamivudine (3TC) and emtricitabine (FTC). *J Virol* 2001; **75:** 4771–79.
53 Perrillo R, Schiff E, Yoshida E, et al. Adefovir dipivoxil for the treatment of lamivudine-resistant hepatitis B mutants. *Hepatology* 2000; **32:** 129–34.
54 Benhamou Y, Bochet M, Thibault V, et al. Safety and efficacy of adefovir dipivoxil in patients co-infected with HIV-1 and lamivudine-resistant hepatitis B virus: an open-label pilot study. *Lancet* 2001; **358:** 718–23.
55 Ying C, De Clercq E, Nicholson W, Furman P, Neyts J. Inhibition of the replication of the DNA polymerase M550V mutation variant of human hepatitis B virus by adefovir, tenofovir, L-FMAU, DAPD, penciclovir and lobucavir. *J Viral Hepat* 2000; **7:** 161–65.
56 van Bommel F, Wunsche T, Schurmann D, Berg T. Tenofovir treatment in patients with lamivudine-resistant hepatitis B mutants strongly affects viral replication. *Hepatology* 2002; **36:** 507–08.
57 Chu CK, Boudinot FD, Peek SF, et al. Preclinical investigation of L-FMAU as an anti-hepatitis B virus agent. *Antivir Ther* 1998; **3** (suppl)**:** 113–21.
58 Colonno RJ, Genovesi EV, Medina I, et al. Long-term entecavir treatment results in sustained antiviral efficacy and prolonged life span in the woodchuck model of chronic hepatitis infection. *J Infect Dis* 2001; **184:** 1236–45.
59 Lai CL, Rosmawati M, Lao J, et al. Entecavir is superior to lamivudine in reducing hepatitis B virus DNA in patients with chronic hepatitis B infection. *Gastroenterology* 2002; **123:** 1831–38.
60 Bryant ML, Bridges EG, Placidi L, et al. Antiviral L-nucleosides specific for hepatitis B virus infection. *Antimicrob Agents Chemother* 2001; **45:** 229–35.
61 Puoti M, Airoldi M, Bruno R, et al. Hepatitis B virus co-infection in human immunodeficiency virus-infected subjects. *AIDS Rev* 2002; **4:** 27–35.
62 Nelson M, Portsmouth S, Stebbing J, et al. An open-label study of tenofovir in HIV-1 and hepatitis B virus co-infected individuals. *AIDS* 2003; **17:** 7–10.
63 Lewin S, Walters T, Locarnini S. Hepatitis B treatment: rational combination chemotherapy based on viral kinetic and animal model studies. *Antiviral Res* 2002; **55:** 381–96.
64 Schalm SW, Heathcote J, Cianciara J, et al. Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomized trial. *Gut* 2002; **46:** 562–68.
65 Liaw YF, Tsai SL, Chien RN, Yeh CT, Chu CM. Prednisolone priming enhances Th 1 response and efficacy of subsequent lamivudine therapy in patients with chronic hepatitis B. *Hepatology* 2002; **32:** 604–09.
66 Lai CL, Leung N, Teo EK, et al. International multicenter trial of LdT (Telbivudine) alone and in combination with lamivudine, for chronic hepatitis B: an interim analysis. *Hepatology* 2002; **36:** 301A.
67 Sung JJY, Lai JY, Zeuzen S, et al. A randomised, double-blind, phase II study of lamivudine (LAM) compared to lamivudine plus adefovir dipivoxil (ADV) for treatment-naive patients with chronic hepatitis B (CHB): week 52 analysis. *J Hepatol* 2003; **38** (suppl)**:** 25–26.