Background: There is a paucity of data on the long-term efficacy of combination lamivudine and adefovir therapy in patients with lamivudine-resistant chronic hepatitis B. Methods: We determined the cumulative virological, serological and biochemical outcomes of 165 lamivudine-resistant chronic hepatitis B patients on lamivudine and adefovir for up to 5 years. Resistance profiles using a line probe assay were determined among patients with detectable viraemia. The significance of different baseline and on-treatment virological parameters was analysed. Results: The median age and duration of follow-up were 45.1 years and 37.1 months, respectively. The cumulative rates of HBV DNA undetectability (<20 IU/ml), alanine aminotransferase normalization and hepatitis B e antigen seroconversion up to 5 years were 74.0%, 95.1% and 44.4%, respectively. One patient achieved hepatitis B surface antigen seroclearance. The 5-year cumulative resistance rate to adefovir was 10.2%. Among different baseline and on-treatment virological parameters, week 24 HBV DNA <200 IU/ml was associated with an increased chance of long-term virological suppression (P<0.001, OR 13.89, 95% CI 3.90, 49.46). Primary non-response and high baseline viral titres were not useful in predicting long-term virological outcomes. The 5-year cumulative rate of serum creatinine elevation >0.5 mg/dl was 4.1%. Conclusions: Combination lamivudine and adefovir therapy for up to 5 years achieved modest rates of virological suppression, but resistance developed in only 10.2% of patients. Week 24 HBV DNA <200 IU/ml was predictive of favourable long-term virological outcomes and could be used to assist treatment decisions on continuing lamivudine and adefovir or switching to more potent therapy.

Introduction

The introduction of nucleoside analogue therapy in the past two decades has revolutionized the treatment of chronic hepatitis B (CHB) [1]. By achieving sustained virological suppression [2], long-term nucleoside analogue therapy has been shown to reduce cirrhotic complications and hepatocellular carcinoma (HCC) for both cirrhotic and non-cirrhotic patients [3–5].

Drug resistance remains an important issue in long-term treatment [6]. For lamivudine, the signature rtM204VI and rtL180M mutations occur in >70% of CHB patients after 5 years of therapy [4,7]. Tenofovir disoproxil fumarate was approved for the treatment of CHB in 2008 and is the most effective treatment for lamivudine-resistant CHB [8]. The worldwide availability of tenofovir for the treatment of CHB was (and in the Asian countries still is) limited; hence, over the years, a large proportion of lamivudine-resistant patients have instead been treated with adefovir dipivoxil.

Both switching to adefovir monotherapy and the addition of adefovir to lamivudine [9–12] have been proven effective in lamivudine-resistant CHB. The addition of adefovir to lamivudine has achieved better rates of virological suppression and lower rates of adefovir resistance for up to 3 years of therapy, although evidence is mainly concentrated in hepatitis B e antigen (HBeAg)-negative patients [13]. Although there is currently data on adefovir monotherapy for up to 5 years in lamivudine-resistant CHB [14], similar long-term data on combination lamivudine and adefovir therapy is lacking. In addition, most studies on
combination lamivudine and adefovir therapy involved Caucasian CHB patients, while Asian CHB patients were under-represented.

Studies of nucleoside analogues have shown that measuring serum HBV DNA levels after a certain period of therapy can be predictive of favourable long-term response [15–17]. For adefovir, an effective viral suppression after 24 weeks of therapy has been shown to be predictive of long-term response [18,19]. However, these studies are limited by the small number of patients and the short durations of follow-up. A more recent study proposed using the HBV DNA level after 1 year to predict long-term outcomes [20]. Whether this holds true for the combination of lamivudine and adefovir therapy remains to be determined.

The aims of our present study were twofold. Firstly, we aimed to determine the degree of viral suppression and resistance following use of combination lamivudine and adefovir therapy for up to 5 years. Secondly, we wanted to establish if the HBV DNA levels after a certain duration of therapy could predict long-term outcomes.

Methods

The Liver Clinic, Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong, is a tertiary referral centre in our locality for patients with chronic liver diseases. Since the availability of adefovir in 2004, combination lamivudine and adefovir was started for patients with evidence of lamivudine resistance. Between February 2004 and October 2009, adefovir was added to the drug regimen of CHB patients on lamivudine on the basis of three criteria: virological breakthrough with or without serum alanine aminotransferase (ALT) elevation; the presence of signature lamivudine mutations rtM204V/I (with or without rtL180M); and >12 months of combination therapy.

All patients were positive for hepatitis B surface antigen (HBsAg) for ≥6 months prior to treatment and were initially on lamivudine monotherapy for a period of ≥12 months. Patients with the following concomitant conditions were excluded: chronic hepatitis C and D infection, autoimmune hepatitis, Wilson’s disease, primary biliary cirrhosis, significant intakes of alcohol (30 g per day for men, 20 g per day for women) and those on immunosuppressive therapy. This study was approved by the Institutional Review Board of the University of Hong Kong and the Hospital Authority Hong Kong Western Cluster, Hong Kong.

Liver biochemistry, a-fetoprotein and serum creatinine were measured at every follow-up. Patients were followed up every 3 to 6 months; all serum samples were stored at -20°C until time of use.

Due to limited government subsidisation of CHB medications in our locality during the study period, patients were allowed to stop lamivudine for financial reasons after ≥6 months of combination lamivudine and adefovir, although this was strongly discouraged. The follow-up duration was censored at the date of stopping lamivudine. Tenofovir has become available in our hospital since November 2008. Patients were given the option of switching to tenofovir monotherapy or tenofovir and lamivudine combination regardless of the degree of viraemia; the follow-up duration was censored at the date of tenofovir commencement.

HBsAg, HBeAg and antibody to HBeAg (anti-HBe) were tested by ELISA (Abbott Laboratories, Chicago, IL, USA). Serum HBV DNA levels were measured by Cobas Taqman assay (Roche Diagnostics, Branchburg, NJ, USA): there was a linear range of 20 to 1.98×10^7 IU/ml. Viral mutational analysis was performed annually for samples with detectable viraemia (≥20 IU/ml) using a line probe assay (LiPA; Innogenetics NV, Gent, Belgium). LiPA DR version 2 was used to identify the amino acids at the codons of rt180, rt181, rt204 and rt236.

The upper limit of normal of ALT was set at 40 U/l. Virological breakthrough was defined as either an increase in serum HBV DNA by ≥1 log IU/ml from the nadir for patients with detectable viraemia or serum HBV DNA≥20 IU/ml for patients with undetectable viraemia during treatment. Primary non-response was defined as a decrease in HBV DNA<2 log IU/ml after 6 months of therapy [21]. Patients who had ≥2 log IU/ml decrease in HBV DNA after 6 months were defined as ‘primary responders’. Genotypic resistance to lamivudine and adefovir were defined as the presence of signature mutations rtM204V/I (with or without rtL180M) and rtA181V/T and/or rtN236T, respectively.

All continuous variables are expressed as median (range). All statistical analyses were performed using SPSS version 18.0 (SPSS Inc, Chicago, IL, USA). The Mann–Whitney U test was used for continuous variables with a skewed distribution. Pearson’s χ² test, or when appropriate Fisher’s exact test, was used for categorical variables. Serum creatinine clearance was measured using the modification of diet in renal disease (MDRD) equation as validated in the Chinese population [22]. The cumulative rates of viral suppression, HBeAg seroconversion and virological breakthrough were calculated using the Kaplan–Meier method; differences were determined by the log-rank test. The cumulative rate of development of viral resistance was calculated by Equation 1 [23]:

\[ P=1-(1-n_1/N_1)(1-n_2/N_2)\ldots(1-n_x/N_x) \]  \[ (1) \]

A two-sided P-value of <0.05 was considered statistically significant.
Results

Between February 2004 and October 2009, 189 CHB patients were started on combination lamivudine and adefovir therapy in our centre. Applying the criteria mentioned above, we excluded patients with no signature lamivudine mutations at baseline (n=12), lamivudine stopped after <12 months of combination therapy (n=7), concomitant immunosuppressive therapy (n=4) and coexisting Wilson’s disease (n=1). A total of 165 patients were included in the present study. The baseline demographics of all patients are listed in Table 1. When compared with HBeAg-negative patients, HBeAg-positive patients had a significantly higher baseline HBV DNA (P<0.001). All patients had signature lamivudine-resistant mutations and no adefovir-resistant mutations at baseline.

The median duration of follow-up was 37.1 (range 12.3–60) months. Altogether 163, 147, 109, 75 and 33 patients were followed-up for 1, 2, 3, 4 and 5 years, respectively. Fifteen patients (9.1%) stopped lamivudine after a median period of 16.7 (range 12.9–28.8) months. Thirty-three patients (19.6%) switched to either tenofovir monotherapy (n=15) or combination tenofovir and lamivudine (n=18) after a median period of 28.9 (range 12.3–59.4) months. The median serum HBV DNA level at the time of switching was 3.24 (range 1.30–5.29) log IU/ml; nine patients (27.3%) had undetectable viral load (<20 IU/ml). Among the 24 patients with detectable HBV DNA at the time of switching, 20 patients (83.3%) achieved undetectable HBV DNA levels after 6 months of tenofovir-based therapy.

Virological and biochemical response

The cumulative rate of viral suppression to HBV DNA undetectability (HBV DNA<20 IU/ml) is shown in Figure 1. For all patients, the cumulative rates of HBV DNA undetectability were 17.0%, 29.5%, 42.5%, 63.2% and 74.0% for years 1, 2, 3, 4 and 5, respectively. HBeAg-positive patients achieved a significantly higher cumulative rate of HBV DNA undetectability when compared with HBeAg-negative patients (P=0.001). Altogether, 93 patients (56.4%) eventually achieved HBV DNA undetectability. The proportion of patients achieving detectable viraemia was also significantly higher in HBeAg-positive patients (n=34, 70.8%) compared with HBeAg-negative patients (n=59, 50.4%; P=0.025).

Many published studies on adefovir efficacy have used a more modest definition of virological suppression (between 40–80 IU/ml) [12,14,20,24]. In our present study, the cumulative rates of HBV DNA<60 IU/ml were 18.2%, 40.1%, 56.3%, 67.0% and 86.3% for years 1, 2, 3, 4 and 5, respectively.

Among 72 patients with detectable viraemia at their last follow-up, 28 (38.9%) had HBV DNA>2,000 IU/ml,
of which 15 (53.6%) were switched to tenofovir-based therapy. The remaining 13 patients did not develop biochemical breakthrough and opted to remain on a combination of adefovir and lamivudine owing to the high financial cost of tenofovir in our locality during the study period. A total of 5 of these 13 (38.4%) patients later admitted to having poor drug compliance.

The cumulative rates of ALT normalization for years 1, 2, 3, 4 and 5 were 62.2%, 77.6%, 85.4%, 90.3% and 95.1%, respectively.

Serological outcomes
The cumulative rate of HBeAg seroconversion among 117 HBeAg-positive patients with combination therapy is depicted in Figure 2. Overall, 31 (26.7%) patients experienced HBeAg seroconversion. The cumulative rate of HBeAg seroconversion up to year 5 was 44.4%. One patient achieved HBsAg seroclearance after 43 months of combination lamivudine and adefovir therapy. This patient achieved undetectable HBV DNA 6 months after commencing therapy. He was kept on both drugs after HBsAg seroclearance due to clinical evidence of cirrhosis.

Figure 3. Cumulative rates of virological breakthrough and resistance with combination lamivudine and adefovir therapy

Percentages depicted are for all patients (n=165). aCalculated using the Kaplan–Meier method. bCalculated as suggested by Pawlotsky et al. [23]. Genotypic resistance to adefovir determined by a line probe assay. HBeAg, hepatitis B e antigen.
Virological breakthrough and resistance

The cumulative rates of virological breakthrough and genotypic resistance to adefovir are depicted in Figure 3. Altogether, 22 patients (13.3%) developed virological breakthrough, of these, 21 (95.5%) patients had virological breakthrough after a follow-up period of ≥3 years. In total, 2 (9.1%) patients had coexisting biochemical breakthrough. The cumulative 5-year rate of virological breakthrough was 40.5%.

Resistance profiling was performed in 113 patients with ≥1 sample of detectable viraemia after commencing lamivudine and adefovir. Lamivudine-resistant mutations were persistent in 98 (86.7%) patients. The remaining 15 (13.3%) patients reverted to wild-type HBV (rt180M and rt204L). Only 5 (3.0%) patients developed adefovir resistance and virological breakthrough after a follow-up period of ≥3 years. In total, 2 (9.1%) patients had coexisting biochemical breakthrough. The cumulative 5-year rate of virological breakthrough was 40.5%.

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Among these five patients with adefovir resistance, four did not develop virological breakthrough and were thus continued on combination therapy. The remaining patient developed adefovir resistance and virological breakthrough after 3 years of lamivudine and adefovir and was switched to tenofovir monotherapy, achieving undetectable HBV DNA after 6 months of tenofovir.

All 13 patients with HBV DNA>2,000 IU/ml at their last follow-up without being changed to tenofovir-based therapy had no genotypic resistance towards adefovir.

Predicting virological suppression

We analysed the association of viral loads after a finite duration of therapy with virological suppression up to years 3, 4 and 5 (Table 2). Among 109 patients with ≥3 years of follow-up, 31.2% (n=34) achieved a week 24 HBV DNA<200 IU/ml. This was associated with long-term virological suppression (P<0.05 for all). The OR of achieving HBV DNA undetectability up to years 3 and 4 were 13.89 (95% CI 3.90, 49.46) and 7.20 (95% CI 1.51, 34.24), respectively. All patients with week 24 HBV DNA<200 IU/ml and with 5 years of follow-up achieved HBV DNA undetectability at year 5. Undetectable HBV DNA at week 24, present in 15.6% (n=17) of patients, was also associated with HBV DNA undetectability up to year 3 (P=0.011) with an OR of 6.03 (95% CI 1.30, 27.89). Undetectable viral load at year 1 was also associated with viral suppression up to years 3 and 4, but with an inferior OR (11.09 and 4.28, respectively) when compared with week 24 HBV DNA<200 IU/ml.

We also analysed the association of ‘primary responders’ and high baseline viral load with long-term virological suppression (Table 2). Neither parameter was
associated with HBV DNA undetectability at years 3, 4 and 5.

Clinical outcomes and safety
Overall, 4 (2.4%) patients developed HCC at 11, 12, 31 and 48 months after commencement of combination lamivudine and adefovir. All four patients were cirrhotic at baseline. In total, 2 (1.2%) patients had a prior history of HCC before commencing combination lamivudine and adefovir; HCC did not recur in these 2 patients. During the study duration, no patients developed hepatic decompensation requiring liver transplantation or resulting in mortality.

Overall, 5 (3.0%) patients had an elevation of serum creatinine of >0.5 mg/dl and a drop in creatinine clearance to <50 ml/min/1.73 m² after a median period of 21.5 months (range 14.9–41.4), which required an adjustment of adefovir dosage. The 5-year cumulative rate of creatinine elevation >0.5 mg/dl was 4.1%. No further deterioration in renal function was noted in these five patients after adjustment of adefovir dosage.

Discussion
Tenofovir is now the most effective nucleotide analogue for lamivudine-resistant CHB. However, given the initial limited worldwide availability of tenofovir for the treatment of CHB, the majority of lamivudine-resistant CHB patients have already been treated with adefovir-based therapy. By contrast to adefovir monotherapy [14], combination therapy of lamivudine and adefovir has been proven effective with low rates of adefovir resistance [13]. The cumulative rate of HBV DNA≥60 IU/ml up to 5 years for adefovir monotherapy is 65.6% [14], considerably lower than the 86.5% achieved by combination adefovir and lamivudine therapy in our study.

An important clinical question would be whether patients on pre-existing combination therapy of lamivudine and adefovir should continue with their current therapy or should be switched to tenofovir. Our present study, using a strict criteria of HBV DNA<20 IU/ml for viral suppression and consisting of >70% HBsAg-positive patients, showed that a combination of lamivudine and adefovir achieved a modest cumulative virological suppression rate of 74% after 5 years of therapy. Hence, the identification of patients with subsequent favourable outcomes at treatment commencement or during an early stage of therapy would help clinicians to decide whether patients should continue with lamivudine and adefovir or should be switched to tenofovir-based therapy.

We analysed and compared several parameters recommended by previous studies to be useful in predicting long-term virological outcomes in nucleoside analogue therapy: high baseline viral load [25], primary non-response [26] and a defined HBV DNA level after a certain period of therapy [16,17,27]. Our present study showed that week 24 HBV DNA<200 IU/ml was the best predictor of long-term virological suppression: 91.2%, 88.9% and 100% of patients who achieved this virological cutoff maintained undetectable viramia up to years 3, 4 and 5, respectively. Hence, in patients achieving week 24 HBV DNA<200 IU/ml, the continuation of combination lamivudine and adefovir would still be effective. In patients failing to achieve this virological cutoff level, a switch to tenofovir-based therapy for long-term virological suppression should be considered.

Other virological cutoff levels, including HBV DNA undetectability at week 24 and year 1, are also useful in predicting long-term virological suppression. A recent Asian study investigating the efficacy of combined lamivudine and adefovir, despite being limited by performing resistance profiles only at baseline and virological breakthrough, concluded that week 24 HBV DNA undetectability was associated with favourable outcomes [28]. The lower OR obtained for week 24 HBV DNA undetectability in our current study could be due to statistical under-power (n=17). However, using a more liberal HBV DNA value of <200 IU/ml rather than undetectability as a marker for week 24 would target a larger patient population, allowing more patients with favourable outcomes to be identified. This is shown in our present study in which, among patients with ≥3 years of follow-up, week 24 HBV DNA<200 IU/ml and HBV DNA undetectability comprise 31.2% and 15.6%, respectively.

The concept of primary non-response was initially designed to identify early nucleoside analogue treatment failure [26], and is still mentioned in current CHB treatment guidelines [21,29]. Although the usefulness of this parameter is limited in more potent nucleoside analogues [17], it has been suggested that it still retains a monitoring role in adefovir-based therapy. Our study showed that determining whether patients had primary non-response was not useful in predicting long-term virological outcomes.

Only five patients developed genotypic resistance towards adefovir. More importantly, the majority of patients with adefovir resistance did not develop virological breakthrough, which is similar to previous findings [13,30]. Hence, resistance is not an important factor impeding virological suppression. The modest drug potency of 10 mg of adefovir [31] and drug compliance [30] were probably more vital factors contributing to suboptimal virological response. Another important finding was that resistance to lamivudine was not persistent among a proportion of patients; reversion to wild-type virus was seen at the rt204 and rt180 loci. This is also consistent with previous findings [13].
A drawback of our study was the limitation of resistance testing to patients with detectable viraemia (>20 IU/ml), thus the rates of adefovir resistance could be underestimated. Nevertheless, according to an internal validation by the manufacturer, the lower limit of LiPA application for testing is an HBV DNA level of 990 copies/ml (or 198 IU/ml) [32]. A previous study found the amplification of positive PCR sequences only possible among 17.8% of CHB patients with undetectable viral load (<12 IU/ml) [25], justifying the testing of resistance profile only among patients with detectable viraemia in our study. The use of LiPA instead of direct sequencing already increases the diagnostic accuracy of resistance. LiPA can detect the presence of mutants when the mutant population constitutes 5% of the total viral population [33], unlike direct sequencing which requires mutants to make up 20–30% of the total viral population before detection is possible [15]. Another limitation was that the majority of our cases (88.5%) started combination lamivudine and adefovir only during ALT elevation, although a previous study has already shown improved outcomes with early addition of adefovir during virological breakthrough [34]. This is again due to the limited government subsidisation of CHB medications making early addition of adefovir difficult in our locality. Serum phosphate and urine protein were also not monitored in our study. Nevertheless, despite concerns of the renal tubular loss of phosphate in long-term adefovir therapy, the changes in serum phosphate levels are likely to be clinically insignificant [35].

In conclusion, combination lamivudine and adefovir therapy among lamivudine-resistant Asian CHB patients resulted in modest improvements in virological outcomes up to year 5. Genotypic resistance to adefovir was uncommon and clinically insignificant. A week 24 HBV DNA<200 IU/ml was able to identify patients with favourable long-term virological outcomes. The use of this virological cutoff level could assist clinicians in deciding whether to continue with lamivudine and adefovir combination therapy or to switch to tenofovir-based therapy.

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Disclosure statement
The authors declare no competing interests.

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