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Treatment of HIV/HBV Coinfection: Clinical and Virologic Issues

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Abstract

Chronic hepatitis B affects nearly 10% of HIV-infected patients. Thus, approximately four million people worldwide are HBV/HIV coinfected. Hepatitis B virus (HBV) infection is a dynamic disease and coinfection with HIV impacts directly on the outcome of HBV infection, considerably complicating its natural history, diagnosis, and management. Hepatic necroinflammation is lower in HBV/HIV coinfection, yet liver damage, especially fibrosis, progresses at a faster rate than in HBV monoinfection. With improved control of HIV disease with HAART, liver disease has emerged as one of the leading causes of death in patients with HIV. Anti-HBV therapy should be considered for all HIV/HBV-coinfected patients with evidence of liver disease, irrespective of the CD4 cell count. In coinfected patients not requiring HAART, HBV therapy should be based on agents with no HIV activity such as adefovir. In contrast, in patients with CD4 counts less than 350 cells/µl, the use of agents with dual anti-HIV and anti-HBV activity should be considered. Combination therapy should ideally be used to avoid or delay the development of antiviral resistance. Regular monitoring of patients is imperative to recognize reactivation and subsequent need for treatment, and to identify drug resistance and viral breakthrough early. Similar close monitoring is required for patients presenting with advanced HIV infection and reduced functional hepatic reserve due to HBV-related cirrhosis. Effective antiviral treatment can precipitate immune reconstitution disease resulting in serious hepatic flare and precipitating liver decompensation. Clearly, more data are needed to more effectively treat HIV/HBV coinfection. **(AIDS Reviews 2007;9:40-53)**

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Key words

HIV. Hepatitis B. Liver. Adefovir. Tenofovir. Entecavir. Lamivudine. Drug resistance.

Introduction: effect of HIV infection on chronic hepatitis B

Serological evidence of hepatitis B virus (HBV) infection is found in up to 90% of HIV-infected individuals worldwide, and 10% of HIV-infected patients are chronically infected with HBV. There is considerable variation in prevalence according to geographic region and exposure risk. Under normal circumstances, HBV replication in hepatocytes is not generally cytopathic[1]; rather, it is the host's immune response, which is either inadequate or inappropriate, that is responsible for the liver disease of chronic hepatitis B (CHB). Coinfection with HIV results in considerable modification of the natural history of HBV infection and is associated with increased hepatitis B e antigen (HBeAg) carriage, higher rates of chronic infection as well as higher HBV-DNA levels, lower serum alanine aminotransferase (ALT) levels and decreased HBeAg seroconversion. Also, milder histologic necroinflammatory scores have been recorded, but despite this, progression to cirrhosis is more common. Most importantly, HIV coinfection leads to increased liver-related mortality from CHB[2]. However, the exact mechanism(s) by which HIV interacts with HBV in this process is presently unknown. This observed increase in liver-disease pro-

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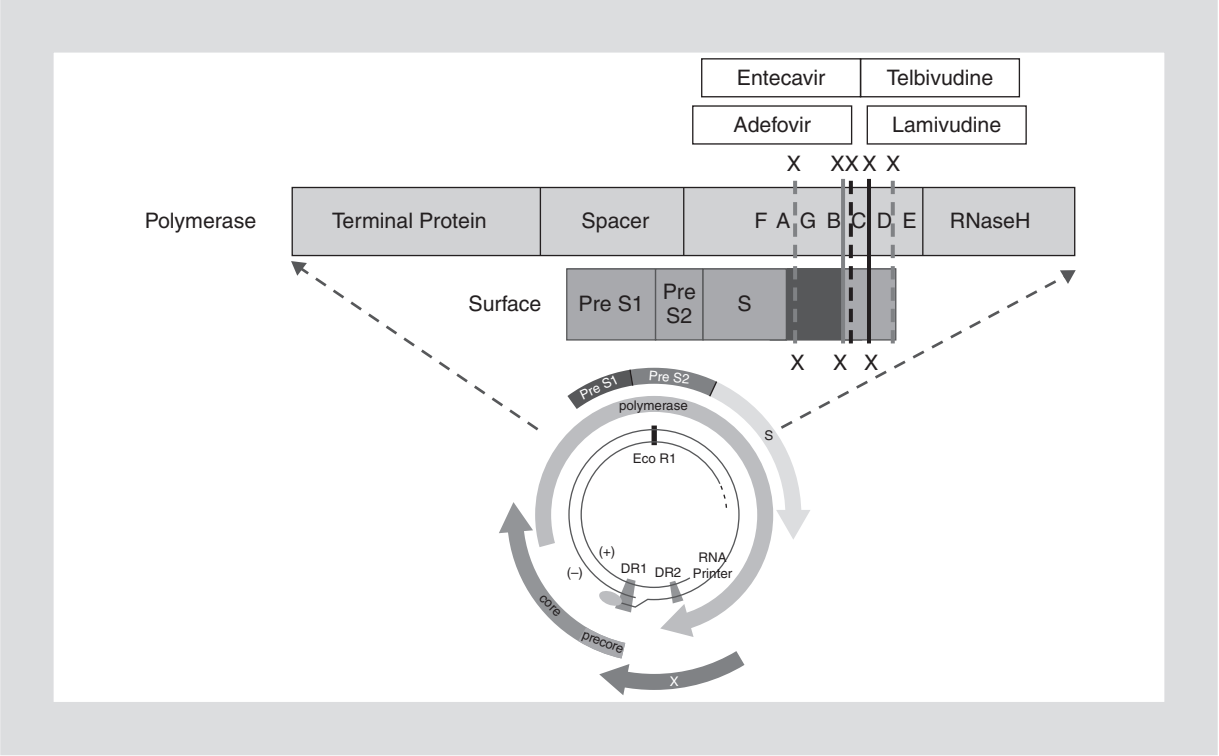

Figure 1. *Hepatitis B virus (HBV) DNA genome showing the overlapping open reading frames, and in particular how the polymerase-envelope overlap can affect each other during the emergence of nucleos(t)ide analog drug resistance.*

gression in HIV-infected patients underscores the need to evaluate persons for hepatitis B treatment.

Molecular virology of hepatitis B

Human HBV is the prototype member of the *Hepadnaviridae* family[3]. Eight HBV genotypes have been identified so far (A-H)[4], and they are unequally distributed worldwide, with genotypes A and D predominating in Western Europe and North America. In Asia, HBV genotypes B and C are prevalent. Genotype D is predominant in Eastern Europe, the Mediterranean basin, the Middle East, and the Indian subcontinent. Genotype E is frequent in Central Africa, and genotype F is found in South America. Immigrants from various origins have brought HBV genotypes A, B, C, and D to North America, whilst other immigrants have brought genotypes E, B, and C to Europe[5]. In Asia, there is increasing evidence that genotypes influence the progression of liver disease, with genotype B having a slower progression to cirrhosis, a lower rate of hepatocellular carcinoma, as well as greater HBeAg seroconversion[6-8] then genotype C.

The HBV partially double-stranded, circular DNA genome (3200 nt) and its encoded polymerase are contained within an icosahedral capsid, itself enveloped by a lipid bilayer into which three different surface proteins are anchored[9]. The genome encodes at least four different overlapping but frame-shifted open reading frames (ORF) (Fig. 1). The pre-S/S ORF codes for the three surface proteins, with translation of the S region producing hepatitis B surface antigen (HBsAg), pre-S2+S region forms the M-protein of HBsAg, whilst, pre-S1+pre S2+S region produces the L form of HBsAg. The L-form is the major envelope protein of the virion, whilst the S-form is the major protein found in the 22 nm subviral particles. The pre-C/C ORF codes for the capsid protein (C region) and translates the hepatitis B core antigen (HBcAg) and, when the full pre-C/C region is translated, a nonstructural protein bearing the HBeAg determinant is made[9]. The HBeAg is exported to the peripheral circulation after posttranslational processing. Nucleotide substitutions in the pre-C region may abrogate the production of the HBe protein translationally, whereas mutations in the basic core promoter region also regulate its expression at the transcriptional level. The polymerase ORF spans a large part of the HBV genome and encodes the HBV polymerase that bears several properties, including a reverse transcriptase activity, an RNaseH activity, and a terminal protein region at the N-terminus, which is involved in protein priming of reverse transcription (rt). Finally, the X

ORF codes the X protein, which is thought to play an important regulatory role, acting as a transactivator of both viral and cellular genes[3,9]. Several proteins of HBV are considered to be involved in HBV-related carcinogenesis, HBx, HBeAg, and truncated Pre-S[10].

The HBV lifecycle starts with virion attachment to an unknown specific receptor complex[3]. The viral envelope then presumably fuses with the cell membrane, releasing the nucleocapsid into the cytoplasm, from where it is transported to the nucleus with the genomic HBV-DNA and HBV-DNA polymerase. The genomic relaxed circular DNA is repaired to yield a fully double-stranded DNA molecule, which forms a viral mini-chromosome. Viral mini-chromosomal DNA is found in a covalently closed circular DNA (cccDNA) form and only within the nuclear compartment of infected cells. The cccDNA is the major transcriptional template of the virus and has a very long intracellular half-life, despite the fact that it is not integrated into the cellular genome. It also serves as a reservoir for "viral reactivation" when nucleos(t)ide analog antiviral therapy is withdrawn. The HBV cccDNA is transcribed by cellular RNA polymerases into messenger RNA for viral protein synthesis, and into a greater than genomic length pre-genomic RNA molecule, which is subsequently encapsidated with HBcAg dimers in the cell cytoplasm together with the HBV-DNA polymerase and host-cell chaperones. The reverse transcriptase (rt) function of the HBV-DNA polymerase catalyzes the synthesis of the negatively stranded genomic DNA, while the pre-genomic RNA is being degraded by the RNaseH activity of the polymerase. The positive-sense DNA strand is then synthesized within the replicating core complex. Newly generated nucleocapsids can be recycled back to the nucleus to replenish the transcriptional pool of cccDNA molecules, keeping the copy number around 10-30 per cell. However, most replicating cores bud into the endoplasmic reticulum, interacting with the newly synthesized viral envelope proteins L, M, and S to form mature virions that are subsequently secreted into the extracellular space[3] along with the other hepatitis B-associated forms, including the long filamentous structures and 22 nm subviral particles.

Antiviral HBV drug resistance

Definitions for primary and secondary antiviral drug failure in CHB have been published[11]. Primary antiviral failure (or nonresponse) can be classified as the inability of the antiviral agent to reduce the serum HBV-DNA viral load by ≥ 1 $\log_{10}$ IU/ml within the first three months of treatment. This is usually due to pharmacologic or antiviral agent efficacy issues[12]. Secondary antiviral treatment failure, which is usually associated with drug resistance, is defined as a ≥ 1 $\log_{10}$ IU/ml increase in viral load from nadir in two consecutive serum samples, typically collected at least one month apart in patients who initially responded to therapy. Thus, viral-load monitoring at the initiation of therapy is recommended before commencing a course of treatment. A sensitive and specific HBV-DNA viral-load assay that has been standardized to read out in IU/ml should be used to detect viral rebounds associated with resistance. This is very relevant in patients with advanced liver disease, as can be found in HIV coinfection.

Antiviral drug resistance reflects the reduced susceptibility of a virus to the inhibitory effect of a drug, and results from a process of adaptive mutations under the selection pressure of antiviral therapy. Two types of mutations have been identified that have been associated with treatment failure to nucleos(t)ide analogs: primary resistance mutations (Fig. 2), which are directly responsible for the associated drug resistance; and secondary or compensatory mutations, which probably occur in order to promote or enhance replication competence. Compensatory mutations emerge because the selection of genetic resistance is usually associated with some cost in replication fitness for the virus. Compensatory mutations are important as they "fix" the discriminatory primary drug-resistant mutations into the genetic archive of the HBV mini-chromosome, thus providing quasispecies memory[13].

With a number of nucleoso(t)ide analogs now approved for the treatment of CHB in many countries (see below), it will become important to describe drug resistance in terms of clinical and laboratory relevance. For example, antiviral drug resistance can be described in terms such as high (> 100-fold increase in EC_{50}), intermediate (10 to 99-fold increase in EC_{50}), or low (2 to 9-fold increase in EC_{50}) level with respect to the fold increase observed in EC_{50} (effective concentration 50%) values derived from *in vitro* studies of resistance and antiviral drug sensitivity testing (Table 1).

Therapies for HBV: efficacy and drug resistance

Six oral nucleos(t)ide analogs are available for inhibiting HBV replication. The L-nucleoside analogs lamivudine (LAM), emtricitabine (FTC) and telbivudine (LdT); the acyclic phosphonates adefovir dipivoxil (ADV) and tenofovir (TDF); and the cyclopenta(e)ne deoxy guanosine analog entecavir (ETV). Of these, only LdT and ADV

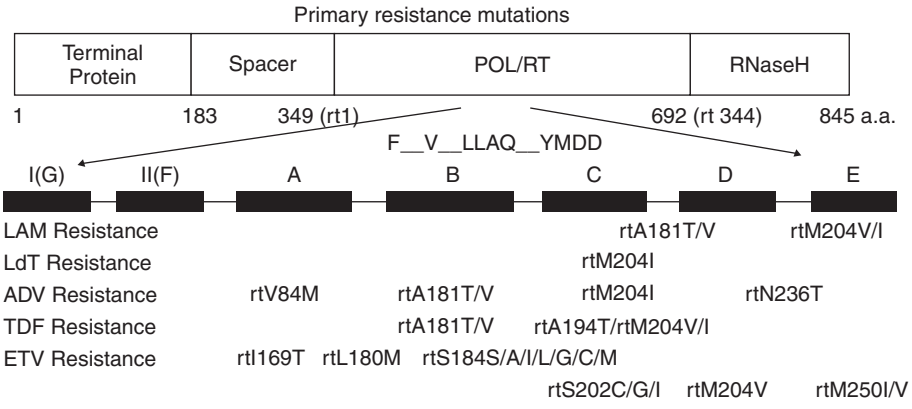

Figure 2. *Location of the primary drug resistance mutations within the HBV polymerase. According to convention and for consistent identification of mutations conferring resistance to antiviral nucleos(t)ide analogs, amino acids are numbered from the beginning of the Pol/RT (rt1 to rt344) domain[22]. Mutations associated with resistance to lamivudine (LAM), telbivudine (LdT), adefovir (ADV), tenofovir (TDF), and entecavir (ETV) are indicated.*

10 mg are not active against HIV. The other drug that is available for HBV treatment is pegylated interferon-alpha-2a (PEG-IFNα-2a). In deciding which of these nucleos(t)ide analogs to use, one must weigh the efficacy of the drugs against the risk for developing drug resistance.

Lamivudine

In several large studies, HBeAg seroconversion rates after one year of lamivudine (LAM) therapy range between 16-18%, which is similar to other oral agents, but

Table 1. Antiviral sensitivity profiles of drug-resistant HBV *in vitro*

	Lamivudine	Clevudine	Telbivudine	Entecavir	Adefovir	Tenofovir
HBV	-Fold Resistance	-Fold Resistance	-Fold Resistance	-Fold Resistance	-Fold Resistance	-Fold Resistance
Wild-type	1	1	1	1	1	1
M204I	> 100[1]	> 100[1]	4[4]	1[4]	< 1-8[1,2,3]	< 1[4]
L180M + M204V	> 100[1]	> 100[1]	NA	5-6[5,6]	< 1-4[1,2,3]	3-6[6,7]
A181T/V	1-2[4]	NA	5-6[4]	1-4[4]	1-3[4]	1[4]
N236T	1[6]	NA	3[4]	< 1[6]	3[6]	5[6]
I169T + V173L + M250V*	> 1000[4]	NA	> 1000[4]	> 700[5]	1[4]	< 1[4]
T184G + S202I*	> 1000[4]	NA	35[4]	> 700[5]	2[4]	6[4]
A194T	NA	NA	NA	NA	NA	2[8]

*(+ L180M + M204I/V); NA: not available; 0-9 fold → no or low level of resistance; 10-99 fold → medium level of resistance; > 100 fold → high level of resistance
[1]Chin, et al. (2001)[55]
[2]Delaney, et al. (2001)[34]
[3]Ono-Nita, et al. (2002)[57]
[4]Sozzi, et al. (2005)[85]
[5]Tenney, et al. (2004)[26]
[6]Brunelle, et al. (2005)[64]
[7]Sheldon, et al. (2005)[49]
[8]Delaney, et al. (2006)[50]

Table 2. Annual prevalent resistance rates for lamivudine, adefovir, entecavir, emtricitabine and telbivudine

	Resistance at year of therapy expressed as percentage of patients				
Drug	**1**	**2**	**3**	**4**	**5**
Lamivudine[a]	23	46	55	71	80
Adefovir[b] (naive HBeAg-neg)	0	3	11	18	29
Adefovir (LAM resistant)	18	–	–	–	–
Entecavir[c] (naive)	0.1	0.4	1.1	–	–
Entecavir[c] (LAM resistant)	6	14	32	–	–
Emtricitabine[c]	9-16	19-37	–	–	–
Telbivudine[d] (HBeAg-pos)[e]	4.4[d]	21.6[e]			
(HBeAg-neg)[e]	2.7	8.6			

[a]Modified and updated from Lai, et al. 2003[30] and Leung, et al. 2001[86]
[b]From Locarnini, et al. 2005[40] and Hadziyannis, et al. 2005[87]
[c]From Perrillo, et al. 2005[53] and Colonno, et al. 2006[88]
[d]In the LAM comparator arm, the percentage was only 8% based on a complex case definition of antiviral drug resistance/treatment failure. One would thus expect a comparable relative level of 10-12% based on genotypic resistance compared with lamivudine (25% per annum).
[e]Lok A and McMahon B[79]

lower than PEG-IFNα-2a[14-17]. This response is durable in about 75% of these individuals. With therapy extended to five years, seroconversion reached 50%[18], but development of LAM resistance reached nearly 85%. Loss of serum HBV-DNA occurred in 40-44%, although some of these studies used hybridization assays with a lower limit of detection of 10^5 copies/ml (~ 2 x 10^4 IU/ml), which is higher than the lower limit for newer PCR assays (< 100 IU/ml). Histologic improvement was noted in about 50% of patients; however, in those patients with drug-resistant HBV, the histology was worse[19]. In HBeAg-negative CHB, DNA loss occurs in the majority; however, rebound is nearly universal upon discontinuation of therapy[20]. There have not been randomized studies in HIV/HBV-coinfected individuals, but efficacy in retrospective studies is similar[21]. Although LAM is potent against HBV, its utility is limited by the rapidity to which resistance develops compared to the other nucleos(t)ides.

Antiviral resistance to LAM has been mapped to the YMDD locus in the catalytic or C domain of HBV Pol[22]. The primary resistance mutations within the Pol gene that have been selected during LAM therapy are designated rtM204I/V/S (domain C) ± rtL180M (domain B)[23]. Other primary mutations include rtA181T/V[24]. Compensatory mutations can be found in other domains of the HBV Pol, such as rtL80V/I[25], rtI169T[26], rtV173L[27], rtT184S/G, rtS202I, and rtQ215S[28], that enhance viral replication levels. The molecular mechanism of LAM resistance is steric hindrance caused by the β-branched side group of the valine or isoleucine amino acids colliding with the oxathiolane ring of LAM altering the deoxynucleotide triphosphate (dNTP)-binding site[29]. This results in a > 100-fold increase in EC_{50} (Table 1).

Lamivudine resistance increases progressively during treatment at rates of 14-32% annually (Table 2), exceeding 70% after 48 months of treatment; after 4-5 years of treatment, this plateaus to around 80% in patients with CHB[30] (Table 2), and to more than 90% in HIV-coinfected patients[31,32]. The three most important factors that increase the risk of development of resistance include high pretherapy serum HBV-DNA and ALT levels and the incomplete suppression of viral replication[30,33]. The main LAM resistance mutations rtM204V/I do not confer cross-resistance to ADV (Table 1), but the rtA181T/V does[28]. The rtI169T, rtT184S/G, and rtS202I contribute to ETV resistance[26] (Table 1). The rtM204V/I is cross-resistant with all other L-nucleoside analogs tested such as FTC, LdT, and clevudine (L-FMAU) (Table 1, Fig. 2, Fig. 3).

Mutations that confer LAM resistance decrease *in vitro* sensitivity to LAM from at least 100-fold to > 1000-fold. The rtM204I substitution has been detected in isolation, but rtM204V and rtM204S are found only in association with other changes in the A or B domains[34]. Five common patterns of resistance have been identified: (1) rtM204I, (2) rtL180M+rtM204V, (3) rtL180M+rtM204I, (4) rtV173L+rtL180M+rtM204V, and (5) rtL80V/I±rtL180M+rtM204I. The dominance of particular patterns tends to be influenced by the HBV genotype[35],

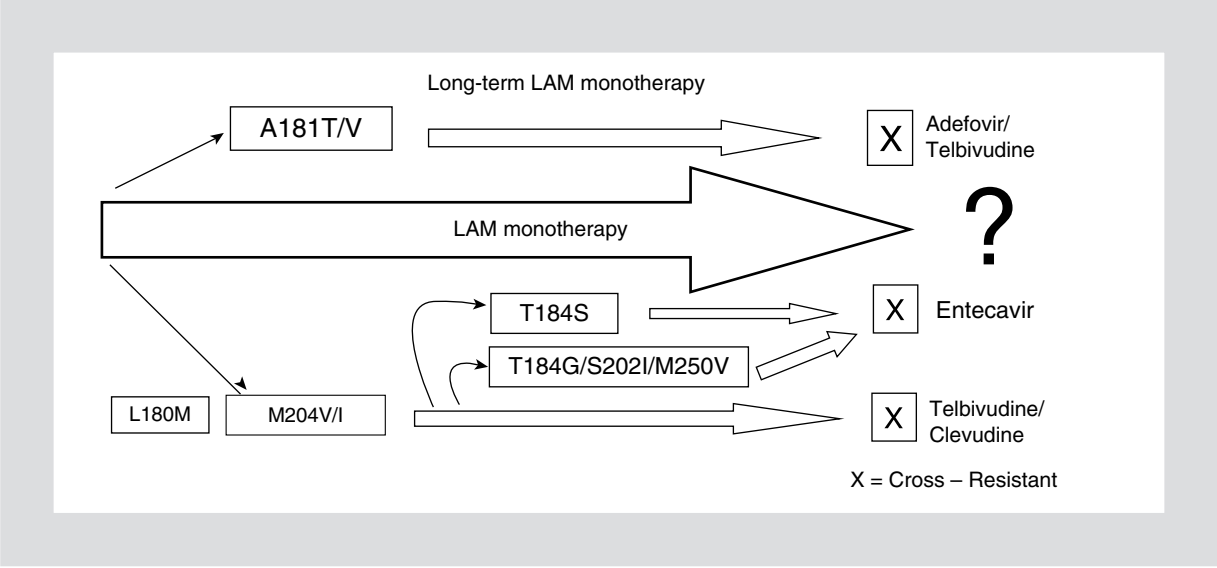

Figure 3. *Pathways of evolution for the HBV Pol during emergence of lamivudine (LAM)-associated resistance in patients undergoing long term LAM monotherapy. If drug selection pressure is maintained once resistance has emerged, then further compensatory mutations can be cumulated, some of which will compromise future rescue therapy options to adefovir, telbivudine, entecavir and clevudine.*

and interestingly, no rtM204V is detected by itself; it is always found in association with rtL180M.

With the widespread use of LAM for anti-HIV therapy, many HIV/HBV-coinfected persons have received long-term LAM therapy. Not surprisingly then, high levels of LAM-resistant HBV have been observed in these patients, with HBV Pol mutations detected in 94% of viremic patients who have received treatment for at least four years[32]. In the study by Mathews, et al. (2006), the LAM-resistant HBV-Pol profile of rtV173L+rtL180M+rtM204V (pattern 4) was found in 17% of viremic patients on long-term LAM. As discussed below (Public Heath Implications), this HBV isolate has the concomitant changes in the envelope of sE164D+sI195M, which exhibits significantly reduced anti-HBs binding properties, similar to the classical vaccine-escape sG145R variant[36].

Adefovir and tenofovir

Adefovir is active against wild-type and LAM-resistant HBV. The 10 mg dose of ADV is not active against HIV, and in monoinfection achieves HBeAg seroconversion in 12% of persons with one year of therapy, the lowest rate of any of the anti-HBV agents[37]. The mean serum decline in HBV-DNA was 3.5 $\log_{10}$ copies/ml, with 48% achieving ALT normalization after one year of therapy. Nephrotoxicity is the major concern, which is seen in 3% of patients after five years of therapy for those with compensated liver disease; thus, monitoring of serum creatinine every three months is essential. In HBeAg-negative CHB, after five years of therapy, 67% are able to achieve an undetectable HBV-DNA using sensitive PCR assays; however, rebound usually occurs with discontinuation of therapy. Adefovir has been studied in 35 HIV/HBV-coinfected patients with ongoing HIV therapy including LAM. After 144 weeks of therapy, 45% reached HBV-DNA < 1000 copies/ml, which is lower than the 56% in HBV monoinfection[37,38].

Although potency is an issue with ADV, the development of resistance is lower than with LAM. Resistance to ADV was initially associated with mutations in the B (rtA181T) and D (N236T) domains of the enzyme[39] (Fig. 2). Resistance of HBV to ADV occurs less frequently than resistance to LAM, with a prevalence of around 2% after two years, 11% after three years, 18% after four years, and 29% after five years[40] (Table 2). These reports of resistance have only occurred in individuals who are on ADV monotherapy for hepatitis B. These ADV-associated mutations in HBV Pol result in only a modest (3 to 8-fold) increase in the EC_{50} (Table 1), and are partially cross-resistant with TDF, probably because the molecular mechanism of resistance is similar in both, with indirect perturbation of the triphosphate binding site between the A and D domains[29,41]. The rtN236T does not significantly affect sensitivity to LAM[39], but the rtA181T/V changes are partially cross-resistant to LAM (Table 1). Recently, another mutation (rtI233V) mapped to the reverse transcriptase domain has been identified that confers resistance to ADV[42]

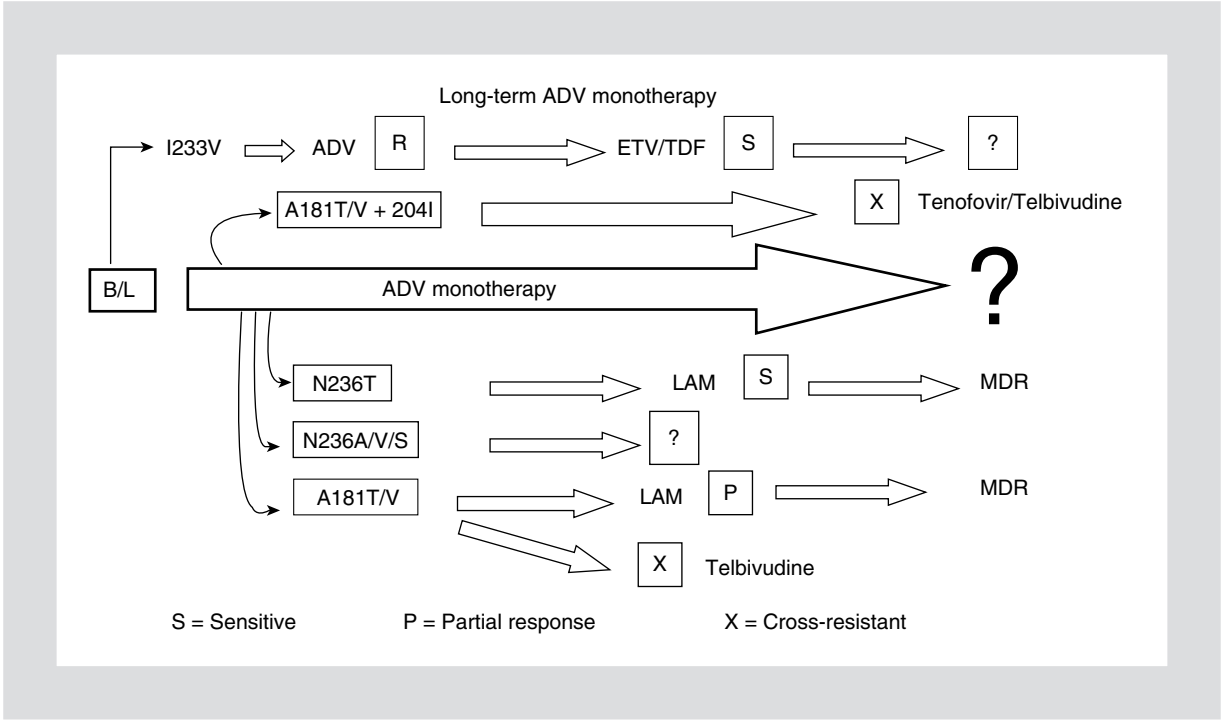

Figure 4. *Pathways of evolution for the HBV Pol during emergence of adefovir (ADV) resistance in patients undergoing long-term ADV monotherapy. As with Figure 3, further accumulation of compensatory mutations will affect future therapeutic rescue options. ETV: entecavir; TDF: tenofovir; LAM: lamivudine; B/L refers to baseline, pre-therapy; MDR: multidrug resistant.*

(Fig. 4). In clinical studies, the rtI233V mutation appears to occur in approximately 2% of all patients with CHB[42,43], and the final significance of this mutation will need independent validation since other groups have not found an association between the rtI233V and ADV resistance[44,45]. At the 10 mg dose, ADV does not appear to select for HIV drug-resistant mutations, but studies have been limited[46].

Tenofovir is approved for the treatment of HIV infection and has also been used to treat the HBV in patients coinfected with HIV. Tenofovir has not been evaluated in large clinical trials for the treatment of CHB, but several retrospective studies and one prospective study support its efficacy as being superior to ADV[47,48]. The prospective study randomized 52 HIV/HBV-coinfected patients to either TDF or ADV, and in an intent-to-treat analysis, the weighted average decline in HBV-DNA was 4.03 $\log_{10}$ copies/ml compared to 3.12 for ADV. Resistance to TDF has been detected in several patients. The amino acid change at rtA194T in association with LAM resistance (Fig. 2) was found to result in a significant increase in EC_{50}[49]. Further studies are required to establish the significance of this mutation, as its contribution to TDF resistance has not been independently confirmed[50]. Studies are also needed to determine the frequency of this mutation since it has only been reported in sporadic cases. Patients rescued with tenofovir following the development of ADV resistance (rtN236T±rtA181T/V) do show viral-load reduction, but continued selection of the rtN236T±rtA181T/V quasispecies[51].

Entecavir (cyclopenta(e)ne sugar)

Entecavir is a guanosine analog that inhibits all three functions of the HBV polymerase including priming, reverse transcriptase, and positive strand synthesis. In a large, randomized clinical trial of ETV compared to LAM in HBV-monoinfected patients, ETV was superior in histologic response (72 vs. 62%), attaining an undetectable HBV-DNA (67 vs. 36%), and biochemical responses (68 vs. 60%)[43]. However, rates of HBeAg seroconversion were similar, with ETV at 21% and LAM at 18%. In those with a virologic response without HBeAg seroconversion, a second year of therapy led to 81% of the ETV group attaining an undetectable HBV-DNA compared to 52% in the LAM group, while about 15% in both groups experienced HBeAg and HBV-DNA loss. In HBeAg-negative patients, ETV was also superior to LAM, with 90% reaching undetectable HBV-DNA[52].

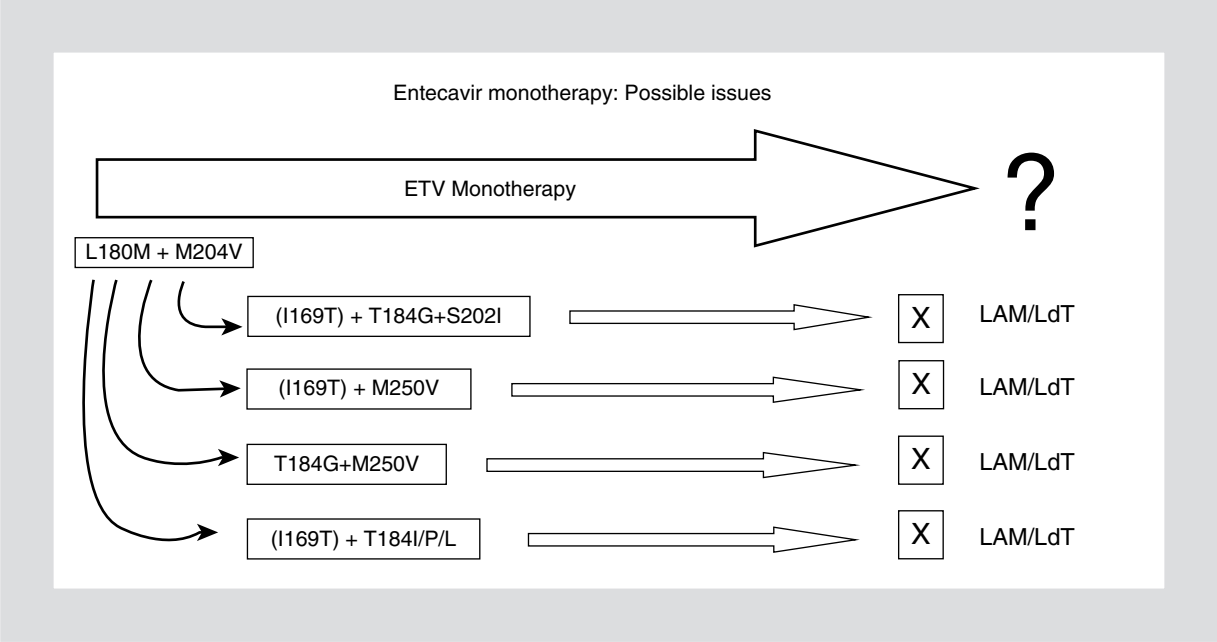

Figure 5. *Possible pathways for evolution of the HBV Pol during the emergence of entecavir (ETV) resistance. These pathways are cross-resistant for lamivudine (LAM) and telbivudine (LdT).*

Resistance to ETV has been observed in patients who are naive to therapy[53] as well as those who have LAM-resistant HBV[26]. Mutations in the viral polymerase associated with the emergence of ETV resistance were mapped to the B domain (rtI169T, rtL180M, and/or rtS184G), C domain (rtS202I and rtM204V), and E domain (rtM250V) of HBV Pol (Fig. 2). In the absence of mutations at rtM204V/I, the rtM250V causes a 9-fold increase in EC_{50}, whereas the rtT184G+rtS202I changes have only a modest effect (Table 1)[26,54-58]. The mechanism of ETV resistance for the rtT184G+rtS202I is an allosteric change with altered geometry of the nucleotide- binding pocket and DNA template binding of the polymerase near the YMDD site[58]. The molecular mechanism of resistance for the rtM250V change is thought to be an alteration of the binding interaction between the DNA primer strand and DNA template strand with the incoming dNTP[58].

The more recent clinical experience with ETV failure has indicated that at least three mutations, rtL180M⊕rtM204V and at least one of rtT184G/S or rtS202I or rtM250V are required in the HBV Pol for ETV resistance to develop (Fig. 5). This accounts for the low resistance rate in naive patients after one year (0.1%), two years (0.4%) and three years (1.1%) of ETV monotherapy (Table 2). In contrast, in LAM-experienced patients, it should be noted that as well as rtL180M and rtM204V, changes at codon 184 occur in 4.5% of patients. Not surprisingly then, the frequency of ETV genotypic resistance changes in LAM-experienced patients to 6% (year 1), 14% (year 2), and 32% (year 3) (Table 2). In this group, viral breakthrough as well as genotypic resistance occur in 1% (year 1), 10% (year 2), and 25% (year 3)[53] of patients.

There is one randomized study of ETV in 68 HIV/HBV-coinfected patients comparing ETV to placebo while continuing their LAM-containing HAART for 24 weeks followed by 24 weeks of open-label ETV (ETV package insert). Since these patients were infected with LAM-resistant HBV, ETV was given at the 1.0 mg dose. In 24 weeks, only 6% of the 51 patients had HBV-DNA < 300 copies/ml and the mean decline in HBV-DNA was 3.65 $\log_{10}$ copies/ml. Of those that received 48 weeks of ETV only 8% had HBV-DNA < 300 copies/ml.

Until recently, these data as well as *in vitro* data did not demonstrate activity of ETV against HIV. However, a recent study demonstrated that ETV has potent *in vitro* activity against HIV, with an IC_{50} between 0.1 and 1 nm as well as *in vivo* activity, with three patients declining at least 1 log in HIV-RNA[59]. This study also demonstrated that the M184V was selected *in vivo* in one patient and that the virus with the M184V was not inhibited by entecavir in an *in vitro* assay. Since ETV is not active against HIV with the M184V, the one clinical study described above in HIV/HBV-coinfected patients would not have detected this activity since they all were LAM-experienced and were on HIV therapy.

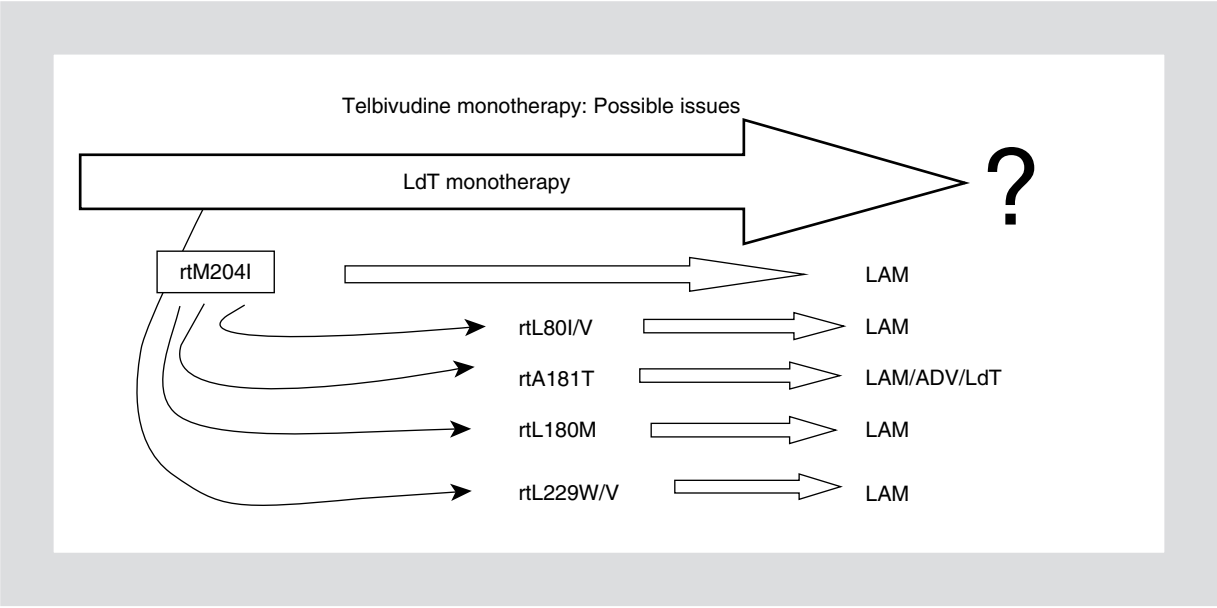

Figure 6. *Pathways of evolution for the HBV Pol during the emergence of telbivudine (LdT) monotherapy. These pathways are cross-resistant for lamivudine (LAM), adefovir (ADV), and tenofovir (TFV).*

Telbivudine

Telbivudine (LdT; TBV) is the L-nucleoside analog of thymidine, and is a potent anti-HBV drug with no HIV activity. A large clinical trial has demonstrated superiority over LAM. In HBeAg-positive hepatitis B, 60% of patients had HBV-DNA undetectable after one year of therapy compared to 40% in the LAM group[60]. This decreased to 54% after the second year due to development of resistance[61]. As with ETV, LdT was not superior to LAM in the rate of HBeAg seroconversion, which was 26 versus 23%, respectively. In patients with HBeAg-negative hepatitis B, 88% of patients on LdT and 71% treated with LAM had undetectable HBV-DNA by PCR at the end of therapy. Telbivudine has not been studied in the HIV/HBV-coinfected setting.

The utility of LdT will be limited by its development of resistance with rtM204I, the major mutation, selected out (Fig. 2). *In vitro*, LdT is active against HBV encoding the rtM204V, supporting the absence of this mutation *in vivo*. However, whether LdT is effective *in vivo* in a patient with preexisting rtM204V is not known as this mutation is rarely found in patients with LAM or FTC resistance. The common resistance profile is pattern 2, rtL180M+rtM204V. As well as the rtM204I, other mutations detected included the rtL80V/I and the rtA181T/V (Fig. 6). The latter is associated with cross-resistance to both LAM and ADV. In the trial above, the rate of resistance in HBeAg-positive patients was 4.4 and 21.6% after one and two years of therapy, respectively (Table 2). However, in those who had undetectable HBV-DNA by PCR at week 24 of therapy, the rate of resistance was lower at 4% after two years. In patients with HBeAg-negative hepatitis B, resistance after one and two years of therapy was 2.7 and 8.6%, respectively. Of the HBeAg-negative patients who had undetectable HBV-DNA by 24 weeks of therapy, only 2% of them had developed resistance to LdT.

Conventional and pegylated interferon alpha

The only nonnucleos(t)ide analog available for treating HBV infections is interferon-alpha (IFNα). Both standard and pegylated forms are available, but with the more convenient dosing schedule and the superiority of the pegylated form, the conventional or standard form is no longer widely used for HBV treatment. Pegylated interferon-alpha has been compared alone and in combination with LAM to LAM alone for 48 weeks[14]. At the end of therapy, the decline in HBV-DNA was superior in the combination group; however, after 24 weeks of continued follow-up, there was no difference since there was greater relapse in the combination arm. The HBeAg seroconversion rates were similar at the end of treatment, but after 24 weeks of follow-up those that received PEG-IFN had HBeAg seroconversion rates of 32% (PEG-IFN) and 27% (PEG-IFN plus LAM) compared to 19% for LAM alone. The LAM resistance rate was less in the combination arm[62,63].

Pegylated interferon-α for 48 weeks for the treatment of HBeAg-negative HBV results in an HBV-DNA response; however, after discontinuation of therapy, this response is sustained in only 15% of patients[63]. Whether longer therapy duration will decrease the relapse rate remains an open question.

Pegylated interferon-alpha has not been studied in HIV-coinfected individuals; thus, its efficacy in this setting is unknown.

Sequential anti-HBV monotherapy and multidrug resistance

Recently, multidrug-resistant HBV has been reported in patients who received sequential treatment with nucleos(t)ide analog monotherapies[26,64-67]. The development of multidrug resistance will certainly have implications on the efficacy of rescue therapy, as in the case of multidrug-resistant HIV[68,69]. Successive evolution of different patterns of resistant mutations have been reported during long-term LAM monotherapy[24,70] (Fig. 3), which affect subsequent sensitivity to ADV (Fig. 4), ETV (Fig. 5), and LdT (Fig. 6). The isolates of HBV with these initial mutations appear to be associated with decreased replication fitness compared with wild-type HBV; however, additional mutations that can restore replication fitness are frequently detected as treatment is continued[25,27,71] (Fig. 3).

A recent study by Yim, et al. in 2006[72] characterized multidrug-resistant HBV in more detail in six patients receiving alternating monotherapies, typically LAM and ADV (Figs. 3 and 4). Using conventional cloning techniques with subsequent PCR sequencing, the majority of the clones (85%) had mutations to both therapies on the same genome. The remainder had LAM-resistant clones only. In three of the patients, analysis of successive samples revealed progressive evolution from single clones with LAM-resistant HBV mutations to mixtures of clones that had multidrug-resistant mutations. These studies strongly support the role for combination therapy in managing patients with CHB[73] (see below).

Public health implications of antiviral drug resistance

As outlined above, the polymerase gene overlaps the envelope gene and changes in the HBV Pol selected during antiviral resistance can cause concomitant changes to the overlapping reading frame of the envelope (Fig. 1). Thus, the major resistance mutations associated with LAM, ADV, LdT, and ETV failure, also have the potential of altering the C-terminal region of HBsAg. For example, changes associated with LAM resistance, such as the rtM204V, result in a change at sI195M in HBsAg, whilst the rtM204I change is associated with three possible changes, sW196S, sW196L, or a termination codon. To date, there has been only one published study that has examined the effect of the main LAM resistance mutations on the altered antigenicity of HBsAg[36]. One of the common HBV quasispecies that is selected during LAM treatment is rtV173L+rtL180M+rtM204V that result in change in the HBsAg at sE164D+sI195M. Approximately 20% of HIV/HBV-coinfected individuals[32] and 10% of monoinfected individuals, encode this "triple Pol mutant"[27]. In binding assays, HBsAg expressing these LAM-resistant substitutions demonstrated reduced anti-HBs binding *in vitro*[36]. This reduction was similar to that observed with the well-known vaccine escape mutant, sG145R.

The codon change rtA181T selected by ADV and/or LAM and/or LdT results in a stop codon mutation at sW172stop. The ADV-resistant mutation at rtA181V results in a change at sL173F. The HBV with mutations that result in a stop codon in the envelope gene such as those for LAM, ADV, and LdT would be present in association with a low percentage of wild-type to enable viral packaging. The ADV resistance mutation rtN236T does not affect the envelope gene and overlaps with the stop codon at the end of the envelope gene.

The ETV-resistant associated change at rtI169T, rtS184G, and rtS202I also affect HBsAg and results in changes at sF161L, sL/V176G, and sV194F. The rtM250V is located after the end of HBsAg. The sF161L is located within the region known as the "a" determinant, a major hydrophilic region, which includes amino acids 90-170 of the HBsAg[74]. This region represents a highly conformational epitope, characterized by multiple disulphide bonds formed from sets of cysteines at residues 107-138, 137-149, and 139-147[74], and acts as the neutralization domain of HBV. Not surprisingly then, distal substitutions such as sE164D can significantly affect anti-HBs binding[36] due to conformational folding effects. The influence of other changes to HBsAg, such as sF161L, needs further investigation to determine its impact on the envelope structure and subsequent anti-HBs binding.

As discussed above, there has been a report of the transmission of LAM-resistant HBV to an HIV patient undergoing LAM as part of antiretroviral therapy[75]. In addition, HBV-encoding LAM-resistant mutations have been found in a cohort of dialysis patients with occult HBV[76]. Therefore, it is important to recognize that both primary and compensatory antiviral-resistant mutations

Table 3. HBV treatment options in HIV-infected patients

Clinical Situation[1]	Preferred[2]	Other options
HIV and HBV need treatment		
• Naive	Tenofovir/emtricitabine	Entecavir with HAART
• LAM-R HBV	Tenofovir/emtricitabine	
HBV only needs treatment	PEG-IFNα-2a, initiate HAART, adefovir	Telbivudine-monitor HBV-DNA at 24 weeks

[1]LAM-R HBV: lamivudine-resistant HBV
[2]Lamivudine can be substituted for emtricitabine

may result in associated changes to the viral envelope that could have substantial public health relevance.

Treatment of chronic hepatitis B in HIV-infected individuals

Treatment goals

The treatment goals of HBV therapy are to stop and even reverse the progression of liver inflammation and fibrosis through sustained suppression of active HBV replication. Since these are long-term endpoints, surrogate markers of the efficacy of therapy that are used include virologic (decrease in HBV-DNA), serologic (anti-HBe or anti-HBs seroconversion), and inflammatory (normalization of ALT or liver histology). It is important to recognize that HBV is probably never cured but rather controlled by antiviral agents since they limit viral replication. The benefits of inhibiting HBV replication are illustrated by studies that demonstrate a direct association between HBV-DNA levels and the risk of developing cirrhosis and hepatocellular carcinoma, independent of HBeAg status[77,78]. Patients who clear HBeAg after IFN therapy have fewer complications and improved overall survival. Loss of HBsAg is associated with the best survival of all, as well as the lowest risk of developing hepatocellular carcinoma and liver-related death.

Who to treat

Since treatment is not curative and has a limited response rate, and since long-term treatment can result in development of resistance, restricting treatment to those patients who are at increased risk for developing cirrhosis or hepatocellular carcinoma is logical. The criteria used in guidelines to identify these patients include evidence of active HBV replication and evidence of liver disease. The former is defined as presence of HBV-DNA levels > 10^4 copies/ml (2 x 10^3 IU/ml). The level of HBV-DNA at which to treat has been debated, and some guidelines still suggest using 10^5 copies/ml (2 x 10^4 IU/ml) as a cutoff[79]. However, recent data from Taiwan suggest that 10^4 copies/ml (2 x 10^3 IU/ml) is the level at which there is significantly increased risk for developing cirrhosis and hepatocellular carcinoma[77,78]. The second criteria for treatment, evidence of liver disease, can be determined either by a liver biopsy or by ALT levels at least twice the upper limit of normal. The liver biopsy is preferred by some since it more accurately stages the disease, which is important as one thinks about treatment options and the ability to stop treatment. The liver biopsy will also determine whether cirrhosis exists, which is a criteria for treatment regardless of HBV-DNA level.

Flares and immune reconstitution

Flares result from changes in the balance between the level of HBV replication and the immune response[80]. Although spontaneous flares and seroconversion occur in HIV-uninfected individuals, this is rarely seen in HIV-infected individuals without HAART[81]. A substantial reduction in HIV viremia and improvement in CD4 cell count after the initiation of HAART, called immune reconstitution, can lead to improved host immune response to HBV and other microbial opportunistic infections. However, in HIV/HBV coinfection, immune reconstitution is often manifested by a flare followed by reduction in HBV viremia and less commonly seroconversion. In addition to immune reconstitution, flares of HBV disease in individuals with HIV can occur when anti-HBV therapy is withdrawn, when anti-HBV drug resistance develops[82,83], or because of HAART-related hepatotoxicity[80].

Occasionally, a severe flare associated with immune reconstitution in patients with high-level HBV-DNA can cause hepatic decompensation and even death[80,82]. This situation highlights the importance of screening all HIV-infected patients prior to initiation of HAART for HBV, and the need to control active HBV replication prior to or in conjunction with initiation of HAART[80]. Control of HBV with an anti-HBV drug that has no anti-HIV activity prior to

initiation of HAART should be considered in patients with advanced fibrosis and cirrhosis who are at higher risk for hepatic decompensation resulting from a flare.

What to treat with

In order to optimize treatment in HIV/HBV-coinfected individuals, one must determine whether only one virus needs treatment or whether both do, since many of the drugs are active against both viruses and can select for resistance (Table 3). The most straightforward situation is when both the HIV and HBV-associated disease meet criteria for treatment in a patient who is naive to therapy. In this case the optimal therapy is TDF and emtricitabine (co-formulated as Truvada®, Gilead, USA) along with either a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor against HIV. Advantages of this option include convenient dosing, long-term safety data, and potency against both HIV and HBV. If TDF or Truvada cannot be used, then an alternative HIV regimen along with ETV can be considered. If this latter option is chosen, it is not known whether an HIV regimen containing LAM will hasten the development of ETV resistance, since the rtM204V and rtL180M are needed as background mutations to develop phenotypic ETV resistance.

In the situation where both viruses need treatment and LAM-resistant HBV exists, then the best option is to include TDF and FTC/LAM in the regimen. Although the FTC/LAM is not active in this regimen, resistance data from ADV to date show no resistance with ongoing LAM[84]. In the one coinfected cohort, no cases of ADV resistance have been seen with five years of follow-up and in all patients LAM was part of the regimen. If TDF cannot be used, the other alternatives are inferior due to issues of cross-resistance as discussed above. One potential choice is to use ETV, recognizing the increased risk for cross-resistance. In this situation, whether discontinuing LAM is beneficial is not known, but should be considered due to the background mutations required for ETV resistance.

The most difficult situation is when only the HBV needs treatment, which is occurring more commonly since it is recognized that not all HIV-infected individuals need immediate therapy. Prior guidelines have recommended ETV in this situation, but given the recent data of its activity against HIV and its potential for selecting the rtM184V, this is not the best option (see discussion above under entecavir). The only drugs that are available to use are those that are not active against HIV, including LdT and ADV, or those that are active but do not produce resistance to either virus, which includes only PEG-IFNα-2a. The risk of LdT is the high rate of development of the mutation at rtM204. One approach if this option is chosen is to check HBV-DNA at week 24, and if it is undetectable, then the risk of developing drug-resistant HBV is 2-4% in two years. The limitation of ADV is that it is the least-potent drug and may not be able to control replication adequately in HIV/HBV-coinfected individuals who tend to have higher levels of replication. Furthermore, with ADV there is also a theoretical risk of developing the HIV mutation K65R, which decreases susceptibility to TDF, but to date this risk has not been realized. The limitation of using PEG-IFNα-2a is the side effects from the drug and lack of efficacy data in HIV/HBV-coinfected patients. The other option is to initiate HAART and include tenofovir and lamivudine/emtricitabine in the regimen.

The last situation to consider then is if only the HIV-associated disease needs treatment. For most cases, it is best just to treat the hepatitis B and assume both viruses need treatment. If one elects to only treat the HIV, then it is important not to use LAM as the only HBV active drug since development of LAM-resistant HBV occurs rapidly.

HBV monitoring

If a patient does not meet the criteria for HIV or HBV treatment, then monitoring both viruses should occur at least every six months. For HBV, monitoring includes obtaining HBV-DNA, ALT, alpha-fetoprotein (AFP) and ultrasound or CT scan (for hepatocellular carcinoma). If a patient meets the criteria for HBV treatment for consecutive visits, then it should be started.

If a patient is on treatment, then testing including HBV-DNA, ALT, HBeAg (if positive at the start of therapy), anti-HBe (if negative at the start of therapy), and AFP and ultrasound or CT scan should be followed every three months. If the level of HBV-DNA suggests that antiviral drug-resistant HBV is emerging, then a repeat HBV-DNA should be obtained and consideration given to changing the regimen. In an HBeAg-positive patient, if anti-HBe develops, then one should also follow anti-HBs every 6-12 months. In the HBeAg-negative patient, monitoring anti-HBs yearly is adequate.

Summary

Chronic hepatitis B affects approximately 10% of the HIV-infected population, and will be a growing problem as anti-HIV therapy is introduced into parts of the globe where HBV is endemic such as Africa and Asia. When treating coinfected patients, one must balance the need to treat both viruses as well as be cognizant of

which drugs have dual activity as well as the resistance patterns that can emerge for both viruses. There are many unanswered questions in treating hepatitis B in the HIV-infected patient, including the utility of PEG-IFNα, the efficacy of combination therapy, and strategies to minimize the development of resistance. Further studies are clearly needed.

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