Research Article
Open Access

Chronic hepatitis B infection: what to do when TDF is not enough to treat?

Abstract

Chronic hepatitis B is a major cause for liver disease worldwide. Until today, a full cure of infection was not achieved and we still can not eliminate the integrated viral cccDNA (covalently closed circular DNA). Nonetheless, a virological, biochemical and histological response is achievable with high genetic barrier nucleos(t)ide analogues such as tenofovir disoproxyl fumarate, tenofovir alafenamide and entecavir, which are associated with a very low risk of long-term resistance. We describe a case of a 76-year-old Portuguese man, with HIV-HBV co-infection who presented with an HBV virologic breakthrough after 10 years of sustained virologic response. Therapeutic adherence was assessed, drug-drug interactions and other coinfections were excluded. Entecavir was added to a triple antiretroviral regimen based on TDF/FTC/EFV that the patient had been taking for the last 15 years. A genotypic resistance test was performed and identified rt M204V and V173L (compensatory) mutations. The patient completed therapy with both TDF and entecavir. After a year has passed, a partial response was achieved.

Keywords: chronic hepatitis B, virologic breakthrough, TDF resistance, HIV co-infection

Volume 13 Issue 6 - 2022

Sara Casanova,[1] Ana Cláudia Miranda,[1] Elizabeth Pádua,[2] Kamal Mansinho,[1] Rita Corte-Real[1]

[1]Department of Infectious Diseases, Centro Hospitalar Lisboa Ocidental, Portugal
[2]Department of Infectious Diseases, National Institute of Health, Portugal

Correspondence: Sara Casanova, Department of Infectious Diseases - Hospital de Egas Moniz, Centro Hospitalar Lisboa Ocidental, Lisbon, Portugal, Tel 00351 917668080, Email saracasanov@campus.ul.pt

Received: November 21, 2022 | **Published:** December 05, 2022

Abbreviations: HCC, hepatocellular carcinoma; RAM, resistance-associated mutations; NA, nucleotide analogues; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide

Introduction

Chronic hepatitis B is a major cause for liver disease worldwide. Globally is estimated that 257million persons, 3,5% of the population, live with chronic HBV infection and of those 2,7million are coinfected with HBV and HIV.[1]

When left untreated HBV chronic infection can lead to life-threatening complications such as cirrhosis or hepatocellular carcinoma (HCC). Cofactors like alcohol, HIV, HCV or HDV co-infection can accelerate the progression to end-stage liver disease. Knowing when and how to treat the infected patients it is of great importance being the main goal, the viral load suppression, halting the disease progression since viral eradication is not yet possible. Consequently, long-term therapy administration is frequently necessary which can result in the virus selection with resistance-associated mutations (RAM) in the viral genome.

Current treatment options are potent nucleotide analogues (NA) with a high barrier to resistance such as tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF) and entecavir (ETV). Combination therapy is not recommended unless multidrug resistance is documented, in which the association of TDF and ETV may be indicated.[2]

There is no current therapeutic approach yet that achieves virological cure, but high genetic barrier resistance to NA, like TDF, have proven to be effective on biochemical and histological response promoting liver fibrosis regression, improving clinical outcomes such as decreasing the progression to Hepatocelular carcinoma and death.[3]

The early emergence of resistance mutations to lamivudine (3TC) and adefovir (ADV) has been well described[2,4] and led to its removal from the first-line treatment strategy. On another hand, resistance to ETV occurs in less than 1% of treatment-naïve patients and TDF or TAF resistance remains very rare.[3] Even though some studies seem to demonstrate no resistance pattern to TDF even after 10 years of therapy, other recent reports start to show evidence of reduced TDF susceptibility in treatment-experienced and treatment-naïve patients with chronic HBV infection.[5]

Here we document the case of a 76-year-old Portuguese male with chronic HIV/HBV coinfection and with iatrogenic immunosuppression due to an anal squamous cell carcinoma, which presented a breakthrough of HBV infection after years of sustained virologic suppression with TDF/FTC.

Case report

We report the case of an HIV/HBV co-infection from a 76-year-old male, born and raised in Portugal, who presented a breakthrough of HBV infection (DNA HBV load of 31.800IU/mL log10 4,50) after being virologic suppressed for more than 10 years with TDF/FTC.

He was diagnosed previously with HIV infection in 2001 (B3 CDC Atlanta, Nadir CD4+ Lymphocytes 19cel/mm3). He is on current ARV medication with the combination of tenofovir disoproxil fumarate (TDF), emtricitabine (FTC) and efavirenz (EFV) taken as a single pill, once a day, and the viral load has been consistently undetectable (HIV RNA <20copies/mL real-time PCR assay) since 2007. He was previously medicated with ddi (didanosine), d4T (stavudine) and EFV (efavirenz) between 2001 and 2004, and with 3TC (lamivudine), ddi and EFV between 2004 and 2006.

By the end of 2005 he was diagnosed with a chronic hepatitis B infection, HBeAg positive, genotype A, presenting with HBV DNA of 17.857.100IU/mL (branched DNA assay). At diagnosic time his liver enzyme levels were 82U/L for AST and 79U/L for ALT, and abdominal sonography revealed a diffuse mild fatty liver with no evidence of cirrhosis. HVD coinfection was excluded. At that point, he was under ddi, EVF and 3TC. The antiretroviral therapy was switched to include TDF (TDF/FTC/EFV) in 2006. He evolved with a partial response as there was an initial decrease of more than 1 log10 in the viral load, but not a virologic suppression at 12 months. The virologic control was achieved only in 2010. He has been undetectable ever since

(DNA VHB<10 IU/mL – real-time PCR assay). AgHBs and AgHBe seroconversion were never attained.

Aside from the HIV-HBV co-infection, his previous medical history included alcohol consumption with abstinence in the past 10years, diabetes mellitus, hypertension, ischemic heart disease, obesity and dyslipidaemia.

In 2019 he was diagnosed with an anal squamous cell carcinoma (T2-3N0M0) and completed treatment with capecitabine and mitomycin until March of 2020. Has been in remission ever since.

In February 2021 the infectious diseases outpatient clinic general revaluation showed a detectable HBV DNA result (31.800IU/mL log10 4,50), and maintenance of HBeAg. At this point, he had no symptoms and no liver enzymes elevation (AST 15U/L, ALT 15U/L). HVD coinfection was ruled out - negative HDV IgM and IgG. Compliance with therapy was assessed as HIV plasma RNA remained <20copies/mL. No history of drug-drug interaction that could influence TDF efficacy or absorption was known and no nutritional supplements or multivitamin combinations were being taken.

Facing the pandemic time and logistic constrains, a new evaluation was only possible 8weeks after which confirmed HBV DNA 37.900IU/mL Log10 4,58. At this point, a screening mutation test was performed using an in vitro reverse hybridization line probe assay (INNO-LIPA HBV multi-DR) which search for wild-type mutations or polymorphisms at codons 80, 173, 180, 181, 184, 194, 202, 204, 236 and 250 of the HBV polymerase gene. The results obtained identified the mutations V173L and M204V, which are non-related to a decreased susceptibility to TDF.

Before knowing the results of the resistance test, and assuming a possible TDF resistance, Entecavir 1g/day was added to the usual therapeutic antiviral regimen. The HBV DNA evaluation at 4, 12, 24 and 48weeks was 37.900IU/mL (Log10 4.58); 5.300IU/mL (Log10 3.72); 3.000IU/mL (Log10 3.48); 40.200IU/mL (Log10 4.60); 315IU/mL (Log10 2.50), respectively. After a year has passed, virologic control is still not achieved.

Discussion

According to EASL guidelines, a virologic breakthrough is defined as a confirmed increase in HBV DNA level of more than 1 log10 IU/ml compared to the nadir (lowest value ever registered) HBV DNA level on therapy. This is a rare outcome and is normally associated with poor compliance, drug-drug interaction with a decrease in NA plasma concentrations, or drug resistance emergence.[3]

Compliance was assessed. It was not possible to measure TDF plasma concentration. The patient was under a single-tablet regimen (TDF/FTC/EFV) to treat HIV and HBV coinfection and the preserved HIV virologic control was observed, implying compliance and efficiency. All other possible drug interactions which included insulin, gliclazide, statin, ramipril, carvedilol, pantoprazole, and acetylsalicylic acid, were ruled out as increasing the TDF rate of metabolism or reducing its absorption.

The possibility of a new liver viral infection was also considered, but the supplementary assessment was negative for acute hepatitis A, C and Delta virus (negative IgM and IgG for anti-VHD; negative anti-VHC and RNA VHC; negative for IgM anti-VHA and positive for IgG anti-VHA).

He was diagnosed with an anal squamous cell carcinoma (T2-3N0M0) in 2019 and completed treatment with capecitabine and mitomycin until March 2020. He has been in remission ever since. The virologic breakthrough occurred about one year after chemotherapy completion. At this time, he denied adhesion failures and maintained an HIV virologic suppression, however the CD4+ T lymphocytes counts decline from 36% to 15% (534cel/ µL to 183cel/µL). Despite a reduced immunological status, a breakthrough would not be expected in a patient who has maintained therapeutic adherence.

Nowadays is still not possible to cure hepatitis B infection. The current therapy available cannot eliminate integrated viral cccDNA from hepatocytes. However, it is possible to suppress the virus by reducing its replication rate to a low level to maintain an undetectable state of DNA HBV. Our patient took almost 4 years to reach virologic control. Nevertheless, HIV and HBV co-infected patients may take longer to get virologic suppression without this being associated with resistance acquisition or therapy inefficiency [6]. Furthermore, higher baseline HBV DNA level, positive baseline HBeAg status as well as its persistence, history of exposure to 3TC and lower nadir CD4+ T cells in the context of HIV infection have been mainly linked to persistent viremia, as was the case described, and not to breakthrough after virologic control.[4]

HBV has a high rate of replication, with 10^{10}-10^{12} virions produced per day and a high mutational rate that combined with the HBV polymerase's lack of proof-reading function, results in approximately 10-point mutations produced per day. Resistance emerges when replication occurs in the presence of the drug selection pressure.

Nucleoside analogues are associated with high virologic and biochemical response. ETV achieves 99% probability of virologic response and 53% probability of HBeAg loss at 5years of treatment, TDF achieves 97% virologic response and 40% probability of Anti-HBe seroconversion at 5years and TAF showed non-inferiority to TDF on the first 48 weeks of treatment.[2] Not being able to cure the infection leads to chronic treatment which, consequently, can result in toxicity, intolerance and periodic suspension that can lead to a selection of resistance-associated mutations (RAM) in the virus. This doesn't seem to be the case here presented.

TDF has a high genetic barrier to resistance and some reports show no resistance development even after numerous years of treatment[5] as well as high efficiency even as monotherapy in multi-drug resistant infection [7]. Some *in vitro* studies propose resistance emergence from the selection of single mutations S78T and A194T on the RT domain of the HBV polymerase, while others demonstrate that two or more RAM are required to confer TDF resistance, and identify a few mutation sites with the strong evidence base, such as L180M, A181T/V, M204I/V, N236T and F221Y when combined with A181T.[8,9]

The RT N236T mutation is associated with resistance to adefovir (ADV) treatment. Given that TDF and ADV are structurally similar, the same mutation may grant reduced susceptibility to both drugs.[5] On the other hand, the RT mutation A181T/V is known to confer resistance to 3TC and may confer a cross path of resistance to TDF, especially if there is previous pharmacological pressure with 3TC[4,8] This could be the case of the patient, who was under ddi+3TC+EFV at the time of HBV diagnosis, nevertheless, this mutation was not identified.

Another recent report showed that a quadruple mutation on the polymerase RT domain might be associated with a decreased susceptibility to TDF. The authors have detected seven mutations in the HBV DNA, including four new substitutions, namely, rtS106C, rtH126Y, rtD134E and rtL269I, which were collectively termed CYEI. The CYE mutation reduced tenofovir-susceptibility (by 3.7-fold)

Citation: Casanova S, Miranda AC, Pádua E, et al. Chronic hepatitis B infection: what to do when TDF is not enough to treat? *Gastroenterol Hepatol Open Access.* 2022;13(6):241–243. DOI: 10.15406/ghoa.2022.13.00529

and the CYEI (quadruple mutation) conferred complete resistance to TDF.[10] Finally, a multidrug-resistant HBV polymerase mutation emerging during TDF + ETV combination therapy was reported, the rt78S/sC69* mutation, that contributed to insufficient response to antiviral treatment and the development of HCC.[7]

On suspicion of a RAM conferring resistance to TDF treatment, a mutation detection test (INNO-LIPA HBV multi-DR) was performed which identified M204V and V173L mutations. Although it may be associated with a resistance pattern, it normally requires other RAM to confer resistance as explained earlier. Unfortunately, we could not test for other combined mutation sites such as the CYEI and rt78S/sC69*, which could have helped to comprehend the current breakthrough.

The barrier to selection of TDF resistance may be lower in certain genotypes of HBV. For instance, genotype C may have higher NA resistant mutation rates than genotype B (63.0% vs. 48.1%, P=0.003) however, the genotype B showed greater evidence of mutation at site 236.[9] M204I appeared in a minority of resistant strains in genotype A.[9] If that was the case for the patient infected with genotype A, it would not be expected to have maintained virologic control for ten years but there would be an expected response to entecavir treatment as shown. Nevertheless, this remains a difficult association to make as HBV genotyping is not routinely done leading to scarce information.

Finally, Thai Hong *et al* showed that TDF reduced susceptibility may not only correlate with specific mutations in RT but with variations across the entire HBV genome and identified 16 sites from different HBV genomic regions strongly associated as a group with the TDF response. These sites are distributed across all HBV genes, nine of them are on genomic regions encoding all four structural domains of the P protein, and only three are on the RT domain.[11] Expecting to find some of these variations, we tried to proceed with the sequencing of the specific genomic regions of virus. Unfortunately, at that time, a partial viral control was achieved (HBV DNA 315UI/mL) which hampered the amplification success and subsequent sequencing analysis to identify other resistance mutations.

Conclusion

There is still much to know about TDF reduced susceptibility and resistance in chronically infected HBV patients. Until today we don't completely understand what could have led to a breakthrough in our patient. However, recent reports are now starting to show evidence of virologic failure or breakthrough in treatment-experienced patients with this first-line therapy that is often prescribed, particularly in HIV co-infected patients. Thus, further studies are needed to assess the extent to which in vivo mutations or genomic alterations

Acknowledgments

None.

Conflict of interest

None.

Funding

None.

References

1. World Health Organization (WHO). Global Hepatitis Report 2017. License: CC BY–NC–SA 3.0 IGO. Geneva: World Health Organization; 2017.
2. Lampertico P, Agarwal K, Berg T, et al. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol*. 2017 Aug;67(2):370–398.
3. Roade L, Riveiro–Barciela M, Esteban R, et al. Long–term efficacy and safety of nucleos(t)ides analogues in patients with chronic hepatitis B. *Ther Adv Infect Dis*. 2021;5;8:2049936120985954.
4. Mokaya J, McNaughton AL, Bester PA, et al. Hepatitis B virus resistance to tenofovir: fact or fiction? A systematic literature review and structural analysis of drug resistance mechanisms. *Wellcome Open Res*. 2020;29;5:151.
5. Lim YS. Management of Antiviral Resistance in Chronic Hepatitis B. *Gut Liver*. 2017; 15;11(2):189–195.
6. Childs K, Joshi D, Byrne R, et al. Tenofovir–based combination therapy for HIV/HBV co–infection: factors associated with a partial HBV virological response in patients with undetectable HIV viraemia. *AIDS*. 2013;1;27(9): 1443–1448.
7. Yim HJ, Suh SJ, Jung YK, et al. Tenofovir–based combination therapy or monotherapy for multidrug–resistant chronic hepatitis B: Long–term data from a multicenter cohort study. *J Viral Hepat*. 2020;27(12):1306–1318.
8. Mokaya J, Maponga TG, McNaughton AL, et al. Evidence of tenofovir resistance in chronic hepatitis B virus (HBV) infection: An observational case series of South African adults. *J Clin Virol*. 2020;129:104548.
9. Zhang X, Chen X, Wei M, et al. Potential resistant mutations within HBV reverse transcriptase sequences in nucleos(t)ide analogues–experienced patients with hepatitis B virus infection. *Sci Rep*. 2019:9(1):8078.
10. Park ES, Lee AR, Kim DH, Lee JH, Jeong–JuYoo, HyunAhn S, et al. Identification of a quadruple mutation that confers tenofovir resistance in chronic hepatitis B patients. *J Hepatol*. 2019;70(6):1093–1102.
11. Thai H, Lara J, Xu X, et al. Complex genetic encoding of the hepatitis B virus on–drug persistence. *Sci Rep*. 2020;23;10(1):15574.