JAC antiviral

Journal of Antimicrobial Chemotherapy (2008) **62**, 1–4
doi:10.1093/jac/dkn175
Advance Access publication 25 April 2008

Why are there different dynamics in the selection of drug resistance in HIV and hepatitis B and C viruses?

Vincent Soriano[1]*, Alan S. Perelson[2] and Fabien Zoulim[3]

[1]Department of Infectious Diseases, Hospital Carlos III, Sinesio Delgado 10, 28029 Madrid, Spain; [2]Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, New Mexico, USA; [3]INSERM, U871, Université Lyon 1, Hospices Civils de Lyon, Lyon, France

The arrival of new antiviral drugs to treat chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections has given rise to great expectations along with concerns regarding the selection of drug-resistant variants. Many lessons learnt from HIV therapeutics can be helpful for designing adequate treatment strategies against viral hepatitis, the avoidance of sequential weak monotherapies being one of them. Although HIV, HBV and HCV share many biological features, including very rapid viral dynamics, distinctive characteristics explain why the speed of selection of drug resistance differs substantially between these viruses, being faster for HCV than for HIV and slower for HBV.

Keywords: HBV, HCV, resistance mechanisms

Chronic infection due to HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) accounts for a substantial proportion of deaths worldwide. Around 36 million people are currently living with HIV. These numbers are approaching 400 million and 200 million for chronic HBV and HCV infections, respectively. Because of similar routes of transmission, these viruses are seen more frequently than expected in co-infection.[1,2] Besides sharing epidemiological niches, HIV, HBV and HCV share several biological similarities, which largely explain the therapeutic difficulties arising when treating any of them, drug resistance being one complication, if not the most challenging.

Viral dynamics are rapid for all three of these viruses. Estimates of the daily production of virions are in the range of 10^{10} for HIV,[3] 10^{12} for HCV[4] and 10^{12}–10^{13} for HBV.[5,6] The half-life of free viral particles is very short, below 1 h for HIV[7] and between 2 and 3 h for HCV.[4,8] For HBV, there is some controversy and estimates vary between 3 and 24 h.[5,6] What is more different is the half-life of infected cells. It has been estimated to be ~1 day for CD4+ T lymphocytes productively infected with HIV,[9] several days or weeks for hepatocytes infected with HCV[4] and up to 100 days for those infected with HBV, with a large lifespan heterogeneity.[10]

Mutations occur frequently during the replication of HIV, HBV and HCV. The reverse transcriptase enzymes of HIV and HBV as well as the RNA-dependent RNA polymerase of HCV are intrinsically error-prone and lack proofreading function, allowing for frequent replication errors to occur. The result is the generation of multiple viral variants, known as a quasispecies, that coexist and reach population densities in direct proportion to their relative replication fitnesses. It has been predicted that every nucleoside of the 3.2 kb HBV genome[6] or the 10 kb HIV[11] and HCV genomes theoretically can be substituted every day within a given infected patient. Table 1 summarizes the main distinctive viral dynamic features of these three viruses.

As any drug pressure may act to select pre-existing drug resistant viral variants, the speed of selecting drug resistance mainly depends on the turnover of the viral nucleic acid acting as a source of new viral genomes. In the case of HIV, it is the proviral DNA integrated within the chromosomes of infected cells. For HBV, it is the cccDNA present within the nucleus of infected hepatocytes as extra-chromosomal (episomal) material. Finally, for HCV, there is no stable reservoir of genetic material and the HCV-RNA strands present in infected hepatocytes serve as templates for producing new HCV virions that soon thereafter are released (Figure 1).

The viral genetic material within infected cells is relatively stable and shows longer half-life for HIV and HBV, in comparison with HCV. Whereas HIV proviral DNA may persist as long as the lifespan of an infected CD4+ T lymphocyte, and the same applies to HBV cccDNA within infected hepatocytes,[12,13] HCV-RNA strands are short-lived molecules with a half-life of ~10 h,[14] in constant renewal replicating within infected hepatocytes associated with cytoplasmic vesicular membraneous structures.[15] Given these facts, it is easy to understand that the time required for selecting drug resistance mutations, present at baseline only as minority genomic variants, to expand and fill a major part of the virus population should be longer for HBV than for HIV and that it must be particularly short for HCV.

*Corresponding author. Tel: +34-91-4532500; Fax: +34-91-7336614; E-mail: vsoriano@dragonet.es

Table 1. Main distinctive viral dynamic features of HIV, HBV and HCV

	HIV	HBV	HCV
Virus			
daily production of virions per day	10^{10}	10^{12}–10^{13}	10^{12}
half-life of free virions (h)	1	3–24	2–3
half-life of intracellular virions	days (dependent on infected cells $t_{1/2}$)	months (dependent on infected cells $t_{1/2}$)	hours (not dependent on infected cells $t_{1/2}$)
mutation rate	very high	high	very high
constraints due to ORFs in targeted viral enzymes	moderate	high	none
immune-mediated escape mutants	frequent	infrequent	frequent
Target cells			
half-life of infected cells	days	months	weeks
size of susceptible cells compartment	large	small	probably large
intracellular viral reservoir	yes (integrated cDNA)	yes (cccDNA)	no

ORFs, overlapping reading frames; cDNA, complementary DNA; cccDNA, covalently closed circular DNA.

This may explain in part why resistance to lamivudine used as monotherapy may be recognized within only 3 weeks in HIV,[16] whereas it only develops after several months to years of therapy in HBV.[17]

The major determinants involved in the selection of drug-resistant mutants for all these viruses are the fitness of the mutants and the replication space available for the spread of mutants.[18] In chronic hepatitis B, the replication space is provided by hepatocyte turnover, which allows the loss of HBV wild-type infected cells and the generation of non-infected hepatocytes that are susceptible to new HBV mutant infections. This process is usually very slow in chronic hepatitis B because the immune-mediated killing of infected cells is slow.[19] In contrast, in HIV, the turnover of CD4+ T lymphocytes is quite rapid, allowing the mutant viruses to expand rapidly. The spread of mutants in the presence of the drug will also depend on the relative fitness of these variants. For HCV, the diversity of the viral genome is greater than that for HBV, and the rate of superinfection of these cells is not well defined, but the extraordinary rapidity of emergence of drug-resistant HCV mutants is in agreement with the short turnover of HCV-RNA molecules in the cytosol of infected hepatocytes.

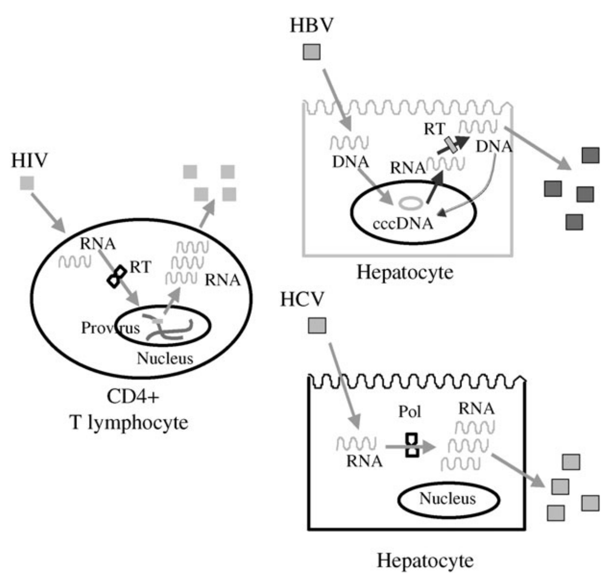

Figure 1. Schematic representation of the virus life cycle for HIV, HBV and HCV.

In a situation in which most potential target cells are already infected and releasing virions, it is clear that infected cells with a long half-life will provide only a minimal opportunity for replacing the original virus population by a new one of drug-resistant variants. This is the case for HBV, whose infected hepatocytes may survive for several weeks or months.[10,20] In contrast, CD4+ T lymphocytes infected with HIV show a shorter half-life (∼1 day).[9] This is why the dynamics of selection of drug resistance are so different comparing HBV and HIV, despite their respective genetic material being archived within the nucleus of infected cells.

Besides the different half-lives of each of the respective viral genetic materials, other factors may explain the relatively slow selection of drug resistance in HBV, compared with HIV and/or HCV (Table 2). Among others are the constraints imposed by the fact that the HBV genome shows overlapping reading frames. In this way, changes at one position may affect the structure and function of more than one viral protein. Indeed, it is well known that some lamivudine-associated resistance mutations may modify the antigenicity of the HBV surface antigen, as a result of the large overlap between the HBV polymerase and envelope genes.[21] HBV escape mutants induced by antiviral therapy have recently attracted much attention as they may represent a public health threat in the near future.[22] Moreover, mutants of the viral polymerase gene may induce mutations in the overlapping surface antigen which may then

Table 2. Factors explaining the slower selection of drug resistance in HBV when compared with HIV and HCV

1. Slower turnover of genetic material acting as source of newly produced viral particles in HBV than HIV and HCV.
2. Constraints in the HBV genome imposed by overlapping reading frames, which do not exist in HIV or HCV.
3. More effective immune escape for HIV and HCV than for HBV.

generate defective or less infectious mutants that may need trans-complementation of the mutant protein by wild-type to package and propagate the mutant virus (some of the M204I and the A181T mutants are examples).[23]

Another mechanism by which HBV may select for drug resistance much slowly than HIV or HCV relies on the immune system. Rapid selection of immune escape mutants has been described for HIV and HCV,[24,25] although immune tolerance may appear to persist throughout the entire course of chronic HBV infection, providing little selective pressure.[26]

Several therapeutic consequences derive from these biological considerations. The first is that eradication of HIV and HBV will not be attainable even after several years of complete virus suppression with current antiviral therapies, as relatively stable reservoirs of viral genetic material may exist waiting to awaken in the event of drug pressure being discontinued. In contrast, the fragile nature of HCV-RNA molecules in continuous turnover may provide a unique opportunity for eradication. Indeed, the vast majority of patients who achieve sustained virological response with interferon-based therapies do not show a rebound in HCV replication thereafter,[27,28] suggesting that the virus has definitively been eliminated. This is true even in HIV–HCV-co-infected persons, in whom immunodeficiency might raise suspicion of possible late relapses.[29,30]

A last therapeutic implication of these differences in the kinetics of selection of drug-resistant mutants is the difficulty in proving the benefit of combination therapy in chronic hepatitis B. It was relatively easier to demonstrate against HIV and is currently being shown against HCV using STAT-C molecules.[31] Although drugs targeting different steps of the life cycle of both HIV and HCV have been developed, and this has not been the case for HBV, it is clear that the relatively slow rate of emergence of drug-resistant HBV mutants in comparison with HIV or HCV leaves room for anti-HBV monotherapy. Clearly, it will not be the case using drugs with relatively low potency, suboptimal dosing and/or low genetic barrier for resistance, such as lamivudine, emtricitabine, telbivudine or adefovir. However, it may apply to drugs such as entecavir or tenofovir, which have much more potent antiviral activity and high genetic barrier to resistance, and for which the annual rate of selection of resistance is below 1% to 2%, at least in drug-naive chronic hepatitis B patients.[32–34]

Acknowledgements

We would like to thank Pablo Barreiro for helpful comments.

Funding

This work was supported in part by grants from Fundación IES (Investigación & Educación en SIDA), Agencia Laín Entralgo, Red de Investigacion en SIDA (RIS, project ISCIII-RETIC RD06/006), the European Commission VIRGIL and NEAT projects and NIH grants RR06555, AI065256 and AI28433. In addition, portions of this work were done under the auspices of the US Department of Energy under contract DE-AC52-06NA25396.

Transparency declarations

None to declare.

References

1. Soriano V, Puoti M, Bonacini M *et al.* Care of patients with chronic hepatitis B and HIV co-infection: recommendations from an HIV–HBV international panel. *AIDS* 2005; **19**: 221–40.

2. Soriano V, Puoti M, Sulkowski M *et al.* Care of patients coinfected with HIV and hepatitis C virus: 2007 updated recommendations from the HCV–HIV International Panel. *AIDS* 2007; **21**: 1073–89.

3. Perelson A, Neumann A, Markowitz M *et al.* HIV-1 dynamics *in vivo*: virion clearance rate, infected cell life-span, and viral generation time. *Science* 1996; **271**: 1582–6.

4. Neumann A, Lam N, Dahari H *et al.* Hepatitis C viral dynamics *in vivo* and the antiviral efficacy of interferon alpha therapy. *Science* 1998; **282**: 103–7.

5. Tsiang M, Rooney J, Toole J *et al.* Biphasic clearance kinetics of hepatitis B virus from patients during adefovir dipivoxil therapy. *Hepatology* 1999; **29**: 1863–9.

6. Murray J, Purcell R, Wieland S. The half-life of hepatitis B virions. *Hepatology* 2006; **44**: 1117–21.

7. Markowitz M, Louis M, Hurley A *et al.* A novel antiviral intervention results in more accurate assessment of HIV-1 replication dynamics and T-cell decay *in vivo*. *J Virol* 2003; **77**: 5037–8.

8. Neumann A, Lam N, Dahari H *et al.* Differences in viral dynamics between genotypes 1 and 2 of hepatitis C virus. *J Infect Dis* 2000; **182**: 28–35.

9. Ramratnam B, Bonhoeffer S, Binley J *et al.* Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis. *Lancet* 1999; **354**: 1782–5.

10. Nowak M, Bonhoeffer S, Hill A *et al.* Viral dynamics in hepatitis B virus infection. *Proc Natl Acad Sci USA* 1996; **93**: 4398–402.

11. Perelson A, Essunger P, Ho D. Dynamics of HIV-1 and CD4+ lymphocytes *in vivo*. *AIDS* 1997; **11** Suppl A: 17–24.

12. Moraleda G, Saputelli J, Aldrich G *et al.* Lack of effect of antiviral therapy in non-dividing hepatocyte cultures on the closed circular DNA of the woodcheck hepatitis virus. *J Virol* 1997; **71**: 9392–9.

13. Werle-Lapostolle B, Bowden S, Locarnini S *et al.* Persistence of cccDNA during the natural history of chronic hepatitis B and decline during adefovir dipivoxil therapy. *Gastroenterology* 2004; **126**: 1750–8.

14. Guo J, Bichko V, Seeger C. Effect of alpha interferon on the hepatitis C virus replicon. *J Virol* 2001; **75**: 8516–23.

15. Dahari H, Ribeiro R, Rice C *et al.* Mathematical modeling of subgenomic hepatitis C virus replication in Huh-7 cells. *J Virol* 2007; **81**: 750–60.

16. Tisdale M, Kemp S, Parry N *et al.* Rapid *in vitro* selection of HIV type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase. *Proc Natl Acad Sci USA* 1993; **90**: 5653–6.

17. Lai C, Chien R, Leung N *et al.* A one year trial of lamivudine for chronic hepatitis B. *N Engl J Med* 1998; **339**: 61–8.

18. Litwin S, Toll E, Jilbert A *et al.* The competing roles of virus replication and hepatocyte death rates in the emergence of drug-resistant mutants: theoretical considerations. *J Clin Virol* 2005; **34** Suppl 1: 96–107.

19. Summers J, Jilbert A, Yang W *et al.* Hepatocyte turnover during resolution of a transient hepadnaviral infection. *Proc Natl Acad Sci USA* 2003; **100**: 11652–9.

20. Lewin S, Ribeiro R, Walters T *et al.* Analysis of hepatitis B viral load decline under potent therapy: complex decay profiles observed. *Hepatology* 2001; **34**: 1012–20.

21. Locarnini S. Molecular virology and the development of resistant mutants: implications for therapy. *Semin Liver Dis* 2005; **25** Suppl 1: 9–19.

22. Sheldon J, Soriano V. Hepatitis B virus escape mutants induced by antiviral therapy. *J Antimicrob Chemother* 2008; **61**: 766–8.

23. Villet S, Pichoud C, Billioud G *et al*. Impact of hepatitis B virus rt181V/T mutants on hepatitis B treatment failure. *J Hepatol* 2008; **48**: 747–55.

24. Petravic J, Loh L, Kent S *et al*. CD4+ target cell availability determines the dynamics of immune escape and reversion *in vivo*. *J Virol* 2008; **82**: 4091–101.

25. Kuntzen T, Timm J, Berical A *et al*. Viral sequence evolution in acute hepatitis C virus infection. *J Virol* 2007; **81**: 11658–68.

26. Lau G, Cooksley H, Ribeiro R *et al*. Impact of early viral kinetics on T-cell reactivity during antiviral therapy in chronic hepatitis B. *Antivir Ther* 2007; **12**: 705–18.

27. Torres-Ibarra R, Cano-Dominguez C. Sustained virological response after more than 3 years of the end of treatment with conventional alpha-2b or pegylated alpha-2a interferon. *J Hepatol* 2007; **46** Suppl 1: 247.

28. Maylin S, Martinot-Peignoux M, Boyer N *et al*. Sustained virological response is associated with eradication of HCV and decrease in anti-HCV titer in patients treated for chronic hepatitis C with interferon alpha-2b or pegylated interferon alpha-2b plus ribavirin. *Hepatology* 2007; **46** Suppl 1: 343–4.

29. Soriano V, Maida I, Nuñez M *et al*. Long-term follow-up of HIV-infected patients with chronic hepatitis C virus infection treated with interferon-based therapies. *Antivir Ther* 2004; **9**: 987–92.

30. Berenguer J, Alvarez-Pellicer J, Lopez-Aldeguer J *et al*. Sustained virological response to interferon plus ribavirin reduces liver-related complications and mortality in HIV/HCV co-infected patients. In: *Abstracts of the Fifteenth Conference on Retroviruses and Opportunistic Infections, Boston, 2008*. Abstract 60. Foundation for Retrovirology and Human Health, Alexandria, VA, USA.

31. Soriano V, Madejon A, Vispo E *et al*. Emerging drugs for hepatitis C. *Expert Opin Emerg Drugs* 2008; **13**: 1–19.

32. Gish R, Lok A, Chang T *et al*. Entecavir therapy for up to 96 weeks in patients with HBeAg-positive chronic hepatitis B. *Gastroenterology* 2007; **133**: 1437–44.

33. Tan J, Degertekin B, Wong S *et al*. Tenofovir monotherapy is effective in hepatitis B patients with antiviral treatment failure to adefovir in the absence of adefovir-resistant mutations. *J Hepatol* 2008; **48**: 391–8.

34. Reijnders J, Janssen H. Potency of tenofovir in chronic hepatitis B: mono or combination therapy? *J Hepatol* 2008; **48**: 383–6.