Combination of nucleoside analogues in the treatment of chronic hepatitis B virus infection: lesson from experimental models

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Owing to the persistence of hepatitis B virus (HBV) and the selection of drug-resistant mutants, a new concept of antiviral therapy for chronic hepatitis B relies on the combination of nucleoside analogues. In experimental models of HBV infection, several key points concerning these combinations were addressed. (i) Is it possible to achieve a synergic antiviral effect with polymerase inhibitors? (ii) Is it possible to impact on intracellular viral covalently closed circular DNA? (iii) What is the impact of the cross-resistance patterns of the different nucleoside analogues? (iv) What is the effect of viral load suppression on the restoration of specific antiviral cellular responses? The clinical impact of these key issues is discussed in the perspective of new clinical trials.

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Introduction

Hepatitis B virus (HBV) is the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma worldwide. Antiviral therapy of chronic HBV infection remains a clinical challenge. Indeed, owing to its unique mechanism of replication, HBV is able to persist in the infected host despite efficient antiviral therapy, and then to escape antiviral drugs. HBV has a DNA genome that is replicated via a reverse transcription step. The template for viral mRNA transcription, viral covalently closed circular DNA (cccDNA), is located in the nucleus of infected cells and its half-life is believed to be long and to be mainly related to the fate of infected cells. As HBV replication does not usually lead to a cytopathic effect, clearance of HBV cccDNA and HBV infection is closely related to the death of infected cells induced by the host immune response. However, in most chronic carriers, the anti-HBV immune response is defective. Altogether, this leads to the development of chronic HBV infection. In the presence of nucleoside analogues that potentiate the viral polymerase, viral production is decreased but the pool of intranuclear cccDNA persists owing to its long half-life. Long-term antiviral therapy is therefore required in most patients with chronic hepatitis B who do not mount a vigorous immune response, to avoid relapse of viral replication after treatment withdrawal. However, the major drawback of prolonged therapy is the selection of drug-resistant mutants generated by the spontaneous error rate of the viral polymerase. Therefore, one of the major research areas is the evaluation of combination therapy to delay or prevent the occurrence of drug-resistant mutants.

The major questions are whether a combination of nucleoside analogues can achieve antiviral synergy, thereby reducing the risk of resistance development? Can it prevent the formation of cccDNA in newly infected cells? Can it decrease the pool of cccDNA in already chronically infected cells? Has the use of drugs that do not share the same cross-resistance profile the capacity to delay the development of multidrug-resistant mutants? And can all these effects on HBV replication result in restoration of immune response to achieve a sustained control of viral replication?

Mechanism of action of nucleoside analogues

Although all orally available drugs target the viral polymerase, nucleoside analogues have different mechanisms of action on the viral genome replication machinery. Several drugs such as adefovir, entecavir and adefovir inhibit the priming of reverse transcription, a unique enzymic reaction that results in the synthesis of a short DNA primer covalently attached to a conserved tyrosine residue of the viral polymerase. Other compounds inhibit the elongation of viral minus-strand DNA, such as lamivudine, emtricitabine and elvucitabine. The mechanism of action of clevudine involves a weak effect on the priming reaction but a stronger inhibitory activity on plus-strand DNA synthesis. The mechanism of action of telbivudine has not been described so far. Based on these distinct modes of action, one may speculate that the combination of drugs targeting different steps of viral genome replication may lead to an additive or synergic effect. Therefore, the evaluation of the combination of drugs such as

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emtricitabine and clevudine,\(^5\) which are under clinical investigation,\(^6,7\) is relevant for the future development of combination therapy.

**Combination of drugs and antiviral synergy**

Although anti-HBV drugs mainly target the viral polymerase activity and not other steps of viral replication, it was shown that antiviral synergy could be obtained by combining adefovir, lamivudine and penciclovir in duck HBV (DHBV)-infected primary hepatocyte cultures.\(^8,9\) In a hepatoma cell line expressing wild-type HBV, an additive effect was also observed with the combination of adefovir and thymidine analogues (lamivudine, emtricitabine and teldivudine).\(^10\) Antiviral synergy was shown against HBV in the 2215 cell line, and in vivo in the woodchuck model.\(^11,12\) However, the few combination trials performed in patients with chronic hepatitis B have not shown a benefit of the combination on the early virological response. Differences may be observed in the long term in terms of the selection of drug-resistant mutants, which may be delayed by using agents that do not share the same cross-resistance profile.

In a recent study, we showed in the DHBV model an additive effect of the combination of drugs acting on the priming of reverse transcription (amdoxovir), elongation of minus-strand DNA (emtricitabine), elongation of plus-strand DNA (clevudine) on the viral polymerase activity, intracellular viral DNA synthesis in primary duck hepatocyte cultures and on virus production in vivo in experimentally infected animals.\(^13\) Antiviral synergy per se was not observed, which may suggest that the combination strategies should use drugs with clearly distinct mechanisms of action, such as polymerase inhibitors and interferon-\(\alpha\) (as demonstrated in recent clinical trials of lamivudine plus pegylated interferon\(^15\)), or the use of new compounds inhibiting viral assembly or morphogenesis or viral entry, etc. In the woodchuck model, we found that the combination of clevudine and emtricitabine resulted in a sharp decrease in viraemia levels,\(^7\) which seemed to be more pronounced than in historical controls receiving single drugs alone. Interestingly, as also observed previously in woodchucks treated with clevudine alone, a slow rebound of viraemia was observed after treatment cessation in animals who received the combination therapy. This unusual evolution of viraemia following drug withdrawal compared with all the other nucleoside analogues tested so far may be attributable to an unknown effect of clevudine.\(^5,15,16\) Furthermore, in Phase II clinical trials this slow rebound of viraemia was also observed in patients.\(^7\) This unique antiviral activity profile may lead to a new concept of antiviral therapy for chronic hepatitis B.

**Effect of combination therapy on viral cccDNA**

One of the major problems of antiviral therapy of chronic HBV infection is the effect of anti-HBV agents on de novo cccDNA formation after viral entry.\(^17-19\) So far, none of the nucleoside analogues that are available was able to inhibit this critical step of viral infection, implying that the residual viraemia during antiviral therapy may still result in the infection of new cells, thus delaying the clearance of viral infection. Even the combination of amdoxovir, emtricitabine and clevudine could not prevent the formation of cccDNA in experimentally infected primary hepatocytes.\(^13\)

Another issue is to determine whether antiviral therapy may have an effect on already formed cccDNA in chronically infected cells, thus resulting in cell curing. As polymerase inhibitors do not have a direct effect on cccDNA formation, this may be due to the inhibition of intracellular recycling of viral nucleocapsid to the nucleus secondary to the inhibition of viral DNA synthesis. This has been observed with single drug administration in the woodchuck model with clevudine and entecavir,\(^16,20\) and in clinical trials with entecavir and adefovir dipivoxil.\(^21\) Interestingly, it was shown that a combination of nucleoside analogues may have an additive effect on the intracellular cccDNA levels in already infected cells, which suggests that the additive effect observed on viral DNA synthesis may also result in an additive effect on cccDNA levels.\(^22\) Therefore, this implies an important role of combination therapy to decrease intrahepatic viral load during long-term therapy.

The decrease in cccDNA levels observed in woodchucks treated with clevudine alone or in combination with emtricitabine may explain the slow relapse of viral replication post-treatment. In some animals this was accompanied by a decline in the number of infected cells to undetectable levels as assessed by immunostaining of liver sections for viral antigens.\(^7\) It is also noteworthy that in one study, the combination of clevudine followed by vaccination against the woodchuck hepatitis virus (WHV) envelope proteins led to the clearance of circulating viral antigens.\(^22\)

**Influence of the cross-resistance profile of nucleoside analogues**

It is important to note that most of the nucleoside analogues administered in monotherapy may select for drug-resistant mutants, as this was shown with HIV.\(^23\) The profile of cross-resistance of these mutants has been studied in vitro after transfection of the mutants in hepatoma cell lines and the study of their susceptibility to other drugs.\(^2\) It was shown that lamivudine and emtricitabine share the same cross-resistance profile with the selection of the M204V or M204I polymerase mutants that are susceptible to adefovir. To date, telbivudine is associated with the emergence of the M204I mutant, which is resistant to lamivudine and emtricitabine. Adefovir selects for the A181V and N236T mutants, which are susceptible to lamivudine. Entecavir is less active against lamivudine-resistant strains and selects for specific resistance mutations on a genetic background of lamivudine resistance mutations. Clevudine is not active against the lamivudine-resistant strains and was shown to select for the same resistant mutants in woodchucks chronically infected with WHV.\(^24\) It also exhibits some antiviral activity against the adefovir-resistant strains in vitro. Elvucitabine is not active against the lamivudine-resistant mutants but is active against the adefovir-resistant strains. Tenofovir shows a good antiviral efficacy against the lamivudine-resistant strains and slightly decreased activity against the adefovir-resistant mutants. A tenofovir-resistant mutant was also recently described. Given the cross-resistance profile of these drugs, the rationale is to combine the drugs that would inhibit the emergence of drug-resistant strains to one or the other drug. This may lead to an improved management of antiviral therapy of chronic HBV infection in the long term.
Restoration of specific anti-HBV immune response

Several studies have shown that the decrease in viral load induced by lamivudine therapy is associated with the subsequent restoration of the CD4 and then the CD8 cellular immune response against HBV. Another recent study showed that the activation of the specific cellular immune response depends on a relatively low level of viral load. However, the recent results of clinical trials with entecavir and tenofovir, which showed a stronger inhibition of viral load compared with lamivudine, did not show a statistical difference in the rate of hepatitis B e antigen (HBeAg) seroconversion.

In our study in the woodchuck model, the intrahepatic delivery of recombinant adenoovirus vectors encoding the woodchuck interferon-γ did not lead to an added benefit to the combination of emtricitabine and clevudine, suggesting that chronically infected woodchuck hepatocytes may be refractory to the anti-WHV activity of interferon-γ. This has been confirmed by other teams, one study showing that interferon-α had more antiviral efficacy than interferon-γ in the woodchuck model.

Perspectives for the treatment of chronic hepatitis B

The current understanding of chronic HBV infection and its treatment suggest that the patients who are more likely to seroconvert anti-HBeAg antibodies should first receive a course of pegylated interferon-α. In this setting, pegylated interferon therapy may be the best option because of the possibility of short-term therapy and the absence of selection of resistant mutants. On the other hand, the majority of patients who are non-seroconverters or are infected with a pre-core mutant will require long-term maintenance therapy to control viral replication and liver disease. In this view, the development of clevudine and emtricitabine opens new avenues in the management of these patients. Clevudine, with its unusual antiviral activity profile, may be the first nucleoside analogue to be used as a relative short-term treatment and to achieve sustained control of viral replication even after treatment withdrawal. On the other hand, emtricitabine, as well as other drugs in development, offers a new option for combination of nucleoside analogues that do not share the same cross-resistance profile. For instance, it may be used in combination with adefovir or tenofovir. The evaluation of such combination strategies will need to rely on accurate endpoints and timing for such analysis.

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References


