Effect of Antiretroviral HIV Therapy on Hepatitis B Virus Replication and Pathogenicity

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Abstract

Coinfections with hepatitis B virus (HBV) and HIV are very frequent. Although HBV is a DNA virus, it replicates via reverse transcription like HIV. Structural similarities between the enzymatic pocket of the HBV DNA polymerase and HIV-1 reverse transcriptase are the basis that certain drugs inhibit both enzymes and thus the replication of both viruses. HBV components increase the pathogenic action of HIV and vice versa directly by certain proteins like HBsAg in the case of HBV and HIV-encoded Tat and Vpr and by disturbing the cytokine balance in affected cells. Antiretroviral therapy is highly beneficial for HIV/HBV-coinfected patients, but carries the risk of drug-induced resistance development and hepatotoxicity. Even with restoration of the immune capacity, signs of hepatic inflammation may develop even after 10 years of treatment.

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Structure of the HIV Reverse Transcriptase and Hepatitis B Virus DNA Polymerase

Structure of the HIV-1 Reverse Transcriptase

The HIV reverse transcriptase (RT) is a heterodimer composed of two subunits, p51 with the RT activity and p66 (p51 plus p15) which harbors mainly the catalytic activity of ribonuclease H. The functionally active RT is an RNA-dependent DNA polymerase. The RT structure corresponds roughly to the shape of the right hand, deduced from the 3.5 Angström resolution electron density map of the crystallized enzyme [1, 2]. The thumb is flexible, has close contact to the finger, and opens to process the HIV RNA strands [2]. HIV particles contain two copies of HIV RNA and transcribe both strands during replication. The RT generates from the first RNA molecule the first DNA strand, and subsequently the first RNA strand is degraded. The second RNA strand used as template for the second DNA strand. The second RNA strand also gets degraded, resulting in an additional double-stranded DNA molecule. By RT-shifting from one RNA strand to the second, recombinant viruses are formed when the cell is infected with two different or mutated viruses [2]. The incoming dNTP (deoxynucleotide triphosphate) is moved by the
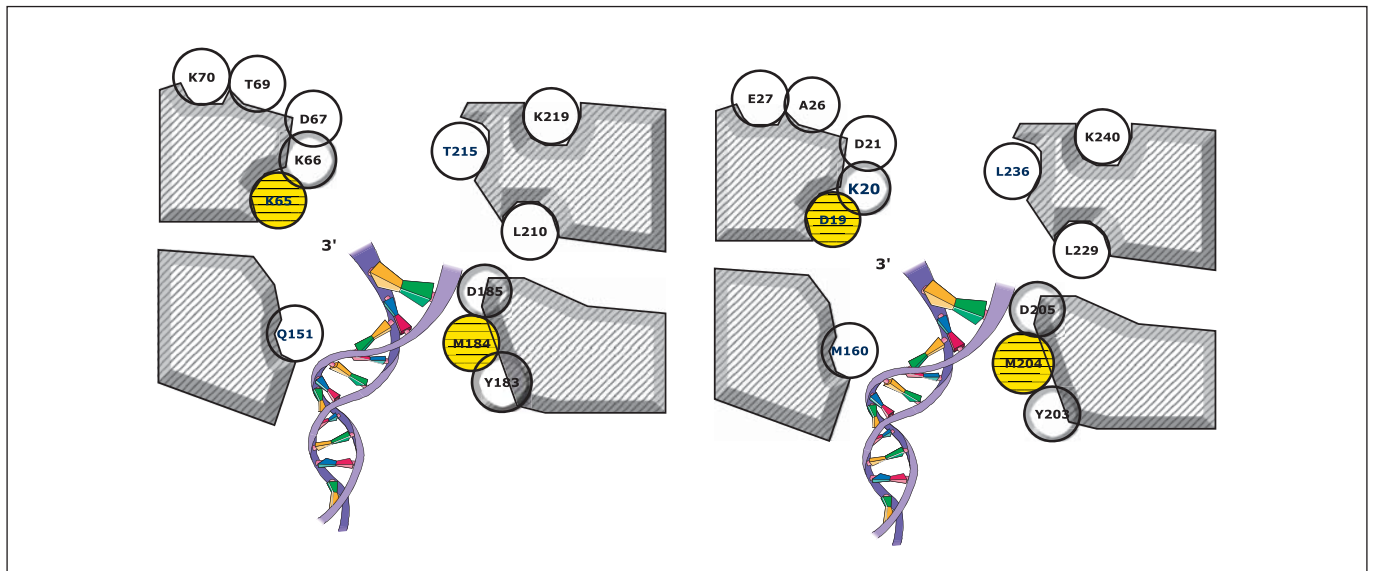
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Fig. 1. Scheme of the enzymatic pockets of HIV-1 (left) and HBV (right). The methionine (M) of the highly conserved YMDD motif is shown hatched in HIV as M184 and in HBV as M204. In the left part of the pocket, the K65 (lysine, also hatched) position may be seen which mutates to K65R (arginine) yielding tenofovir resistance in HIV. This amino acid is replaced in the HBV polymerase by an aspartic acid (D19). In the center of the pocket is the growing DNA strand with its 3′ end.

fingers of the RT of HIV-1 – amino acid positions K65 (lysine) and R72 (arginine) – and incorporated in the growing DNA strand. The enzymatic pocket is built by a loop of YMDD (tyrosine-methionine-aspartic acid-aspartic acid) in positions Y183, M184, D185, and D186 (not shown), and by the amino acids Y115 and Q151 (glutamine) [3] – as schematically presented in figure 1.

New DNA is synthesized at an approximate velocity of 50–70 nucleotides per minute [2]. The enzymatic machinery of HIV leads after years of infection to a virus titer of 10^4 to 10^5 per ml plasma, with a turnover rate of around 10^{10} particles daily [4]. The action of the RT is error prone since there is no proofreading activity and 1–10 mutations may be introduced with each replication cycle [4], summing up to around 10^6 mutations per day. Most of the mutations are silent.

The enzymatic pocket structure, as shown in figure 1, is very conserved and found with corresponding loop structures in other DNA polymerases as those of diverse viruses including other retroviruses, hepatitis B virus (HBV), and even bacteria as *Escherichia coli* [5].

Structure of the HBV Polymerase
Currently, an accurate spatial structure of the HBV DNA polymerase, which also harbors RNA-dependent DNA polymerase activity and ribonuclease H activity, is not available. Due to the functional similarity, the structure of the HIV RT is used for modelling the spatial structure of the HBV polymerase [6, 7]. Comparison of HBV polymerase and HIV-1 RT on an amino acid sequence basis revealed only around 14% homologous sites [6], but the YMDD motif is conserved (HBV pol sequence position 203–206 according to the nomenclature of Stuyver et al. [8]). The enzymatic pocket is formed by certain structurally exposed amino acids (described above), which enable a possible identical conformation of the pockets of HIV-1 and HBV polymerases (fig. 1). The commonly used drugs effective for HBV and HIV treatment are the nucleos(t)ide RT inhibitors (NRTI), which act by chain termination. Specific substances suitable for HBV and HIV treatment are lamivudine and tenofovir. Telbivudine, adefovir, and entecavir are licensed only for HBV (see van Bömmel [this issue, pp. 171–180]).

As described for the HIV-1 RT, the M204V or I mutation in the YMDD motif of HBV results in complete resistance against lamivudine and emtricitabine [6]. The prominent amino acids that build the enzymatic pocket of the HBV RT are in relation to HIV-1 as shown in figure 1: D19 (in yellow instead of K65 in HIV) in the upper left part, M160 in the motif PM(160)G in HBV and PQ(151)G in HIV in the lower left part, M204 in yellow the lower right part, and finally L229 in the upper right
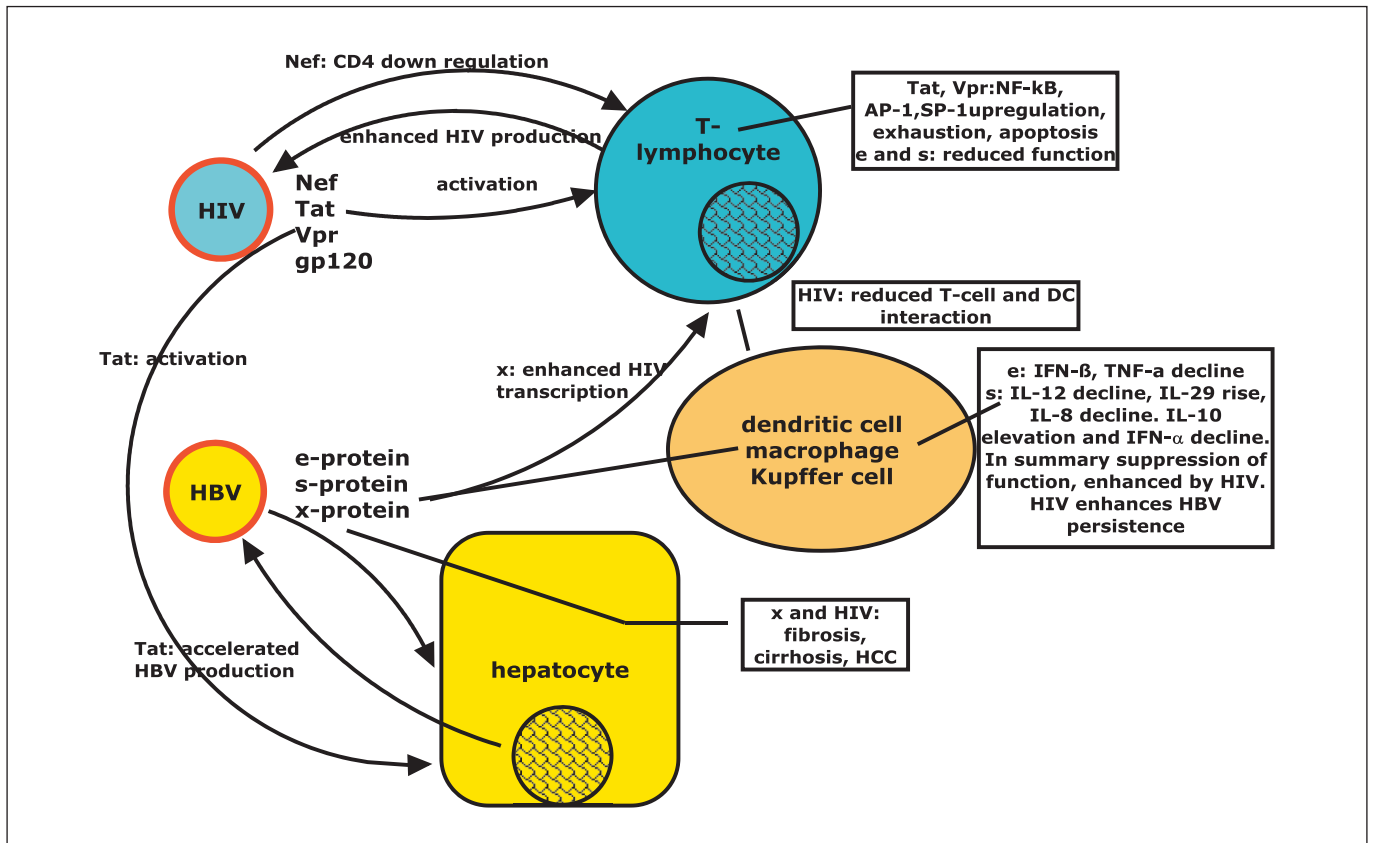
Fig. 2. Scheme of the interactions of HBV and HIV-1, either by the virus itself or by viral proteins that enhance immunosuppression and accelerate viral turnover and thus increase pathogenicity [30]. e-protein = HBeAg; s-protein = HB surface Ag; x-protein = HBx protein; gp120 = HIV-1 surface glycoprotein with a molecular weight of 120.000; Nef = negative regulating factor; Tat = transcription-mediated transactivator, Vpr = viral protein R; HCC = hepatocellular carcinoma. All viral proteins may be found inside and outside of a cell.

part. HBV L180, which is selected as a compensatory mutation to M204V or I, does not have a corresponding amino acid in the HIV-1 sequence.

In a patient with chronic hepatitis B, 10^{12} HBV particles are produced and eliminated daily [7]. Assuming a mutation rate of 10^{-5} per cycle within the process of HBV RT, sufficient mutations could be generated leading to rapid drug resistance as found in HIV monotherapy. However, since the HBV genome is condensed and uses overlapping reading frames, mutations in the RT open reading frame must be tolerated as well in the preS/S open reading frame; thus, many of the theoretically possible mutations are not viable. However, lamivudine resistance of HBV develops very often at a rate of around 14–24% per year [7], while clinically relevant tenofovir resistance has not been found after >10 years of drug therapy of hepatitis B.

Pathogenicity

Major Targets of HIV-1 Pathogenicity

There are multiple factors and potential therapeutic targets for HIV pathogenicity [9]; however, only parts of those HIV-1 proteins that interact with the HBV replication cycle are shortly described here. Generally HIV-1 infection worsens the course of hepatitis B and HBV pathogenicity more than vice versa [10]. The main HIV proteins for activation of the cell metabolism and viral turnover, Tat, Vpr, and gp120, are released from HIV-infected cells and are detectable in the plasma of AIDS patients [11]. They are taken up by several cell types, including hepatocytes and accelerate the pathogenic action of HBV like induction of fibrosis and hepatocellular carcinoma [12, 13]. Nef downregulates CD4 and HLA molecules in HIV-infected cells and induces by this action a

certain degree of immune incompetence on a cell-cell co-operation level [14]. This is supported by the toxic action of Tat on T lymphocytes, which in the beginning leads to activation of the cell metabolism. In the long term, Tat activation of lymphocytes and hepatic cells is followed by exhaustion and finally apoptosis [11, 15]. HIV itself is cytotoxic and leads to lysis of the infected cell, partially associated with giant cell formation. A scheme of this HBV and HIV-1 component interaction is shown in figure 2.

The destruction of helper T lymphocytes by virus release, viral components, cytotoxic T cell-mediated immunity, antibody-dependent cellular cytotoxicity, and impaired restoration of lost helper T lymphocytes are specific for HIV pathogenicity [9]. The weakened immune function hampers the inhibition of HBV replication from the covalently closed circular DNA form in the nuclei of hepatocytes and generate HBV and cellular components, such as IL-10, which further support the immunosuppressive action.

Major Targets of HBV Pathogenicity

While HIV itself is cytotoxic for T lymphocytes, HBV usually does not induce liver cell cytotoxicity by itself. Hepatocyte destruction and associated clinical signs of hepatitis are dependent on the function of the host immune system. Necrosis of hepatocytes is accomplished by primed cytotoxic T lymphocytes, and antibody-dependent cellular cytotoxicity [16]. Under insufficient immune control, HBV titers in blood will increase, as will the concentration of HBsAg and HBeAg and potentially the HBx protein. When the HIV-induced immunodeficiency improves under antiretroviral treatment, symptoms of hepatitis may aggravate severely – a phenomenon described under immune reconstitution inflammatory syndrome [4, 10]. HBV induces the liberation of IL-10, which has an immunosuppressive action by inhibiting the production of interferon (IFN)-γ and stimulating cytokines such as IL-2, IL-3, and TNF-α [17], and by inhibiting IFN-α production, which supports apoptosis of dendritic cells [16].

HBV proteins disturb the host immune function in several ways. In monocytes, dendritic cells, and Kupffer cells, HBsAg inhibits the synthesis of IL-12, which itself stimulates the production of IFN-γ; therefore, HBsAg acts in an immunosuppressive manner [17]. HBeAg suppresses IFN-β and TNF-α synthesis, and thus leads to impaired immune function [17]. The HBx protein inhibits the synthesis of IFN-β [17]. As mentioned above, the HBx protein enhances RNA transcription in the HIV-1 long terminal repeat, which leads to higher HIV replication

and sustains the immune dysfunction through the action of HIV-1 components [18, 19]. Finally, the HBx protein may enhance liver disease progression associated with cell fibrosis and (after decades) cirrhosis and tumorigenesis (hepatocellular carcinoma) [12].

Copathogenicity of HBV and HIV-1

HBV may be cleared from blood and body fluids by the function of an intact immune system; therefore, clinically typical signs of liver disease are not apparent in around 85–95% of infected patients. However, the virus remains persistently in the liver cell due to the high stability of the HBV covalently closed circular DNA. HIV causes permanent infection after the proviral DNA has been integrated in the host cell genome of T-helper lymphocytes, macrophages, and microglia cells. Both viruses induce immunodeficiency. HIV acts directly by impairing the function of T-helper lymphocytes, and by their destruction until in some AIDS patients their total depletion occurs. HBV acts more subtly by secretion of the proteins HBsAg, HBeAg, and potentially HBx, which may act themselves tolerogenic or even immunosuppressive and strengthen the HIV-induced immunodeficiency [10]. Unfortunately, the HIV-associated immunodeficiency is neither sufficient to block immune pathogenesis of hepatitis B, nor to interfere with the pathogenic action of HBV and HIV components on the cytokine cascades. Thus, HIV-1 worsens the outcome of chronic hepatitis B and, on a cellular level, enhances its own replication and that of HBV. The higher viral burden is associated with higher toxicity and extended pathogenicity. The vicious cycle can be interrupted by a selected antiretroviral treatment which hampers replication of both viruses and, finally, has the potential to stop virus production [20].

Treatment of HIV-1/HBV-Coinfected Patients with NRTI

Only NRTI are active against HBV. Non-NNRTI will not block the activity of the HBV polymerase by changing the substrate binding site of the enzymatic pocket as in HIV-1 RT [4]. Substances that inhibit the HIV protease or integrase cannot be used since both enzymes are absent in HBV.

The first HBV drug used was lamivudine (3TC) which is no longer recommended due to frequent (around 20% per year) mutations in M204V/I causing resistance [21]. A specific problem was that lamivudine remained active against HIV due to the combination therapy, while HBV – treated only with lamivudine – became rapidly resistant and caused severe hepatitis B. Further drugs

were developed and today the preference to treat HIV-1/HBV is the combination of tenofovir plus emtricitabine since no HBV drug resistance has been detected in the patients after 10 years of application [22]. However, tenofovir treatment is possibly less effective in HIV patients coinfected with HBV genotype G after pretreatment with lamivudine [23]. Long-term tenofovir exposure might reduce kidney function in 63% of the treated patients [24]. A further side effect is that a combination of various ART drugs given to suppress the replication of HIV-1 in a chronic hepatitis B patient may lead to deadly acute liver failure due to severe hepatotoxicity [25]. Treatment of a recently coinfected patient shortly after HIV-1 seroconversion doubles the hazard for an AIDS or death event [26], indicating that not all of the pathogenic interactions of HBV and HIV-1 coinfection are known.

Influence of Host Factors

Finally, there are several host genetic factors (not discussed in this article) that influence the course of the disease, such as CCR5Δ32, a mutation in the CCR5 (chemokine receptor 5), the coreceptor for HIV entry, and SNPs in the HBV receptor sodium-dependent taurocholic cotransporting polypeptide, which is a G7 protein [27, 28] spanning the cell membrane as CCR5. Additionally, both HIV and HBV RNA stability are partially controlled by the APOBEC3 system.

Future Aspects

There are two ways to influence the outcome of patients infected with HBV and HIV: the development of new drugs and vaccination to prevent infection.

New Drugs

Inhibition of the HBV polymerase is achieved usually at lower drug concentrations than necessary for the inhibition of the action of the HIV RT. Nearly all of the drugs used today for HBV treatment were initially developed for HIV, including the combination of tenofovir plus emtricitabine, which is as effective as tenofovir monotherapy for the long-term treatment of hepatitis B [22]. The main restriction of the present tenofovir therapy is still hepatotoxicity [29] and nephrotoxicity [24]. As shown for the inhibition of HCV enzymes, there is a high pharmaceutical potential to design new drugs, which should open some aspects for a better quality of life and outcome of coinfected patients.

Vaccination

The immune reaction against HBV induces clinical resolution of hepatitis B and control of the occult intrahepatic HBV in around 85–95% of those with natural infection [30]. HBV childhood vaccination, and with a somewhat lower efficacy adulthood vaccination, has been shown to protect against HBV disease and HBV chronicity after exposure. Thus, extended HBV vaccination of a population will limit the spread of HBV. Complications of chronic hepatitis B like liver fibrosis, cirrhosis, and HCC are absent in successfully vaccinated persons. There are still the limitations of HBV escape variants and immunological failure in around 5% of those vaccinated to produce a sufficient amount of anti-HBs.

An effective vaccine against HIV-1 or HIV-2 does not exist and, according to all the trials performed over the last 30 years, will be very hard to design. Thus, the presence and spread of HIV in certain parts of a population will persist while the circulation of HBV can be reduced to a very low level.

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