

Hepatocarcinoma in HBV infection is controlled by the use of entecavir, interferon and tenofovir: viral suppression and restoration of hepatic decompensation

O hepatocarcinoma na infecção do HBV é controlado pelo uso de entecavir, interferon e tenofovir: supressão viral e restauração da descompensação hepática

El hepatocarcinoma en la infección por VHB se controla mediante el uso de entecavir, interferón y tenofovir: supresión viral y restauración de la descompensación hepática

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RESUMO

Objetivo: Elucidar como as terapias com interferons e análogos de nucleotídeos em pacientes infectados pelo vírus da hepatite B ocasionam a reversão de cirrose hepática e hepatocarcinoma. **Revisão bibliográfica:** O entecavir demonstrou benefícios virológicos e bioquímicos, reduzindo a incidência de mutações resistentes aos medicamentos, sendo associado a um risco menor de óbito. Os interferons (IFNs) têm sido usados para tratar hepatite B/C crônica, IFNs induzem a expressão de APOBEC3G, com ativação do STAT3 inibindo o HBV, complexos desses IFNs ativam o transdutor de sinal da quinase ativada por Janus (JAK) e o ativador da via de transcrição (STAT), levando a expressão de genes estimulados por IFN (ISGs). Estudos com tenofovir para hepatite B sugerem que ele reverte a fibrose hepática, o tenofovir é hidrolisado pelas esterases intestinais e plasmáticas, sendo metabolizado principalmente intracelularmente pela catepsina A. **Considerações finais:** Os IFNs ativam células do sistema imunológico responsáveis pela resposta antiviral, o entecavir atua como terminador de cadeia de DNA do HBV e o tenofovir está associado a reversão da fibrose hepática e restauração da função renal.

Palavras-chave: Fármacos anti-HBV, Infecção pelo HBV, Carcinoma hepatocelular.

ABSTRACT

Objective: To elucidate how therapies with interferons and nucleotide analogues in patients infected with hepatitis B virus cause reversal of liver cirrhosis and hepatocarcinoma. **Literature review:** Entecavir has demonstrated virological and biochemical benefits, reducing the incidence of drug-resistant mutations, and being associated with a lower risk of death. Interferons (IFNs) have been used to treat chronic hepatitis B/C, IFNs induce APOBEC3G expression with activation of STAT3 inhibiting HBV, complexes of these IFNs activate the Janus-activated kinase signal transducer (JAK) and the transcription pathway activator (STAT), leading to the expression of IFN-stimulated genes (ISGs). Studies with tenofovir for hepatitis B suggest that it reverses

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hepatic fibrosis, tenofovir is hydrolysed by intestinal and plasma esterases, and is metabolized mainly intracellularly by cathepsin A. **Final considerations:** IFNs activate immune cells responsible for the antiviral response, entecavir acts as the HBV DNA strand terminator, and tenofovir is associated with reversal of liver fibrosis and restoration of kidney function.

Keywords: Anti-HBV drugs, HBV infection, Hepatocellular carcinoma.

RESUMEN

Objetivo: Dilucidar cómo las terapias con interferones y análogos de nucleótidos en pacientes infectados con el virus de la hepatitis B causan la reversión de la cirrosis hepática y el hepatocarcinoma. **Revisión de la literatura:** Entecavir ha demostrado beneficios virológicos y bioquímicos, reduciendo la incidencia de mutaciones resistentes a los medicamentos y asociándose con un menor riesgo de muerte. Los interferones (IFN) se han utilizado para tratar la hepatitis B/C crónica, los IFN inducen la expresión de APOBEC3G con la activación de STAT3 inhibiendo el VHB, los complejos de estos IFN activan el transductor de señal quinasa activado por Janus (JAK) y el activador de la vía de transcripción (STAT), lo que conduce a la expresión de genes estimulados por IFN (ISG). Los estudios con tenofovir para la hepatitis B sugieren que revierte la fibrosis hepática, el tenofovir es hidrolizado por esterases intestinales y plasmáticas, y se metaboliza principalmente intracelularmente por cathepsina A. **Consideraciones finales:** Los IFN activan las células inmunitarias responsables de la respuesta antiviral, el entecavir actúa como terminador de la cadena de ADN del VHB y el tenofovir se asocia con la reversión de la fibrosis hepática y la restauración de la función renal.

Palabras clave: Fármacos anti-VHB, infección por VHB, carcinoma hepatocelular.

INTRODUCTION

Viral hepatitis is a major public health problem, being responsible for more than 1.3 million deaths annually, potentially fatal complications such as liver cirrhosis and hepatocellular carcinoma (HC) arise when chronic viral infections are not diagnosed or treated, it is estimated that hepatitis B virus (HBV) infection affects almost 300 million people worldwide, of which only 10% are diagnosed and an even smaller proportion are receiving the treatment, the availability of a prophylactic vaccine has led to a significant reduction in new HBV infections, however, a cure for individuals chronically infected with HBV is still undefined (MOHD-ISMAIL NK, et al., 2019).

HBV is a hepatotropic virus, where its envelope is formed by lipid bilayer with the antigens S-HBsAg, M-HBsAg, L-HBsAg, being incorporated as transmembrane proteins, the viral envelope involves a nucleocapsid, which stores the circular DNA genome and DNA polymerase. This virus is a member of the *Hepadnaviridae* family, genus *Orthohepadnavirus*, the establishment of chronic infection is the result of the synergy between the host and the viral factors, occurring when viral shedding is ineffective, defined by persistence of serum HBsAg for at least 6 months, followed by symptoms such as anorexia, nausea, jaundice, abdominal discomfort, where serum biochemical markers are characterized by elevated alanine transaminase (ALT) and total bilirubin activity (CAMPOS-VALDEZ M, et al., 2021).

HBV can affect key factors of oncogenic signaling pathways such as mitogen-activated protein kinase (MAPK), HBV structural proteins drive the regulation of many important pathways in disease progression, some proteins such as Hbx can induce the production of reactive oxygen species and oxidative stress, with a role in replication, disease severity or malignant transformation, CH is a multi-step process that may involve epigenetic and genetic changes, inactivation of p53 tumor suppressor genes, pRb, activation of proto-oncogenes including Ras, c-myc, c-fos, telomere shortening, single nucleotide variants, mutations in TERT and CTNNB1 (SHORAKA S, et al., 2023).

Nucleotide reverse transcriptase inhibitors have been used as a component of combination antiretroviral therapies for patients, and are currently being used to treat chronic HBV infections. Nucleotide analogues (ANs) have been the most widely used option for patients with chronic hepatitis B, entecavir an analogue of deoxyguanosine targets DNA polymerase and reverse transcriptase, is one of the most used antiviral ANs

against HBV, this drug has potent activity and a low rate of acquisition of drug resistance by HBV (TAKAMATSU Y, et al., 2015).

The use of injectable type I Interferons (IFN I) are used in therapy, have been tested as an alternative strategy to stimulate antiviral pathways, when host-associated pattern recognition receptors detect pathogen-associated molecular patterns, IFNs signal autocrine and paracrine, HBV-infected hepatocytes can induce a robust IFN response, resulting in reduced HBV replication or suppression of virus covalently locked DNA activity, the therapeutic use of IFNs alone or in combination with entecavir may lead to viral suppression of HBV positive patients and increased immune response, targeting activity specifically for the liver as potential therapeutic strategies (NOVOTNY LA, et al., 2021).

Tenofovir is an ANs recommended as first-line treatment for HBV, in long-term follow-up, sustained virologic suppression has been associated with histological improvement, regression of cirrhosis, as well as reduced risk of hepatic decompensation in HC, the safety and efficacy of tenofovir have been reported in two phase III clinical trials, virologic suppression rates were 76% and 93% at week 48 in HBV e antigen (HBeAg) positive and HBeAg negative patients, tenofovir is effective in treatment-naïve patients as well as in patients already undergoing treatment (LOVETT GC, et al., 2017).

It is well established that interferon-alpha, pegylated interferon-alpha, ANs (lamivudine, entecavir, and telbivudine), and nucleotide analogues adefovir dipivoxil and tenofovir disoproxil fumarate, are being used in clinical treatment with good recovery rates, in terms of three parameters (i) HBV DNA reduction, (ii) HBeAg seroconversion, and (iii) resistance rate have indicated that entecavir and tenofovir (both as monotherapy) are superior to other treatment regimens as well as monotherapy (CLERCQ ED, et al., 2010).

Thus, this research is justified by investigating how these drugs, specifically interferon, entecavir and tenofovir, are related to viral load suppression and response of affected individuals. In view of the above, the objectives of this research are to define neutralizing molecular mechanisms by the use of interferon, entecavir and tenofovir against the virus; assess how the process of restoring liver function occurs; and to analyze the immune response profile on the pathogenesis of HBV infection.

LITERATURE REVIEW

Entecavir in viral clearance

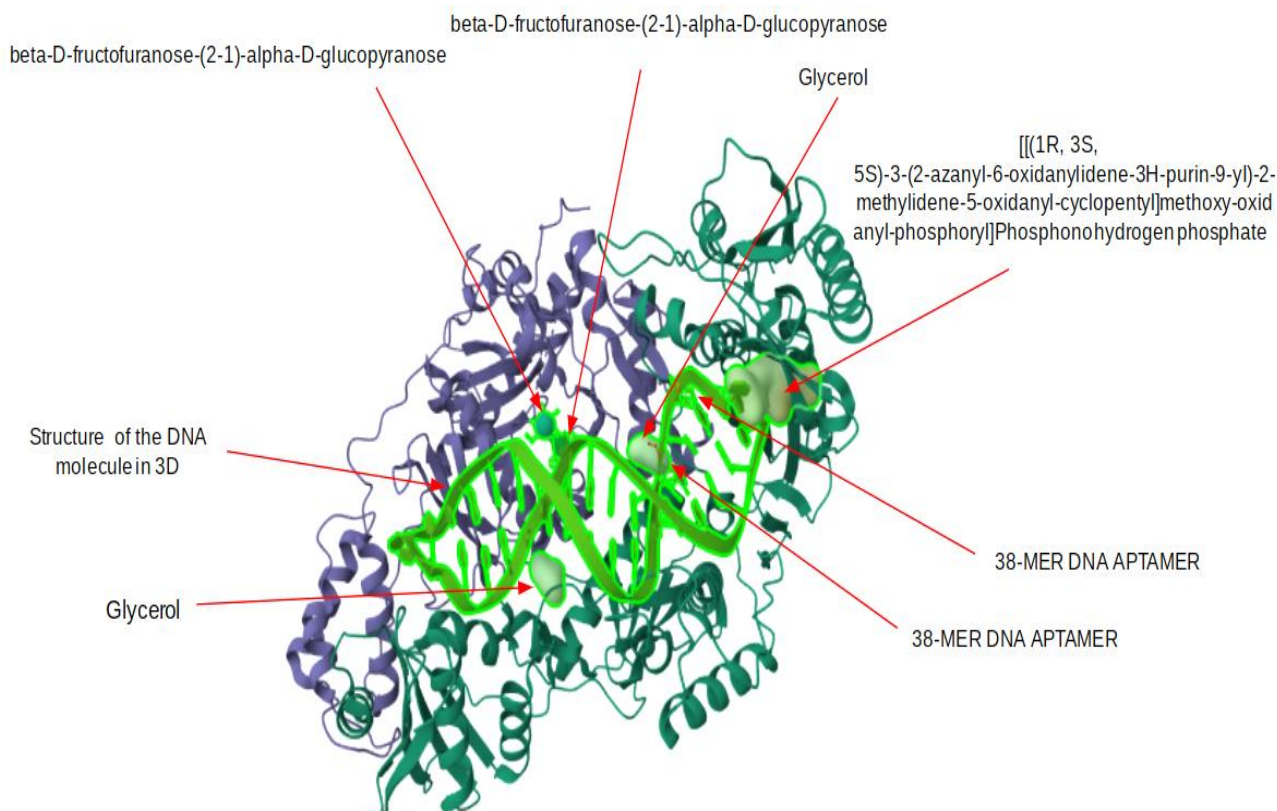
Entecavir is a potent drug that has demonstrated superior virological and biochemical benefits compared to lamivudine, it has been found to be associated with a lower risk of death or transplantation, and the use of ANs such as entecavir is directly associated with restoration of liver function in patients with HBV CH, the complete virologic response rate can be achieved in up to 1 year, in most cases without requiring salvage therapy, ANs are an essential component in patients with HBV related CH, the antiviral should be a preferred choice in patients with CH, given the superior overall survival, decompensation-free survival compared to patients treated with lamivudine (KIM JH, et al., 2016).

Yin G-Q, et al. (2021) argue that rapid viral suppression of HBV within 12 weeks of entecavir treatment reduces the incidence of drug-resistant mutations by prolonging the duration of therapy, in NA-naïve patients with cirrhosis or hepatic decompensation, increasing the dose of entecavir to 1.0 mg/d has been observed to improve response and reduce drug-resistant mutations, compared to approved or recently developed NAs have the strongest ability to inhibit HBV and the broadest safety range, clinical trials for multiple doses of entecavir, 0.1 mg /d up to 20 mg/d, were performed and the long-term safety of 1.0 mg/d entecavir was determined in the treatment of patients with refractoriness to lamivudine or cirrhosis.

During viral DNA replication, endogenous entecavir nucleosides are phosphorylated by various host cell kinases into their active triphosphate form, and are then taken up by the polymerases and incorporated into the growing DNA or RNA strands (**Figure 1**), ANs require activation by the kinases to become active metabolites, to effectively deliver their active triphosphate form within infected cells, kinases responsible for activation can be a virus-encoded specific thymidine kinase or non-virus-encoded cellular kinases, the solute

transporter (SLC) family also play important roles in drug disposition, including organic anion transporter proteins (OATPs), organic anion and cation transporters (OATs and OCTs), peptide transporters (PEPTs), and microbial extrusion (YANG M and XU X, 2022).

Figure 1 - Ternary complex of the entecavir triphosphate antiviral molecule, acting as an inhibitor of HIV-1 and HBV reverse transcriptase, the drug molecule binds to 5'-triphosphate.

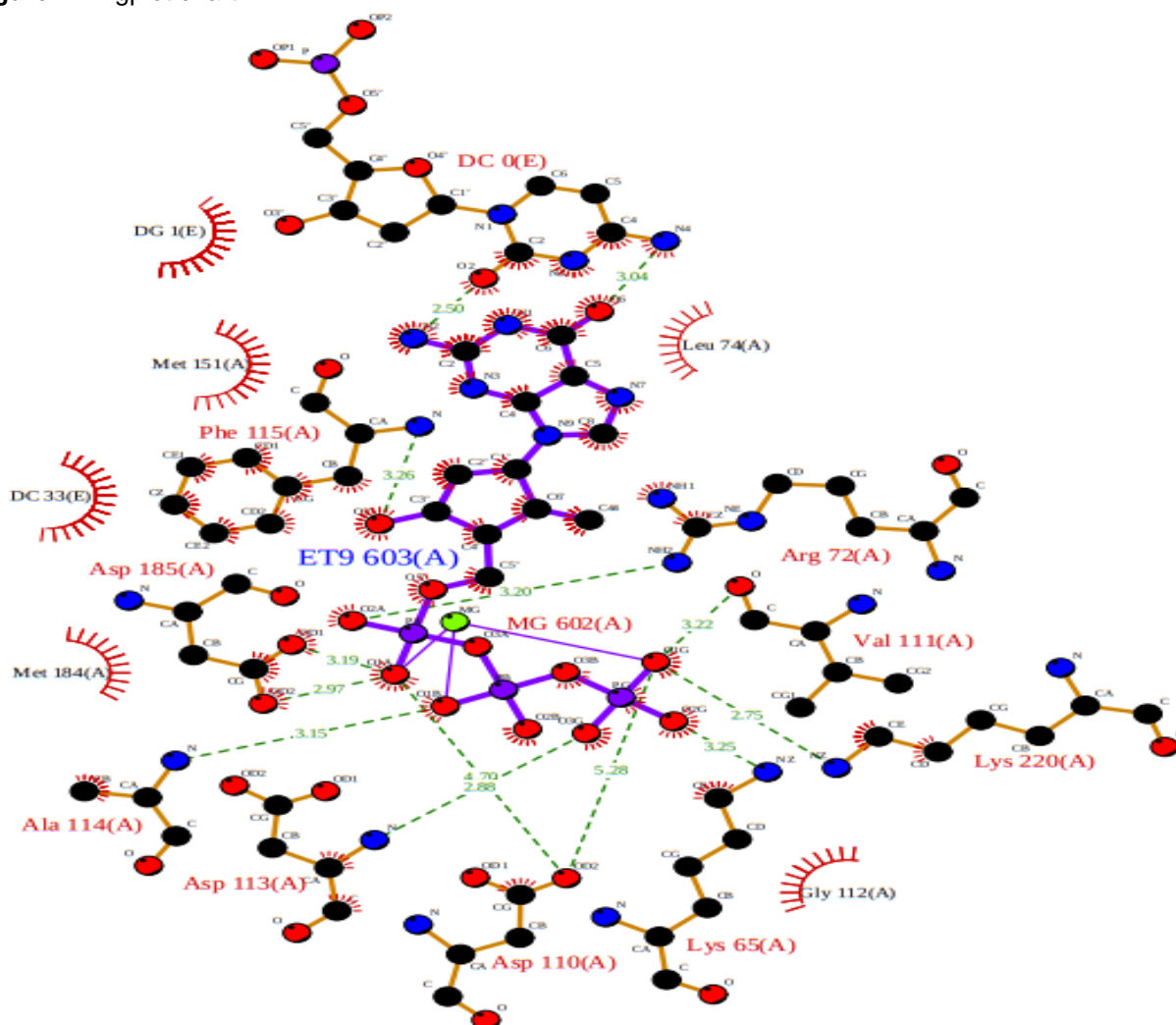


Description: Beta-D-fructofuranose-(2-1)-alpha-D-glucopyranose are metabolites produced through hepatic gluconeogenesis through hepatic gluconeogenesis and the breakdown of polymeric forms of glucose. Glycerol exhibits antiviral activity, acts as an osmolyte and can assume any conformation for protein stabilization [[[1R,3S,5S)-3-(2-azanyl-6-oxidanylidene-3H-purin-9-yl)-2-methylidene-5-oxidanyl-cyclopentyl]methoxy-oxidanyl-phosphoryl] Phosphono hydrogen phosphate is the name of the ET9 ligand that carries out molecular iterations in the 5' position of the DNA line, inhibiting the viral DNA replication process in the cell. The 38-MER DNA APTAMER are domains of short DNA molecules that can bind to a specific site, adjusting to the molecular target with a blocking function.

Source: Padilha MDM, et al., 2024. Image from the RCSB PDB (RCSB.org) of PDB ID 5XN1 (Yasutake Y, et al. HIV-1 reverse transcriptase Q151M:DNA:entecavir-triphosphate ternary complex (2018) Proc. Natl. Acad. Sci. USA 78: 2179-2183). Adapted from rcsb.org/3d-view/5XN1 (Accessed December 2023).

The Pol enzyme has terminal protein (TP), spacer, polymerase, and ribonuclease H (RH) domains, which performs RNA-dependent DNA polymerization via a tyrosine residue derived from the TP domain as a primer, ANs such as entecavir (**Figure 2**) are phosphorylated to nucleotides, acting as chain terminators binding to the deoxynucleotide-triphosphate site inhibiting HBV reverse transcriptase (YASUTAKE Y, et al., 2018).

Figure 2 - Ligplot chart.



Description: Among the three ligands of entecavir, the ET9 603 ligand (A) is the molecule that has the greatest chemical structure interacting with the Mg²⁺ ion in light green in the center, which reacts with the oxygen atoms (O) highlighted in a red circle, the amino acids Methionine (Met) 151 (A), 184 (A), Leucine (Leu) 74 (A), Glycine (Gly) 112, indicate hydrophobicity.

Source: Padilha MDM, et al., 2024. Image from the EMBL-EBI. Adapted from <https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=5xn1&template=ligands.html&l=2.1> (Accessed December 2023).

Interferon therapy

Interferons are a group of immunomodulatory cytokines produced by the immune system in response to the presence of pathogens, these molecules have been used to treat a variety of diseases, including chronic hepatitis B/C, these proteins can activate natural killer cells, macrophages, and regulate the presentation of antigens to T lymphocytes (MERTOWSKA P, et al., 2023). The expression of apolipoprotein B mRNA editing enzyme similar to 3G catalytic polypeptide APOBEC3G in patients is lower compared to uninfected controls, APOBEC3G induces hypermutation from G (guanine) to A (adenine) in HBV DNA inhibiting its replication, IFNs can induce the expression of APOBEC3G with activation of signal transducer and transcription activator 3 (STAT3), inhibiting HBV (WOO ASJ, et al., 2017).

The covalently closed circular HBV DNA persists in the nucleus of the infected cell, interferons eliminate this DNA through APOBEC3 or IFN stimulated genes ISG20 nuclease mediated deamination, in addition to cytokine signaling controlling HBV transcription and epigenetic status, it also limits the stability of transcripts

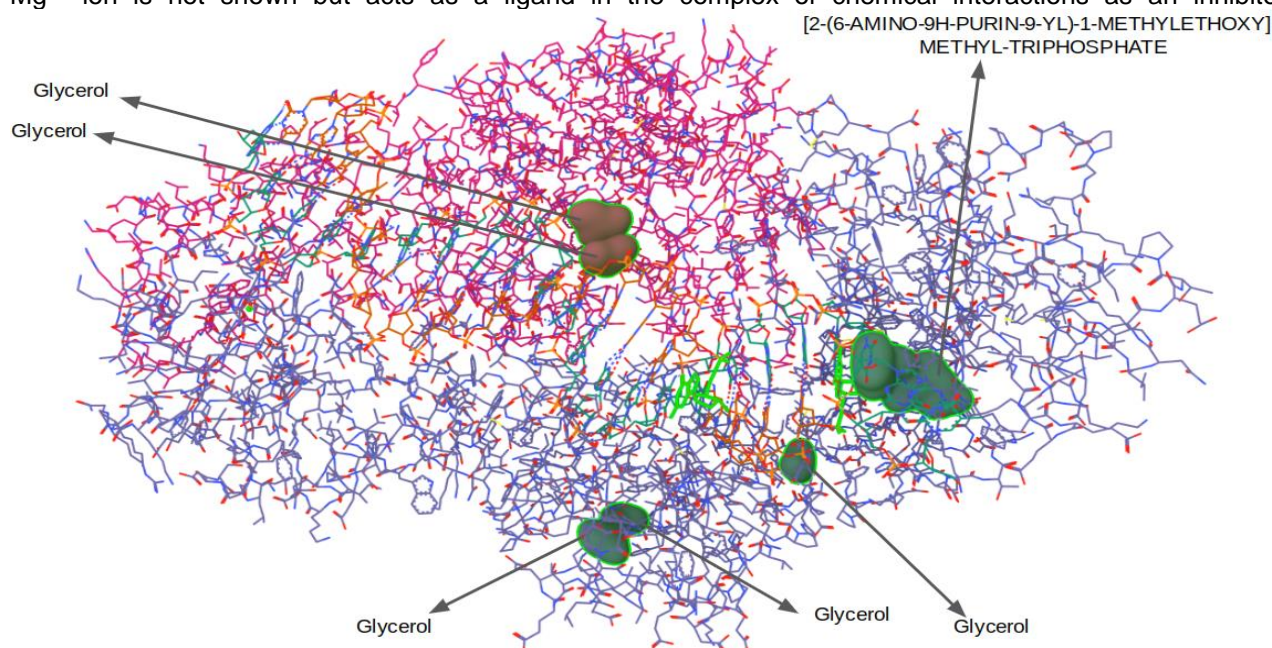
and pregenomic capsids containing HBV RNA (STADLER D, et al., 2021). In the research of Xu F, et al. (2019) they identified that the nucleus-binding factor (CBF β) subunit β increases the steady-state level of viral infectivity factor (Vif) protein, type III-triggered, IL-10-induced CBF β has also been shown to be able to inhibit HBV replication. According to Li Q, et al. (2022) these regulators are defined in three types, being IFN type I (α/β), type II (γ) or type III (λ), where the complexes of these IFNs activate the signal transducer of Janus-activated kinase (JAK), and the STAT, which leads to the expression of IFN stimulated genes (ISGs). This antiviral therapeutic effect is potentiated when combined with other drugs.

2'-5'-oligoadenylate is an IFN-type I-induced enzyme that activates the Ribonuclease L (RNaseL) nuclease that mediates the degradation of viral RNA, another enzyme, the RNA-activated protein kinase, prevents the recycling of guanidine diphosphate, which, in turn, blocks the translation of viral RNA, and PEG-IFN- α 2 has been shown to effectively eliminate the HBeAg and decrease the HC rate with improved survival (LIN F-C and YOUNG HA, 2014). According to Tan G, et al. (2018) the expression of the TRIM gene is induced in response to IFN stimulation and is necessary for the control of viral infection, TRIM25 expression is increased by the induction of type I IFN, in an interleukin 27 (IL-27) dependent manner, inhibiting HBV replication through increased IFN production, and TRIM22 has also been shown to suppress the activity of the HBV central promoter.

Tenofovir in viral suppression and restoration of liver function

Disoproxil fumarate is an acyclic diester phosphonate nucleoside analogue of adenosine monophosphate and is initially converted to tenofovir by hydrolysis of the diester in the intestine, liver, or plasma, is absorbed by cells and phosphorylated by cellular kinases in the active form of the drug, if tenofovir (**Figure 3**) is incorporated into viral DNA instead of deoxyadenosine 5' triphosphate chain termination occurs (MACBRAYNE CE, et al., 2018).

Figure 3 - HIV-1 reverse transcriptase cross-linked to template primer with bound tenofovir-diphosphate as input nucleotide substrate, separate DNA-binding complexes are used as HBV DNA suppression mechanism, Mg²⁺ ion is not shown but acts as a ligand in the complex of chemical interactions as an inhibitor.



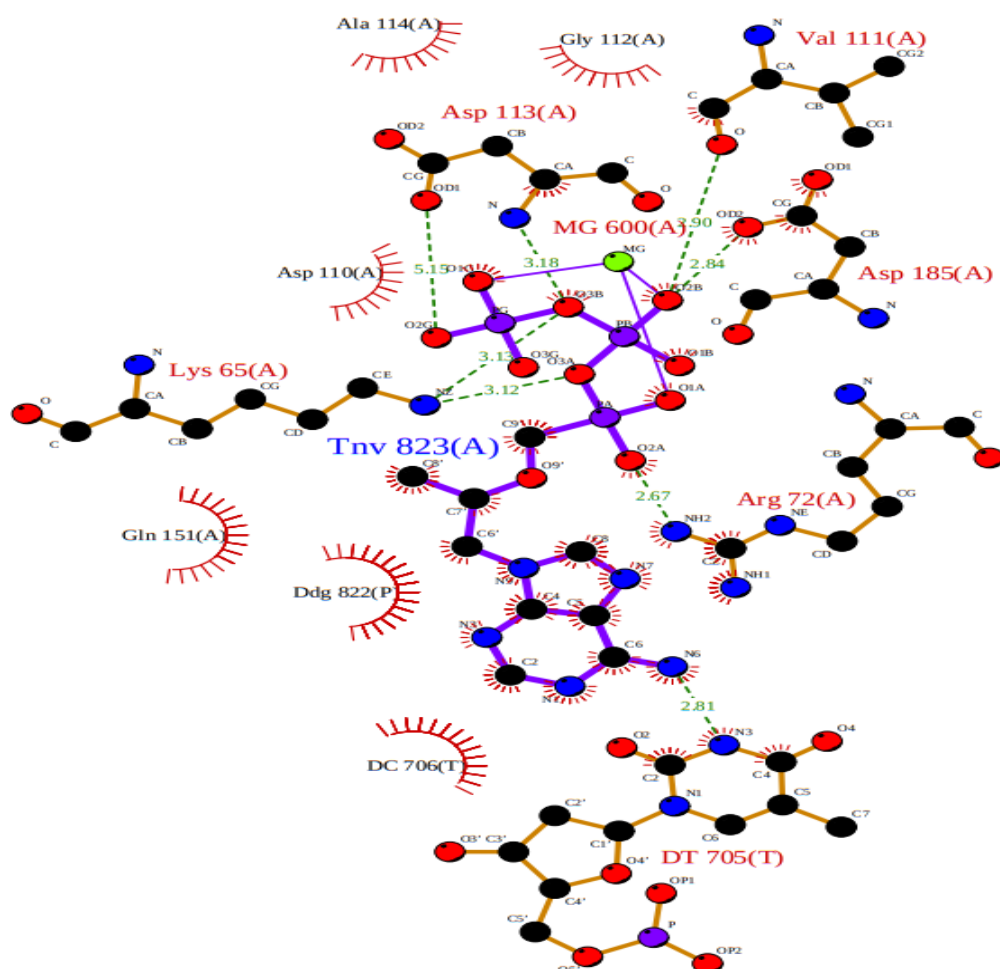
Description: Glycerol acts as a molecule stabilizer in the DNA-tenofovir complex. [2-(6-AMINO-9H-PURIN-9-IL)-1-METHYLETHOXY]METHYL-TRIPHOSPHATE is the name of the tenofovir diphosphate ligand bound as an entry molecule associated with reverse transcriptase as an entry nucleotide substrate.

Source: Padilha MDM, et al., 2024. Image from the RCSB PDB (RCSB.org) of PDB ID 1T05 (Tuske S, et al. HIV-1 reverse transcriptase crosslinked to template-primer with tenofovir-diphosphate bound as the incoming nucleotide substrate (2004) Proc. Natl. Acad. Sci. USA 78: 2179-2183). Adapted from <https://www.rcsb.org/3d-view/1T05> (Accessed December 2023).

Studies have looked at what tenofovir treatment for hepatitis B suggests that it reduces or reverses liver fibrosis, in contrast to other antiviral agents, histological data from a prospective study in HIV/HBV co-infected patients treated with tenofovir for just over 2 years demonstrate a decline in fibrosis scores, a trend not seen with other antivirals, once tenofovir is internalized into cells it is subsequently phosphorylated into the active metabolite, tenofovir diphosphate, as effects it is possible to highlight in murine models that: (i) Tenofovir prevents the development of hepatotoxin-induced liver fibrosis; (ii) protects against bleomycin-induced dermal fibrosis and (iii) blocks the release of cellular ATP mediated by Pannexin-1, leading to a decrease in adenosine levels in the extracellular space, which decreases A2AR-mediated fibrosis (FEIG JL, et al., 2017).

In its original form, tenofovir (**Figure 4**) is a dianion with physiological pH, after its oral administration, tenofovir is hydrolyzed by intestinal and plasma esterases, being metabolized mainly intracellularly by cathepsin A, the active form tenofovir diphosphate is similar for the treatment of HIV and HBV, with an improved pharmacokinetic profile, this improvement is attributed resulting in the increase of active intracellular tenofovir diphosphate, tenofovir diphosphate works by inhibiting HIV replication by competing with the natural substrate deoxyadenosine 5'-triphosphate for incorporation into DNA during HIV transcription. Tenofovir diphosphate inhibits HBV replication by inhibiting HBV polymerase (WASSNER C, et al., 2020).

Figure 4 - Ligplot chart. Among the three ligands of Tenofovir, the ligand Tnv 823 (A) is the molecule that has the greatest chemical structure interacting with the Mg²⁺ ion in light green in the center, which reacts with the oxygen atoms (O) highlighted in a red circle, the amino acids Alanine (Ala) 114 (A), Asparagine (Asp) 110 (A), Glycine (Gly) 112 (A), Glutamine (Gln) 115 (A), indicate hydrophobicity.



Source: Padilha MDM, et al., 2024. Image from the EMBL-EBI. Adapted from <https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl?pdbcode=1t05&template=ligands.html&l=1.1> (Accessed December 2023).

A variety of enzymes and drug transporters are involved in the pharmacokinetics of tenofovir, including carboxylesterase 1, P-glycoprotein, breast cancer resistance protein, organic anions carrying polypeptide 1B1 and 1B3, underscoring that between-patient variations contribute to its pharmacokinetics, efficacy, and safety in individual patients (LI X, et al., 2021). The AK2 enzyme has been implicated in carrying out the first phosphorylation step required for activation in all cells and tissues examined, this enzyme may be a key participant in the regulation of tenofovir response, AK2 is located in the mitochondria, playing a crucial role in cellular homeostasis and being a target in pharmacology through drug phosphorylation (HAMLIN AN, et al., 2019).

Immunological influence on the pathogenesis of infection

Promoter elements that influence the persistence of covalently bonded DNA in HBV cells include binding sites for transcription factors and enriched nuclear receptors in the liver, those that activate viral transcription include HNF1, HNF3/FoxA, HNF4, C/EBP, RXR α /PPAR α , FXR, NF1, SP1, AP-1, and CREB (CRT1) functions as a transcriptional coactivator. IFN α / γ and tumor necrosis factor (TNF α) and activation of the lymphotoxin- β receptor, are capable of inducing the degradation of covalently closed HBV DNA, and causing apoptosis through the activation of nuclear deaminases APOBEC3A and 3B (MOHD-ISMAIL NK, et al., 2019).

Smc5/6 is known for its key role in the mechanisms of DNA repair by homologous recombination, which is impaired by the HBV Hbx protein, several other transduction pathways such as NF- κ B, Wnt/ β -catenin, Janus kinase/signal transducer and activator of transcription (JAK/STAT), phosphoinositide-3-kinase-protein kinase B (PI3K/AKT), Ras/Raf, MAPK, Src-kinase, p53 and others are modulated by cytosolic Hbx, promoting tumorigenesis, migration and survival of CH (SCHOLLMEIER A, et al., 2023). Potential factors contributing to the persistence of HBV in infection include mutational escape leading to inactivation of B and T cell epitopes, with specific inhibition of the adaptive immune response by viral proteins. HBsAg also acts by suppressing the immune clearance of infected cells, since serum HBsAg levels are often observed in chronic patients (CHISARI FV, et al., 2010). In these processes, the adaptive immune response of TCD4 $^{+}$ and TCD8 $^{+}$ cells as well as neutralizing antibodies is significantly more involved than the innate immunity, specific TCD8 $^{+}$ cells are the main effectors of viral shedding in cases of infection and TCD4 $^{+}$ act by inducing and favoring the persistence of TCD8 $^{+}$ cells and antibody responses (NEVOLA R, et al., 2023).

The inhibitory effect of HBV on innate and adaptive immune cells leads to obstacles in the recognition and elimination of the virus, which aggravates HBV-induced chronic inflammation, in CH patients, HBsAg can inhibit the expression and activation of STAT3 in NK cells, impaired NK cells have a defective clearance effect, which accelerates the progression of HC (JIANG Y, et al., 2021). Decreased HBV-specific TCD8 $^{+}$ cell functions in patients with chronic hepatitis B (CHB) may occur, in addition to high levels of expression of inhibitory receptors such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein (PD-1), or T cell immunoglobulin mucin receptor 3 (TIM-3), with higher levels of TIM-3 in patients with active CHB compared to inactive CHB, suggesting that TCD8 $^{+}$ cells are functionally depleted in HBV infection (CHEN Y and TIAN Z, 2019). In patients with long-term HBV infection, the population of virus-specific TCD8 $^{+}$ cells is often insufficient or depleted, this ineffective harmful immune response is due to immunosuppressive mediators such as interleukin (IL-10), these events may reduce the influx of HBV-specific T cells into the liver, in this microenvironment TCD8 $^{+}$ cells are depleted and unable to eradicate infection (WANG S-H, et al., 2021).

FINAL CONSIDERATIONS

IFNs limit HBV DNA replication, these cytokines can act as key regulators of cellular pathways such as APOBEC, STAT, CBF β , ISGs, JAK, which restrict viral activity. These molecules can control HBV signaling and transcription, epigenetic status, as well as limit the stability of viral transcripts. The use of ANs as a drug therapy against HBV HC can reverse liver cirrhosis, the main effect of these drugs is on HBV reverse transcriptase, entecavir has an effect on the phosphorylation of kinases that act as DNA or viral RNA strand terminators. It has been established that tenofovir reduces and can reverse liver fibrosis, kinase phosphorylated becomes the active form of the drug, and when integrated with viral DNA chain termination occurs, and variations in patient responses contribute to the pharmacokinetics of tenofovir.

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