New therapeutic options for persistent low-level viremia in patients with chronic hepatitis B virus infection: Increase of entecavir dosage

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Abstract

Chronic hepatitis B virus (HBV) infection (CHB) is a public health concern worldwide. Current therapies utilizing nucleos(t)ide analogs (NA) have not resulted in a complete cure for CHB. Furthermore, patients on long-term NA treatment often develop low-level viremia (LLV). Persistent LLV, in addition to causing the progression of liver disease or hepatocellular carcinoma, may shed light on the current plight of NA therapy. Here, we review the literature on LLV, NA treatment, and various doses of entecavir to find a strategy for improving the efficacy of this antiviral agent. For LLV patients, three therapeutic options are available, switching to another antiviral monotherapy, interferon-α switching therapy, and continuing monotherapy. In real-world clinical practice, entecavir overdose has been used in antiviral therapy for CHB patients with NA refractory and persistent LLV, which encouraged us to conduct further in-depth literature survey on dosage and duration related entecavir studies. The studies of pharmacodynamics and pharmacokinetics show that entecavir has the maximal selected index for safety, and has great potential in inhibiting HBV replication, in all of the NAs. In the particular section of the drug approval package published by the United States Food and Drug Administration, entecavir doses 2.5-20 mg/d do not increase adverse events, and entecavir doses higher than 1.0 mg/d might improve the antiviral efficacy. The literature survey led us to two suggestions: (1)
Hepatitis B virus (HBV) can lead to a debilitating liver infection; chronic HBV infection (CHB) is a public health concern worldwide, affecting about 240 million people. Current therapies for CHB include nucleos(t)ide analogs (NA) and interferon (INF). However, while these therapies are effective treatments, they cannot cure HBV. Since 2016, four significant articles regarding the treatment of CHB have been published by the following authors, Terrault NA et al, Kim JH et al, Terrault NA et al, and Lok AS et al, respectively1–4. These articles shed light on the current plight of treating CHB and the efforts of clinicians and researchers.

Recently, it was shown that the incidence of persistent low-level viremia (LLV)(HBV DNA < 2 × 10^3 IU/mL) gradually increased in some patients during NA treatment, contributing to the progression of liver disease. The American Association for the Study of Liver Diseases (AASLD) 2016 guidelines suggest that LLV patients on entecavir or tenofovir monotherapy should continue monotherapy, regardless of alanine aminotransferase status5. This suggestion is different from the previous NA therapy strategy and a bit confounding to researchers. Subsequently, Kim et al6 reported that persistent LLV patients on continued entecavir monotherapy were at a higher risk of hepatocellular carcinoma (HCC)6. Their results were contrary to the AASLD recommendation. However, the 2018 AASLD recommendation upheld its 2016 recommendation6.

While the recommendations and reports may differ, these articles do confirm that the current problem for patients is the entecavir refractory HBV. Currently, several researchers are developing novel antiviral therapies for HBV. Unfortunately, these newly developed therapies cannot eradicate the intrahepatic covalently closed circular DNA (cccDNA) or integrated HBV DNA. In a report by Lok AS et al, a complete HBV cure was defined as “undetectable hepatitis B surface antigen in the serum and eradication of HBV DNA in the hepatocytes, including intrahepatic cccDNA and integrated HBV DNA”. Thus, the current therapies relying on NAs and/or INF cannot achieve a complete HBV cure6–8.

The molecular mechanisms of LLV or NA refractoriness have been partially discovered. In an important investigation regarding NA mediated development of...
resistance, Coffin CS and colleagues analyzed the prevalence of HBV DNA, cccDNA, HBV resistant mutations, and gene diversity in different tissues including plasma, peripheral blood mononuclear cells (PBMCs), and liver explants in patients undergoing NA treatment and liver explants. The results showed 100% and 83% wild type HBV in the plasma and PBMCs. Moreover, 66% of drug-resistant mutations were observed in the liver explants. Notably, the adefovir-treated patients who exhibited adefovir resistant mutation received the combination lamivudine plus tenofovir rescue therapy. While this rescue treatment resulted in undetectable HBV DNA and 100% wild type HBV in the plasma, detectable HBV DNA, cccDNA, and HBV resistant mutations correlating with adefovir and lamivudine were retained in the liver explants. Thus, the rescue lamivudine plus tenofovir therapy could not eradicate the previous adefovir drug-resistant virus and instead induced the new lamivudine-resistant mutation, in the liver of these patients[5]. Hence, the presence of this mutation in the liver suggested that the LLV patients possessed intrahepatic viruses that were resistant or less sensitive to NAs.

Molecular evolutionary analysis can reconstruct phylogenetic trees and infer phylogenetic histories[6]. The evolutionary patterns of HBV resistant mutations during NA treatment have been studied by combining molecular phylogenetic analysis with the NA resistant mutation profile[6,10]. These studies indicate that prolonged entecavir or tenofovir treatment leads to development of LLV status, NA resistance, virology breakthrough, and biochemical breakthrough resulting in progression of liver disease or HCC in the majority of patients. Thus, LLV should be considered as the early stage of NA resistance.

Currently, the primary goals of NA therapy are to suppress HBV replication for as long as possible and to avoid the progression of liver disease; and the ultimate goal of antiviral treatment is to prolong survival of CHB patients and to provide them with better quality of life[13,14]. Therefore, prolonged treatment with NAs could result in life-extending goals in most patients. Moreover, carefully balancing between the risks and benefits required for prolonging life and reducing the HCC risk, support the AASLD statement.

Here, we investigate the literature on persistent LLV patients receiving NA combination therapy (nucleoside plus nucleotide analogs). The regimens of IFN-α therapy, namely, switching from NAs to INF-α and subsequent re-treatment with IFN-α, were applied to treat these patients. IFN-α switching therapy resulted in safe NA cessation, and IFN-α re-treatment caused sustained immune controls[13]. However, IFN-α therapy caused stronger adverse effects and was not suitable for cirrhosis patients. Moreover, our previous study and the research by Reijnders et al indicated that increasing the entecavir dose when treating persistent LLV patients resulted in no observed side effects[14,15]. Therefore, the current study scoured the literature to shed light on the possibility of improving the antiviral effect of entecavir by increasing the dose and prolonging the treatment time.

**LITERATURE SEARCH STRATEGY**

All HBV treatment articles available since 1995 on the MEDLINE/PUBMED database and United States Food and Drug Administration (FDA) website were searched. In addition, information on prescriptions of the NA drugs entecavir, lamivudine, adefovir, telbivudine, tenofovir, and tenofovir alafenamide (TAF) was also retrieved. Furthermore, all available literature relating to LLV, partial virological response, virological breakthrough, and NA resistance were collated together to find the optimum therapy strategy for LLV.

The aim was to find the correlation between entecavir doses and efficacy. Here, search terms applied to enable identification of doses and antiviral efficacy were chronic hepatitis B, entecavir, tenofovir, adefovir, telbivudine, lamivudine, and tenofovir alafenamide. The articles retrieved by this strategy included the studies on pharmacokinetics, pharmacodynamics, pre-marketing randomized, placebo-controlled trials, as well as case reports and real-world clinical observation, randomized, contrast studies, and the therapy guidelines for CHB. In addition, to compare the antiviral efficacy of entecavir with newly developed drugs, the literature on the novel CHB therapeutic agents was retrieved.
THERAPY FOR PERSISTENT LLV

Persistent LLV and partial virological response in NA therapy

Persistent LLV and partial virological response are common phenomena and may be found in all types of NA therapy. In studies prior to 2016, LLV was referred to as a partial virological response, when primarily detectable HBV DNA was < 2 × 10^6 IU/mL following 48 wk of treatment. However, LLV in entecavir and tenofovir therapy was defined when detectable HBV DNA was < 2 × 10^6 IU/mL during therapy. The AASLD guidelines defined LLV in entecavir and tenofovir therapy as < 2 × 10^6 IU/mL detectable HBV DNA, with or without viral rebound during therapy. These definitions were examined in the paper by Zoutendijk et al, who, in a cohort of 333 HBV patients (293 naïve and 90 experienced) treated with entecavir at standard doses, showed that by weeks 48, 96, and 144 of entecavir treatment (standard doses without changes in therapy), NA-naïve patients reached 48%, 76%, 90% and 89%, 98%, 99% of hepatitis B e antigen (HBeAg)-positive and HBeAg-negative virological response, respectively.

LLV patients have two types, those with partial virological response and those with viral breakthrough. The incentives of LLV are caused by the following: (1) Patients with high basal viremia may show a slow decline of plasma HBV-DNA under antiviral treatment; (2) Patients exposed previously to lamivudine ± adefovir may harbor resistance mutations and are more prone to develop resistance to entecavir or tenofovir; and (3) Suboptimal adherence to treatment. Resistance testing in LLV patients might not be technically possible because of low viral levels. Therefore, the LLV patients evaluated included patients treated with NAs that were resistant to NA therapy, and a few patients with detectable resistance mutations.

Incidence of LLV

The incidence of LLV with entecavir and tenofovir treatment is similar. Previous studies showed that the incidences of partial virological response during entecavir monotherapy were 45% at week 48 in the Liu et al’s study, 17% at year 2 in Chang et al’s paper and 23.6%, 11.3%, and 22% at 96 wk in studies by Lok AS et al, Zoutendijk R et al, and Choi HN et al, respectively. Kim et al presented an article in Seoul, Korea, in which the total proportion of LLV was 43% (377/875). Kim’s data is similar to Liu’s results in Shanghai, China, but different from other studies. In addition, the incidence of partial virological response to tenofovir monotherapy ranged from 18.1% to 45.3%, similar to that of entecavir.

Current therapeutic options for LLV

For patients with partial virological response to NAs, or LLV, three therapeutic options are available: (1) Traditional treatment, which is switching to another antiviral monotherapy with a high genetic barrier, or the addition of a second antiviral with a complementary resistance profile. The disadvantage of this traditional treatment is that it results in the failure of combined therapy with nucleoside plus nucleotide, which shortens the total course of the NA treatment; (2) The AASLD guidelines recommend continued monotherapy for LLV patients who received entecavir or tenofovir monotherapy. Continuation of monotherapy prolongs the period of NA therapy. However, prolonged treatment with entecavir or tenofovir increases the LLV incidence, resulting in HBV DNA > 2 × 10^6 IU/mL and the emergence of detectable drug-resistant mutations, which ultimately leads to the progression of liver disease; and (3) The use of the IFN-α therapy as used by Yin GQ and Zhong B to treat the failure of the combined nucleoside plus nucleotide therapy and subsequent virological breakthrough in LLV patients (HBV DNA < 2 × 10^6 IU/mL). The IFN-α therapy freed the patients from the predicament of multidrug resistance. However, IFN-α therapy in LLV or NA-refractory patients is problematic as it causes significant adverse events. Moreover, most patients have to undergo IFN-α re-treatment twice or more to achieve immune control. Twelve years ago, we started using IFN-α on patients with a focus of alleviating LLV in NA-refractory patients. However, we found that some patients were not suitable candidates for IFN-α therapy. Thus, the focus was shifted to treating LLV by increasing the entecavir dose.
ATTEMPTS TO INCREASE THE DOSE OF ENTECAVIR IN CLINICAL THERAPY

Entecavir overdose has been used in antiviral therapy for CHB patients with NA refractoriness and persistent LLV. In real-world clinical practice, Reijnders et al. increased the entecavir dose in excess of the Baraclude prescribing information recommended dose to treat the LLV patients. Reijnders et al. increased the entecavir dose to 2.0 mg/d to treat patients with NA refractoriness and LLV, which resulted in a decline of HBV DNA and was tolerated well. In our clinical work, we used the entecavir at a dose higher than that recommended by Baraclude to treat HBV patients with NA complications such as multidrug-resistant mutations and renal impairment, resulting in a decrease of viral load and stable creatinine clearance. The 2015 WHO CHB guidelines stated that ‘in unstable persons with deteriorating renal function, entecavir can be used at a recommended dosage of 1 mg daily and the patient should be monitored for lactic acidosis’. The doses of entecavir utilized in the Reijnders et al. clinical study and our clinical report, as well as the dose recommended by the 2015 WHO CHB guidelines, were higher than the Baraclude recommended dosage. This information of entecavir overdose has been noted by Bristol-Myers Squibb Company and the FDA. Baraclude prescribing information (2015) states that “there is limited experience of entecavir overdosage reported in patients. Healthy subjects who received single entecavir doses up to 40 mg or multiple doses up to 20 mg/d for up to 14 d did not experience unexpected or adverse events. If an overdose occurs, the patient must be monitored for evidence of toxicity, and standard supportive treatment applied as necessary”. The results of the clinical investigation encouraged us to conduct further in-depth literature survey on studies of the pharmacodynamics, pharmacokinetics, and treatment dosage and duration of entecavir.

PHARMACOKINETICS AND PHARMACODYNAMICS OF ENTECAVIR

Generally, NA incorporation into the mitochondrial DNA (mtDNA) via mitochondrial polymerase γ results in host mitochondrial dysfunction and morphological changes. However, entecavir triphosphate is a poor substrate for mitochondrial polymerase γ and does not significantly inhibit the mitochondrial respiratory function or alter the mtDNA synthesis. In the cell line HepG2 and the HBV-transfected cell lines 2.2.15, entecavir showed efficacy at a 50% effective concentration (EC_{50}) of 3.8 ± 1.4 nmol/L (1.1 ± 0.41 ng/mL), and 50% cytotoxic concentration (CC_{50}) of 30 μmol/L. The CC_{50}/EC_{50} ratio of entecavir was 8000, which was the maximal selected index for safety in all of the NAs. The poor binding of entecavir to mitochondrial polymerase γ leads to the selected index.

An in vitro investigation by Liu et al. showed that the NA concentration varied between the cells and the supernatant samples; thus, the variability in entecavir and lamivudine concentrations encompasses a wide linear range of measurements. Therefore, the plasma entecavir concentration was generally thought to predict the antiviral efficacy in the pharmacokinetic studies. The pharmacokinetic parameters of the entecavir dose were reported by Yan et al. For a 0.1 mg dose, the mean steady-state (day 14) peak plasma concentration (C_{max}) was 0.60 ng/mL, and the area under the concentration-time curve (AUC) was 2.51 ng/h/mL. For doses of 0.5 mg and 1.0 mg, the corresponding C_{max} and AUC values were 4.23 ng/mL and 14.78 ng/h/mL, and 8.24 ng/mL and 26.38 ng/h/mL, respectively. In addition, steady-state (drug-input/clearance) entecavir plasma concentrations were approximately 0.2 ng/mL and 0.4 ng/mL for the 0.5 mg dose and 1.0 mg dose, respectively. As the entecavir dose increased from 0.1 mg to 1.0 mg, the AUC increased, suggesting that an increase in entecavir dose was associated with an increase in efficacy.

Yurdadayin et al. compared the EC_{50} of entecavir with that of lamivudine, adefovir, tbelivudine, and tenofovir. They found that the EC_{50} of entecavir was lower than that of other NAs (Table 1). TAF is a pro-drug of tenofovir and has been used to treat CHB. The EC_{50} of TAF was lower than that of tenofovir but higher than that of entecavir. Tenofovir conjugating a lipid moiety (CMX-157), a lipid conjugated tenofovir moiety, is in development for treating CHB. The EC_{50} of CMX-157 is 1.6 μmol/L, which is nearly a 5-fold increase in potency over tenofovir, but 1/420 of the efficacy for entecavir. Thus, entecavir is the most effective treatment among all of the current and developing therapies (Table 1).

The pharmacokinetics and pharmacodynamics data indicate that entecavir is the
Table 1 Relative potencies of various nucleos(t)ide analogs for inhibiting hepatitis B virus

| Drug       | EC_{50} (nM), mean ± SD | Difference (fold) from entecavir EC_{50} | Ref.                     |
|------------|--------------------------|------------------------------------------|--------------------------|
| Entecavir  | 5.3 ± 2.5                | 1                                        | Yurdaydin et al\(^{[31]}\), 2008 |
| TAF        | 86.6                     | 16                                       | Gibson et al\(^{[32]}\), 2016 |
| Lamivudine | 1491 ± 1033              | 281                                      | Yurdaydin et al\(^{[31]}\), 2008 |
| CMX-157    | 1600                     | 420                                      | Pei et al\(^{[33]}\), 2017   |
| Tenofovir  | 2482 ± 1938              | 468                                      | Yurdaydin et al\(^{[31]}\), 2008 |
| Adefovir   | 2636 ± 1549              | 497                                      | Yurdaydin et al\(^{[31]}\), 2008 |
| Telbivudine| 8950 ± 4803              | 1689                                     | Yurdaydin et al\(^{[31]}\), 2008 |

EC_{50}: Effective concentration of nucleos(t)ide analogs that results in 50% inhibition of HBV DNA production; TAF: Tenofovir alafenamide; CMX-157: Tenofovir conjugated with a lipid moiety.

The safest treatment and has great potential in inhibiting HBV replication.

EFFICACY AND SAFETY OF ENTECAVIR AT VARIOUS DOSES (0.1-20 MG/D) IN CLINICAL STUDIES FROM BERGMAN’S REVIEW

Bergman KL and Zheng JH presented a review entitled “Clinical pharmacology and biopharmaceutics review 021797/S-000”, which was published in the United States FDA website in 2005\(^{[34]}\). In this particular section of the drug approval package published by the FDA, the authors submitted 37 placebo-controlled phases 1, 2, or 3 clinical trials of entecavir, with the registration numbers A1436001-A1436066 (Table 2).

Some of main findings in Bergman’s review are:

**FDA has approved using entecavir at doses of 0.5 mg/d and 1.0 mg/d to treat nucleoside-naïve patients and lamivudine refractory patients**

Prior to determining the pivotal entecavir dose required for treating CHB, researchers first investigated the characteristics of the exposure-response relationship for safety. The incidence of headache and nausea after taking the medicine (which were the most common treatment emergent adverse events) was defined as the index of safety in the clinical studies A1463004 and A1463005. According to these safety indicators, the incidence of adverse events was greater as the dose of entecavir increased, ranging from 0.5 mg to 40 mg (Table 2a). Considering the greater antiviral activity vs the 0.1 mg dose, superiority over lamivudine for antiviral activity response, reduction of HBV DNA to < 0.7MEq/mL after 22 wk, and an acceptable safety profile, entecavir 0.5 mg/d was selected for treating nucleoside-naïve patients. According to the observation that 1.0 mg/d entecavir exhibited significantly greater antiviral activity than the 0.5 mg/d, with the reduction of HBV DNA to < 400 copies/mL after 24 wk, and an acceptable safety profile, entecavir 1.0 mg/d was determined to treat lamivudine refractory patients. Researchers, however, chose the lower limit of the effective dose to treat CHB (Table 2b and c).

**Increase in entecavir dose from 0.1 mg/d to 20 mg/d entails increased efficacy (higher AUC value)**

Researchers investigated the pharmacokinetic parameters for multiple doses of entecavir (0.1, 0.5, 1.0, 2.5, 5, 10, and 20 mg/d) in healthy volunteers, for up to 14 d; or single doses up to 40 mg/d in the clinical studies. The trough concentrations were used for analysis. Thus, during a 14-d course of treatment with multiple dosing, a steady-state was attained approximately 9 to 10 d following once-daily dosing (Table 2d). The doses of 0.1, 0.5, 1.0, 2.5, 5.0, 10, and 20 mg per day, and their AUC values are depicted in Table 3. The AUC for the 2.5-5.0 mg dose was significantly higher than the 1.0 mg dose. In addition, the AUC for 10-20 mg/d was significantly higher than that of the 2.5-5.0 mg/d (Table 2d).
Table 2 Summary of principal findings from Bergman’s review on the Food and Drug Administration website

| Research project in Bergman’s review | Content of research project | Registration number of clinic studies | Page in Bergman’s review |
|--------------------------------------|----------------------------|--------------------------------------|--------------------------|
| (a) Treatment of emergent adverse events | Treatment of emergent adverse events in the clinical studies in which entecavir doses from 0.5 to 40 mg/d were used to select the pivotal doses of entecavir | A1463004, A1463005 | Pages 17 and 18 |
| (b) Entecavir 0.5 and 1.0 mg/d for current treatment | The dose and dose regimen of 0.5 mg/d for NA naïve patients and 1 mg/d for lamivudine refractory patients were determined to treat CHB | A1463002, A1463005, A1463014, A1463022, A1463027 | Pages 23 and 24 |
| (c) Exposure-response: HBV DNA changes for 0.1 up to 1.0 mg/d | HBV DNA changes for 0.1, 0.5, and 1.0 mg/d were reported | A1463004, A1463005, A1463014, A1463017 | Pages 11-17 |
| (d) Pharmacokinetics of multiple doses | Entecavir multiple dose pharmacokinetic parameters from 0.5 to 20 mg/d were presented | A1463002, A1463003 | Page 25 |
| (e) Overall incidence of adverse events for doses 0.5 and 1.0 mg/d | No apparent dose-response relationship in the overall incidence of adverse events for doses of 0.5 and 1.0 mg/d was found | A 1463004, A1463005, A1403014, | Pages 18 and 19 |
| (f) Doses and adverse events for multiple dose therapy, 0.5 mg up to 20 mg/d | The dose and adverse events with entecavir multiple doses, 0.5 mg up to 20 mg daily, were reported in nine clinical trials | A 1463001, A1463002, A1463003, A1463004, A1463005, A1463010, A1463033, A1463034, A1463041 | Pages 17, 21, and 23 |
| (g) Cardiovascular safety | The studies for cardiovascular safety included in vitro investigations and six clinical studies, in which the safety of entecavir was assessed at doses of 0.5 mg/d to 20 mg/d and a single dose of 40 mg | A1463001, A1463002, A1463003, A1463004, A1463010, | Pages 21 and 23 |

NA: Nucleos(t)ide analogs; CHB: Chronic hepatitis B virus infection; HBV: Hepatitis B virus.

Table 3 Pharmacokinetic parameters of entecavir multiple doses in Bergman’s review

| Dose (mg/d) | AUC (ng/h/mL) | C_{max} (ng/mL) | T_{max} (h) | T_{1/2} (h) |
|-------------|---------------|-----------------|-------------|-------------|
| 20          | 545.6 ± 57.9  | 179.8 ± 34.8    | 1.0         | 142.5 ± 55.5|
| 10          | 304.3 ± 35.6  | 99.9 ± 13.7     | 0.75        | 127.5 ± 41.8|
| 5.0         | 145.8 ± 28.4  | 46.2 ± 6.4      | 0.88        | 91.3 ± 57.9 |
| 2.5         | 71.6 ± 10.3   | 22.8 ± 5.7      | 0.75        | 115.7 ± 37.2|
| 1.0         | 26.38 ± 12    | 8.24 ± 16       | 0.75        | 148.89 ± 39.5|
| 0.5         | 14.78 ± 17    | 4.23 ± 9        | 1.0         | 129.9 ± 17.28|
| 0.1         | 2.5 ± 21      | 0.6 ± 29        | 1.0         | 127.69 ± 91.44|

Data are presented as the mean ± SD. Source: A1463002 and A1463033 clinical studies; AUC: Area under the concentration-time curve.

Dosage related adverse events in entecavir multiple-dose studies (0.5 mg up to 20 mg)

Researchers have assessed the integrated safety data of the entecavir treatment. In the clinical studies A1463001, A1463002, A1463003, A 1463004, A1463005, A1463010, A1463033, A1463034, and A1463041, no relationship between the doses administered and the severity of the adverse events was observed (Table 2e and f), which was different from the data regarding the treatment of emergent adverse events in the clinical studies A 1463004 and A1463005 (Table 2a); and no relationship between the predicted entecavir exposure (C_{max}, AUC, or C_min) and the severity of adverse events was found for the central nervous system, gastrointestinal, or the digestive system. The overall incidence of adverse events with an oral entecavir dose of 0.5 mg or 1.0 mg was comparable to that of the placebo, for example, 40% in the entecavir treatment vs 38% in the placebo group. In addition, no apparent dose-response relationship was found for the overall incidence of adverse events observed in this double-blind, randomized clinical study (Table 2e). In addition to the 0.5 mg or 1.0 mg dose data, entecavir retained a wide margin of safety at a much higher dose (2.5-20 mg/d;
Table 2). Surprisingly, in the clinical studies A1463004 and A1463005, the subjects participated in both the trials (1) most common treatment emergency adverse event study; and (2) the overall incidence of adverse events research. This indicated that the emergency adverse event only occurred at the initial stage of entecavir treatment, and it disappeared with continued entecavir treatment.

Further studies focused on cardiovascular adverse events during entecavir treatment. First, researchers conducted in vitro studies to assess the cardiovascular safety of entecavir. The researchers evaluated the possibility of entecavir to interfere with cardiac calcium and potassium currents or the electrophysiological parameters. Entecavir (30 μmol/L, > 10000 x the steady-state human plasma concentrations) did not have any significant biological effect on the L-type calcium currents or the Purkinje fiber action potential parameters [including the resting membrane potential, overshoot, maximal upstroke velocity, or 50%-90% repolarization (APD₀ and APD₉₀)].

Furthermore, researchers carried out clinical trials using entecavir doses to evaluate untoward cardiac effects (Table 2). Electrocardiogram (ECG) parameters were evaluated over a range of entecavir doses (0.1-40 mg). The retrospective ECG analysis parameters were used to evaluate the effects of entecavir. The results were as follows: (1) No dose-or concentration-dependent relationships between the QT interval or changes in the QTc were observed for entecavir doses of 0.5-20 mg for up to 14 d or as a single dose of 0.5-40 mg; and (2) For each additional 10 ng/mL of plasma concentration, an estimated increase in the change in QTcB ranged from 1.22 to 0.22 msec. The estimated slope of the linear regression at day 1 was slightly greater than zero, whereas the estimated slope on days 7 and 14 was negative (Table 2g). The in vitro data and the ECG data from six clinical studies suggested that QT or QTc interval prolongation potential of entecavir was minimal. Thus, entecavir therapy was considered safe for the cardiovascular system.

In 2005, 0.5 mg/d entecavir for nucleoside-naïve patients and 1.0 mg/d for lamivudine-refractory patients were approved by the FDA for treating CHB, and these doses have been used to this day.

**Insights from Bergman’s review**

Double-blind, randomized clinical studies showed that entecavir doses 2.5-20 mg/d for 14 d did not increase adverse events. In addition, three main findings were presented: (1) The researchers had completed phases 1 and 2 clinical studies for entecavir over a range of doses (0.1 up to 20 mg/d); (2) The correlation between AUC values in the plasma and the entecavir doses of 0.1, 0.5, 1.0, 2.5, 5, 10, and 20 mg/d implied that using doses higher than 1.0 mg/d might improve the antiviral efficacy; and (3) The maximum dose of entecavir can exceed 20 mg/d.

Thus, Bergman’s review indicated that increasing the dose of entecavir to 2.5-20 mg/d may improve its antiviral efficacy, and its use was safe over a broad range of doses (0.5 up to 20 mg/d).

**INCREASING DOSE OF ENTECAVIR IN LLV PATIENTS**

In summary, data from clinical, pharmacokinetics, and pharmacodynamics studies and the Bergman’s review showed that entecavir doses at over 1.0 mg/d improved efficacy and were safe. We hypothesized that patients with prior NA treatment, partial virological response, or LLV should continue monotherapy, at higher doses of 2.5-5.0 mg/d.

The goal of increasing the dose was to maximally inhibit HBV replication during entecavir treatment for 15 years or longer. Compared to the approved NAs and NAs being newly developed, entecavir has the strongest ability to inhibit HBV and the broadest safety range. The clinical trials for multiple doses of entecavir, 0.1 mg/d up to 20 mg/d (Tables 2 and 3), have been conducted. The long-term safety of 1.0 mg/d entecavir has been determined in the clinical treatment of patients with lamivudine refractoriness or cirrhosis. However, no controlled clinical trials have been conducted to assess the long-term toxicity of ingesting entecavir at doses above 1.0 mg/d. Thus, we recommend that clinical trials assessing the long-term toxic effects of entecavir at doses of 2.5 and 5.0 mg/d should be conducted to determine if these doses could improve the efficacy of entecavir. Moreover, compared to the development of novel NAs and the HBV inhibitors utilizing an available treatment is cost-effective and potentially a more viable option. However, these views should be evaluated by researchers.

Liu and colleagues demonstrated that rapid suppression of HBV within 12 wk of
Entecavir treatment reduced the incidence of drug-resistant mutations and prolonged the duration of entecavir therapy (seeing the Figure 1 in Liu’s paper)\(^{2,3}\). In addition, NA naïve patients with cirrhosis or hepatic decompensation were treated with entecavir at a dose of 1.0 mg/d to improve the entecavir response and to reduce drug-resistant mutations\(^{2,3}\). Inspired by the above research, we suggest the entecavir dose should be increased to 1.0 mg/d for the treatment of NAs naïve patients with HBV DNA > 2 × 10^6 IU/mL.

NAs have been used to treat CHB for the last 20 years. Approximately 150 NA derivatives have been examined, including 17 NAs (such as lamivudine, telbivudine, entecavir, adefovir, tenofovir, TAF, and GS-7340)\(^{33}\). Entecavir, tenofovir, and TAF are the most commonly used drugs and are recommended as the first-line therapy based on their highest potency and lowest frequency of developing resistance\(^{33,34,35}\). Because NAs cannot eradicate cccDNA, half of the patients with entecavir or tenofovir monotherapy will eventually present with LLV. Therefore, increased doses of entecavir could be a viable option for treating the patients with prior NA treatment, partial virological response, or LLV, and could be used in over 50% of the population requiring entecavir treatment. We deduce that increasing the dose of entecavir is appropriate for patients on entecavir monotherapy. However, increasing the tenofovir or TAF doses is not a suitable option as their doses could not be safely increased\(^{32,36}\).

**CONCLUSION**

Persistent LLV sheds light on the current plight of NA therapy. The majority of patients on long-term entecavir or tenofovir treatment eventually reach LLV status resulting in progression of liver disease or HCC. For patients with partial virological response to NA, or LLV, three therapeutic options are available, namely, switching to another antiviral monotherapy, IFN-α switching therapy, and continuing monotherapy.

Literature survey indicates that entecavir possesses a strong capacity for inhibiting HBV and a broad dose-range safety. Moreover, entecavir overdose has been used in antiviral therapy in limited patients; thus, an increased dose could be utilized to treat LLV or NA refractory patients. The results of placebo-controlled phase 1, 2, or 3 clinical trials indicate that increasing the dose of entecavir to 2.5-20 mg/d might improve the antiviral efficacy, and higher entecavir doses (up to 20 mg/d) were considered safe. To decrease the incidence of LLV, we suggest increasing entecavir dose to 1.0 mg/d for the treatment of NA naïve patients with HBV DNA > 2 × 10^6 IU/mL. In addition, clinical trials should be conducted to assess the long-term toxic effects of entecavir at doses of 2.5-5.0 mg/d in patients with prior NA treatment, partial virologic response, or LLV state.

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