Title: Pharmacokinetic evaluation of besifovir for the treatment of HBV infection

Structured abstract

Introduction: Besifovir (LB80380) is a relatively new oral acyclic nucleotide phosphonate. We reviewed the pharmacokinetic characteristics of LB80380 and discussed its role in the treatment of chronic hepatitis B infection.

Areas covered: LB80380 is a prodrug of LB80331 and LB8031. It is rapidly absorbed when taken orally. Escalating doses of besifovir produce linear increase of the plasma concentration. Doses above 60mg are effective for inhibiting HBV in human. Using 60mg as an example, the maximal concentration of LB80331 in plasma is 397 ng/mL. The time required to reach maximal concentration in plasma and elimination half-life are 2.0 and 3.0 hours, respectively. Besifovir and its metabolites are mainly excreted via the kidneys. Its antiviral efficacy is non-inferior to ETV 0.5mg daily. It is generally safe in terms of renal and bone toxicity. The most common adverse event is carnitine depletion which affects almost all patients on besifovir requiring carnitine supplementation.

Expert opinion: Besifovir demonstrated predictable pharmacokinetic characteristics in human subjects. Few clinical studies on besifovir have been conducted. More data are expected particularly for special populations. The adverse events upon long term exposure should be monitored. Large scale head-to-head trials comparing besifovir with existing NA, especially tenofovir alafenamide, should be conducted.

Keywords: adverse effects, besifovir, carnitine, efficacy, hepatitis B virus, humans, nucleotide analogue, pharmacokinetics, renal toxicity

1. Introduction

Despite aggressive medical therapy and newborn vaccination, the burden of chronic hepatitis B virus (HBV) infection is enormous, affecting as many as 257 million people worldwide. In year 2015, chronic HBV infection (CHB) caused 887,000 deaths from HBV-related complications, including cirrhosis and hepatocellular carcinoma (HCC) [1]. Currently available therapies include two classes of drugs, namely nucleos(t)ide analogues (NA) or pegylated interferon (PEG-IFN). The European Association for the Study of the Liver (EASL) recommends 3 NAs as first-line treatments for patients with CHB: entecavir (ETV), tenofovir disoproxil fumarate (TDF) or tenofovir alafenamide (TAF), while PEG-IFN can be considered in patients with mild to moderate CHB [2]. The 3 NAs demonstrate similar potency in term of antiviral efficacy, although only short-term 96-week results are available for TAF up to date. Long-term ETV or TDF have been shown to halt progression of liver disease, improve histological necroinflammation, cause regression of fibrosis and reduce risk of HCC [3,4]. However, existing therapies are not yet ideal. Liver decompensation or HCC can still develop in some patients despite achieving undetectable HBV DNA [5]. Also, for patients taking ETV, there is the potential of developing drug resistance especially in lamivudine-exposed patients. Moreover, TDF is not without side effects such as bone loss and renal toxicities [6,7]. Therefore, new therapeutic agents are being continually searched for, including newer NA or drugs that act on alternative steps of viral replication. This review will focus on one of the newer nucleotide analogue, besifovir.

2. Overview of the market

Existing treatment for CHB include PEG-IFN or NA. The advantage of PEG-IFN is a finite duration of use (48 weeks). However, it is relatively contraindicated in patients with cirrhosis. Moreover, it is poorly tolerated and requires weekly subcutaneous injection. NAs are more potent in terms of suppressing serum HBV DNA and normalizing serum alanine aminotransferase (ALT), and with much better tolerability profile. However, NAs need to be used on a long term basis. Also, ETV is associated with 1.2% cumulative incidence of HBV resistance up to 7 years [8], and prior exposure to lamivudine significantly increases the ETV resistance rate to 8%, while the rate is even higher (51%) with documented lamivudine resistance [9,10]. Safety concerns over bone loss and renal impairment are valid for long term TDF users. Rare cases of Fanconi syndrome associated with TDF have been reported in CHB subjects. Studies using sensitive markers of glomerular and tubular kidney function and of bone mineral density have reported decline in glomerular filtration rate, chronic tubular damage and reduction in bone mineral density in TDF-treated patients [6,7]. Moreover, the rate of HBsAg loss have been disappointing and long-term complications can still develop in many patients on NA with apparent good viral control.

In view of these unmet needs, a few competitor compounds are in active development. TAF is recently approved by the United States Food and Drug Administration (FDA) in 2016. It showed similar antiviral efficacy and superior renal and bone safety profiles compared to TDF. CMX157 (tenofovir exalidex, TXL^{TM}) is another NA with high *in vitro* antiviral potency and is currently in phase II development. Therapeutic agents targetting alternative viral replicative steps include viral entry inhibitor, RNA interfering molecules, capsid assembly inhibitor, HBsAg

release inhibitor, cyclophilin inhibitor, immunomodulators and therapeutic vaccines, and these agents are in various phases of preclinical and clinical development. Detailed discussion of these agents is beyond the scope of this paper. The following sections will focus on discussion of besifovir.

3. Introduction to the compound

3.1 Chemistry

Besifovir (LB80380) is an acyclic nucleotide phosphonate. It is a prodrug of LB80317, which is the active metabolite confering antiviral effect as a nucleotide analogue of guanosine triphosphate. Its chemical structure is 3-[({1-[2-amino-9H-purin-9-yl)methyl]cyclopropyl}oxy)methyl]-8,8-dimethyl3,7-dioxo-2,4,6-trioxa-3λ5-phosphanon-1-yl pivalates. It is formulated into tablets (60mg or 90mg) to be taken orally. (Table 1)

3.2 Pharmacodynamics

Besifovir is a DNA polymerase inhibitor. After phosphorylation to di- and tri-phosphate forms, LB80317 is incorporated into the viral DNA inside the nucleocapsid, which inhibits further DNA synthesis. In *in vitro* studies using cell line models, the drug concentrations causing 50% reduction in HBV DNA produced (EC_{50}) were 0.500 μM for LB80317, compared to 1.3 μM for adefovir, 0.006 μM for lamivudine, 0.004 μM for ETV, and 0.14-1.5 μM for tenofovir.

3.3 Pharmacokinetics and metabolism

The pharmacokinetics of besifovir in human were illustrated in two phase I studies [11,12]. Since LB80380 is a prodrug and is not detected in plasma, its metabolites

namely LB80331 (intermediate metabolite) and LB80317 (the active metabolite) were measured.

Following oral administration of LB80380, it is absorbed and deacetylated to LB80331 in the intestine and liver, and further oxidized to LB80317. From the plasma concentration-time profiles of LB80331 in subjects taking different doses of besifovir (10, 30, 60, 120, 240, 480mg), the plasma LB80331 concentrations increase with the dose of besifovir, producing a linear increase in the area under the plasma concentration-time curve (AUC). For an eight-fold dose increase from 30mg to 240mg, the mean maximal concentration in plasma (C_{max}) and AUC values increase 5.4- and 5.9- fold, respectively, resulting in a dose-proportionality factor of 0.7. The time required to reach C_{max} (T_{max}) of LB80331 is observed at 1.0 to 2.0 hours post-dose, while the T_{max} is delayed to 4-6 hours if besifovir is taken after ingestion of a high-fat meal. The ratio of the mean AUC from time zero to infinity ($AUC_{0-\infty}$) in the fed group (AUC_{fed}) to the mean $AUC_{0-\infty}$ in the fasting group ($AUC_{fasting}$) is 1.10, which is close to unity, i.e. the degree of absorption is not affected despite a delay in drug absorption in subjects who have taken a high-fat meal.

The plasma concentrations of LB80331 decline in a mono-exponential manner with an elimination half-life ($t_{1/2}$) of 2.5 – 3.3 hours. However for higher dose group (240mg and 480mg) the decline is bi-exponential with longer elimination half-life of 3-4 hours. The mean clearance (CL/F) values are 244 – 304 ml/min for lower doses (30mg, 60mg, 120mg) and 363 – 416 ml/min for 240mg.

The AUC and C_{max} are similar in subjects who receive daily doses of besifovir achieving steady state (2-4 weeks of therapy) compared to subjects who receive only a single dose, suggesting no accumulation of LB80331 in the body upon repeated dosing with an observed accumulation index of <11%.

Measurements for LB80317 are also performed, although only doses higher than 60mg can yield detectable plasma levels. In contrast to LB80331, the systemic exposure to LB80317 increases to 2- to 3- fold after taking multiple doses when compared to taking a single dose. The $t_{1/2}$ is much longer (45 – 62 hours) compared to LB80331. The AUC and C_{max} between LB80317 and LB80331 correlate strongly (r=0.9181 and r=0.8998, respectively) [12]. The pharmacokinetic parameters of LB80331 and LB80317 following single dose and multiple doses administration are summarized in Table 2 and Table 3, respectively.

The bioavailability of besifovir has been studied in animals, which ranges from 13% in Cynomolgus monkeys to 53% in Beagle dogs. The *in vivo* protein binding of LB80331 in human plasma are 17.7%, 12.0% and 12.0% at concentrations of 0.2, 2 and 20 μg/mL, respectively. Tissue distribution between serum and liver of LB80331 and LB80317 is studied in mice. The amounts of LB80331 and LB80317 in the mice livers as the phosphorylated forms are 39% and 39 – 41 %, respectively. The main route of excretion is via the kidneys, which accounts for 80% of drug excretion.

3.4 Clinical efficacy

The antiviral activity of besifovir has been evaulated in a few randomized controlled clinical trials. In a phase I/II placebo-controlled dose-finding study involving 29

HBeAg-positive CHB patients receiving 4 weeks of besifovir, the mean maximum HBV DNA reduction was 3.05, 4.20, 3.67 and 3.68 log copies/mL for 30, 60 120 and 240mg of besifovir, respectively. Doses higher than 60mg provided a high degree of viral inhibition [11]. As the plasma LB80317 level is too low at lower doses (including 30 and 60 mg, see Table 3), doses higher than 60mg were henced used in the subsequent randomized clinical trials.

In a phase IIb multicentre trial involving 114 CHB patients, head-to-head comparisons of antiviral efficacies were made between besifovir (90mg or 150mg) and ETV 0.5mg daily. At week 48, the proportion of patients achieving undetectable HBV DNA were 63.6%, 62.9% and 58.3% for besifovir 90mg daily, 150mg daily and ETV 0.5mg daily, respectively ($p>0.05$). The mean decline of serum HBV DNA from baseline were 5.84, 5.91 and 6.18 log copies/mL ($p>0.05$), respectively, for HBeAg-positive patients. For HBeAg-negative patients, the mean decline of serum HBV DNA from baseline were 4.65, 4.55 and 4.67 log copies/mL, respectively ($p>0.05$). Rates of normalization of serum ALT between the 3 groups were similar (91.7%, 76.9%, 89.7%, respectively, $p>0.05$). There were no significant differences in treatment-induced HBeAg-seroconversion (ESC) rates in HBeAg-positive patients (11.11%, 15%, 9.52%, respectively, $p>0.05$). The authors concluded there was non-inferiority of besifovir (either 90mg or 150mg) to ETV [13]. They subsequently reported the 2-year outcome of these patients in a roll-over study. At 96 weeks of besifovir 90mg daily, 150mg daily or ETV 0.5mg daily, rates of ALT normalization and serum HBV DNA undetectability were maintained, while the cumulative treatment-induced ESC rates in HBeAg-positive patients increased compared to 48 weeks (20%, 21.4% and 22.4%, respectively, $p>0.05$). Loss of HBsAg was not

observed in any patient up to 96 weeks of follow-up. Only one patient taking besifovir 90mg daily (out of 31) developed virological breakthrough at week 16 due to drug non-compliance, and there was no identifiable drug resistance in the reverse transcriptase genome of HBV [14].

Besifovir was shown to be effective in reducing viral load in 65 CHB patients with lamivudine-resistant virus. The extent of serum HBV DNA reduction after adding besifovir for 8 weeks at variable doses (30, 60, 90, 150, 240mg) was dose-dependent (2.81, 3.21, 3.92, 4.16 and 4.00 log copies/mL, respectively, $p<0.001$). Overall, 93.4% of patients had a >2 logs reduction of serum HBV DNA and 11.5% of patients had undetectable HBV DNA [15].

The antiviral efficacy of besifovir has also been compared with TDF in a phase III randomized study involving 193 treatment-naïve CHB patients. These patients were randomized to take besifovir 150mg daily or TDF 300mg daily. At week 48, 85.33% vs. 88.75% achieved undetectable serum HBV DNA, respectively ($p>0.05$). In addition, liver biopsies were performed in 29 patients at baseline and at week 48. This showed that significantly more patients taking besifovir than TDF (77.78% vs. 36.36%, respectively, $p=0.0482$) demonstrated histological improvement of necroinflammation, i.e. reduction in Knodell necroinflammatory score of ≥ 2 without worsening of fibrosis, as defined by the authors. No patients in either group developed resistance to treatment [16]. A phase III open-label extension study is underway (NCT01937806). From these studies, besifovir 150mg demonstrates at least non-inferiority when compared to ETV 0.5mg daily or TDF 300mg daily in terms of virological suppression.

3.5 Safety and tolerability

3.5.1 General tolerability

In the dose-finding study, no dose-related clinical or laboratory adverse events (AE) were reported. In the lamivudine-resistance study, 44.6% of patients experienced AE, including cough (10.77%), headache (9.23%), influenza (6.15%) abdominal discomfort (3.08%), dyspepsia (3.08%), fatigue (3.08%), myalgia (3.08%) and rhinorrhea (3.08%), but none of these were considered to be related to study medication or serious AE [11]. In the phase IIb study comparing besifovir and ETV, none of the patients in any treatment arm reported severe AE. One patient developed HCC while taking besifovir 150mg daily which was treated with transarterial chemoembolisation, and one patient developed ventricular tachycardia which was treated with radiofrequency ablation [13]. In the subsequent 2-year outcome study, one patient had inguinal hernia and one had cerebral aneurysm. These were considered not related to study treatment and the study medications were continued without interruption in all these patients [14].

3.5.2 Renal and bone safety

Besifovir demonstrates overall safety in terms of renal function and bone mineral density. In the phase IIb study, no patients had serum creatinine increment of ≥0.5mg/dL or significant reduction of estimated glomerular filtration rate (eGFR) from baseline. Two patients taking besifovir had grade 2 hypophosphataemia (serum phosphate 1.5-1.9mg/dL, normal >2mg/dL) which were asymptomatic and transient without need of additional treatment [13]. These findings were reinforced in the

subsequent 2-year outcome study, where grade 1 elevation of serum creatinine (increase in creatinine level of >0.3mg/dL or 1.5-2.0 times above baseline) happened in 11 (35.5%), 5 (17.9%) and 10 (33.3%) patients receiving besifovir 90mg, 150mg and ETV 0.5mg, respectively, and the creatinine levels returned to normal spontaneously without treatment interruption. Hypophosphataemia developed in 4 (12.9%), 3 (10.7%) and 3 (10.0%) patients, respectively, and again, none was symptomatic or required treatment interruption [14]. Compared to TDF, besifovir 150mg daily had a smaller decrease of bone mineral density at week 48 (besifovir -0.02 ±0.44, TDF -0.10±0.86, p=0.0248) [16].

3.5.3 Carnitine depletion

The most common side effect related to besifovir is carnitine depletion. L-carnitine is required for the transport of fatty acids from the cytosol into the mitochondria during lipid breakdown for generation of metabolic energy. Similar to adefovir, besifovir has a pivalic moiety. When pivalate enters cells, pivaloyl-CoA will be formed and the pivaloyl moiety will be transferred from coenzyme A to carnitine to form pivaloylcarnitine, which is directly excreted in the urine instead of the usual pathyway of energy generation. Normal ranges of total and free L-carnitine are 37-78 μmol/L and 25-54 μmol/L, respectively. In the phase IIb study, 94.1% patients taking besifovir had depletion of serum L-carnitine, and occurred in more patients taking 150mg compared to 90mg. The lowest total and free L-carnitine level were 23.6 μmol/L and <20 μmol/L, respectively. L-carnitine supplement (660mg daily) was given to most patients (61 out of 64 patients with carnitine depletion). This adverse effect was monitored in the subsequent 2-year outcome study, which showed 83.9% and 100% of patients taking besifovir 90mg and 150mg daily, respectively, had

sustained carnitine depletion requiring supplementation. The serum L-carnitine levels subsequently normalized and none of the patients developed symptoms of carnitine deficiency eg. hypoglycaemia, hypoketosis, encephalopathy [13,14]. Of note, the serum carnitine levels were not reported in patients receiving ETV.

3.6 Toxicity

There are no reports of drug toxicity from human use of besifovir so far. In animal studies, a single oral dose of 2000mg/kg of besifovir to rats results in weight loss, soft stool and diarrhea, but no mortality or macroscopic changes are seen. In term of reproductive toxicity, besifovir does not affect fertility, early embryonic development or implantation in rats given at the dose of 250mg/kg/day. No studies on drug concentration in breast milk have been performed.

3.7 Drug-drug interactions

There are no reported drug-drug interactions for besifovir at the moment. *In vitro* studies show that LB80380, LB80331 and LB80317 had negligible inhibitory effects on CYP1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1 and 3A4 up to 50 μM. These 3 compounds cause no induction effects on CYP1A2 and CYP3A4 except for CYP3A4 by LB80380 at 10 μM. However, as metabolism of LB80331 and LB80317 do not require catalytic activity of microsomal CYP450, LB80380 and its metabolites were considered unlikely to cause *in vivo* drug interactions.

3.8 Dosing routes

Besifovir is administered orally as tablet form.

3.9 Regulatory affairs

Besifovir is approved by The Ministry of Food and Drug Safety (MFDS) of Korea in 2017 for treatment of CHB. It is not yet approved by the U.S. FDA at the time of writing.

4. Conclusion

Besifovir is a nucleotide analogue (NA) with potent antiviral efficacy against HBV. It is rapidly absorbed during fasted state and it will not be accumulated upon repeated ingestion. Doses above 60mg are effective for inhibiting HBV in human. When given at oral doses of 90mg or 150mg daily, the antiviral efficacies are non-inferior to ETV 0.5mg daily. It is generally safe in terms of renal and bone toxicity. The most common adverse event is carnitine depletion which affected almost all patients on besifovir requiring carnitine supplementation. Preliminary results from a phase III study comparing with TDF showed histological improvement in a higher proportion of patients on besifovir 150mg daily. However, the number of patients having histological evaluation was small (N=29) and the baseline serum ALT and baseline necro-inflammatory score were not reported. The same issue applied to the apparently better bone safety, as the baseline bone mineral densities were not reported. Moreover, the changes in bone mineral density at 48 weeks of therapy were quite small in magnitude (-0.02 for besifovir vs. -0.01 for TDF) and the effects on longer duration of therapy were unknown. Most importantly, clinical events of fracture should be dictated before concluding its ‘bone-friendliness’. More studies are needed to confirm this histological advantage and assess the long term effects of besifovir, including treatment-related adverse events and other important endpoints such as HBsAg seroconversion, liver decompensation and HCC.

5. Expert opinion

Besifovir is another potent oral NA in treating CHB as proven by the clinical trials. The antiviral efficacy of besifovir in CHB is non-inferior to ETV and TDF. Moreover, besifovir demonstrates better bone safety in terms of bone mineral density compared to TDF. The safety profile is also favourable, except carnitine depletion occurs in almost every patient taking besifovir. This adverse event is subclinical and is reversible with oral carnitine supplementation.

Data on long term outcomes of besifovir especially HBsAg loss and HCC risk are anticipated. Since the mechanism of action is inhibition of relaxed circular double-stranded DNA synthesis, just like other NAs, besifovir is unlikely to directly result in silencing of covalently-closed circular DNA (cccDNA), although other NAs were shown to lead to undetectable cccDNA in 49% (21/43) of patients at >6 years of therapy [17]. The mechanisms for decline in cccDNA in NA-treated patients are thought to be related to both depletion of intra-nuclear cccDNA amplification pathway and hepatocyte death, rather than by a direct action of NA [18]. Therefore, cccDNA persistence is expected in besifovir-treated patients and clinical outcomes should be evaluated. The favourable histologic effects of besifovir compared to TDF should be further validated by larger scale studies. It should also be compared with the other potent NAs (TAF and ETV). The histological benefits should be proven to be long-lasting beyond 48 weeks in future studies with longer follow-up and paired liver biopsies. Similarly, the apparently superior bone safety for besifovir when compared to TDF should be further studied after longer exposure to the drug, and especially by head-to-head trials with TAF. Since besifovir is mainly excreted via the

kidneys, its pharmacokinetic characteristics should be studied in patients with renal dysfunction as dose adjustments may be required. Moreover, its safety in patients with advanced liver disease should be studied, since cases of lactic acidosis have been reported in patients taking ETV with decompensated liver disease [19]. The significance of carnitine depletion remains to be defined. These trials only reported serum carnitine level replenishment while tissue carnitine levels were not investigated. Also, the consequences of carnitine depletion were only assessed by clinical assessment, while more objective evaulations e.g. serum glucose, have not been performed. As carnitine is via renal route in the urine (after formation of pivaloylcarnitine from the pivaloyl moiety of the drug), other more sensitive markers of renal dysfunction apart from serum creatinine and eGFR should be assessed as well, such as urine protein-to-creatinine ratio, retinol-binding-protein-to-creatine ratio and beta-2-microglobulin-to-creatinine ratio. Safety profile in pregnancy and breast feeding should also be evaluated. A phase III trial is currently underway which aims to evaluate the safety and efficacy of besifovir 150mg daily compared to TDF 300mg daily in CHB patients with resistance to NA (NCT02792088). The results of this trial may help to confirm the efficacy of besifovir in patients with LAM or ETV resistance, though questionable superiority over TDF would be expected in terms of efficacy, as TDF-resistance virtually does not exist.

Besifovir is currently only approved and available in Korea. The best candidates for besifovir may be CHB patients who had prior lamivudine exposure or documented ETV resistance, whom are at risk of renal dysfunction or bone loss, and TAF is not available. In countries where TAF is available, one would need to see more advantages of besifovir over the existing NAs.

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