Dear. Editor and reviewers

Dr. Lian-Sheng Ma,

Company Editor-in-Chief,

Journal of World Gastroenterology

We thank the reviewers for their valuable advice, and are grateful to you for all the favor you have done us.

We have responded to all the questions by the reviewers with answers or explanations, and have revised our paper accordingly. We hope they will be satisfied. But, we are willing to provide further information if they think it necessary. The answer attached on the end this letter.

According to your requirements, the revision has been editing again by **Wiley English Language Editing Service** at Dec 14, 2020. (Order No OQINZ_1_5)

In here, we are submitting the revised manuscript **' New therapeutic options for persistent low-level viremia in patients with chronic HBV infection: Increase of enticavir dosage (Manuscript NO: 60189)**' to you.

We wish our paper be accepted and published in your journal as soon as possible.

Yours respectfully;

Dr. Guo-Qing Yin, Jun Li, Bei Zhong, Yong-Fong Yang and Mao-Rong Wang

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Reviewers' Comments to Author:

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: 1) In page 5, line 22, the authors claim that "Unfortunately, these developing new therapies also are not designed to eradicate the intrahepatic covalently closed circular DNA (cccDNA) or integrated HBV DNA". This is not accurate as there are several treatments currently in trial for cccDNA and integrated HBV DNA as reported by Geish R, 2020 for example and other reviews. These treatment options should be discussed

and explain why would increasing the dose of Enticavir woud be superior. 2) The review needs more updated references and more in text citations. The number of references used in the past 10 years is little given that the authors are suggesting new treatment module based on literature review. 3) The authors highlighted the efficacy of enticavir at higher doses by providing higher AUC values in plasma and in page 14 line 1 they mention “by increasing the dose over 1.0 mg/day might improve the antiviral efficacy”. What is meant by antiviral efficacy? Was the mechanism by which enticavir might target cccDNA or integrated DNA examined? 4) In page 14, line 27, the authors are discussing a study by Liu et al. as a reference for suggesting increasing the dose of enticavir for LLV, however in the study, this conclusion was never reached. This paragraph needs to be rephrased and supported by more studies. Minor comments 5) Page 6, line 34; “Therefore, the current study aimed to discover a viable way to improve the antiviral effect of entecavir by increasing the dose and prolonging the treatment time”. It should be changed to “ suggest” or “ shed the light on” not “discover”. 6) The manuscript needs grammatical and spelling revision.

Question:

1) In page 5, line 22, the authors claim that “Unfortunately, these developing new therapies also are not designed to eradicate the intrahepatic covalently closed circular DNA (cccDNA) or integrated HBV DNA”. This is not accurate as there are several treatments currently in trial for cccDNA and integrated HBV DNA as reported by Geish R, 2020 for example and other reviews. These treatment options should be discussed and explain why would increasing the dose of Enticavir woud be superior.

Answers:

While highly effective treatment options for hepatitis C virus have emerged recently, HBV therapy remains challenging. This is partly due to the viral replication strategy of HBV. After infection of hepatocytes through the sodium taurocholate cotransporting polypeptide, viral DNA is transported to the nucleus and converted into a minichromosome (covalently closed circular DNA (cccDNA)) to serve as a template for viral transcription. Some parts of the viral DNA are even integrated into the genomic hepatocyte DNA. cccDNA as well as integrated viral DNA persist in the nucleus and represent a reservoir for reactivation even after spontaneous viral clearance in serum. In the hepatocyte nucleus, cccDNA and the host genome share the gene transcription system and coexist with the host liver cell. Currently, there is no inhibitor that can inhibit cccDNA replication without inhibiting host gene transcription. Although a few researchers have studied the inhibitory effect for cccDNA, there are no studies that cccDNA inhibitor can inhibit HBV replication in clinical trial, let alone cure HBV infection. Current, elimination of cccDNA and integrated viral DNA remain

challenging.

The advent of several novel antiviral and immune modulatory therapies for chronic hepatitis B now necessitates a standardized appraisal of the efficacy and safety of these therapies, and definitions of new or additional endpoints to inform clinical trials. To move the field forward, and to expedite the pathway from discovery to regulatory approval, the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver jointly organized the Hepatitis B Treatment Endpoints Workshop. In the article by AS Lok et al, the consensus reached was that a complete sterilizing cure, i.e., viral eradication from the host, is unlikely to be feasible because of cccDNA. Currently, the clinical real-world state is that no antiviral drugs, NAs and interferon, can completely cure HBV infection, and LLV is the difficulty of ANs treatment. Based on the above consensus, we will not discuss the study of cccDNA clearance in this article.

Therefore, the sentence, 'Unfortunately, these developing new therapies also are not designed to eradicate the intrahepatic covalently closed circular DNA (cccDNA) or integrated HBV DNA' was changed to 'Unfortunately, these developing new therapies cannot eradicate the intrahepatic covalently closed circular DNA (cccDNA) or integrated HBV DNA' in Page 5, line 23.

Increasing the dose of enticavir with improving the antiviral efficacy is answered in the question 3.

References

Gehring AJ, Protzer U. Targeting Innate and Adaptive Immune Responses to Cure Chronic HBV Infection. Gastroenterology. 2019 Jan;156(2):325-337.

Lang J, Neumann-Haefelin C, Thimme R. Immunological cure of HBV infection. **Hepatol Int.** 2019 Mar;13(2):113-124.

Lok AS, Zoulim F, Dusheiko G, Ghany MG. Hepatitis B cure: From discovery to regulatory approval. Hepatology 2017; 66: 1296-1313.

Pei Y, Wang C, Yan SF, Liu G. Past, Current, and Future Developments of Therapeutic Agents for Treatment of Chronic Hepatitis B Virus Infection. J Med Chem. 2017;60:6461–6479.

Question:

2) The review needs more updated references and more in text citations. The number of references used in the past 10 years is little given that the authors are suggesting new treatment module based on literature review.

Answers:

On early phase of NAs therapy, partial virologic response and LLV were found, and clinical studies have shown that LLV is closely related to viral rebound and NAs resistant mutation. Most of these researches were published before 5-10 years. In the past 5 years, the incidence of LLV has gradually increased, which has become a problem in NAs therapy. Since 2016, the AASLD guidelines 2016 and 2018 have twice recommended LLV treatment options. We started researching LLV treatment in 2007 and published papers on LLV treatment. Therefore, papers on 2008-2016 are cited in this manuscript.

The studies of pharmacokinetics and pharmacodynamics were published before 2000. Subsequently, biopharmaceutical and biotechnology companies developed Entecavir. Review, Clinical pharmacology and biopharmaceutics review 021797/S-000, was published on the US Food and Drug Administration (FDA) website in 2005. These literatures were published before 10 years. Different from basic research, the literature collection time for clinical research is generally longer. The strategy of collecting documents in the present paper is that the documents related to the research purpose should be collected completely, and the documents not related to the research should be excluded.

Question:

3) The authors highlighted the efficacy of enticavir at higher doses by providing higher AUC values in plasma and in page 14 line 1 they mention “by increasing the dose over 1.0 mg/day might improve the antiviral efficacy”. What is meant by antiviral efficacy? Was the mechanism by which enticavir might target cccDNA or integrated DNA examined?

Answers:

The area under the concentration-time curve (AUC) is pharmacological parameter that is positively correlated with efficacy of drugs, and can be inferred antiviral efficacy by detecting AUC. In page 10, Liu YT reported that AUC was correlated with the antiviral efficacy of entecavir in the dose of 0.1mg/day, 0.5mg/day and 1.0 mg/day.

In the clinical studies in page 12, the sentences were stated as follows, ‘Considering the greater antiviral activity versus the 0.1 mg dose, superiority over lamivudine for antiviral activity response, reduction of HBV DNA to <0.7MRq/mL after 22 weeks, and an acceptable safety profile, the entecavir 0.5 mg/day was selected for treating nucleoside-naïve patients. According to that 1.0 mg/day entecavir exhibited significantly greater antiviral activity than the 0.5 mg/day, with the reduction of HBV DNA to < 400 copies/mL after 24 weeks, and an acceptable safety profile, entecavir 1.0 mg/day was determined to treat lamivudine refractory patients. Researchers chose the lower limit of the effective dose to treat CHB (Table 2. b. c).’,

which indicated that enticavir dose from 0.1 mg/day, 0.5mg/day to 1.0 mg/day is gradually increasing the efficacy of enticavir. In addition, it also predicts that increasing the dose of entecavir by more than 1 mg/day will further increase the antiviral efficacy.

All NAs, including Entecavir, cannot be target cccDNA or integrated DNA.

Question:

4) In page 14, line 27, the authors are discussing a study by Liu et al. as a reference for suggesting increasing the dose of enticavir for LLV, however in the study, this conclusion was never reached. This paragraph needs to be rephrased and supported by more studies.

Answers:

Ok! The sentence, 'Liu F et al. demonstrated that rapid suppression of HBV within 12 weeks of entecavir treatment reduced the incidence of drug-resistant mutations and prolonged the duration of entecavir therapy [8].' was changed to 'Liu F, (Fig 1), showed that rapid suppression of HBV within 12 weeks of entecavir treatment reduced the incidence of drug-resistant mutations and prolonged the duration of entecavir therapy [8].'.

Question:

5) Page 6, line 34; "Therefore, the current study aimed to discover a viable way to improve the antiviral effect of entecavir by increasing the dose and prolonging the treatment time". It should be changed to " suggest" or " shed the light on" not "discover".

Answers:

Thanks for your suggestion. The sentence was changed to 'Therefore, the current study scoured literature to shed light on the possibility of improving the antiviral effect of entecavir by increasing the dose and prolonging the treatment time.' in Page 6, line 3.

Question:

6) The manuscript needs grammatical and spelling revision.

Answers:

Ok.

The manuscript was again editing again by Language Editing Service.