Reviewer's report

Title: High-dose clevudine impairs mitochondrial function and glucose-stimulated insulin secretion in INS-1E cells

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Reviewer: Nazzareno Capitanio

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The study by Y-O Jang and co-workers provides evidence that the NRTI clevudine induces, at relatively high concentrations, impairment of mitochondrial functions in INS-1E and HepG2 cells. In particular, as expected, clevudine treatment causes a significant reduction of the mtDNA copy number/cell likely due to inhibition of the mitochondrial DNA polgamma. Moreover, in INS-1E cells the drug causes a marked decrease of insulin release upon high glucose-stimulation. The authors link this defective insulin release to alteration of the ATP level in clevudine-treated cells.

All these effects are attained at non-pharmacological concentrations of the drug therefore they are of limited clinical relevance as correctly stated by the authors. However, individual differences in the genetic background of the DNA polgamma may render some subjects more susceptible to the mitotoxicity of the drug thereby accounting for the limited number of cases of miopathy and diabetes reported as side-effect in patients long-treated with clevudine.

The manuscript is well written, the data clearly presented and the results of interest. However, the following specific points/concerns are raised and required to be satisfied to make the paper acceptable for publication.

Major points

1. The reported decrease in the Cox-1 mRNA (Fig. 1B,D) does not necessarily mean a decrease of the protein and therefore of the related function. Measurement of the activity of the cytochrome c oxidase on cell lysate may help to strengthen the authors’ conclusions.

2. The MTT assay is extremely vague to measure the mitochondrial enzymatic activity. A more specific assay like measurement of the citrate synthase activity is suggested (Barrientos A. Methods 2002;26:307-316). If the authors had the possibility to measure the oxygen consumption rates in intact cells by respirometry this would very clearly make the point on the oxidative phosphorylation capacity.

3. The cause-effect relationship between the decreased ATP level and the impaired insulin release does not appear very straightforward. Comparing Figs. 2B and 3 it seems that some inhibition of insulin release is observable already at 100 microM clevudine (high-glucose setting) which however did not change the ATP level. The authors are asked to clarify this point. If the release of insulin by INS-1E cells is sensitive to the mitochondrial ATP, oligomycin-treatment in
control cells should mimic the observed clevudine-mediated decrease of insulin release therefore proving the cause-effect relationship.

Minor points
4. The authors should clarify the reason of the long 4 weeks-treatment with clevudine. A time course of the reported effects has been established? Is there some effect on the cell growth?
5. Fig. 1A. The normalization of the mtDNA should be better defined in Materials and Methods. In the same Figure the significance (P value) of the differences should be included (same for Fig. 2C).
6. In the MTT assay the absorbance should have been normalized to the cell number per well. If the number of cells/well is the same why the authors state (in the Results Section) that the observed decrease implies a reduction in cells number.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests