Reviewer's report

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

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Reviewer: Bernard Fromenty

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The article submitted by Dr Jang and collaborators shows that high concentrations of the anti-HBV nucleoside analogue clevudine induce cytotoxicity, deplete mitochondrial DNA (mtDNA), reduce Cox-1 mRNA levels, lower cellular ATP content and impair insulin release under high glucose concentration, in the rat pancreatic beta cell line INS-1E. Moreover, high concentrations of clevudine are able to decrease mitochondrial DNA (mtDNA) and Cox-1 mRNA levels in the human hepatoma cell line HepG2. Although these findings are interesting and novel, there are several important issues which need to be addressed in order to strengthen the manuscript.

Major issues (major compulsory revisions):

1- Statistical analysis is not appropriate since the Student’s t test is used to compare two groups of data. Because the authors are comparing several groups, a one-way ANOVA must be used (Figure 1 and 2A). Moreover, when the effects of clevudine and glucose levels are simultaneously studied, a two-way ANOVA must be employed.

2- The data regarding the effect of 100 micromolar clevudine on PGC-1alpha, Tfam and Nrf1 expression are quite interesting and seem to be related to mitochondrial adaptation and proliferation. However, the authors should also present the data for the 1 mM concentration. Moreover, it would be of interest to determine the expression (or the activity) of the mitochondrial enzyme succinate dehydrogenase (SDH) in order to determine whether the adaptive up-regulation of these transcription factors is associated with mitochondrial proliferation. Such an event has already been observed in muscle of patients suffering from clevudine-induced myopathy (ref 10). A novel Figure could be added if these new data are informative.

3- One metabolic consequence of mtDNA depletion and OXPHOS deficiency is an impairment of fatty acid oxidation, thus leading to lipid accumulation. The finding that lipid droplets accumulate within treated cells will reinforce the notion that high concentrations of clevudine have deleterious metabolic consequences beyond the reduction of ATP content. Lipid accretion could be detected with oil red O staining or the fluorescent probe Nile red.

4- In this study, clevudine induced a 50-60% reduction in the mtDNA levels. In most tissues, however, the mtDNA copy number must fall below 20-40% of the basal levels in order to provoke mitochondrial dysfunction (DiMauro and Schon,
Moreover, different investigations showed that nucleoside reverse transcriptase inhibitors such as stavudine and zidovudine can also induce mitochondrial dysfunction without significantly depleting mtDNA (Barile et al., Gen Pharmacol 1998; Pan-Zhou et al., Antimicrob Agents Chemother 2000; Igoudjil et al., Antivir Ther 2007; Igoudjil et al., Toxicol in Vitro 2008). Thus, the authors cannot exclude the possibility that clevudine could impair mitochondrial function through mechanisms other than mtDNA depletion. This concept should be introduced in the discussion.

Other comments (discretionary revisions):

1- More pieces of information should be provided on the INS-1E and HepG2 cell lines. In particular, the authors should specify that these cell lines correspond to rat pancreatic beta cells and human hepatoma cells, respectively.

2- The authors should specify that clevudine-induced effects on glucose-stimulated insulin secretion have been studied after 4 weeks of treatment.

3- Several articles have shown that uridine is able to prevent NRTI-induced mitochondrial dysfunction (including mtDNA depletion) in different tissues (see for instance Lebrecht et al., Hepatology 2007; Lebrecht et al., Arthritis Rheum 2008; Venhoff et al., AIDS 2010). It would be interesting to test whether this molecule could be able to prevent clevudine-induced mitochondrial dysfunction in the INS-1E cell line.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests