Review of: "M-cell targeting acid-resistant oral vaccine delivery for immunization against Hepatitis B infection using cationic solid lipid nanoparticles"

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Potential competing interests: No potential competing interests to declare.

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The manuscript contains numerous convoluted sentences with multiple adjectives and mismatch between the subject and verb. The manuscript needs to be extensively edited. Several aspects of the methodology are unclear and numerous claims must be made accurate or removed. The work in the manuscript does not provide basis for solid nanoparticles being effective against Hepatitis B virus infection, only that they could modestly induce IgA and IgG production.

Abstract

Purpose. The stated transmission of HBV is incomplete. HBV can be transmitted through percutaneous, permucosal and parenteral means. Having this information in the very first sentence of the Abstract is a bit much. Better to leave it out?

Purpose. The word “prime” has several meanings. Better to be direct and replace “prime” with “most important”.

Purpose. Should define “M-cell” (*should be M cells, no dash*). Did the authors specifically test microfold cells? If not, this claim must be removed throughout the manuscript. It is inappropriate to conclude that the integrity of the M cells was maintained after treatment with SLN. The authors did not examine the integrity of the cells.

Purpose. “M-cell targeting acid-resistant oral vaccine delivery” is quite a mouthful of adjectives for the subject “delivery”. Also, the subject “delivery” does not match “have been formulated”. This sentence needs to be changed.

Methods. SDS-PAGE was used to analyze the amount and form of the HBsAg presence, not to evaluate “acid degradation protection of prepared formulation”.

Methods. “Hepatitis B surface antigen (HBsAg) loaded alginate coated cSLNs” is another mouthful of words describing the subject, cSLNs. Should be “alginate-coated”. In addition, Figure 1 shows that LPS coating the cSLNs and not contacting the HBsAg. The abstract suggests that LPS anchors cSLNs. Anchoring may not be an appropriate word. Later in the manuscript, the authors used the term “decorated”. This term is more appropriate.

Results. Change “prepared nanoparticles” to “cSLNs”. Assessment of IgG/IgA production using ELISAs should be in the Methods, not the Results. The sentence “Induction of immunity produced by prepared nanoparticle for Hepatitis B was determined on female Balb/c mice… “ is both awkwardly phrased and inaccurate. The authors assessed only antibodies levels, not the entirety of immunity.

Results. Amounts of antibodies produced were stated as IU/ml. It would be better to state these results as folds over the control.

Running title. It is inappropriate to claim that SLNs prevented HBV infection, as they did not examine infection. They did not even examine whether the antibodies can neutralize HBV.

Main text

Please edit each sentence carefully. Unlike the Abstract, I will not address specific sentences here.

The first reference cited does not directly address the number of acute hepatitis B infections. It is a paper on solid nanoparticles.

HBsAg exists in several forms (Large, Medium and Small) that differ substantially. While the authors received HBsAg as a gift, it is really important to specify its form and quality, as it will affect the production of neutralizing antibodies. The image in Figure 3 suggests that only the S form was used, although there may be oligomers.

Induction of immunity. Again, immunity is multi-faceted and the authors only examined the production of some antibodies. The title for this section should be changed. In addition, description of the “Blank” needs to be improved. At present, it is unclear as to the composition of the various Blanks.

The time for each stage of nanoparticle preparation should be included. At present, only a range (1 to 6 hour) is given. According to the TEM, the NPs contain many aggregates that are smaller in size to the AcSLNs. These aggregates could affect transcytosis by M cells and the subsequent activation of immunity. Were these removed?

Analysis of HBsAg integrity. I would not conclude that HBsAg was in the native form. In Fig. 3, the molecular mass of the markers needs to be added. Lane 4 appears to be from a separate gel as Lanes 1-3. This should be stated.

The mucoadhesion results are not shown and only qualitative description of adhesion such as “good binding” was provided. These results should be shown.

MTT assays. Both cSLNs and LPS-HB-cSLNPs clearly reduced the viability of RAW264.7 cells in a concentration-dependent manner.

The interpretation that “LPS-HB-cSLNs was non-toxic” needs to be removed. In terms of safety, the authors did not provide any information on the well-being and side effects on the mice. Observtions on the mice should be provided.

Stability studies. Table 3. In all of the formulations, the particle sizes increased with time, indicating that they changed. I disagree that the results be interpreted as the particles being stable. Also, the method (spectroscopy?) used to assess particle size should be clarified. In Table 4, HBsAg was also decreased with time, likely due to contamination with proteases. It is likely that these are BCA results and that the HBsAg could be cleaved. The interpretation that as the NP protected the HBsAg needs to be more nuanced.

Collection of blood and fluids. 100 mM PMSF is approximately 1000X fold higher than is typically used. Is this correct?

It is interesting and perplexing that the Blank SLNs significantly increased immunoglobulins that bind HBsAg (ca. 8 to 9-fold for IgA and over 2000-fold for IgG). If the data were normalized to the Blanks, the induction of IgA and IgG by HBsAg was actually modest, a few folds. The authors need to explain this in the main text.