Reviewer's report

Title: Preventive Effects of the Novel Antimicrobial Peptide Nal-P-113 in a Rat Periodontitis Model by Limiting the Growth of Porphyromonas gingivalis and Modulating IL-1β and TNF-α Production

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Reviewer: Marianna Kulka

Reviewer's report:

In this work, Wang et al have evaluated the antimicrobial effect of a modified peptide Nal-P-113 that was derived from histatin 5 in a periodontitis rat model induced by Porphyromonas gingivalis.

They investigated the effect of a series of Nal-P-113 concentrations on alveolar bone loss and IL-1β and TNF-α expression. The study shows an interesting in vivo result, which significantly limited periodontitis by decreasing the amount of Porphyromonas gingivalis. However, the authors have not adequately examined the inflammatory cytokine expression to fully ascertain its effect on inflammation. Furthermore, some of the data represented is unclear, or missing from the manuscript.

1. The figure resolution in some cases is poor. Please insert higher resolution figures.

2. The treatment group "f", is described as "f, without P. gingivalis W83 or Nal-P-113." (P2 L 41 and P6 L125). However, the authors also mentioned this group to be "without P. gingivalis W83 and Nal-P-113" in P20 469…Please check the use of "or" and "and" in these cases.

3. Figure 1, a better explanation of panel B is required. How were the images obtained and under what circumstances? In the current figure legend it is unclear which information pertains to panel A and B.

4. Figure 2. The SEM images in this figure are impressive. However, the notations below the figures are too small to read and the scale bar is very unclear. The authors should provide labels to indicate the bacteria and the tissue morphology that they wish the reader to focus on.

5. Figure 3. The authors did not provide information of how IL-1β and TNF-α expression was measured. What kind of staining method and which color represents which cytokine in the images? The graph in section C is very poor resolution and the X and Y axis labels are not legible.

6. The authors mentioned that they observed "significantly higher numbers of neutrophils and other inflammatory cells", yet there is no discussion or explanation of how this was
determined. The authors should at least mention how they characterized these different cell populations and how they define "significantly higher" in this context. Also, what kind of inflammatory cells were they expecting to see and why?

7. Figure 4: This figure shows a linear correlation curve of the amount of P. gingivalis' DNA copy and cytokines in periodontal tissue (IL-1β and TNF-α). A significant positive linear correlation was identified for DNA copy of P. gingivalis and cytokines levels in periodontal tissue.

In the figure legend, the authors need to better explain panel A and panel B.

8. The SEM protocol used in this study relied on washing with PBS and then dehydration. This could lead to salt crystals, which can be seen in e/f. One step of washing in water can avoid this.

9. P13, L289 "intro"? Should it be "in vitro"?

10. The mechanism behind the therapeutic effect is unclear. What is the effect of Nal-P-113 without P. gingivalis? Does it upregulate the production of these cytokines on its own?

11. Does the peptide directly interact with P. gingivalis? Or can this peptide also interact with immune cells? Although the authors have mentioned "One of the reasons may be that antimicrobial peptides themselves can induce a slight up-regulation of TNF-α expression.[30]", so the question is, up regulated by which cell, through what possible receptor? There are many antimicrobial peptides and signaling pathways that could be involved and examining at least one of these would be very helpful to this study. One possibility are macrophages and mast cells, both of which express antimicrobial peptide receptors and are able to fight bacterial infections in tissues.

12. What happens when the authors use the unmodified version of this peptide? Does it have the same effect?

13. What happens to the production of other cytokines and chemokines such as IFN-gamma and MCP-1? What about the production of other host-produced antimicrobial peptides?

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
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I am able to assess the statistics

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