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OPEN PEER REVIEW

Design and Evaluation of a Novel Anti-microbial Peptide from Cathelicidin-2: Selectively Active Against *Acinetobacter baumannii*

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1 Original submission

3 comments · 26 Oct 2023 – 30 Oct 2023

Referee 1 Reviewer

MAJOR REVISION 26 Oct 2023

The idea is quite interesting, but the authors are advised to make the following major revisions before acceptance can be considered.
1- The toxicity of the claimed peptides in human kidney and liver cell lines must be tested. HepG2 and HEK293 can be convenient suggestions. Apparently, concentrations above those causing significant toxicity are not worth further investigation.
2- Colistin and polymyxin have poor agar diffusion, so the agar/disk diffusion methods are not applicable. (ref: /www.ncbi.nlm.nih.gov/pmc/articles/PMC6440778)
3- Control standards must also be applied in biofilm metabolism and formation.
4- (optional) Having an animal model of such an infection and the subsequent treatment will make the efficacy and selectivity of the claimed peptide unquestionable.

Referee 2 Reviewer

MINOR REVISION 27 Oct 2023

Reviewer's remarks to the authors:
Below I have provided various remarks on the text, as it is often vague and has some shortcomings that deserve improvement. Here are some details the authors may want to address.
1. The abbreviations do not need to be included in the abstract or keyword section. It is preferable to describe the abbreviations once in the text or in a separate section with the same title.
2. Considering the novel nature of the peptide sequence and the lack of approved data for identification and purification methods (HPLC and LC/mass), it is better to briefly mention the details of these methods.
3. It is also recommended to describe the details of the method used to lyophilize the peptide.
4. What were the main reasons for using nonidentical concentrations of peptides in anti-bacterial and anti-fungal tests?
5. Write OD instead of DO in line 133.
6. It is recommended to prepare figures and tables with a more appropriate format and higher quality prior to publication.
7. The results of the hemolytic activity test should be presented in a more complete manner. It is necessary to provide a table for the test results and microscopic images of healthy RBCs after exposure to different concentrations of the peptide to confirm its lack of hemolytic activity.
8. It seems necessary to provide images of the plates for the anti-bacterial agar well diffusion test.
9. Write Figure 4 instead of Figure 3 in line 286.
10. It is recommended that the URL links referred to in the manuscript also be given in the form of cited references.

Associate Editor

MAJOR REVISION 30 Oct 2023

Major revision

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor

Thank you for the opportunity to revise the manuscript *"* ***Design and Evaluation of a novel Anti-Microbial Peptide from Cathelicidin-2: Selectively Active against Acinetobacter baumanii*** *"*. We appreciate the careful review and constructive comments of respected reviewers. The manuscript was revised according to their valuable suggestions and it substantially improved. We hope the revised manuscript could be satisfied the publication criteria of ***Iranian Journal of Pharmaceutical Sciences.***

The revised parts of the manuscript were highlighted in text and a point-by-point response to the reviewers' comments were presented below.

Thank you in advance

Best Regards

Corresponding author

Response to the Comments:

We greatly thank the respected reviewers and their time and effort to evaluate our manuscript and we tried to exert all needed revisions according to the reviewers' comments.

Reviewer 1:

The idea is quite interesting but the authors are advised to make below major revisions before considering acceptance.

1- The toxicity of claimed peptides on human kidney and liver cell lines must be performed. HepG2 and HEK293 can be convenient suggestions. Apparently, the concentrations above significant toxicity do not worth further investigations.

Thank you for your respected suggestion. As a primary cytotoxicity study, only the cytotoxic effect of the novel AMP on human fibroblast cell line (DHF) was performed and these data were added to the manuscript. As more optimization of peptide sequence might be needed, study of its cytotoxicity on different human cell lines will be performing in the future studies.

2- Colistin and polymyxin have poor agar diffusion and so the agar/disk diffusion methods are not applicable. (ref; /www.ncbi.nlm.nih.gov/pmc/articles/PMC6440778)

Thank you for your notice. The agar well diffusion study has shown small growth inhibitory zone for novel AMP and even, polymyxin B, however, as the microdiltuin antimicrobial tests were also provided data, according to the suggestion, these parts were removed from the manuscript.

3- Control standards must also be applied in biofilm metabolism and formation.

According to reference 28, growth control and sterile control were adequate for the test. In addition, polymyxin B could not show promising antibiofilm activity through our studies, and an appropriate alternative positive control could not be discovered to verify the novel peptide's biofilm capabilities. As a result, the positive control was not used in this experiment.

4- (optional) Having an animal model of such infection and the subsequent treatment will make the efficacy and selectivity of claimed peptide unquestionable.

Thank you for your suggestion. This study is ongoing and the effectiveness of novel designed AMP in animal models will be studying in the future. However, now, we have had not any data according to the in vivo studies.

Reviewer 2:

Reviewer's remarks to the authors:

Below I have provided various remarks on the text as it is often vague and has some shortcomings that deserve improvements. Here are some details the authors may want to address.

1. The abbreviations do not need to be included in the abstract or keyword section. It is preferable to describe the abbreviations once in the text or in a separate section with the same title.

Thank you. They were revised.

2. Considering the novel nature of the peptide sequence and the lack of approved data for identification and purification methods (HPLC and LC/mass), it is better to briefly mention the details of these methods.

Thank you for your suggestion. Peptide synthesis was outsourced and a Chinese company (GenScript) synthesized, purified and characterized the novel AMP; we have received a lyophilized peptide powder with respected certificate confirmed peptide identity and purity. Related data of peptide synthesis was completed in methods.

3. It is also recommended to describe the details of the lyophilization method of the peptide.

Thank you for your suggestion. Peptide synthesis was outsourced and a Chinese company (GenScript) synthesized, purified and characterized the novel AMP; we have received a lyophilized peptide powder with respected certificate confirmed peptide identity and purity.

4. What were the main reasons for using non-identical concentrations of peptides in anti-bacterial and anti-fungal tests?

For all tests including antibacterial and antifungal activity tests, the initial value of 1 $mg.mL^{-1}$ was considered and a serial dilution was applied. As the antifungal activity was not observed in the concentrations less than 31.25 $\mu g.mL^{-1}$, therefore, more dilutions were not applied.

5. Write OD instead of DO in line 133.

Thank you for your point. It was revised.

6. It is recommended to prepare figures and tables with a more appropriate format and higher quality prior to publication.

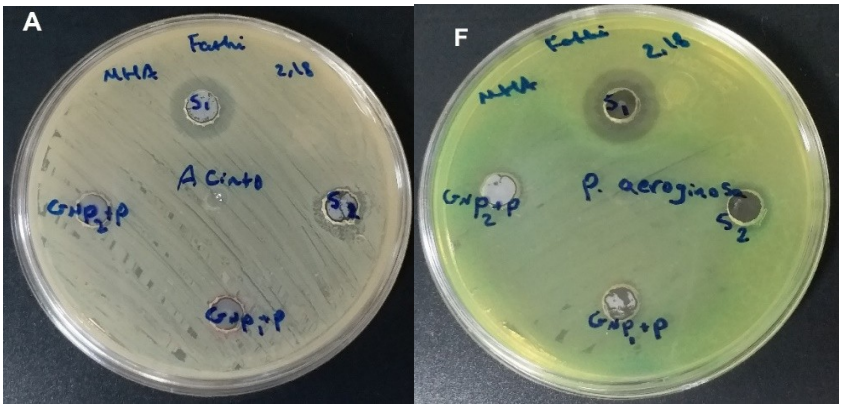
Thank you for your suggestion. They were revised.

7. The results of the hemolysis activity test should be presented in a more complete manner. It is necessary to provide a table for the test results and microscopic images of healthy RBCs after exposure to different concentrations of the peptide to confirm its lack of hemolytic activity.

Thank you for your suggestion. The data of absorption measurement at 567 nm and the hemolysis percentages calculated based on Eq. 4 were reported in Table 5. Unfortunately, the samples images before and after the exposure to the peptide were not captured.

8. It seems necessary to provide images of the plates for the anti-bacterial agar well diffusion test.

Thank you for your suggestion. These data were removed from the manuscript according to the other reviewer's suggestion. However, example images of test plates were as:



9. Write Figure 4 instead of Figure 3 in line 286.

Thank you for your suggestion. All figures and Tables numbers were checked, corrected and confirmed.

10. It is recommended that the URL links referred to in the manuscript are also given in the form of cited references.

Thank you for your suggestion. The URLs were presented in Table 1, and the related references of each tools and databases as previously provided, were presented in References.

2 Revision 1

2 comments · 16 Nov 2023 – 16 Nov 2023

Referee 1 Reviewer

ACCEPT 16 Nov 2023

Accepted

Associate Editor

ACCEPT 16 Nov 2023

Accept