Iranian Journal of Pharmaceutical Research
Volume 23 · Issue 1 · e144901

OPEN PEER REVIEW

RadioLabeling of the Mutant form of Anti-PlGF Nanobody by ^{99m}Tc-Tricarbonyl

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SUBMITTED	REVISED	ACCEPTED	DOI
10 Jan 2024	9 Apr 2024	30 Apr 2024	10.5812/ijpr-144901

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1 Original submission

2 comments · 17 Feb 2024 – 27 Feb 2024

hossein behnam-manesh Reviewer

MINOR REVISION 17 Feb 2024

1. This study includes two parts, biological and radio-chemical studies, and the authors have focused more on the second part, although the part related to Nano antibody synthesis is very important. Please mention the reference title if the production method is general; otherwise, give more details about the Nano body synthesis method.
2. In the discussion section, most of the authors' efforts have focused on the titles of studies published in related fields without effectively connecting them to their own work. In this section, please try to compare the investigated parameters and effective conditions with those in other scientifically conducted studies, for example, studies of other labeled Nano bodies.
3. Please replace the HPLC graphs in this manuscript with the graphs obtained from the HPLC device, if you have them.
4. What is the innovation in this study? If this is the first time this Nano body has been labeled with technetium tricarbonyl and its parameters have been checked, please mention it.

Associate Editor

MAJOR REVISION 27 Feb 2024

Based on the reviewers' comments, this manuscript cannot be published in the current format, and substantial work is required prior to re-submission (major revision).

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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 reviewers,

I would like to first express our deep appreciation towards you for reviewing this manuscript.

Undoubtedly, your sound comments will add to the credibility of our work.

In accordance with the comments of the reviewers, a list of revisions has been provided below with the corresponding numbers. It must be noted that all revisions have been highlighted in the manuscript too.

Reviewers' Comments:

Reviewer 1:

1. This study includes two parts, biological and radio-chemical studies, and the authors have focused more on the second part. While the part related to Nano antibody synthesis is very important. Please mention the reference title if the it production method is general, otherwise, gives more details about the Nano body synthesis method.

-As it was suggested by reviewer, more details about the nanobody synthesis were added to the manuscript in sections 3.1.

2. In the discussion section, most of the authors' efforts have been on the titles of studies published in related fields without effective communication with their own work. Please, in this section, try to check the investigated parameters and effective conditions with other scientifically conducted studies, for example with other labeled Nano bodies.

-As it was suggested by reviewer, correction was done in the manuscript in sections 5.

3. Please replace the graphs related to HPLC placed in this manuscript with the graphs obtained from the HPLC device if you have.

-The HPLC radiochromatogram was obtained by injection of 20 µL of 99mTc-tricarbonyl. Since the HPLC wasnot equipped with gamma detector, the outlet of HPLC column was connected to a fraction collector. Thirty fractions (1 mL/min) were collected, activity of fractions were measured and drawn against the number of fractions (Figure 2b).

4. What is innovation in this study? If for the first time this Nano body has been labeled with the technetium tricarbonyl and its parameters have been checked, please mention it.

-As it was suggested by reviewer the innovation was mentioned in the manuscript section 1.

Reviewer 2:

1- In the abstract, the authors mentioned, “The purified nanoparticles were confirmed by SDS-PAGE and western blotting.”. I think they mean nanobody instead of nanoparticles.

-As it was suggested by reviewer the correction was done in the manuscript abstract.

2- Section “3.4. Stability studies of radiolabeled Nb-mut2” stability test by dilution is usually performed by more than 10 times dilution to give a crud assumption upon the dilution effect during injection to an animal or a human while the author diluted the radiotracer 2 folds. Please indicate the reason behind this. Furthermore, I suggest that the authors perform stability in blood serum.

-The radiolabeled nanobody was purified using a PD-10 column and purified radiolabeled nanobody was used for stability studies. Since the concentration of radiolabeled nonobody was very low, 2 times dilution was used. Otherwise, the sample should be more diluted. This was a preliminary study and for future studies, stability in blood serum would be determined as well.

3- Please change the title of “4.2. Confirmation of the constructed technetium tricarbonyl” to quality control.

-As it was suggested by reviewer the correction was done in the manuscript section 4.2.

4- The authors claim that the RCP after purification was 100% in Fig.4, while in Fig. 4 the RCP is below 90% due to the broad peak at 5-10min.

-The TLC-chromatogram was wrong. It was corrected in the manuscript (Figure 4). The method is cut and count.

5- Please provide all the TLC chromatograms. The provided figures for the TLC do not convey any specific information.

-As it was suggested by reviewer, the TLC-chromatograms were inserted in the manuscript. The method is cut and count.

6- Please revise the conclusion section based on the data obtained from your study.

-As it was suggested by reviewer, the conclusion was revised.

7- The study lacks conviction. To improve this, this study needs to add specific binding on different cell lines, and biological distribution in normal mice or potential xenograft models.

-This was a very preliminary study, and more effort was put on radiolabeling. The study is not finished and more experiments including biological evaluation would be done.

8- affinity studies including KD, IC50, and Bmax calculation are vital for such a study.

-It was explained in previous question of respected reviewer.

2 Revision 1

1 comment · 28 Apr 2024

Associate Editor

ACCEPT 28 Apr 2024

This manuscript can be accepted in our journal. The authors have responded to most of the reviewers' comments.