Journal of Clinical and Translational Research
Peer review process file 03.201702.002

Scorpion and spider venoms in cancer treatment: state of the art, challenges, and perspectives

Catarina Rapôso

Corresponding author:
Catarina Rapôso
Department of Structural and Functional Biology, Institute of Biology, State University of Campinas (UNICAMP), Campinas, SP, Brazil

Handling editor:
Michal Heger
Department of Experimental Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
Review timeline:

Received: 4 October, 2016
Editorial decision: 9 November, 2016
Revision received: 1 March, 2017
Editorial decision: 9 April, 2017
Revision received: 7 May, 2017
Editorial decision: 7 May, 2017
Published online: 24 May, 2017

1st editorial decision
Date: 29-May-2017

Ref.: Ms. No. JCTRes-D-16-00032
Animal Venoms for Cancer Therapy: State of the Art, Challenges and Perspectives
Journal of Clinical and Translational Research

Dear Dr Rapôso,

Two reviewers and the editor have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you resubmit your work.

Your revision is due by Dec 09, 2016.

To submit a revision, go to http://jctres.edmgr.com/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Editor's comments:

Please see the document attached.

Reviewer #1:

Introduction - too long and largely redundant. I would suggested cutting all but the first two paragraphs and the last paragraph.
Figure 1 - all abbreviations need spelling and all molecular pathways need referencing in the text.
cancer cell targets - the scope of the paper is too large, which results in a superficial discussion on multiple topics. this contributes very little to the current body of knowledge. i would trim down dramatically or omit all together this section.
effect of scorpion and spider venoms on cancer therapy - this is the true story. i would suggest adding a figure of a cell showing where the different toxins act.
i recommend to review also Cohen-Inbar et al
(https://www.ncbi.nlm.nih.gov/pubmed/27452128)

Reviewer #2:

There are some grammatical and spelling errors throughout the manuscript. Attention to articulation and punctuation is required.
A systematic review should provide information in the introduction section, how the literature was searched and which papers have been selected? Which databases have been searched? Which quality criteria for inclusion and exclusion have been applied? The author should avoid the impression that they just used a staple of copied papers collected over time more or less by chance.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

********Authors response********

Ref.: Ms. No. JCTRes-D-16-00032

Journal of Clinical and Translational Research

Editor-in-Chief

I am pleased to submit the enclosed revised manuscript, now entitled "**The Use of Scorpion and Spider Venoms in Cancer Treatment: State of the Art, Challenges and Perspectives".**

The manuscript was extensively restructured at the request of the editor and reviewers. The main objective of this review was to identify the effects and mechanisms of scorpion and spider venoms and toxins in cancer cells, and the difficulties of moving forward to clinical trials with these molecules.

This manuscript complies with the ethics in publishing and the ethical guidelines for journal publication for manuscripts submitted to the Journal of Clinical and Translational Research.

I confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. I have no conflicts of interest relevant to the subject of the article to declare.

Sincerely yours,

Catarina Rapôso
Corresponding author

Department of Structural and Functional Biology
Institute of Biology, State University of Campinas (UNICAMP), Campinas, SP, Brazil
Tel. 55 19 983798091
E-mail: cataraposa@gmail.com

Editor's comments Raposo2016JClinTranslRes

1. As suggested by reviewer 1, the Introduction would benefit from more brevity and restructuring in accordance with the following suggestions:

- Please start the first paragraph with “Animal venoms are a mix…” (p. 3, line 10)

R – This change has been made.

- Eliminate the entire second paragraph (starting p. 3, line 34). Everyone knows that cancer is a big medical problem and that effective treatments are still lacking. This draws away from the central theme of the paper. The same applies to the third paragraph (starting p. 4, line 5), which can be summarized in one or two referenced statements attesting that many clinically prescribed anti-cancer drugs have a natural origin.

R – The second paragraph has been removed and the third paragraph has been summarized.

- The fourth paragraph (starting p. 4, line 29) should list all hallmarks of cancer and preferably be accompanied by a figure. The hallmarks should be numbered in consecutive order. Table 1 should subsequently specify which hallmarks are inhibited by every listed compound, e.g., by inserting the hallmark number from the suggested figure (and further specified in the table legend). Please make sure that proper references are used.

R – Four hallmarks have been numbered in the text (Introduction section, second paragraph) and in Figure 1. In addition, the same hallmarks are now mentioned in Table 1.

- Reference 14 (p. 4, line 39) is inappropriate to address the statement that “the changes in cell behavior are [a] consequence of complex signals, signaling pathways[,] and cross-talk between signaling pathways.” The Hanahan and Weinberg *Cell* papers are more appropriate.

R – The reference has been replaced.

- Please study [Lee KW, Bode AM, and Dong Z (2011) Molecular targets of phytochemicals for cancer prevention. Nat Rev Cancer 11:211–218] in support of the utility of phytochemicals with respect to targeting multifarious intracellular pathways/loci. See for example the introduction of our paper on curcumin [Pharmacol Rev. 2013 Dec 24;66(1):222-307] on how to sell the message.

R – The suggested literature was extremely helpful.

2. Figure 2 excludes a very important cause of cancer, namely chronic inflammation and corollary pervasive oxidative stress. Please include this in the figure.

R – Chronic inflammation and oxidative stress have been added to the figure and to the text (Introduction, section, second paragraph).

3. Please reference key statements in the text, such as those in the paragraph on p. 6, lines 20-32. This is just one example which should be extended throughout the entire text. Another example is on p. 19, lines 25-28: insert references to studies proving that MMP isoforms are overexpressed in the indicated cell types.

R – References have been inserted in the above examples and in other parts of the text.

4. Please indicate which biological/biochemical triggers activate the PI3K/AKT/mTOR pathway.

R – This is now indicated. See subsection 2.1.1, third paragraph.

5. Another way to reduce the length of the text is to make figures of the key pathways in terms of cancer development and progression/metastasis (e.g., PI3K/AKT/mTOR pathway, pRb pathway, apoptosis pathways, angiogenesis pathways, metastasis and invasion pathways, etc.). This will enhance the readability and understandability of the text. See again our curcumin review for examples [Pharmacol Rev. 2013 Dec 24;66(1):222-307]. Other good examples on how to illustrate these pathways are provided in our Cancer Metastasis Rev. 2015 Dec;34(4):643-90 paper. The figures can be reused to graphically pinpoint intervention sites of the various toxins addressed in later sections of the paper. Please let me know if I need to provide the pdfs of the abovementioned papers. Also, please note that I don't want you to cite these papers but to adopt the presentation strategy to make your text more accessible. Molecular pathways that are described textually are disastrous to readers not familiar with the pathways, so graphical aids are important tools to ensure that everyone can follow what you are relaying.

R – The pathways are now presented schematically (Figure 2A-D). In addition, the section "Cancer cells targets: from malignant transformation to metastasis" has been removed from the text and each mechanism has been discussed throughout the current section 2 ("Effects of scorpion and spider venoms in cancer therapy).

6. Since you address only scorpion and spider venoms, you should change the title accordingly.

R – The title has been changed.

7. p. 14, line 39: the phrasing "emphasized selectivity" is incorrect syntax. In the last sentence of this paragraph the phrases "rational engineering" and "improving the guide molecules" are elusive. Please rephrase.

R – An English revision has been carried out by a native speaker

8. Top paragraph p. 14, please provide examples of conventional cytotoxic drugs and more selective drugs. The latter especially is rather vague; do you mean immunotherapy, antibodies, kinase inhibitors? Also, I recommend that you refrain from using the phrasing “new targets” because in a few years these targets will not be new anymore but your paper will still be read. It is better to provide a table of these targets that are relevant in the context of scorpion and spider venoms.

R – The top paragraph p. 14 was removed, as has the expression: “new targets”. Table 1 and Figure 2A-D show the targets of venoms.

9. The paragraph starting with scorpion venom (p. 15, line 5) should fall under a separate section on scorpion venom. Similarly, a separate section on spider venom should be included in the paper (starting on p. 22, line 10). Please number the main sections as well as the subsections for clarity.

R – The separated sections for scorpions and spider have been added. Sections and subsections are now numbered.

10. p. 15, lines 24-29: please specify whether these models of cancer are cell lines or animal models (which should be further specified; e.g., mouse, rats, etc).

R – This has been done throughout the text. Figures 3-5 show the cancer cell lines and animal models separately.

11. One very important element that is often missing from in vitro studies using cancer cell lines is the addition of the compound/extract/lyophilized powder in non-cancerous (i.e., slow-to-non-proliferating, metabolically slow cell types such as endothelial cells (HUVECs), primary hepatocytes, fibroblasts, immune cells. If more information on the effect of venoms on these or similar cell types is available, I would very much like you to address this more extensively than already done. A venom for cancer therapy is useless if the non-cancerous cells die to an equal extent as a result of the venom. That would place the venom in the category conventional chemotherapeutics, which you alluded to before and essentially condemned the utility of these drugs because of their widespread toxicity.

R – The results in non-cancerous cells in each study (if have) have been added throughout the text. A conclusion regarding this issue has been added to the last paragraph of section 2.2.

12. Please make sure throughout the manuscript that it is clear which addressed study was performed in cell lines and which was performed in vivo. For the in vivo sections (e.g., “Angiogenesis inhibition of scorpion venoms”), you should address (1) dosage/concentration; (2) dosage route (e.g., oral, iv, ip); and the systemic/toxicological effects of the venoms on the

organism. In other words, animal studies reveal whether the use of the venom is reducible to practice, which is critical for future clinical implementation.

R – Cell lines and *in vivo* models have been clearly indicated throughout the text. The same has been carried out for dosage/dosage route concentrations and systemic/toxicological effects, where available.

13. Please ensure that the species and tissue origin of all cell lines is specified (e.g., HCT is derived from colorectal carcinoma). Preferably use the following denotation: "…venom induced apoptosis in human hepatocellular carcinoma (HepG2) cells…"

R – This has been done.

14. p. 19, line 7: reduced colony formation is elusive mechanistically. Was this owing to inhibition of cell proliferation or induction of cell death?

R – This has been clarified.

15. Given the large amount of abbreviations, I would like to receive a list of abbreviation that will be placed at the beginning of the manuscript.

R – An abbreviation list is provided at the beginning of the manuscript.

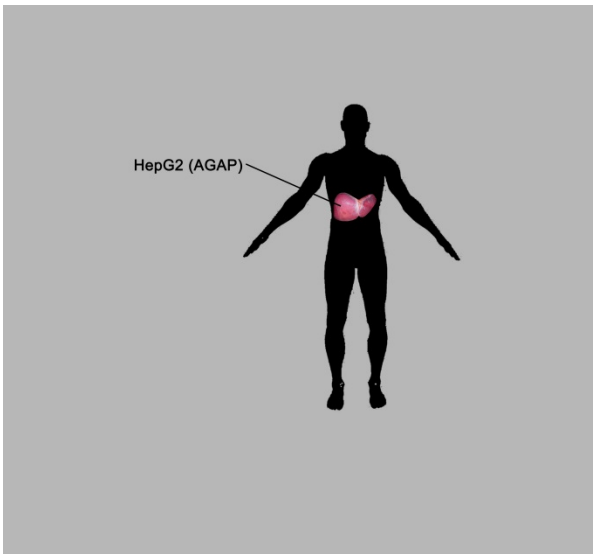
16. p. 20, line 7: CTX-based unities is incorrect syntax.

R – This has been corrected.

17. Please include LD50 values where these values were determined.

R – These have been included.

18. What would really help the readers is to include an anatomical map of a human being showing the organs where scorpion and spider venoms were effective in killing the respective human cancer cells in model systems. Prepare such a map for in vitro studies and for in vivo studies, so 2 sets per type of venom. Follow the example below:



Place this figure at the end of the section on scorpion and snake venom. Only include cell lines (in this example: HepG2) and venom components (in this example: AGAP) that were shown to be effective.

R – Figures 3 and 4 have been included, representing in vitro models of human cancer cell lines in studies using scorpion and spider venoms/toxins, respectively. Figure 5 represents in vivo models of human cancer cell lines in studies using scorpion and spider venoms.

19. Please extend my comments on the scorpion venom section to the spider venom section.

R – The spider venom section has been reformulated according to the recommendations.

20. I encourage you to include as much published and unpublished data as possible, as you have done in Figure 2, to complement the text. A stipulation is that the data *de facto* support the information you are relaying. We have recently published a review in which we included results from a mouse study and a clinical cohort to furnish empirical corroboration of the text [Antioxid Redox Signal. 2014 Sep 1;21(7):1098-118]. This format is very useful and elucidating.

R – Studies using *Phoneutria nigriventer* spider venom in cancer models are a recent undertaking for our lab. For this reason, we have little data to include. However, results can be published in the future.

21. Could you elaborate on biotech approaches to produce peptides and proteins? Could the DNA of the proteinaceous venoms be transfected into bacterial/fungal expression systems or even expression systems of vertebrate (chicken eggs) to produce bulk quantities of the venom?

R – Yes, it is possible to produce peptides and proteins from libraries of spider venoms and scorpions. In addition, it is possible to transfect DNA into other organisms to produce and modify components of venom. Some studies with these approaches have been detailed in the manuscript. See, for example, the last paragraph of section 2.1.3 and the second paragraph of section 2.1.4.

Reviewer #1:

Introduction - too long and largely redundant. I would suggested cutting all but the first two paragraphs and the last paragraph.

R – The Introduction has been summarized.

Figure 1 - all abbreviations need spelling and all molecular pathways need referencing in the text.

R – The abbreviations have been spelt in Figure 1, and a list of abbreviations has been made available at the beginning of the manuscript.

cancer cell targets - the scope of the paper is too large, which results in a superficial discussion on multiple topics. this contributes very little to the current body of knowledge. i would trim down dramatically or omit all together this section.

R – This section has been omitted.

effect of scorpion and spider venoms on cancer therapy - this is the true story. i would suggest adding a figure of a cell showing where the different toxins act. i recommend to review also Cohen-Inbar et al (https://www.ncbi.nlm.nih.gov/pubmed/27452128)

R – Four new figures (Figures 2-5) have been added to the manuscript, detailing the mechanisms and tumors/cell lines that scorpion and spider venoms target. In addition, Table 1 also summarizes the mechanisms and cell lines.

Reviewer #2:

There are some grammatical and spelling errors throughout the manuscript. Attention to articulation and punctuation is required.

R – An additional English review has been carried out.

A systematic review should provide information in the introduction section, how the literature was searched and which papers have been selected? Which databases have been searched? Which quality criteria for inclusion and exclusion have been applied?

R – This information has been provided in section 2, first paragraph.

The author should avoid the impression that they just used a staple of copied papers collected over time more or less by chance.

R – The studies have been discussed more fully.

2nd editorial decision
Date: 9-Apr-2017

Ref.: Ms. No. JCTRes-D-16-00032R1
The Use of Scorpion and Spider Venoms in Cancer Treatment: State of the Art, Challenges and Perspectives
Journal of Clinical and Translational Research

Dear author(s),

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your responses.

Your revision is due by Apr 23, 2017.

To submit a revision, go to http://jctres.edmgr.com/ and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Dear Catarina,

As agreed via email, please change the figures according to my instructions and implement textual modifications as per my instructions in the main text. The text has been attached to this email.

Thank you for your contribution to JCTR.

Kindest regards,

Michal.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.
****

3rd editorial decision
Date: 7-May-2017

Ref.: Ms. No. JCTRes-D-16-00032R2
Scorpion and spider venoms in cancer treatment: state of the art, challenges, and perspectives
Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

Comments from the editor and reviewers can be found below.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers:

Dear Catarina,

Thank you for your revision. I have made some more modification to your manuscript, which you may find attached to this email.

The paper is now suitable for publication.

Congratulations and thank you for introducing us to this very exciting world of medicinal spider and scorpion venoms and toxins!

Kindest regards,

Michal.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.
****