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OPEN PEER REVIEW

Oxilipin, a New Anti-cancer Phospholipase A2-like Protein from Iranian Caspian Cobra, *Naja Naja* Oxiana

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

1 comment · 11 Aug 2022

Referee 1 Reviewer

MINOR REVISION 11 Aug 2022

Review comments

The authors of the manuscript "Oxilipin, a new anti-cancer phospholipase A2-like protein from Iranian Caspian cobra, Naja naja oxiana" report good anticancer activity, first of a Caspian cobra venom fraction and then of Oxilipin, which is the most effective fraction. The results showed that there is no necrosis and that the sensitivity of normal cells (HEK-293) is significantly lower than that of colorectal cancer cells. This is a result that speaks of a good potential for selective action.

This manuscript is a good initial analysis, but in the future it is necessary to elaborate on the mechanism of proapoptotic action, as well as some other mechanisms responsible for the initiation and progression of cancer. Also, there is a need to examine the potential of Oxilipin towards the development of resistance.

To return to the manuscript, I think that the title is adequate and that the authors have provided enough information about the methodology and procedure of venom purification. Also, the graphs and pictures are clear and transparent. However, I will point out some omissions below:

1. Lines 75, 80, 81 - there are some spelling mistakes here (the space after the percentage should be deleted; in CO2, put the 2 in subscript). In general, revise the entire text and correct spelling mistakes;
2. Line 79 – Cell viability assay - it is necessary to describe which concentrations were used;
3. There are a lot of chemicals in the paper that are listed in the methodology but not in the materials section;
4. Line 114 - Which concentrations of Oxilipin were used?
5. Line 196 – Is Fraction 5 a mistake? Fraction 4 has the best anticancer activity, as you mentioned previously;
6. Line 323 – The species name must be italicized;
7. Line 456 – Reference 26 is in a different font.

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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.

Reviewer 1:
In this manuscript, the cytotoxicity of Oxilipin was evaluated in two cell lines in vitro. Based on the results presented, it was proposed that the toxicity of Oxilipin was higher in colon cancer cell line than those in normal cell. There are several concerns and open questions depicted below that can help author's interpretate/discuss methods and results.

Q1. Please check spelling, mistyping, and language.

R1: Well done.

Q2. The densitometry data related to PI staining was missed.

R2: The intensity checked by using a fluorescent microscope.

Q3- MTT assay for evaluation of viability is enough, please explain why the author perform trypan blue exclusion assay.

R3: It is a very good suggestion and it was removed.

Q4. The hemolysis data was missed.

R4: Well done. Fig. 10 was added:

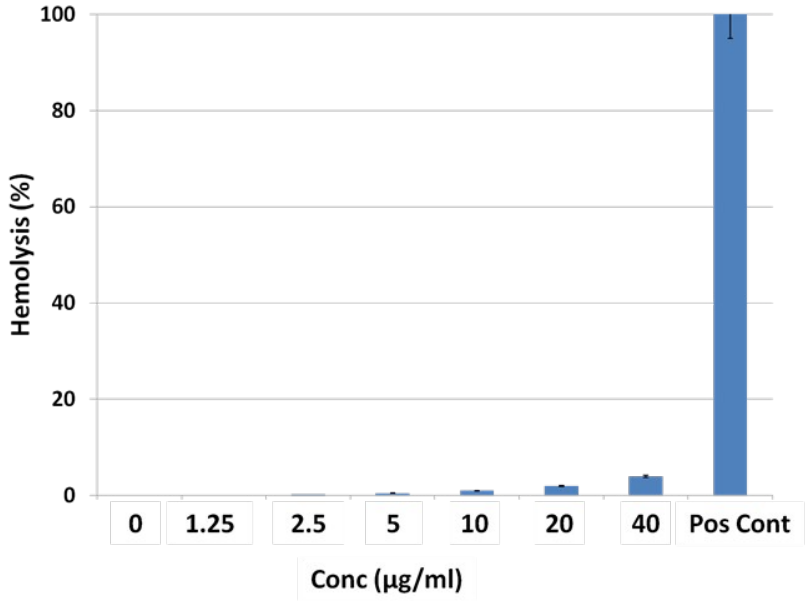


Fig. 9. Hemolytic activity of Oxilipin on human RBCs. Different concentration of Oxilipin was used to determine the hemolysis activity, but no considerable hemolysis was shown. Triton□100 used as positive control.

Reviewer 2:
Review comments

The authors of the manuscript "Oxilipin, a new anti-cancer phospholipase A2-like protein from Iranian Caspian cobra, Naja naja oxiana", indicate a good antitcancer activity of Caspian cobra venom fraction in first, and then of Oxilipin, which is the most effective fraction. The results showed that there is no necrosis, and that the sensitivity of normal cells (HEK-293), is significantly lower than colorectal cancer cells. This is a result that speaks of a good potential for selective action.
This manuscript is a good initial analysis, but in the future it is necessary to elaborate the mechanism of proapoptotic action, as well as some other mechanisms responsible for the initiation and progression of cancer. Also, there is a need to examine the potential of Oxilipin towards the development of resistance.
To return to the manuscript, I think that the title is adequate, that the authors have provided enough information about the methodology and procedure of venom purification. Also, the graphs and pictures are clear and transparent. However, I will point out some omissions below:

Q1. Lines 75., 80., 81, - there are some spelling mistakes here (the space after the percentage should be deleted; CO2 - 2 put in subscript). In general, revise the entire text and correct spelling mistakes;

R1: Well done.

Q2. Line 79 – Cell viability assay - it is necessary to describe which concentrations were used;

R2: It was mentioned: (0.7-50 µg/ml from SEC, 0.3-40 µg/ml from IEX).

Q3. There are a lot of chemicals in the paper that are listed in the methodology but not in the material section;

R3:The list of materials were added: Trypsin, ammonium acetate, NaCl, Tris buffer, Dialysis tube, Coomassie blue G250, Polyacrylamide, Propidium iodide, LDH release kit, PBS (phosphate buffer saline) were bought from Sigma (Saint Louis, MO, USA).

Q4. Line 114 - Which concentrations of Oxilipin were used?;

R4: The amount of 0.3-40 µg/ml were used that were added.

Q5. Line 196 – Fraction 5 is a mistake? Fraction 4 has the best anticancer activity, as you mentioned previously;

R5: Thank you for your comment, it was changed to peak 5 (P5).

Q6. Line 323 – The species name must be italicized;

R6: Well done: Daboia russelli russelli

Q7. Line 456 – Reference 26. is of a different font.

R7: It was corrected.

2 Revision 1

0 comments

No public comments in this round.

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Revision 2

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 Respected Reviewer,

Grammatical corrections and final edition in the revised manuscript 129616 were done as following:

Line 6: “necessitated” changed to “necessary”.

Line 7: “With respect to the above” changed to “Concerning the aforementioned” and “the purpose of” was added.

Line 12: “Oxilipin, an 11 kDa protein” changed to “Oxilipin, a 13 11kDa protein”

Line 16: “The amount of 40 µg/ml concentration of toxin” changed to “The amount of 40 µg/ml of Oxilipin”

Line 21: “paptide” changed to “peptide”

Line 27: “More than 1.93 new cases of colon cancer are reported globally.” Changed to “There are annually more than 1.93 million newly reported cases of colon cancer worldwide.”

Line 29: “A large variety” changed to “A wide variety”

Line 30: “can” changed to “have the potential to”

Lines 31-32: “mainly originating from the large intestine's internal 32 lining” changed to “coming primarily from the inner wall of the large intestine.”

Line 32: “non-cancerous” changed to “not 33 cancerous”

Lines 33-34: “conventional approaches to cancer treatment” changed to: “are traditional methods of treating cancer”

Line 34: “Generally,” was deleted

Lines 34-35: “the main method of 35 cancer treatment.” Changed to “usually the primary method for treating cancer.”

Line 35: “cancerous cells are” changed to “cancer cells become”

Line 36: “lead to a hard” changed to “present a major”

Line 39: “Concerning” changed to “As regards”

Line 40: “can act” changed to: “may be:

Line 42: “one of the precious sources of” changed to: “a valuable source of new drug”

Line 43: “During the last” changed to: “Over the past”

Line 44: “drug lead regarding” changed to “pharmaceutical lead with respect to”

Lines 44-45: “in comparison” changed to “compared”

Lines 43-46: “anticancer peptides (ACPs), have 44 successfully presented as potential drug lead regarding their low toxicity in 45 comparison to chemical agents (7, 10). 46 During the past decades,” was removed.

Line 49: “14” changed to “12”

Line 50: “These two” changed to”Both”

Lines 50-52: “cancerous cell lines of HepG2, MCF7, P and DU145.” Changed to “HepG2, MCF7, P and DU145 cancer cell lines.”

Line 52: “14” changed to “12”

Line 281: “(17, 18)” changed to “(15-16)

Lines 283-284: “(19-21) changed to “17-18)

Line 285: “22” changed to “19”

Line 288: “and nominated as Oxilipin.” Was added.

Line 302: “(14)” changed to “12”

Line 304: “(23-25)” changed to “(20-21)

Line 306: “(26)” changed to “(22)”

Lines 306-310: “A study conducted by Castro Damsio and colleagues on colon cancer cells in 2014 found that the lectin BJcuL of Bothrops jararacussa snake venom induces apoptosis through mitochondrial membrane permeability in HT29 cell colon adenocarcinoma cell line (27)” was deleted.

Line 312: “(28)” changed to “(23)”

Lines 314-316: “The crude venom of Naja naja oxiana can induce cytotoxicity and apoptosis in different cancer cell lines in a dose-dependent manner.” Was deleted.

Line 318 “(15)” changed to “(13)”

Line 322: “(24) was added.

Line 322: “(24” was added

Lines 329-330: “The release of LDH into the culture medium is used as a marker to investigate the cytotoxicity of cytotoxins (29-30).” Was deleted.

Line 335: “(14)” changed to “(12)”

Lines 387-398” Ref 11-13 were deleted.

Lines 424-427: Ref 22 was deleted.

Line 440: Ref 23 “Zakraoui O, Marcinkiewicz C, Aloui Z, Othman H, Grépin R, Haoues M, et al. A snake venom disintegrin, suppresses human colon cancer cells proliferation and tumor-induced angiogenesis through cell cycle arrest, apoptosis induction and inhibition of VEGF expression. Mol Carcinog. 2017 Jan;56(1):18-35. doi: 10.1002/mc.22470. [PubMed: 26824338].” Was added.

Lines 445-460: Ref 27-30 were deleted.

3 Revision 2

0 comments

No public comments in this round.