For decades, venoms have been emerging as a new source of therapeutic leads, bioinsecticides and pharmaceutical tools. To date, six venom-derived drugs and one venom-derived insecticide are approved and are now available on the market. Ant venoms exhibit a complex molecular diversity and harbor a great potential for the discovery of new bioactive molecules, although they remain understudied in comparison with other animal venoms. Also, recent studies revealed a broad array of biological effects such as antimicrobial, anti-inflammatory, ion channel modulation1, 9. Herein, we focused on Myrmex-Tb1a (i.e. Bicarinalin) and U9-MYRTX-Tb1a, two venom peptides characterized from the venom of the ant Tetramorium bicarinatum, displaying low amino acid sequence identity but sharing similar physicochemical properties.

Previous fundamental investigations revealed that Bicarinalin is an amphipathic α-helical peptide able to form pores in bacterial membranes1. In order to assess the potential of ant venoms for the discovery of new bioactive peptides, we investigated the biological activity of Bicarinalin and U9 on a D. melanogaster cell line. Considering the physicochemical properties of both toxins, we hypothesized that they display similar biological effects and mode of action, even though their predicted three-dimensional structure are different. Our preliminary results confirmed that Bicarinalin is a membrane-active peptide and suggest that U9 display a cytotoxic effect through a different pathway.

Materials and Methods

LC-MS analysis - The venom composition was analyzed by LC-MS (LCQ- ion trap Advantage equipped with an ESI-LC system). Peptides were separated using a Luna-C18 column and eluted using a mobile phase composed of 0.1% aqueous formic acid and 0.1% formic acid in acetonitrile.

Cell culture - S2 cell line of D. melanogaster (ThermoFisher) was maintained in Schneider’s insect medium (+ 10% Fetal Bovine Serum, + 1% Penicillin/Streptomycin) at 25°C without CO2.

Peptide synthesis - Peptides were chemically synthesized by GenScript® with C-terminal amidation.

Cytotoxicity assays - Cells were counted by using trypsin blue dye exclusion. They were then seeded in 96-well plates at a density of 2.10^4 and 6.10^3 cells/mL and incubated at 25°C for 24h before being treated with Bicarinalin or U9 for 24h. In order to assess their effect on cell viability, the absorbance was measured after performing of CCK-8 (450nm) and LDH (490 nm) assays at 2.10^3 and 6.10^3 cells/mL densities, respectively.

Image kinetic of peptide effect - Cells were treated with 50 μM of peptides and photos were acquired every second for 5 min (Bicarinalin) and every 5 seconds for 60 min (U9). Absorbance measurements and photos were acquired with the Biotek Cyto 4 (Biotek®).

Presence of a turn in predicted U9 structure

Different mode of action between U9 and Bicarinalin

Conclusion

- Conserved prepropeptide sequences, similar physicochemical features of mature regions, and predicted 3D structures dominated by α-helices with the presence of a turn for U9 were observed.
- Two distinct IC50 were observed for U9, suggesting of cytotoxic effect at low concentration without membrane disruption.
- U9 induced an increase in S2 cell volume, a condensation of cell nucleus followed by its expulsion from the cell.
- Several pathways including autophagy and apoptosis was investigated to further explain this unusual observation.

References


Acknowledgments

This work was supported by a regional funding of Occitane. We are grateful to all people providing me with technical support for this work and particularly Arthur Luhar from DGRC for his numerous advices on drosophila cells culture.