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Bradykinin in *Hemipepsis ustulata:* a novel method for safely milking wasps

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Abstract

Wasp venom characterization is of interest across multiple disciplines such as medicinal chemistry and evolutionary biology. A simple method is described herein to milk wasp venom without undue risks to the researcher. The wasps were immobilized by cooling for safe handling, restrained, and their venom was collected on parafilm. Bradykinin from *Hemipepsis ustulata* was identified by LC-MS/MS during method verification.

Keywords

Wasp; bradykinin; venom; milking; *Hemipepsis ustulata*; LC-MS/MS

Wasp venoms have been affected by evolutionary processes based on their changing molecular targets and contain a variety of biologically active substances (Lee et al., 2016) that help define metazoan taxa. In particular, solitary wasps contain neurotoxins such as bradykinins and pompilidotoxins that attack sodium channels, as well as a myriad of other diverse functioning compounds (Palma, 2006). Exploration of venom across families is important for biomedical research (lead drug candidates), although only a few species of solitary wasps have been studied (Konno et al., 2002; Baek et al., 2015). Methods of venom collection for social Hymenopterans (Benton et al.,1963) cannot be applied to solitary wasps. This short communication focuses on a novel milking technique suitable for large-sized, solitary wasps.

The method was tested on three individuals of *Hemipepsis ustulata*-large wasps (up to 5 cm) with rust colored wings and iridescent blue bodies. *Hemipepsis ustulata* are ectoparasites that use paralyzed spiders as a food source for their young. These wasps are not aggressive, in general, but their sting has a fierce reputation (Schmidt, 2004) causing extreme, but short-lived pain. Although they are distributed worldwide, they are particularly well known in the southwest U.S., where they enjoy the status of being the state insect of New Mexico.

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The safest method for venom collection involves killing the insect and removing the venom sac (Piek, 1986). This method has the disadvantage of producing a more complex sample than that acquired from an external secretion. Three other methods in the literature describe external venom collection, but all of these unnecessarily subject the researcher to the risk of being stung. For instance, one method has the researcher using a piece of tape on their finger to try to immobilize the active wasp by contact with its wings and then crushing the head with a pin (Deyrup and Matthews, 2003). This method also carries the limitation of a one-time milking. Another method suggests small wasps, such as jewel wasps (*Ampulex compressa*), can be milked by forcing them stinger first into a narrow tube with a hole at the end and then confined with a piston that serves also as a source of blunt trauma to induce venom secretion (Mukhopadhyay, 2014). Other researchers suggest picking up the wasp between the thumb and forefinger (Sahayaraj et al., 2006). Most recently, an agar block-based method has been published (Bhagavathula, et al., 2016), but collecting the venom in agar is unnecessary and introduces an extra extraction step. The novel method described here uses a reduced-temperature incubation to induce a state of immobilization to limit the exposure of the researcher to being stung. Parafilm is used to receive the venom droplets.

Specimens were collected in Grant County, NM in the City of Rocks State Park and from the campus of the Chiricahua Desert Museum in Rodeo, NM. The motor function of the wasps was reduced by placing the cage in a refrigerator at 4 °C. The wasp was checked on every 2 minutes until immobilized and then removed from the refrigerator. At this point, the wasp was handled with tongs and its wings clipped (the wing venation was preserved for identification purposes), an operation which facilitates ease of handling after the wasp reinvigorates. Narrow strips of duct tape were used to secure the wasp in a supine position on a suitably-sized tube. The platform was securely taped down to the work surface to avoid the unexpected movement of the stinger during milking. The wasp was ready for milking after 5-10 min.

At this point, a piece of parafilm (4×6 cm) was held over the end of the abdomen to collect venom while the head of the wasp was pressed on without crushing to induce secretion (Fig. 1). At this point, the stinger (up to 6 mm long) was easily observed and the parafilm was moved in contact with it. The stinger punctured the parafilm and deposited venom on the top side. The droplets were easily observed and were collected rapidly using a micropipetter and a small amount of water (5-10 μL) to help dissolve the venom. After milking, the wasp was immobilized again using the refrigeration protocol. The tape was then carefully removed and the wasp returned to its cage. Cut grapes and wet paper towels were provided to sustain the wasps during captivity. This process was repeated for 2-4 days before the cumulative effect of the procedure became fatal.

The droplets secreted by *Hemipepsis* were around 1 μL with about 4-8 droplets able to be collected in a single session. Venom samples were purified and preconcentrated with C-18 spin columns, lyophilized, and stored frozen until the time of analysis. Thawed venom was redissolved in 20 μL of 0.1 % formic acid solution and a trypsin digestion was performed to reduce coelution issues with proteins. A sample injection of 2 μL was then analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Q Exactive mass spectrometer with an Easy NanoLC-1000 system (Thermo Fisher Scientific, U.S.A).

Separation was conducted using a C18 column (75 μm x 150 mm). Reverse phase chromatography was performed with (A) 0.1 % formic acid/water and (B) 0.1 % formic acid/acetonitrile. A four-step, linear gradient was used for the LC separation (column pre-equilibration with 2% B for 10 min; 2% to 30% B in the first 47 min.; followed by 80% B for 13 min.). Bradykinin was targeted in the data sets since bradykinin and related compounds have been found in other solitary wasp species (Konno et al., 2002; Picolo et al., 2010).

These samples were concentrated enough to generate a large number of peaks in the main chromatogram (Fig. 1). A mass range filter from 530.77-530.79 amu was applied to the total ion chromatogram (Fig. 2a) to search for bradykinin (RPPGFSPFR), resulting in a strong peak at retention time 15.6 min. A match for the parent ion (MH^{2+}_2) at monoisotopic mass 530.787 amu was found (Fig. 2b) as the base peak in the mass spectrum (the online resource from University of California San Francisco, Protein Prospector, was used to calculate the theoretical value of the bradykinin peak at 530.788 amu). The doubly-charged state was deduced from the 0.5 amu spacing of the envelope.

This paper presents a novel, effective (validated by LC-MS/MS) approach for milking large wasps safely without the need for an agar block receiver. In addition, this method can provide multiple samples from a single individual over time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Highlights

- A novel method of venom collection from wasps is presented
- The method is safe and can be used multiple times on a single individual
- Bradykinin is identified in *Hemipepsis ustulata* by LC-MS/MS

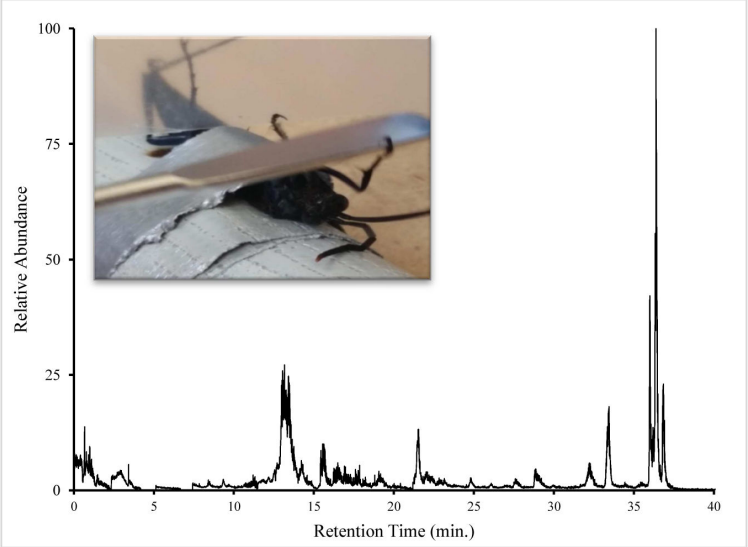

Figure 1.

Total ion chromatogram for *Hemipepsis ustulata* venom after trypsinolysis. Wasps were restrained in a supine position and agitated to sting parafilm.

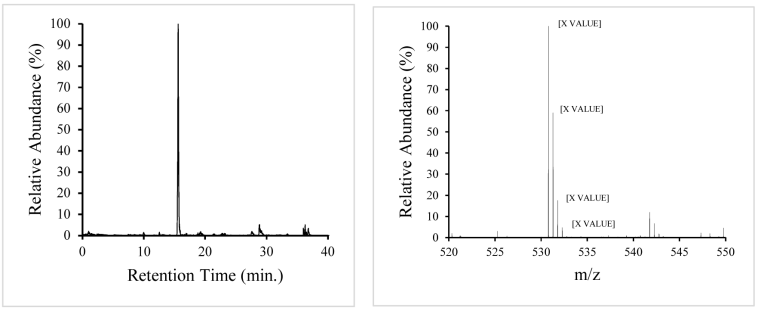

Figure 2.

a Selected ion chromatogram for bradykinin at mass 530.79 (±0.5) amu.

b. Mass spectrum for retention time 15.6 min. (bradykinin) showing the isotopic envelope for the doubly-charged, parent MH^{2+}_2 peak.