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Evaluation of the antimicrobial activity of the mastoparan Polybia-MPII isolated from venom of the social wasp *Pseudopolybia vespiceps testacea* (Vespidae, Hymenoptera)

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ABSTRACT

Mastoparans, a class of peptides found in wasp venom, have significant effects following a sting as well as useful applications in clinical practice. Among these is their potential use in the control of micro-organisms that cause infectious diseases with a significant impact on society. Thus, the present study describes the isolation and identification of a mastoparan peptide from the venom of the social wasp *Pseudopolybia vespiceps* and evaluated its antimicrobial profile against bacteria (*Staphylococcus aureus* and *Mycobacterium abscessus* subsp. *massiliense*), fungi (*Candida albicans* and *Cryptococcus neoformans*) and in vivo *S. aureus* infection. The membrane pore-forming ability was also assessed. The mastoparan reduced in vitro and ex vivo mycobacterial growth by 80% at 12.5 μM in infected peritoneal macrophages but did not affect the shape of bacterial cells at the dose tested (6.25 μM). The peptide also showed potent action against *S. aureus* in vitro (EC_{50} and EC_{90} values of 1.83 μM and 2.90 μM, respectively) and reduced the in vivo bacterial load after 6 days of topical treatment (5 mg/kg). Antifungal activity was significant, with EC_{50} and EC_{90} values of 12.9 μM and 15.3 μM, respectively, for *C. albicans*, and 11 μM and 22.70 μM, respectively, for *C. neoformans*. Peptides are currently attracting interest for their potential in the design of antimicrobial drugs, particularly due to the difficulty of micro-organisms in developing resistance to them. In this respect, Polybia-MPII proved to be highly effective, with a lower haemolysis rate compared with peptides of the same family.

1. Introduction

Wasps possess highly toxic venom that contains a cocktail of pharmacologically active substances, including biogenic amines, polyamine toxins, and high- and low-molecular-mass proteins such as enzymes, allergens and bioactive peptides [1–3]. Moreover, ca. 70% of the dry weight of social wasp venom is composed mainly of peptides [4], representing important biotools in defending the colony against predators and prey capture. Antimicrobial peptides (AMPs) are part of a wasp's highly conserved innate immune system, thus promoting powerful action in the control of infections caused by bacteria, fungi, viruses and parasites [5,6]. Although pathogenic bacteria have evolved mechanisms to circumvent the effects of AMPs, they are still considered an indispensable and significant barrier [7,8].

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The majority of AMPs isolated from social wasp venom and extensively studied belong to the mastoparan and chemotactic classes [2,3,9]. They are formed primarily by polycationic peptides composed of 14 amino acid residues with an amidated C-terminal leucyl residue, two to four lysinyl residues, complete absence of cysteinyl residues, and a secondary α-helix conformation in the presence of lipid bilayers [10]. Mastoparans are directly involved in several pathophysiological sting effects, such as mast cell degranulation, inflammation, cell membrane lysis, haemolysis, platelet activation and histamine release (for a review, see References [1,11]). The multifunctional properties of mastoparans are due in part to their ability to mimic G protein-coupled receptors, activating these proteins, and to produce pores in lipid membranes [1,3,12].

Furthermore, mastoparans also show potent antimicrobial activity against Gram-positive and Gram-negative bacteria, a broad spectrum of antiviral effects, and powerful antitumour activity [13–16]. A number of authors have also reported the ability of mastoparans to translocate biological membranes, classifying them as cell-penetrating peptides, which are useful in the development of carriers (transportan) to intracellular sites or even across the blood–brain barrier [17,18]. The use of mastoparans alone or in combination with classic antibiotics may be a good therapeutic alternative to control multidrug-resistant infections in clinical practice [3,19]. In this respect, the present study describes the isolation and identification of a mastoparan peptide from the Neotropical social wasp *Pseudopolybia vespiceps* and evaluated the antimicrobial profile against bacteria (*Mycobacterium abscessus* subsp. *massiliense* and *Staphylococcus aureus*), fungi (*Candida albicans* and *Cryptococcus neoformans*) and in vivo infection by *S. aureus*. Interaction with the lipid bilayer was also assessed to detect its possible mechanism of action.

2. Materials and methods

2.1. Specimen collection and venom extraction

P. vespiceps females were collected in Brasília (Brazil) and were identified by Prof. Fernando B. Noll [Universidade Estadual Paulista (UNESP), São Paulo, Brazil]. Following euthanasia by freezing (–20 °C), a total of 50 venom sacs were dissected, macerated in a 1:1 acetonitrile/deionised water (ACN/H_2O) solution and centrifuged at 5000 × *g* for 10 min at 4 °C. The supernatant was collected and was filtered using an ultrafilter (Millipore, Billerica, MA) with a 3 kDa cut-off for 30 min at 5000 × *g*.

2.2. Venom fractionation, peptide purification and identification

The filtrated extract was lyophilised, weighed and re-suspended in 1:1 ACN/H_2O containing 0.07% trifluoroacetic acid (TFA) and was submitted to reverse-phase high-performance liquid chromatography (RP-HPLC) (C_{18} ODS; Phenomenex, Torrance, CA) at a flow rate of 5.0 mL/min and a linear gradient elution of 5% ACN/H_2O (v/v) (containing 0.07% TFA) for 20 min, followed by 5%–60% for 40 min. Absorbance was monitored at 216 nm and 280 nm.

2.2.1. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/TOF MS)

The peptide of interest was submitted to MALDI-TOF/TOF MS (UltraFlex III; Bruker Daltonik GmbH, Bremen, Germany) under reflector (MS) and LIFT™ (MS/MS) positive modes to determine purity and identity, respectively. Similarity searches were performed using BLASTP and FASTA, and the ClustalO alignment program for sequences comparison.

2.2.2. Peptide synthesis

After the amino acid sequence was determined, the peptide was synthesised by AminoTech Development and Technology (Diadema, SP, Brazil) using the F-MOC stepwise solid phase (N-9-fluorophenylmethoxy-carbonyl) strategy.

2.3. Antimicrobial assays

In vitro antimicrobial assays were performed with the bacterial species *M. abscessus* subsp. *massiliense* (GO06) and *S. aureus* (ATCC 29213) and the fungal species *C. albicans* (SC5314) and *C. neoformans* var. *grubii* (serotype A, H99). For each biological assay, serial dilution of the peptide was performed using sterilised saline. Moreover, all experiments were conducted in triplicate and according to Clinical and Laboratory Standards Institute (CLSI) guidelines [20] with some modifications.

2.3.1. Antibacterial activity

Prior to the assay, *M. abscessus* was grown overnight at 37 °C in 5 mL of Mueller–Hinton broth (MHB) for 5 days. An aliquot of 100 μL of bacteria was suspended in 5 mL of MHB (1:50 dilution) and was incubated at 37 °C for 3–5 days until mid-log phase of the culture. The mid-log phase was adjusted to obtain an inoculum of 1500 CFU/mL and then 50 μL of this suspension was incubated with different concentrations of the peptide (1.5, 12.5 and 100 μM) for 24 h. Next, each well was diluted in phosphate-buffered saline (PBS) (1:3 dilution) and then 50 μL of this suspension was plated on Mueller–Hinton agar (MHA) for 3–5 days [21]. Clarithromycin was used as a positive control.

Scanning electron microscopy (SEM) was performed to determine whether the mycobactericidal effect of the peptide could alter the shape of the cell envelope. Colonies grown on MHA were removed from the plates and were exposed to 6.25 μM of the peptide for 24 h. Following incubation, cells were fixed with modified Karnovsky solution (1% paraformaldehyde and 3% glutaraldehyde in 0.07 M cacodylate buffer, pH 7.2) for 30 min at 4 °C. The fixative solution was subsequently removed and the samples were dehydrated by increasing concentrations of ethanol (30%, 50%, 70%, 90% and 100%) for 10 min, followed by acetone and hexamethyldisilazane (HMDS) (v/v) for an additional 5 min. Images were obtained using a JEOL JSM-6610 microscope (JEOL, Akishima, Japan) equipped with an energy dispersive spectrometer (NSS Spectral Imaging; Thermo Fisher Scientific, Waltham, MA).

For the *S. aureus* strain, an aliquot from a frozen stock was plated on MHA. Following 24 h of agar plate incubation, three isolated colonies were transferred to 1 mL of MHB. The broth culture was incubated overnight (12–16 h) at 37 °C with agitation. To evaluate bacterial growth, the microdilution technique in MHB was used with an initial inoculum of 5×10^5 of *S. aureus* cells in untreated polystyrene microtitre plates.

2.3.2. Antifungal activity

C. albicans and *C. neoformans* were maintained in Sabouraud dextrose broth (SDB) supplemented with 35% glycerol. For experiments with *C. albicans*, cells were grown overnight in SDB at 30 °C in a shaker at 150–200 × *g*. For *C. neoformans*, an aliquot was streaked on Sabouraud dextrose agar (SDA) plates for single-colony isolation and was incubated at 30 °C for 48 h. Finally, a single colony was inoculated in SDB and was grown for 24 h at 30 °C in a shaker at 200 × *g*. Following incubation, cells were suspended in RPMI-1640 medium (Invitrogen Corp., Carlsbad, CA) supplemented with 0.165 M MOPS [3-(N-morpholino)propanesulphonic acid].

Peptide solution was added to the wells (50 μL/well to final concentrations of 6–24 μM for *C. albicans* and 2–22 μM for *C. neoformans*). Following inoculation (final volume of 100 μL/well, 2×10^3 CFU/mL for *C. albicans* or 10^4 CFU/mL for *C. neoformans*), the