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ORIGINAL ARTICLE

Antinociceptive Activity of the Peptide Fraction from the Venom of Social Wasp *Pseudopolybia vespiceps* Testacea

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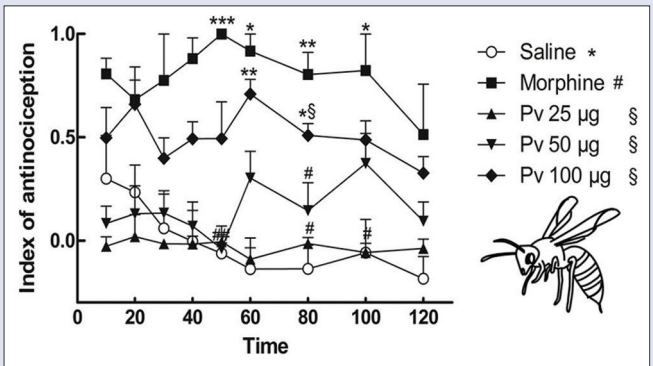
ABSTRACT

Background: Chronic pain is an urgent medical problem worldwide, resulting in long-term sick leave, low quality of life, and high socioeconomic costs. Despite the physiological, emotional, and financial burden of chronic pain, there is still a lack of efficient treatments. In this context, toxins isolated from arthropods are considered powerful tools because they have targets that are compatible with the impulse transmission of pain and can provide an interesting alternative in the development of more efficient analgesic treatments. Wasps are arthropods with potent venom that contains a complex mixture of compounds, including biogenic amines, proteins, and peptides. For decades, chemical identification and biological characterization of wasp venom peptides have accumulated considerable attention from researchers. **Objective:** The objective of this work was to evaluate the antinociceptive activity of the peptide fraction (F_{pep}) from low-molecular-weight compounds (LMWCs) from the venom of the Brazilian social wasp *Pseudopolybia vespiceps testacea*. **Materials and Methods:** *P. vespiceps* females were collected, and after euthanasia by freezing (–20°C), a total of 148 venom sacs were used to acquire only compounds with LMWCs. The antinociceptive effect of the F_{pep} from LMWCs was observed by two thermal tests: tail flick and hot plate measured in male *Swiss* mice. **Results:** F_{pep} showed dose-dependent antinociceptive activity in mice submitted to intracerebroventricular injection in two different models. **Conclusion:** These results revealed the significant potential impact of the LMWCs of the venom of *Pseudopolybia vespiceps testacea*, including neuroactive peptides that can be used as pharmacological resources for antinociceptive drug research.

Key words: Antinociception, pain, peptides, toxin, wasp venom

SUMMARY

- The venom from wasp *Pseudopolybia vespiceps* testacea has neuroactive compounds
- Peptide fraction from the venom of *P. vespiceps* induced a dose-dependent antinociceptive effect in the tail flick
- Antinociceptive effect was also observed in the hot-plate test
- Three peptides with antinociceptive effect were found in social wasp venom from previously reported literature.



Peptide fraction from the venom of *P. vespiceps* (Pv) induced a dose-dependent antinociceptive effect in the tail flick test after intracerebroventricular injection.

Abbreviations used: ACN/H_2O: Acetonitrile/deionized water; CEUA: Committee for Ethics in Animal Use; CNS: Central nervous system; F_{pep}: Peptide fraction; i.c.v: Intracerebroventricular; LMWCs: Low-molecular-weight compounds; *P. vespiceps*: *Pseudopolybia vespiceps testacea*.

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INTRODUCTION

Pain is defined by the International Association for the Study of Pain as "an unpleasant sensory or emotional experience associated with actual or potential tissue damage."[1,2] Nowadays, pain is described as a multidimensional entity with the equal involvement of the central nervous system (CNS), involving the perception of nociception, emotion, and cognition.[2]

Chronic pain is an urgent medical problem worldwide, resulting in long-term sick leave, low quality of life, and high socioeconomic costs.[3-6] Estimates suggest that one-fifth of the world's adult population suffer from pain, and 10% are newly diagnosed with chronic pain every year.[7]

Despite the physiological, emotional, and financial burden of chronic pain, there is still a lack of efficient treatments.[8] Use of analgesics, particularly opioids, is the foundation of treatment for most types of pain; although in many cases, especially in patients with neuropathic pain, more aggressive treatments are needed, and only 30%–50% of patients achieve clinically significant pain relief.[9,10] Moreover, side effects of these therapies often limit their usefulness.[10]

Unrelieved pain affects the psychological state of the patient and family members. A large gap exists between the increasingly sophisticated

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comprehension of the pathophysiology of pain and the extensive inadequacy of its treatments.[11,12]

In this context, toxins isolated from arthropods are considered powerful tools because they have targets that are compatible with the impulse transmission of pain and can provide an interesting alternative in the development of more efficient analgesic treatments.[13]

Wasps are arthropods with potent venom capable of paralyzing prey that belong to the *Hymenoptera* order. Their venom contains a complex mixture of compounds, including biogenic amines, proteins, and peptides, which are answerable for a range of biological activities, such as antimicrobial, anxiolytic, allergic reactions, hemolytic effects, anticonvulsant, antinociceptive, and others.[14-16]

As regards peptides, they are responsible for most of the activities described in wasp venom, representing 70% of the dry weight. The small size of these molecules enables them to interact with different cell types and tissues, capable of causing muscle contraction, hypertension, cell lysis, and polymorphonuclear cell chemotaxis.[14] For decades, the chemical identification and biological characterization of wasp venom peptides have accumulated considerable attention from researchers. Peptides with neuroactive profile isolated from these venoms when applied directly to the CNS of mammals demonstrate potent antinociceptive, anxiolytic, anticonvulsant, and antipanic effects.[17-22]

Pseudopolybia vespiceps testacea is a genus of neotropical swarm-founding wasps (tribe *Epiponini*) and belongs to the family *Polistinae*.[23] The genus comprises four species, which are widely distributed from Nicaragua to southern Brazil, and they are found mainly in rainforest.[23-25] In 2017, Silva *et al.* described the isolation and identification of a mastoparan peptide from the venom of the social wasp *P. vespiceps* and evaluated its antimicrobial profile.[26] However, the venom of this wasp has not been evaluated for the presence of neuroactive compounds. In this respect, the objective of this work was to evaluate the antinociceptive activity of low-molecular-weight compounds (LMWCs) (peptide fraction) from the venom of the Brazilian social wasp *Pseudopolybia vespiceps testacea*.

MATERIALS AND METHODS

Wasp collection and venom extraction

P. vespiceps females were collected in accordance with the normative instruction nº 154 from the Brazilian Institute of the Environment (IBAMA) and authorized by Chico Mendes Institute for Biodiversity Conservation of Brazil (license number 21723-1, date of issue 10/27/2009) in Brasília (Brazil) and were identified by Prof. Fernando B. Noll (Universidade Estadual Paulista, São Paulo, Brazil). After euthanasia by freezing (−20°C), a total of 148 venom sacs were dissected, macerated in a 1:1 acetonitrile/deionized water solution, and centrifuged at 5000 ×*g* for 10 min at 4°C. The material obtained was filtered using an ultrafilter (Millipore, Billerica, MA) with a 3 kDa cutoff for 30 min at 5000 ×*g*, in order to acquire only LMWCs. Finally, the LMWC samples were lyophilized, quantified, and stocked at −80ºC until use.

Animal care

Animals were preserved in accordance with the Ethical Principles on Animal Experimentation stipulated by the Brazilian National Council for the Control of Animal Experimentation (BRASIL, 2008), the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised in 1978), and the Brazilian Arouca Law (No. 11.794/2008).[27] All experimental procedures were approved by the Committee for Ethics in Animal Use of the University of Brasília, under license number 63878/2011.

Surgery

Male *Swiss* mice (16–22 g) were anesthetized intraperitoneally (i.p.) with ketamine hydrochloride (60 mg/kg, Syntec, Brazil) and xylazine hydrochloride (10 mg/kg, Syntec, Brazil) and fixed in a stereotactic frame (Insight Equipments®, Brazil). After a subcutaneous (s.c.) local injection (0.05 mL) of lidocaine hydrochloride (30 mg/mL) with norepinephrine hemitartrate (0.04 mg/mL) (Lidostesim® 3%, Dentsply, Brazil), the cranium was exposed to implant a stainless steel cannula (10 mm long and 0.7 mm in external diameter, fixed with dental acrylate) in the lateral ventricle, using the following coordinates: 0.8 mm posterior to bregma, 1.6 mm lateral from the midline, and 3.4 mm ventral from the surface of the skull according to the atlas of Paxinos and Watson.[28] After 4–6 days of recovery, the bioassays were performed.

Independent groups of animals (n = 4–6) received an intracerebroventricular (i.c.v.) injection with F_{pep} at 25, 50, and 100 µg/animal for tail-flick test and 50 and 100 µg/animal for hot-plate test (we choose these two doses because the lowest dose did not show antinociceptive effect on tail-flick test and the amount of F_{pep} was limited), with the aid of a Hamilton syringe driven by an infusion pump (Harvard Apparatus), injecting a volume of 1 µl over 1 min. Morphine (12 nmol) and saline (NaCl – 150 mM) were also administrated through i.c.v.

Antinociceptive assays

The antinociceptive effect of the F_{pep} was observed by two thermal tests, namely tail flick and hot plate.

For the hot-plate test, experiments were carried out based on the protocol described by Bannon and Malmberg.[29] Animals were placed on an aluminum hot plate (AVS Projetos®, Brazil) at a temperature of 55.5°C ± 0.5°C. The latency until they jumped or licked their hind paws was recorded. Before the test, the mice were evaluated, and a 16-s cutoff time was established. Those who did not respond to the thermal stimulus within this time range were not used in the experiment. Prior to F_{pep} administration, basal responses (mean of three latencies at 5-min intervals) were obtained from the mice.[30] Escape latencies were recorded after treatment, at the following time intervals: 20, 40, 60, 90, and 120 min.

All motor response latencies (antinociception latencies [ALs]) were normalized by the Analgesia Nociception Index (AI) using the following equation:

$$\frac{AL - BML}{TL - BML}$$

Where BML: basal mean latency and TL: time limit of the experiment (50 s). The results were expressed as AI averages ± standard error of mean and area under the curve (AUC).

In the tail-flick test, the assays were carried out according to the instruction manual for the equipment and in line with the protocol described by Bannon and Malmberg, with modifications.[29] The mouse tails were positioned on the top of a Ni-chrome filament on digital analgesimeter (Insight®, Brazil), which heats at a rate of 10°C/s, reaching a maximum temperature of 75°C (limit for the equipment). Prior to treatment, basal measurements (three times, at 5-min intervals) were performed. Escape latencies were recorded after treatment at the following intervals: 20, 40, 60, 90, and 120 min.

Histology

To verify the correct positioning of the cannula, at the end of the experiments, animals were euthanized by an inhalation of carbon monoxide (CO_2) inside a chamber (insight, Brazil) and injected i.c.v.

with 3 µL of methylene blue (Anachemia®, Canada) through the cannula to check the exact site of injection. The brain was then removed and fixed in 4% formaldehyde solution. Appropriately located cannulas were considered to be those that would enable a complete blue coloration of the lateral ventricles. Data from mice with guide cannula tips located outside the lateral ventricle were not included in behavioral and statistical analyses.

Statistical analysis

Results with normal distribution were submitted to two-way repeated measures analysis of variance (two-way ANOVA) and the Bonferroni correction. The AUC was analyzed by one-way ANOVA, with $P < 0.05$, followed by Tukey's test. All statistical analyses were performed using GraphPad Prism® software version 6.0 for Mac (GraphPad Software, Inc., USA).

RESULTS

The F_{pep} from the venom of *P. vespiceps* induced a dose-dependent antinociceptive effect in the tail-flick test after i.c.v. administration ($F_{(4,17)} = 8.752$; $P < 0.0004$) [Figure 1]. Significant differences in time ($F_{(9,21)} = 7.27$; $P < 0.0001$) and treatment-versus-time interaction ($F_{(36,79)} = 1.612$; $P < 0.02$) were observed. One-way ANOVA showed a significant effect for the treatment at the highest dose (100 µg/animal) in 60 and 80 min of the test when compared to animals treated with saline.

A tendency of dose-dependent response was also observed in the AUC for tail-flick test ($F_{(4,14)} = 17.31$; $P < 0.0001$). Another result that can be observed from this graph is the similarity between morphine and the LMWCs (Pv) at the dose of 100 µg/animal [Figure 2].

An antinociceptive effect was also observed in the hot-plate test. There were significant effects of treatment ($F_{(4,17)} = 57.49$; $P < 0.0001$) and treatment-versus-time interaction ($F_{(4,17)} = 2.417$; $P < 0.0018$). One-way ANOVA showed that the highest dose produced significantly increased hot-plate latencies of the mice when compared to the saline group [Figure 3]. The AUCs from the hot-plate test revealed significant differences at the dose of 100 µg/animal in comparison to its lowest dose, as well as compared to the control animals ($F_{(4,17)} = 91.61$; $P < 0.0018$) [Figure 4].

DISCUSSION

Chronic pain is a highly prevalent condition for which there is an absence of effective treatments.[8] Since the isolation of morphine from opium in 1800, the first treatment for chronic pain targeting the opioid receptors in the CNS has been the use of both natural and synthetically produced opioids.[31] Despite that, this treatment produces many side effects that contribute to the reduced quality of life among chronic pain patients, and it lacks long-term efficacy.[32]

Representing a source of neuroactive compounds that can be useful tools in neuroscience and pharmacological investigations, arthropod venoms are considered powerful tools and have attracted interest from researchers because they have congruent targets of the impulse transmission of pain and may provide an attractive alternative to opioid treatments.[33]

Wasp venom contains a cocktail of substances such as biogenic amines (<1 kDa), peptides (1 kDa–10 kDa), and proteins (>10 kDa), which are responsible for several biological activities from the venom.[34]

Peptides have distinct functions and a significant presence in wasp venom. They are divided into classes according to their most characteristic biological activity, including kinins, chemotaxic peptides, antimicrobials, neuroactive peptides, and mastoparans.[35]

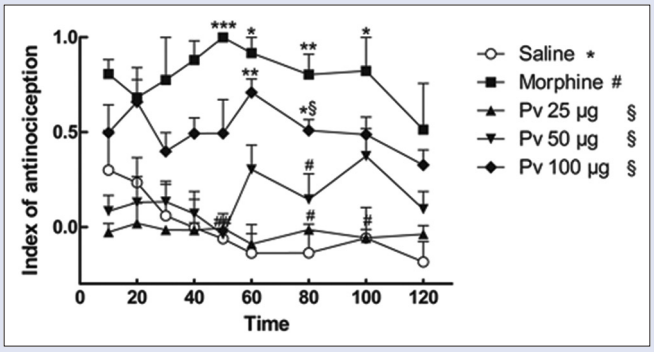

Figure 1: Tail-flick times after intracerebroventricular injections of low-molecular-weight compound, morphine or saline in mice. (a) Time course of the increase in latencies in the tail-flick test induced by intracerebroventricular injection of low-molecular-weight compound (100, 50, and 25 µg), morphine (20 µg/animal), or saline (NaCl -150 mM). Antinociception index were analyzed by two-way analysis of variance, followed by the Bonferroni post hoc test. *Significant differences compared to saline. #Significant differences compared to morphine; §Significant differences compared to 100-µg dose. *$P < 0.05$, **$P < 0,01$, ***$P < 0,001$

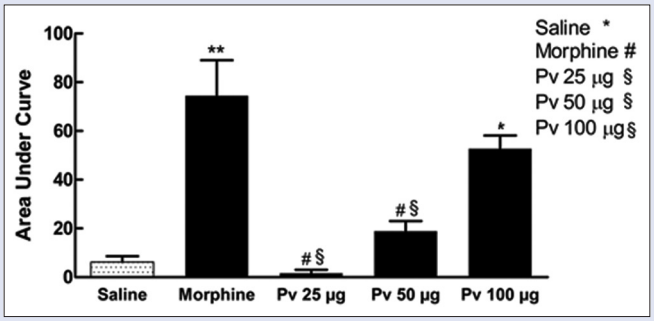

Figure 2: Area under the antinociception index curve. Data were analyzed by analysis of variance, followed by Tukey's post hoc test. *Significant differences compared to saline. #Significant differences compared to morphine; §Significant differences compared to 100-µg dose. **$P < 0,01$, ***$P < 0,001$

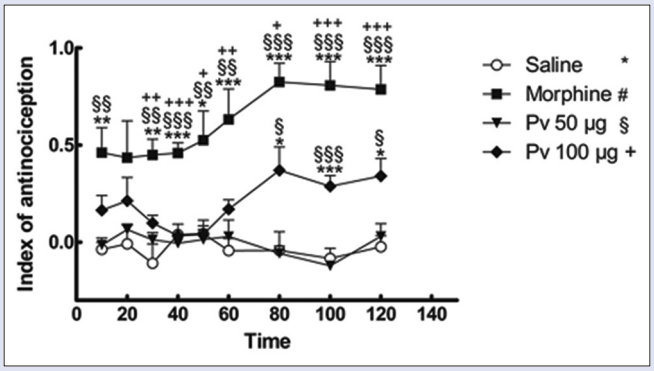

Figure 3: Hot-plate escape latencies after intracerebroventricular administration of low-molecular-weight compound, morphine or saline in mice. (a) Time course of the increase in latencies in the tail-flick test induced by intracerebroventricular injection of low-molecular-weight compound (100 and 50 µg), morphine (20 µg/animal) or saline (NaCl - 150 mM). The Antinociception index were analyzed by two-way analysis of variance, followed by the Bonferroni *post hoc* test. *Significant differences compared to saline $P < 0.05$. §Significant differences compared to 50-µg dose; +Significant differences compared to 100-µg dose. **$P < 0,01$, ***$P < 0,001$. §§($P < 0,01$), §§§($P < 0,001$), ++($P < 0,01$), +++($P < 0,001$)

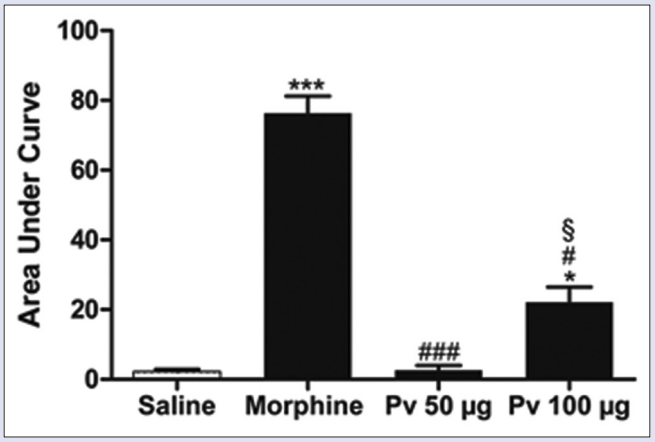

Figure 4: Area under the antinociception index curve. Data were analyzed by analysis of variance, followed by Tukey's *post hoc* test. *Significant differences compared to saline. #Significant differences compared to morphine; §Significant differences compared to 100-µg dose. ***$P < 0{,}001$, ###$P < 0{,}001$

Mastoparans are the most abundant peptide components in wasp venom[36] and are involved in cell membrane lysis, inflammation, mast cell degranulation and histamine release, as well as neutrophil and T-helper chemotaxis.[37] Mastoparans also activate phospholipase A2[38] and G-protein-coupled receptors when administrated through i.c.v., resulting in a long-lasting reduction of the supraspinal antinociception induced by opioids.[39,40]

In this article, we used only the compounds below 3000 Da, called peptide fraction (F_{pep}). F_{pep} presented relevant antinociceptive activity when injected directly into the CNS of mice, in both tail-flick and hot-plate tests. F_{pep} showed activity after 80 min, which lasted until the end of experiment.

Three peptides with antinociceptive effect were found in social wasp venom. Threonine-6 bradikinin (Thr^6–BK), isolated from *Polybia occidentalis* wasp, exerts a dose- and time-dependent antinociceptive effect when injected directly into the CNS of mice in hot-plate and tail-flick tests, and it is three times more potent than morphine and four times more potent than BK in the tail-flick test.[30]

Pallipin-III, isolated from the social wasp *Agelaia pallipes pallipes*, exhibited antinociceptive and anti-inflammatory effects when injected peripherally into mice in the von Frey test.[14]

The mastoparan Agelaia MP I isolated from *Parachartergus fraternus* social wasp venom showed dose-dependent antinociceptive activity in mice submitted to i.c.v. injection in two different models (tail flick and hot plate) and induced partial and reversible blockade of the amplitude of action potential, probably interacting with voltage-gated sodium channels. The antinociceptive effect described by Gonçalves *et al*. is a novel activity for mastoparans.[36] According to these results, mastoparans present in LMWCs from *P. vespiceps testacea* are probably interfering in the antinociceptive activity, as does Agelaia MP I.

The significant potential impact on the CNS of compounds isolated from wasp venom reveals the great potential for the study of antinociceptive compounds. Studies on the application of wasp venom in the treatment of pain are scarce. The results obtained from this study indicate that wasp venom and its components are promising tools for experimental pharmacology. Further studies will be conducted in order to improve the understanding of their effects and exploit their potential.

CONCLUSION

Chronic pain is a highly prevalent condition for which there is a lack of effective treatments. Our investigation demonstrated the significant antinociceptive effect of compounds isolated from wasp venom that acts on the CNS. Further research is needed to clarify the analgesic effect of F_{pep} on the CNS to provide novel alternatives in the study and treatment of pain-related disorders.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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