

Pharmacological Perspectives of Wasp Venom

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Abstract: Venoms of several animals have been used to study various physiopathologic processes, and also to offer opportunity to design and develop new therapeutic drugs. We briefly review certain wasp venom components and their biological effects, which may be potential sources of novel pharmacologically active compounds.

Keywords: Biological activity, Hymenoptera venom, wasp venom, allergens.

INTRODUCTION

Hymenoptera venoms are rich sources of biological active compounds, which contain complex mixture of amines, small peptides and high molecular weight proteins such as enzymes, allergens and toxins [1-5]. The importance of venoms as the allergen source in Hymenoptera allergy has been known for a long time [6-11]. The prevalence of sensitization against these insects was estimated between 9.3% and 28.5% in the adult population [12,13]. Reactions to Hymenoptera stings are classified into normal local reactions, large local reactions, systemic anaphylactic reactions, systemic toxic reactions [9,14,15], and unusual reactions [9,16-18]. The most frequent clinical patterns are large local and systemic anaphylactic reactions. Knowledge of the composition of venoms and structure of allergens is a prerequisite for the accurate diagnosis and treatment of insect venom allergy [19]. The sequences and structures of the major venom allergens have been cloned and sequenced; several major allergens have been expressed in recombinant form (<http://www.allergen.org>). The antigen 5 and phospholipases A2 (PLA2) are recognized as major allergens from the honeybee and wasp venoms, respectively [7,20,21]. The majority of the allergens are glycoproteins of 10–50 kDa containing 100–400 amino acid residues [10,22]. However, some venom components are smaller, such as honeybee venom melittin (Api m 4), a peptide of 2.9 kDa with 26 amino acid residues [10,23], and an allergen described Api m 6 with the molecular weight of 7.9 kDa [19,24]. The amount of venom which is released during a sting varies in different groups: bee stings release an average of 50 µg [25] up to 140 µg [26] of venom protein per sting; however, venom sacs may contain up to more than 300 µg of venom [27]. Bumblebee stings release 10–31 µg of venom [25]. In contrast, the Vespinae subfamily, which are capable of repeated stings, generally inject less venom per sting:

Vespula stings release 1.7–3.1 µg of venom, *Dolichovespula* stings 2.4– 5.0 µg and *Polistes* stings from 4.2 to 17 µg of protein [25]. The dry weight of venom per sac was found to be 260 µg [19,28].

The Vespidae family includes hornets (genera *Vespa* and *Dolichovespula*), yellow jackets (genus *Vespula*) and paper wasp (genus *Polistes*). They possess highly toxic venom, which is rich in toxins, enzymes and biologically active peptides [1,2]. They are important venomous animals endangering human life, causing fatalities in serious cases. The clinical symptoms induced by wasp stings include local reactions (such as pain, wheal, edema and swelling), immunological reactions usually leading to anaphylaxis with subsequent anaphylactic shock, and systemic toxic reactions caused by large doses of venom, resulting in hemolysis, coagulopathy, rhabdomyolysis, acute renal failure and hepatotoxicity [29-35]. Aortic thrombosis and cerebral infarction is also one of the clinical symptoms after massive wasp stings [35]. In general, the venom components can act on the cardiovascular system, nervous system and immunological system of mammals. Allergic reactions induced by wasp venoms have been extensively studied. The major allergens of wasp venom are phospholipase A₁ (PLA₁), hyaluronidase, antigen 5 and serine proteases. Local reactions may result from biologically active peptides such as bradykinin-like peptides, chemotactic peptides and mastoparans [1,2,36] (Table 1).

ALLERGENS AND IMMUNOTHERAPY

Venom allergy may be mediated by immunologic mechanisms (IgE-mediated or non-IgE-mediated venom allergy), and also by non-immunologic mechanisms [19,37]. The clinical features of wasp venom allergy are widespread, from painful local swelling to systemic anaphylactic reactions and shock symptoms. With one sting up to 17 µg of the vespids venom can be injected into the skin, which can be sufficient for sensitization in man [38]. Today venom immunotherapy (VIT) (i.e. subcutaneous injection of increasing doses of allergen preparations) is the only curative

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Table 1. Pharmacological Properties Exhibited by Wasp Venom Components

Components	Main Action (s)	References
Antigen 5	Most potent allergen	[7,10,20,21,51-57]
Hyaluronidase	Allergenic activity Degradation of extracellular matrix, Increase endothelial permeability	[7,22, 64, 65, 67, 69, 70,163-170]
Phospholipase A1 (PLA ₁)	Allergenic and inflammatory action Hemolytic activity Platelet aggregation and thrombosis induction,	[1, 64, 67, 71, 75, 81,82,84, 86-90]
Phospholipase A2 (PLA ₂)	Allergic reactions, Hemolytic actions	[7, 72,79,80]
Mastoparans	Mast cell degranulation; Antimicrobial activity; Calmodulin binding; Induce release of histamine, cytokine, GABA, serotonin, and other chemical substances, such as hormones, from several cell types; Inflammation with production of TNF- α , IL-1 β , NO and PGE ₂ by peritoneal cells of mice; Phospholipase-A2, -C and -D activation; G-protein and guanylate cyclase activation; Increase the intracellular free calcium concentration; Cell lysis induction by necrosis and apoptosis; Hemolysis induction; Selectively inhibit proliferation of prostate and bladder cancer cells.	[95,97-99,103-105, 107, 108,110,112-123,126- 129,132,171,172]
Protonectin	Potent hemolytic activity; Neutrophil migration induced; Mast cell degranulating activity; Potent antimicrobial action;	[91,110, 176]
Neurotoxins	Blocks the synaptic transmission of nicotinic acetylcholine receptor and ionotropic glutamate receptor of insects and vertebrates; Sodium channel inactivation; Blocks the release of neurotransmitters from presynaptic nerve membranes; Presynaptic inhibition of cholinergic neurotransmission; Anti-nociceptive effect; Anticonvulsant and behavioural effects.	[101,143,146,147,148,154,155,160,162,173-176]

GABA, aminobutyric acid; TNF- α , tumor necrosis factor-alpha; IL-1 β , interleukin-1 beta; NO, oxide nitric; PGE₂, prostaglandin E2.

treatment against allergies, and beneficial effects can persist for many years [39,40]. Vaccines used for immunotherapy are based on extracts derived from natural allergen sources [41]. New findings on the immunological mechanisms of efficacy of VIT suggest that IL-10 has a prominent role reducing the IL-4 production [42]. Furthermore, increased numbers of both Tr1 cells (source of IL-10 and TGF- β) and CD4⁺CD25⁺ Treg cells have been demonstrated in allergic patients after treatment with VIT [43,44]. The production of such vaccines, in particular of vespidae venom, is laborious and cost intensive, as it is difficult to obtain large amounts of natural allergen preparations with homogeneous/ standar-

dized allergen composition [45-47]. Moreover, not all patients are allergic to the whole panel of allergens presented in the vaccine, which might cause a risk of sensitization to other allergens within the preparations used for immunotherapy. Also anaphylactic side reactions might be a particular problem during venom immunotherapy, as insect venoms contain – besides the allergens – a variety of pharmacologically active amines and enzymes that can trigger immunoglobulin E (IgE)-independent mast cell mediator release that present a fatal risk to Hymenoptera venom-allergic patients [48-50].

ANTIGEN 5

Vespid venoms contain a homologous set of three major allergens: antigen 5, hyaluronidase and phospholipase A₁ (Table 1). The antigen 5 is member of a superfamily composed of secreted proteins, has 23 kDa, and the major proteic component and most potent allergen from wasp venom [7,20,21], but is not found in honeybee venom, and has not yet been defined its biological function [10,51-53]. Different antigen 5s have been isolated and their sequence similarity and allergenic activity widely studies [21,52,54-57]. Most vespid-allergic patients show multiple reactions to more than one vespid venom [6,7,58], suggesting partial antigenic identify of the component proteins [54,59]. Bees, fire ants and vespids each have unique as well as homologous venom allergens. One of the four known bee allergens is homologous to vespid hyaluronidases with about 50% sequence identify. Two of the four known fire ant allergens are homologous to vespid antigen 5 and phospholipases. Fire ant antigen 5 has about 35% sequences identify with vespid antigen 5 [10]. The vespid antigen 5 has partial sequence identify with other proteins from diverse sources, such as human and mouse testis proteins, human glioma protein and proteins from tomato, nematode and lizard, in their C-terminal region [10,60-63]. Antigen 5, or phospholipases of hornets, yellow jackets, and wasps have 44-68% sequence identify, and their hyaluronidases have 73-92% sequence identify [10]. Sequence similarity is 98% for antigen 5 from species of the same genus and drops to 57% when antigen 5s from different genera, for example *Vespula* and *Polistes*, are compared [21]. All of them have some immunological cross-reactivity degree which is relevant to define the composition of mixtures for desensitization treatments [21,56].

HYALURONIDASE

Hyaluronidase, one of the three major allergens of venom wasp, is a glycoprotein of approximately 43 kDa, which is largely responsible for the cross-reactivity of wasp and bee venoms with sera of allergic patients [64,65]. The enzyme belongs to family 56 of the glycosyl hydrolases [66], the enzymatic specificity of vespid hyaluronidase is of the endo-*N*-acetylhexosaminidase type [59], as it catalyzes the release of reducing groups of *N*-acetylglucosamine from hyaluronic acid, a polymer of repeating disaccharides of D-glucuronic acid and *N*-acetyl-*D*-glucosamine, found in the extracellular matrix of almost all tissue as a component of the substance that connects protein filaments, collagen fibers and the connective tissue cells [64]. The hyaluronidase in the venom is thus responsible for the breakdown of the hyaluronic acid after the insect sting. Several studies have demonstrated the high sequence similarity of different hyaluronidases contained in bee or wasp venoms [64,65,67,68]. The role of venom hyaluronidases as major cross-reactive allergens in honeybee and *Vespula* venom has been recognized previously [64,69,70] and a significant sequence identity ($\approx$ 50%) between these allergens has been observed [67]. This similarity of the glycan structures on wasp and bee venom hyaluronidases can explain their allergological cross-reactivity.

PHOSPHOLIPASES (PLA)

PLAs are relatively common in Hymenoptera venoms, occurring mainly as A and B types [71,72]. The PLA₁ and PLA₂ catalyses the hydrolysis in the positions sn-1 and sn-2, respectively, of the ester bonds 1,2-diacyl-3-sn-glycerophospholipids, converting these substrates into their corresponding lyso compounds with the release of fatty acids such as arachidonic acid, that is important substrate in the production of prostaglandins and leukotrienes [73-75]. PLAs also are able to disrupt the phospholipid packing of several types of biological membranes, leading to pore formation and/or cell lysis [75,76]. PLA₂, such as polybitoxins and agelotoxin, have been characterized in honeybees [77,78] and in some neotropical social wasps, and are glycoproteins found in many different oligomeric forms and that presented high hemolytic activities [79,80]. In addition, the PLA₂ was recognized as one of the most lethal peptides and the major allergens from honeybee venom, it is a glycoprotein with 134 amino acid residues, which represented 12-15% of the dry weight of bee venom [1,19,78,81]. The PLA₁s comprises 6-14% of the total dry weight of vespid venom [82], and they were initially characterized in some wasp species from Northern Hemisphere [83,84] and ants [85]. Recently, a PLA₁, containing 304 amino acid residues and molecular mass of $\approx$ 34kDa, was purified and characterized from the venom of the wasp *Polybia paulista* [75]. This *P. paulista* PLA₁ has 53-82% sequence identity with phospholipase A₁ from wasp species from Northern Hemisphere, and it presents direct hemolytic activity against washed red blood cells. However, the hemolytic activity of this protein is much less potent than the PLA₁'s from other vespid venoms, such as the verotoxins from *V. verutina* and the lethal toxin from *V. basalis*, and it is also less potent than the vespid PLA₂'s, as PbTXs isolated from the venom of same wasp species [75]. Several PLA₁s have been identified as one of the major allergens from wasp venoms, and also inflammatory processes [64,67,84,86-89]. Recently, a PLA₁ named magnifin was isolated from venoms of *V. magnifica* wasp, it induced strongly platelet aggregation at slow concentrations and induced thrombosis *in vivo*, which may result from its enzymatic activity in hydrolyzing phospholipids to produce 1-acyl-lysophosphatidic acids that can mediate platelet aggregation and this can accelerate thrombus formation [90].

POLYCATIONIC PEPTIDES

Extensive research over the last several decades has shown that wasp venoms include peptides of pharmacological interest. The polycationic peptides represent the major group of components of vespid venoms; the most important families of cationic peptides are represented by the mastoparans, chemotactic peptides and wasp kinins [91]. These α -helical cationic peptides present amphipathic properties and in general contain from 12 to 50 amino acids residues, with a net positive charge between +2 and +7 due to the excess of basic amino acid residues and also present more than 50% of their amino acid sequences constituted of hydrophobic residues, generally interact with the cell membranes [91-93]. The cationic parts of the peptides are capable of interacting with negatively charged

structures of the microbial membrane and promote transmembrane pore assembly, resulting in membrane collapse and death of the bacteria [94,95]. It is known that the dual cationic and hydrophobic nature of the peptide is important for the initial interaction between the peptide and bacteria membrane [96,97]. Cationicity permit wasp peptides to interact specifically with the anionic components of the cell or bacterial membranes, triggering a cascade of biological effects. These major actions were inflammation induction, including initial lysis of cell membrane or mast cell degranulation, leading to histamine release and consequent vasodilatation, increasing neutrophils and T helper cells chemotaxis, and some *in vitro* antimicrobial effects [91,95,98]. The mastoparans family forms an important peptides group with different biological responses, principally because these peptides have structures characteristics to assemble zwitterionic phospholipids [91,98].

MASTOPARANS

Mast cell degranulating peptides and chemotactic peptides are among the major components of vespid venoms [99], amongst which are included mastoparans [95,100-112]. The mastoparans are tetradecapeptides with an amidated C-terminal rich in hydrophobic and basic amino acids, which exert for several biological activities, including antimicrobial activity [91,95,106,108,111,113,114], calmodulin binding [115] and activation of phospholipase A₂, C and D, G-proteins and guanylate cyclase, causing cell lysis [81,116-123]. In addition, they induce the release of histamine and other mediators from mast cells, production of serotonin by platelets, secretion of catecholamines and adenylic acids from adrenal chromaffin cells [97,98,103], exocytosis in rat pituitary cells [124] and pancreatic β -cells [125], and stimulates the release of inflammatory mediators, such as TNF- α , IL-1 β , nitric oxide and prostaglandin E₂ into mice peritoneal cavity [97]. Furthermore, mastoparans are thought to cause the formation of ion channels in lipid membranes leading to cell lysis [126], and they are also known to increase permeability of ions and small molecules through the biological membranes by forming pores at high peptide concentrations [127]. These peptides also are responsible for mobilization of Ca²⁺ ions from sarcoplasmic reticulum, activation of the ryanodine receptor, modulation of various enzymes, for example, the Na⁺-K⁺-ATPase of rat brain [128,129], induction of the mitochondrial permeability transition [130], induction of cell death by necrosis and apoptosis [119], causing hemolysis [95,104,105,107,110] and myotoxicity [131]. Recently, a mastoparans (Polybia-MPI) isolated from the venom of the social wasp *Polybia paulista*, were shown to be potent antimicrobial against both Gram-positive and Gram-negative [108], and it can selectively inhibit the proliferation of prostate and bladder cancer cells, but has lower cytotoxicity to normal murine fibroblasts [132].

NEUROTOXIC KININS

Arthropod venoms, such as spiders, scorpions and wasps have attracted much attention for years as rich source of bioactive substances, in particular of neurotoxins. Some of these neurotoxins are highly selective to mammalian nervous

tissues, being extensively used for studying neuronal mechanisms, such as elucidating ion channels or characterizing receptor functions [133-136]. In addition, several researches have described that these neurotoxins could be applied in the development of natural insecticides and drugs potentially useful for the treatment of neuropathologies [137-140]. Solitary wasps paralyze insects or spiders with their stinging venoms, and feed the paralyzed prey to their larvae. Therefore, the solitary wasp contains a variety of compounds in the venom, principally neurotoxins [141]. The first neurotoxic component characterized were kinins, Threonine⁶-bradykinin (Thr⁶-BK) and megascoliakinins (Thr⁶-BK-Lys-Ala), isolated from the venom of European solid wasp *Megascolia flavifrons* [142-144]. A Thr⁶-BK was also isolated from the venom of another scoliid wasp *Colpa interrupta* [145]. These kinins irreversibly block the synaptic transmission of the nicotinic acetylcholine receptor in the insect central nervous system [143,146-148]. Many neurotoxins have been isolated from the venom of solitary wasps, such as Pompilidotoxins (PMTXs) and peptides similar to bradykinin that have been isolated from spider wasps *Anoplius samariensis* [149,150], *Batozonellus maculifrons* [150] and *Cyphononyx dorsalis* [151] and δ -Philanthotoxins were isolated from venom of solitary wasp *Philanthus triangulum* [152,153]. PMTXs affect both vertebrate and invertebrate nervous systems, which are due to the slowing or blocking of sodium channel inactivation [154,155] and δ -Philanthotoxins mainly inhibit neuromuscular transmission by block of the postsynaptic glutamate and nicotinic receptors [156,157]. Kinin-related peptides also have been isolated from the venoms social wasp [158-161]. Recently, a Thr⁶-BK was isolated and sequenced from the venom of the social wasp *P. occidentalis*. The authors observed that this kinin was most active than others kinins isolated from venoms of social wasps and it induced anti-nociceptive effects more potent than morphine and bradykinin [162].

ACKNOWLEDGMENTS

The authors express their gratitude to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil, for financial support.

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