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IgE cross-reactivity of phospholipase A_2 and hyaluronidase of *Apis dorsata* (Giant Asian Honeybee) and *Apis mellifera* (Western Honeybee) venom: Possible use of *A. mellifera* venom for diagnosis of patients allergic to *A. dorsata* venom

Peshala Gunasekara [a, *], S.M. Handunnetti [a], Sunil Premawansa [b], E.W.R.A. Witharana [c], W.M.D.K. Dasanayake [d], Indra P. Ratnayake [e], Suranjith L. Seneviratne [f, g], R.K.S. Dias [h], G.A.S. Premakumara [i], Rajiva de Silva [d]

[a] Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Colombo, Sri Lanka
[b] Department of Zoology and Environment Sciences, Faculty of Science, University of Colombo, Colombo, Sri Lanka
[c] Base Hospital, Deniyaya, Sri Lanka
[d] Department of Immunology, Medical Research Institute, Colombo, Sri Lanka
[e] District Hospital, Bandarawela, Sri Lanka
[f] Royal Free Hospital, United Kingdom
[g] Department of Surgery, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka
[h] Department of Zoology and Environmental Management, Faculty of Science, University of Kelaniya, Kelaniya, Sri Lanka
[i] Herbal Technology Section, Industrial Technology Institute, Colombo, Sri Lanka

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ABSTRACT

Diagnostic and therapeutic reagents are unavailable for anaphylaxis arising from stings by *Apis dorsata*. Venom profiles and cross-reactivity of *A. dorsata* and *Apis mellifera* were compared, to ascertain whether venom of *A. mellifera* can be used for diagnosis in *A. dorsata* allergy. Both venom profiles were similar by High Performance Liquid Chromatography and SDS-PAGE. Sera of 29 of 30 (96.7%) patients with anaphylaxis to *A. dorsata* stings had IgE to the phospholipase-2 (PLA_2) doublet (15 and 16 kDa) of *A. dorsata* venom by immunoblot, compared to 26 of 30 (86.7%) with the PLA_2 of *A. mellifera* and a purified preparation of PLA_2. Twelve patients (40%) with severe anaphylaxis had IgE reactivity to a 39 kDa protein band of venom of both species, a third band, identified in immunoblot as hyaluronidase. The cross-reactivity of PLA_2 and hyaluronidase of *A. dorsata* and *A. mellifera* were further confirmed by immunoblot inhibition results. Twenty five of 30 (83.3%) of our patients had positive venom specific IgE (>0.35 KU_A/L) reactivity to Phadia ImmunoCAPs of *A. mellifera* venom. The observed IgE cross reactivity suggests the possibility of using *A. mellifera* venom as a diagnostic test for *A. dorsata* venom allergy.

1. Introduction

Apis dorsata Fabricius, the Giant Asian honey bee (*Sinhala-Bambara, Tamil-Kulangei Kulavi*), is frequently found in rural wilderness areas in South Asian countries including Sri Lanka (Budagoda et al., 2010; Lao-araya et al., 2013; Witharana et al., 2015). It is the most aggressive stinging insect in Sri Lanka, responsible for the most number of anaphylactic incidents recorded in rural areas (Budagoda et al., 2010; Witharana et al., 2015). A prospective observational study which was conducted at Deniyaya Base Hospital, Sri Lanka, during the two-year period from September 2011 to August 2013, reported 322 cases of stinging insect where 292 of them were due to *A. dorsata* stings and the prevalence of anaphylaxis reactions against *A. dorsata* was estimated to be around 4–5% (Witharana et al., 2015. Another case study carried out in Teaching Hospital, Peradeniya, Sri Lanka, reported 83 cases of stinging insects in the year of 2008 (Budagoda et al., 2010).

A. dorsata and *Apis mellifera* Linnaeus (Western honey bee)

* Corresponding author. Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo, Colombo, Sri Lanka.
E-mail address: Peshala12@gmail.com (P. Gunasekara).

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belong to family Apidae of order Hymenoptera. *A. dorsata* is larger than *A. mellifera* in body size. Compared to *A. mellifera* which is a cavity nesting bee species, *A. dorsata* builds a single, large, exposed comb under tree branches or under cliffs (Koeniger and Koeniger, 1980). During migratory swarm, worker bees of *A. dorsata* in Sri Lanka travel for a month or so, up to 200 km in each direction, resting in trees along the way to reach a new destination (Koeniger and Koeniger, 1980).

Stings of *A. dorsata* and *A. mellifera* are a major cause of life threatening allergic reactions in humans (Biló et al., 2005). However, in some patients, apart from allergic reactions, due to excessive amounts of venom injected to the body, direct toxic effects may occur. In these cases, most prevalent clinical symptoms are generalized oedema, arterial hypotension, hemolysis, rhabdomyolysis and acute renal failure (Vetter et al., 1999; Budagoda et al., 2010). IgE mediated *A. mellifera* venom allergy is relatively common in the West (Portnoy et al., 1990 Treudler et al., 2008; Vincent et al., 2010; Zalat et al., 2005). Most patients develop only a local reaction after a sting, but in some, systemic reactions may occur (Bilò, 2011; Shkenderov, 1974. Systemic reactions may range from mild (urticaria and angioedema) to moderate or severe reactions, and sometimes result in death (Krishna et al., 2011).

In the West, the purified venom of *A. mellifera* is well characterized and commercially available (Hoffman and Jacobson, 1984; Henriksen et al., 2001; King et al., 1983, 1984). Patients with systemic reactions to *A. mellifera* stings, are diagnosed using *in vivo* (skin prick and intra dermal tests) and *in vitro* (venom specific IgE levels by Phadia ImmunoCAPs and component resolved diagnostics (CRD)) (Ebo et al., 2014; Kohler et al., 2014) methods. Appropriate patients are offered venom immunotherapy (VIT) using the purified venom of *A. mellifera* (Bonifazi et al., 2005; Hamilton et al., 1993; Schwartz et al., 1990). However, diagnostic tests and VIT are not available for patients allergic to *A. dorsata* stings as the pure venom is not properly characterized or commercially available. If the venoms of *A. mellifera* and *A. dorsata* are similar, it may be possible to use commercial preparations of *A. mellifera* venom for diagnosis and immunotherapy of patients allergic to *A. dorsata* venom. Our study aims to identify allergenic components of the venom of *A. dorsata* and to compare this with the commercially available venom of *A. mellifera.*

2. Materials and methods

2.1. Ethical statement

Ethical approval for this study was obtained from Ethics Review Committee, Medical Research Institute, Colombo, Sri Lanka (No: 46/2013). Informed written consent was obtained from all patients and healthy individuals. Permission to extract venom from *A. dorsata* was obtained from the Department of Wildlife Conservation of Sri Lanka (WL/3/2/71/14).

2.2. Patients and controls

Patients who developed anaphylaxis following a sting by *A. dorsata*, and were treated at Base Hospital, Deniyaya, Sri Lanka and District Hospital, Bandarawela, Sri Lanka, were selected. The stinging insect was identified when it was brought to the hospital or in instances where the insect was not available, patient was asked to identify the insect from a series of dead specimens of stinging hymenopterans. A questionnaire was filled obtaining relevant information from the patients and 5 ml of blood was taken from each patient and 5 ml of blood samples were collected from 4 healthy individuals. Serum were separated from each blood sample and were kept in −20 °C.

2.3. Insect identification and venom collection

Insects were identified using their morphological characteristics as described previously (Gullan and Cranston, 2010). External morphology of identified worker bee was shown in Fig. 1a and b.

To collect the venom from *A. dorsata* hives, electrical stimulation was performed as described previously (Rybak, 2008) with the following modifications; 2 s (s) impulse duration and 2 s impulse interval were maintained manually and the voltage difference of the apparatus was kept at 30 V. This voltage stimulated *A. dorsata* to eject venom from the stinger on to a glass plate without killing the bees. Dried venom was scraped out from the glass plate (Fig. 1c). Total venom proteins were quantified by the Bradford method using bovine serum albumin (BSA) as the standard (Bradford, 1976), after dried venom dissolved in 0.13 M PBS.

2.4. High Performance Liquid Chromatography (HPLC)

The crude venom of *A. mellifera* and three pure venom components: PLA_2, apamin and melittin were purchased from Sigma Aldrich (Germany). Following standard solutions were used in HPLC analysis: 1 mg/ml of crude venom of *A. dorsata* and *A. mellifera*, purified PLA_2, apamin and melittin. Ten μl of solution (filtered using 0.45 μm membrane filter) was injected to the column in each analysis. To establish the HPLC method, the following conditions used previously for separation of *A. meliffera* venom, were tested (Szokan et al., 1994): chromatographic column with C18 packing materials of 100 Å. flow rate of 1.5 ml/min and gradient elution with 5% B − 80% B, 60 min. Chromatographic separation was performed using the following mobile phases; A − 0.1% trifluoroacetic acid (TFA) in deionized water and B − 0.1% TFA in deionized water (80:20). The separated venom components were detected using Photo Diode Array (PDA) detector at 220 nm wavelength (Szokan et al., 1994; Packova et al., 1995).

2.5. SDS-PAGE and immunoblot

Venom profile of *A. dorsata*, based on the molecular weight of the venom components, was obtained and compared with *A. mellifera* venom using SDS-PAGE techniques. SDS-PAGE was performed using a mini protein R- apparatus (Bio-Rad). Five μl of protein standards (106–18.5 kDa) was added to the first well of the 15% gel. Total of 15 μl (10 μl of venom and 5 μl of 3X Lammeni sample buffer) venom samples which was incubated at 95 °C for 5 min, added to the remaining wells of the gel and electrophoresis through the gel at 70 V at 4 °C for 3 h. Gels were stained with Coomassie blue stain for 1 h and destained for about 5 h. Standard curve was plotted using log molecular weights of the standards and calculated R_f values. Using the standard curve, molecular weights of the protein bands in the gel were estimated.

Immunoblot, as described previously (Thomson et al., 1991), was carried out to determine the presence of venom specific IgE to venom. Briefly, the venom proteins which were separated be SDS-PAGE were transferred to a nitrocellulose membrane using a mini protein tetra system (Bio-Rad) for 2 h at 70 V constant voltage. The membrane was blocked with PBST containing 5% nonfat milk at 4 °C for 1 h, after washing the membrane with phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST). The membrane was reacted with 1:40 dilution of patient's serum in antibody diluting buffer (5% nonfat milk in PBST) at 4 °C for overnight incubation, after washing with PBST for 5 min for three times. Washing step was repeated with PBS for 5 min for three time. Washed membrane then reacted at 4 °C for 2 h with the 1:1000 dilution of peroxidase-labeled goat anti-human IgE antibody. After washing with PBST for 5 min for three time, the membrane was visualized using 4-cloro-