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Neutralization of *Apis mellifera* bee venom activities by suramin

Camila Z. El-Kik [a], Fabrício F.A. Fernandes [a], Marcelo Amorim Tomaz [a], Glauco A. Gaban [a], Tatiane F. Fonseca [a], Sabrina Calil-Elias [b], Suellen D.S. Oliveira [a], Claudia L.M. Silva [a], Ana Maria Blanco Martinez [c], Paulo A. Melo [a,*]

[a] *Programa de Farmacologia e Química Medicinal, Instituto de Ciências Biomédicas, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*
[b] *Departamento de Farmácia e Administração Farmacêutica, Faculdade de Farmácia, UFF, Niterói, RJ, Brazil*
[c] *Programa de Pesquisa em Neurociência Básica e Clínica, Instituto de Ciências Biomédicas, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil*

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ABSTRACT

In this work we evaluated the ability of suramin, a polysulfonated naphthylurea derivative, to antagonize the cytotoxic and enzymatic effects of the crude venom of *Apis mellifera*. Suramin was efficient to decrease the lethality in a dose-dependent way. The hemoconcentration caused by lethal dose injection of bee venom was abolished by suramin (30 μg/g). The edematogenic activity of the venom (0.3 μg/g) was antagonized by suramin (10 μg/g) in all treatment protocols. The changes in the vascular permeability caused by *A. mellifera* (1 μg/g) venom were inhibited by suramin (30 μg/g) in the pre- and posttreatment as well as when the venom was preincubated with suramin. In addition, suramin also inhibited cultured endothelial cell lesion, as well as *in vitro* myotoxicity, evaluated in mouse *extensor digitorum longus* muscle, which was inhibited by suramin (10 and 25 μM), decreasing the rate of CK release, showing that suramin protected the sarcolemma against damage induced by components of bee venom (2.5 μg/mL). Moreover, suramin inhibited the *in vivo* myotoxicity induced by i.m. injection of *A. mellifera* venom in mice (0.5 μg/g). The analysis of the area under the plasma CK vs. time curve showed that preincubation, pre- and posttreatment with suramin (30 μg/g) inhibited bee venom myotoxic activity in mice by about 89%, 45% and 40%, respectively. Suramin markedly inhibited the PLA_2 activity in a concentration-dependent way (1–30 μM). Being suramin a polyanion molecule, the effects observed may be due to the interaction of its charges with the polycation components present in *A. mellifera* bee venom.

1. Introduction

African and European subspecies of the honeybee (*Apis mellifera*) were mixed in Brazil and the hybrid, the Africanized honeybee, is now spread all over Americas. This new subspecies has made Brazil one of the major producers and exporters of honey, but it is considered extremely aggressive and is associated with various types of accidents,

* Corresponding author. Laboratório de Farmacologia das Toxinas, Avenida Brigadeiro Trompowski, s/n – Centro de Ciências de Saúde – CCS, bloco J, sala 18, Ilha do Fundão, CEP 21941-590 Rio de Janeiro, RJ, Brazil. Tel./fax: +55 21 2610 3726.
E-mail address: melo.pa@gmail.com (P.A. Melo).

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including mass attacks, resulting in serious and fatal accidents in animals and humans, although in most cases, by being stung by only one or few insects, the victim does not seek medical attention (França et al., 1994; Clarke et al., 2002; Almeida et al., 2011; Ferreira et al., 2012; Funayama et al., 2012).

A. mellifera venom is composed of a mixture of components that include melittin, phospholipase A_2, apamin and hyaluronidase (Ferreira et al., 2012; Okamoto et al., 1995; Schumacher et al., 1992). Melittin, which is the main component of the venom, is a cationic polypeptide that presents cytotoxic effects inducing hemolysis, cardiotoxicity and myotoxicity. This component has a potent destructive action on biological membranes mainly when acting synergistically with the venom phospholipase A_2 in the membrane phospholipids (Fletcher and Jiang, 1993; Ownby et al., 1997). Phospholipase A_2 present in *A. mellifera* venom hydrolyzes phospholipids of biological membranes and induces pore formation and lysis (Ferreira et al., 2012). The proteolytic activity of the venom from Africanized honeybee has been detected by Lima et al. (2000), with possible occurrence of necrosis associated to the stings. Another important venom component is apamin, a potassium channel blocker that interferes on the membrane potential properties and is a valuable pharmacological tool in electrophysiological studies (Habermann, 1972; Catterral et al., 2002).

Envenomations by multiple stings by honeybee cause many dysfunctions described in clinical conditions (Vetter et al., 1999). Manifestations include leukocytosis, elevated clotting time, severe disturbances such as respiratory depression, acute kidney injury, severe anemia, rhabdomyolysis, tachycardia, headache, vomiting, abdominal cramps and elevated creatinine levels (Almeida et al., 2011; Ferreira et al., 2012; Funayama et al., 2012). These serious manifestations are the result of both direct and indirect toxic effects of the large amount of venom received. Sometimes multiple bee stings are fatal due to the lack of a specific antidote for the venom, the complexity of its components or the lack of information about the systemic effects. Some reports described that the acute anaphylaxis is an important consequence of massive insect sting, mainly by honeybee (Ellis and Day, 2003; Reisman, 1994). Venom components present pharmacologic and allergic effects producing local pain, edema and erythema caused by the increase of the vascular permeability (Nabil et al., 1998; Ownby et al., 1997; Smith et al., 1980). Victims of multiple stings of Africanized honeybee manifest nauseas, generalized weakness, cardiotoxicity and pulmonary edema (Bresolin et al., 2002; Ferreira et al., 1995). Late manifestations include acute renal failure due to acute tubular necrosis as well as changes in arterial pressure, hypotension and rhabdomyolysis (Azevedo-Marques et al., 1992). In addition, skeletal muscle damage or myotoxicity has been described as an effect of bee venom (Azevedo-Marques et al., 1992; Fletcher et al., 1996; Nabil et al., 1998; Ownby et al., 1997). Prado et al. (2010) have described the mouse as a model to characterize the main alterations induced by bee venom when injected by subcutaneous route to induce a sublethal, severe envenoming. They defined the mouse model as useful to evaluate preclinically the efficacy of antivenoms or other inhibitors.

Recent studies have reported that suramin, a polysulfonated naphthylurea derivative, is an enzymatic inhibitor that prevents, due to polyanion properties, the effects produced by polycationic toxins present in snake venoms, some of which present similarities with components of the *A. mellifera* venom (Arruda et al., 2002; Murakami et al., 2004, 2005; Oliveira et al., 2003; Sifuentes et al., 2008). Suramin is known by its anti-trypanosomal activity. It is also an antagonist of P2 purinoceptor subtypes and has anti-enzymatic activity by binding basic proteins (Freissmuth et al., 1996; Nakazawa et al., 1990).

In this work, we evaluated the cytotoxic and enzymatic activities of *A. mellifera* venom and the ability of suramin to antagonize these effects.

2. Material and methods

2.1. Material

A. mellifera venom and suramin were purchased from Sigma Chemical Co (St Louis, MO, USA). Bee venom and suramin were dissolved in physiological saline solution (PSS). All other reagents, substances and the diagnostic kit for creatine kinase were purchased from BIOCLIN® (Brazil), and all reagents were of analytical grade.

2.2. Animals

Adult male Swiss mice weighing 20–25 g were maintained under a 12 h light–dark cycle in a temperature controlled environment (22–28 °C). Food and water were freely available. All the animal procedures were in accordance with international guidelines for the use of animals and were approved by the Ethics Committee for the Use of Animals of the Federal University of Rio de Janeiro.

2.3. Lethality and hematocrit

The systemic effect of *A. mellifera* venom was evaluated by measurement of lethality and alterations on the hematocrit. Different groups of animals ($n = 6$ each) received intraperitoneal bee venom injection of increasing doses (3–10 μg/g) to calculate the dose that would cause the death of 50% and 100% of the animals (LD_{50} and LD_{100}). A group received suramin (3–30 μg/g) preincubated during 30 min at 37 °C with the LD_{100} of the venom (6.5 μg/g), before the i.p. injection. For pre- and posttreatment protocols, two groups of animals received suramin (3–30 μg/g) i.p. 15 min before and after venom injection (LD_{100}), respectively. Animals were observed during 24 h and the survivors were quantified. In all groups, the animals were anesthetized under diethyl ether for blood collection just before and 2 h after venom injections to evaluate the hematocrit. The blood was collected from the orbital plexus with heparinized capillaries, centrifuged and analyzed using a percentage hematocrit ruler.

2.4. Vascular permeability

To investigate the vascular permeability and plasma extravasation on the mouse skin vessels induced by *A.*