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Antiplasmodial effect of the venom of *Crotalus durissus cumanensis*, crotoxin complex and Crotoxin B

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ABSTRACT

The antiplasmodial activity of phospholipases A_2 (PLA_2) isolated from different animals has been studied. We explored the *in vitro* anti *Plasmodium falciparum* effect of a fraction containing crotoxin, Crotoxin B and whole venom of the rattlesnake *Crotalus durissus cumanensis*. Fraction II (crotoxin complex) was obtained by size exclusion chromatography, whereas Crotoxin B was purified by RP-HPLC. The whole venom is active against the parasite at concentrations of 0.17 ± 0.03 μg/ml, fraction II at 0.76 ± 0.17 μg/ml and Crotoxin B at 0.6 ± 0.04 μg/ml. Differences were observed in the cytotoxic activity against peripheral mononuclear cells, with Crotoxin B exhibiting the highest cytotoxicity. The concentration of Crotoxin B required to exert cytotoxic activity was higher than that required to exert antiplasmodial activity. Lethality in mice confirmed the higher toxicity and neurotoxicity of whole venom and fraction II, whereas Crotoxin B was not lethal at the doses tested. These results suggest the potential of Crotoxin B as a lead compound for antimalarial activity.

1. Introduction

Malaria is caused by parasites of the genus *Plasmodium* and is a public health problem in tropical and sub-tropical regions of the world. Annually malaria provokes approximately 1.5 million deaths. Over 85% of them occur in Africa with *P. falciparum* as the leading species involved in most fatalities (Breman et al., 2004; WHO, 2008). The W.H.O. report confirmed almost 1 million deaths during the previous year (WHO, 2010). The most widely used treatment consists of artemisinin-based combined therapies (WHO, 2010). High rates of antimalarial treatment failure have led to the investigation of possible therapeutic alternatives, among which toxins and poisons derived from animals and plants are promising candidates (Abdel-Sattar et al., 2010; Ayuko et al., 2009; Gao et al., 2010; Karunamoorthi et al., 2010; Milhous and Weina, 2010; Muller et al., 2010).

Rattlesnakes (genus *Crotalus*, family Viperidae) are pit vipers with widespread distribution in the Americas (from North America to northern Argentina), being classified into many species and subspecies. Among them, *Crotalus durissus cumanensis* is distributed in Colombia and Venezuela (Campbell and Lamar, 2004). The venoms of South American subspecies of *C. durissus* are composed of a complex mixture of peptides, enzymes and toxins, such as crotamine, gyroxin, convulxin, thrombin-like serine proteinase (Bucaretchi et al., 2002; Oshima-Franco et al., 1999) and the crotoxin complex (a heterodimer composed of two subunits, A and B). Crotoxin complex is responsible for the high toxicity of the venom due to neurotoxic, nephrotoxic and myotoxic activities (Azevedo-Marques et al., 1985; Martins et al., 2002; Oshima-Franco et al., 1999), which may provoke death by neurotoxic paralysis or acute renal failure.

Phospholipases A_2-type (PLA_2, EC 3.1.1.4) are a superfamily characterized by their ability to hydrolyze phospholipids at the *sn*-2 ester bond of fatty acids to produce lysophospholipids and fatty acids. Secreted phospholipases A_2 ($sPLA_2$) share several characteristics: low molecular mass (13–18 kDa), numerous disulfide bridges, histidyl and aspartyl catalytic residues and a highly conserved calcium binding region (Ca^{2+}) (Six and Dennis, 2000; Talvinen and Nevalainen, 2002). PLA_2s from snake venoms

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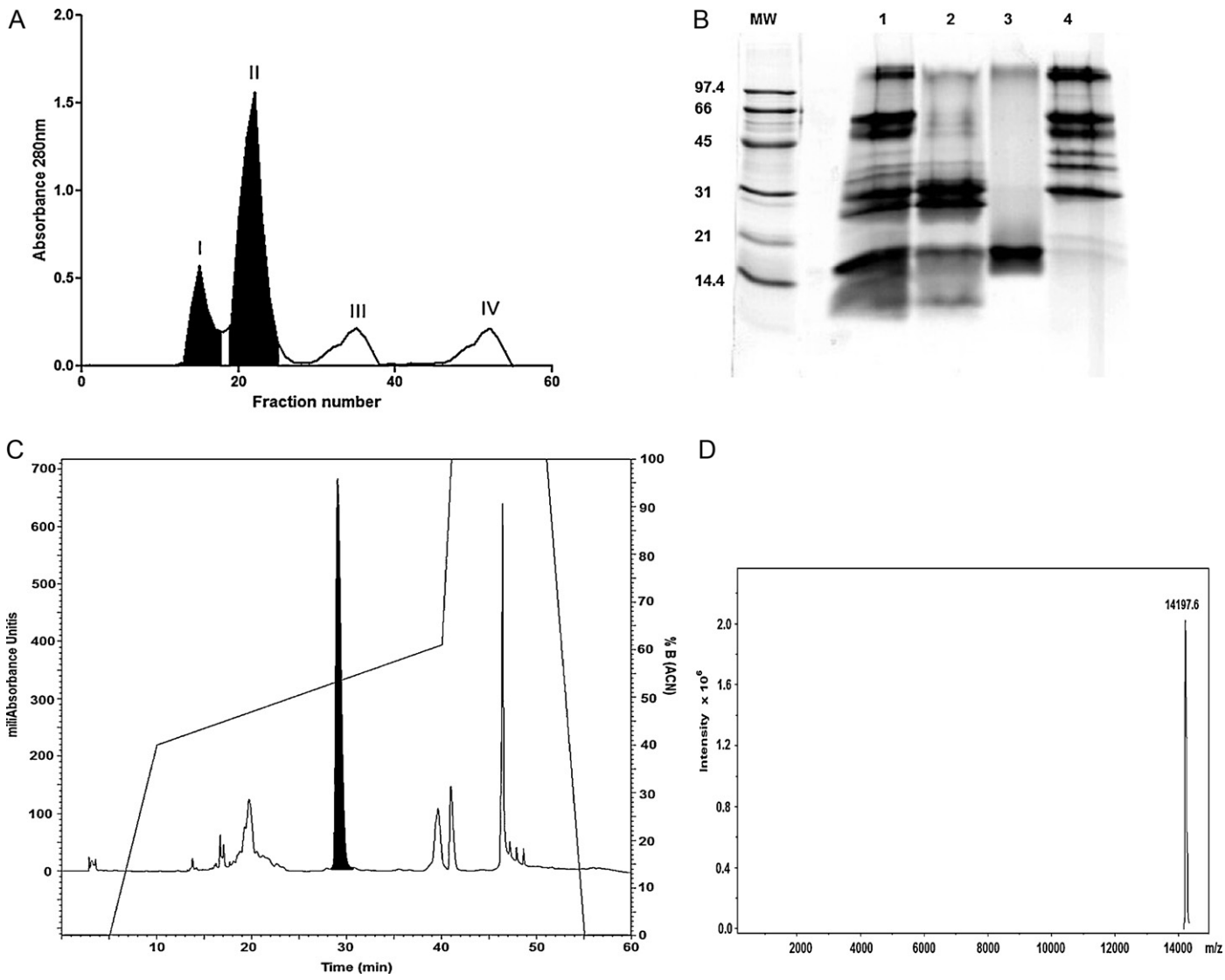

Fig. 1. (A) Chromatographic elution profile on Sephacryl S-200 of the venom of *C. d. cumanensis*. The shaded fractions correspond to the fractions used, (B) SDS-PAGE (12%) separation of crude *C. d. cumanensis* venom (lane 1), fraction II from gel filtration chromatography (lane 2), Crotoxin B (lane 3), and fraction I from gel filtration chromatography (lane 4). MW correspond to molecular weight markers, (C) reverse-phase HPLC separation on a C-18 column of fraction II from gel filtration chromatography. The shaded area corresponds to Crotoxin B, (D) molecular mass determination of Crotoxin B by mass spectrometry.

exhibit a variety of toxicological and pharmacological activities, such as myotoxicity, neurotoxicity, anticoagulant activity, edema inducing-activity, cardiotoxicity, bactericidal activity, antiparasitic effect, and various effects on platelet aggregation (Andriao-Escarso et al., 2000; Barbosa et al., 2005; Costa Torres et al., 2010; Evangelista et al., 2010; Gutierrez and Lomonte, 1995; Harris et al., 2000; Kini, 2003; Kini and Evans, 1987; Landucci et al., 2000; Murakami et al., 2005).

In recent years, several pharmacological applications for PLA_2 have been described, including a potential activity against parasites (Costa Torres et al., 2010; Deregnaucourt and Schrevel, 2000; Guillaume et al., 2004; Passero et al., 2008). Owing to the high concentration of Crotoxin B, a PLA_2, in the venom of *C. d. cumanensis*, this venom constitutes a potential source of antimalarial activity. The aim of this study was to characterize the Crotoxin B from the venom of *C. d. cumanensis*, comparatively with a fraction containing the whole crotoxin complex and with the crude venom, for its *in vitro* antiplasmodial activity against *P. falciparum*, as well as its cytotoxicity on a human cell line and its acute toxicity in mice.

2. Materials and methods

2.1. Venom and reagents

The venom was obtained by manual milking of 15 specimens from different regions of Colombia held in captivity at the Serpertarium of the University of Antioquia (Medellín, Colombia). Venoms were pooled, centrifuged (3000 rpm, 15 min), and the resulting supernatants were lyophilized and stored at −20 °C until use.

Acetonitrile (CH_3CN) and trifluoroacetic acid (CF_3COOH) HPLC grade were purchased from Fisher Scientific (Loughborough, UK), Histopaque®-1077, RPMI-1640 medium culture, Thiazolyl Blue Tretrazolium Bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Sigma (Sigma–Aldrich, St. Louis, USA). Water for HPLC was deionised to a degree of purity of 17 Ω.

2.2. Venom fractionation

Crotoxin B was purified from the venom of *C. d. cumanensis* using size molecular exclusion chromatography on a BioRad