A comparative study of the effects of venoms from ve rear-fanged snake species on the growth of Leishmania major: Identi cation of a protein with inhibitory activity against the parasite

María E. Peichoto [a,b,*], Flávio L. Tavares [b], Gregory DeKrey [b], Stephen P. Mackessy [b]

[a] Cátedra de Farmacología, Facultad de Ciencias Veterinarias, Universidad Nacional del Nordeste, Sargento Cabral 2139, 3400 Corrientes, Argentina
[b] School of Biological Sciences, University of Northern Colorado, 501 20th St., CB 92, Greeley, CO 80639-0017, USA

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a b s t r a c t

Leishmania parasites of several species cause cutaneous and visceral disease to millions of people worldwide, and treatment for this vector-borne protozoan parasite typically involves administration of highly toxic antimonial drugs. Snake venoms are one of the most concentrated enzyme sources in nature, displaying a broad range of biological effects, and several drugs now used in humans were derived from venoms. In this study, we compared the effects of the venoms of the South American rear-fanged snakes Philodryas baroni (PbV), Philodryas olfersii olfersii (PooV) and Philodryas patagoniensis

drug amphotericin B (a polyene macrolide antibiotic) is considered an ef cient treatment option for many antimonial-resistant strains of Leishmania (Rosenthal et al., 2009). Other drugs, such as allopurinol, miltefosine and pentamidine also represent important options for leishmaniasis treatment (Loiseau and Bories, 2006). However, effective new anti-leishmanial compounds are needed due to the high costs, development of parasite resistance and side effects related to those drugs currently in use. Importantly, no licensed human vaccine for leishmaniasis is available.

A productive, more recent approach to the search for more ef cient and less toxic chemotherapeutic agents has been the screening of natural compounds able to inhibit protistan growth. Snake venoms, one of the most concen-

0.1% TFA was spotted onto a MALDI sample holder, mixed with an equal volume of 10 mg/mL sinapinic acid in 50% ACN containing 0.1% TFA, and allowed to dry. Mass spectrum was obtained using a Bruker Ultra ex II MALDI-TOF/TOF mass spectrometer.

For unequivocal identi cation of the puri ed PLA_2, gel bands of interest were excised, destained and subjected to reduction with DTT, alkylation with iodoacetamide, and then in-gel digestion with mass spectrometry grade Trypsin Gold (Promega, Madison, WI, USA), following the manufacturer 's instructions. The tryptic peptide mixtures were puri ed and concentrated using ZipTip C18 pipette tips (Millipore Corporation, Billerica, MA, USA). The peptides eluted from the ZipTip tips were dried in a Speed-Vac and redissolved in 5 mL of 50% ACN containing 0.1% TFA. Digests (1 mL) were spotted onto a MALDI sample holder, mixed with an equal volume of 10 mg/mL a-cyano-4-hydroxycinnamic acid in 50% ACN containing 0.1% TFA, e,

growth (Fig. 3), and a very high nal concentration of 1.7 mg/mL was necessary to inhibit parasite proliferation by only 51.5 3.6%. This venom represents an example of non-speci c effects against parasite growth and results provide rationale for not considering it further for possible drug screening. Venoms from the other three species (PbV, PooV and HttV), at nal concentrations of 562, 524 and 438 mg/mL respectively, had no signi cant effect on L. major

Leishmania amazonensis promastigotes (Passero et al., 2007). However, it is less potent than amphotericin B, a standard anti-leishmanial drug, which showed an IC_{50} of 0.2 mg/mL against L. major (Sabina et al., 2005), though on a molar basis it shows comparable potency. The amount of puri ed trimorphin required to eliminate parasite viability is approximately 3.3% of the amount of crude venom required to accomplish the same decrease in viability; signi cantly, PLA_2 represents about 3.2% of the total protein of the venom. Thus, it is likely that the anti-leishmanial activity of TlbV can be accounted for solely by the mass of trimorphin present in this venom.

Snake venom PLA

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