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Short communication

Antiparasitic effect of *Dinoponera quadriceps* giant ant venom

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ABSTRACT

Neglected tropical diseases (NTD) are treated with toxic therapy of limited efficacy. Previously, we studied the antimicrobial effect of *Dinoponera quadriceps* venom (DqV) against bacteria. To continue the study, we report in this short communication the antimicrobial effect of DqV against *Leishmania amazonensis* and *Trypanosoma cruzi*. DqV inhibits the promastigote forms of *L. amazonensis* and all *T. cruzi* developmental forms, with low toxicity in host cells. DqV causes cell death in *T. cruzi* through necrotic and apoptotic mechanisms observed by staining the cells with annexin V-FITC (AX) and propidium iodide (PI), loss of mitochondrial membrane potential by flow cytometry analyses and confocal microscopy and morphological alterations, such as loss of membrane integrity and cell shrinkage by scanning electron microscopy (SEM). In conclusion, we suggest there is an antimicrobial effect also on parasites.

1. Introduction

The World Health Organization (WHO) estimates that the Neglected Tropical Diseases (NTDs) affect proximally 37% of the world's poorest individuals (WHO, 2010b; Berkowitz et al., 2015). The NTDs cause significant disfigurement, morbidity and mortality, accounting for 1% of the global burden of disability-adjusted life years lost in 2010 (Hotez et al., 2014).

Chagas disease and Leishmaniosis are the first and third most prevalent NTDs in the world, respectively. It is estimated that 7 to 8 million people are infected with *T. cruzi* and 1.3 million are infected with Leishmania (WHO, 2010b; WHO, 2013). The treatment of parasitic diseases is of limited effectiveness and has severe collateral effects (WHO, 2010a; Murcia et al., 2012; Silva et al., 2014). Therefore, several studies have sought to discover novel antiparasitic substances from natural sources (Passero et al., 2007; Adade et al., 2011, 2013; 2014).

Recently, our group produced a *Dinoponera quadriceps* venom gland cDNA library to investigate the toxin repertoire present in the venom used in this study. This venom has 30% of dinoponeratoxins in its composition and has high homology with ponericins, antimicrobial peptides (Torres et al., 2014). Antimicrobial peptides from venoms of many organisms have shown antiparasitic effect on Leishmania and Trypanosoma genera (Mcgwire and Kulkarni, 2010). Recently, we demonstrated the antimicrobial action of *D. quadriceps* venom against bacteria (Lima et al., 2014). To continue the study of the antimicrobial effect of *D. quadriceps* venom (DqV), we report in this short communication its action on *Trypanosoma cruzi* and *Leishmania amazonensis*.

2. Material and methods

2.1. Venom

D. quadriceps venom (DqV) was obtained as described by Sousa et al. (2012). For the experimental assays, final concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 μg/mL were utilized, with Sterile PBS being used as negative control (pH 7.4).

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2.2. Effect of D. quadriceps venom on promastigote forms of L. amazonensis

Promastigote forms of *L. amazonensis* (IFLA/BR/67/PH8) were cultivated in NNN/Schneider medium (Sigma–Aldrich™, St. Louis, USA) supplemented with 20% of fetal bovine serum (FBS) and antibiotics with different concentrations of DqV and amphotericin B (Cristália, São Paulo, Brazil) at 26 °C for 72 h. Parasite growth inhibition was quantified in a Neubauer chamber (Torres et al., 2010).

2.3. Effect of D. quadriceps venom on epimastigote forms of T. cruzi

Epimastigote forms of *T. cruzi* strain Y were plated in Liver Infusion Tryptose medium supplemented with antibiotics and 10% of FBS with different concentrations of DqV and Benznidazole (Bz), incubated at 28 °C for 24 and 48 h. Parasite growth inhibition was quantified in a Neubauer chamber (Abe et al., 2002; Gonçalves et al., 2002).

2.4. Effect of D. quadriceps venom on trypomastigote forms of T. cruzi

The trypomastigote forms of *T. cruzi* obtained by infecting LLCMK2 cells, were incubated at 37 °C in an atmosphere with 5% CO_2 in DMEM medium (Vitrocell, São Paulo, Brazil) supplemented with antibiotics and 2% of FBS (Aparicio et al., 2004). Cells were incubated with different concentrations of DqV and Bz for 24 h. Parasite growth inhibition was quantified in a Neubauer chamber (Abe et al., 2002; Gonçalves et al., 2002).

2.5. Cytotoxicity to mammalian cells (MTT assay)

Cell viability was also measured using a standard MTT assay (Mosmann, 1983). LLC-MK2 cells were plated in the DMEM medium, treated with different concentrations of DqV and incubated at 37 °C for 24 h. MTT (Amresco, Ohio, USA; 5 mg/mL) was added and the cells were incubated for 4 h, when 10% Sodium dodecyl sulphate (SDS; Vetec, São Paulo, Brazil) was added to solubilize the formazan product. Cell viability measurements were performed at 570 nm on a microplate reader (Biochrom® Asys Expert Plus). Selectivity index (SI) was calculated by the ratio of cytotoxic/trypanocidal activity (Gallé et al., 2013).

2.6. Effect of D. quadriceps venom on amastigote forms of T. cruzi

LLC-MK2 cells were seeded in 24-well plates containing glass coverslips (13-mm diameter) cultivated in DMEM supplemented with 10% FCS, and maintained at 37 °C in a 5% CO_2 atmosphere for 24 h. Cells were infected with trypomastigote forms (parasite: host cell ratio of 20:1) in DMEM medium containing 2% FCS. After 48 h of infection, the non-internalized parasites were removed and the cells were cultivated in 2% FCS DMEM medium with or without DqV (1.98 and 3.96 μg/mL) and Bz (73.4 and 146.8 μg/mL). The coverslips were collected up to 24 h and 48 h, washed with PBS, fixed in Bouin's solution and stained with Giemsa (Adade et al., 2011). The percentage of infected cells and the number of intracellular amastigotes per 100 cells was determined by counting 300 cells in triplicate.

2.7. Cytometry flow analysis

Epimastigote forms treated with IC50 of DqV (28.32 μg/mL) incubated for 24 h were stained with FITC-conjugated to annexinV/propidium iodide according to the manufacturer's instructions (BD Pharmigen, California, USA). The population of AX-PI viable cells was evaluated. Mitochondrial transmembrane potential and swelling of reservosomes were also determined. Epimastigote forms were treated with IC50 (28.32 μg/mL) and 2 x IC50 (56.64 μg/mL) of DqV for 24 h and stained with Rhodamine 123 (10 μg/mL) and Acridine Orange (5 μg/mL) according to the manufacturer's instructions (Sigma–Aldrich™, St. Louis, USA). The results were established by determining the fold change (treated/non-treated cell ratio) of the geometric mean of fluorescence. At the end of each incubation period, the cells were washed and submitted to cytometry flow analysis. All data were collected in a FACSCalibur system and analyzed using the Cell Quest software (Becton-Dickinson, California, USA).

2.8. Confocal microscopy

Epimastigote forms were incubated with IC50 (28.32 μg/mL) and 2 x IC50 (56.64 μg/mL) of DqV for 24 h at 28 °C. The parasites were washed and labeled with Rhodamine 123 (10 μg/mL) to observe the mitochondrial transmembrane potential. At the end of the incubation time, the cells were washed and the slides were mounted. The cells were analyzed under a LSM 710 confocal laser scanning microscope (Zeiss, Germany).

2.9. Scanning electron microscopy (SEM)

Epimastigotes forms were treated with IC50 (28.32 μg/mL) and 2 x IC50 (56.64 μg/mL) of DqV for 12 h. After incubation, the parasites were fixed for 2 h with 2.5% glutaraldehyde (Electron Microscopy Sciences, Hatfield, Pennsylvania) washed, dehydrated, dried with CO2, coated with gold and observed in a FEG Quanta 450 scanning electron microscope (FEI, Oregon, USA). Digital images were acquired and stored in a computer.

2.10. Statistical analysis

All tests were performed in triplicate. The statistical analysis was performed using the GraphPad Prism 5 program (GraphPad Software, San Diego, CA, USA). IC50 values were determined by nonlinear regression. Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's post-test. Significance was defined as $^{*}p < 0.05$.

3. Results and discussion

Venom components are of great biotechnological interest, as they are useful to identify new therapeutic targets and establish molecular models for the development of new drugs, with improved efficacy and less toxicity (Harvey, 2014).

Benznidazole and nifurtimox are the only drugs currently being used to treat Chagas' disease; both are highly toxic and rarely beneficial during the chronic phase, with a cure rate of approximately 20% (Urbina and Docampo, 2003). Meglumine antimoniate, sodium stibogluconate, amphotericin B, pentamidine, and miltefosine are the current therapeutic options used to treat Leishmaniasis, which are also highly toxic (D'Annessa et al., 2015).

Leishmanicidal activity by animal venom has been demonstrated (Passero et al., 2007; Pinto et al., 2014). In this study, the growth of promastigote forms of *L. amazonensis* was inhibited by DqV with IC50 of 63.76 ± 20 μg/mL whereas IC50 of amphotericin B was 0.18 ± 0.2 μg/mL after 72 h of incubation.

The treatment of *T. cruzi* epimastigotes with DqV resulted in dose-dependent growth inhibition, with an IC50/24 h treatment of 28.32 ± 5 μg/mL and IC50/48 h of 20.82 ± 5 μg/mL, while BZ showed IC50/24 h treatment of 56.76 ± 15 μg/mL and IC50/48 h of 15.91 ± 3 μg/mL. Treatment of trypomastigote forms also resulted