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Original article

Trypanosoma cruzi: analysis of two different strains after piplartine treatment

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ABSTRACT

The hemoflagellate protozoan, *Trypanosoma cruzi*, mainly transmitted by triatomine insects through blood transfusion or from mother-to-child, causes Chagas' disease. This is a serious parasitic disease that occurs in Latin America, with considerable social and economic impact. Nifurtimox and benznidazole, drugs indicated for treating infected persons, are effective in the acute phase, but poorly effective during the chronic phase. Therefore, it is extremely urgent to find innovative chemotherapeutic agents and/or effective vaccines. Since piplartine has several biological activities, including trypanocidal activity, the present study aimed to evaluate it on two *T. cruzi* strains proteome. Considerable changes in the expression of some important enzymes involved in parasite protection against oxidative stress, such as tryparedoxin peroxidase (TXNPx) and methionine sulfoxide reductase (MSR) was observed in both strains. These findings suggest that blocking the expression of the two enzymes could be potential targets for therapeutic studies.

Introduction

Chagas' disease, caused by the parasite *Trypanosoma cruzi*, is an endemic illness in Latin America, mainly prevalent in Brazil, Argentina, Chile, Colombia, and Venezuela. The current treatment for Chagas' disease is based on benznidazole and nifurtimox, which are effective against acute infections, but poorly effective during the chronic phase. In Brazil, nifurtimox had its production and use discontinued. In this context, major efforts are necessary to identify new targets and develop novel trypanocidal drugs.[1–5]

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Phytochemical studies of *Piper* species have led to the isolation of typical classes of secondary metabolites such as amides, terpenes, benzoic acid derivatives, and hydroquinones, lignans, neolignans, and flavonoids.[6–9] Piplartine (Fig. 1) is an amide isolated from *Piper* species. An excellent review described its biological activities,[10] such as cytotoxic, genotoxic, antitumor, antiangiogenic, antimetastatic, antiplatelet aggregation, antinociceptive, anxiolytic, antidepressant, anti-atherosclerotic, antidiabetic, antibacterial, antifungal, leishmanicidal, trypanocidal, schistosomicidal, and larvicidal activities.[11–19] Administration of piplartine inhibited solid tumor development in mice transplanted with Sarcoma 180 cells and histopathological analysis showed that liver and kidneys of treated animals were only slightly and reversibly affected.[20] Anticancer activity of piplartine was reinforced when it reduced human glioblastoma (SF-295) and colon carcinoma (HCT-8) cell viability.[21] Acute toxicity studies performed in rats and mice treated with different piplartine doses by oral route demonstrated no obvious clinical alterations.[22] Viability assay carried out against *T. cruzi* epimastigote forms demonstrated growth inhibition higher than that observed with benznidazole,[23] and activity against *Leishmania donovani* was observed, with considerable reduction in parasitic burden and spleen weight *in vivo*.[24]

The low systemic cytotoxicity of piplartine associated with activity against trypanosome cells brought new questions about the possibility of its use as a trypanocidal drug. Therefore, the objective of this study was to evaluate protein expression changes in response to piplartine treatment on the epimastigote form of *T. cruzi*. The capacity of the parasite to grow, associated to high level of cells obtained, allow for performing differential proteomic and mass spectrometry assays. The obtained results are consistent with those described in the literature and may contribute with future similar analysis using other forms of *T. cruzi*.

In this paper, two-dimensional gel electrophoresis (2-DE) and mass spectrometry were used to identify different expressed proteins as a result of piplartine effect on two *T. cruzi* strains, Y[25] and Bolivia,[26] respectively.

Materials and methods

Piplartine isolation

Isolation of piplartine from *Piper tuberculatum* leaves was carried out according to a methodology previously published.[23]

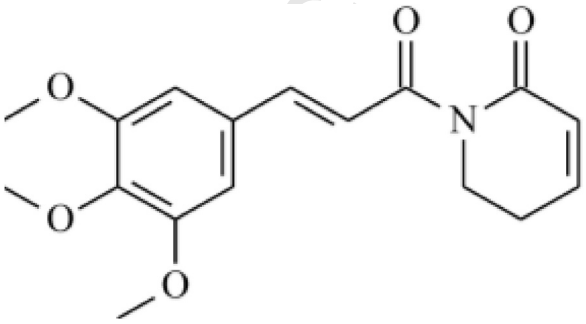

Fig. 1 – Chemical structure of piplartine.

Briefly, specimens of *Piper tuberculatum* were cultivated from seeds under greenhouse conditions at the Institute of Chemistry, Universidade Estadual Paulista, Araraquara, São Paulo State, Brazil. Plant material was collected in May 2005 and identified by Dr. Guillermo E. D. Paredes from Universidad Pedro Ruiz Gallo, Kambayeque, Peru. Voucher (Cordeiro-1936) was deposited at the herbarium of the Institute of Biosciences, Universidade de São Paulo, São Paulo, São Paulo State, Brazil.

Shade-dried and powdered leaves of *P. tuberculatum* (200 g) were extracted with ethyl acetate (Synth) and methanol (Synth) (9:1), for three weeks at room temperature. After filtering, the solvent was evaporated under reduced pressure to yield crude extract (11 g). Crude extract (10 g) was subjected to chromatography column over silica gel 60 (70–230 mesh, Merck) and eluted with hexane (Synth) and ethyl acetate (Synth) (0–100% ethyl acetate), and ethyl acetate (Synth) and methanol (Synth) (0–100% methanol) to afford 12 fractions (E1–E12). Fractions E6 and E7 (2.17 g) was further purified by chromatography column over silica gel 60 (70–230 mesh, Merck) and eluted with hexane (Synth) and acetone (Synth) (9:1) to provide 21 fractions (F1–F21), including piplartine (fraction F11, 532 mg). Structure of piplartine was identified by ^{1}H and ^{13}C NMR spectra analysis.

Trypanosoma cruzi cell viability assay

Epimastigote forms (Y and Bolivia strains) were maintained in Liver Infusion Tryptose (LIT) medium (68.4 mM NaCl; 5.4 mM KCl; 56.3 mM Na_2HPO_4; 111 mM Dextrose; 0.3% Liver Infusion Broth; 0.5% Tryptose)[27] and the cells were harvested during the exponential growth phase. The parasites (1×10^7 cells/mL) were treated for 48 h in LIT at 28 °C with 13.7 μM (Y strain) and 10.5 μM (Bolivia strain) piplartine, which corresponds to half of IC_{50}/72 h previously determined for this compound. Triplicates of treated and control (without piplartine) parasites were prepared. Calculated IC_{50} in 72 h of experiment for piplartine in Y and Bolivia strains were 27.45 μM and 20.95 μM, respectively. For Balb/c macrophages IC_{50} was 305.79 μM. Safety index was 11.14 and 14.59, in comparison with piplartine IC_{50} for Y and Bolivia strains, respectively (data not published). The chosen concentration of the piplartine for 2DE-DIGE tests was half the IC_{50} previously determined, since the intention was to induce protein expression changes, maintained considerable number of viable parasites.

Sample preparation, labeling and two-dimensional electrophoresis

Treated and control epimastigotes parasites from both strains were centrifuged at 3000 × *g* for 5 min at 4 °C, the pellet was washed three times with *tryps wash* buffer (100 mM NaCl, 3 mM $MgCl_2$, 20 mM Tris–Hcl, pH 7.5) and incubated in lysis buffer (7 M Urea, 2 M Thiourea, 4% CHAPS, 40 mM Tris, 0.1 mg/mL PMSF; 1 M DTT, 1 mg/mL Pepstatin, 10 mg/mL Aprotinin and 10 mg/mL Leupeptin; all enzyme inhibitors from Sigma-Aldrich) for two hours. Protein extraction was performed using three independent biological replicates of each sample (treated and control). To obtain the soluble protein fraction, the cell lysate was centrifuged at 14,000 × *g* for 10 min

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