

Repositioning of HIV aspartyl peptidase inhibitors against the neglected pathogen *Trypanosoma cruzi*

Sangenito, L.S^{a,*}; d'Avila-Levy, C.M^b; Branquinha, M.H^{a,‡};
Santos, A.L.S^{a,c,‡}

* ibastefano@hotmail.com ‡ Both authors share senior authorship.

a Laboratório de Investigação de Peptidases, Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes (IMPG), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil;

b Laboratório de Estudos Integrados em Protozoologia, Instituto Oswaldo Cruz (IOC), Fundação Oswaldo Cruz (FIOCRUZ), Rio de Janeiro, Brazil.

c Programa de Pós-Graduação em Bioquímica, Instituto de Química, UFRJ, Rio de Janeiro, Brazil;

ABSTRACT:

There is an urgent need to implement new strategies to combat Chagas' disease. In this context, repositioning of clinically approved drugs appears as a viable tool to combat neglected pathologies. An example is the use of aspartic peptidase inhibitors (PIs) currently applied in the HIV treatment (HIV-PIs) against different infectious agents. In fact, since introduction of HIV-PIs, in AIDS therapy, the incidence, morbidity and mortality of protozoan co-infections decreased dramatically. Therefore, with this in mind, the main objective of this work was to verify the effects of HIV-PIs against *Trypanosoma cruzi*. Our results showed that the majority of the HIV-PIs ($n=6/9$) were able to drastically decrease the viability of trypomastigotes after 4 h of treatment, being nelfinavir and lopinavir the most effective compounds presenting LD₅₀ values of 8.6 and 10.6 μM , respectively. The treatment with nelfinavir and lopinavir caused many morphological/ultrastructural alterations in trypomastigotes such as shrinkage and cell rounding as well as shortening of the flagellum and damage in plasma membrane. Both compounds were able to decrease the aspartyl peptidase and proteasome activities, two predicted targets for HIV-PIs. However, another possible targets can not be ruled out. In this sense, nelfinavir and lopinavir also impaired the mitochondrial functions in *T. cruzi* trypomastigotes by inhibiting the mitochondrial dehydrogenases and reducing the mitochondrial transmembrane electric potential ($\Delta\Psi_m$). In addition, a decompensation in generation of reactive oxygen species was also detected in nelfinavir/lopinavir-treated parasites. Another observed fact was the presence of many lipid bodies positive to Nile Red and randomly distributed throughout the parasite's cytoplasm, indicating alterations on lipid metabolism. The double staining of treated cells with annexin-V and propidium iodide may be correlated to late events of apoptosis-like death or necrosis. Cytotoxicity assays with LLC-MK₂ epithelial cells and RAW macrophages allowed the evaluation of the effects of HIV-PIs on the interaction between trypomastigotes and these cells as well as the survival of intracellular amastigotes. The pre-treatment of trypomastigotes with nelfinavir and lopinavir inhibited the association index with LLC-MK₂ and RAW cells in a dose- and time-dependent manner. In addition, nelfinavir and lopinavir also reduced significantly the number of intracellular amastigotes in both cell lineages, particularly when administered in daily doses. Our results open the possibility for the use of HIV-PIs as a palpable alternative in the treatment of Chagas' disease. In addition, these data contribute to better understanding how HIV-PIs could act in protozoan parasites, however, further studies should be conducted to confirm these findings and more studies using *in vivo* models must be conducted.

Keywords: *Trypanosoma cruzi*, Chagas' disease, HIV-PIs, anti-*T. cruzi* activity, mode of action

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