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

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There is an urgent need to implement new strategies and to search new chemotherapeutic targets to combat Chagas' disease. In this context, repositioning of clinically approved drugs appears as a viable tool to combat this and several other 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, sinceintroduction 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 is to verify the effects of HIV-PIs against the trypomastigote and amastigote forms of *Trypanosoma cruzi* using *in vitro* models.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 LD_{50} values of 8.6 and 10.6 µM, respectively. Additionally, both HIV-PIs demonstrated to be effective in a time- and cell density-dependent manner.The treatment with nelfinavir and lopinavir caused many morphological/ultrastructural alterations in trypomastigotes. Those main responses to the treatment were 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 anexin-V and propidium iodidemay be correlated to late events of apoptosis-like death or necrosis. Cytotoxicity assays with LLC-MK_2 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-MK_2 cells and RAW macrophages in a dose- and time-dependent manner. In addition, nelfinavir and lopinavir also reduced significantly the number of intracellular amastigotes in both mammalian cell lineages, particularly when administered in daily doses. Both compounds had no effect on nitric oxide production in infected RAW cells. 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.