International Journal of Antimicrobial Agents 48 (2016) 703–711

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Nelfinavir and lopinavir impair *Trypanosoma cruzi* trypomastigote infection in mammalian host cells and show anti-amastigote activity

Leandro S. Sangenito [a], Claudia M. d'Avila-Levy [b], Marta H. Branquinha [a,*,1], André L.S. Santos [a,c,*,1]

[a] *Departamento de Microbiologia Geral, Laboratório de Investigação de Peptidases, 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*

ARTICLE INFO

Article history:
Received 22 June 2016
Accepted 15 September 2016

Keywords:
Trypanosoma cruzi
HIV-PI
Nelfinavir
Lopinavir
Cellular interaction
Amastigotes

ABSTRACT

There is an urgent need to implement new strategies and to search for 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 human immunodeficiency virus (HIV) treatment against different infectious agents. Therefore, the main objective of this work was to verify the effects of the HIV-PIs nelfinavir and lopinavir against *Trypanosoma cruzi* using *in vitro* models of infection. Cytotoxicity assays with LLC-MK_2 epithelial cells and RAW macrophages allowed an evaluation of the effects of HIV-PIs on the interaction between trypomastigotes and these cells as well as the survival of intracellular amastigotes. 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 significantly reduced 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 macrophages. These results open the possibility for the use of HIV-PIs as a tangible alternative in the treatment of Chagas' disease. However, the main mechanism of action of nelfinavir and lopinavir has yet to be elucidated, and more studies using *in vivo* models must be conducted.
© 2016 Elsevier B.V. and International Society of Chemotherapy. All rights reserved.

1. Introduction

Chagas' disease, also known as American trypanosomiasis, is an endemic neglected tropical disease in 18 countries in Latin America caused by the parasite *Trypanosoma cruzi*. The disease is one of the leading causes of morbidity, long-term disability and mortality, with estimates of more than 8 million infected individuals [1]. Although Chagas' disease is considered an emblematic Latin American problem, the influx of immigrants from endemic areas has led to the disease also becoming a health problem in North America and Europe [2], facilitating co-infection with human immunodeficiency virus (HIV) in areas with a high viral prevalence [3].

Despite the wide distribution and severe symptoms, treatment of Chagas' disease is based on only two drugs approved for around half a century, namely benznidazole and nifurtimox. Both treatments have many side effects and their therapeutic efficacy is still unclear, requiring careful monitoring. In Brazil, nifurtimox was withdrawn from the market owing to the great uncertainty regarding its effectiveness and its severe side effects. Therefore, benznidazole is used as the unique first-line treatment for Brazilian patients with Chagas' disease [4]. Promising new drug candidates for treatment include inhibitors of the sterol biosynthesis pathway such as the azoles ravuconazole and posaconazole [5]. In addition, fexinidazole was selected for development by the Drugs for Neglected Diseases Initiative (DNDI) as a new drug candidate for sleeping sickness [6] and showed promising effects in oral treatment of acute and chronic experimental Chagas' disease [7].

Although some progress in drug discovery has been made in recent years, there are still gaps to be filled regarding the correct and efficacious treatment against Chagas' disease. Moreover, the high cost and financial uncertainties discourage pharmaceutical companies to invest time and money to search for new chemotherapeutics against this parasitic illness [8,9]. In the face of this,

* Corresponding authors. Departamento de Microbiologia Geral, Laboratório de Investigação de Peptidases (LIP), Instituto de Microbiologia Paulo de Góes (IMPG), Centro de Ciências da Saúde (CCS), Universidade Federal do Rio de Janeiro (UFRJ), Bloco E-subsolo, Sala 05, Rio de Janeiro, RJ 21941-902, Brazil. Fax: +55 21 3938 8344.
E-mail addresses: mbranquinha@micro.ufrj.br (M.H. Branquinha); andre@micro.ufrj.br (A.L.S. Santos).
[1] These authors share senior authorship.

http://dx.doi.org/10.1016/j.ijantimicag.2016.09.017
0924-8579/© 2016 Elsevier B.V. and International Society of Chemotherapy. All rights reserved.

drug repositioning appears as an interesting strategy to discover new tangible treatments for Chagas' disease and other neglected diseases [10,11]. Since the prevalence of opportunistic protozoan infections in patients with acquired immune deficiency syndrome (AIDS) during the highly active antiretroviral therapy (HAART) era was dramatically decreased, some groups have focused on the use of HIV aspartic peptidase inhibitors (HIV-PIs), commonly administered during HAART [12], against pathogenic protozoa [13,14], including *Leishmania* spp. [15–18] and *T. cruzi* [19]. For instance, the viability of *T. cruzi* trypomastigotes was powerfully inhibited by treatment with HIV-PIs, mainly by nelfinavir and lopinavir. These two HIV-PIs induced irreversible morphological damage and blocked both aspartic-type peptidase and proteasome activities, two potential targets for these drugs [19]. In the current study, the effects of the HIV-PIs nelfinavir and lopinavir on the interaction process between trypomastigotes and mammalian cells and on replicative intracellular amastigotes were evaluated.

2. Methods

2.1. Mammalian cells and parasite cultivation

LLC-MK_2 epithelial cells and RAW 264.7 murine macrophages were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% of foetal bovine serum (FBS) at 37 °C in an atmosphere containing 5% CO_2. Tissue culture-derived trypomastigotes of *T. cruzi* Y strain were harvested (500 × *g*, 5 min) from culture supernatants of 5-day-old infected LLC-MK_2 cells [20].

2.2. Mammalian viability assays

The effect of the HIV-PIs on the viability of LLC-MK_2 and RAW 264.7 cell lineages was evaluated by MTT assay [21]. First, mammalian cells (10^5 cells/mL) were allowed to adhere to 96-well plates in DMEM supplemented with 10% FBS for 24 h at 37 °C in a 5% CO_2 atmosphere. Cells were then incubated with single doses (at time 0 h) of increasing concentrations of the HIV-PIs (0.78–400 μM) for a period of 4 h (both cells), 48 h (RAW macrophages) and 72 h (LLC-MK_2 cells). Alternatively, cells were incubated with daily doses of each drug in fresh DMEM for a total period of 48 h (RAW macrophages) and 72 h (LLC-MK_2 cells). Subsequently, formation of formazan was measured by incubating the wells for 3 h in the dark at 37 °C with MTT (5 mg/mL in phosphate-buffered saline). Plates were then centrifuged (300 × *g*, 8 min), the pellet was dissolved in dimethyl sulphoxide (DMSO) and the absorbance at 570 nm was measured in an enzyme-linked immunosorbent assay (ELISA) microplate reader. Concentrations of nelfinavir and lopinavir capable of maintaining 95% cellular viability were used in subsequent interaction assays. In addition, the 50% cytotoxic concentration (CC_{50}) for each cell lineage was determined by linear regression analysis after 4 h of treatment with each inhibitor. The selectivity index (SI) was calculated by dividing the CC_{50} value of each mammalian cell lineage by the 50% lethal dose (LD_{50}) of trypomastigotes. SI values considered satisfactory for each compound were those >10 [22].

2.3. Effects of nelfinavir and lopinavir on T. cruzi–mammalian cell interactions

To determine the effects of HIV-PIs on the interaction process, the following systems were adopted. Trypomastigotes were incubated in DMEM with 2% FBS and were treated for 1 h with different concentrations of nelfinavir or lopinavir (1, 5 and 10 μM) or were treated with each inhibitor at 1 μM for different incubation intervals (1, 2 and 4 h). Untreated and HIV-PI-treated trypomastigotes were added to 24-well tissue plates to interact with RAW macrophages (for 3 h) or LLC-MK_2 cells (for 24 h) at a parasite/cell ratio of 10:1 at 37 °C in a 5% CO_2 atmosphere. Subsequently, non-adhered parasites were removed by washing with DMEM. Infected cells were fixed in Bouin solution and were stained with Giemsa. The percentage of infected cells was determined by randomly counting ≥200 cells on each of triplicate coverslips using bright-field microscopy. The association index was obtained by multiplying the percentage of infected cells by the number of amastigotes per infected cell.

2.4. Effects of nelfinavir and lopinavir on intracellular amastigotes

Trypomastigotes were added to 24-well tissue plates to infect RAW macrophages for 3 h or LLC-MK_2 cells for 24 h at a parasite/cell ratio of 10:1 at 37 °C in a 5% CO_2. After the respective periods of incubation, free parasites were removed by washing with DMEM, and then fresh medium with 2% FBS was added. Mammalian cells infected with *T. cruzi* were then subjected to two different schemes of treatment with nelfinavir and lopinavir at concentrations able to preserve ca. 95% of host cell viability. The first system consisted of treating the infected cultures at time 0 with different concentrations of nelfinavir or lopinavir in single doses for a period of 48 h for RAW macrophages and 72 h for LLC-MK_2 cells. The second system consisted of treating both mammalian cell lines with fresh daily doses of each HIV-PI up to 2 days or 3 days for RAW macrophages and LLC-MK_2 cells, respectively. To observe this last experiment, infected cultures had their supernatants collected every 24 h, the cells were washed with DMEM, and then fresh medium with 2% FBS and containing each drug was added. The percentage of infected cells and the association index were determined as previously described. The 50% inhibitory concentration (IC_{50}) for amastigotes was determined by linear regression analysis. Images were obtained under a ZEISS Axioplan microscope.

2.5. Effects of nelfinavir and lopinavir on nitric oxide (NO) production

Supernatants from control (macrophages alone in culture) and trypomastigote-infected macrophages non-treated or treated with single and daily doses of nelfinavir and lopinavir were analysed for their nitrite contents by the Griess reaction [23]. Lipopolysaccharide (LPS) was used as a positive control for stimulation of NO production in uninfected macrophages. Absorbance at 540 nm was measured and the concentration of nitrite was calculated using linear regression of a standard curve.

3. Results and discussion

3.1. Cytotoxicity of nelfinavir and lopinavir to mammalian cells

Initially, the cytotoxicity of lopinavir and nelfinavir to mammalian cells was evaluated after 4 h of treatment. The CC_{50} values of lopinavir were >400 μM both for LLC-MK_2 and RAW cells (Table 1). The same result was obtained for RAW macrophages after treatment with nelfinavir, whilst the CC_{50} value for LLC-MK_2 cells was 166.7 μM (Table 1). The direct effect of nelfinavir and lopinavir on the viability of tissue culture trypomastigotes was previously demonstrated by our group, with LD_{50} values of 8.6 μM and 10.6 μM, respectively, after only 4 h of parasite–drug contact [19]. Under these experimental conditions, both HIV-PIs showed higher toxicity to *T. cruzi* trypomastigotes compared with mammalian cells, resulting in excellent SI values (Table 1).

Cytotoxicity to mammalian cells was also determined for long periods of time with single-dose treatment of each HIV-PI (Fig. 1). In 72-h-treated LLC-MK_2 cultures, a significant deleterious effect was observed only with nelfinavir at concentrations >3.12 μM, whilst lopinavir presented these effects at >12.5 μM (Fig. 1, upper panel).

Download English Version:

https://daneshyari.com/en/article/5666864

Download Persian Version:

https://daneshyari.com/article/5666864

Daneshyari.com