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Antileishmanial activity of HIV protease inhibitors

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Abstract

The proteasomes of some protozoa are possible targets for chemotherapy. Leishmaniasis is a major health problem in human immunodeficiency virus (HIV) co-infected subjects. Two HIV protease inhibitors (PI), indinavir and saquinavir, have been shown to block proteasome functions; we therefore investigated their effects on the growth of two Leishmania spp. (Leishmania major and Leishmania infantum). After 24 h of treatment, both drugs exhibited a dose-dependent antileishmanial activity, with 50% lethal dose (LD 50 ) values of, respectively, 8.3 μM and 7 μM on L. major; minor activity was observed on L. infantum. These results add new in vitro insights into the wide-spectrum efficacy of PI and suggest studying their action on amastigote forms of leishmania within macrophages in order to validate their potential contribution against opportunistic infections in treated seropositive patients.

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Keywords: Leishmania major; Leishmania infantum; Protease inhibitors; Proteasomes

1. Introduction

Leishmaniasis currently affects an estimated 12 million people, in every continent except Australia and Antarctica. Moreover, the emergence of visceral leishmaniasis as an opportunistic infection [1], particularly in patients infected with human immunodeficiency virus (HIV), has given rise to the need for new therapeutic strategies. The control of leishmaniasis infection is T-cell dependent and requires a Th1 response. There has therefore been an increased incidence of kala azar in HIV co-infected patients in Bihar (India) [2], and in the Mediterranean basin up to 9% of acquired immune deficiency syndrome (AIDS) patients suffer from newly acquired or reactivated visceral leishmaniasis [3]. Treatment with antimonials, the mainstay of therapy, is less satisfactory in immunocompromised patients than in immunocompetent subjects [1]. New therapies, such as lipid formulations of amphotericin B or miltefosine, are very expensive. Therefore, there is a pressing need for new antileishmanial drugs. The incidence of HIV–Leishmania co-infections has been decreasing since the introduction of highly active antiretroviral therapy (HAART) [4,5]. Some antiretroviral agents exhibit significant inhibitory effects on purified human proteasome functions [6,7]. In recent years several studies have documented the antiproteasomal activity of some proteasome inhibitors. Proteasomes are large, non-lysosomal, multi-subunit protease complexes. They are characterised by evolutionarily conserved proteins and are present in Trypanosoma brucei [8], Trypanosoma cruzi [9], Toxoplasma gondii [10], Plasmodium spp. [11], Entamoeba histolytica and Entamoeba invadens [12], as well as in Leishmania mexicana [13]. A molecular similarity was found between the human 20S proteasome α-type subunit and a Leishmania donovani cDNA clone [14] and between a gene encoding a 20S proteasome β subunit in Plasmodium falciparum [15]. Proteasomes play a key role in differentiation and replication of protozoa, and lactacystin, a specific inhibitor of proteasomes, has a negative impact on the replication of L. mexicana promastigotes, although only at a high concentration [13].

We explored whether indinavir and saquinavir, two HIV protease inhibitors (PI) commonly used in infected individuals that share potent inhibitory effects on purified human proteasomes [6,7], have antileishmanial activity.
The activity of Nt.-α-difluoromethylornithine (DFMO), a polyamine analogue inhibiting protozoan ornithine decarboxylase [16], was also assessed.

2. Materials and methods

2.1. Leishmaniae

*Leishmania major* (LRC-L137 strain) and *Leishmania infantum* (MHOM/TN/80/IPT1 strain) were maintained in vitro at 25 °C in Tobe’s diphagous medium. Before use, promastigotes were grown in medium 199 (Invitrogen, CA, USA), modified as previously indicated [17], at 25 °C for 4 days to reach late-log phase growth.

2.2. In vitro studies

The effects of the two PI, indinavir (Merck; a kind gift of S. Vella, Rome, Italy) and saquinavir (BS 00120083; a kind gift of Roche, Basel, Switzerland), on promastigotes of *L. major* and *L. infantum* were assessed by a method similar to that previously described [17]. Promastigotes were counted using a haemocytometer (Thoma chamber) and re-suspended in fresh medium to a final concentration of 5 × 10^5 viable (showing motile behaviour and/or lack of staining after vital staining with trypan blue) promastigotes/mL. The two compounds were added to the cultures at final concentrations of 6.25, 12.5, 25 and 50 mM (starting from a 5 mM solution in dimethylsulfoxide (DMSO) that was serially diluted in medium). Dilutions of DMSO corresponding to those used to prepare the drug solutions were assessed in parallel. Nt.-α-DFMO (Sigma, Milan, Italy) was assayed at final concentrations of 3, 6, 12 and 24 mM. After 24, 48 and 72 h incubation at 25 °C with occasional agitation, the number of viable, motile promastigotes was quantified. At the end of 24 h incubation, the reversibility of the effect was assessed by adding fresh complete medium at a 10:1 ratio; the same dilution was made for the control. After a further 48 h incubation, the number of live promastigotes was evaluated. The experiments were performed in triplicate and the standard deviations were ≤10% of the means obtained at each point. The 50% lethal dose (LD₅₀), i.e. the drug concentration that caused a 50% reduction in survival/viability in comparison with that in identical cultures without the compound, was evaluated after 24 h. This value was determined by non-linear regression analysis, by plotting the number of viable promastigotes versus log drug concentrations by use of GraphPad Prism 3 software.

3. Results and discussion

The results demonstrate that after 1 day of treatment, starting from a concentration of 6.25 μM, both indinavir and saquinavir induced ~50% growth inhibition of *L. major* promastigotes (Table 1). At this time point, DMSO also exhibited a mild inhibitory activity, although only at dilutions corresponding to a drug concentration of ≥25 μM. After 2 days (and likewise after 3 days), the inhibitory effects of saquinavir on parasite growth persisted, ranging from 17% to 65% inhibition in a dose-dependent manner (Table 1).

<table>
<thead>
<tr>
<th>Leishmaniae spp.</th>
<th>Days of treatment</th>
<th>Dose</th>
<th>30μM</th>
<th>25μM</th>
<th>12.5μM</th>
<th>6.25μM</th>
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<tr>
<td><em>L. major</em></td>
<td>1</td>
<td>35°</td>
<td>60</td>
<td>38°</td>
<td>72</td>
<td>43°</td>
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<td>2</td>
<td>47°</td>
<td>71</td>
<td>66</td>
<td>88</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>48°</td>
<td>83</td>
<td>78</td>
<td>58</td>
<td>95</td>
</tr>
<tr>
<td><em>L. infantum</em></td>
<td>1</td>
<td>69</td>
<td>94</td>
<td>72</td>
<td>70</td>
<td>95</td>
</tr>
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<td>71</td>
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</tbody>
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* Statistically significant difference, *P* < 0.05.
pounds may be due to their effects on proteasome activity. As observed on \textit{L. mexicana} [13], these effects may depend on the stage of the parasite; for example, amastigotes developing within macrophages during human infection are more sensitive to lactacystin than promastigotes developing in culture medium. If the same applies to indinavir and saquinavir, their leishmanicidal effects should be higher in vivo than in vitro. Potent antiretroviral therapy with PI decreases the expression of virulence enzymes of \textit{Candida albicans} [19]; furthermore, it has a negative impact on the yield of \textit{Cryptococcus neoformans} [20], \textit{Pneumocystis carinii} [21] and \textit{Cryptosporidium parvum} [22]. In addition to the immune restoration, HIV-infected patients receiving PI may thus benefit from their direct inhibitory activity on a large array of microorganisms.

These results add new in vitro insights into the wide-spectrum efficacy of HIV PI and suggest studying their action on amastigote forms of leishmania within macrophages to validate their potential contribution against opportunistic infections in treated seropositive patients.

References


