2005

**Antimalarial activity of human immunodeficiency virus type 1 protease inhibitors**

Sunil Parikh  
*University of California - San Francisco*

Jiri Gut  
*University of California - San Francisco*

Eva Istvan  
*Washington University School of Medicine in St. Louis*

Daniel E. Goldberg  
*Washington University School of Medicine in St. Louis*

Diane V. Havlir  
*University of California - San Francisco*

*See next page for additional authors*

Follow this and additional works at: [https://digitalcommons.wustl.edu/open_access_pubs](https://digitalcommons.wustl.edu/open_access_pubs)

**Recommended Citation**  
[https://digitalcommons.wustl.edu/open_access_pubs/2360](https://digitalcommons.wustl.edu/open_access_pubs/2360)

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
Antimalarial Activity of Human Immunodeficiency Virus Type 1 Protease Inhibitors

Sunil Parikh, Jiri Gut, Eva Istvan, Daniel E. Goldberg, Diane V. Havlir and Philip J. Rosenthal


Updated information and services can be found at: http://aac.asm.org/content/49/7/2983

REFERENCES

This article cites 14 articles, 6 of which can be accessed free at: http://aac.asm.org/content/49/7/2983#ref-list-1

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: http://journals.asm.org/site/misc/reprints.xhtml
To subscribe to another ASM Journal go to: http://journals.asm.org/site/subscriptions/
Antimalarial Activity of Human Immunodeficiency Virus Type 1 Protease Inhibitors

Sunil Parikh, Jiri Gut, Eva Istvan, Daniel E. Goldberg, Diane V. Havlir, and Philip J. Rosenthal

Department of Medicine, San Francisco General Hospital, University of California, San Francisco, San Francisco, California, and Department of Medicine and Department of Molecular Microbiology, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, Missouri

Received 21 January 2005/Returned for modification 9 March 2005/Accepted 18 March 2005

Aspartic proteases play key roles in the biology of malaria parasites and human immunodeficiency virus type 1 (HIV-1). We tested the activity of seven HIV-1 protease inhibitors against cultured Plasmodium falciparum. All compounds inhibited the development of parasites at pharmacologically relevant concentrations. The most potent compound, lopinavir, was active against parasites (50% inhibitory concentration [IC50], 0.9 to 2.1 μM) at concentrations well below those achieved by ritonavir-boosted lopinavir therapy. Lopinavir also inhibited the P. falciparum aspartic protease plasmepsin II at a similar concentration (IC50, 2.7 μM). These findings suggest that use of HIV-1 protease inhibitors may offer clinically relevant antimalarial activity.

The human immunodeficiency virus type 1 (HIV-1) pandemic has emerged in many regions of the developing world already suffering from the burden of malaria (5). In developed countries, HIV-1 protease inhibitors have dramatically improved the outcome of HIV disease. The target of these inhibitors is the HIV-1 protease, a member of the aspartic protease family (6). Plasmodium falciparum, the most virulent human malaria parasite, expresses a number of aspartic proteases, known as plasmepsins (2). Recent studies suggest that three HIV-1 protease inhibitors, saquinavir, ritonavir, and indinavir, inhibit the growth of Plasmodium falciparum parasites in vitro at clinically relevant concentrations (13). In addition, evidence suggests that HIV-1 protease inhibitors may protect against malaria through the inhibition of CD36-mediated cytoadherence of P. falciparum-infected erythrocytes (9). We hypothesized that HIV-1 aspartic protease inhibitors exert antimalarial activity by acting against plasmepsins. To test this hypothesis, we investigated the effects of seven available HIV-1 protease inhibitors on the in vitro development of cultured malaria parasites and on the P. falciparum aspartic protease plasmepsin II.

To evaluate the antiparasitic effects of HIV-1 protease inhibitors, we incubated cultured parasites with multiple concentrations of seven inhibitors. P. falciparum parasites were cultured with human erythrocytes (2% hematocrit) in RPMI medium and 10% human serum (11). Four laboratory strains of P. falciparum (acquired from the Malaria Research and Reference Reagent Center) with a wide range of sensitivities to standard antimalarial drugs were studied (12). Parasites were synchronized by serial treatments with 5%D-sorbitol (11). Microwell cultures of synchronized parasites were incubated with HIV-1 protease inhibitors (from 1,000× stocks in dimethyl sulfoxide [DMSO]; final concentrations ranged from 100 μM to 25 nM) for 48 h beginning at the ring stage. The effects of inhibitors upon P. falciparum morphology were assessed by light microscopy of Giemsa-stained smears. After 12 h of incubation, beginning at the late ring stage, synchronized parasites treated with concentrations of lopinavir achievable with standard dosing (10 μM) exhibited markedly altered morphology (Fig. 1A). Parasite abnormalities were more marked after 24 h, and after 48 h, when control cultures contained normal rings, treated cultures contained only very abnormal pyknotic parasites. The morphological changes caused by the protease inhibitors were rather nonspecific, but similar to those caused by the generic aspartic protease inhibitor pepstatin (1, 10).

To quantify antimalarial activity, new ring stage parasites were counted after incubating parasites with protease inhibitors for one 48-hour life cycle, beginning at the ring stage. Ring parasitemias were assessed by fluorescence-activated cell sorter analysis and compared with those of control cultures incubated with the same concentration of DMSO, as previously described (11, 12). Fifty percent inhibitory concentrations (IC50s) were calculated by nonlinear regression with the Prism 3.0 program (GraphPad Software). All tested HIV-1 protease inhibitors demonstrated antimalarial activity at low micromolar concentrations (Table 1). Results were similar for all four tested P. falciparum strains. Calculated IC50s were higher than those previously reported for the HIV-1 protease inhibitors saquinavir, ritonavir, and indinavir (13), probably due to differences in assay methods, but nonetheless all tested compounds exerted antimalarial activity at concentrations near those achievable in the bloodstream with standard dosing. Importantly, combination regimens that take advantage of the boosting of levels of other protease inhibitors by the strong cytochrome P450 inhibitor ritonavir are increasingly advocated for standard antiretroviral therapy (8). In this regard, it is of interest that the most potent antimalarial protease inhibitor was lopinavir, which demonstrated an IC50 nearly 10-fold below the trough blood concentration achieved with standard...
TABLE 1. Activity of HIV-1 protease inhibitors against cultured *P. falciparum*.

<table>
<thead>
<tr>
<th>Drug</th>
<th><em>P. falciparum</em> IC50 (μM) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HB3</td>
</tr>
<tr>
<td></td>
<td>D6</td>
</tr>
<tr>
<td></td>
<td>Dd2</td>
</tr>
<tr>
<td></td>
<td>W2</td>
</tr>
<tr>
<td>Saquinavira</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>Ritonavirb</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>Indinavirc</td>
<td>5.8 ± 0.9</td>
</tr>
<tr>
<td>Nelfinavird</td>
<td>15.2 ± 0.1</td>
</tr>
<tr>
<td>Amprenavire</td>
<td>51.9 ± 22.4</td>
</tr>
<tr>
<td>Lopinavire</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Atazanavire</td>
<td>6.8 ± 0.3</td>
</tr>
</tbody>
</table>

**Legend**

a Based on saquinavir 1,200 mg three times daily (as free base) in HIV-infected individuals coadministered saquinavir soft gel capsule 1,000 mg/ritonavir 100 mg b.i.d. in HIV-infected individuals (Roche prescribing information) (14).

b Based on ritonavir 600 mg b.i.d. in healthy HIV-infected individuals (Abbott prescribing information).

c Based on indinavir sulfate 800 mg every 8 h and coadministered indinavir 800 mg/ritonavir 100 mg b.i.d. in healthy individuals with low-fat meal (3).

d Based on nelfinavir mesylate 1,250 mg b.i.d. in HIV-infected individuals; Cmin was determined prior to morning dosage (Agouron prescribing information).

e Based on amprenavir 1,200 mg b.i.d. in healthy individuals and coadministered amprenavir 600 mg/ritonavir 100 mg b.i.d. in HIV-infected individuals (GlaxoSmithKline prescribing information) (7).

f Based on lopinavir 400 mg/ritonavir 100 mg b.i.d. in HIV-infected individuals (Abbott prescribing information).

g Based on atazanavir sulfate 400 mg once a day (q.d.) in HIV-infected subjects and coadministered atazanavir 300 mg/ritonavir 100 mg q.d. (Bristol-Myers Squibb prescribing information); serum concentrations are given as geometric means of atazanavir, as free base.

h IC50 data are means ± standard deviations from four experiments. Serum concentrations are from published information. Cmax and Cmin are the mean maximum and minimum serum levels achieved under standard dosing intervals, respectively. Ritonavir-boosted serum concentrations are those achieved with coadministration with ritonavir, as indicated in the other footnotes. NA, not applicable, as these formulations are not used clinically.
vir regimen. Due to concerns regarding cost, toxicity, and potential selection of resistant viruses, it is unlikely that currently available HIV-1 protease inhibitors will gain roles as standard treatments for malaria. Nonetheless, it seems likely that, for select protease inhibitors, the concentrations achieved during chronic antiretroviral therapy will offer some protection against malaria. If standard regimens for HIV-1 offer chemoprophylaxis against malaria, particularly in children, in whom the burden of malaria is greatest, the clinical consequences of this effect will be great. However, it is unclear if in vitro results showing antimalarial activity of HIV-1 protease inhibitors predict clinical efficacy. Therefore, clinical trials to test the hypothesis that HIV-1 protease inhibitors confer protection against malaria are urgently needed.

We thank members of the Rosenthal laboratory (Puran Sijwali, Kailash Pandey, Julie Lehman, and Anthony Lau) and Jun Liu of the Goldberg laboratory for their expert technical assistance. Financial support was provided by the National Institutes of Health. Protease inhibitors were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: lopinavir, ritonavir, saquinavir (as free base), atazanavir, indinavir, nelfinavir, and amprenavir.

REFERENCES


