Antimalarial effects of human immunodeficiency virus type 1 protease inhibitors differ from those of the aspartic protease inhibitor pepstatin

Sunil Parikh  
University of California - San Francisco

Jun Liu  
Washington University School of Medicine in St. Louis

Puran Sijwali  
University of California - San Francisco

Jiri Gut  
University of California - San Francisco

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

See next page for additional authors

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Recommended Citation

Parikh, Sunil; Liu, Jun; Sijwali, Puran; Gut, Jiri; Goldberg, Daniel E.; and Rosenthal, Philip J., "Antimalarial effects of human immunodeficiency virus type 1 protease inhibitors differ from those of the aspartic protease inhibitor pepstatin." Antimicrobial Agents and Chemotherapy. 50,6. 2207. (2006).  
https://digitalcommons.wustl.edu/open_access_pubs/2363
Antimicrobial Agents and Chemotherapy

Antimalarial Effects of Human Immunodeficiency Virus Type 1 Protease Inhibitors Differ from Those of the Aspartic Protease Inhibitor Pepstatin

Sunil Parikh, Jun Liu, Puran Sijwali, Jiri Gut, Daniel E. Goldberg and Philip J. Rosenthal


Updated information and services can be found at:
http://aac.asm.org/content/50/6/2207

These include:

REFERENCES
This article cites 15 articles, 10 of which can be accessed free at: http://aac.asm.org/content/50/6/2207#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/
NOTES

Antimalarial Effects of Human Immunodeficiency Virus Type 1 Protease Inhibitors Differ from Those of the Aspartic Protease Inhibitor Pepstatin

Sunil Parikh,1* Jun Liu,2 Puran Sijwali,1 Jiri Gut,1 Daniel E. Goldberg,2 and Philip J. Rosenthal1

Department of Medicine, San Francisco General Hospital, University of California, San Francisco, California,1 and Department of Medicine and Department of Molecular Microbiology, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, Missouri2

Received 6 January 2006/Returned for modification 30 January 2006/Accepted 6 March 2006

Human immunodeficiency virus type 1 protease inhibitors (HIVPIs) and pepstatin are aspartic protease inhibitors with antimalarial activity. In contrast to pepstatin, HIVPIs were not synergistic with a cysteine protease inhibitor or more active against parasites with the cysteine protease falcipain-2 knocked out than against wild-type parasites. As with pepstatin, HIVPIs were equally active against wild-type parasites and against parasites with the food vacuole plasmepsin aspartic proteases knocked out. The antimalarial mechanism of HIVPIs differs from that of pepstatin.

Human immunodeficiency virus type 1 (HIV-1) and malaria are coendemic throughout much of the developing world. HIV-1 expresses an aspartic protease, which is an important drug target (3). In addition to their important antiretroviral activity, HIV-1 protease inhibitors (HIVPIs) are active against Plasmodium falciparum in vitro and against rodent malaria parasites in murine models (7, 9, 13). The predicted targets of these inhibitors are plasmepsins, a family of aspartic proteases of malaria parasites. A number of plasmepsins act in concert with falcipain cysteine proteases and other enzymes to hydrolyze hemoglobin in the P. falciparum food vacuole (5, 8). Several HIVPIs inhibit the food vacuole protease plasmepsin II (7) and a homologous protease of the rodent parasite Plasmodium chabaudi (6). Pepstatin, the most-studied aspartic protease inhibitor, also exhibits activity against cultured malaria parasites and inhibits several plasmepsins (2, 6). As the antimalarial activity of HIVPIs may have important implications in areas where those treated for HIV-1 infection are at risk of malaria, and as both HIVPIs and pepstatin may serve as leads for new antimalarial agents, it was of interest to compare their antimalarial mechanisms of action.

Insight into the antimalarial mechanisms of protease inhibitors came from studies that showed that cysteine protease inhibitors [N-(trans-epoxysuccinyl)-l-leucine-4-guanidino-butyramide (E-64)] and aspartic protease inhibitors (pepstatin) display marked synergy against malaria parasites (1, 10). Further supporting an important interaction between these two classes of proteases, pepstatin had markedly enhanced activity against P. falciparum parasites in which the gene for the cysteine protease falcipain-2 was disrupted (11). It was of interest to determine if HIVPIs had effects similar to those of pepstatin.

We evaluated the HIVPI lopinavir for synergy with E-64. P. falciparum (W2 strain) parasites were cultured in RPMI medium supplemented with 10% serum and synchronized with 5% d-sorbitol as previously described (11). Ring stage parasites were incubated with study drugs (0.039 to 10 μM, from stock solutions concentrated 1,000-fold in dimethyl sulfoxide [DMSO]) or with equivalent concentrations of DMSO for 48 h, fixed with 1% formaldehyde in phosphate-buffered saline for 48 h, and labeled with 1 nM YOYO-1 dye (Molecular Probes) in 0.1% Triton X-100 in phosphate-buffered saline. Parasitemias were determined from dot plots acquired with a FACSort flow cytometer, and 50% inhibitory concentration (IC50) values were calculated as previously described (11, 12). Potential synergy was evaluated as the sum of the fractional inhibitory concentrations (sum FIC) by the following equation: sum FIC = [(IC50 drug A in combination)/(IC50 drug A alone)] + [(IC50 drug B in combination)/(IC50 drug B alone)]. The sum FIC value for lopinavir and E-64 was 2.04 ± 0.48 (mean ± standard deviation of results from two experiments, each done in triplicate). Thus, lopinavir and E-64 (Sigma-Aldrich) showed no evidence of synergism, but rather borderline antagonism. In contrast, E-64 and pepstatin have shown marked synergy with a sum FIC value of 0.54 ± 0.16 (10).

To further characterize the antimalarial mechanism of HIVPIs, we tested the compounds against P. falciparum parasites with disrupted food vacuole proteases. For plasmepsin knockout parasites, previously described 3D7 strain parasites were used (5). For falcipain-2 knockout parasites, procedures very similar to those previously described were used (11). Briefly, 3D7 strain parasites were transfected with the pHKΔFP2 plasmid, selected with WR99210 until integration of the plasmids was detected, enriched for recombinant parasites through negative selection with ganciclovir, and cloned to obtain pure recombinant parasites. Wild-type 3D7 and plasmepsin knockout parasites were incubated in microwell cultures in the presence of serial dilutions of lopinavir, ritonavir, and saquinavir (0.025 to 150 μM, from 1,000-fold-concentrated
TABLE 1. Activity of HIV-1 protease inhibitors against \textit{P. falciparum} plasmepsin knockout parasites

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC$_{50}$ ($\mu$M) for \textit{P. falciparum}$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>12.2 ± 0.3</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>12.2 ± 0.4</td>
</tr>
<tr>
<td>Lopinavir</td>
<td>3.0 ± 0.4</td>
</tr>
</tbody>
</table>

$^a$ IC$_{50}$ data are means ± standard deviations of results from three experiments. Abbreviations: PMP, plasmepsin; KO, knockout; HAP, histoaspartic protease.

TABLE 2. Activity of HIV-1 protease inhibitors against \textit{P. falciparum} falcipain-2 knockout parasites

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC$_{50}$ ($\mu$M) for \textit{P. falciparum}$^a$</th>
<th>Knockout IC$<em>{50}$ ($%$ of wild-type IC$</em>{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>Falcipain-2 knockout</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>5.7 ± 0.8</td>
<td>7.6 ± 1.7</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>7.9 ± 1.1</td>
<td>8.9 ± 0.9</td>
</tr>
<tr>
<td>Indinavir</td>
<td>25.2 ± 4.4</td>
<td>26.6</td>
</tr>
<tr>
<td>Nelfinavir</td>
<td>21.8 ± 2.4</td>
<td>24.7</td>
</tr>
<tr>
<td>Amprenavir</td>
<td>71.2 ± 16.1</td>
<td>84.7</td>
</tr>
<tr>
<td>Lopinavir</td>
<td>2.5 ± 0.4</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td>Atazanavir</td>
<td>35.0 ± 7.4</td>
<td>42.0</td>
</tr>
<tr>
<td>Pepstatin</td>
<td>7.5 ± 0.4</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>E-64</td>
<td>2.4 ± 0.4</td>
<td>1.4 ± 0.1</td>
</tr>
</tbody>
</table>

$^a$ IC$_{50}$ data are means ± standard deviations of results from three experiments.

IV), another quite different aspartic protease, plasmepsin V, was recently characterized (4). This protease is not located in the food vacuole and does not bind pepstatin. The HIV-1 protease is also quite different from the food vacuole plasmepsins (15). These structural differences may offer insight into the differential effects of the HIVPIs and pepstatin. Previously we showed that HIVPIs inhibit recombinant plasmepsin II (7), but the intracellular target of these inhibitors is unknown. Thus, pepstatin may target food vacuole plasmepsins, which are dependent on food vacuole cysteine proteases for maximal activity, while HIVPIs may target other aspartic proteases, such as plasmepsin V. In addition to differential inhibition of parasite proteases, various effects may be due to differential access to the food vacuole or other cellular compartments. Alternatively, either pepstatin or HIVPIs might exert antimarial effects that are unrelated to protease inhibition. Our results do not yet identify a specific mechanism of action for HIVPIs, but they offer the surprising finding that HIVPIs do not, as would have been predicted, act in the same manner as pepstatin. Further research into the precise mode of action of HIVPIs and other antimarial aspartic protease inhibitors is warranted to provide insight into the development of protease inhibitors as new antimarial drugs and to understand the means by which antiretroviral drugs may offer protection against malaria.

We thank members of the Rosenthal and Goldberg laboratories for their expert technical assistance. The following 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 ampranavir. \textit{P. falciparum} strains were obtained from the Malaria Research and Reference Reagent Center (Manassas, Va). Financial support was provided by the National Institutes of Health. P.J.R. is a Doris Duke Charitable Foundation Distinguished Clinical Scientist.

REFERENCES


