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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

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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

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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 protease inhibitors (HIVPIs) are antiretroviral drugs that target the HIV-1 protease, which is essential for HIV replication. However, the mechanism by which HIVPIs exert antimalarial effects is not well understood. In this study, the antimalarial effects of HIVPIs were compared to those of pepstatin, a well-studied aspartic protease inhibitor. The results showed that HIVPIs did not exhibit synergistic effects or increased activity against parasites with specific protease knockouts, unlike pepstatin. This finding suggests that the antimalarial mechanism of HIVPIs is different from that of pepstatin.

* Corresponding author. Mailing address: University of California San Francisco, Box 0811, San Francisco, CA 94110. Phone: (415) 206-8687. Fax: (415) 648-8425. E-mail: sparikh@medsgh.ucsf.edu.
stocks in DMSO) or with equivalent concentrations of DMSO for 44 h, beginning at the ring stage, and then 0.5 μCi of [3H]hypoxanthine (178.7 Ci/mmol; Perkin Elmer) was added. The incubation was continued for 16 h, the parasites were harvested, the hypoxanthine uptake rates of treated and control parasites were compared, and IC₅₀ values were generated as previously described (5). The antimalarial activities of seven HIVPIs against 3D7 wild-type and falcipain-2 knockout parasites were evaluated by assessing the fluorescence of YOYO-1-stained parasites and determining IC₅₀ values using fluorescence-activated cell sorter-based analysis as described above (11, 12).

HIVPIs had similar activities against control, plasmepsin knockout (Table 1), and falcipain-2 knockout (Table 2) parasites. Discrepancies between reported IC₅₀ values in Tables 1 and 2 likely reflect differences between the [3H]hypoxanthine uptake and fluorescence-activated cell sorter-based assay methods. Considering the actions of other protease inhibitors, E-64 was about twice as active against falcipain-2 knockout and plasmepsin knockout parasites as it was against control parasites, as previously described (5). Pepstatin (Sigma-Aldrich) was about equally active against control and plasmepsin knockout parasites but was much more active against falcipain-2 knockout parasites, all consistent with prior findings (5, 11).

Our results identify major differences between the antimalarial activities of pepstatin and HIVPIs. Pepstatin, the most widely available broadly active aspartic protease inhibitor (3), inhibits multiple plasmepsins and rapidly kills cultured parasites, but its antimalarial mechanism of action is uncertain. For both pepstatin and HIVPIs, activities were similar against wild-type and plasmepsin knockout parasites. These results suggest that the functional redundancy of plasmepsins but do not shed light on the mechanism of HIVPIs. In contrast, the activities of pepstatin and HIVPIs differed markedly when evaluated for antimalarial synergy with the cysteine protease inhibitor E-64 or for activity against falcipain-2 knockout parasites. Pepstatin shows strong synergy with cysteine protease inhibitors (10), and it is remarkably more potent against falcipain-2 knockout parasites than against wild-type parasites (11). These results suggest important biological interactions between falcipains and plasmepsins. Surprisingly, the HIVPIs were not synergistic with E-64 and were not more active against falcipain-2 knockout parasites than against wild-type parasites. Thus, although we cannot yet identify specific biological targets for either pepstatin or HIVPIs in malaria parasites, our results strongly suggest that the compounds act differently.

*P. falciparum* encodes 10 predicted aspartic protease genes (14). In addition to the four food vacuole plasmepsins (1 to 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 antimalarial 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 antimalarial aspartic protease inhibitors is warranted to provide insight into the development of protease inhibitors as new antimalarial drugs and to understand the means by which antiretroviral drugs may offer protection against malaria.

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