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Letter to the Editor

May the Drug Transporter P Glycoprotein Affect the Antiviral Activity of Human Immunodeficiency Virus Type 1 Proteinase Inhibitors?

Several studies have demonstrated that human immunodeficiency virus (HIV) proteinase inhibitors (PIs) are substrates of the multidrug transporter P glycoprotein (P-gp) (2, 3, 5).

In a recent article Srinivas and coworkers (4), in addition to confirming previous observations, demonstrated for the first time that HIV PIs interact also with MRP1, another multidrug transporter protein (1). They have also observed that HIV PIs are equally effective against HIV in wild-type cells and in a P-gp-expressing cell line, suggesting that cellular resistance to HIV PIs might not be a major therapeutic concern. It is our opinion that the possibility that P-gp expression may affect the antiviral activity of PIs should be further addressed.

In the framework of a project aimed to address the role played by cellular resistance in HIV infection treatment failure, we have performed similar experiments. The results, however, are not perfectly consistent with those reported by Srinivas et al. We found that the cell line CEMVBL 100, expressing a high level of P-gp (as measured by fluorescein-activated cell sorter analysis with a specific monoclonal antibody and by reverse transcriptase PCR designed to detect mRNA for P-gp), is less sensitive to PI antiviral activity than the parental cell line, not expressing P-gp.

Figure 1 shows the results. It can be seen that saquinavir and indinavir display at the indicated concentrations significantly reduced antiviral activities in CEMVBL100 cells compared to CEM cells. Specifically, 50 and 5 nM saquinavir reduced HIV yield in the parental cell line by 90 and 70%, respectively. In contrast, the same compound in CEMVBL100 at the same concentrations inhibited HIV yield by 80 and 40%. In repeated experiments this small difference becomes significant ($P < 0.05$).

Basically, the same results were obtained using indinavir. Importantly, both drugs partially recover the ability to inhibit replication of HIV-1 in the presence of nontoxic concentratioin of verapamil, an inhibitor of P-gp function (6), thus indicating that P-gp expression may affect the antiviral activity of PIs.

The inhibitory effect exerted by P-gp against PIs, however, occurs only at intermediate dosage, which approximately corresponds to the 90% effective dose (ED_{90}) reported by Srinivas et al. At higher doses PIs are able to inhibit HIV replication in CEMVBL100 cells also, whereas at lower doses they do not affect HIV replication.

Together these findings suggest that P-gp expression may influence the antiviral activity of PIs at least at a certain dosage.

Srinivas et al. reported a higher ED_{90} for CEMVBL100 cells than that for the parental line, but the difference was not significant. Using a different calculation—comparing percent viral-yield reduction—the difference between the two cell lines becomes significant. We do not want to overemphasize such a small difference. However, in light of the recent demonstration that all PIs are first-rate substrates of the P-gp pump, we believe that the question of whether P-gp mediated cellular resistance to anti-HIV drugs exists is still open.

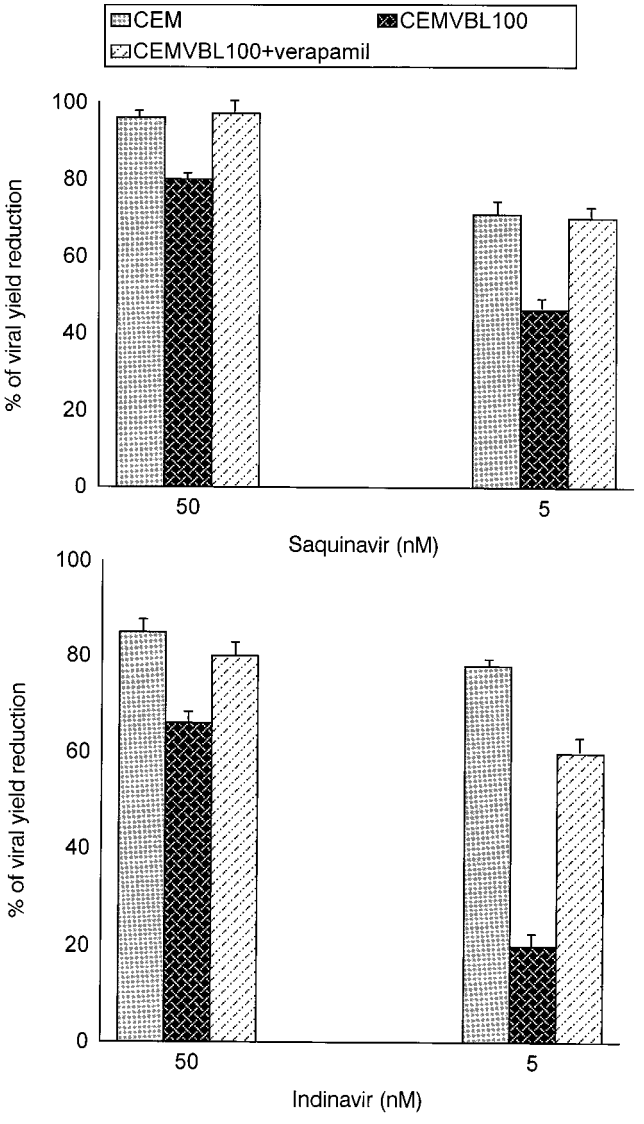

FIG. 1. Antiviral activities of saquinavir and indinavir in CEM and CEMVBL100 cells in the presence or absence of verapamil (2 μM). Cells of both types were infected with HIV_{pnl43} at a multiplicity of infection of 0.1. After 5 days of incubation, percent viral-yield reduction was determined by measuring the level of p24 antigen. Data are means with standard deviations for three independent experiments.

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Author's Reply

Turriziani and coworkers show a significant, albeit small, reduction in the antiviral efficacy of the HIV PI saquinavir in MDR1-overexpressing CEM-VBL100 cells compared to wild-type CEM cells using virus yield reduction assays. Indeed, we also observed a slight reduction in the antiviral efficacy of various HIV PIs in CEM variants that over express the multidrug transporters, as evidenced by slight increases in the 90% inhibitory concentrations of various HIV-PIs in CEM-VBL100 cells. These differences, although reproducible, were not statistically signficant due to the large interassay variations. While it is clear that the differences observed by Turriziani and coworkers were statistically significant, the biological signficance of these small differences in virus yields is hard to evaluate.

The impact of multidrug transporter overexpression in lymphocytes, the primary targets of HIV, on HIV PI therapy is hard to evaluate since MDR overexpression per se can modulate the dynamics of HIV infection in lymphocytes. For example, we have noted that cells that overexpress MRP-4 are less sensitive to HIV infection (7). Similar findings have been observed with MDR-1-overexpressing cells. Furthermore, MDR overexpression among cells of hematopoietic cell lineage may have other consequences relevant to HIV biology as well. Unlike normal hematopoietic stem cell (HSC), MDR-1-transduced HSC are capable of ex vivo expansion and show signficantly greater immune reconstitution (2). Therefore, while we do not dispute the notion that MDR-1 overexpression may adversely affect response to PI therapy, we believe that such effects are more likely to be due to reduced plasma and tissue PI concentrations resulting from reduced oral uptake and increase hapatobiliary clearance, rather than selective exclusion from the target lymphocytes.

While the interaction of MDR-1 P glycoprotein with various HIV PIs is well documented, the interaction of P glycoprotein with nucleoside reverse transcription inhibitors (NRTI) has been controversial. Several investigators, including Turriziani and coworkers, have suggested that P glycoprotein may confer resistance to zidovudine and other NRTI (1, 3, 4, 6; H. W. Doerr, J. Cinatl, Jr., B. Weber, and J. Cinatl, Abstr. 94th Gen. Meet. Am. Soc. Microbiol. 1994, abstr. T-14, 1994). MDR-1 is a member of a large family of related multidrug resistance-associated transporters, and other members of this family may play a role as well. Earlier we showed that like MDR-1, MRP-1 also interacts with HIV PIs (8). More recently, we showed that MRP-4, a newly characterized member of the MDR family, does not interact with HIV PIs but confers cellular resistance to HIV inhibition by multiple NRTI (5). Given the complexities of these drug transporter interactions, we agree with Turriziani and coworkers that further studies are needed to clarify the role of multidrug transporters in cellular resistance to antiviral therapy.

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