PROTEASE INHIBITORS

Protease inhibitors were discussed at the Los Angeles conference by Steven A. Miles, MD, from the University of California, Los Angeles, and at the Atlanta conference by Kathleen E. Squires, MD, from the University of Alabama at Birmingham. At the time of these conferences, saquinavir was the only protease inhibitor approved for use in the treatment of HIV disease. Shortly thereafter, the protease inhibitors ritonavir and indinavir were granted accelerated approval by the FDA.

Saquinavir

Saquinavir (Ro31-8959) was the first protease inhibitor to be approved for use in the treatment of HIV disease. Initial studies of the current formulation of this drug used alone showed beneficial effects of relatively small magnitude on CD4+ cell counts, p24 antigen levels, and plasma HIV RNA levels in both antiretroviral-naive and -experienced patients in a regimen of 600 mg tid, the highest dose examined. In the AIDS Clinical Trials Group (ACTG) protocol 229, the triple combination of saquinavir, zidovudine, and zalcitabine was associated with beneficial changes in peripheral blood mononuclear cell (PBMC) HIV titers and CD4+ cell counts that were somewhat better than those observed in patients given zidovudine/zalcitabine over 24 weeks of study. Saquinavir is a potent agent in vitro, and it appears that the modest magnitude of benefit observed with this drug is due to the exceedingly poor bioavailability of the current formulation (4%). One group has recently examined use of higher doses of saquinavir; at dosages of 3600 mg/d and 7200 mg/d, reductions of 0.85 to 1.3 log in plasma HIV RNA levels have been observed in association with increases in CD4+ cell counts of 80/μL to 120/μL. A soft gelcap formulation of saquinavir intended to improve bioavailability is under development. This new formulation is expected to provide drug serum levels comparable to those produced by the 7200 mg/d dosage with the current formulation. Such a formulation would likely improve the efficacy of saquinavir.

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Ritonavir

In early studies of ritonavir monotherapy (ABT 538), dosages of 500 mg and 600 mg bid for 12 weeks were associated with reductions in plasma HIV RNA levels of greater than 1 log and with increases in CD4+ cell counts of 60/μL to 100/μL. Longer-term monotherapy studies with a range of doses have shown that 600 mg bid of ritonavir is associated with median increases in CD4+ cell counts of 150/μL to 200/μL sustained for at least 30 weeks. At 30 weeks, the increases in CD4+ cell counts observed with lower doses had been lost or dramatically reduced (Figure 1). These data suggest that higher doses are associated with more persistent drug effects, as well as a greater magnitude of effect.

Ritonavir, added to whatever antiretroviral regimen study subjects were already receiving, was recently shown to significantly prolong survival in patients with advanced HIV disease who had received extensive prior antiretroviral treatment. In this trial, patients with advanced disease were randomized to ritonavir 600 mg bid (median CD4+ cell count, 18/μL) or placebo (median CD4+ cell count, 22/μL) and were continued on their other nucleoside analogue medications. During approximately 5.5 months of study, ritonavir treatment was associated

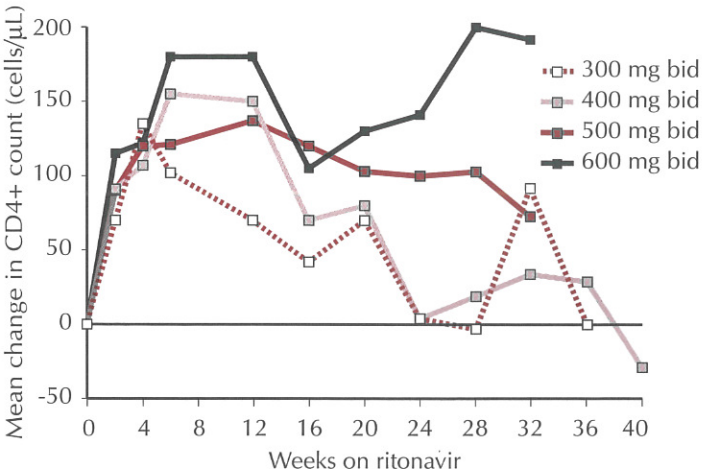

Figure 1. Median CD4+ cell count changes from baseline over time in patients given ritonavir at 300, 400, 500, or 600 mg bid.

with a significant reduction in mortality, with death occurring in 26 (4.8%) of 543 ritonavir-treated patients and in 46 (8.4%) of 547 patients given placebo ($P = .02$), and with a significant reduction in the rate of AIDS-defining conditions or death (12.7% vs 27.3%; $P < .001$). Adverse events occurred in 17% of ritonavir-treated patients and in 6% of those given placebo. In ritonavir-treated patients, a mean peak increase of 47 CD4+ cells/μL occurred at 16 weeks, compared with a virtual absence of change in those given placebo. A mean peak decrease of 1.3 log in plasma HIV RNA levels occurred at 2 weeks. In some cases, increased CD4+ cell counts were accompanied by improved immunologic function, skin test reactivity, and clearance of opportunistic infections and neoplasms. The ritonavir-treated group showed a mean peak increase in CD8+ counts of 314 cells/μL at 8 weeks.

Indinavir

In a trial that compared indinavir (L735-524, MK639), zidovudine, and the combination of the two in zidovudine-naive patients with plasma HIV RNA levels greater than or equal to 20,000 copies/mL, indinavir monotherapy and the indinavir/zidovudine combination were both associated with reductions in plasma HIV RNA levels of at least 1.5 log. Indinavir alone was

associated with a reduction of greater than 2 log that was maintained for more than 24 weeks (Figure 2). Assessment of zidovudine-resistant mutations during the course of therapy showed that approximately 60% of patients given zidovudine monotherapy yielded virus with zidovudine-resistant mutations by 24 weeks compared with less than 10% of patients who were given combination treatment; this finding suggests that concomitant use of indinavir may delay development of resistance to zidovudine. In a trial comparing indinavir, zidovudine/lamivudine, and the combination of the three in 97 patients with at least 6 months of prior treatment with zidovudine, CD4+ cell counts of 50/µL to 400/µL, and plasma HIV RNA levels greater than or equal to 20,000 copies/mL, indinavir alone was associated with a 1.5-log reduction in plasma HIV RNA levels. The combination of indinavir, zidovudine, and lamivudine produced a 2-log decrease in plasma HIV RNA levels, while the zidovudine/lamivudine combination resulted in a decrease of approximately 1 log. The triple combination group experienced a median increase in CD4+ cell counts of 146/µL at 24 weeks (Figure 3).

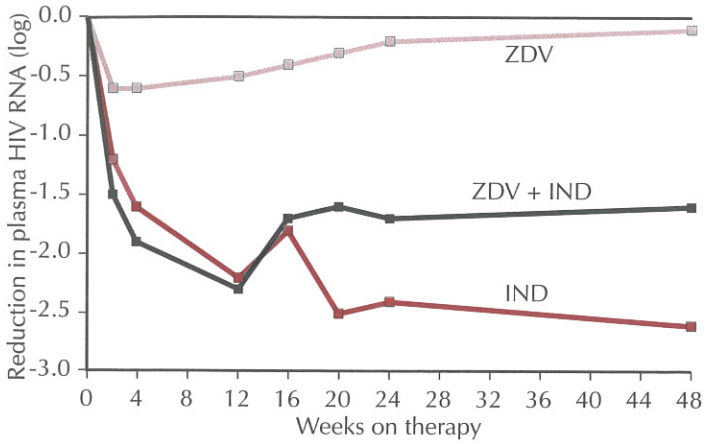

Figure 2. Log reduction in plasma HIV RNA in zidovudine-naive patients given zidovudine alone, indinavir alone, or zidovudine/indinavir.

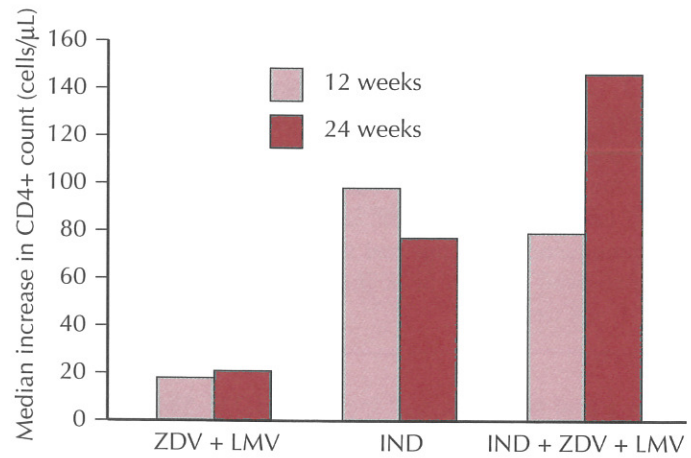

Figure 3. Median CD4+ cell count increases from baseline in zidovudine-experienced patients after 12 and 24 weeks of treatment with zidovudine/lamivudine (ZDV + LMV), indinavir (IND) alone, or with the triple combination of indinavir/zidovudine/lamivudine (IND + ZDV + LMV).

Resistance

Resistance has been demonstrated with all protease inhibitors. However, high-level resistance to both indinavir and ritonavir is not conferred by any single mutation or pair of mutations, but is rather the result of stepwise accumulation of multiple mutations. Indinavir-resistant virus is cross-resistant to ritonavir and many other protease inhibitors in development but not to saquinavir; moreover, neither resistance to ritonavir nor to saquinavir is associated with broad-class resistance. Figure 4 shows reported data on resistance mutations to saquinavir, ritonavir, and indinavir in clinical HIV isolates. Since saquinavir was the first protease inhibitor approved for use and since it was feared that resistance to a particular protease inhibitor would confer broad-class resistance, there was concern that using a compound formulation associated with marginal benefit would compromise later use of compounds that appeared to have much greater potency. The data on the distribution of resistance mutations in vivo and those on in vitro sensitivity of virus with saquinavir resistance mutations (codons 48 and 90 mutations) to other protease inhibitors suggest that resistance to saquinavir may not lead to resistance to other compounds. Resistance to saquinavir at higher and more effective doses appears to occur at approximately the same rate as that seen with lower doses. In the small-scale studies to date using higher doses, 25% to 45% of patients have yielded virus with either the codon 48 or the codon 90 mutation by 24 weeks. Longer-term follow-up has indicated that 40% to 45% of patients develop the mutations associated with resistance after 1 year of treatment.

Effect of Ritonavir on Blood Drug-Levels

One potential motivation for using protease inhibitors in combination is the interaction between ritonavir and virtually all other protease inhibitors developed to date; ritonavir increases blood levels of other protease inhibitors by inhibiting the cytochrome P450 3A4 isoenzyme. Studies in rats have indicated that a single dose of ritonavir increases levels of saquinavir in the blood by more than 290-fold. Smaller but significant increases have reportedly been observed with virtually all other protease inhibitors currently in clinical development. Given the absence of cross-resistance between saquinavir and ritonavir, it may make sense to take advantage of this interaction to increase blood levels of saquinavir. Further elucidation of the pharmacokinetic interaction in terms of toxic effects is necessary before this approach can be used clinically.

Adverse Effects

The protease inhibitors currently available have both shared and distinctive adverse effects. Although saquinavir has thus far been associated with the fewest side effects at currently recommended doses, use of higher doses has been associated with a significant incidence of stomach distress (ie, bloating and gas). Indinavir has been associated with nephrolithiasis attributable to precipitation of indinavir in the urine. Ritonavir has been associated with significant nausea; the soft gelcap formulation of ritonavir is somewhat better tolerated. Most patients given ritonavir have reported perioral dysesthesia during the first 1 or 2 weeks of treatment, typically described as a numbness or tin-

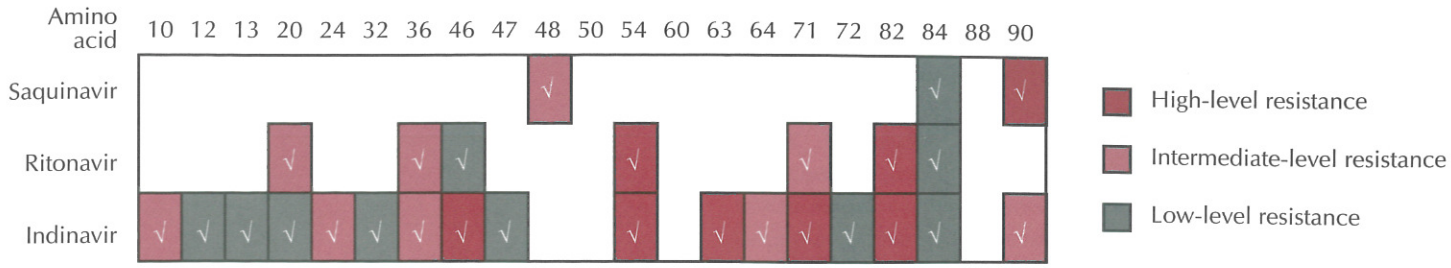

Figure 4. Patterns of clinical HIV isolate resistance mutations to saquinavir, ritonavir, and indinavir. Adapted from Molla et al. International Workshop on HIV Drug Resistance—Sardinia. *1995; and from Chondra et al.* Nature. *1995.*

gling. Ritonavir is also associated with diarrhea. Most of the compounds evaluated to date have been associated with hepatitis, with clinical development of a number of potentially useful compounds having been halted due to a significant incidence of this adverse effect. Lipid abnormalities have also been observed with use of most of these compounds. Pancreatitis is common in patients with HIV disease, and with the burgeoning use of liposomal preparations of a variety of frequently used medications, the lipid interactions of protease inhibitors with liposomal drugs need to be investigated. Less common significant adverse effects include rash and fever, which have been observed in patients given indinavir. Most studies have shown that adverse effects occur in 15% to 20% of patients with advanced HIV disease who are treated with protease inhibitors. The effect of ritonavir on hepatic cytochrome P450 induction also affects the metabolism of other medications, and, therefore, careful observation is required for potential drug-drug interactions.

Drugs Under Development

Newer protease inhibitors include second-generation compounds being developed by Agouron, Glaxo-Wellcome, Upjohn, Ciba-Geigy, and other manufacturers. The Agouron compound, currently in phase III testing, is furthest along in development; early studies have indicated that this drug produces substantial decreases in viral load when used alone or in combination with stavudine. According to Drs Miles and Squires, the drugs in development have either superior pharmacologic characteristics or superior manufacturing processes that may translate into increased therapeutic benefit and reduced treatment costs.

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Kathleen E. Squires is Assistant Professor of Medicine and Associate Director of the AIDS Outpatient Clinic at the University of Alabama at Birmingham.

Suggested Readings

Cameron B, Heath-Chiozzi M, Kravcik S, et al. Prolongation of life and prevention of AIDS in advanced HIV immunodeficiency with ritonavir. Presented at Third Conference on Retroviruses and Opportunistic Infections; January 28–February 1, 1996; Washington, DC.

Chondra JH, et al. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. *Nature.* 1995;374:569–571.

Collier AC, Coombs RW, Schoenfeld DA, et al, for the AIDS Clinical Trials Group Protocol 229 team. A comparative study of saquinavir (SAQ) and zidovudine (ZDV) vs. ZDV and zalcitabine (ddC) vs. SAQ, ZDV and ddC. Presented at Thirty fourth Interscience Conference on Antimicrobial Agents and Chemotherapy; October 4–7, 1994; Orlando, Fla.

Collier AC, Coombs RW, Schoenfeld DA, Bassett R et al. Extended treatment with saquinavir (SAQ), zidovudine (ZDV) and zalcitabine (ddC) vs SAQ and ZDV vs ddC and ZDV. Presented at Thirty fifth Interscience Conference on Antimicrobial Agents and Chemotherapy; September 17–20, 1995; San Francisco, Calif.

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Heath-Chiozzi M, Leonard J, Henry D, et al. Anti-HIV activity and lymphocyte surrogate marker response dynamics to ritonavir therapy in advanced HIV immunodeficiency. Presented at Third Conference on Retroviruses and Opportunistic Infections; January 28–February 1, 1996; Washington, DC.

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Massari F, Conant M, Mellors J, et al. A phase II open-label, randomized study of the triple combination of indinavir, zidovudine and didanosine versus indinavir alone and zidovudine/didanosine in antiretroviral naive patients. Presented at Third Conference on Retroviruses and Opportunistic Infections; January 28–February 1, 1996; Washington, DC.