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Evaluation of Reverse Transcriptase and Protease Inhibitors in Two-Drug Combinations against Human Immunodeficiency Virus Replication

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Current treatments for human immunodeficiency virus (HIV) include both reverse transcriptase and protease inhibitors. Results from in vitro and clinical studies suggest that combination therapy can be more effective than single drugs in reducing viral burden. To evaluate compounds for combination therapy, stavudine (d4T), didanosine (ddI), or BMS-186,318, an HIV protease inhibitor, were combined with other clinically relevant compounds and tested in a T-cell line (CEM-SS) that was infected with HIV-RF or in peripheral blood mononuclear cells infected with a clinical HIV isolate. The combined drug effects were analyzed by the methods described by Chou and Talalay (Adv. Enzyme Regul. 22:27–55, 1984) as well as by Prichard et al. (Antimicrob. Agents Chemother. 37:540–545, 1993). The results showed that combining two nucleoside analogs (d4T-ddI, d4T-zidovudine [AZT], and d4T-zalcitabine [ddC]), two HIV protease inhibitors (BMS-186,318–saquinavir, BMS-186,318–SC-52151, and BMS-186,318–MK-639) or a reverse transcriptase and a protease inhibitor (BMS-186,318–d4T, BMS-186,318–ddI, BMS-186,318–AZT, d4T–saquinavir, d4T–MK-639, and ddI–MK-639) yielded additive to synergistic antiviral effects. In general, analysis of data by either method gave consistent results. In addition, combined antiviral treatments involving nucleoside analogs gave slightly different outcomes in the two cell types, presumably because of a difference in phosphorylation patterns. Importantly, no strong antagonism was observed with the drug combinations studied. These data should provide useful information for the design of clinical trials of combined chemotherapy.

Five reverse transcriptase (RT) inhibitors, zidovudine (AZT), didanosine (ddI), stavudine (d4T), zalcitabine (ddC), and lamivudine (3TC), have been approved for treatment of human immunodeficiency virus (HIV) infection. In AIDS patients, these drugs initially reduce viral load; however, adverse side effects are associated with extensive use of nucleoside analogs (7, 13, 21, 30, 35, 48). In addition, the appearance of virus with reduced sensitivity renders the drugs less effective over time (11, 15, 28, 31, 41). HIV protease inhibitors (saquinavir, indinavir, SC-52151, and BMS-186,318 [BMS-PI]) represent a new class of anti-HIV agents (1, 9, 18, 27, 42, 46). Saquinavir and indinavir are currently under clinical investigation and show effective antiviral activity during initial treatment; however, protease-resistant mutants have also been isolated in patients over time (6). Because of limitations of monotherapy, the potential for combining anti-HIV drugs is being explored to determine if concurrent drug treatment will enhance anti-HIV activity (3, 19).

Synergy can result from combining drugs that affect more than one cell type, affect cells in different stages of activation, or inhibit virus replication at different steps (8, 10, 12, 20, 22–24, 38, 44). Synergy may also be observed when combining antiviral agents with compounds that enhance drug uptake, affect cell activation levels, or increase the level of an active metabolite (2, 16, 26, 34, 36). Examples of successful drug combination therapies include antibiotics against tuberculosis, cancer therapy with different antineoplastic drugs, and nucleoside analogs for AIDS treatment (14, 37).

Convergent drug therapy, with compounds effective against a single target, may select for multiple mutations in one protein, thus compromising its function. Unfortunately, this approach was not effective in one in vitro study combining AZT, ddI, and nevirapine because of the emergence of multiply resistant mutants (32). However, a recent clinical trial has shown promising results with the combination of AZT and 3TC. This synergistic effect may result from a restoration of AZT sensitivity in the presence of the 3TC-resistant mutation (45). Divergent therapy involving drugs with different targets is a more conventional approach. Only recently, with the development of protease inhibitors, has this become a feasible option in the treatment of HIV infection. Combining RT and protease inhibitors not only targets two different proteins but also affects two different stages of the viral life cycle, suggesting that together they may offer a therapeutic advantage. Given the growing number of new drugs, in vitro analyses are necessary to identify the combinations that give enhanced antiviral effects and to exclude compounds that may antagonize the activity of others. Moreover, in vitro assays offer the potential to test many combinations with a range of drug concentrations, drug ratios, and cell types.

This report describes the results from various two-drug combination experiments which involved two RT inhibitors, two protease inhibitors, or a combination of both. The effects of host cell type and data analysis methods were also compared. The cell systems used included the continuous T-cell line

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TABLE 1. CIs for two-drug combinations in CEM-SS cells with RT inhibitors

Drug combination[a]	Molar ratio[b]	CI at the following levels of HIV inhibition (%)[c]:				Overall result
		50	75	90	95	
d4T-ddI	1:2	1.06	1.26	1.49	1.67	
	1:10	0.74	0.74	0.73	0.73	Additive
	1:20	1.29	1.13	0.99	0.90	
d4T-AZT	500:1	0.96	1.11	1.24	1.35	
	100:1	1.2	0.85	0.65	0.54	Additive
	20:1	1.10	0.90	0.74	0.65	
d4T-ddC	1:1	0.70	0.61	0.57	0.62	
	1:5	0.64	0.50	0.46	0.45	Synergy
	1:25	0.67	0.51	0.43	0.42	

[a] The EC_{50}s for each monotherapy were as follows: d4T, 0.32 μM; ddC, 0.048 μM; ddI, 2.5 μM; and AZT, 4 nM.

[b] The highest concentrations of each drug used were as follows: d4T, 50 μM; ddC, 25 μM; ddI, 100 μM; and AZT, 1 μM.

[c] CIs were calculated according to the method described by Chou and Talalay, where values of <1, 1, and >1 indicate synergism, an additive effect, and antagonism, respectively.

CEM-SS infected with the laboratory strain HIV-RF, as well as human peripheral blood mononuclear cells (PBMCs) infected with the clinical isolate 006 (33). PBMCs were included since they more closely represent infection in vivo, and combinations most likely to be used in clinics were selected for study in both CEM-SS cells and PBMCs. In addition, the data were analyzed by the following two methods. Combination index values (CIs) were calculated according to the method described by Chou and Talalay, with CIs of <1, 1, and >1 indicating synergistic, additive, and antagonistic drug effects, respectively (5). The second analysis method calculates synergy or antagonism in volumes with the MacSynergy program (40). Synergy and antagonism are displayed as peaks above or below the predicted additive plane in a 3-dimensional graph.

The two-drug combinations described in the present paper gave additive to synergistic effects regardless of the cell type used and yielded no significant antagonism. Previous in vitro studies with the combinations ddI-AZT and ddC-AZT displayed combined synergy in MT4 cells, macrophages, and PBMCs and were later shown to significantly reduce viral load in AIDS patients (3, 12, 14, 24, 43). Therefore, the drug combinations with additive or synergistic activity described in the present report warrant further clinical consideration.

MATERIALS AND METHODS

Viruses and cells. The RF strain of HIV type 1 (HIV-1) and the CEM-SS human T-cell line were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, contributed by R. Gallo and P. Nara, respectively. The HIV clinical isolate 006 has been previously described and contains no known drug resistance mutations (33). PBMCs were isolated from healthy seronegative donors by Ficoll-Hypaque density gradient centrifugation. The cells were stimulated for 3 days with phytohemagglutinin (2 μg/ml) in the presence of interleukin 2 (2 U/ml) before infection.

Compounds. BMS-PI (1), SC-52151 (Searle) (18), indinavir (Merck) (46), saquinavir (Roche) (42), ddI, and d4T were synthesized at Bristol-Myers Squibb. AZT was purchased from Burroughs Wellcome.

HIV inhibition assays. The inhibitory effects of the compounds on HIV-1 replication in CEM-SS cells infected with HIV-RF were measured by the XTT dye reduction method (47). Dilutions were made in half-log steps for both individual drugs and constant-ratio drug combinations. Drug ratios based on the 50% effective concentrations (EC_{50}s) of the individual drugs were chosen. One ratio corresponded to equivalent EC_{50}s (1:1), while the other ratios used EC_{50} ratios of 2:1, 1:2, 1:3, 3:1, 5:1, or 1:5. Cytotoxicity in CEM-SS cells was assessed by the XTT assay.

PBMCs were infected with a clinical isolate of HIV (006) at a multiplicity of infection of 0.001 50% tissue culture infective dose per cell. The cells were seeded into 96-well microtiter plates in the presence or absence of drug. The drugs were diluted in twofold steps in a checkerboard (five by six wells). On day 4 postinfection, one-half of the medium was replenished with fresh medium and drug. Supernatants were harvested on day 7 for quantitation of p24 by enzyme-linked immunosorbent assay (NEN-Dupont). Cytotoxicity was assessed by the trypan blue exclusion method with uninfected PBMCs and the highest drug concentrations used in the antiviral assays.

Analysis of drug combination effects. To assess the antiviral effects of different combination drug treatments, CIs were calculated according to the method described by Chou and Talalay (5) and volumes of synergy or antagonism were assessed according to the method described by Prichard et al. (40). For calculation of CIs, drugs were diluted in a fixed ratio and more than one ratio was analyzed. Dose-response curves were determined for each individual drug and each combination by the median-effect equation. The equation was fit by using the nonlinear regression routine (Proc Nlin) in PC SAS version 6.08. Numerically, CIs of <1, 1, or >1 indicate synergism, an additive effect, or antagonism, respectively.

The extent of synergy or antagonism was determined by using the MacSynergy program. For this analysis, drugs were diluted twofold in a matrix (5 by 6 wells). The theoretical additive interactions from the monotherapy groups were determined by the independent effect equation and were plotted as a plane in a 3-dimensional graph. The data from the experimental drug combination assay were then compared with the predicted additive interaction. Points above the additive plane represent synergistic interactions, while points below the plane represent antagonism. The extent of the synergy or antagonism is determined by the volume of the area above or below the additive plane. According to Prichard et al., volumes of synergy greater than 50 $\mu M^2\%$ may be considered significant, as may volumes of antagonism of less than -50 $\mu M^2\%$. The data shown were obtained at the 99% confidence level and were plotted with DeltaGraph.

RESULTS

Two-drug combinations with RT inhibitors. The antiviral effects of combining the RT inhibitors d4T, ddI, AZT, and ddC in CEM-SS cells and PBMCs were evaluated. CIs were calculated at the EC_{50}, EC_{75}, EC_{90}, and EC_{95} levels since these are the most representative of in vivo circumstances. However, in some PBMC assays, viral replication was not inhibited to 95% and CIs were not determined at that level. The combination of d4T-ddI in CEM-SS cells at a ratio of 1:20 yielded an additive response with CIs of approximately 1 at each inhibition level (Table 1). Synergistic CIs were obtained at the 1:10 ratio, and antagonistic CIs were obtained at the 1:2 ratio (Table 1). This set of results show that in CEM-SS cells, the combined effect of d4T-ddI ranges from moderate synergy to weak antagonism, depending on the ratios of the two compounds used. In PBMCs, the CIs for the d4T-ddI combination at ratios of 1:25

TABLE 2. Two-drug combinations in PBMCs

Drug combination[a]	Molar ratio[b]	CI at the following levels of HIV inhibition (%)[c]:				Overall result
		50	75	90	95	
d4T-ddI	1:25	0.47	0.33	0.23	NC[d]	Synergy
	1:50	0.36	0.27	0.20	NC	
d4T-AZT	5:1	0.76	0.70	0.63	NC	Synergy
d4T–BMS-PI	0.67:1	0.81	1.11	1.51	NC	Additive
	1.3:1	0.66	0.74	0.83	NC	
d4T-saquinavir	10:1	0.91	0.67	0.50	NC	Synergy
	20:1	0.91	0.66	0.51	NC	
BMS-PI–saquinavir	13:1	0.68	0.82	0.99	1.13	Additive
	27:1	0.80	0.88	0.97	1.04	
BMS-PI–indinavir	8:1	1.24	1.26	1.07	NC	Slight antagonism
	16:1	0.76	1.18	1.16	NC	

[a] The EC_{50}s for each monotherapy were as follows: d4T, 0.36 μM; ddI, 2.2 μM; AZT, 9 nM; BMS-PI, 0.038 μM; saquinavir, 4.9 nM; and indinavir, 3.2 nM.

[b] The highest concentrations of each drug used were as follows: d4T, 0.4 μM; ddI, 5 μM; AZT, 25 nM; BMS-PI, 0.4 μM; saquinavir, 40 nM; and indinavir, 50 nM.

[c] CIs were calculated according to the method described by Chou and Talalay, where values of <1, 1, and >1 indicate synergism, an additive effect, and antagonism, respectively.

[d] NC, CIs not calculated at this effective level.

TABLE 3. CIs from two-drug combinations in CEM-SS cells with RT and protease inhibitors

Drug combination[a]	Molar ratio[b]	CI at the following levels of HIV inhibition (%)[c]:				Overall result
		50	75	90	95	
d4T–BMS-PI	0.5:1	1.07	0.99	1.00	1.03	
	0.2:1	1.27	0.98	1.03	1.19	Additive
ddI–BMS-PI	2:1	0.73	0.48	0.32	0.25	
	10:1	1.24	0.92	0.70	0.61	Synergy
AZT–BMS-PI	1:500	0.86	0.54	0.36	0.29	
	1:100	0.85	0.78	0.77	0.71	Synergy
d4T-indinavir	20:1	1.31	1.27	1.26	1.26	
	40:1	1.22	1.05	0.92	0.84	Slight antagonism
ddI-indinavir	120:1	1.64	1.20	0.89	0.73	
	240:1	1.54	1.19	0.93	0.79	Additive

[a] The EC_{50}s for each monotherapy were as follows: d4T, 0.22 μM; ddI, 2.25 μM; BMS-PI, 0.1 μM; AZT, 3 nM; and indinavir, 4 nM.
[b] The highest concentrations of each drug used were as follows: d4T, 50 μM, ddI, 50 μM; BMS-PI, 50 μM; AZT, 1 μM; and indinavir, 1 μM.
[c] CIs were calculated according to the method described by Chou and Talalay, where values of <1, 1, and >1 indicate synergism, an additive effect, and antagonism, respectively.

and 1:50 were consistently less than 1, indicating a synergistic response (Table 2).

The results from combining d4T with AZT in CEM-SS cells at a ratio of 500:1 gave CIs of close to 1, indicating an additive drug interaction (Table 1). Combination of the drugs at ratios of 100:1 and 20:1 ratios yielded a moderate synergistic effect, suggesting that lower levels of d4T relative to AZT may be more effective. In contrast, in PBMCs, the d4T-AZT combination at a ratio of 5:1 gave CIs of consistently less than 1, indicating a synergistic response (Table 2).

Another combination tested in CEM-SS cells, d4T with ddC, resulted in high levels of synergy, with CIs ranging from 0.42 to 0.70 (Table 1). Cytotoxicity studies were performed with each of the above combinations in parallel to the antiviral assays, and no toxicity was observed at the highest drug concentrations used in these studies.

Two-drug combinations with inhibitors of RT and HIV protease. The two-drug combination BMS-PI–d4T was studied in CEM-SS cells and PBMCs. The CIs obtained with this combination in CEM-SS cells at two different ratios tested, 0.5:1 and 0.2:1, were nearly equal to 1 at each effective level, indicating an overall additive effect (Table 3). In PBMCs, synergistic values were seen at the 1.3:1 ratio, while weak antagonistic values were found at the 0.67:1 ratio at the higher effective doses (Table 2). Higher levels of HIV inhibition (CIs of <1) were observed at the higher ratios of d4T to BMS-PI.

A second combination, d4T-saquinavir, showed synergy in PBMCs at ratios of 10:1 and 20:1 (Table 2). CIs of near 1 were seen at the 50% effective dose, while values of as low as 0.5 were found at the higher effective levels. Therefore, in PBMCs, d4T-saquinavir showed a slightly better combined antiviral effect than d4T-BMS-PI.

Four additional combinations with one nucleoside analog and one protease inhibitor were examined in CEM-SS cells. BMS-PI with ddI or AZT showed a synergistic response, with CIs of significantly less than 1 at nearly all effective doses and at two different drug ratios (Table 3). Combining the protease inhibitor indinavir with d4T resulted in additive values at the 40:1 ratio and weak antagonistic values at the 20:1 ratio (Table 3). Similar results were found with the indinavir-ddI combination, although at both ratios (120:1 and 240:1), synergistic values were seen at the higher levels of HIV inhibition and moderate antagonistic values were seen at the lower effective levels. In general, higher drug concentrations yielded higher levels of combined antiviral effects. No cytotoxicity was observed when these drug combinations were tested on uninfected cells at the highest concentrations used for the antiviral assays.

Two-drug combinations with HIV protease inhibitors. The combined antiviral effects with two protease inhibitors were studied in infected CEM-SS cells and PBMCs. The BMS-PI–saquinavir combination in CEM-SS cells showed antiviral synergy at ratios of both 75:1 and 25:1 (Table 4). At each effective level, CIs were significantly less than 1 and as low as 0.28 at the higher doses. In PBMCs, the BMS-PI–saquinavir combination showed an overall additive effect, with some CIs of <1 at the lower effective doses (Table 2).

The combination BMS-PI–indinavir resulted in an overall additive response in both cell types (Tables 2 and 4). In CEM-SS cells, CIs of >1 were found at the 10:1 ratio, yet values of <1 were obtained at the ratio of 20:1, showing that antagonism is not a general outcome of this combination in this T-cell line (Table 4). In PBMCs, all CIs obtained for the BMS-PI–indinavir combination were nearly 1 or just greater than 1, indicating a slightly antagonistic response (Table 2).

One additional combination, BMS-PI with SC-52151, was tested in CEM-SS cells and showed significant synergy at both ratios studied (Table 4). CIs were consistently less than 1 at all effective levels and as low as 0.44 at the highest effective doses. Therefore, in both cell types, BMS-PI–indinavir was essentially additive while BMS-PI with saquinavir was additive in PBMCs. BMS-PI was very effective in CEM-SS cells when it was combined with the protease inhibitor saquinavir or SC-52151. No cytotoxicity was found with any of the concurrent treatments studied.

Two-drug combinations in PBMCs analyzed by two different methods. The data from each combination tested in PBMCs were analyzed by the methods described by Chou and Talalay (5) as well as Prichard et al. (40). For these assays, the drugs were titrated in a checkerboard so that CIs and the extent of synergy (volumes) could be determined from the same data sets. Volumes of greater than +50 $\mu M^2\%$ can be considered significant (above background), while volumes of greater than +100 $\mu M^2\%$ may be biologically meaningful (39). The two-drug combination d4T-ddI was synergistic, according to the technique described by Prichard et al., with a volume of 120 $\mu M^2\%$ (Fig. 1A). The CIs for this combination were significantly less than 1 (Table 2), also indicating synergy. Analysis of the d4T-AZT combination by MacSynergy gave a volume of 67 $\mu M^2\%$, indicating a low level of synergy (Fig. 1B). For this

TABLE 4. CIs from two-drug combinations in CEM-SS cells with HIV protease inhibitors

Drug combination[a]	Molar ratio[b]	CI at the following levels of HIV inhibition (%)[c]:				Overall result
		50	75	90	95	
BMS-PI–saquinavir	75:1	0.60	0.44	0.33	0.28	
	25:1	0.50	0.49	0.51	0.61	Synergy
BMS-PI–indinavir	10:1	1.53	1.14	1.05	1.13	
	20:1	1.30	0.84	0.61	0.51	Additive
BMS-PI–SC-52151	5:1	0.64	0.54	0.47	0.44	
	1:1	0.86	0.67	0.51	0.48	Synergy

[a] The EC_{50}s for each monotherapy were as follows: BMS-PI, 0.18 μM; saquinavir, 6 nM; indinavir, 4 nM; and SC-52151, 0.06 μM.
[b] The highest concentrations of each drug used were as follows: BMS-PI, 75 μM; saquinavir, 1 μM; indinavir, 1 μM; and SC-52151, 50 μM.
[c] CIs were calculated according to the method described by Chou and Talalay, where values of <1, 1, and >1 indicate synergism, an additive effect, and antagonism, respectively.

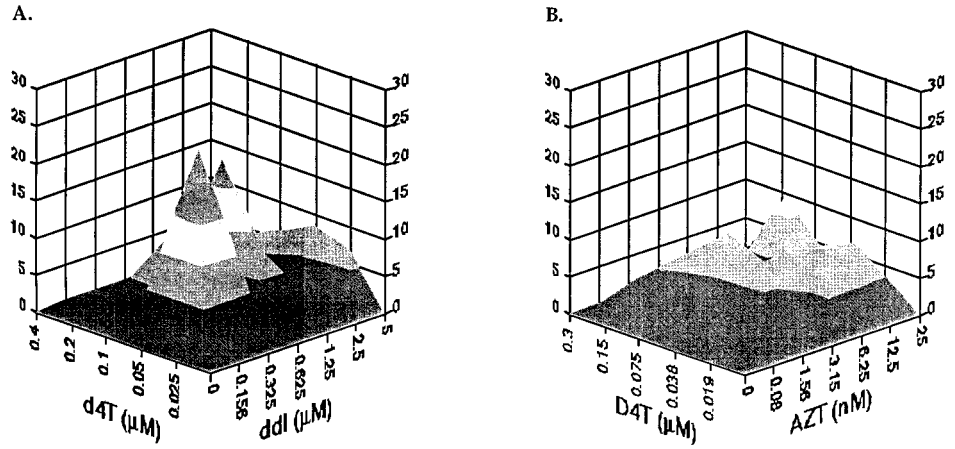

FIG. 1. Analysis of two-drug interactions (with MacSynergy) with two RT inhibitors and PBMCs. The drug concentrations are labeled on the *x* and *y* axes, and the *z* axis values are percent drug interaction values. (A) d4T and ddI; (B) d4T and AZT. The EC_{50}s for d4T, ddI, and AZT were 0.036 μM, 2.2 μM, and 9 nM, respectively.

combination, the CIs were also <1, suggesting a synergistic effect (Table 2). Therefore, both methods arrived at similar conclusions for the two combinations d4T-ddI and d4T-AZT. Since all three of these compounds are available, clinical testing of these combinations warrants consideration.

Analysis of the combinations d4T–BMS-PI and d4T-saquinavir with MacSynergy is shown in Fig. 2. The plot of the combination d4T–BMS-PI gave a volume of 62 $\mu M^2\%$, indicating a low level of synergy. Although the CIs suggested an overall additive effect, several values were less than 1 (Table 2). Therefore, both analyses show that at some ratios and effective levels, a low level of synergy may exist but additivity appears to be the general outcome from combining these two compounds in PBMCs. For d4T-saquinavir, a volume of 20 $\mu M^2\%$ was found, indicating additivity (Fig. 2B). By the analytical method described by Chou and Talalay, the combined effects with these two compounds showed an overall synergistic response in PBMCs, although CIs of approximately 1 were seen at the EC_{50} level. This demonstrates that a complete understanding of combined drug effects requires analysis of data at several drug ratios and effective levels.

Analyses by MacSynergy of combinations involving two protease inhibitors are shown in Fig. 3. The plots show that BMS-PI–saquinavir gave essentially an additive antiviral effect with a volume of 52 $\mu M^2\%$. This number can indicate a low level of synergy; however, in this case the peak above the additive plane is very broad and at any point the volume is very small. The area under the plane, which is equal to -3.5 $\mu M^2\%$, does not represent notable antagonism. The CIs for BMS-PI–saquinavir also indicate an overall additive response in PBMCs (Table 2). For the combination BMS-PI–indinavir, a volume of 36 $\mu M^2\%$ (Fig. 3B) was obtained, suggesting additivity, and, as with BMS-PI–saquinavir, the small area under the additive plane is not significant. CI analysis (Table 2) suggests a very low level of antagonism for the BMS-PI–indinavir combination; however, most values were near 1. Since the MacSynergy plots included outcomes from drug interactions at many drug ratios and anti-HIV levels while CIs were determined at selected drug ratios, the results generally agreed when the same ratios were examined. Indeed, the two methods showed very similar trends regarding the combined antiviral drug effects.

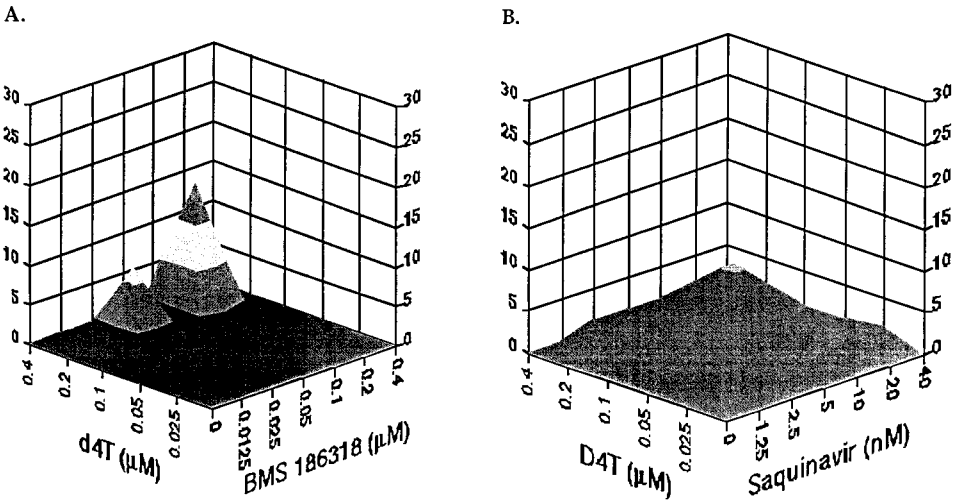

FIG. 2. Analysis (with MacSynergy) of two-drug combinations with one RT and one protease inhibitor. The drug concentrations are labeled on the *x* and *y* axes, and the *z* axis values are percent drug interaction values. (A) d4T and BMS-PI; (B) d4T and saquinavir. The EC_{50}s for d4T, saquinavir, and BMS-PI were 0.035 μM, 4.9 nM, and 0.038 μM, respectively.

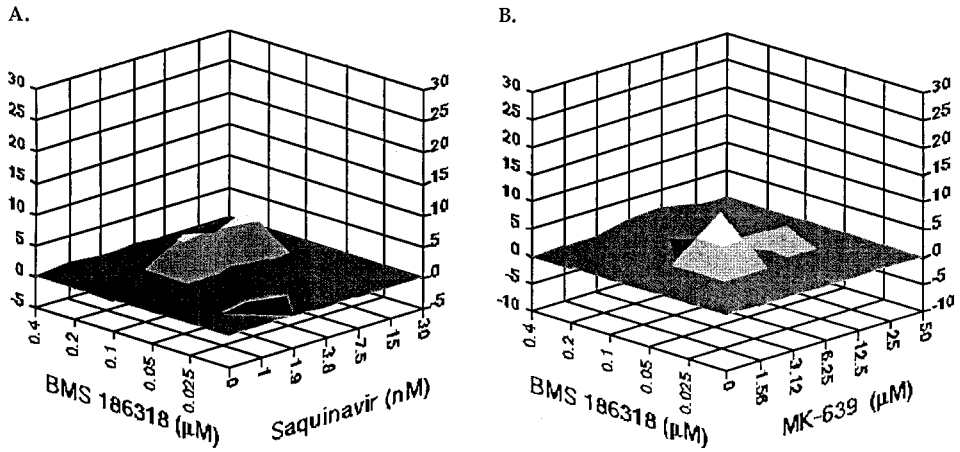

FIG. 3. Two-drug interaction analysis (with MacSynergy) of combinations of two protease inhibitors. The drug concentrations are labeled on the *x* and *y* axes, and the *z* axis values are percent drug interaction values. (A) BMS-PI and saquinavir; (B) BMS-PI and indinavir. The EC_{50}s for BMS-PI, saquinavir, and indinavir were 0.037 μM, 4.4 nM, and 3.2 nM, respectively.

DISCUSSION

In this study, two-drug combinations with RT and protease inhibitors in both CEM-SS cells and PBMCs were examined and two analysis methods were compared. In agreement with published reports (4, 38), the two analysis methods gave similar outcomes, while some differences were found when the two cell types were compared.

The combinations with RT inhibitors gave slightly different results for the CEM-SS cells and PBMCs, presumably because the anti-HIV activity of the nucleoside analogs requires phosphorylation by cellular enzymes (17). The results showed that the outcome of combining d4T and ddI in CEM-SS cells ranged from slight antagonism to moderate synergy, depending on the drug ratios used. In PBMCs, d4T-ddI was overall synergistic. The results from previous studies of the d4T-ddI combination in MT4 cells infected with HIV-IIIB also yielded a synergistic effect (43). Combination of d4T with AZT was additive to synergistic in CEM-SS cells, depending on the drug ratios, and was synergistic in PBMCs. Although antagonism might be expected from the AZT-d4T combination, on the basis of the dependence of both drugs on cellular thymidine kinase for activation, the drug concentrations used in these antiviral assays may be too low to observe a competitive effect. The different results observed with the RT inhibitors in CEM-SS cells and PBMCs were relatively small, and no combination appeared to be antagonistic in one cell type and synergistic in the other. However, drugs which depend on cell activation should be tested in combination in several cell types before a final outcome is determined. Finally, in CEM-SS cells, combining the nucleoside analogs d4T and ddC showed strong synergy; however, overlapping neurotoxicity must be taken into consideration in the design of clinical trials.

For combinations with two protease inhibitors, in both PBMCs and CEM-SS cells, BMS-PI with saquinavir was synergistic at the lower effective levels, and the BMS-PI–indinavir combination showed some antagonism at 50% inhibition of virus replication. In general, these two protease inhibitor combinations gave similar responses in both cell types, although the final outcome was slightly different. Significant synergy was observed with the BMS-PI–SC-52151 combination in CEM-SS cells; however, unfortunately, SC-52151 is no longer in development.

In CEM-SS cells, the combination ddI-indinavir was, in general, additive while d4T-indinavir showed slight antagonism. BMS-PI combined with ddI or AZT was synergistic yet additive with d4T. Interestingly, with the combinations BMS-PI–ddI and BMS-PI–AZT, higher levels of synergy were observed at higher protease inhibitor-to-nucleoside analog ratios. In both PBMCs and CEM-SS cells, BMS-PI–d4T was additive, and one additional combination, d4T-saquinavir, showed synergy in PBMCs. Others have also published reports showing additive to synergistic interactions with different HIV protease inhibitors in combination with AZT or ddC in vitro (9, 23, 25, 29, 44). In one clinical trial, the combination AZT-ddC-saquinavir resulted in a significant and sustained reduction in viral load (19).

The in vitro drug combination assay is a useful tool for prescreening drug pairs for clinical investigation, although this type of short term assay does not address the emergence of multiple drug resistance and cannot fully predict combined clinical toxicity that may arise in patients. While each of the combinations tested were overall additive or synergistic, the combinations d4T-saquinavir, d4T-AZT and d4T-ddI yielded significant levels of combined antiviral responses, are currently available, and thus deserve further clinical evaluation.

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