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Combinations of 3′-Azido-3′-Deoxythymidine (Zidovudine) and Phosphonoformate (Foscarnet) against Human Immunodeficiency Virus Type 1 and Cytomegalovirus Replication In Vitro

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Combinations of 3′-azido-3′-deoxythymidine and phosphonoformate produced a moderate synergistic inhibitory effect against human immunodeficiency virus type 1 in vitro at concentrations that are easily achieved in humans. The synergistic effect was more pronounced with increasing concentrations and was not secondary to toxic effects of the drugs. 3′-Azido-3′-deoxythymidine neither inhibited the replication of human cytomegalovirus in human embryonic lung fibroblasts nor interfered with the anticytomegalovirus effect of phosphonoformate. By using partially purified reverse transcriptase of human immunodeficiency virus type 1 and human cytomegalovirus DNA polymerase, various combinations of 3′-azido-3′-deoxythymidine-5′-triphosphate and phosphonoformate produced strong indications of additive interactions. The synergistic interactions in infected cells and the additive effects observed at the reverse transcriptase level indicate that mechanisms other than the reverse transcriptase may be of importance for the inhibition of human immunodeficiency virus replication by these two compounds. A concomitant treatment of cytomegalovirus infections, such as cytomegalovirus retinitis, with phosphonoformate in patients with acquired immunodeficiency syndrome receiving 3′-azido-3′-deoxythymidine may be appropriate, and this combination may also be useful in controlling human immunodeficiency virus infection.

Several reports have indicated that a number of agents can affect human immunodeficiency virus type 1 (HIV-1) replication in cell culture (4, 17, 23, 32, 43). 3′-Azido-3′-deoxythymidine (zidovudine; AZT), a modified nucleoside, has received considerable attention because it is the first agent shown in a multicenter, double-blind, placebo-controlled study to prolong life and provide clinical improvement in certain patients with acquired immunodeficiency syndrome (AIDS) and advanced AIDS-related complex who had been treated during a period up to 6 months (10). However, AZT is associated with pronounced toxicities which have limited its use in individuals with established AIDS (27, 38). Opportunistic human cytomegalovirus (HCMV) infections are one of the major problems in individuals with AIDS. Cytomegalovirus retinitis has been described in approximately one-third of this population (26). In the absence of therapy, this infection inevitably leads to irreversible retinal necrosis and can progress to permanent blindness. Only two drugs, 9-(1,3-dihydroxy-2-propoxymethyl)guanine (ganciclovir, DHPG, 2′-NDG, or BIOLF-62) and phosphonoformate (foscavir, Foscarnet, or PFA), have been investigated with some success as possible treatments against HCMV infections (8, 20, 21, 25, 28, 35, 40). PFA, an analog of pyrophosphate, has the unique property of being effective against both HIV-1 and HCMV in vitro (5, 24, 30, 31, 39, 42). Recent studies by Jacobson et al. (19) have also demonstrated a significant reduction of HIV-1 p24 antigen concentrations in patients after a 14-day treatment with PFA. Since a concomitant treatment of PFA against CMV retinitis is currently being considered in HIV-infected individuals undergoing AZT therapy, it was important to determine the type of interaction produced by these drugs against HIV-1 and HCMV.

The use of combinations of compounds with different modes of action is an attractive and logical extension of any therapeutic approach to enhance drug efficacy. Lower doses of the drugs might be used, which also may reduce the toxicity caused by either drug alone and the appearance of drug-resistant virus. In fact, almost all of the currently available antiretroviral agents have been evaluated in combinations with AZT (4, 13, 32). For example, using a rigorous definition of synergy for drug interactions, Hartshorn et al. (15) demonstrated synergistic effects against HIV-1 in cell culture by combinations of PFA and recombinant alpha-A interferon. In a similar in vitro system, combinations of AZT and alpha-A interferon or granulocyte-macrophage colony-stimulating factor have been reported to produce synergy (14, 16).

In this report, the effects of different combinations of AZT and PFA were examined on the replication of HIV-1 in human peripheral blood mononuclear (PBM) cells and HCMV Ad169 in human embryonic lung (HEL) fibroblasts. Since the major mode of action of PFA and the triphosphate derivative of AZT (AZT-TP) is an inhibition of the HIV-1 reverse transcriptase (RT) activity by different mechanisms (6, 7, 12, 41, 42), the effects of several combinations of the two compounds were also examined on the partially purified HIV-1 RT. For comparison, the effects of combinations of PFA and AZT-TP on the partially purified HCMV DNA polymerase were studied.

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MATERIALS AND METHODS

Compounds. PFA was provided by Astra ALab, Södertälje, Sweden. AZT and AZT-TP were synthesized and

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purified in our laboratory according to previously described methods (22, 41, 44). The purity of AZT and AZT-TP was established by reverse-phase and anion-exchange high-performance liquid chromatography methods and spectrophotometric analysis.

Cells, virus strains, and cell culture assays. PBM cells from healthy HIV-1- and hepatitis B virus-seronegative donors were isolated and propagated as described previously (33). HIV-1 (strain LAV-1) was obtained from the Centers for Disease Control, Atlanta, Ga., and propagated in phytohemagglutinin-stimulated human PBM cells as described previously (33). The details of the methods used for infection and assaying the anti-HIV-1 effect in infected human PBM cells have been reported previously (33).

HCMV Ad169 was a gift from Fred Rapp, Hersey, Pa. Human embryonic lung (HEL) cells, obtained from the American Type Culture Collection, Rockville, Md., were cultured in Dulbecco modified Eagle medium as described previously (37). Viable cells were counted microscopically by using a hemacytometer and the trypan blue exclusion method. Plaque reduction assays were performed in confluent monolayers of HEL cells in 6-well plates (Costar, Cambridge, Mass.) with 100 to 200 PFU of HCMV Ad169. After an adsorption period of 1 h, unadsorbed virus was removed and the monolayers were overlaid with Dulbecco modified Eagle medium containing 0.75% SeaPlaque-agarose (FMC BioProducts, Rockland, Maine), 2% heat-inactivated fetal calf serum, and the appropriate concentration of drug. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 10 to 12 days. The monolayers were then fixed with 10% buffered Formalin (Fisher Scientific Co., Pittsburgh, Pa.) and stained with crystal violet, and the number of plaques were counted.

Cell proliferation. The drugs alone and in combination were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells. Flasks were seeded so that the final cell concentration was 3×10^5/ml. The cells were cultured with or without drug for 6 days, at which time equal samples were counted for cell viability by using the trypan blue exclusion method.

RT activity assay with disrupted virions. Supernatant (6 ml) from each culture was clarified from cells at 300 × *g* for 10 min. Virus particles were then pelleted from 5-ml samples at 40,000 rpm for 30 min by using a rotor (70.1 Ti; Beckman Instruments, Inc., Fullerton, Calif.) and suspended in 200 μl of virus-disrupting buffer (50 mM Tris hydrochloride [pH 7.8], 800 mM NaCl, 20% glycerol, 0.5 mM phenylmethylsulfonyl fluoride, 0.5% Triton X-100).

The RT assays were performed at 37°C in 96-well microdilution plates by methods described previously (33, 36). The results were expressed as disintegrations per minute per milliliter of the originally clarified supernatant.

RT activity assay with partially purified enzyme. HIV-1 RT was isolated from detergent-disrupted virions obtained from the cell-free supernatant of infected phytohemagglutinin-stimulated PBM cells. The enzyme was purified by passing the extract through ion-exchange chromatography columns as described previously (12). The enzyme was characterized as HIV-1 RT on the basis of its specific requirements according to previous descriptions (1, 18). The standard reaction mixture (100 μl) contained 100 mM Tris hydrochloride (pH 8.0), 50 mM KCl, 2 mM $MgCl_2$, 5 mM dithiothreitol, 400 μg of bovine serum albumin per ml, 0.05 U of $(rA)_n \cdot (dT)_{12-18}$ per ml (3.1 μg/ml), 1 μM [^{3}H]dTTP (specific activity, 18,000 cpm/pmol), and 10 μl of HIV-1 RT. The reaction mixtures were incubated and processed as previously described (7).

CMV DNA polymerase assay. The partially purified HCMV-specific DNA polymerase was a generous gift from B. Wahren, National Bacteriological Laboratory, Stockholm, Sweden. The procedures for the purification of HCMV DNA polymerase by sequential chromatographic steps on DEAE-cellulose (DE-52; Whatman, Inc., Clifton, N.J.) and phosphocellulose (P-11; Whatman) and the measurement of enzyme activity have been described previously (5).

Calculation of synergy. To determine whether synergistic, additive, or antagonistic antiviral effects were achieved in virus-infected cell cultures treated with combinations of AZT and PFA or in enzyme assays with combinations of AZT-TP and PFA, the multiple drug effect analysis developed by Chou and Talalay (2, 3) was used. Briefly, the method involves plotting of dose-effect curves for each compound alone and in combinations. It is essential to use a fixed ratio of the agents in multiply diluted combinations in order to use the median-effect equation $f_a/f_u = (C/C_m)^m$. In this equation, C is the concentration, f_a and f_u are the fractions of the system which are affected and unaffected, respectively, by concentration C. C_m is the concentration required to produce the median effect (analogous to the more familiar 50% inhibitory concentration and 50% effective dose values) and m is a Hill-type coefficient signifying the sigmoidicity of the dose-effect curve. The slopes (m) of the dose-effect plots, obtained for the studied compounds, provided information as to whether the compounds are mutually exclusive (i.e., similar modes of action) or mutually nonexclusive (i.e., different modes of action). When the plots of both compounds and their combination were all parallel, the effects of the two compounds were mutually exclusive. The interaction between two compounds were determined by calculating the combination index (CI) with assumptions, when appropriate, of either mutually exclusive or nonexclusive interactions. When uncertainty existed as to whether the drugs acted in similar or independent manners, CI values were calculated under each assumption and compared. Values of CI less than 1 indicated synergy, a CI equal to 1 indicated additive effects, and a CI greater than 1 indicated antagonism. A computer program obtained from Elsevier-Biosoft, Cambridge, United Kingdom, was used for automatic analysis of all dose-effect data. Additional details on using this method have been reported previously (34).

RESULTS

Effects in HIV-1-infected and uninfected PBM cells. The effects of AZT and PFA, alone and in combinations, on the HIV-1 replication in human PBM cells (measured as RT activity of disrupted virions) are shown in Table 1. The supernatant of untreated virus-infected human PBM cells contained a mean RT activity of 772 kdpm/ml (equivalent to 11.8 pmol of dTMP incorporated into acid-insoluble product). A concentration-dependent decrease in RT activity was observed in cultures treated with each compound alone and their combinations. A 50% reduction of HIV-1 replication (median effective concentration [EC_{50}]) was observed at 0.006 μM AZT and 22 μM PFA when the drugs were tested alone and at 3.5 μM and 8.5 μM when combinations of AZT and PFA were tested at ratios of 1:1,000 and 1:4,000, respectively. The ratios of AZT to PFA were selected according to the approximate ratio of their EC_{50}s. The upper part of Table 2 summarizes the slopes, median-effect values,

TABLE 1. Effects of AZT and PFA alone and in combinations at ratios of 1:1,000 and 1:4,000 on HIV-1 replication in human PBM cells

Treatment (drug ratio)	Concn (μM)	RT activity (kdpm/ml)[a]	Inhibition (%)
AZT	0.002	750	3.0
	0.004	584	24.5
	0.008	343	55.7
	0.016	35.9	95.5
PFA	8	703	9.1
	16	455	41.1
	32	190	75.5
	64	93.1	88.1
	128	35.9	95.5
AZT/PFA (1:1,000)	0.001/1	797	−3.1
	0.002/2	668	13.6
	0.004/4	247	68.1
	0.008/8	68.4	91.3
AZT/PFA (1:4,000)	0.001/4	635	17.8
	0.002/8	551	28.8
	0.004/16	238	69.3
	0.008/32	4.6	99.5

[a] The activity of uninfected PBM cells was 5,100 dpm/ml. All values are corrected for the mean value of the blanks (995 dpm). The mean value of RT activity ± the standard deviation of triplicate untreated HIV-infected cells was 772 ± 50.8 kdpm/ml.

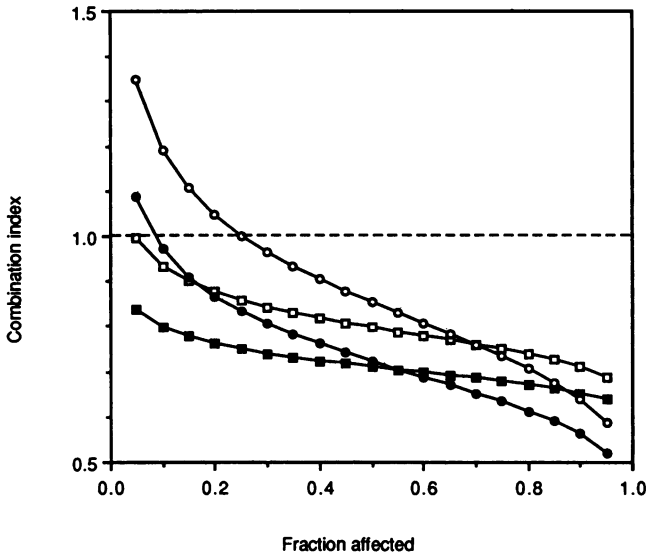

FIG. 1. Computer-generated presentation of the CI with respect to the fraction affected (f_a) for the inhibition of HIV-1 multiplication in human PBM cells. Combinations of AZT and PFA at ratios 1:1,000 and 1:4,000 were analyzed under both mutually exclusive (■ and ●) and mutually nonexclusive (□ and ○) assumptions, respectively.

and correlation coefficients obtained from the median-effect plots of AZT, PFA, and their combinations, respectively. The observed slope values (m) were greater than 1 for both compounds and their combinations in HIV-1 infected cells, which indicated a sigmoidal rather than a hyperbolic nature of the dose-effect curves (m = 1). This is in accordance with what is normally observed in more organized biological systems in which the dose-effect relationships of inhibitors are frequently sigmoidal rather than hyperbolic (2). Since the median-effect plots for AZT, PFA, and their combinations were not parallel to each other in HIV-1-infected cells (data not shown), the exclusivity of the combined effects could not be established. Therefore, the CI values were calculated under both mutually exclusive and mutually nonexclusive assumptions. As shown in Table 2, the CI values of both combinations giving a 50, 70, or 90% reduction of the HIV-1 replication were all less than 1, which suggests synergistic effects. The computer-generated CI values for f_a values ranging from 0.05 to 0.95 for the studied combinations of AZT and PFA are shown in Fig. 1. Both combinations produced similar synergistic effect patterns which were concentration dependent and increased with increasing concentrations.

At a ratio of 1:4,000, the combination of AZT and PFA produced no toxicity greater than the agents alone to uninfected phytohemagglutinin-stimulated PBM cells; the highest combined concentration tested was 128.032 μM. PFA

TABLE 2. Median effective concentration and CI values for AZT (AZT-TP) and PFA alone and at different drug ratios against HIV-1 replication in human PBM cells and purified HIV-1 RT

Treatment (drug ratio)	Parameter[a]			CI at f_a of[b]:		
	m ± SE	EC_{50} (μM)	r	0.50	0.70	0.90
HIV-1-infected cells						
AZT	3.02 ± 0.27	0.006	0.99			
PFA	1.89 ± 0.17	22.0	0.99			
AZT/PFA (1:1,000)	3.03 ± 0.10	3.5	0.99	0.71 (0.80)	0.68 (0.76)	0.65 (0.71)
AZT/PFA (1:4,000)	3.20 ± 0.90	8.5	0.93	0.72 (0.85)	0.65 (0.76)	0.56 (0.64)
HIV-1 RT						
AZT-TP	0.87 ± 0.03	0.003	1.00			
PFA	0.91 ± 0.03	0.14	1.00			
AZT-TP/PFA (1:20)	0.89 ± 0.04	0.043	0.99	0.98	0.97	0.96
AZT-TP/PFA (1:50)	0.92 ± 0.02	0.074	1.00	1.02	0.99	0.93
AZT-TP/PFA (1:100)	0.87 ± 0.02	0.084	1.00	0.89	0.91	0.95
AZT-TP/PFA (1:200)	0.92 ± 0.02	0.11	1.00	0.95	0.93	0.90

[a] m is the slope (the standard errors of the mean are given when four or more concentrations were used in the median-effect plot), EC_{50} is the median effective concentration, and r is the correlation coefficient as determined from the median-effect plot.

[b] CI < 1, Synergy; CI = 1, additivity; CI > 1, antagonism (see Materials and Methods). f_a is a component of the median-effect equation referring to the fraction of the system affected (e.g., 0.50 means the CI at a 50% reduction of activity). CI values for HIV-1-infected cells were determined under both mutually exclusive and mutually nonexclusive (numbers in parentheses) assumptions.

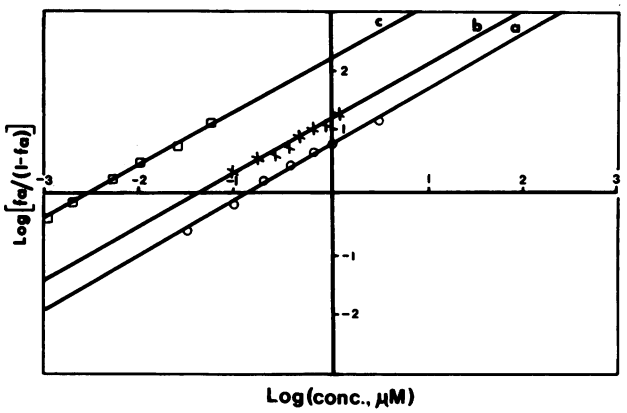

FIG. 2. Median-effect plots for the inhibition of HIV-1 RT by AZT-TP (line c) and PFA (a) alone and a representative combination of the two compounds at a ratio of 1:20 (b). Slope values: ± the standard errors of the mean AZT-TP, 0.87 ± 0.03; PFA, 0.91 ± 0.03; combination, 0.89 ± 0.04.

was not toxic to PBM cells when tested up to 640 μM (data not shown).

Effects on HIV-1 RT. The synergistic effect observed in HIV-1-infected PBM cells by combinations of AZT and PFA could be a consequence of the different mechanisms involved in inhibiting the RT activity. A combination of AZT-TP and PFA may therefore produce synergistic effects at the RT level. To study this hypothesis, the effects of several different combinations of AZT-TP and PFA were investigated by using partially purified HIV-1 RT. The median-effect plots for the inhibition of HIV-1 RT by AZT-TP, PFA, and a representative combination of the two components at a molar ratio of 1:20 are shown in Fig. 2. Both inhibitors followed first-order kinetics (i.e., m values were close to 1), and from the parallel lines it was apparent that AZT-TP and PFA were mutually exclusive inhibitors. The slopes, median-effect concentrations, and CI values calculated for AZT-TP and PFA alone and in the four different combinations studied (1:20, 1:50, 1:100, and 1:200) against the HIV-1 RT are presented in the lower part of Table 2. In contrast to what was observed in HIV-1-infected cells, slope values closer to 1 were observed. The CI values were found to be close to 1 for f_a values at 0.50, 0.70, and 0.90, which strongly suggests that all studied combinations produced additive effects. The computer-generated calculation of CI values for combinations of AZT-TP and PFA at ratios of 1:20 and 1:200 were found to be close to 1 over the entire range of f_a values (Fig. 3).

Effects on HCMV plaque formation. To study whether AZT would interfere with the anti-HCMV effect of PFA, the effects of four appropriate combinations (1:1, 1:2, 1:5, and 1:10) of the two compounds on the multiplication of HCMV Ad169 in HEL fibroblasts were studied. Whereas AZT was found to be virtually without inhibitory effect even at concentrations up to 200 μM (data not shown), a dose-dependent inhibition was obtained for PFA with an EC_{50} of 34 μM (Table 3). Although AZT did not significantly inhibit HCMV multiplication, an EC_{50} of 383 μM was extrapolated (data not shown) in order to calculate the CI values for the studied combinations. The obtained CI values indicated an additive effect for all combinations of AZT and PFA examined (Table 3). Furthermore, the EC_{50}s obtained indicated that the contribution of PFA in each combination caused the antiviral effect.

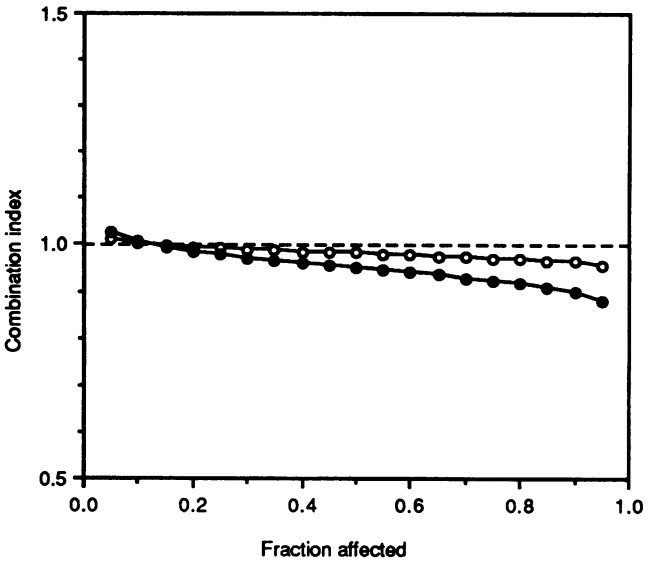

FIG. 3. Computer-generated presentation of CI values versus f_a values for combinations of AZT-TP and PFA at molar ratios of 1:20 (○) and 1:200 (●) against HIV-1 RT. The CI values were analyzed under mutually exclusive assumptions.

Effects on the HCMV DNA polymerase. Since AZT-TP and PFA are structurally different and presumably inhibit the HCMV DNA polymerase activity by different mechanisms, the interaction of the two drugs was investigated for four different combinations, at ratios of 5:1, 10:1, 25:1, and 50:1. As summarized in the lower part of Table 3, AZT-TP was found to inhibit the HCMV DNA polymerase activity with an observed EC_{50} of 25 μM. For PFA the corresponding EC_{50} of 0.78 μM was more than 30 times lower. All combinations of AZT-TP and PFA produced CI values close to 1 when the HCMV DNA polymerase activity was inhibited between 50 and 90%. This indicated that all combinations of the two compounds produced an additive effect also at the HCMV DNA polymerase level.

DISCUSSION

An effective chemotherapeutic treatment of HIV-1 infections is a formidable challenge. Not only does the virus infect and multiply in several different cells, it also establishes latency. A considerable genetic variability within and among clinical isolates has also been reported (9, 29). Since individuals infected with HIV-1 will have to be treated for prolonged periods of time, drug-resistant variants of virus may be selected. A combination of chemotherapeutic agents may be used to enhance the distribution of drugs and their antiviral effects, decrease toxicity, and reduce the potential risk of drug resistance development, and in the case of HIV-1-infections, both suppress virus replication and help to restore the immune system. Since AZT has only a documented effect against HIV-1 infections, a concomitant therapy may be necessary to treat the opportunistic infections which occur in AIDS patients. Therefore, it was important to study to what extent PFA, a compound which not only has been shown effective against CMV retinitis but which also inhibits HIV replication, may interact with AZT against HIV-1 infections.

The results presented here demonstrate that the interaction of AZT and PFA against HIV-1 replication in human

TABLE 3. Median effective concentration and CI values for AZT (AZT-TP) and PFA alone and at different drug ratios against CMV plaque formation in human fibroblasts and purified CMV DNA polymerase

Treatment (drug ratio)	Parameter[a]			CI at f_a of[b]:		
	$m \pm$ SE	EC_{50} (μM)	r	0.50	0.70	0.90
CMV-infected cells						
AZT	5.95	383	0.97			
PFA	1.88 ± 0.10	34	1.00			
AZT/PFA (1:1)	2.16 ± 0.39	61	0.97	0.97 (1.04)	0.94 (1.03)	0.91 (1.03)
AZT/PFA (1:2)	1.71 ± 0.32	41	0.95	0.82 (0.85)	0.87 (0.91)	0.97 (1.05)
AZT/PFA (1:5)	2.65 ± 0.22	52	0.99	1.29 (1.32)	1.14 (1.17)	0.94 (0.97)
AZT/PFA (1:10)	2.30	33	0.92	0.89 (0.90)	0.82 (0.83)	0.72 (0.74)
CMV DNA polymerase						
AZT-TP	0.88 ± 0.09	25	0.99			
PFA	0.79 ± 0.02	0.78	1.00			
AZT-TP/PFA (5:1)	0.74 ± 0.05	3.4	0.99	0.84	0.92	1.07
AZT-TP/PFA (10:1)	0.70 ± 0.02	6.1	1.00	0.93	1.10	1.45
AZT-TP/PFA (25:1)	0.78 ± 0.03	11	1.00	0.93	1.00	1.13
AZT-TP/PFA (50:1)	0.87 ± 0.08	16	0.99	1.01	0.98	0.94

[a] m is the slope (the standard errors of the mean are given when four or more concentrations were used in the median-effect plot), EC_{50} is the median effective concentration, and r is the correlation coefficient as determined from the median-effect plot.

[b] CI < 1, Synergy; CI = 1, additivity; CI > 1, antagonism (see Materials and Methods). f_a is a component of the median-effect equation referring to the fraction of the system affected (e.g., 0.50 means the CI at a 50% reduction of activity). CI values for CMV-infected cells were determined under both mutually exclusive and mutually nonexclusive (numbers in parentheses) assumptions.

PBM cells produced a moderate synergy, which was more pronounced with increasing f_a values. In contrast, when combinations of AZT-TP and PFA were studied against HIV-1 RT activity, a clear additive effect was indicated. The latter result suggested that both drugs cannot simultaneously enter their binding sites on the enzyme and, therefore, the binding of and inhibition caused by one compound will prevent the other compound from exhibiting its antiviral effect. This so-called mutually exclusive inhibition suggests that the binding sites for PFA and AZT-TP on the HIV-1 RT molecule may be overlapping. Mutually exclusive interactions have previously been observed for combinations of 9-(2-hydroxyethoxymethyl)-guanine (acyclovir) and PFA against herpesvirus DNA polymerase (11). The observed synergistic effect of AZT and PFA in HIV-1-infected cells and the additive effect of AZT-TP and PFA against HIV-1 RT activity indicate that mechanisms other than the HIV-1 RT may be of importance in the inhibition of HIV-1 multiplication by these two compounds. In these studies, the synergistic effect of AZT and PFA against HIV-1 was observed without any indication of increased toxicity to uninfected human PBM cells.

The observed lack of synergistic effects of combinations of AZT and PFA against HCMV replication in cell culture could be predicted, since AZT was shown not to possess any anti-HCMV effect. The observed additive effect resulted merely from the contribution of increasing concentrations of PFA in each of the different mixtures tested. The EC_{50}s for PFA against HCMV Ad169 replication in cell culture and the partially purified HCMV DNA polymerase were in agreement with previous observations (5, 39). Although no inhibitory effect could be observed for AZT in HCMV-infected cells, AZT-TP was shown to be a weak inhibitor of the HCMV DNA polymerase activity, with an EC_{50} of 25 μM. The additive effect observed at the DNA polymerase level suggests that in analogy to what was discussed above for HIV-1 RT, the binding sites for PFA and AZT-TP may be overlapping. However, since concentrations of AZT in the millimolar range would have to be administered to HCMV-infected cells in order to achieve a concentration of AZT-TP close to its EC_{50} against the HCMV DNA polymerase activity, this may explain why no effect for AZT could be demonstrated at the concentrations tested. An administration of such high levels of AZT in attempts to control an HCMV-infection will cause cell-toxic effects in vitro and will probably not be clinically meaningful because of possible side effects.

The results presented demonstrate that at concentrations easily attained in vivo, combinations of AZT and PFA interacted synergistically in inhibiting HIV-1 replication in human PBM cells. Furthermore, AZT had no effect against HCMV infections in cell culture, and AZT (or AZT-TP) did not antagonize the anti-HCMV effect of PFA. Although laboratory data do not necessarily predict what will happen in humans, our results support not only the concomitant use of PFA in AZT-treated individuals suffering from CMV infections, such as CMV retinitis, but also the consideration of clinical trials with combinations of AZT and PFA in HIV-1-infected individuals.

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