ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1991, p. 1060–1065
0066-4804/91/061060-06$02.00/0

Vol. 35, No. 6

Three-Dimensional Analysis of the Synergistic Cytotoxicity of Ganciclovir and Zidovudine

MARK N. PRICHARD,[1] LYNN E. PRICHARD,[2] WILLIAM A. BAGULEY,[2] M. REZA NASSIRI,[2] AND CHARLES SHIPMAN, JR.[1,2]*

Department of Microbiology and Immunology, School of Medicine,[1] and Department of Biologic and Materials Sciences, School of Dentistry,[2] The University of Michigan, Ann Arbor, Michigan 48109

Received 10 December 1990/Accepted 19 March 1991

The combined cytotoxicity of zidovudine and ganciclovir in three cell lines of human origin was examined. The data were generated by a new rapid cell proliferation assay and a more sensitive plating efficiency assay. A three-dimensional analytical approach was used to evaluate the drug-drug interactions, and the results were compared with those obtained by two conventional methods of analysis. Synergistic cytotoxicity was observed in all cell lines examined and by both assays. Moreover, this synergistic cytotoxicity was statistically significant at physiologically relevant concentrations. It is not known whether these drug-drug interactions manifest themselves in vivo as granulocytopenia or other untoward side effects. These results, however, indicate that further investigation is warranted and that the coadministration of zidovudine and ganciclovir may be contraindicated.

Cytomegalovirus retinitis is a common sequela of AIDS and occurs in at least 25% of patients with human immunodeficiency virus disease (3, 15). Ganciclovir (DHPG) therapy impedes the progress of this sight-threatening condition but requires lifelong therapy to prevent recurrent infections (9, 13, 18), and neutropenia is seen in 30% of patients (17). Zidovudine (AZT) is useful in treating AIDS (10, 29), but like DHPG, it causes at least some bone marrow suppression in most patients (23). Although the coadministration of AZT with drugs which interfere with erythrocyte and leukocyte number or function is not recommended (3a), the concomitant administration may be inevitable because few alternatives currently exist. Recently, clinical studies have suggested that combination AZT and DHPG therapy causes severe toxicity in patients, confirming initial concerns (14, 16, 21).

We examined the cytotoxicity of combinations of AZT and DHPG in vitro and analyzed the drug-drug interactions as part of a larger effort to evaluate and develop potent combinations of antiviral drugs. The data were obtained in three human cell lines, including one primary cell line. Cytotoxicity was evaluated by two methods, a cellular proliferation assay and a plating efficiency assay. The cellular proliferation assay was developed to measure efficiently the cellular growth rate in the presence of many drug combinations. The data obtained from this assay identified concentrations of AZT and DHPG at which anomalous effects were seen. These concentrations were then specifically targeted by a plating efficiency assay, which is a more sensitive indicator of cytotoxicity. The data obtained from both of these assays were analyzed by a new three-dimensional (3-D) analytical method. The 3-D method was used because it can identify concentrations at which synergistic or antagonistic interactions occur, and then it is able to quantitate and statistically analyze the interactions (22). This analysis revealed that AZT and DHPG are synergistically cytotoxic in vitro. In addition, it demonstrated that the observed effects are statistically significant at physiologically relevant concentrations.

* Corresponding author.

MATERIALS AND METHODS

Chemicals. AZT and DHPG were provided through the courtesy of Sandra Lehrman of the Burroughs Wellcome Co. and Thomas Matthews of Syntex Laboratories, respectively.

Cell culture. KB cells, an established line of human epidermoid carcinoma cells, were grown in monolayers and in suspension cultures as described previously (26). Primary human foreskin fibroblasts (HFFs) and WI-38 cells, a human embryonic diploid cell line, were grown in minimal essential medium with Earle's salts (MEM[E]) supplemented with 10% fetal bovine serum. Cells were routinely passaged with 0.05% trypsin plus 0.02% EDTA in HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-buffered saline (HBS) (25).

Dye binding assay. A rapid method for quantitating cells in 96-well plates was developed and is similar to the methods described by others (1, 19). To evaluate this method, KB cells were grown in MEM(H) supplemented with reduced (1%) calf serum for 24 h at 37°C in a humidified 3% CO_2–97% air atmosphere. Twofold dilutions of these stationary-phase cells were made in 96-well plates in the reduced-serum medium and incubated for an additional 24 h to allow the cells to adhere. The plates were washed with HBS, fixed with 95% ethanol, and stained with 100 μl of 0.01% (wt/vol) crystal violet in 20% (vol/vol) methanol. The unbound dye was shaken out, and the plates were washed four times with distilled water. The bound stain was eluted by adding 150 μl of 95% ethanol containing 1% (vol/vol) 1 N HCl to each well. The optical densities (ODs) of the solutions containing the eluted dye were determined directly in a microplate reader (Bio-Tek Instruments, Inc., Winooski, Vt.) at 570 and 405 nm to correct for light scattering by the cell sheet. When the experiments were conducted with WI-38 cells and HFFs, fetal bovine serum was substituted for the calf serum.

Cell proliferation assay. The proliferation of KB cells was measured by the dye-binding assay. Briefly, 2×10^3 KB cells grown in MEM(E) supplemented with 10% calf serum were

seeded in each well of quadruplicate 96-well plates (Costar Corp., Cambridge, Mass.) and incubated for 24 h at 37°C in a humidified 3% CO_2–97% air atmosphere to allow the cells to adhere. Checkerboard dilutions of the drugs, including the drugs used individually, then were added to the inner 60 wells of three plates in a 6-by-10 matrix. The remaining plate was washed with HBS, fixed with 95% ethanol, and later used to obtain the initial cell concentration. Plates were incubated for an additional 48 h, washed with HBS, and fixed as described above. The fixed plates, including the plate fixed at 24 h, were stained with 0.01% (wt/vol) crystal violet in 20% (vol/vol) methanol and washed four times with distilled water, and the bound stain was eluted with 150 μl of 95% ethanol containing 1% (vol/vol) 1 N HCl. The ODs of the solutions containing the eluted dye then were determined directly in a microplate reader at 570 and 405 nm. The proliferation of WI-38 cells and HFFs was quantitated in the same manner with the following modifications. Cells grown in MEM(E) supplemented with 10% fetal bovine serum were harvested while they were still subconfluent and were seeded at 5×10^3 cells per well in 96-well plates. The cells were incubated for only 6 h to allow adherence before the drugs were added. The plates then were incubated for 40 h, fixed with 95% ethanol, rinsed with HBS, and stained as described above. The experiments were conducted two or more times in all cell lines and yielded similar results.

The population doubling time (PDT) for cells growing in the presence of each drug combination was subsequently calculated from the data by the equation $PDT = 0.301\ (t)/(\log OD_0) - (\log OD_t)$, where t is equal to the elapsed time (in hours) between fixing the initial plate at time 0 and drug-containing plates at time t, OD_0 is equal to the OD values in each well of the first plate fixed at time 0, and OD_t is equal to the average OD in triplicate wells after growing for t hours in the presence of the drug.

Plating efficiency. Logarithmic-phase KB cells from suspension cultures were diluted with MEM(E) supplemented with 10% fetal bovine serum, and 500 to 650 cells were seeded in plastic tissue culture dishes (150 by 25 mm; Falcon, Oxnard, Calif.). The drugs, both alone and in combination, were immediately added from stock solutions which were prepared in the same medium. The plates were incubated for 12 to 14 days at 37°C in a humidified 3% CO_2–97% air atmosphere. The medium was then aspirated; and the plates were rinsed with HBS, fixed in situ with absolute methanol for 1 to 2 min, and stained with 0.1% crystal violet in 20% (vol/vol) methanol. Clones of ≥1 mm in diameter were scored and enumerated (24).

Synergy analysis. To characterize the drug-drug interactions that were present, a new 3-D method was used (22). Briefly, theoretical additive interactions were calculated from the dose-response curves of the individual drugs. This calculated additive surface, which represented predicted cytotoxicity, was then subtracted from the experimental surface to reveal regions of greater than expected cytotoxicity (synergy). The resulting surface would appear as a horizontal plane at 0% inhibition above the calculated additive surface if the interactions were merely additive. Any peaks above this plane would be indicative of synergy. Similarly, any depression in the plane would indicate antagonism.

The 95% confidence intervals around the experimental dose-response surface were used to evaluate the data statistically. If the lower 95% confidence limit of the experimental data was still greater than the calculated additive surface, the synergy was considered to be significant. Conversely, if the upper 95% confidence limit of the experimental data was still less than the calculated additive surface, the antagonism was considered to be significant.

We used a Macintosh computer (Apple Computer, Inc., Cupertino, Calif.) with the spreadsheet Microsoft Excel (Microsoft Corp., Redmond, Wash.) to perform the required calculations and the graphics program Deltasoft (Deltapoint Inc., Monterey, Calif.) to plot the resulting data. We also used the IBM PC-compatible statistics and graphics program Statgraphics (STSC, Inc., Rockville, Md.) to perform the calculations and to graph the results. The Microsoft Excel spreadsheet was programed to perform all the calculations automatically. This spreadsheet calculates the theoretical additive interactions and locates and quantitates synergistic or antagonistic interactions that are significant at the 95 and 99% confidence levels.

To evaluate this new method of analysis, isobolograms were plotted from the raw data and compared with our results (9). The commercially available combination analysis program by J. Chou and T.-C. Chou (Biosoft, Cambridge, United Kingdom) also was used to analyze the data, and the results were compared with the results from the 3-D analysis (5–7).

RESULTS

To adequately evaluate drug-drug interactions, it is essential to thoroughly explore the biological effects of many combinations over a wide range of concentrations. To facilitate the analysis of the interactions between AZT and DHPG, we developed a rapid new cell proliferation assay. This technique allowed us to determine in triplicate the cytotoxicity of every drug combination in a 6-by-10 dilution matrix. The data obtained in this manner were analyzed by a new 3-D analytical method which not only characterized the drug interactions but also identified the drug concentrations at which these effects were observed. The regions where these effects were observed were subsequently targeted by a plating efficiency assay, which is a more sensitive indicator of toxicity. This two-step approach allowed us to examine rigorously and efficiently the synergistic cytotoxicity between AZT and DHPG.

Cell proliferation assay. The new dye-binding assay for quantitating cells in 96-well plates yielded a linear relationship between the number of KB cells and OD (data not shown). The relationship was linear between 40 and 40,000 cells per well and was efficient enough to perform a large number of assays. Similar results were obtained in both primary HFFs and WI-38 cells (data not shown). The growth rates derived from these data are considered to be sensitive indicators of cytotoxicity.

The 3-D plot (Fig. 1) represents cell growth in the 6-by-10 dilution matrix and is expressed as the PDT of cells grown in concentrations of AZT and DHPG represented by the x and y axes, respectively. Figure 1 reveals that whereas 900 μM AZT and 300 μM DHPG increase the PDT of KB cells by 3- and 1.4-fold, respectively, the combination increases the PDT by a factor of 20.

Synergy analysis. An isobologram of the proliferation data was constructed (Fig. 2) (9, 20). This conventional plot indicated that synergistic cytotoxicity was present. The shift of the isobologram to the left of the predicted cytotoxicity, which is represented by the dashed line, is indicative of synergy. The limited amount of information obtainable by the isobologram method, however, led us to use a more powerful method of analysis.

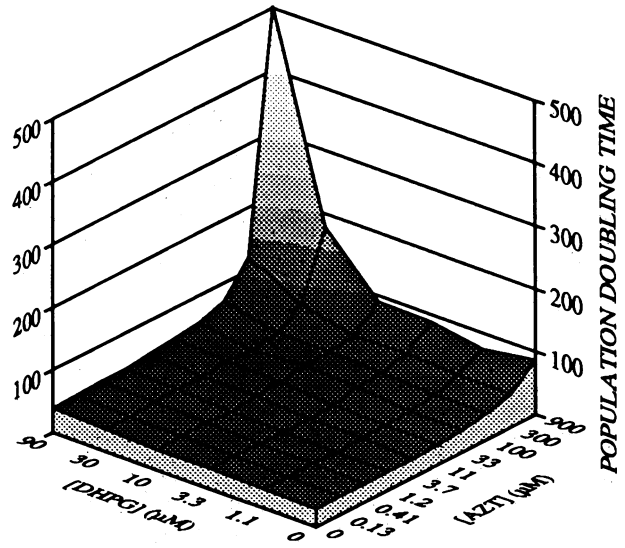


FIG. 1. PDTs of KB cells in the presence of increasing concentrations of DHPG and AZT.

When the data were rigorously analyzed by the 3-D model, the synergistic cytotoxicity of AZT and DHPG was confirmed. The peak represents greater than expected cytotoxicity and is located at the drug concentrations at which this effect was seen (Fig. 3A). A contour plot of the 3-D synergy plot (Fig. 3B) is also shown to facilitate the identification of concentrations at which the synergistic cytotoxicity occurred. The maximal synergistic cytotoxicity was seen at concentrations of 2 to 30 μM AZT and 5 to 20 μM DHPG (Fig. 3B). These synergistic interactions occurred at biologically relevant concentrations of ≤30 μM DHPG and ≤5.5 μM AZT (calculated from the Burroughs Wellcome Co. package insert [3a] and the Syntex Laboratories, Inc. [Palo Alto, Calif.], package insert [27a]). Although this effect was less dramatic than that at higher drug concentrations, the potentiation of cytotoxicity was nonetheless statistically significant, as determined by the 95% confidence intervals around the experimental surface.

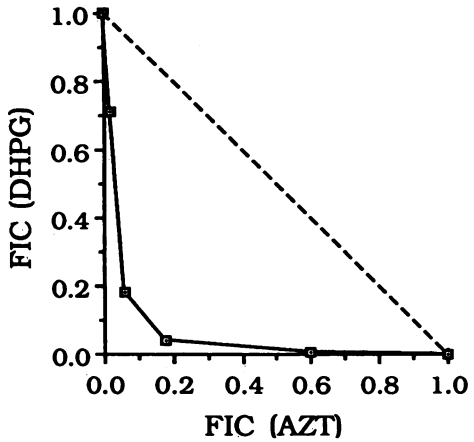
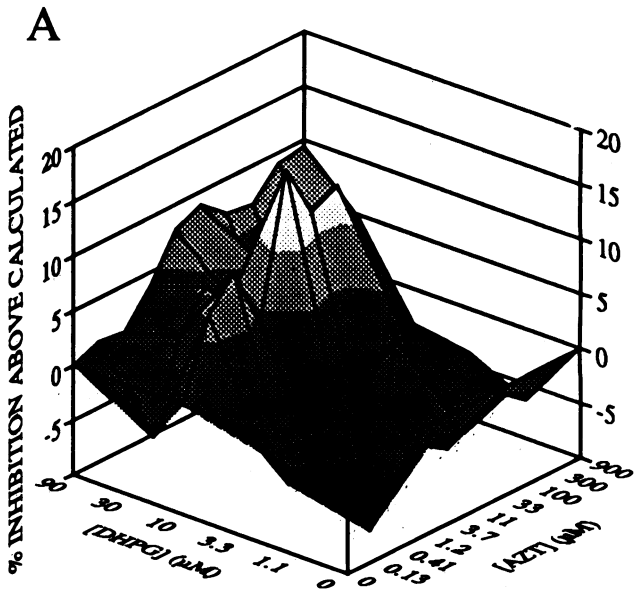


FIG. 2. Isobologram of the synergistic cytotoxicity between AZT and DHPG in KB cells. The fractional inhibitory concentrations (FICs) for both AZT and DHPG represent the concentrations required to inhibit cell growth by 50%.



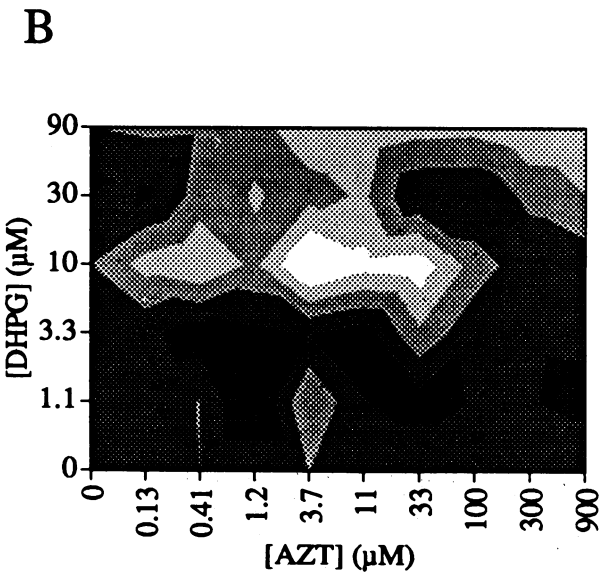


FIG. 3. Synergistic cytotoxicity of DHPG and AZT determined by KB cell growth. Theoretical additive cytotoxic effects were subtracted from the observed effects to reveal synergistic interactions and are expressed as percent inhibition on the z axis (A). A contour plot of the 3-D figure is also shown (B). The region shown in white contains significant synergistic cytotoxicity and occurs at physiologically relevant concentrations of ≤30 μM DHPG and ≤5.5 μM AZT.

The data were also analyzed by the combination index method of Chou and Talalay (5–7) on the basis of the mutually nonexclusive assumption. The results obtained with three drug ratios, which is the maximum number allowed by the program, are shown in Fig. 4. The constant molar ratio requirement imposed by the program restricts analysis to diagonal lines across the dose-response surface. The AZT:DHPG molar ratio of 0.013, which resulted in a diagonal line that was to the left of the region of synergy shown in Fig. 3B, shows some synergistic cytotoxicity at

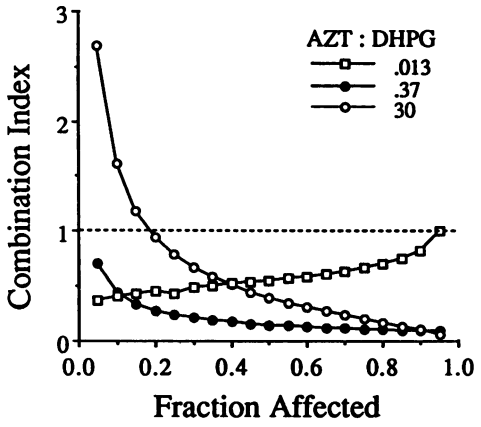


FIG. 4. Combination index analysis of the combined cytotoxicity between DHPG and AZT. A combination index value of <1 would be indicative of synergy, while a value of >1 would be indicative of antagonism. Fixed molar ratios are indicated.

low effect levels (fraction affected) and gradually becomes additive at higher values. The molar ratio of 0.37 resulted in a diagonal which intersected the region of intense synergy we identified in Fig. 3B and displayed potent synergy at all effect levels. Molar ratios of 30 and above resulted in diagonals which were to the right of the region of synergy and indicate marked antagonism. The antagonism identified by a molar ratio of 90 was so intense that it could not be plotted with the data in Fig. 4 and is not shown. The apparent antagonism identified by the Chou and Talalay method (5–7) can be seen as a depression in the lower right corner of Fig. 3B. The combination index method was unable to analyze the data statistically, which makes interpretation of the results questionable. The 3-D analysis revealed that this antagonism was statistically insignificant.

These results demonstrate that the combination index method currently used by many investigators can be misleading and must be plotted at many different molar ratios in an attempt to reveal the full extent of the interactions involved. Even when multiple molar ratios were plotted, however, it was difficult to obtain any coherent relationship among them (Fig. 4). Results from analyzing the data obtained by the combination index method are often ambiguous and should be reported with caution (2, 12).

The experiments were repeated in primary HFFs and WI-38 cells, a human embryonic diploid cell line. Combinations of DHPG and AZT were shown again to interact in a synergistic manner, producing more than expected cytotoxicity (data not shown).

Plating efficiency. To analyze the areas of interest identified by the proliferation assay more thoroughly, a plating efficiency assay was performed. The experiment, designed in a 4-by-4 matrix, focused on concentrations that were seen as synergistically cytotoxic by the cell proliferation assay. This more sensitive assay revealed synergy at the same concentrations identified by the cell proliferation assay (Fig. 5A). The combination of AZT and DHPG produced >40% more inhibition than that which was predicted, and maximal effects were seen at concentrations of 2 to 20 μM AZT and 10 to 50 μM DHPG. Again, these effects were seen at physiologically meaningful concentrations (Fig. 5B).

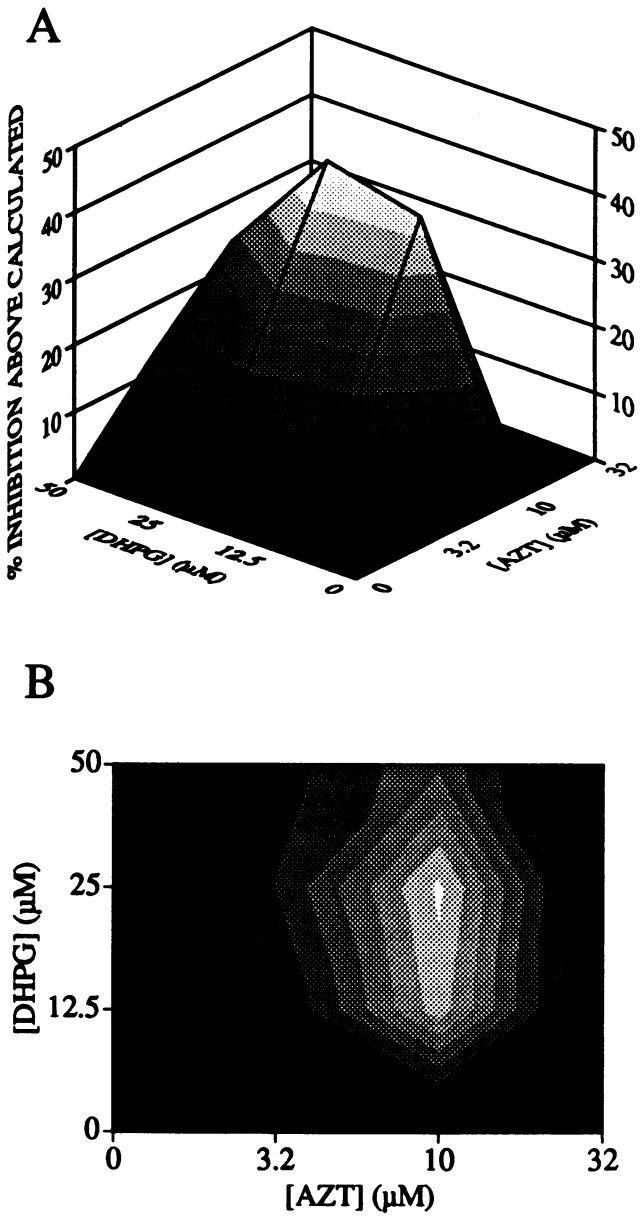


FIG. 5. Synergistic cytotoxicity of DHPG and AZT determined by KB cell plating efficiency. Theoretical additive cytotoxic effects were subtracted from the observed inhibition of colony formation to reveal synergistic interactions and are expressed as percent inhibition on the z axis (A). A contour plot of the 3-D figure is also shown (B).

DISCUSSION

Both AZT and DHPG are efficacious antiviral drugs and are useful in treating human immunodeficiency virus and cytomegalovirus infections, respectively. Both drugs, however, have significant bone marrow toxicity when administered independently and, theoretically, should not be administered concurrently. Unfortunately, large numbers of patients with AIDS being treated with AZT develop cytomegalovirus retinitis. The potential toxicity of the drug interactions between AZT and DHPG must be balanced with the potential therapeutic benefits (14, 16, 21).

To evaluate the interactions between AZT and DHPG, we

used a new analytical method for evaluating these drug-drug interactions. This 3-D concept, unlike other currently used analytical methods, allowed us both to evaluate the interactions and to determine the concentrations of drugs at which the effects were observed (22). A rapid assay was initially used to identify regions of synergy. Once these concentrations were identified, they subsequently were examined by a more sensitive assay to characterize the interactions. This approach revealed that AZT and DHPG are synergistically cytotoxic in vitro. Human hematopoietic progenitor cells could be used for experiments of this type. The cell lines used in these experiments are typically less susceptible to drug toxicity than are hematopoietic progenitor cells. Consequently, the drug toxicity measured in this manner should be considered to be an underestimate of potential bone marrow toxicity.

The recently developed 3-D analytical method used to analyze the data is more accurate than the two-dimensional combination index method of Chou and Talalay (5–7) in three key respects. First, it does not require an experimental design of fixed ratios of the two drugs. Because fixed drug ratios examine effects along diagonal lines across the dose-response surface, data gathered in this way could reveal synergy, antagonism, or additivity, depending on the drug ratios chosen by the investigator (Fig. 4). Second, it is easy to interpret the data obtained from an analysis. The synergy plot immediately reveals the quantity of both synergistic and antagonistic interactions and identifies the drug concentrations at which they are observed (Fig. 3). Finally, this new method is able to evaluate statistically the results, unlike the isobologram or the combination index method. Using the 3-D method, we were able to demonstrate that the combination of AZT and DHPG is synergistically cytotoxic in vitro and that this synergy is statistically significant at physiologically relevant concentrations.

There are at least two plausible explanations for the observed synergistic cytotoxicity. One explanation is that the DNA repair enzyme, DNA polymerase β, is inhibited by AZT triphosphate (AZT-TP) (28). When DHPG, a chain terminator (4), is used alone, the modest amount of DNA damage that occurs may be partially corrected by the repair enzyme. The inhibition of DNA polymerase β by AZT, however, may compromise the ability of the cell to tolerate DHPG. One other report in the literature asserted that AZT-TP has no affect on DNA polymerase β, but the concentrations of AZT-TP used were insufficient to observe the effect seen by White and coworkers (28). The inhibition of DNA polymerase β by AZT-TP has been confirmed recently (21a).

Deoxynucleotide triphosphate pool perturbations also could account for the synergistic affect. AZT has been shown to markedly decrease dGTP pools in several cell lines (11). As DHPG triphosphate competes with dGTP for cellular polymerases, the increased ratio of DHPG triphosphate: dGTP could increase the probability of cellular DNA chain termination (3, 16, 27).

We conclude that combinations of AZT and DHPG interact adversely in vitro and that the interactions occur at physiologically relevant concentrations. It is not known whether these drug-drug interactions manifest themselves in vivo as granulocytopenia, neutropenia, anemia, or other untoward side effects. However, these results indicate that further investigation is warranted and that the coadministration of these drugs may be contraindicated.

ACKNOWLEDGMENTS

We thank Sandra Lehrman of the Burroughs Wellcome Co. and Thomas Matthews of Syntex Laboratories for their generous gifts of AZT and DHPG, respectively. In addition, we owe special thanks to William M. Shannon for providing unpublished data.

This work was supported by Public Health Service grants DE-08510 from the National Institute of Dental Research and AI-25739 from the National Institute of Allergy and Infectious Diseases and the National Cancer Institute.

REFERENCES

1. **Barer, M. R., H. Lyon, and B. S. Draser.** 1985. Quantitation of dye binding by cell monolayers in a microtiter system. Histochem. J. **18**:122–128.
2. **Berman, E., R. Osterndorf-Duigu, S. E. Krown, M. P. Fanucchi, J. Chou, M. S. Hirsch, B. D. Clarkson, and T.-C. Chou.** 1989. Synergistic cytotoxic effect of azidothymidine and recombinant interferon alpha on normal human bone marrow progenitor cells. Blood **74**:1281–1286.
3. **Buhles, W. C., Jr., B. J. Mastre, A. J. Tinker, V. Strand, and S. H. Koretz.** 1988. Ganciclovir treatment of life- or sight-threatening cytomegalovirus infection: experience in 314 immunocompromised patients. Rev. Infect. Dis. **10**(Suppl. 3):S495–S506.

3a. **Burroughs Wellcome Co.** 1990. Retrovir package insert. Burroughs Wellcome Co., Research Triangle Park, N.C.

4. **Cheng, Y.-C., S. P. Grill, G. E. Dutschman, K. B. Frank, J.-F. Chiou, K. F. Bastow, and K. Nakayama.** 1984. Effects of 9-(1,3-dihydroxy-2-propoxymethyl)guanine, a new antiherpesvirus compound, on synthesis of macromolecules in herpes simplex virus-infected cells. Antimicrob. Agents Chemother. **26**:283–288.
5. **Chou, T.-C., and P. Talalay.** 1980. Generalized equations for the analysis of inhibitors of Michaelis-Menten and higher order kinetic systems with two or more mutually exclusive and nonexclusive inhibitors. Eur. J. Biochem. **249**:2928–2931.
6. **Chou, T.-C., and P. Talalay.** 1983. Analysis of combined drug effects: a new look at a very old problem. Trends Pharmacol. Sci. **4**:450–454.
7. **Chou, T.-C., and P. Talalay.** 1984. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv. Enzyme Regul. **22**:27–55.
8. **Collaborative DHPG Treatment Study Group.** 1986. Treatment of serious cytomegalovirus infections with 9-(1,3 dihydroxy-2-propoxymethyl)guanine in patients with AIDS and other immunodeficiencies. N. Engl. J. Med. **314**:801–805.
9. **Elion, G. B., S. Singer, and G. H. Hitchings.** 1954. Antagonists of nucleic acid derivatives. J. Biol. Chem. **208**:477–488.
10. **Fischi, M. A., D. D. Richman, M. H. Grieco, M. S. Gotlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, R. T. Schooley, G. G. Jackson, D. T. Durack, D. King, and the AZT Collaborative Working Group.** 1987. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. N. Engl. J. Med. **317**:185–191.
11. **Frick, L. W., D. J. Nelson, M. W. St. Claire, P. A. Furman, and T. A. Krenitsky.** 1988. Effects of 3′-azidothymidine on the deoxynucleotide triphosphate pools of cultured human cells. Biochem. Biophys. Res. Commun. **154**:124–129.
12. **Hayashi, S., R. L. Fine, T.-C. Chou, M. J. Currens, S. Broder, and H. Mitsuya.** 1990. In vitro inhibition of the infectivity and replication of human immunodeficiency virus type 1 by combination of antiretroviral 2′,3′-dideoxynucleosides and virus-binding inhibitors. Antimicrob. Agents Chemother. **34**:82–88.
13. **Henderly, D. E., W. R. Freeman, D. M. Causey, and N. A. Rao.** 1987. Cytomegalovirus retinitis and response to therapy with ganciclovir. Ophthamology **94**:425–434.
14. **Hochster, H., D. Dieterich, S. Bozzette, R. C. Reichman, J. D. Connor, L. Liebes, R. L. Sonke, S. A. Spector, F. Valentine, C. Pettinelli, and D. R. Richman.** 1990. Toxicity of combined ganciclovir and zidovudine for cytomegalovirus disease associated with AIDS. Ann. Intern. Med. **113**:111–117.
15. **Jabs, D. A., C. Enger, and J. G. Bartlett.** 1989. Cytomegalovirus retinitis and acquired immunodeficiency syndrome. Arch. Oph-

thalmol. **107**:75–80.

16. **Jacobson, M. A., P. De Miranda, S. M. Gordon, M. R. Blum, P. Volberding, and J. Mills.** 1988. Prolonged pancytopenia due to combined ganciclovir and zidovudine therapy. J. Infect. Dis. **158**:489–490.
17. **Jacobson, M. A., J. J. O'Donnell, H. R. Brodie, C. Wofsy, and J. Mills.** 1988. Randomized prospective trial of ganciclovir maintenance therapy for cytomegalovirus retinitis. J. Med. Virol. **25**:239–249.
18. **Jacobson, M. A., J. J. O'Donnell, D. Porteus, H. R. Brodie, D. Fiegal, and J. Mills.** 1988. Retinal and gastrointestinal disease due to cytomegalovirus in patients with acquired immunodeficiency syndrome. Q. J. Med. **254**:473–486.
19. **Kueng, W., E. Silber, and U. Eppenberger.** 1989. Quantification of cells cultured on 96-well plates. Anal. Biochem. **182**:16–19.
20. **Loewe, S.** 1953. The problem of synergism and antagonism of combined drugs. Arzneimittelforschung **3**:285–320.
21. **Millar, A. B., R. F. Miller, G. Patou, A. Mindel, R. Marsh, and S. J. Semple.** 1990. Treatment of cytomegalovirus retinitis with zidovudine and ganciclovir in patients with AIDS: outcome and toxicity. Genitourin. Med. **66**:156–158.

21a. **Parker, W. B., E. L. White, S. C. Shaddix, L. J. Ross, R. W. Buckheit, Jr., J. M. Germany, J. A. Secrist III, R. Vince, and W. M. Shannon.** J. Biol. Chem., in press.

22. **Prichard, M. N., and C. Shipman, Jr.** 1990. A three-dimensional model to analyze drug-drug interactions. Antiviral Res. **14**:181–206.
23. **Richman, D. D., M. A. Fischi, M. H. Grieco, M. S. Gotlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, M. S. Hirsch, G. G. Jackson, D. T. Durack, S. Nusinoff-Lehrman, and the AZT Collaborative Working Group.** 1987. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. N. Engl. J. Med. **317**:192–197.
24. **Schwartz, P. M., C. Shipman, Jr., R. H. Carlson, and J. C. Drach.** 1974. Thermal inactivation as a means of inhibiting the serum-associated deamination of 9-β-D-arabinofuranosyladenine in tissue culture media. Antimicrob. Agents Chemother. **5**:337–343.
25. **Shipman, C., Jr.** 1969. Evaluation of 4-(2-hydroxyethyl)-1-piperazineëthanesulfonic acid (HEPES) as a tissue culture buffer. Proc. Soc. Exp. Biol. **130**:305–310.
26. **Shipman, C., Jr., S. H. Smith, R. H. Carlson, and J. C. Drach.** 1976. Antiviral activity of arabinosyladenosine and arabinosylhypoxanthine in herpes simplex virus-infected KB cells: selective inhibition of viral deoxyribonucleic acid synthesis in synchronized suspension cultures. Antimicrob. Agents Chemother. **9**:120–127.
27. **St. Claire, M. H., W. H. Miller, R. L. Miller, C. U. Lambe, and P. A. Furman.** 1984. Inhibition of cellular alpha DNA polymerase and herpes simplex virus-induced DNA polymerase by the triphosphate of BW759U. Antimicrob. Agents Chemother. **25**: 191–194.

27a. **Syntex Laboratories, Inc.** 1990. Cytovene package insert. Syntex Laboratories, Inc., Palo Alto, Calif.

28. **White, E. C., W. B. Parker, L. J. Macy, S. C. Shaddix, G. McCaleb, J. A. Secrist III, R. Vince, and W. M. Shannon.** 1989. Comparison of the effect of carbovir, AZT, and dideoxynucleoside triphosphates on the activity of human immunodeficiency virus reverse transcriptase and selected human polymerases. Biochem. Biophys. Res. Commun. **161**:393–398.
29. **Yarchoan, R., R. W. Klecker, K. J. Weinhold, P. D. Markham, H. K. Lyerly, D. T. Durack, E. Gelmann, S. N. Lehrman, R. M. Blum, D. W. Barry, G. M. Shearer, M. A. Fischl, H. Mitsuya, R. C. Gallo, J. M. Collins, D. P. Bolognesi, C. E. Myers, and S. Broder.** 1986. Administration of 3'-azido-3'-deoxythymidine, an inhibitor of HTLV-III/LAV replication, to patients with AIDS or AIDS-related complex. Lancet **i**:575–580.