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Modeling Combinations of Antiretroviral Agents In Vitro with Integration of Pharmacokinetics: Guidance in Regimen Choice for Clinical Trial Evaluation

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We propose a method for the selection of doses and dosing schedule for drugs to be used in combination. This approach uses the simulation of steady-state concentrations of the drugs in the combination and overlays these concentrations onto a three-dimensional effect surface. The MacSynergy II program is used to construct the three-dimensional drug interaction surface from the direct evaluation of drug combination effect in vitro. The study examined the combination of an inhibitor of the human immunodeficiency virus protease, A-77003, and the nucleoside analog zidovudine. Zidovudine concentrations from a steady-state interval were simulated on the basis of the administration of 100 mg every 12 h by mouth, while for A-77003 simulation profiles were for intravenous administration of 800 mg every 4 h as well as a continuous infusion of 200 mg/h. The average percentage of the maximal effect was taken as a measure of regimen effectiveness. Three different schedules of administration were examined. If both drugs were to be administered simultaneously, the model predicts a mean maximal effect of a steady-state interval (12 h) of 67%. If the drug doses were offset by 2 h, the mean maximal effect predicted was 71%. If A-77003 was to be given by continuous infusion, the mean maximal effect predicted was 90%. This method holds promise as a way of quickly evaluating potential combinations of agents that takes into account the drug interaction in a mathematically robust way and that allows the evaluation of the effect of each drug's pharmacokinetic profile.

Multiple classes of antiretroviral drugs targeting different areas of the life cycle of human immunodeficiency virus (HIV) have been produced over the last several years (10, 11, 19). In each instance, however, each class of agents has had significant limitations because of toxicity, the rapid emergence of resistance, or less than optimal antiretroviral activity (8, 15, 18).

Combination antiviral chemotherapy is attractive for a number of reasons. (i) Combinations may allow attainment of a greater effect than the effect of any single agent alone. (ii) Combinations may allow attainment of an effect with less toxicity than that from the use of single agents because lower doses of each agent can be used. (iii) Combinations may, most importantly, prevent or delay the emergence of viral resistance.

While these purported advantages of combination chemotherapy have long been recognized, the choice of dose and dosing schedule for each of the drugs in the combination is a problem which has not been approached to date in a rational and precise manner. Certainly, much innovative work has gone into quantitating and displaying the way in which multiple agents interact. Numerous investigators have published evaluations of the rational quantitation of the effects obtained with multiple agents (1, 6, 7, 13, 16). Each of these approaches is based on a different principle. For instance, Chou (7) and Be'lankii and Schinazi (1) have based their approach on the median effect principal, while other investigators have used Loewy additivity as the null reference model. Prichard et al. (16) have based their interaction program on the Bliss Independence null reference model. While each of these approaches is insightful and displays the interaction of agents in a regimen in a mathematically robust fashion, none of these approaches, by itself, is useful in guiding combination trial design, because none of these approaches incorporates the pharmacokinetic profile of the regimen into the evaluation.

Regimen design is particularly important for phase I/II trials of combination antiretroviral chemotherapy. As can be seen by examining early trials of combination therapy with zidovudine plus interferon (14, 20), it can be concluded that matrix designs are rational but require very large numbers of subjects for evaluation of all therapeutic regimens. For example, three dose levels of one drug to be evaluated and three dose levels of a second drug give nine combination regimens per evaluation and two to six single-agent regimens. Even large, multicentered phase I/II trials would likely be inadequate for the evaluation of a regimen. As an example, the 11 single-agent regimens described above would require a study with in excess of 200 patients if the size of each cohort was a modest number of 20. The intensive nature of phase I/II evaluations means that extensive resources would need to be expended to make a rational choice of dose and dosing schedule possible for later evaluation in a phase III trial.

In order to aid the regimen design process for phase I/II trials, we propose a rational method for the evaluation of combination regimens to limit the number of regimens requiring study. This method explicitly incorporates the pharmacokinetics of each agent into the evaluation and can easily accommodate the evaluation of between-patient variance in pharmacokinetics through the use of Monte Carlo simulation.

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MATERIALS AND METHODS

Agents. The reverse transcriptase inhibitor zidovudine was kindly supplied by Glaxo-Wellcome. A-77003 and A-80987, inhibitors of HIV protease, were kindly supplied by Abbott Laboratories Abbott Park, IL). ^{14}C-A-80987 (36.7 Ci/mmol;

purity, >97%) was prepared by the Radiochemistry Group, Pharmaceutical Products Division, Abbott Laboratories.

Cells and viruses. The MT-2 cell line and HIV type 1 (HIV-1) strain HIV-1_{IIIB} were obtained from the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

HIV cytopathic effect assay. MT-2 cells were infected with HIV-1_{IIIB} in RPMI 1640 medium (Paragon Biotech, Baltimore, Md.) with 4 μg of polybrene per ml. After 1 h, the cells were centrifuged and suspended in RPMI 1640 medium with 10% fetal bovine serum (FBS). Cell suspensions of 100 μl were added to 100 μl of medium with and without twofold the indicated concentrations of drugs in 96-well plates. Cell viability was correlated to the formation of formazan in an MTT (methyltetrazole) assay as described previously (5). The mean ± standard deviation of five replicates was used for the calculation of viral inhibition.

Efflux assay. Efflux kinetics were measured by an oil-stop method (4). MT-2 cells were infected with HIV-1_{IIIB} at a multiplicity of infection of 1. Cells were incubated for 1 h at 37°C and were then centrifuged and suspended at 2×10^6 cells per ml in medium containing 10% FBS. After 10 min at 37°C, 0.1 ml of 100 μM ^{14}C-A-80987 was added per 0.9 ml of suspension, and incubation was continued for 1 h. The cells were centrifuged at 1,000 × *g* and were suspended in medium without label to measure the efflux of labeled drug from cells at 0, 15, 30, and 60 min. At the indicated time points, 500-μl aliquots were pipetted into 1.7-ml Eppendorf tubes containing 500 μl of oil mixture (1 part corn oil [Best Foods, Englewood Cliffs, N.J.]:5 parts dibutyl phthalate [Sigma, St. Louis, Mo.]). Samples were centrifuged at 9,500 rpm in an Eppendorf microcentrifuge (Westbury, N.Y.) for 5 min. The tips of the tubes containing the cell pellet were removed with a safety device and were transferred to a vial. Cell pellets were solubilized with 1% sodium dodecyl sulfate (SDS); this was followed by the addition of Ecoscint-H scintillation fluid (National Diagnostics, Manville, N.J.). The radioactivity in each sample was measured by liquid scintillation spectrometry.

Drug interaction modeling. The method of Prichard et al. (16) was used to evaluate the interaction of A-77003 and zidovudine. The program MacSynergy II was used for this purpose (16). Synergy versus additivity versus antagonism was evaluated by estimating the 95% confidence bound about the interaction surface. The theoretical additive surface was constructed from data for the wells containing zidovudine alone and A-77003 alone. If the 95% confidence bound around the interaction surface did not touch the theoretical additive and was above the additive surface, synergy was considered to be present in that area. If the interaction surface and its 95% confidence bound were below the additive surface, antagonism was considered to be present. Otherwise, the interaction was considered additive.

The measure of effect was plotted on the *z* axis as a percentage of the maximal effects seen.

Closed-loop vector plots. Closed-loop vector plots were constructed as overlays to the effect surface. For their construction, the plasma concentration-time profiles of both zidovudine and A-77003 were simulated for a 12-h period. For simulation purposes, the dosage of zidovudine was chosen to be 100 mg administered orally every 12 h, which is somewhat below the maximal effect dosage for this drug and which was chosen for illustrative purposes only. For A-77003, the dosage was chosen to be 800 mg given intravenously as a 1-h infusion every 4 h (4,800 mg/day) or the equivalent amount per hour by continuous intravenous infusion.

The values of the pharmacokinetic parameters for the simulations were drawn from the report of Gitterman et al. (12) for zidovudine. For A-77003, the values of the pharmacokinetic parameters were taken from the report of Reedjik et al. (17). Simulations were accomplished with the ADAPT II package of programs of D'Argenio and Schumitzky (9).

RESULTS

Simulations were constructed for hourly intervals through 12 h of a steady-state dosing interval. Because of the schedules involved, the simulation for this time period would then repeat itself for the next 12-h interval. Hence, only data from this time period were necessary for the evaluation.

The following three regimens were simulated at steady state: regimen A, zidovudine administered at hours 0 and 12 of the steady-state dosing interval, with A-77003 administered at hours 0, 4, and 8; regimen B, zidovudine administered at hours 0 and 12 of the steady-state dosing interval with A-77003 administered at hours 2, 6, and 10; and regimen C, zidovudine administered at hours 0 and 12 of the steady-state dosing interval, with A-77003 given as a continuous intravenous infusion.

The actual concentration-time curves produced by the simulation are displayed in Fig. 1. The pairs of concentrations of zidovudine and A-77003 for each of the three regimens described above were sited on the effect surface and were connected by vectors to indicate the flow of time during the dosing interval (hence, a closed-loop vector plot, because this was done as a repeating interval at steady state). The percent maximal inhibition was determined for each of the 12 hourly points of the plot for each of the regimens. The average of the percent maximal inhibitions at each of the 12 evenly spaced hourly points was taken as the estimator of the regimen effect. Because the points were evenly spaced, the estimator should be unbiased relative to drug pharmacokinetics.

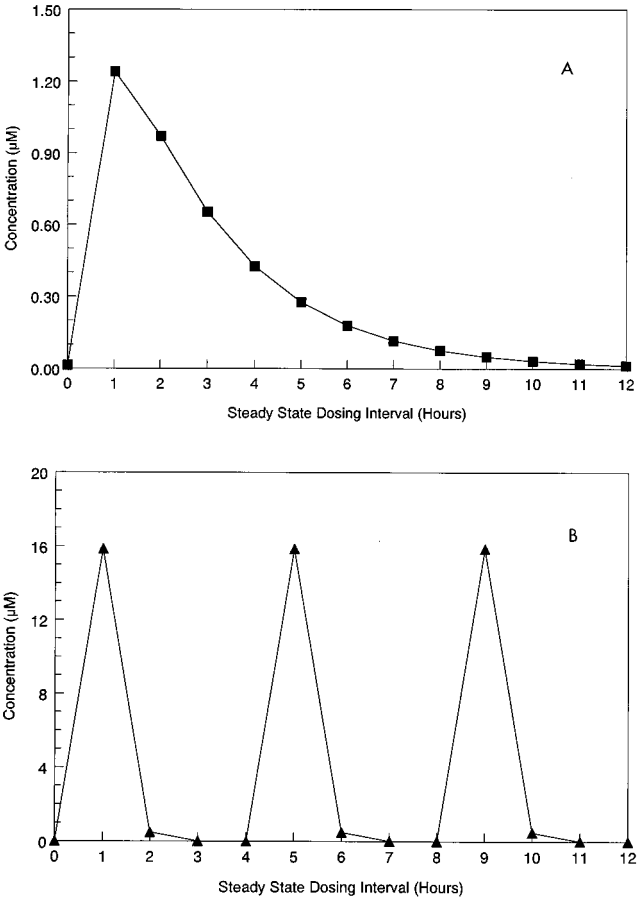

FIG. 1. Simulated steady-state plasma concentration-time profiles (12 h) for combination therapy with zidovudine and A-77003 when the drugs are administered intermittently. (A) Profile for zidovudine. (B) Profile for A-77003.

Regimen A (both drugs given together initially) produced a mean effect of 67% of the maximal effect. This can be seen in Fig. 2. It should be noted that as A-77003 is redosed, the largest maximal effect is seen (with the highest concentration of A-77003). However, these sequentially decrease with each of the three administrations modeled because the concurrent concentrations of zidovudine are lower with each redosing of A-77003.

Regimen B (drugs administered with a 2-h offset) produced a mean effect of 71% of the maximal effect. This can be seen in Fig. 3. Of interest, the closed-loop vector plot traverses a quite different portion of the effect surface because of the drug administration time offset.

Regimen C (A-77003 administered as a continuous infusion) produced a mean effect of 90% of the maximal effect. This can be seen in Fig. 4.

Fig. 2 to 4 show that the schedule on which the drugs are

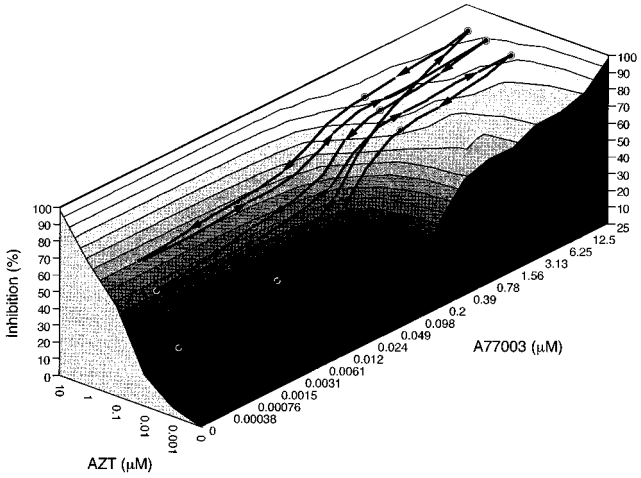

FIG. 2. Concentrations of zidovudine (100 mg given orally every 12 h) and A-77003 (800 mg given intravenously every 4 h) in plasma over steady-state dosing intervals. The simulation is for drugs administered at the same times, and the results are sited on a three-dimensional drug interaction surface for the two drugs. The *z* axis is the percent maximal antiviral effect seen for any combination of drug concentrations in the in vitro system. Consequently, any pair of concentrations will have a specific effect on the surface. The average of the effects for the 12 simulated concentrations pairs (simulated at hourly intervals) is taken as a measure of regimen effectiveness. The mean maximal effect for this regimen was 67%.

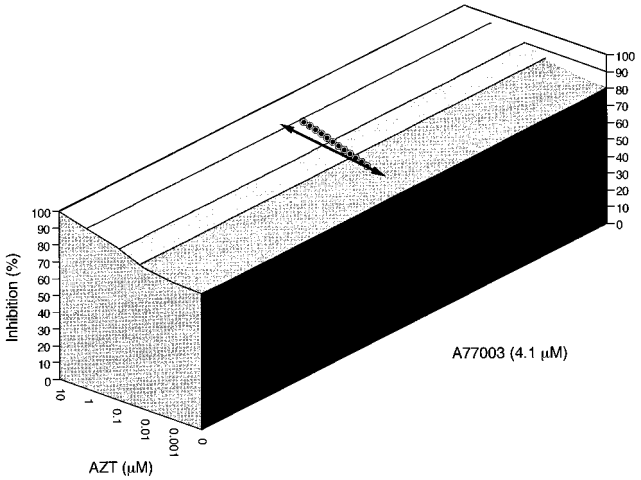

FIG. 4. A-77003 administration by continuous intravenous infusion (see Fig. 2 and 3 legends). The mean maximal effect for this regimen was 90%.

administered can profoundly alter the effectiveness of a regimen. The vector plots traverse quite different parts of the effect surfaces as a function of the administration schedule, and consequently, the measure of effectiveness of the regimens is different for each of the regimens examined.

DISCUSSION

We have described a simple method of integrating pharmacokinetics into the evaluation of regimens with combinations of antiretroviral agents. The method uses a simulation of plasma concentration-time profiles of the agents in the combination at arbitrary time points for a steady-state interval and siting them upon a robustly determined interaction surface.

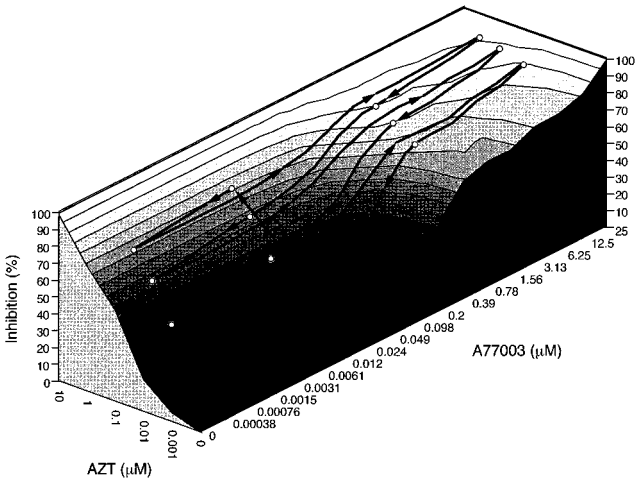

FIG. 3. Zidovudine and A-77003 administration schedules, which were offset by 2 hours (see Fig. 2 legend). The mean maximal effect for this regimen was 71%.

In the evaluation, which we have performed, we used the MacSynergy II program of Prichard et al. (16) for the analysis. The program uses the Bliss Independence null reference model. A number of other very innovative and mathematically robust programs and approaches are available for the evaluation of multidrug interactions. Those approaches use not only Bliss Independence but Loewy Additivity or the median effect principle as the null reference model. Any one of these approaches can be adapted to the method that we have used to integrate pharmacokinetics into the evaluation.

The endpoint for the evaluation of the regimen is simply the arithmetic mean of the percent maximal effects for the time points evaluated. Because the time points are simulated in the present study at equal spacings, this should be an unbiased estimator of the effectiveness of a regimen. Certainly, as the spacing between the time points of evaluation decreases, it is likely that the outcome will approach an asymptote of the true mean maximal effect. This approach allows the effects of both dose and schedule of administration to be determined in a straightforward manner. It should be noted that we have used the effect surface and not the interaction surface for our evaluation. It is straightforward to mathematically subtract the theoretical additive surface from the effect surface, leaving the interaction (synergistic or antagonistic) surface. However, while one could easily perform the same evaluation on the synergy surface, it is theoretically possible that optimally synergistic regimens may still not develop the maximal effect. Consequently, we chose to use the effect surface for regimen evaluation.

We used the mean population pharmacokinetic parameter values for the simulations displayed in Fig. 2 through 4. However, true between-patient variance in the values of important pharmacokinetic parameters exists. Consequently, in order to most appropriately assess a dose and schedule for a combination of agents, the full effect of variability in population pharmacokinetic parameter values needs to be taken into account. This can be accomplished in a straightforward manner by the method that we have set forth here. The ADAPT II package of programs has a simulation module which allows Monte Carlo simulation to be accomplished by using the population mean parameter vector and the full covariance matrix. Consequently, large numbers of concentration-time profiles for patients receiving each regimen can be evaluated on the effect surface

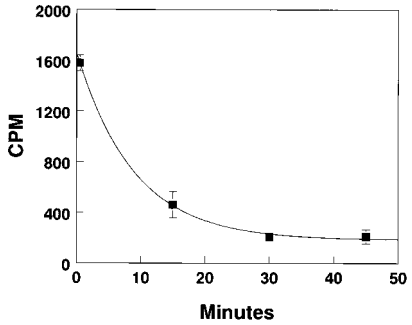

FIG. 5. Efflux of A-80987 from preloaded HIV-1-infected peripheral blood mononuclear cells. Peripheral blood mononuclear cells infected with HIV-1 were preincubated with 10 μM ^{14}C-A-80987 for 90 min. They were then centrifuged and suspended in medium without A-80987 at 2×10^6 cells per ml. At the indicated time points, duplicate 500-μl samples were layered onto an oil mixture, and the mixture was centrifuged. Radioactivity was determined by liquid scintillation spectrometry. Each point is the mean of two experiments.

and the mean percent maximal effect can be ascertained across the population in a Monte Carlo simulation. This allows robust statistical testing of differences in the mean percent maximal effect between doses and dosing schedules.

The effect of the dosing schedule for a regimen with equal doses (the three regimens evaluated above) was ascertained in the present evaluation, and the greatest average effect was shown to be present for a regimen of continuous infusion of the protease inhibitor A-77003. Because this effect was shown with a regimen with equal doses, this lends credence to the ability of this approach to determine accurately less subtle influences, such as a change in the dose.

The ability of a static evaluation, such as that performed here, to predict the outcome of a dynamic system, such as that seen with drug administration in humans, can be called into question. Indeed, an underlying assumption in the present evaluation is that there would be the same response at each dosing interval (i.e., no emergence of resistance). Some idea of the correctness of the evaluation presented here can be obtained by examining the phase I evaluation of A-77003 (17) and the time course of efflux of a closely related protease inhibitor, A-80987, from HIV-infected cells (the only radiolabelled inhibitor available to us) from HIV-infected cells. Once the drug is removed from the area outside of the cell and the gradient is maximized, the efflux of A-80987 from the cell is remarkedly rapid, with a half-time on the order of 8 min (Fig. 5). Consequently, with a drug such as A-77003, which has a very rapid half-life in the plasma of humans (on the order of 20 min), it should come as no surprise that continuous infusion or frequent administration (more frequently then every 4 h) should provide better inhibition of the virus even when A-77003 is combined with a nucleoside analog.

Our approach allows drug interaction and the pharmacokinetics of each drug to be factored into a decision on evaluations of dose and dosing schedule for phase I/II trials with combinations of antiretroviral agents. However, other issues must also be considered. While the antiviral effect is critical, the toxicities of the regimens and the possibility of suppressing the emergence of resistance must also be considered in the selection of regimens for evaluation. For instance, regimens which produce the predicted effects on the flat part of the response surface are certainly desirable from an antiviral effect point of view. However, these regimens may also produce an excess risk of toxicity, which would force patients to stop therapy more quickly. Consequently, the toxicity of a regimen needs to be taken into account in the decision-making process.

In a counterbalancing way, suppression of the emergence of resistance also needs to be taken into account. It is likely that there is a hierarchy of endpoint effects. For instance, a 2-log decrease in the RNA PCR result is an impressive accomplishment for any antiviral regimen. However, such a demonstration of antiretroviral effect may still be inadequate for preventing the emergence of viral resistance. It is likely that the doses of both drugs in a combination regimen which would produce a 2-log decrease in the RNA PCR result would have to be escalated in order to significantly prolong the time to the emergence of viral resistance. So, while our approach may be a start on a rational approach to the design of combination regimens, it should be remembered that other endpoints such as suppression of resistance may require larger doses of drugs than would be required to optimize the antiviral effect per se. Therefore, it is important that predictions from this model be tested in other settings such as our previously described in vitro hollow fiber model system (2, 3) and, ultimately, in clinical trials to validate these predictions for endpoints of interest.

Finally, we have examined combinations of antiretroviral agents in the present study. However, there is no a priori reason why this general approach cannot be applied to combinations of antibacterial agents, antifungal agents, or even anticancer agents. Indeed, any area of investigation in which drugs are administered in combination and in which there is a continuous variable output can potentially be approached in the manner set forth here.

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