Effects of Cytokines on Antiviral Pharmacokinetics: an Alternative Approach to Assessment of Drug Interactions Using Bioequivalence Guidelines

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The effects of cytokines on the pharmacokinetics of nucleoside analogs were evaluated in two separate studies using zidovudine in combination with interleukin-2 and didanosine in combination with alpha interferon. In each study, drug interactions were evaluated by using both a standard method (Student’s t test) and bioequivalence testing. Serial blood samples were collected from human immunodeficiency virus-infected patients prior to and during cytokine therapy for determination of nucleoside analog concentrations. Concentrations were fit separately to a two-compartment model by using the iterative two-stage approach to population analysis. No alterations in area under the curve or oral clearance were observed for either drug during combination therapy. In general, there was good agreement between statistical methods for determining if antiviral pharmacokinetic parameters were altered by concomitant cytokine therapy. However, large individual changes in the maximum concentration of zidovudine in serum were detected by bioequivalence testing but no difference was found by Student’s t test. For didanosine, significant but clinically irrelevant decreases determined by standard hypothesis testing were seen for both the volume of the central compartment (1.91 to 1.86 liters) and the absorption rate constant (0.79 to 0.73 h⁻¹) in the presence of alpha interferon. No interaction was noted for these parameters by using bioequivalence guidelines. Bioequivalence testing may provide an alternative approach to assessment of drug interactions. Interleukin-2 and alpha interferon do not alter the pharmacokinetics of zidovudine and didanosine, respectively.

Nucleoside analogs are currently the only approved therapies for human immunodeficiency virus (HIV) infection and are widely used both alone and in combination. Despite short-term improvement, a survival benefit past 2 years has not been demonstrated to date (2, 5, 22). Thus, drug development for new agents against HIV has turned towards alternative approaches. One focus involves attempts to utilize cytokines for their antiviral properties or to enhance the patient’s immune system. Therapy with two cytokines, alpha interferon (IFN-α) and interleukin-2 (IL-2), is being evaluated in clinical trials since these agents demonstrate both in vitro properties and clinical results which suggest that they may provide benefit to patients infected with HIV (7, 8, 11, 16, 18, 19).

In vitro studies have shown that IFN-α possesses activity against HIV and that it is synergistic with other antiretroviral agents (8). Clinical trials have also demonstrated anti-HIV activity (13). Currently, patients often receive IFN-α combined with nucleoside analogs such as didanosine for treatment of Kaposi’s sarcoma or primary HIV infection (7, 12).

Preliminary clinical studies with IL-2 have shown a potential sustained beneficial effect on CD4 counts (11). However, activation of T cells by IL-2 may also accelerate HIV replication. A transient increase in viral burden immediately following IL-2 administration has been observed, with markers of viral activity subsequently returning to baseline levels, usually within 1 week (11). Consequently, patients receiving IL-2 also receive concomitant antiretroviral therapy, commonly zidovudine, in an effort to counter this apparent increase in viral activity.

A primary limitation to cytokine therapy is an extensive adverse effect profile potentially affecting multiple organ systems. Several side effects of IL-2 and IFN-α therapy, such as nausea, vomiting, and diarrhea, could impair the absorption of zidovudine or didanosine. Therapy with these agents also may result in hepatic and renal impairment, which may alter metabolism and elimination of antiviral drugs, or cause a capillary leak syndrome which may alter their volume of distribution (7, 12, 24). In addition, release of other cytokines during therapy, such as tumor necrosis factor or IL-6, could inhibit hepatic metabolism of nucleoside analogs (1). On the basis of this potential for interaction, we studied the effects of these cytokines on antiviral pharmacokinetics in two separate trials. The first study examined the effects of prolonged IL-2 infusions on the pharmacokinetics of zidovudine, and the second examined the effects of subcutaneous IFN-α on the pharmacokinetics of didanosine.

Traditional methods to evaluate drug interactions examine if there is a statistically significant difference in pharmacokinetic parameters (i.e., area under the concentration-time curve [AUC]) for a drug given alone and the drug given in combination with another. However, this may not be the most appropriate method of analysis for these types of studies. A result of “no difference” does not necessarily imply that there is no interaction, since small sample sizes or large variability may lead to this finding. While hypothesis testing is appropriate to demonstrate differences, it does not prove that the pharmacokinetics of a drug in combination is equivalent to that when given alone. This is crucial, since the primary question of a drug interaction study is whether a second drug alters the

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pharmacokinetics of the drug in question out of its normally accepted range. To accurately demonstrate that there is no interaction, the pharmacokinetics of a drug given alone and in combination must be shown to be equivalent.

Guidelines for testing bioequivalence have been adopted by the U.S. Food and Drug Administration (FDA) for the approval of generic drugs (6). Bioequivalence testing evaluates if the pharmacokinetic parameters of a generic (test) product fall within ±20% of those of the reference drug. Steinijans et al. (21) have previously suggested that bioequivalence guidelines could also be used to evaluate drug interactions in which the drug in combination is the “test” and the drug administered alone is the “reference”. This method may be a useful alternative or adjunctive statistical procedure for drug interaction studies, since it overcomes some of the limitations of hypothesis testing. The purpose of this study was twofold: (i) to determine if there is a drug interaction between zidovudine and IL-2 or between didanosine and IFN-α and (ii) to compare bioequivalence testing with the results of standard hypothesis testing.

MATERIALS AND METHODS

Study design. (i) IL-2 and zidovudine. Patients included in the IL-2 and zidovudine trial participated in a phase I-II study examining the safety of escalating doses of IL-2 combined with zidovudine (11). Patients with HIV infection were eligible for the study if they had a CD4 count of >200 cells per mm³ and no ongoing opportunistic infection. All patients received zidovudine (Retrovir; Burroughs Wellcome), 200 mg every 4 h, for at least 6 weeks prior to receiving IL-2. The patients were hospitalized and received recombinant IL-2 (Proleukin; Chiron Corporation) for 21 days by continuous infusion via a central line. Immediately prior to beginning IL-2 therapy and again after approximately 14 days of IL-2 therapy, serial blood samples for determination of zidovudine levels were drawn at 0 h (predadministration) and 0.25, 0.5, 0.75, 1, 2, 3, and 4 h after administration of a dose of zidovudine. Zidovudine was administered on an empty stomach prior to pharmacokinetic studies. IL-2 was administered at doses ranging from 1.8 × 10⁶ to 1.0 × 10⁷ IU/day, with an individual patient receiving the same dose for the entire 21 days unless toxicities necessitated dosage reduction or discontinuation of IL-2 therapy.

(ii) IFN-α and didanosine. Patients included in the IFN-α and didanosine trial participated in a phase I-II study examining the safety and efficacy of escalating doses of IFN-α combined with didanosine (11). Patients with HIV infection were eligible for the study if they had a CD4 count of >200 cells per mm³ and no ongoing opportunistic infection. All patients received didanosine (Videx; Bristol-Myers Squibb) in the fasting state at a dose of either 100, 250, or 375 mg (sachet formulation) twice daily for at least 6 weeks prior to receiving IFN-α. The patients were given IFN-α (Intron A; Schering-Plough) by daily subcutaneous injection, and the infusion was administered for 28 weeks. Immediately prior to beginning IFN-α and again during IFN-α therapy, serial blood samples for determination of didanosine levels were drawn at 0 h (predadministration) and 0.5, 1, 2, 4, 6, and 8 h after administration of a dose of didanosine. IFN-α was administered at doses ranging from 1 × 10⁶ to 1.5 × 10⁶ units/day, with an individual patient receiving the same dose for the entire study period unless toxicities necessitated dosage reduction or discontinuation of IFN-α therapy. Both studies were approved by the NIAID Institutional Review Board, and patients gave written informed consent.

Analytical methods. Serum zidovudine concentrations were determined by a direct equilibrium assay which utilizes a zidovudine derivative for both tracer and standards. (ZDV-Trac radioimmunoassay; Incstar Corporation, Stillwater, Minn.). The assay was linear over the dose range of 0.3 to 6.000 ng/ml, and the sensitivity was 0.30 ng/ml. The overall intra- and interday variabilities of the assay were 10.2 and 8.4%, respectively.

Plasma samples were assayed for didanosine by a contract facility (Clinical Pharmacokinetics Laboratory, School of Pharmacy, University of Maryland at Baltimore) using a high-performance liquid chromatography method based on the method of Knupp et al. (10). In brief, samples were extracted by using SRA-1 cartridges conditioned with 1 ml each of methanol and water. After addition of the sample (250 μl) and internal standard (500 μl) mixture, the column was washed with 1 ml of phosphate buffer, and then protein was eluted with two 250-μl portions of 40% methanol-water. After evaporation under nitrogen, reconstitution was performed with 250 μl of mobile phase, which consisted of 25 ml of 0.1 M ammonium phosphate with 2.4% acetonitrile and 1.6% methanol and water to a total volume of 1 liter. The flow rate was 1.0 ml/min, and retention times were 15.2 and 17.7 min for didanosine and the internal standard (stavudine), respectively. The assay was linear over the concentration range of 20 to 11,070 ng/ml. Concentrations below 20 ng/ml were reported as not detectable. The within- and between-day percent coefficient of variation for the standard curves and quality controls was <8%.

Pharmacokinetic analysis. Pharmacokinetic analyses were performed separately for each antiviral drug. Antiviral pharmacokinetic parameters, in the presence and absence of cytokine therapy, were determined by using a mixed-effects nonlinear regression model consisting of population parameter values, a covariance matrix, and a model for the residual variance of the serum concentrations. The iterative two-stage approach to population analysis (20) was used to obtain the zidovudine and didanosine population estimates. The interactive two-stage approach was implemented by using subroutines from the Adapt II program (4). Additionally, individual pharmacokinetic parameter point estimates and asymptotic covariance matrices were also determined.

A two-compartment model was selected for both drugs on the basis of Akaike’s information criterion (25). Each of the six pharmacokinetic parameters used to describe the model (apparent volumes of the central [Vc] and peripheral [Vp] compartments, distributional clearance [CLd], oral total body clearance [CLB], absorption rate constant [ka], and lag time to absorption [Tlag]) could be independently increased or decreased by the presence of IL-2 or IFN-α. Two rate inputs were included to account for dosing in the presence or absence of cytokines.

The empiric variance model assumed that the residual standard deviations (SDs) of the observations (σ) were linearly related to the true values (Y): σ = v₁Y + v₂, in which v₁ and v₂ are the variance parameters, v₁ is the intercept, and v₂ is the slope of a regression line. The v₁ is closely related to the observation coefficient of variation. Initial estimates of the variance parameters were based on assay performance. Later in the process, the variance parameters were determined from the data.

For noncompartmental analysis, the steady-state AUC was determined by dividing the dose by the fitted CLr. The maximum concentration (Cmax) and time to maximum concentration (Tmax) of antiviral drugs were determined directly from concentration-time profiles.

Statistical analysis. The approach to bioequivalence testing, suggested by FDA guidelines (6), involves testing two one-sided hypotheses (17). To determine bioequivalence, 90% confidence intervals were constructed by using the natural log (ln)-transformed data of each ratio of means (i.e., AUC of zidovudine with IL-2/AUC of zidovudine alone). The ln-transformed data are recommended for testing since they are most robust to assumptions of normality. FDA guidelines use an interval of acceptability of ±20% for evaluating bioequivalence of pharmaceutical agents (e.g., generic versus reference). However, since zidovudine and didanosine pharmacokinetic parameters demonstrate large intrapatient variability (3, 15, 14), a wider acceptability interval of ±30% was utilized to define whether cytokines alter antiviral pharmacokinetics beyond that which is expected because of normal variation. Thus, bioequivalence, or a result of no interaction, was concluded when the probability that the true mean was within our interval of acceptability was 0.9 or higher. Although the FDA uses only AUC and Cmax in the assessment of bioequivalence, we also evaluated the fitted parameters of Vc, CLd, ka, and Tlag in our analysis.

For comparison, standard hypothesis testing comparing pharmacokinetic parameters for antiviral agent used alone and in combination with cytokines was performed, using a paired Student’s t test. A P value of <0.05 was considered statistically significant.

RESULTS

Demographics. (i) IL-2 and zidovudine. Nine patients (all male) with a mean age (± SD) of 37.4 ± 6.2 years were evaluated for zidovudine-IL-2 interactions. The mean CD4 count (± SD) prior to the study was 445 ± 159, and the mean percent CD4 (± SD) was 30 ± 12%. All patients receiving IL-2 experienced grade I and II toxicities, including fever, chills, nausea, arthralgias, or insomnia. One patient (patient 4) developed grade IV neutropenia, and IL-2 was discontinued after 17 days. Samples were collected for determination of zidovudine pharmacokinetics prior to the discontinuation of IL-2. Patients did not receive any concomitant medications known to alter zidovudine disposition.

(ii) IFN-α and didanosine. Twenty-six patients (25 male and 1 female) with a mean age (± SD) of 33.4 ± 6.9 years were evaluated for didanosine-IFN-α interactions. Eleven, nine, and six patients received didanosine regimens of 100, 250, and 375 mg twice a day, respectively. The dose of didanosine remained constant throughout the pharmacokinetic study in all patients. The daily doses of IFN-α during the combination period were 1 MIU for eight patients, 5 MIU for seven patients, 10 MIU for eight patients, and 15 MIU for three patients.
tients. At study enrollment, the mean CD4 count (± SD) was 456 ± 138 cells per mm³ and the mean percent CD4 (± SD) was 27 ± 7%. Patients did not receive any concomitant medications known to alter the pharmacokinetics of didanosine.

Pharmacokinetics. Pharmacokinetic parameters for zidovudine and didanosine are given in Tables 1 and 2, respectively. Mean concentration-time curves for zidovudine and didanosine are shown in Fig. 1 and 2, respectively. For zidovudine, mean concentration-time curves for zidovudine and didanosine are given in Tables 1 and 2, respectively.

The pharmacokinetic model fit the data with good precision and a lack of bias for both drugs. The plot of the observed versus fitted (model-predicted) concentrations was best described by the line y = 1.06x + 0.003, which did not differ from the line of identity by Student’s t test. The fitted parameter values for the residual variance model for zidovudine were 0.210 for V1 and 10 ng/ml for V2. For didanosine, the line describing observed versus fitted concentrations was y = 0.99x + 0.0006. The fitted parameter values for the residual variance model for didanosine were 0.170 for V1 and 14 ng/ml for V2.

The results of bioequivalence and standard statistical testing are shown in Tables 1 and 2. In general, the two statistical approaches yielded similar results. For zidovudine, AUC, Vmax, CLt, and Tlag were bioequivalent as determined by the two methods, and differences were not statistically significant. Tlag did not meet bioequivalence guidelines and was statistically different. Small but significant differences were seen for both Vmax and V1. Vmax decreased from 1.91 to 1.86 liters, and k1 decreased from 0.79 to 0.73 h⁻¹ in the presence of IFN-α. Despite being significantly different, these parameters met our guidelines for bioequivalence.

DISCUSSION

Our results suggest that IFN-α and IL-2 do not markedly alter the pharmacokinetics of nucleoside analogs used to treat HIV infection. The greatest difference in any zidovudine pharmacokinetic parameter was a 24% decrease in the rate of absorption when combined with IL-2. AUCmax values also did not achieve equivalence because of large variability between treatment phases. However, the biological significance of these changes is probably minimal, since the overall extent of absorption (AUC) and apparent total body clearance were equivalent in the presence of concomitant IL-2. Therefore, it is unlikely that there is a significant interaction between zidovudine and IL-2 at these doses of IL-2. Similarly for didanosine, only Tlag failed to meet bioequivalence guidelines when values with and without IFN-α were compared. Since AUC and clearance were also equivalent for didanosine, this difference in time to absorption is unlikely to have any clinical relevance.

Therefore, IFN-α does not appear to alter didanosine pharmacokinetics and can be concomitantly administered to HIV-infected patients.

This study also illustrates the contrast between demonstrating differences versus equivalence in pharmacokinetic parameters. The true intent of a drug interaction study is to demonstrate whether the pharmacokinetics of a drug when administered alone is the same as in the presence of another agent. It is important to distinguish this from demonstrating whether a significant difference exists. As shown in this study, bioequivalence testing can also be used to assess drug interactions and may overcome some common limitations of standard hypothesis testing.

Two particular situations in which hypothesis testing may not provide appropriate results are when a small difference is significant but is of no clinical relevance and when a large

### Table 1. Pharmacokinetic parameters for zidovudine alone and in combination with IL-2

<table>
<thead>
<tr>
<th>Zidovudine treatment</th>
<th>Mean value ± SD (CV%)&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>AUC_{0–4} (ng · h/ml)</td>
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<tr>
<td>Alone</td>
<td>1,577 ± 539 (34.2)</td>
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<tr>
<td>With IL-2</td>
<td>1,412 ± 339 (24.0)</td>
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<sup>a</sup> CV%, coefficient of variation (expressed as a percentage); AUC_{0–4}, AUC from time zero to 4 h after the dose.

### Table 2. Pharmacokinetic parameters for didanosine alone and in combination with IFN-α

<table>
<thead>
<tr>
<th>Didanosine treatment</th>
<th>Mean value ± SD (CV%)&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>AUC_{0–4} (ng · h/ml)</td>
</tr>
<tr>
<td>Alone</td>
<td>1,631 ± 968 (59.4)</td>
</tr>
<tr>
<td>With IFN-α</td>
<td>1,606 ± 1,250 (77.8)</td>
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</table>

<sup>a</sup> CV%, coefficient of variation (expressed as a percentage); AUC_{0–4}, AUC from time zero to 8 h after the dose.

<sup>b</sup> Significantly different compared with didanosine alone at P < 0.05 by paired t test.
difference is not significant because of a small sample size or large variability (23). An additional problem with hypothesis testing is that it provides an all-or-none approach, establishing either a definitive presence or absence of a drug interaction and providing no information on the degree or extent of the pharmacokinetic changes. By using guidelines for equivalence, the probability that the pharmacokinetic parameters of a drug given alone versus in combination are within specified guidelines of normal variability can be determined. This probability can give a relative indication of the degree of the drug interaction.

However, bioequivalence testing to assess drug interactions raises its own set of problems. The variability in a drug’s pharmacokinetics and what the investigator perceives as important raises its own set of problems. The variability in a drug’s pharmacokinetics can be determined. This probability can give a relative indication of the degree of the drug interaction.

The successful treatment of HIV-infected patients, especially those with advanced disease, requires polytherapy. Many of the agents used concomitantly with antiretroviral agents are inhibitors or inducers of hepatic cytochrome P-450 enzymes (i.e., rifabutin and clarithromycin), while others (IL-2 and IFN-α) have effects on multiple organ systems which may result in changes in the disposition of other drugs. In addition to prescribed drugs, many patients also self-administer alternative agents, including herbs, extracts, and vitamins for which no pharmacologic data are available (9). Therefore, the typical HIV-infected patient is prone to multiple-drug interactions which may lead to adverse effects, noncompliance, or reduced efficacy. The study of drug interactions remains an important aspect of AIDS research. However, the current statistical methods to assess these interactions may not be adequate. As this component of HIV research expands, additional studies evaluating improved methods for analysis and interpretation of drug interactions are warranted.

REFERENCES


FIG. 1. Mean concentration-time profiles of zidovudine (ZDV) at steady state when administered alone or with IL-2.

FIG. 2. Mean concentration-time profiles of didanosine (DDI) at steady state when administered alone or with IFN-α.


