The Effect of Probenecid on the Pharmacokinetics of Zalcitabine in HIV-Positive Patients

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Purpose. The purpose of this study was to determine the potential effect of probenecid on the pharmacokinetics of zalcitabine in HIV-positive patients.

Methods. Twelve patients received single oral 1.5 mg doses of zalcitabine alone and during probenecid treatment (500 mg at 8 and 2 hours before and 4 hours after zalcitabine dosing) in an open-label, randomized two-way crossover study with a one-week washout period between treatments. Serial blood and urine samples were collected over a 24 hour period and assayed for zalcitabine by a modified GC/MS method.

Results. Coadministration of probenecid with zalcitabine resulted in a decrease in mean (%CV) renal clearance of zalcitabine from 310 (28%) ml/min when zalcitabine was given alone to 180 (22%) ml/min with probenecid and a prolonged half-life from 1.7 hours to 2.5 hours. Mean AUCs increased from 59 ng·h/ml when zalcitabine was given alone to 91 ng·h/ml when given with probenecid. Considering the short half-life of zalcitabine (1–3 hours) relative to its dosing schedule, the pharmacokinetic changes observed in this study are not expected to result in significant accumulation during chronic dosing.

Conclusions. The results of this study show that co-administration of probenecid with zalcitabine results in a moderate decrease in renal clearance of zalcitabine due to inhibition of renal tubular secretion and a 50% increase in drug exposure. Although well tolerated in this single-dose study, patients taking this combination should be monitored closely for signs of toxicity and dosage reduction should be considered if warranted.

KEY WORDS: pharmacokinetics; zalcitabine; probenecid; interaction; HIV-infection.

INTRODUCTION

Zalcitabine (formerly ddC or dideoxycytidine) is one of several pyrimidine nucleoside analogs (others are zidovudine and dideoxyinosine) that has been shown to inhibit HIV replication "in vitro" and "in vivo". It has been approved for the treatment of HIV infection in adults with advanced HIV disease who either are intolerant to zidovudine (ZDV, Retrovir®) or who have disease progression while receiving zidovudine. It is also approved for use in combination with zidovudine for the treatment of selected patients with advanced HIV disease (CD4 cell count $\leq$300 cell/mm^3). Pharmacokinetic studies in adult HIV-positive patients or patients with AIDS or ARC (AIDS-related complex) show that zalcitabine is rapidly and extensively absorbed after oral administration, and subsequently eliminated with a half-life ranging from 1 to 3 hours. Renal excretion of unchanged drug is the primary route of elimination, accounting for 60 to 70% of an oral dose within 24 hours after dosing. Renal clearance of zalcitabine exceeds glomerular filtration rate, suggesting that tubular secretion contributes to the renal elimination of the drug. (1–4).

Probenecid is a uricosuric agent that is primarily used for the treatment of gout and as an adjunct to prolong blood levels of antibiotics such as penicillins and cephalosporins. Probenecid is an effective inhibitor of renal tubular secretion of organic anions (5,6) and glucuronide formation (7,8). Previous studies in which 500 mg of probenecid was given in combination with zidovudine showed that probenecid significantly inhibited the glucuronidation of zidovudine and renal clearance of its glucuronide metabolite (9–11). The authors of these reports suggested that less frequent dosing of zidovudine may be possible if it is co-administered with probenecid. Since zalcitabine is similar in structure to zidovudine and undergoes renal tubular secretion as its primary elimination pathway, it is possible that probenecid may inhibit the excretion of zalcitabine resulting in increased efficacy/toxicity and/or require less frequent dosing. The present study examines the potential effect of probenecid on the pharmacokinetics of zalcitabine.

MATERIALS AND METHODS

Patient Selection

Ten male and two non-pregnant female HIV-positive patients completed the study. They were all ambulatory ranging in age from 26–46 years (mean age 36 years; mean weight 78 kg) and diagnosed as asymptomatic or had symptomatic AIDS or ARC. At the time of entry into the study, each patient was free of opportunistic infection and demonstrated normal renal and hepatic function. In addition, a hemoglobin of at least 9.5 gm/ml, absolute neutrophil count $\geq$1000 cells/mm^3, a platelet count $\geq$100,000 platelets/mm^3 and an estimated creatinine clearance $\geq$75 ml/min were required. Patients who received drugs with a potential to cause peripheral neuropathy were excluded as were active drug or alcohol abusers. Patients with a fever >38.5°C or with significant cardiac, liver, renal, pancreatic or gastrointestinal disease were ineligible for the study. Patients with intolerance to nucleoside analogs or probenecid, or patients with elevated serum amylase or lipase were also excluded. The protocol was reviewed and approved by the Investigational Review Board of the Newark Beth Israel Medical Center and each patient gave written informed consent to participate in the study.

Experimental

Within the period of 1 month to 2 weeks prior to the start of the study, a medical history, physical examination, vital signs, ELISA/Western Blot Analysis for detection of HIV-antibody and laboratory tests were performed on each patient. The laboratory tests consisted of blood chemistries, hematology and urinalysis. Alcohol was excluded for 72 hours prior to each dosing and until discharge from the unit. A negative urinary pregnancy

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test was required in the two women volunteers. The patients were confined to the study unit approximately 12 hours prior to the start of each study interval and remained in the study unit until the 24 hour urine specimen had been collected. A light snack was served 10 hours prior to dosing after which an absolute fast except for water was maintained. In the morning, patients received a single oral dose of 1.5 mg zalcitabine alone (Treatment A) or with 500 mg probenecid at 8 and 2 hours prior to and 4 hours after the zalcitabine dose (Treatment B) according to a randomized 2-way crossover schedule. Each zalcitabine and probenecid dose was given with 240 ml water. For Treatment A, 240 ml of water was given at the times corresponding to probenecid administration. No food was allowed until after the 4 hour blood samples had been collected after which a standard lunch was served. No food was allowed between lunch and dinner. Dinner was served 10 hours after dosing; thereafter, food was allowed *ad libitum*. Water was permitted *ad libitum* throughout the study. A minimum one-week washout period separated each treatment.

Fourteen ml blood samples were drawn into heparinized Vacutainer® tubes prior to zalcitabine administration (pre-dose) and 7 ml samples were collected at 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 10 hours after zalcitabine dosing for analysis of zalcitabine. The total volume of urine voided was collected for two hours prior to zalcitabine dosing and at 0–4, 4–8, 8–12 and 12–24 hour intervals post zalcitabine dose. Plasma and urine samples were stored at −70°C until analysis. Samples were handled in accordance to the CDC criteria (12).

Sample Analysis

Plasma and urine concentrations of zalcitabine were determined by a modified gas chromatographic/negative chemical ionization mass spectrometric (GC/NCIMS) method (13). The assay for zalcitabine in plasma showed overall inter- and intra-assay precisions of 1.8% and 7.5%, respectively, and a lower limit of quantification of 2 ng/ml. The assay for zalcitabine in urine showed overall inter- and intra-assay precisions of 5% and 9.1%, respectively, and a lower limit of quantification of 30 ng/ml.

Data Analysis

Pharmacokinetic parameters of zalcitabine were calculated from the plasma concentration-time data. Maximum plasma concentration (C_{max}) and the time of maximum plasma concentration (t_{max}) were read directly from the plasma concentration-time data. The area under the plasma concentration-time curve from time zero to infinity (AUC) was determined by conventional linear trapezoidal summation and extrapolation methods. The elimination rate constant (β) was estimated by linear least-squares regression analysis on the terminal log-linear portion of the plasma concentration-time curve. The elimination half-life ($t_{1/2}$) was calculated as ln $2/\beta$. The concentration of drug in urine and the total urine output were used to calculate the urinary excretion of zalcitabine during each collection interval and the overall excretion of drug during the entire collection period and the fraction of dose excreted unchanged in the urine (f_e). Apparent oral clearance (Cl/F) was estimated by dividing Dose by AUC and the renal clearance (Cl_r) was determined as the total urinary recovery divided by AUC (14). The standard analysis of variance for crossover designs including a test for a first-order carryover effect was used to analyze the pharmacokinetic variables of zalcitabine (15,16).

RESULTS

Overall, single oral doses of zalcitabine were generally well tolerated. Headache was the most frequent adverse experience reported in both treatment groups.

A summary of the pharmacokinetic parameters [Mean (%CV) and Range] is presented in Table 1. The mean plasma concentration-time curves for each treatment are illustrated in Figure 1.

Administration of zalcitabine with and without probenecid resulted in similar mean values for C_{max} and t_{max} (Table 1). Mean AUC values increased 54% and the half-life was prolonged from 1.7 to 2.5 hours when zalcitabine was given with probenecid. Probenecid decreased the renal clearance of zidovudine significantly by 42%. Statistical analysis of these data showed that a significant treatment difference was found from the analysis of variance for AUC, β and CL_r ($p < 0.01$).

DISCUSSION

The data following zalcitabine administration alone were consistent with those of previous studies with zalcitabine (1–4) and indicate that the drug was rapidly and extensively absorbed following oral administration in these patients. Similar mean values for C_{max} and t_{max} were observed for both treatments suggesting a similar rate and extent of absorption when zalcitabine is administered alone and in combination with probenecid.

Plasma concentrations declined more slowly and the half-life was prolonged significantly ($p < 0.01$) when zalcitabine was given during probenecid treatment compared to zalcitabine given alone. Mean apparent oral clearance and renal clearance declined 37% and 42%, respectively. The reduction in renal

Table I. Summary of Zalcitabine Pharmacokinetic Parameters [Mean (%CV) and Range, N = 12]

Parameters	1.5 mg Zalcitabine Alone	1.5 mg Zalcitabine during Probenecid	ANOVA
C_{max} (ng/mL)	20.2 (36) [9.0–32.5]	22.8 (20) [15.2–29.2]	NS
t_{max} (hr)	1.1 (57) [0.5–3.0]	1.3 (42) [0.5–2.0]	NS
AUC (ng·hr/mL)	59 (27) [31–85]	91 (22) [65–125]	$p < 0.01$
$t_{1/2}$[a] (hr)	1.7 [1.2–2.6]	2.5 [2.0–3.3]	$p < 0.01$[b]
CL/F (mL/min)	457 (31) [294–806]	287 (21) [200–385]	—
CL (mL/min)	310 (28) [184–477]	180 (22) [121–240]	$p < 0.01$
$\%f_{e\ (0\text{–}24\ hr)}$	71 (26) [22.8–99.0]	64 (22) [32.1–81.9]	—

NS = not significant.
[a]Harmonic mean.
[b]From analysis of β.

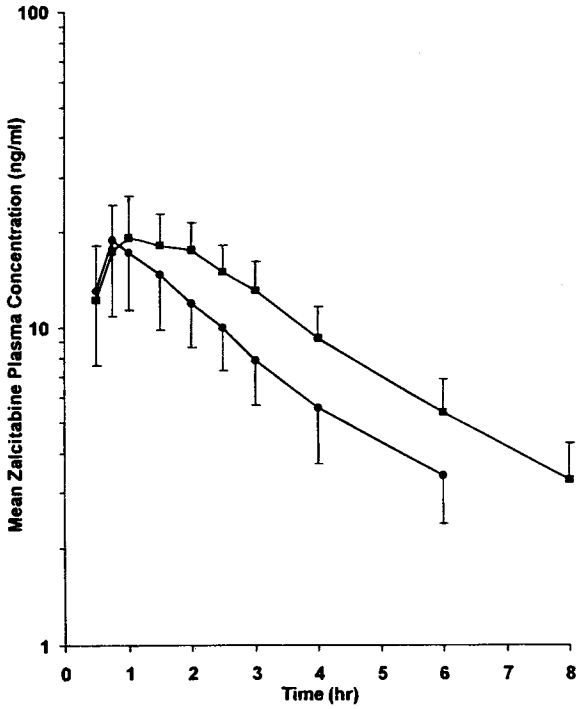

Fig. 1. Mean (±SD) plasma concentration-time profiles of zalcitabine alone (●) and during treatment with probenecid (■).

clearance can be attributed to inhibition of tubular secretion, a well-described characteristic of probenecid.

The effect of probenecid on zalcitabine disposition observed in this study is greater than anticipated. Probenecid is usually regarded as an inhibitor of glucuronidation and renal excretion of organic anions. However, zalcitabine does not undergo glucuronidation to a measurable degree (Data on File, HLR). It is weakly basic with a pKa of 4.4 and is therefore present primarily in unionized form.

The results of this study therefore demonstrate the involvement of probenecid in the renal transport of a molecule which is neither anionic nor cationic. The mechanism for this interaction can only be speculated upon. Probenecid has been generally considered to competitively inhibit the transport of organic anions in the proximal renal tubule (5,6,17). However, its specificity and selectivity for anions may not be so complete. In rats, Aiba et al (17) showed that probenecid inhibited zidovudine (primarily a cation at physiological pH) uptake at this site. Other studies in humans showed that probenecid decreased the renal clearance of the cationic molecules cimetidine (18) and famotidine (19). Increasing evidence therefore suggests the possibility of overlapping transport systems with different specificities. More recently, specific nucleoside transport systems present in renal tubule brush border membranes have been described. Although early studies showed that only naturally occurring nucleosides were substrates of these systems (20,21), later reports have suggested that nucleoside analogs may also be involved (22,23). It is not known if probenecid has an inhibitory effect on this mechanism.

The results of this study are in contrast to those of previous studies (8–10) evaluating the interaction of probenecid with another nucleoside analog, zidovudine. Whereas the interaction of probenecid with zalcitabine appears to be simply inhibition of renal tubular secretion of parent drug, the interaction with zidovudine is relatively complex. Probenecid pre-treatment significantly inhibits both glucuronidation of zidovudine and renal clearance (presumably by inhibition of tubular secretion) of the glucuronide metabolite. However, it has no effect on renal clearance of the parent drug. Overall, there was a two-fold increase in exposure (AUC) to both the parent drug and the glucuronide metabolite and a wider range of AUC values for each component in the seven subjects receiving the combination compared to ZDV alone. By comparison, there is an approximate 50% increase in drug exposure, based on AUC, and a similar degree of variability for zalcitabine alone relative to zalcitabine with probenecid. Thus, the magnitude and variability of the probenecid interaction with zalcitabine is quantitatively less and not as complex as that with zidovudine. Considering the short half-life of zalcitabine (<2 hr alone, 2.5 hr with probenecid) relative to its dosing schedule (t.i.d.), the decrease in clearance and prolonged half-life observed in this study would not be expected to result in significant accumulation during long-term dosing. The therapeutic implications of these differences, if any, are not known. We also note that, although probenecid has been typically associated with its effects on renal transport, recent literature also indicates its ability to inhibit the drug transport into other cell types such as T lymphocytes (24), human intestinal epithelial cells (25), and human hepatoma cells (26). The potential for probenecid to modify the uptake by cells responsible for phosphorylation to its active moiety, thereby diminishing its efficacy, should also be considered.

In conclusion, plasma concentrations of zalcitabine were higher and clearance slower when the drug was given during probenecid dosing due to inhibition of its renal elimination pathway. Physicians should be aware of this interaction and closely monitor patients taking this combination for signs of toxicity and consider a dosage reduction if warranted. Conversely, this interaction could be employed as an advantage whereby co-administration of this combination is used to increase drug exposure to individual doses of this rapidly-eliminated drug without leading to significant drug accumulation during long-term use. Although a possible therapeutic benefit, the potential pharmacokinetic and pharmacodynamic interaction of probenecid with other drugs taken by AIDS patients must also be taken into consideration. Further studies are needed to address the efficacy and safety of this combination.

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