Renal Disposition and Drug Interaction Screening of (−)-2′-deoxy-3′-thiacytidine (3TC) in the Isolated Perfused Rat Kidney

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Purpose. Dideoxynucleoside bases are used for the treatment of acquired immune deficiency syndrome (AIDS), acting by inhibiting reverse transcriptase and preventing human immunodeficiency virus (HIV) replication. Currently, AZT (zidovudine), ddC (zalcitibine), and ddI (didanosine) are available to the medical community to prevent the onset of AIDS in HIV-infected individuals. 3TC (−)-2′-deoxy-3′-thiacytidine, lamivudine), a new dideoxynucleoside base, is currently undergoing Phase II/III trials, and has exhibited anti-HIV replication activity, a favorable adverse event safety profile, and is eliminated via renal mechanisms. Concomitantly administered drugs could potentiate the effects of 3TC due to interaction in the kidney.

Methods. An isolated perfused rat kidney (IPK) technique was used to screen several clinically relevant drugs for potential interaction with 3TC. The following perfusions were performed: baseline 3TC; and 500 ng/mL 3TC with clinically relevant concentrations of AZT, ddC, ddI, probenecid, trimethoprim, sulfamethoxazole, ranitidine, and cimetidine.

Results. Renal clearance of 3TC was nonlinear between 500 and 5000 ng/mL, decreasing from 3.06 to 1.74 mL/min. Excretion ratio also decreased, from 3.67 (500 ng/mL) to 2.49 (5000 ng/mL), consistent with a decrease in 3TC secretion. AZT, ddI, and ddC elicited no or minimal effects on 3TC elimination at the concentrations studied. However, trimethoprim caused significant reductions in 3TC elimination parameters: clearance and excretion ratio decreased to 1.25 mL/min and 1.43, respectively.

Conclusions. These results indicate that caution should be exercised when the combination of 3TC and trimethoprim are administered to AIDS patients.

KEY WORDS: 3TC; AIDS; drug interactions; renal clearance; excretion ratio; secretion.

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is a devastating and lethal disease in which the retrovirus, human immunodeficiency virus (HIV), attacks helper T4 white blood cells leading to an immunologically compromised host defense system (1). This leaves the infected person susceptible to several opportunistic infections including *pneumocystis carinii,* cytomegalovirus, and toxoplasmosis (2). Ultimately, the infected person dies as there is no known cure for this disease.

A new class of drugs has been introduced in an attempt to retard the progression of AIDS. These compounds are dideoxynucleoside bases, which act to inhibit reverse transcriptase, thereby preventing HIV replication (3). Three dideoxynucleosides are currently available to the medical community in the battle to prevent the progression of AIDS: AZT (zidovudine), ddC (zalcitibine), and ddI (didanosine). A similar compound, 3TC (−)-2′-deoxy-3′-thiacytidine, lamivudine), is currently undergoing Phase II/III trials.

Structurally, 3TC is similar to ddC, incorporating a sulphur atom in the furanose ring in place of a methylene group. The mechanism of action of 3TC is similar to marketed dideoxynucleosides, that being inhibition of reverse transcriptase, with selective inhibition of HIV (types I and II) replication *in vitro* (4). The inhibition of HIV replication is less potent than AZT and ddC, but the cellular toxicity profile is less severe and 3TC exhibits antiviral activity against AZT-resistant strains of HIV (5,6). Ongoing clinical trials have reported that 3TC is well tolerated in humans with no serious drug related toxicity associated with 3TC administration (7,8,9).

This present research was undertaken to screen several clinically relevant drugs for potential drug interaction with 3TC. The major elimination pathway of 3TC is excretion of unchanged drug into the urine with a net secretory component. An isolated perfused rat kidney (IPK) was chosen as a model for identification of renal elimination mechanism and significant drug interactions. The rationale for this includes utility in focusing on renal mechanisms of elimination and drug interaction (10,11) and a demonstrated correlation of IPK results and human data (12,13,14).

MATERIALS AND METHODS

An IPK was employed for all drug interaction studies. Additionally, a clearance-dose proportionality study was conducted using three nominal initial concentrations of 3TC representative of those expected in clinical use: 500 ng/mL 3TC, 1000 ng/mL 3TC, and 5000 ng/mL 3TC. Interaction studies were performed with 500 ng/mL 3TC and clinically relevant concentrations of AZT (1 mcg/mL), ddC (2 mcg/mL), ddI (1 mcg/mL), probenecid (50 mcg/mL), trimethoprim (4 mcg/mL) and sulfamethoxazole (80 mcg/mL), the components of Bactrim/Septra, ranitidine (400 ng/mL), and cimetidine (2 mcg/mL).

Materials

3TC was supplied by Glaxo Inc. Research Institute; AZT, ddC, ddI, probenecid, trimethoprim, sulfamethoxazole, ranitidine, cimetidine, fraction V bovine serum albumin, dextran (chemical grade, MW 60,000–90,000), creatinine, and L-amino acids were obtained from Sigma Chemical Co. (St. Louis, Missouri).

Surgical Technique

Kidneys were isolated from male Harlan (Indianapolis, Indiana) Sprague-Dawley rats (300–400 gm). All rats were

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housed in stainless steel cages and acclimated to their environment for at least four days before any surgery was performed. The surgical technique used in all IPK experiments was the Bowman (15) modification of the Nishiitsutsuji-Uwo procedure (16) as outlined by Statkevich et al. (14).

Perfusion Design

The IPK perfusion was performed according to the method as described by Bekersky (10). Recirculating perfusate (37°C) consisted of supplemented Krebs-Henseleit buffer containing 5% bovine serum albumin (BSA), 0.67% dextran, 110 mg/dl glucose, 54.3 mg/dl creatinine for the determination of glomerular filtration rate (GFR), and 20 L-amino acids (17). Perfusate was oxygenated and pH maintained at 7.4 by bubbling a mixture of 95% O_2/5% CO_2 gas through the perfusate for 1 hour prior to initiating each perfusion and continuously throughout an experiment. The total perfusate volume was initially 80 mL. This volume was maintained throughout each experiment by addition of perfusate diluted 1:1 with distilled water, equivalent to the total volume of urine collected and perfusate sampled in each collection period. Immediately upon collection, urine volume was determined gravimetrically and urine pH measured. Perfusate was sampled at the midpoint of each collection interval.

A 10-minute stabilization period preceded any pharmacokinetic experimentation, and a 10-minute distribution period followed the addition of drug as a bolus to the perfusion reservoir. The remainder of the two hour perfusion was divided into a maximum of ten 10-minute collection periods. Perfusion pressure was maintained at 100 ± 10 mm Hg by adjusting perfusate flow rate. Parameters monitored to assess kidney function throughout an IPK perfusion included glomerular filtration rate (GFR), fractional reabsorption of glucose (FR_{GLU}) and sodium (FR_{Na}), urine flow rate and pH.

Study Design

Baseline control studies were performed in the absence of 3TC to establish the viability of the kidney preparation (3 kidneys). Urine and perfusate samples were collected for the determination of sodium, glucose, and creatinine concentration, permitting calculation of viability parameters.

Perfusions were then performed to determine the relationship between initial 3TC concentration and the elimination parameters, renal clearance and excretion ratio, in an effort to determine whether 3TC exhibited linear pharmacokinetic behavior over this concentration range. Three kidneys were perfused with each initial 3TC concentration. During drug interaction studies, a clinically relevant concentration of each interactant drug was added to the perfusate following the stabilization period, and allowed to distribute. 3TC (500 ng/mL) was then introduced into the reservoir, and up to nine 10-minute urine collection intervals followed.

Concentration of 3TC was measured in perfusate and urine for determination of renal clearance. All 3TC determinations were performed by Glaxo Inc. Research Institute using a sensitive and specific HPLC assay (18). The assay was validated between 3TC concentrations of 25 and 10,000 ng/mL, with a lower limit of sensitivity of 25 ng/mL. Renal clearance (CL_R) of 3TC was calculated for each collection interval as the ratio of drug excretion rate into the urine and midpoint 3TC concentration in perfusate. CL_R was corrected for glomerular filtration rate (GFR) during each interval and binding of drug, which is negligible for 3TC over the concentration range studied (19). This corrected value, referred to as excretion ratio (ER), provided insight into the net mechanism of renal elimination (20).

Data Collection

Several indices of viability were monitored over each perfusion, including cannula tip pressure, perfusate flow rate, urine pH, and urine flow rate. Additionally, several other parameters were calculated using glucose, electrolyte, and creatinine concentrations in perfusate and urine. These secondary parameters included GFR, fractional reabsorption of glucose (FR_{GLU}), and fractional reabsorption of sodium (FR_{Na}). Elimination parameters determined for mechanistic and drug interaction interpretation were renal clearance and excretion ratio.

Statistical Analysis

The data set was reduced using several criteria which ensured that only data from viable collection periods were used in subsequent analyses. First, only collection intervals exhibiting greater than 85% glucose reabsorption, a specific marker of proximal tubular function, were included in the final data set. Second, those collection intervals having greater than 80% sodium reabsorption were included. Lastly, drug excretion rate was calculated. Since a lag-time between equilibration of drug in the recirculating perfusate, kidney tissue, and urine may exist, only those early collection intervals within 25% of peak excretion in each perfusion were included into the data set. All collection intervals following maximum excretion rate were included for analysis.

Univariate analysis of variance (ANOVA, Proc GLM, SAS) was used to determine whether there existed a significant difference among treatment groups for each of the following parameters: GFR, FR_{GLU}, CL_R, and ER. If a significant difference was detected by ANOVA, Tukey's multiple range test was employed to determine which treatment group differed from control values. A significance level of 0.05 was set for all analyses.

RESULTS

All relevant data from perfusions in which 3TC was added to the perfusate are presented in Tables I and II. GFR and FR_{GLU} are presented for assessment of viability. CL_R and ER are presented for assessment of clearance-dose linearity, mechanism of renal elimination, and identification of significant drug interaction.

A set of perfusions were performed as controls in order to get an estimate of GFR and FR_{GLU} in the absence of any drug. Mean glomerular filtration rate (sd) was 0.71 (0.092) mL/min and mean FR_{GLU} (sd) was 0.97 (0.024). These perfusions were done in order to obtain representative values for these viability parameters with the specific perfusate used in these experiments (5% BSA/0.675 DEX). These values were within the expected range for this technique.

Mean CL_R (sd) was 3.06 (0.79) and 3.64 (0.93) mL/min

Table I. Mean (sd) of Lamivudine (3TC) Perfusion Parameters for the 500, 1000, and 5000 ng/mL Perfusion Groups

Group	GFR (mL/min)	FR_{GLU}	CL_R (mL/min)	ER	N
3TC 1	0.89	0.97	3.06	3.67	21
(500 ng/mL)	(0.22)	(0.028)	(0.79)	(1.34)	
3TC 2	0.99	0.98	3.64	3.70	32
(1000 ng/mL)	(0.14)	(0.012)	(0.93)	(0.98)	
3TC 3	0.70[a]	0.96	1.74[a]	2.49[a]	30
(5000 ng/mL)	(0.21)	(0.026)	(0.60)	(0.34)	

[a] Significantly different from 500 ng/mL baseline as indicated by Tukey's test: GFR = glomerular filtration rate, FR_{GLU} = fractional reabsorption of glucose, CL_R = renal clearance of 3TC, ER = excretion ratio = renal clearance/filtration clearance, N = number of clearance intervals.

for the 500 and 1000 ng/mL 3TC perfusion groups, respectively (Table I). Mean ER for these two groups was 3.67 and 3.70, respectively. These clearance and excretion ratio values were not statistically different. Mean CL_R (sd) and ER (sd) decreased significantly to 1.74 (0.60) mL/min and 2.49 (0.34), respectively, when kidneys were perfused with 5000 ng/mL 3TC.

All parameters from drug interaction perfusions are presented along with the 500 ng/mL 3TC group in Table II. Mean CL_R for the trimethoprim/3TC group was statistically different from baseline 3TC (1.25 *versus* 3.06 mL/min, respectively). Mean excretion ratio for the ddC/3TC and trimethoprim/3TC groups were statistically different from 3TC baseline (2.50 and 1.43 *versus* 3.67, respectively). All other CL_R and ER values were not statistically different from baseline 3TC.

DISCUSSION

Zidovudine was the first dideoxynucleoside base to be approved for the treatment of AIDS. Unfortunately, AZT monotherapy is limited by the development of hematologic toxicity and resistance of the HIV virus to AZT (21). The predominant adverse events of the other dideoxynucleosides involves neurotoxicity. Therefore, rational drug therapy includes administration of reduced doses of AZT and another dideoxynucleoside. This reduces the frequency of adverse events and helps prevent the onset of drug resistance (21). Lamivudine offers the advantages of reduced toxicity and demonstrated activity *versus* AZT-resistant strains of HIV (5).

This investigation tested several clinically relevant drugs for interaction with 3TC in an isolated perfused rat kidney. GFR for all drug perfusion groups was between 0.70 and 1.09 mL/min (Tables I and II), and the FR_{GLU} was relatively constant at 96%. The global means (sd) were 0.91 (0.18) mL/min and 0.96 (0.023) for GFR and FR_{GLU}, respectively. While statistically significant differences were detected among treatment groups and the 500 ng/mL 3TC baseline group, these were not deemed to be of great importance and attributable to the small standard deviation of the parameters (coefficient of variation of 19.8% and 2.40%, respectively, for GFR and FR_{GLU}).

The clearance of 3TC decreased between 500 and 5000 ng/mL, as indicated by the drop from 3.06 and 3.64 at 500 and 1000 ng/mL 3TC, respectively, to 1.74 mL/min at 5000 ng/mL 3TC. In part, this difference may be explained by the significant difference in GFR for the 5000 ng/mL perfusion group. Nevertheless, there was a change in net excretory mechanism, as indicated by ER (GFR-corrected CL_R), a decline from 3.67 and 3.70 for the 500 and 1000 ng/mL 3TC groups, respectively, to 2.49 for the 5000 ng/mL 3TC group. The mechanism for elimination in the kidney appears to be one of filtration and net secretion (73%, 73%, and 60% secretion at 500, 1000, and 5000 ng/mL 3TC, respectively). FR_{GLU} was excellent in all three perfusion groups (>95%, Table I).

Assuming a single transporter for 3TC secretion in the proximal tubule and minimal reabsorption, total excretion rate would equal the sum of filtration and secretion rates. Binding of 3TC to plasma proteins is minimal (19), therefore, unbound and total 3TC concentrations are equivalent. The following equation describes CL_R:

$$CL_R = GFR + Tmax/\{Km + C\} \quad (1)$$

where

CL_R = renal clearance of 3TC

Tmax = 3TC secretory transport maximum

Km = 3TC concentration at half Tmax

C = 3TC concentration in perfusate

Equation 1 was fitted to CL_R *versus* 3TC concentration and GFR data (NONMEM, Version IV, 1992) using a combination error structure, resulting in a 19.8% and 18.7% re-

Table II. Mean (sd) of Lamivudine (3TC) Perfusion Parameters for Baseline 500 ng/mL 3TC and Drug Interaction Perfusion Groups

Group	GFR (mL/min)	FR_{GLU}	CL_R (mL/min)	ER	N
3TC	0.89	0.97	3.06	3.67	21
(500 ng/mL)	(0.22)	(0.028)	(0.79)	(1.34)	
3TC + AZT	0.96	0.94[a]	2.55	2.72	22
	(0.18)	(0.039)	(0.74)	(1.00)	
3TC + ddI	0.77	0.95[a]	2.33	3.02	23
	(0.19)	(0.023)	(0.83)	(0.84)	
3TC + ddC	0.98	0.96	2.50	2.50[a]	22
	(0.14)	(0.026)	(1.06)	(0.90)	
3TC + PBN	1.09[a]	0.97	3.45	3.21	18
	(0.17)	(0.018)	(0.63)	(0.59)	
3TC + TMP	0.87	0.97	1.25[a]	1.43[a]	27
	(0.092)	(0.017)	(0.33)	(0.35)	
3TC + SMX	0.95	0.98	3.35	3.54	21
	(0.30)	(0.0081)	(1.34)	(0.76)	
3TC + RAN	0.87	0.97	3.27	3.89	20
	(0.17)	(0.018)	(1.12)	(1.62)	
3TC + CIM	1.05	0.97	3.85	3.62	17
	(0.11)	(0.0064)	(1.96)	(1.70)	

[a] Significantly different from 500 ng/mL (Tukey's test): GFR = glomerular filtration rate, FR_{GLU} = fractional reabsorption of glucose, CL_R = renal clearance of 3TC, ER = excretion ratio = renal clearance/filtration clearance, N = number of clearance intervals, PBN = probenecid, TMP = trimethoprim, SMX = sulfamethoxazole, RAN = ranitidine, CIM = cimetidine.

sidual error at 25 and 5000 ng/ml, respectively. GFR was that observed measurement corresponding to each interval renal clearance. Estimates of Tmax (SE) and Km (SE) were 2080 ± 3.25 ng/min and 687 ± 135 ng/mL, respectively. Based on log-likelihood difference, the inclusion of a linear reabsorption term into equation 1 did not significantly improve the fitting, and the estimate of the fraction reabsorbed was not significantly different from zero. Point estimates of Tmax and Km were then used to predict excretion rate of 3TC as a function of 3TC perfusate concentration (figure 1).

The dependency of renal clearance on concentration is supported by data following 3TC administration to humans and other dideoxynucleoside data reported in the literature. Clearance decreased 15% in humans when dosed 0.25 mg/kg and 1.0 mg/kg, over a maximum concentration range of 386 to 1615 ng/mL (19). Additionally, the renal clearances of AZT (22) and ddI (23) have been shown to decrease with increasing doses administered to rats. Interestingly, in the same animal species, ddC renal clearance was independent of dose (24).

In drug interaction perfusion groups, AZT, ddI, and ddC appeared to have minimal or no effect on the renal handling of 3TC at the concentrations studied since CL_R and ER values were all similar to the 3TC 500 ng/mL data. However, ddC did cause a significant reduction in ER, from 3.67 to 2.50, indicating a slight inhibition of secretion. This outcome is not surprising since both are cytidine-based compounds which are highly secreted in the proximal tubule of rats and are expected to compete for the same transporter. These data would support the combination of AZT and 3TC in clinical trials in an effort to minimize drug toxicity and resistance while maximizing therapeutic outcome. Data from a clinical trial in AIDS patients correlated well with these IPK data as no significant effect between AZT and 3TC was observed (25).

Data in the literature involving the interaction between dideoxynucleosides are limited. Studies indicate that current results are consistent with those published by other investigators as no interaction between AZT and ddI was observed in rats (26), and no interaction between AZT and either ddC or ddI was found in monkeys (27,28).

Trimethoprim elicited a highly significantly effect on the CL_R of 3TC (1.25 mL/min), as evidenced by an ER of 1.43 (from approximately 73% net secretion to 30% net secretion). This is consistent with an inhibition of the 3TC secretory component of renal elimination by the basic cation, trimethoprim. This would indicate that caution should be exercised in patients dosed Bactrim/Septra and 3TC concomitantly, as a significant increase in serum concentration of 3TC may result due to inhibition of secretion. The second component of Bactrim/Septra, the weakly acidic antibiotic sulfamethoxazole, did not inhibit 3TC secretion at the concentrations studied. These data were confirmed by results from a follow-up clinical trial in AIDS patients where trimethoprim caused a significant decrease in the clearance of 3TC (29).

At interactant concentrations studied, probenecid and the H_2-antagonists, ranitidine and cimetidine, exhibited no effect on the elimination of 3TC. Information regarding the effect of probenecid on the elimination of other dideoxynu-

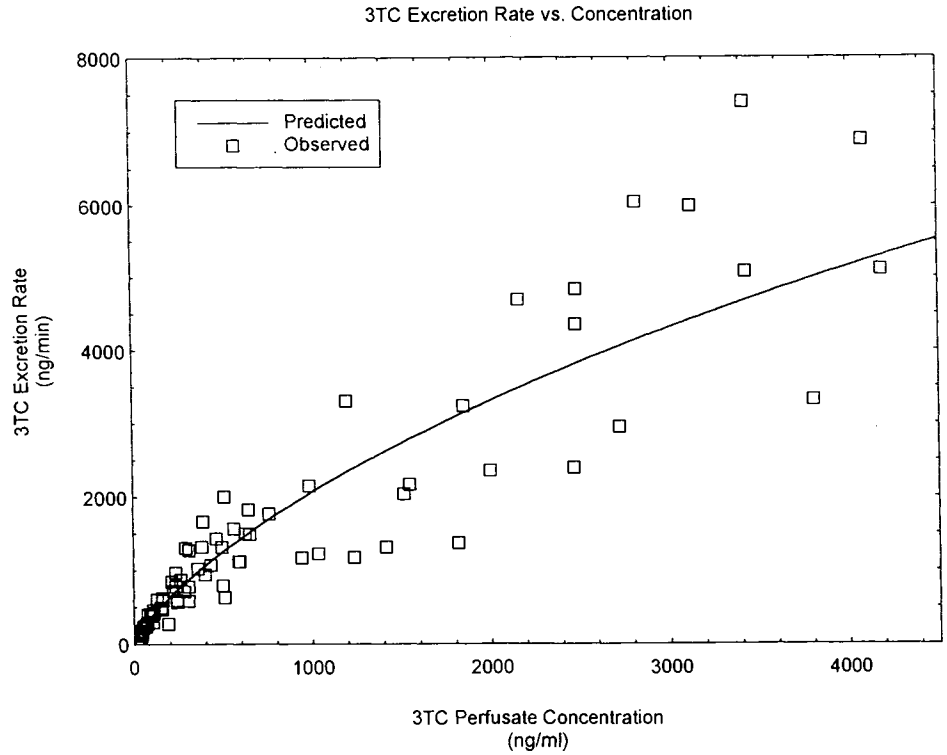

Fig. 1. 3TC excretion rate (ng/min) versus 3TC perfusate concentration (ng/mL). Open squares are observed data and the solid line is predicted from transport maximum (2080 ng/min) and Km (687 ng/mL) estimates from fitting renal clearance as a function of 3TC perfusate concentration.

cleosides is contradictory. While it is well established that probenecid inhibits the metabolic conversion of AZT to AZT-glucuronide (30–36), the effect on the renal component is not as clearly established. Probenecid has been reported to either have no effect on AZT renal elimination in humans (34) or elicit a decrease in renal clearance through inhibition of secretion in rabbits, rats, and monkeys (31,32,35,37,38). Cimetidine caused a significant decrease in the secretion of AZT in rats (38), but the concentration of cimetidine used was 1000 times that of AZT on a molar basis. The only report of an H_2-antagonist/dideoxynucleoside interaction in humans was published by Knupp et al. (39), indicating that ranitidine caused no effect on the elimination of ddI.

Another possibility for differences in the interaction profiles of the dideoxynucleosides is that they are secreted by different transporters in the proximal tubule. This may account for the reported effects of probenecid and cimetidine on AZT while no effect of either of these interactants was observed in this current investigation with 3TC. It has been established that nucleoside transporters exist in the brush border membranes of the proximal tubule which function in reabsorption of nucleosides from the tubular fluid of the nephron (40–42). Two transporters have been identified in bovine brush border membrane vesicles (BBMV). In contrast, only one Na^+-dependent transporter has been identified in human BBMVs which interacts with uridine, thymidine, and cytidine.

Investigations into secretory transporters on the basolateral membrane are limited. AZT was found to inhibit PAH and probenecid transport across basolateral membrane vesicles (BLMV) in rats while having no effect on cationic markers NMN and TEA (43). Further studies of the interaction between the dideoxynucleosides and common markers of secretion for anionic and cationic transporters in the renal proximal tubule need to be performed to better elucidate the mechanisms involved in elimination of these drugs.

CONCLUSIONS

Lamivudine (3TC) was investigated at three nominal initial concentrations in the IPK. CL_R and ER values of 3TC were moderately reduced at the highest 3TC concentration studied. This reduction in clearance was statistically significant and consistent with human data under similar concentration exposure. Trimethoprim was found to markedly inhibit the renal secretion of 3TC, indicating that caution should be exercised when this combination is administered to humans. These IPK data accurately predicted the effect of AZT and trimethoprim on 3TC elimination in AIDS patients and demonstrated it as a viable tool to screen potential renal drug interactions in humans.

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