Competitive Inhibition of Zidovudine Clearance by Probenecid During Continuous Coadministration

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The pharmacokinetics of zidovudine in the rabbit were studied during coadministration of probenecid at two infusion rates. Each animal ($n = 6$) served as its own control during an initial 8-hr infusion of zidovudine. In the second 8-hr infusion period, probenecid was coadministered with zidovudine. Urine samples were collected by bladder flush hourly for 19 hr. Plasma samples were taken at the midpoint of the urine collection interval and at predetermined intervals for 3 hr postinfusion. Plasma concentrations of zidovudine reached steady state during control periods but showed incomplete attainment of steady state during the infusions of probenecid at the higher rate. Total and renal clearance of zidovudine were reduced by 24.0 ± 4.0 and 20.7 ± 15%, respectively, during low-dose probenecid treatment and 48.9 ± 7.4 and 55.7 ± 3.4%, respectively, with high-dose probenecid treatment. Plasma probenecid concentrations during low-dose and high-dose infusion were 56.9 ± 12 and 248 ± 42 μg/ml. Postinfusion data showed that the zidovudine terminal half-life during high-dose probenecid treatment was longer than that with low-dose probenecid treatment (58.2 ± 4.6 vs 39.0 ± 9.1 min). The volume of distribution of zidovudine also decreased (1.76 ± 0.27 vs 1.10 ± 0.095 L/kg) as a result of probenecid coadministration. The results are consistent with competitive inhibition of renal and nonrenal clearances. A drug interaction model relating zidovudine clearances to plasma probenecid concentrations was derived. Michaelis-type constants for probenecid inhibition of zidovudine renal and nonrenal clearances were 73 and 55 μg/ml, respectively. The maximum proportion of AZT's renal clearance subject to inhibition is significantly greater (72%) than that of the nonrenal clearance (54%) and agrees closely with the fraction not filtered.

KEY WORDS: zidovudine; probenecid; competitive inhibition; pharmacokinetics.

INTRODUCTION

AZT, or 3'-azido-2',3'-dideoxythymidine (also known as azidothymidine or zidovudine), is the only drug currently approved for the treatment of acquired immunodeficiency syndrome (AIDS). As an analogue of nucleoside thymidine, AZT undergoes phosphorylation in the infected cell to form AZT triphosphate, which inhibits the production of viral DNA by competitive inhibition and chain termination at the level of reverse transcriptase (1).

Following intravenous administration of AZT in AIDS patients, 19% of the parent drug is excreted unchanged in urine, with more than 60% of the given dose eliminated as the 5'-*O*-glucuronide (GAZT) metabolite of zidovudine. The bioavailability of AZT is approximately 65%, with 14% of the orally administered dose recovered as AZT and 75% as GAZT in the urine (2,3).

In AIDS patients, AZT exhibits a total-body clearance (Cl_{tot}) measured in plasma of 1900 ml/min, a renal clearance (Cl_r) of 350 ml/min, and a half-life of 1.1 hr (2). The high nonrenal clearance ($Cl_{nr} = Cl_{tot} - Cl_r$), far exceeds the value of hepatic plasma flow, suggesting extrahepatic metabolism. The relatively high oral bioavailability of AZT in humans lends further support to this interpretation. Moreover, that Cl_r is three times the glomerular filtration rate demonstrates that this drug is actively secreted in the renal tubule.

Because of the rapid elimination of AZT in patients, frequent dosing appears necessary to maintain therapeutic plasma levels. However, a marked reduction in the clearance of this agent during coadministration of probenecid (PBD) has been reported in normal volunteers (4) and in patients with AIDS (5,6). Coadministration of PBD (500 mg, every 6 hr) with AZT increased the area under the curve by two- to threefold in both groups with a corresponding decrease in Cl_{tot} (4,5). In normal volunteers, PBD was shown (4) to inhibit both renal and nonrenal clearances of AZT, whereas in a report which examined this interaction in AIDS patients, metabolic clearance was reduced, but renal clearance was unchanged (6). Discrepancies in the results of these studies may stem from intersubject variability or from differences in study design. For example, differences in the timing of AZT and PBD doses in these trials may affect outcome. A previous report showed that peak plasma concentrations of PBD occurred only after 3 hr following oral administration of 500 mg (7). Thus, concurrent oral administration of PBD and AZT may result in different degrees of inhibition of hepatic metabolism of AZT than when these agents are given in staggered regimens.

It was reasoned that a steady-state interaction study utilizing intravenous infusion of AZT and PBD might circumvent problems associated with fluctuating plasma levels of these agents and allow for better characterization of this interaction. The present study was undertaken to examine the extent to which the pharmacokinetics of AZT in the rabbit are affected by the coadministration of PBD, under conditions where plasma concentrations of both compounds are maintained close to steady state.

MATERIALS AND METHODS

Chemicals

Zidovudine (3'-azido-3'-deoxythymidine; AZT) was a gift from Burroughs Wellcome, Research Triangle Park, NC, and the following chemicals were purchased and used as received: probenecid (PBD), β-hydroxyethyl theophylline, and β-hydroxypropyl theophylline from Sigma Chemical, St. Louis, MO: α-phenylcinnamic acid (cis-form) from Aldrich Chemical Company, Inc., Milwaukee, WI; sodium bicarbonate from Fisher Scientific, Fair Lawn, NJ; acetonitrile, chloroform, and isopropyl alcohol from Burdick and Jackson

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Labs, Muskegon, MI; and ammonium phosphate monobasic, acetic acid, and methyl alcohol from Mallinckrodt, St. Louis, MO. All solvents were HPLC grade, and the chemicals were AR grade.

Drug Solution Preparation

AZT and PBD solutions were prepared directly before the administration. AZT or PBD was dissolved in 5% sodium bicarbonate in normal saline at room temperature. Aliquots of both dosing solutions were taken before and after administration to an animal and analyzed by HPLC.

Animal Preparation

Male New Zealand White rabbits (3.31 ± 0.21 kg) were studied (n = 6). A Foley pediatric catheter (Bard, Murray Hill, NJ), size 8 FR, was inserted into the bladder through the urethra without anesthesia. Both marginal ear veins were cannulated (I-Cath Delmed, New Brunswick, NJ), and the catheters were positioned in the anterior vena cava (8). One catheter was used for blood sampling, while the other was reserved for drug administration. During the experimental period, the animal was conscious but restrained (8) and had free access to water.

Drug Administration

The animals were divided into two groups (A and B) and studied in crossover fashion. During an initial 8-hr control phase (no PBD), all animals received a loading intravenous bolus of AZT (1 mg/kg) followed by a constant-rate infusion of AZT (1 mg/kg · hr) at a flow rate of 3 ml/hr using a variable-rate infusion pump (Harvard Apparatus, Millis, MA). This infusion of AZT was continued throughout a 16-hr period, i.e., during two consecutive 8-hr phases. At the end of the first 8-hr period, Group A rabbits (n = 3) received an intravenous loading dose of PBD (10 mg/kg), followed by a constant-rate of infusion of 8 mg/kg · hr (low-dose PBD treatment) for an additional 8-hr treatment phase. Animals in Group B (n = 3) received (after the initial 8-hr control infusion) an intravenous loading dose of PBD (20 mg/kg), followed by a constant-rate infusion of 20 mg/kg · hr (high-dose PBD treatment) for an additional 8 hr. In both groups, constant-rate intravenous infusion of AZT was uninterrupted, allowing for continuous administration at a constant rate for 16 hr, after which infusions were terminated.

Urine and Plasma Sampling

During the 16-hr intravenous infusion period, total urine was collected every hour via the catheter by irrigating the bladder with four 15-ml aliquots of normal saline, maintained at 37°C, at 20, 15, 10, and 5 min before the end of each urine collection interval. Postinfusion urine samples were collected similarly. During the infusion blood samples (0.5 ml) were drawn into heparinized tubes at the midpoint of each urine collection period and immediately centrifuged to obtain plasma. Blood samples were also drawn at 5, 10, 20, 30, 45, 60, 90, 120, 150, and 180 min postinfusion. The total blood loss represented less than 8% of the blood volume (9). Samples were stored at −20°C until analysis.

Sample Analysis

AZT concentrations in plasma and urine were determined according to an HPLC method reported previously (10). The method involves a liquid–liquid extraction step and only requires 100 μl of plasma or urine. Reconstituted samples were injected from an autosampler onto the HPLC (Hewlett-Packard 1084B, Palo Alto, CA), where separation was achieved using a flow rate of 1.5 ml/min on a Supelcosil (Supelco, Bellefonte, PA) ODS column (15 × 0.46 cm, 5 μm) fitted with a 2-cm Supelguard LC-18 precolumn. The coefficient of variation between days was 1.6–6.6 and 3.4–6.9% for plasma (0.2–4.0 μg/ml) and urine (0.5–50.0 μg/ml), respectively.

Analysis of PBD in plasma and urine was performed using HPLC (Model 1090, Hewlett-Packard). The method was modified from a previous report (11), with α-phenylcinnamic acid used in the present method as the internal standard. Plasma or diluted urine (100 μl) was mixed with 1 ml phosphate buffer (McIlvaine's, citric acid–phosphate, pH 4) and extracted with 8 ml of 5% isopropyl alcohol in chloroform. The residue obtained after evaporating the organic layer was reconstituted in 100 μl of mobile phase [0.5% acetic acid in water and methyl alcohol (45:55 by volume)] which was mixed on line and delivered at 1.5 ml/min. Separation was performed on an ODS Supelcosil column (15 × 0.46 cm, 5μm). Column effluent was monitored at 244 nm with a variable-wavelength UV detector (Model SPD-6A; Shimadzu, Kyoto, Japan) and peak heights for PBD and internal standards were calculated using electronic integration. The day-to-day coefficient of variation was 7.0–13.7 and 4.4–13.3% for plasma (5.0–400.0 μg/ml) and urine (5.0–400.0 μg/ml), respectively, using 100 μl of sample.

Pharmacokinetic Analysis

During the control infusion period, when only AZT was infused, the renal clearance (Cl_r) of AZT in each rabbit was calculated as the rate of AZT urinary excretion divided by the corresponding midpoint plasma concentration. Total clearance (Cl_{tot}) was estimated by Eq. (1) for each of the last five-hourly intervals during the control period:

$$Cl_{tot} = k_0/C_{p_{mid}} \quad (1)$$

Cl_{tot} for AZT was estimated during treatment phases (low-dose and high-dose PBD coadministration) over consecutive sampling periods using Eq. (2), which permits interval-to-interval estimates when clearance is changing or when steady state has not yet been achieved (8):

$$Cl_{tot}{}_{t1}^{t2} = \frac{k_0 * (t_2 - t_1) - V_d * (C_{p_{t2}} - C_{p_{t1}})}{AUC_{t1}^{t2}} \quad (2)$$

Here k_0 is the infusion rate, V_d is the volume of distribution at steady state (11), and t_1 and t_2 are consecutive sampling times.

The fraction excreted unchanged (f_e) was calculated by dividing the Cl_r by the Cl_{tot} estimated in the corresponding interval. Nonrenal clearance (Cl_{nr}) was obtained as

$$Cl_{nr} = Cl_{tot} - Cl_r \quad (3)$$

Terminal half-lives for AZT were estimated by regres-

sion analysis of the postinfusion data, fitting at least the last five data points. V_{d_β} was estimated from these data as

$$V_{d_\beta} = t_{1/2} * Cl_{tot}/0.693 \quad (4)$$

Using the treatment period data, relative changes in Cl_r and Cl_{nr} of AZT for all rabbits were simultaneously fitted to the corresponding plasma PBD concentrations using PCNONLIN (12) to examine the inhibitory effect elicited by PBD on renal and nonrenal clearances of AZT, according to Eqs. (5) and (6), respectively (see Appendix):

$$\frac{\Delta Cl_r}{Cl_r} = \left(\frac{P}{K_i + P}\right) * f_s \quad (5)$$

$$\frac{\Delta Cl_{nr}}{Cl_{nr}} = \left(\frac{P}{K_i' + P}\right) * f_m \quad (6)$$

Cl_r and Cl_{nr} are the baseline renal and nonrenal clearances of AZT, respectively; ΔCl_r and ΔCl_{nr} represent decreases in these clearances from their corresponding baseline values; K_i is the dissociation of the PBD–transport complex characterizing the renal secretion system; P is the plasma concentration of PBD; the f_s is the fraction of the renal clearance of AZT subject to PBD inhibition. The parameter K_i' is the dissociation constant for the nonrenal (presumed to be metabolic) eliminating system common to PBD and AZT; f_m is the fraction of nonrenal clearance of AZT susceptible to PBD inhibition.

RESULTS

AZT Pharmacokinetic Data

The mean AZT plasma concentration–time profile observed in the rabbits is given in Fig. 1. AZT plasma concentrations from the control period for all the rabbits are almost identical. However, during the treated period there was an approximately twofold difference in plasma levels between the two groups at steady state. During the postinfusion period, the AZT elimination half-lives in the high-dose PBD-treated animals were significantly longer than those in the low-dose animals.

Figure 2 compares AZT plasma concentrations and total clearances for each rabbit at steady state (control vs treated)

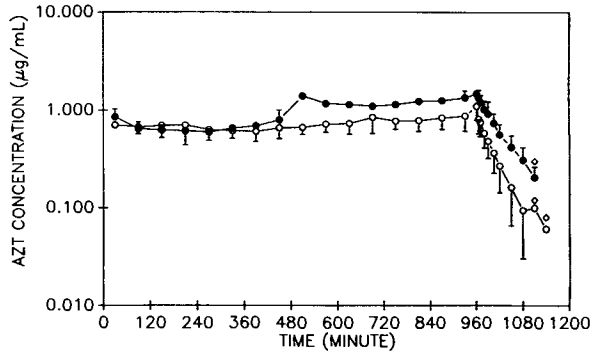

Fig. 1. Semilog plot of mean (±SD) AZT plasma concentration-time profile. (○——○) Low-dose PBD treatment; (●——●) high-dose PBD treatment. PBD administration begins at 480 min. (◇) The mean of only two rabbits.

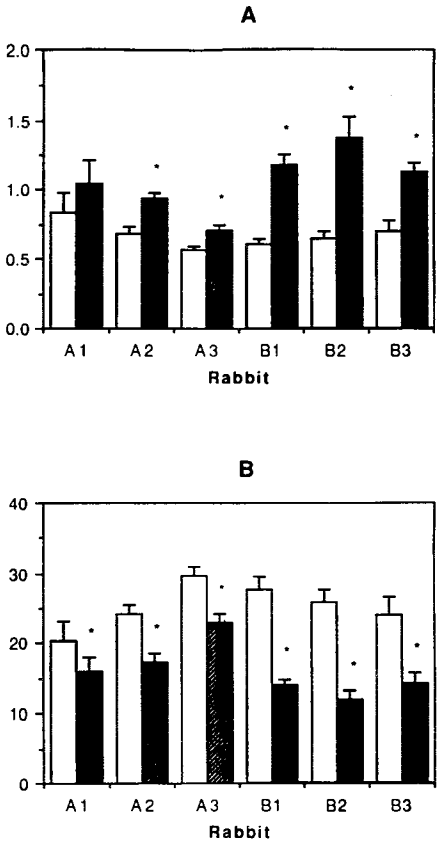

Fig. 2. AZT plasma concentrations (mean ± SD; A) and Cl_{tot} (mean ± SD; B) for each rabbit in the low-dose (Group A) and high-dose (Group B) groups during control (open bar) and treatment (shaded bar) period. (*) $P < 0.05$.

based on five data points measured during each period. For the low-dose PBD-treated rabbits (Group A), the Cl_{tot} during treatment was statistically different (t test) from the corresponding control values ($P < 0.05$). The parameters $C_{p_{ss}}$ and Cl_{tot} were statistically different from the corresponding control values ($P < 0.05$) for all high-dose PBD-treated rabbits (Group B). The fraction (%) of baseline renal and nonrenal clearances remaining during each treatment phase is summarized by animal in Fig. 3.

Results of the paired t test on the mean parameters (control vs treated) for each animal are shown for low-dose and high-dose treatments in Table I. AZT pharmacokinetic parameters during the control period were similar to values previously reported in untreated animals (11). In low-dose-treated rabbits, Cl_r and Cl_{nr} were reduced by approximately 20–25% during the coadministration of PBD. Cl_{tot} was decreased by 24.0 ± 4.0%, while $C_{p_{ss}}$ was increased by 29.3 ± 5.8%, with both sets of values statistically different from controls. With the high-dose PBD treatment all AZT pharmacokinetic parameters were substantially affected; Cl_r and Cl_{tot} were decreased by 55.7 ± 3.4 and 48.9 ± 7.4%, respectively, and $C_{p_{ss}}$ was increased by 87.5 ± 26%. These decreases in clearance strongly suggest that PBD inhibits both the renal and nonrenal elimination pathways. The fraction excreted in the urine unchanged, f_e, did not show any significant change in either low-dose or high-dose PBD treatment, supporting the observation that both renal and nonre-

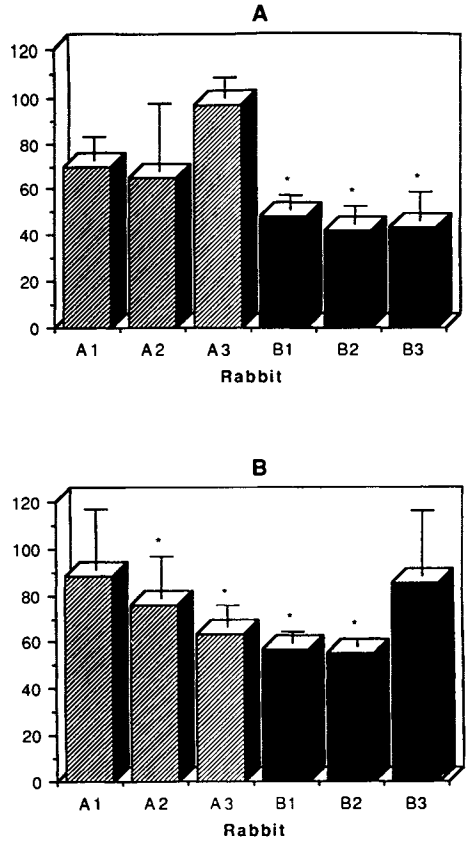

Fig. 3. Fraction (%) of baseline renal (mean ± SD; A) and nonrenal (mean ± SD; B) clearance remaining for each rabbit during low-dose (Group A) and high-dose (Group B) treatment. (*) $P < 0.05$.

nal pathways were affected to approximately the same degree.

A comparison of the terminal AZT half-life in untreated rabbits previously studied in our laboratory and low-dose PBD-treated animals in this study showed no significant difference (*t* test). However, differences in half-life were found between these untreated and the high-dose-treated rabbits, as shown in Table II. Figure 4 depicts the observed linear relationship between the terminal elimination rate constant and Cl_{tot} of AZT.

PBD Plasma Concentrations

Plasma concentrations of PBD during low-dose and high-dose infusion were 56.9 ± 12 and 248 ± 42 μg/ml, respectively, while the fluctuation about the mean ranged from 12.1 to 21.1% in individual rabbits. Although the plasma

Table I. Percentage Change (Mean ± SD) in AZT Pharmacokinetic Parameters During PBD Treatment

	Low dose (n = 3)	High dose (n = 3)
Cl_r	−20.7 ± 15	−55.7 ± 3.4*
Cl_{nr}	−24.3 ± 12	−37.2 ± 20
Cl_{tot}	−24.0 ± 4.0*	−48.9 ± 7.4*
C_{Pss}	+29.3 ± 5.8*	+87.5 ± 26*

* Significantly different from control (paired *t* test, $P < 0.05$).

Table II. AZT Pharmacokinetic Parameters (Mean ± SD) in Control and PBD-Treated Rabbits During Postinfusion Phase

		Treated	
	Untreated	Low dose (n = 3)	High dose (n = 3)
$t_{1/2}(\beta)$ (min)	48.7 ± 3.4 (n = 3)	39.0 ± 9.1	58.2 ± 4.6*
V_{d_β} (L/kg)	1.76 ± 0.27 (n = 4)	1.04 ± 0.076**	1.16 ± 0.065**
Cl_{tot} (ml/min · kg)	25.2 ± 3.2 (n = 6)	18.8 ± 3.8**	13.1 ± 1.2**

* $t_{1/2}(\beta)$ following high-dose treatment was significantly longer than following low-dose treatment or in untreated animals ($P < 0.05$).
** The values for PBD-treated animals were significantly less than for untreated animals ($P < 0.05$).

PBD levels during low-dose treatment were close to steady state during the last 5 hr of the treatment period, the high-dose PBD treatment produced plasma levels that increased with time (Fig. 5). The nonrenal clearance of PBD represented more than 90% of the total clearance, as has been observed in human and monkey (13,14).

Parameter Estimates

The relative inhibition of AZT renal clearance, $\Delta Cl_r/Cl_r$ as a function of PBD plasma concentration observed during control and PBD treatment periods was fitted to Eq. (5). Model parameters were obtained using PCNONLIN, with f_s and K_i estimated as 0.72 and 73 μg/ml, respectively. Figure 6 shows the observed and predicted relationship between relative inhibition (%) of AZT renal clearance and PBD plasma concentration. These results are consistent with those previously reported (11) and, further, suggest that the effect of PBD on AZT Cl_r is due to competitive inhibition of the secretion of AZT in the renal tubule (see Appendix).

The parameters of Eq. (6) estimated using PCNONLIN were 0.54 and 55 μg/ml for f_m and K_i', respectively. The observed and model-predicted relationship between relative inhibition of the nonrenal clearance of AZT and PBD plasma concentrations appears in Fig. 7. Estimates of the model parameters and the standard error of these estimates ob-

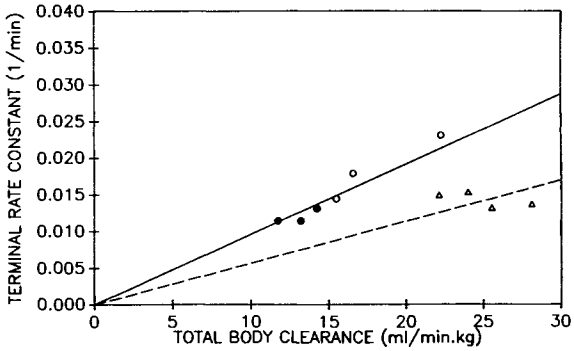

Fig. 4. Relationship between elimination rate constant and Cl_{tot} in untreated and PBD-treated rabbits: (○——○) Low-dose PBD treatment; (●——●) high-dose PBD treatment; (△——△) untreated rabbit. (-----) Regression line for untreated rabbits; (——) regression line for treated rabbits.

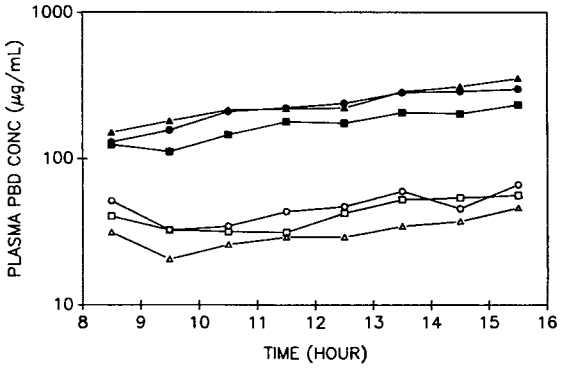

Fig. 5. Plasma probenecid concentrations during the 8-hr low-dose and high-dose probenecid infusions: open and filled symbols represent low- and high-dose treatments, respectively. Rabbit A1 (○—○); A2 (△—△); A3 (□—□). Rabbit B1 (●—●); B2 (▲—▲); B3 (■—■).

tained in the nonlinear regression analysis are summarized in Table III.

DISCUSSION

Probenecid, a drug with high therapeutic index, is often used in the treatment of gout. Because of its potent inhibitory effects on organic anion renal transport, this drug has also been used in combination with penicillin and amoxicillin to prolong effective plasma concentrations of these antibiotics (15,16). Probenecid has also been shown to reduce the renal clearance of a number of acidic drugs including carprofen (17), indomethacin (18), ketoprofen (19), naproxen (20), and zomepirac (21). Further, this drug is also reported to inhibit renal tubule secretion of organic cations such as cimetidine (22) and famotidine (23) in humans, demonstrating some overlap of specificity in renal transport inhibition. Because PBD is extensively metabolized in the liver, giving rise to side-chain oxidized products and their glucuronides (24), it is capable of inhibiting the metabolic clearance of drugs which are metabolized via ester or ether glucuronidation (17–21,25).

The Cl_r of AZT, calculated from the fraction of drug excreted unchanged, is 56.1 ± 13.8% of the Cl_{tot} in the control period of this study. Although the nonrenal elimination pathway for AZT in the rabbit has not yet been elucidated, it is not possible to detect the glucuronide metabolite in the

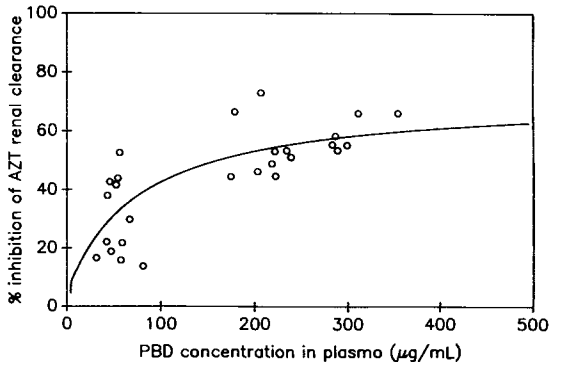

Fig. 6. Percentage inhibition of AZT renal clearance by PBD. Each datum point (○) represents one observation; (——) fitted curve.

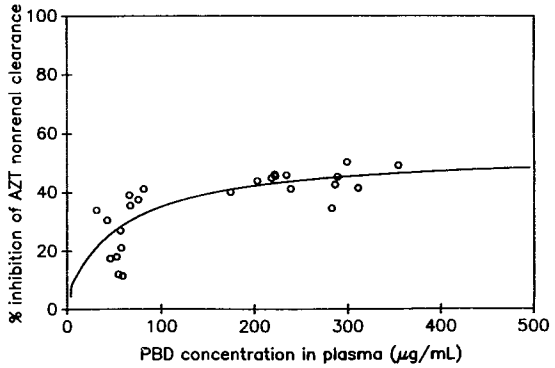

Fig. 7. Percentage inhibition of AZT nonrenal clearance by PBD. Each datum point (○) represents one observation; (——) fitted curve.

plasma or urine of rabbits receiving AZT intravenously (11). Nevertheless, the relative extents of inhibition in both Cl_r and Cl_{nr} brought about by PBD are similar (Table I). In humans, however, where glucuronidation is the predominant elimination pathway, it appears that the metabolic clearance of AZT is slightly more susceptible than renal clearance to PBD inhibition (4).

Although steady state was achieved for AZT plasma levels as early as the first hour in the control period, data from the first 3 hr of each infusion phase were omitted in the calculation of steady-state parameters. During the treatment period, incomplete attainment of steady state was often noted, and this was characterized by slowly increasing plasma levels of AZT during the infusion periods, particularly those associated with the high-dose PBD treatment. This likely resulted from the slow and continuous increase in PBD plasma levels (Fig. 5), which presumably produced gradual decreases in AZT clearances over this time course. Thus, estimates of AZT clearance over consecutive intervals in both low-dose and high-dose treatment phases were made using Eq. (2).

All animals in the high-dose treatment group showed a marked reduction in AZT Cl_r. In theory, this decrease may be attributable to an increase in plasma protein binding, decreased renal plasma flow, increased tubular reabsorption, or inhibition of active secretion in the renal tubule. AZT is 93 ± 3% unbound in human plasma over a concentration of 0.1–2.5 μg/ml and there was no significant change of this free fraction in the presence of PBD (4). In rabbit plasma AZT is greater than 95% unbound (11). Therefore, the decrease in Cl_r cannot be attributed to changes in AZT plasma binding induced by PBD. In theory, PBD could increase the reab-

Table III. Estimates[a] of Model Parameters for Competitive Inhibition of Renal [Eq. (5)] and Nonrenal [Eq. (6)] Clearances of AZT by PBD

	Inhibition constant (μg/ml)	Fraction subject to inhibition by PBD
Renal	K_i = 73 ± 24	f_s = 0.72 ± 0.078*
Nonrenal	K_i' = 55 ± 16	f_m = 0.54 ± 0.046*

[a] Parameter estimate ± SE.

* f_s and f_m are significantly different ($P < 0.05$).

sorption of AZT by providing an acidic medium on the luminal side of the renal tubule. However, because Cl_r of AZT is approximately three times the GFR in the rabbit, the most likely mechanism which explains the decrease in Cl_r is inhibition of active secretion.

One of the animals (B3) did not show a significant decrease in nonrenal clearance of AZT relative to baseline during high-dose probenecid treatment (Fig. 3). That this animal also exhibited the lowest plasma PBD levels (Fig. 5) suggests that the lack of treatment effect may have been due in part to a high PBD clearance. Nevertheless, the renal clearance of AZT in this animal was inhibited to approximately the same degree as that in the other rabbits during high-dose treatment.

A comparison of the terminal AZT half-life in untreated rabbits previously studied in our laboratory and low-dose PBD-treated animals in this study showed no significant difference (t test). However, differences in half-lives were found between the untreated and the high-dose-treated rabbits, as shown in Table II. The slopes of the regression lines in Fig. 4 are the reciprocals of the average V_{d_β} for untreated and PBD-treated animals. Although there was no difference between the average V_{d_β} in low-dose- and high-dose-treated rabbits (Table II), there was a significant difference in V_{d_β} between untreated and PBD-treated rabbits. Thus, the observed changes in AZT half-life may be attributed to a reduction in V_{d_β} as well as inhibition of Cl_{tot} brought about by PBD.

Probenecid has also been reported to reduce the volume of distribution of indomethacin (18), famotidine (23), and several penicillins (26) in man. The reduction in V_{d_β} of AZT in the current study in both treatment groups relative to untreated rabbits cannot be explained by changes in plasma protein binding caused by PBD. Alteration of tissue binding or changes in membrane transport of AZT due to PBD are possible mechanisms. The increase in steady-state plasma concentrations of AZT results from a significant reduction in Cl_{tot}, but the observed change in half-life, primarily the consequence of a reduced clearance, is tempered by an opposing effect of PBD on the volume of distribution. Although decreases in V_{d_β} do not reflect a corresponding change in $V_{d_{ss}}$ (27), these results are consistent with similar observation in humans (4) in which the $V_{d_{ss}}$ of AZT was decreased when PBD was coadministered.

In this study, PBD plasma concentrations are 30- to 300-fold higher than AZT, and its inhibition of AZT elimination is therefore much greater than the effect of AZT on PBD. The model-fitted parameters f_s and K_i are similar to those derived from nonsteady state experiments (11). The estimated fraction of renal clearance which is subject to inhibition (f_s) is 0.72, and this relative reduction in renal clearance was approached in high-dose-treated rabbits. Since there is no evidence that PBD changes the GFR of AZT in humans (4), the 28% of renal clearance which is not subject to PBD inhibition clearance corresponds closely to GFR. This suggests that secretion clearance is subject to complete inhibition by PBD in the rabbit. The model assumes that AZT and PBD are transported by the same carrier system in the tubular cells and that the same T_m characterizes the transport of both drugs.

The significant inhibition of AZT renal excretion produced by PBD is due to the relatively low K_i (73 μg/ml), which represents the plasma concentration of PBD at which 50% inhibition of the secretion clearance of AZT occurs. Plasma concentrations of PBD greater than 300 μg/ml were obtained during the high-dose treatments.

The parameter f_m, which is the fraction of nonrenal clearance of AZT subject to inhibition by PBD, was estimated as 0.54. This susceptible clearance represents about 25% of the Cl_{tot} in the rabbit. That this parameter is significantly less than f_s demonstrates that the extent to which the nonrenal clearance of AZT can be maximally inhibited is less than that for renal clearance. The low value of K_i' (55 μg/ml) was not significantly different from K_i, indicating that enzymes involved in the nonrenal elimination of AZT show a similar susceptibility to PBD inhibition as the renal secretion system. It is assumed here that a portion of the nonrenal elimination pathway is common for both AZT and PBD, with the same enzyme system being responsible for the elimination of both drugs.

In summary, the coadministration of PBD and AZT by intravenous infusion in the rabbit markedly elevates the plasma concentration of AZT at steady state by decreasing Cl_{tot}. The observed increase in the half-life of AZT occurs in spite of the reduction in volume of distribution since the decrease of Cl_{tot} is even greater. This decrease in Cl_{tot} is attributed to competitive inhibition of both renal secretion and nonrenal clearance of AZT. Although the proportion of the renal clearance which can be maximally inhibited by probenecid is higher than the corresponding fraction of nonrenal clearance, both show similar sensitivities to probenecid.

APPENDIX

Renal Clearance

The renal clearance of AZT can be represented by filtration (αGFR) and secretion clearance (Cl_s) in Eq. (A1). The secretion clearance can be further defined as $T_m/(K_s + C_p)$, which is subject to competetive inhibition by PBD. The renal clearance of AZT can be written as Eqs. (A2) and (A3) during control and treatment periods, respectively:

$$Cl_r = \alpha * GFR + Cl_s \quad \text{(A1)}$$

$$Cl_r = \alpha * GFR + \frac{T_m}{K_s + C_p} \quad \text{(A2)}$$

$$Cl_r' = \alpha * GFR + \frac{T_m}{K_s * (1 + [P]/K_i) + C_p} \quad \text{(A3)}$$

Here GFR is the glomerular filtration rate, α is the unbound fraction of AZT in the plasma, T_m is the maximum rate of transport, and K_s is the dissociation constant between AZT and the transport system. $[P]$ is the plasma concentration of PBD, K_i is the dissociation constant between PBD and the transport system, and C_p is the plasma concentration of AZT.

Previous studies have shown that AZT exhibits linear pharmacokinetics and constant renal clearance at plasma concentrations much higher than those in the present study, indicating that K_s is much greater than C_p. Thus, Eqs. (A2) and (A3), respectively, become

$$Cl_r = \alpha * GFR + \frac{T_m}{K_s} \quad (A4)$$

$$Cl_r' = \alpha * GFR + \frac{T_m}{K_s * (1 + [P]/K_i)} \quad (A5)$$

Assuming that αGFR is not affected by the presence of PBD (4), Eqs. (A6) and (A7) are obtained by subtracting Eq. (A5) from Eq. (A4), where $Cl_s = T_m/K_s$ in the absence of PBD (control period):

$$Cl_r - Cl_r' = \frac{T_m}{K_s} * \left(\frac{P}{K_I + P}\right) \quad (A6)$$

$$\frac{Cl_r - Cl_r'}{Cl_s} = \left(\frac{P}{K_i + P}\right) \quad (A7)$$

Multiplying both sides of Eq. (A7) by Cl_s/Cl_r (ratio of secretion clearance to total renal clearance), Eqs. (A8) and (A9) are obtained:

$$\frac{Cl_r - Cl_r'}{Cl_r} = \left(\frac{P}{K_i + P}\right) * \frac{Cl_s}{Cl_r} \quad (A8)$$

$$\frac{\Delta Cl_r}{Cl_r} = \left(\frac{P}{K_i + P}\right) * f_s \quad (A9)$$

Here, ΔCl_r is the decrease of renal clearance during the coadministration of PBD relative to the baseline renal clearance during the control period. The parameter f_s, equivalent to Cl_s/Cl_r, represents the fraction of renal clearance in control animals attributed to active secretion of AZT and susceptible to inhibition by PBD.

Nonrenal Clearance

The nonrenal clearance of AZT represents 70–80 and 40–50% of the total-body clearance in the human (2–4) and rabbit (11), respectively. Although the metabolic fate of AZT in the rabbit has not yet been elucidated, a previous report (11) and the current results demonstrate that the nonrenal clearance is inhibited by PBD. To model the extent of nonrenal clearance inhibition, it was assumed that only a portion of AZT's nonrenal clearance could be inhibited by PBD. This is reflected in Eq. (A10):

$$Cl_{nr} = Cl_i + Cl_n \quad (A10)$$

Cl_{nr} represents the total nonrenal clearance, whereas Cl_i and Cl_n represent the components of nonrenal clearance that are subject and not subject to inhibition by PBD, respectively.

Thus Eqs. (A11) and (A12) define the nonrenal clearance of AZT during control and treatment periods, respectively:

$$Cl_{nr} = Cl_n + \frac{V_m}{K_m} \quad (A11)$$

$$Cl_{nr}' = Cl_n + \frac{V_m}{K_m * (1 + [P]/K_i')} \quad (A12)$$

Because linear pharmacokinetics of AZT are observed over a broad range of plasma concentrations, Cl_i may be represented by V_m/K_m in the control situation. V_m is the maximum rate of nonrenal elimination of AZT via the pathway which is subject to inhibition by PBD. K_m and K_i' are Michaelis-type dissociation constants for AZT and PBD with the nonrenal eliminating system, respectively.

Subtracting Eq. (A12) from Eq. (A11) and dividing both sides by Cl_{nr}, Eqs. (A12) and (A13) are obtained:

$$\frac{Cl_{nr} - Cl_{nr}'}{Cl_{nr}} = \left(\frac{P}{K_i' + P}\right) * \frac{Cl_i}{Cl_{nr}} \quad (A12)$$

$$\frac{\Delta Cl_{nr}}{Cl_{nr}} = \left(\frac{P}{K_i' + P}\right) * f_m \quad (A13)$$

Here, ΔCl_{nr} is the decrease in nonrenal clearance during the coadministration of PBD relative to the baseline nonrenal clearance during the control period. The parameter f_m, which is equivalent to Cl_i/Cl_{nr}, represents the fraction of nonrenal clearance that is assumed to share the same enzymatic system and, hence, that is susceptible to inhibition by PBD.

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