Interaction of Zidovudine (Azidothymidine) with Isoprinosine and Probenecid in *Macaca fascicularis*

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INTRODUCTION

Zidovudine (ZDV; azidothymidine, AZT) is an anti-human immunodeficiency virus (HIV) drug approved in the United States for the treatment of AIDS. Due to the toxicity of ZDV and the potential for resistance (1,2) to its anti-HIV effect, combined therapy with other anti-HIV drugs has been advocated repeatedly. One proposed combination is isoprinosine and ZDV.

Isoprinosine (inosine pranobex, inosiplex, methisoprinol; ISO) is a synthetic immunomodulator formed from *p*-acetamidobenzoate salt of *N,N*-dimethylamino-2-propanol and inosine at a 3:1 molar ratio. *In vivo,* ISO exhibits antiviral, antitumor, and cytotoxic activities (3). Its antiviral activity is due, in part, to inhibition of HIV reverse transcriptase and immunopotentiation of HIV-infected lymphocytes (4). Several studies have demonstrated that ISO increases lymphocyte activity and production of interleukin I and II (5–7). Thus, this drug may have an important role in the treatment of AIDS, particularly in combination with other anti-HIV drugs (e.g., ZDV). ISO is rapidly metabolized in the body via several pathways, including oxidation and glucuronidation (3). It has been reported that ZDV plasma concentrations are increased when administered together with ISO (8). The mechanism(s) of this increase has not been delineated. The present study was designed (i) to confirm the findings of this interaction study in a representative animal model, the nonhuman primate (*Macaca fascicularis*), and (ii) to establish the mechanistic basis of this interaction. To do so, we have used probenecid, a drug which inhibits the metabolism of ZDV to glucuronide (9), as a positive control.

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MATERIALS AND METHODS

Materials

ZDV and ZDVG were gifts from Burroughs Wellcome Co. (Research Triangle Park, NC), and ^{3}H-ZDV (15 Ci/mmol) was purchased from Moravek Biochemical, Inc. (Brea, CA). PRO and 5-ethyl-5-(p-hydroxyphenyl)barbituric acid were purchased from Sigma (St. Louis, MO). ISO was supplied by Newport Pharmaceuticals International, Inc. (Laguna Hills, CA). All other solvents and reagents were of high-performance liquid chromatography (HPLC) grade (J. T. Baker, Inc., Phillipsburg, NJ).

The HPLC unit consisted of Waters solvent delivery system Model 501, Tunable absorbance detector Model 484, Gilson autosampler (Model 232), and Hewlett-Packard integrator (Model HP 3396A). The analytical column (C18; 5-μm cartridge; 250 × 4.6 mm) and the guard column (C18; 5 μm) were adsorbosphere (Alltech Associates, Inc., Deerfield, IL).

Study Design

Four healthy adult male monkeys (*M. fascicularis,* 4–5 kg) were used. Each animal had a femoral or a jugular vein cannulated under anesthesia more than 2 weeks prior to the study. On the study day, after overnight fasting, the animals were given 5 mg/kg ZDV (as a solution in water containing 5 μCi ^{3}H-ZDV) by gavage (under ketamine sedation, 5 mg/kg) alone or together with 25 mg/kg ISO or 15 mg/kg PRO in a randomized crossover design. ISO (25 mg/kg) or PRO (15 mg/kg) was administered (as a solution) twice daily (between 0900 and 1000 and between 1700 and 1800) by gavage, for 3 days prior to the study day. On separate occasions, three animals were also given an iv dose of ZDV (5 mg/kg, in normal saline) alone. Any two phases of the study were separated by at least 2 weeks. Blood samples (2 mL, in EDTA tubes) were collected over 6 hr, and plasma was stored at −20°C until analysis. Urine collected over 24 hr as described previously (10).

Plasma and Urine Analysis

To an aliquot (100 μL) of plasma was added 125 ng of the internal standard [5-ethyl-5-(*p*-hydroxyphenyl)barbituric acid]. Samples were then extracted with a 500-μL mixture of methylene chloride:ethyl acetate (1:1), vortexed for a few seconds, vigorously agitated for 15 min, and centrifuged for 5 min at 1500 *g*. The organic phase was evaporated under a stream of nitrogen at 50°C. The residue was reconstituted with 50 μL deionized water, and 40 μL was injected onto the HPLC. The analytes were detected at 266 nm after elution from the column at 1 mL/min with the mobile phase [acetonitrile:methanol:buffer (0.05% phosphoric acid and 0.15% ammonium hydroxide), 9:1:90, pH 3.2].

To determine ZDV concentrations in urine, 100 μL of urine was extracted as described for the plasma. To determine ZDVG concentration in urine, samples were diluted 1:50 (in deionized water) and 20 μL was assayed by direct injection, a method previously established in our laboratory (10). It was necessary to use two procedures to analyze urine

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samples, since occasionally it was noted that ISO or its metabolites (but not PRO) coeluted with the ZDV peak in the diluted blank urine samples but not in the extracted urine or plasma samples. The percentage of the dose recovered in 24-hr urine was estimated by counting an aliquot (100 μL) of urine using a Packard liquid scintillation analyzer (Packard, Downers Grove, IL).

RESULTS

The retention times of ZDV, ZDVG, and the internal standard in this system were 12.8, 8.9, and 16.2 min, respectively. The calibration curves of ZDV and ZDVG were linear ($r^2 > 0.999$) over a wide range of concentrations (0.01–40 μg/mL). The intraday coefficient of variation was 5.1% (n = 9), 1.2% (n = 10), and 3.9% (n = 10) at ZDV plasma concentrations of 0.1, 1, and 2 μg/mL, respectively.

Figure 1 illustrates the plasma concentration–time profiles for each animal following oral administration of ZDV with and without ISO or PRO. The rate of absorption of ZDV is variable, resulting in a wide range of peak plasma concentrations and times to reach peak. Since the animals often demonstrated a "hump" (possibly due to enterohepatic recycling) in the terminal portion of the plasma concentration–time profile, the total area under the curves (AUC) to time infinity could not be estimated by extrapolation. Thus, a truncated AUC ($AUC_{0-6\ hr}$) was estimated using the trapezoid rule (Table I) (11). However, we believe that $AUC_{0-6\ hr}$ is probably a reasonable estimate of $AUC_{0-\infty}$, since by 6 hr the ZDV plasma concentration had reached a small fraction (<10%) of the peak plasma concentration in most of the animals. Although the mean truncated AUC for the ISO arm is not significantly different ($P > 0.05$, paired t test) from that after ZDV alone, the mean truncated AUC for the PRO arm is 81% greater ($P = 0.048$) than that obtained after ZDV alone and 96% greater ($P = 0.042$) than that obtained after ZDV plus ISO.

Although the ZDVG/ZDV ratios are similar for the ISO- and ZDV-alone arms, there is a decrease ($P > 0.05$, paired t test) in the molar concentration ratio of ZDVG/ZDV for the PRO arm (Table II). In addition, the percentage of total radioactivity recovered was comparable for the three treatments (ZDV alone, 64.5 ± 8.4%; ZDV and ISO, 67.4 ± 6.0%; ZDV and PRO, 65.6 ± 7.0%).

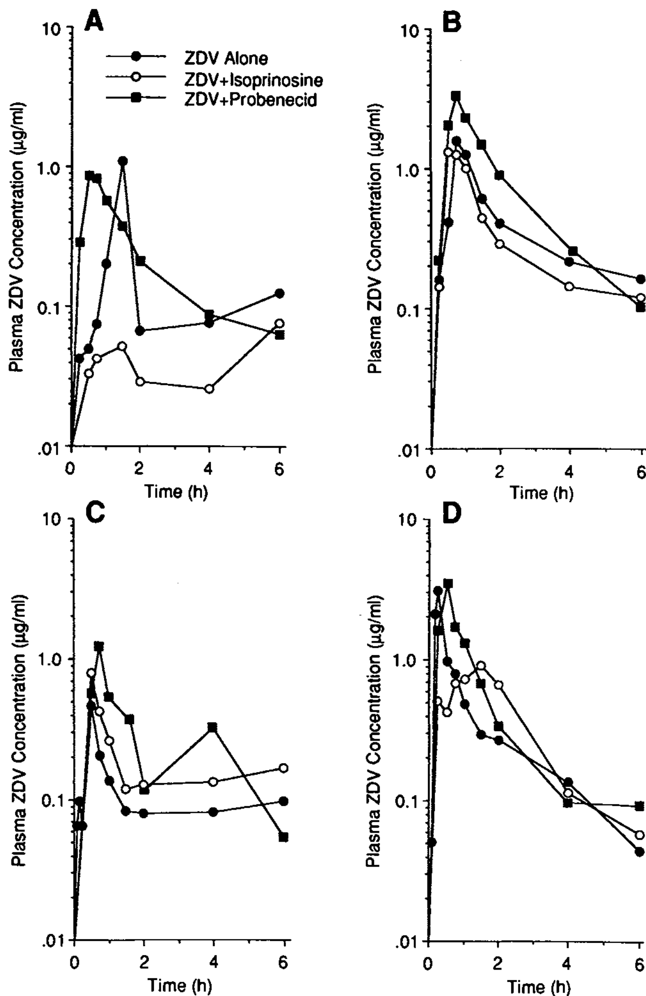

Fig. 1. Plasma concentration–time profile of zidovudine (ZDV) following oral administration alone (●), with isoprinosine (○), or with probenecid (■) in *M. fascicularis*.

DISCUSSION

The mean percentage ZDV dose recovered in the urine as radioactivity did not significantly differ among the three treatment arms and was similar to that observed after iv administration (77.2 ± 0.4%). Thus, any difference in mean AUC values between treatment arms cannot be attributed to differences in the fraction of dose absorbed. Based upon this argument, PRO (used here as a positive control) decreased ZDV clearance. The mechanisms responsible for this change may be a decrease in the metabolism of ZDV to ZDVG, the renal clearance of ZDV, or both. That the metabolism of ZDV to ZDVG may be decreased by PRO is supported by the decrease in the mean urinary ratio of ZDVG/ZDV in the presence of PRO. The latter did not reach statistical significance compared with ZDV alone, probably because PRO has been shown to inhibit the renal clearance and the metabolic clearance of ZDV and because of the significant interanimal variability. However, our findings do agree with those reported in humans (9,12), rabbits (13), and rats (14).

When ZDV is administered with ISO, absorption of ZDV is delayed in two of four animals (A, D). It is possible that ISO may interact with ZDV in the gastrointestinal tract or slow its absorption by an, as yet, unknown mechanism(s).

Table I. Area Under the Plasma Concentration–Time Profile ($AUC_{0-6\ hr}$) of ZDV, Following Oral Administration of ZDV with and Without Isoprinosine or Probenecid

	$AUC_{0-6\ hr}$ (μg · hr/mL)		
Animal	ZDV alone	ZDV + ISO	ZDV + PRO
A	1.028	0.230	1.466
B	2.387	2.047	4.682
C	0.648	1.067	1.754
D	2.104	2.250	3.260
Mean	1.541	1.398	2.790*
SD	0.835	0.934	1.486

* Significantly higher than that after ZDV alone or ZDV + ISO.

Table II. Molar Ratio of ZDVG/ZDV in 24-hr Urine, Following Oral Administration of ZDV with and Without Isoprinosine

Animal	ZDV alone	ZDV + ISO	ZDV + PRO
A	6.53	8.10	1.72
B	4.22	2.01	1.26
C	0.32	4.86	1.99
D	1.80	3.23	2.13
Mean	3.22	4.55	1.77
SD	2.73	2.64	0.38

However, ISO does not appear to reduce the extent of absorption of ZDV since there was no change in the percentage of radioactivity recovered in the urine compared with that obtained with administration of ZDV alone.

In contrast to the findings of DeSimone *et al.* (4, 8) in humans, ISO does not decrease the clearance of ZDV in the macaque. It is not clear why our findings differed from those of DeSimone *et al.* However, several elements of the study raise questions about the validity of their results. First, a radioimmunoassay was used for the measurement of ZDV. No data are provided on the cross-reactivity of the antibodies with ISO or its metabolite(s). Second, their study was not a crossover study, and thus a period effect cannot be neglected. It should be noted that in the DeSimone study, 1 g of ISO was given every 6 hr for 7 days, and the dose of ZDV was 100 mg given every 6 hr. Although the ratios of ZDV/ISO and ZDV/PRO doses in our study were kept identical to that used in the human studies (4,12), it is possible that both the dose and the duration of ISO treatments in these monkeys were not sufficient to demonstrate the interaction clearly. The latter is an unlikely hypothesis, since the half-life of ISO in humans is extremely short (50 min) (3) and should not lead to accumulation of the drug. Alternatively, the macaque may not be a good animal model in which to reproduce this interaction, despite the fact that it is an excellent animal model for the disposition of ZDV (10,15,16).

In conclusion, our study failed to demonstrate that ISO decreases the clearance of ZDV in macaques.

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