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Pharmacokinetics of Anti-HIV Nucleosides in Microswine

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Abstract—The objective of the study was to determine if the micropig (*Sus scrofa*) would serve as an animal model for the anti-HIV nucleosides 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI), and 2′,3′-dideoxycytidine (ddC). Four adult male micropigs were administered i.v. 20 mg kg^{-1} AZT, 20 mg kg^{-1} ddI, both as 10 min infusions, and 5 mg kg^{-1} ddC, as an i.v. bolus. At least 12 days separated each drug administration. Following each drug administration, blood samples were collected by venipuncture and urine was collected by placement of the animals in metabolism cages. Concentrations of parent drug and AZT's glucuronide metabolite were quantitated in plasma and urine by HPLC. Data were analysed by non-compartmental methods to obtain pharmacokinetic parameters for each drug. Total and renal clearances for AZT, 0·482 ± 0·058 and 0·326 ± 0·075 L h^{-1} kg^{-1}, respectively, and for ddI, 0·500 ± 0·057 and 0·337 ± 0·100 L h^{-1} kg^{-1}, showed that these drugs were eliminated primarily by renal excretion rather than by liver metabolism as in man. ddC's clearances were similar to rates in man. Volume of distribution at steady state values were 0·784 ± 0·071, 1·192 ± 0·288 and 0·886 ± 0·199 L kg^{-1} for AZT, ddI and ddC, respectively. Half-life values for AZT, ddI and ddC were 1·39 ± 0·127, 2·585 ± 0·243 and 1·832 ± 0·380 h, respectively. Based on these findings, the micropig could be an appropriate model for the study of ddC in man, but not for AZT of ddI.

Animal populations provide a controlled environment in which to study new drugs and determine pharmacokinetic characteristics useful in the design of dosing regimens in man, as well as in the assessment of drug toxicity. Identification of appropriate animal models for anti-HIV drugs in man is important for obtaining comprehensive pharmacokinetic data. A variety of animal species has been used for anti-HIV nucleoside pharmacokinetic studies, with monkeys having the most similar pharmacokinetic attributes to man. As monkeys are expensive, and more hazardous to work with than other animals, it was desirable to use an alternate species for pharmacokinetic investigations. Although rodents are relatively inexpensive and easy to study, they do not metabolize 3′-azido-3′-deoxythymidine (AZT) or 2′,3′-dideoxyinosine zidorudine, (ddI) to the same extent as in man (Patel et al 1990; Ray et al 1990) and, therefore, are unsatisfactory for examining factors affecting metabolism. Similarly, rabbits, cats and dogs do not produce the glucuronide metabolite, 3′-azido-3′-deoxy-5′-*O*-β-D-glucopyranuronosyl thymidine (AZTG) of AZT to the same extent as primates. Swine have several similarities to man in anatomy, physiology, biochemistry and pharmacology suggesting their usefulness as experimental animals (Bustad 1966; Dodds 1982), and are already recognized as valuable animal models in many areas of biomedical research. Miniature swine (*Sus scrofa*) offer the added benefits of small size, early reproductive maturity and ease of handling (Panepinto & Phillips 1986). The micropig is the same species as the popular Yucatan miniature pig, selected for its even smaller size, 16–20 kg, at six months of age.

The objective of the current study was to determine in what capacity micropigs would serve as animal models for the pharmacokinetics of anti-HIV nucleosides. AZT, ddI, and 2′,3′-dideoxycytidine (ddC) were studied as they are currently the most important anti-HIV nucleosides.

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Materials and Methods

Materials

AZT and AZTG were kindly provided by Burroughs Wellcome (Research Triangle Park, NC, USA). ddI was similarly provided by Bristol-Meyers Squibb (Wallingford, CT, USA). ddC was provided by Dr Karl Flora of the National Cancer Institute (Bethesda, MD, USA). AZddU (3′-azido-2′3′-dideoxyuridine) was provided as an internal standard by Dr David Chu, University of Georgia, USA. HPLC grade methanol and acetonitrile were obtained from J T Baker Inc. (Phillipsburg, NJ, USA). All other chemicals were reagent grade.

Four adult male micropigs (*Sus scrofa*) were used throughout the study. The pigs, 9–12 kg, were obtained from Charles River Laboratories (Wilmington, MA, USA) at three months of age and were housed at the farm facilities of the University of Georgia College of Veterinary Medicine. The mean weights of the pigs during the AZT, ddC and ddI parts of the study were 14·19 ± 2·61, 18·53 ± 4·07 and 19·43 ± 5·12 kg, respectively.

Methods

Sample collection. For dosing and blood collection, each pig was transferred to a Panepinto sling, a hammock-like device which provided access to the ear and jugular veins without anaesthesia. Blood samples of approximately 1 mL were collected by venipuncture in either the ear or jugular vein. Between blood collection times, the pigs were kept in metabolism cages to facilitate the collection of urine. Blood samples were placed in heparinized microcentrifuge tubes and centrifuged to yield plasma. Plasma and urine were stored at −20°C until analysis by HPLC.

AZT. Each pig was administered 20 mg kg^{-1} AZT, prepared in 0·9% NaCl (saline) i.v. as a zero-order rate infusion over 10 min. Blood samples were collected at 10, 25, 40, 70, 100,

130, 190, 250, 370, 490 and 610 min after the start of the infusion.

AZT and AZTG were analysed in plasma and urine by the solid phase extraction method described by Qian et al (1991). To 200 μL of plasma, 10 μL of internal standard (AZddU, 30 μg mL^{-1}) was added, and the mixture vortexed for 30 s. Bond Elut C-18 cartridges (Analytichem International, Harbor City, CA, USA) (3 mL capacity) were activated by rinsing with 3 mL of methanol, followed by 2 × 3 mL of phosphate buffered saline (PBS). The plasma samples were loaded on the cartridges, allowed to equilibrate for 10 min, then rinsed with 2 × 1 mL PBS. Finally, AZT, AZTG and the internal standard were eluted with 2 × 1 mL methanol. The eluents were evaporated to dryness under a stream of nitrogen at 50°C and reconstituted with 200 μL mobile phase. An aliquot was injected onto the HPLC system.

Urine volume was measured and filtered through 0·22 μm filters (Millex-GS, Millipore Products Division, Bedford MA, USA). Filtered urine was stored at −20°C until analysis. Ten μL of AZddU (1 mg mL^{-1}) and 970 μL of deionized water were added to 20 μL of filtered urine. The sample was vortexed for 30 s and 20 μL was injected directly onto the HPLC system.

The HPLC system included a 150 mm × 4·6 mm (i.d.) C18 analytical column (Hypersil, Alltech Assoc., Deerfield, IL, USA) preceeded by a guard column packed with 30–40 μm pellicular RP-18 Perisorb material (Upchurch Scientific, Inc., Oak Harbor, WA, USA). For the separation of AZT, AZTG and AZddU the mobile phase consisted of 8% (v:v) acetonitrile:deionized water, with the final pH adjusted to 2·5 with H_3PO_4. Mobile phase was pumped at 2·0 mL min^{-1}. The UV detector was set at 267 nm. Retention times for AZT, AZTG and AZddU were 4·3, 6·2 and 8·0 min, respectively. The lower limit of quantitation for both AZT and AZTG in plasma was 100 ng mL^{-1}.

ddI. Each pig received 20 mg kg^{-1} of ddI, prepared in normal saline, i.v. as a constant rate infusion over 10 min. Blood samples were collected at 10, 20, 30, 40, 55, 70, 100, 130, 190, 250, 370, 490 and 600 min from the start of the infusion. Urine was collected in the metabolism cage for the entire 10 h study period.

Analysing ddI in plasma again required the use of solid phase extraction. To 100 μL of plasma, 10 μL of internal standard (AZddU, 50 μg mL^{-1}) was added and the sample vortexed. Bond Elut cartridges (1 mL capacity) were activated by rinsing with 1 mL methanol followed by 2 × 1 mL of deionized water. Samples were loaded, equilibrated for 10 min, rinsed with 2 × 1 mL deionized water and eluted with 2 × 1 mL methanol. Eluents were evaporated to dryness under nitrogen at 50°C and reconstituted with 200 μL of mobile phase. A sample was injected into the HPLC system. Urine samples were filtered and prepared as for AZT analysis.

Mobile phase of 4% (v:v) acetonitrile:30 mM Na_2HPO_4 in water, final pH 7·0, was pumped at 2 mL min^{-1}. UV detection was at 254 nm. Retention times were 5·7 and 10·4 min for ddI and AZddU, respectively. The lower limit of quantitation was 100 ng mL^{-1}.

ddC. Each pig received 5 mg kg^{-1} of ddC by i.v. bolus injection. Blood samples were collected at 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480 and 600 min after the injection. Urine was collected over the 10 h study period.

To a 100 μL ddC plasma sample, 10 μL of internal standard (ddI, 100 μg mL^{-1} was added, vortexed and transferred to a Centrifree tube (Amicon Division, W-R Grace and Co.-Conn, Beverly, MA, USA). The tubes were centrifuged at 4000 rev min^{-1} for 25 min and a sample of the ultrafiltrate was injected onto the HPLC system.

Filtered urine (20 μL) was vortexed with 10 μL of internal standard (ddI, 400 μg mL^{-1}) and 970 μL of deionized water, and 20 μL was injected onto the HPLC.

The mobile phase for ddC determination in plasma and urine consisted of 3% (v:v) acetonitrile:20 mM Na_2HPO_4 in water, final pH 7·0. The flow rate was 1·5 mL min^{-1} and the UV detector was set for 273 nm. Retention times for ddC and ddI were 4·7 and 11·5 min, respectively. The sensitivity limit for ddC in plasma was 100 ng mL^{-1}.

Data analysis. Non-compartmental analysis was used to determine the following pharmacokinetic parameters from the plasma concentration-time and urine data: total systemic clearance (CL_t renal clearance (CL_r) elimination half-life ($t_{\frac{1}{2}}$), volume of distribution at steady state (V_{SS}) and percent of dose excreted unchanged in urine (f_e). The equations for these parameters are as follows:

$$CL_t = \text{dose}/AUC \quad (1)$$

$$CL_r = X_u/AUC \quad (2)$$

$$t_{\frac{1}{2}} = 0{\cdot}693/\lambda_N \quad (3)$$

For AZT and ddI:

$$V_{SS} = \frac{\text{dose}\cdot AUMC}{AUC^2} - \frac{\text{dose}\cdot T}{X\cdot AUC} \quad (4)$$

For ddC: $V_{SS} = \text{dose}\cdot AUMC/AUC^2$ (5)

$$f_e = X_u/\text{dose}\cdot 100$$
(adjusted for mol. wt in the case of AZTG) (6)

X_U is equal to the total amount of drug in urine at 10 h. T is the infusion duration. The area under the plasma concentra-

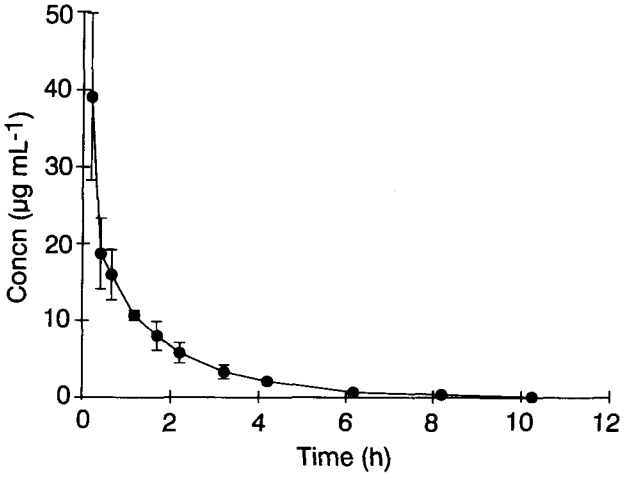

FIG. 1. Plasma concentrations (mean ± s.d.) of AZT in micropigs after administration of 20 mg kg^{-1} of AZT as a constant rate infusion over 10 min.

Table 1. Pharmacokinetic parameters of AZT and GAZT in micropigs following administration of 20 mg kg^{-1} as a 10 min i.v. infusion.

	AZT					GAZT		
Animal	CL_t (L h^{-1} kg^{-1})	CL_r (L h^{-1} kg^{-1})	V_{SS} (L kg^{-1})	$t_{\frac{1}{2}}$ (h)	f_e (%)	CL_r (L h^{-1} kg^{-1})	$t_{\frac{1}{2}}$ (h)	f_e (%)
B	0·522	0·384	0·798	1·543	71·3	0·258	1·577	3·7
K	0·429	0·223	0·716	1·366	51·8	0·258	1·576	4·1
L	0·436	0·319	0·746	1·417	72·4	0·270	1·191	6·3
E	0·541	0·379	0·877	1·235	71·7	0·510	1·577	8·4
Mean	0·482	0·326	0·784	1·390	66·8	0·338	1·480	5·6
s.d.	0·058	0·075	0·071	0·127	10·0	0·117	0·193	2·175

tion-time curve (AUC) and first non-normalized moment (AUMC) were determined by polynomial interpolation and integration from time zero to the last measured sample time with extrapolation to infinity using the least square terminal slopes (Rocci & Jusko 1983). For the short term infusions, C_o was set equal to zero, whereas, for the bolus dose of ddC, C_o was set equal to the first measured concentration. λ_N is the terminal disposition rate constant, and was obtained from the terminal slope of the natural log of concentration vs time curve.

Table 2. Pharmacokinetic parameters of ddI in micropigs following administration of 20 mg kg^{-1} as a 10 min i.v. infusion.

Animal	CL_t (L h^{-1} kg^{-1})	CL_r (L h^{-1} kg^{-1})	V_{SS} (L kg^{-1})	$t_{\frac{1}{2}}$ (h)	f_e (%)
B	0·561	0·388	1·602	2·921	65·0
K	0·530	0·435	1·145	2·497	78·5
L	0·474	0·321	1·088	2·575	65·1
E	0·434	0·204	0·933	2·347	45·2
Mean	0·500	0·337	1·192	2·585	63·45
s.d.	0·057	0·100	0·288	0·243	13·72

Results and Discussion

Pharmacokinetic results for AZT (Fig. 1) and AZTG are presented in Table 1. The total systemic clearance in micropigs. 0·482 ± 0·058 L h^{-1} kg^{-1}, was much less than the value of 1·3 ± 0·3 L h^{-1} kg^{-1} in man (Klecker et al 1987). The renal clearance is greater in pigs and the amount of AZT excreted in the urine of pigs, 66·8 ± 10·0%, far surpasses that found excreted in urine by man, 18% (Klecker et al 1988). In man AZT is metabolized to AZTG followed by renal excretion, whereas pigs excrete very little AZTG; AZTG recovered in the urine of man and pig is 63 and 5·6%, respectively. These results are consistent with the report of Haumont et al (1990) that showed in-vitro pig liver microsomal glucuronidation rates of AZT to AZTG were half those measured in monkeys and human microsomes. The volume of distribution in pigs, 0·784 ± 0·071 L kg^{-1}, is half that of man, 1·4 L kg^{-1} (Klecker et al 1987), and suggests more extensive drug binding to tissue components in man. The combined reductions in volume and clearance in pigs results in an elimination half-life of AZT (1·39 ± 0·127 h) that is comparable with values (1·1 h) in man (Klecker et al 1987).

Total clearance of ddI was lower in pigs (0·500 ± 0·057 L kg^{-1} h^{-1}) (Fig. 2) than in man (1·01 L kg^{-1} h^{-1} (Hartman et al 1990)). Renal clearance, however, was comparable in man and in pigs; 0·36 L kg^{-1} h^{-1} in man vs 0·337 ± 0·100 L kg^{-1} h^{-1} in pigs (Hartman et al 1990). Nonetheless, humans excrete only 36% of the ddI dose in urine compared with 63·45 ± 13·72% excreted by pigs. This indicates that in man ddI is primarily eliminated by non-renal pathways, similar to AZT, whereas the renal pathway is more important for pigs.

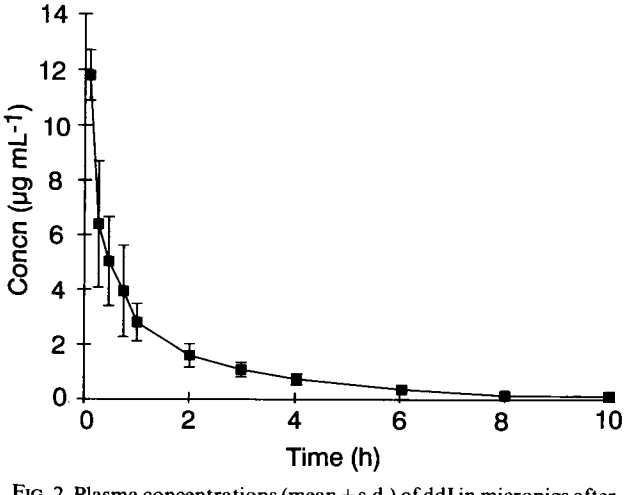

FIG. 2. Plasma concentrations (mean ± s.d.) of ddI in micropigs after administration of 20 mg kg^{-1} of ddI as a constant rate infusion over 10 min.

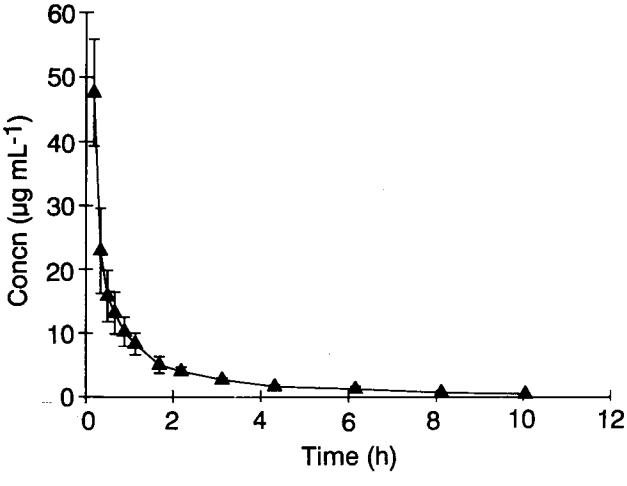

FIG. 3. Plasma concentrations (mean ± s.d.) of ddC in micropigs after administration of 5 mg kg^{-1} as an i.v. bolus.

Table 3. Pharmacokinetic parameters of ddC in micropigs following administration of 5 mg kg^{-1} as an i.v. bolus.

Animal	CL_t (L h^{-1} kg^{-1})	CL_r (L h^{-1} kg^{-1})	V_{SS} (L kg^{-1})	$t_{\frac{1}{2}}$ (h)	f_e (%)
B	0·413	0·381	0·903	1·772	90·89
K	0·511	0·395	0·955	1·514	72·71
L	0·315	0·281	0·618	1·661	88·24
E	0·423	0·315	1·099	2·379	69·37
Mean	0·416	0·343	0·886	1·832	80·30
s.d.	0·080	0·054	0·199	0·380	10·84

The steady-state volume of distribution of ddI in micropigs is similar to that in man, $1·192 \pm 0·288$ L kg^{-1} compared with 1·01 L kg^{-1} (Hartman et al 1990). The pharmacokinetic parameters for ddI are shown in Table 2.

Total and renal clearances of ddC in pig (Fig. 3) and man (Gustavson et al 1990) are more similar than for AZT or ddI (see Table 3). The fraction excreted unchanged in the urine was $80·3 \pm 10·84$% for pig and 75% for man (Klecker et al 1988), and indicates that both pig and man eliminate ddC primarily by the renal pathway. The volume of distribution in pigs is $0·886 \pm 0·199$ L kg^{-1} and in man, 0·54 L kg^{-1} (Klecker et al 1988). Half-life in pig and man is respectively $1·832 \pm 0·380$ and $1·2 \pm 0·6$ h (Klecker et al 1988). In conclusion, AZT and ddI undergo greater metabolism in man than in pigs, and this limits the usefulness of pigs as animal models, particularly when metabolic interactions are investigated. In the case of ddC, however, this study suggests comparable elimination pathways in man and micropig. Thus, the micropig may be a valuable model for studies on the pharmacokinetics of ddC.

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