Comparative pharmacokinetics of Racivir®, (±)-β-2′,3′-dideoxy-5-fluoro-3′-thiacytidine in rats, rabbits, dogs, monkeys and HIV-infected humans

Selwyn J Hurwitz[1], Michael J Otto[2] and Raymond F Schinazi[1*]

[1]Department of Pediatrics, Emory University School of Medicine and Veterans Affairs Medical Center, Decatur, GA, USA
[2]Pharmasset Inc, Tucker, GA, USA

*Corresponding author: Tel: +1 404 728 7711; Fax: +1 404 728 7726; E-mail: rschina@emory.edu

Racivir® is a 50:50 racemic mixture of the (–)- and (+)-β-enantiomers of 2′-deoxy-3′-thia-5-fluorocytosine (FTC), which is being developed for the treatment of HIV and hepatitis B virus (HBV). The (+)-enantiomer of FTC is approximately 10–20-fold less potent than (–)-FTC, but it selects for a different HIV mutation in human lymphocytes. Plasma concentrations from a group of 54 rats, 12 pregnant rabbits and 60 dogs enrolled in large toxicity studies using a wide variety of oral doses, were compared using non-compartment pharmacokinetic modelling versus dose, treatment duration, species and gender. The pharmacokinetics of Racivir® were also compared with those of a previously published pharmacokinetic study in rhesus monkeys and with data from HIV-infected human male volunteers. The (+)-FTC, but not the (–)-enantiomer, can be deaminated to the non-toxic inactive metabolite (+)-FTU. Therefore, the plasma exposure to (+)-FTU was also determined. The order of relative plasma exposure to (+)-FTU was rhesus monkeys > humans > pregnant rabbits > dogs > rats. Allometric scaling was performed to relate systemic clearance/fraction of drug absorbed (Cl/F) and terminal phase volume of distribution (V_β/F) versus species body weights. No individual animal species mimicked the Cl/F values in humans. However, allometric scaling using a combination of rats, pregnant rabbits and monkeys predicted the mean human Cl/F value better than a combination of rats and rabbits only (within 0.24 and SD of mean vs 0.81 SD of the observed mean value). Similarly, human V_β/F values were best predicted using a combination of rat and monkey data (within 0.64 SD of mean value). Species demonstrating greater deamination to (+)-FTU tended to have greater than predicted Cl/F values. The C_{max} values of dogs were the closest to humans, but were statistically different. This study highlights the importance of selecting animal species that demonstrate similar cytidine deaminase activity to humans when performing preclinical dosing studies on Racivir® and other antiviral agents that are substrates for mammalian cytidine deaminases.

Keywords: Racivir, FTC, (+)-FTU, interspecies pharmacokinetics, allometric scaling, antiretroviral nucleoside, cytidine deaminase

Introduction

Racivir® (RCV) is a 50:50 racemic mixture of the (–)- and (+)-enantiomers of 2′-deoxy-3′-thia-5-fluorocytosine (FTC) and is being developed by Pharmasset Inc for use in the treatment of HIV and hepatitis B virus (HBV) infection. The (–)-enantiomer of β-2′,3′-dideoxy-5-fluoro-3′-thiacytidine [(–)-FTC; emtricitabine] has potent and selective antiviral activity against HBV and HIV-1 *in vitro* and *in vivo* (Darque *et al.*, 1999; Furman *et al.*, 1992; Korba *et al.*, 2000; Molina *et al.*, 2000; Schinazi *et al.*, 1992, 1994), and is approved for use in combination with other antiretroviral agents for the treatment of HIV (Emtriva™, Gilead Sciences, Foster City, CA, USA). Mechanistic studies indicated that the triphosphate of (–)-FTC is a more potent inhibitor of HIV-1 reverse transcriptase than the triphosphate of lamivudine (3TC) (Feng *et al.*, 1999; Ray *et al.*, 2002, 2003). Testing of the resolved enantiomers has revealed (+)-FTC to be a non-toxic and approximately 40-fold less potent inhibitor of HBV replication than (–)-FTC (Doong *et al.*, 1991; Furman *et al.*, 1992). However, the difference in the anti-HIV activities in culture with human PBMCs is only 10-fold (Schinazi *et al.*, 1992a). Furthermore, the triphosphates of (–)-FTC and (+)-FTC have comparable K_i values (2.8 and 8.6 μM, respectively) for purified HIV-1 reverse transcriptase (Schinazi *et al.*, 1992a). Preliminary studies indicate that, when (+)-FTC is incubated in the whole blood of various mammals, it can be

converted to varying extents to the non-toxic metabolite (+)-β-2′,3′-dideoxy-5-fluoro-3′-thiauridine [(+)-FTU], due to varying cytidine deaminase levels and substrate specificity of this enzyme (Schinazi *et al.*, 1992b; Frick *et al.*, 1993, 1994; Cui *et al.*, 1996; Moore *et al.*, 1997). (–)-FTC was stable to deamination, regardless of the mammalian species tested, although some deamination by bacterial cytidine deaminase has been observed (Furman *et al.*, 1992).

Since the livers of most species may have greater cytidine deaminase levels than the blood, *in vivo* pharmacokinetic studies were considered pertinent to determine the extent of deamination of RCV between species (Camiener & Smith, 1965). Cell-based studies suggest that (+)-FTC may potentiate the anti-HIV activity of (–)-FTC. Studies in the HuPBMC-SCID mouse model of HIV infection indicate that administration of RCV resulted in a higher peak plasma concentration (C_{max}) value for the (–)-FTC enantiomer and an enhanced inhibition of HIV levels than a comparative dose of (–)-FTC alone (Ussery *et al.*, 1995; Black *et al.*, 2000). Furthermore, *in vitro* resistance studies suggest that the RCV, like (–)-FTC, selects for M184V, but at a slower rate. In addition, RCV inhibited the development of the T215Y resistance mutation that is associated with resistance to antiretroviral agents such as zidovudine (AZT) and stavudine (d4T) (Schinazi *et al.*, 1997; Marcelin *et al.*, 2004). Therefore, treatment regimens with RCV may offer some advantages over (–)-FTC in the clinical setting.

The single-dose pharmacokinetics of RCV have previously been studied in a small number of male rats after intravenous administration (10, 50 and 100 mg/kg, *n*=6 per group) and in male rhesus monkeys (33.3 mg/kg, *n*=3) (Abobo *et al.*, 1994; Schinazi *et al.*, 1992b). The primary objective of this study was to describe the pharmacokinetics obtained from preclinical studies in rats, rabbits, dogs, monkeys and HIV-infected humans enrolled in a Phase I clinical study, and to determine if any one species could be used to predict the pharmacokinetics of RCV in humans.

Materials and methods

RCV and (+)-FTU were synthesized in our laboratories or by Samchully Pharmaceutical Co, Seoul, South Korea, using a previously described method (Choi *et al.*, 1991). The detailed synthesis and characterization of these compounds are described elsewhere (Hoong *et al.*, 1992). 3′-Azido-2′,3′-deoxyuridine (AZdU) was synthesized in our laboratories or by Microbiologica, Rio de Janeiro, Brazil, using a previously described method (Chu *et al.*, 1989). The chemical structures of all compounds were confirmed by ^{1}H nuclear magnetic resonance spectra and the HPLC purity was >99%. Acetonitrile (HPLC grade) and all other chemicals (analytical grade) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Adenosine was obtained from Sigma Chemical Co (St Louis, MO, USA).

Approval by ethics commitees

Human data were from an US FDA-approved double-blind, dose-escalation study in HIV-infected male volunteers that took place at the Immunologische Tagesklinik, Auguste-Viktoria-Klinikum, Berlin, Germany, under the supervision of Dr Med Keikawus Arasteh. The study was approved by the human subject ethics committee at that institution.

All previously unpublished animal studies took place at BAS Evansville, IN, USA and the protocols were approved by the BAS Evansville Animal Care and Use Committee. The entire study was designed to conform to all applicable Good Laboratory Practice regulations and to all applicable sections of the Animal Welfare Act regulations (US Code of Federal Regulations, 1998). The animal studies were approved by the BAS Evansville Quality Assurance Unit in accordance with BAS Evansville standard operating procedures. Wherever possible, procedures used in these studies were designed to avoid or minimize discomfort, distress and pain to the animals.

Assessment of RCV deamination to (+)-FTU in the whole blood of humans and dogs

RCV (100 mM) was incubated in 1 ml of human and dog whole blood at 37°C in tubes containing potassium ethyl diamine tetra-acetic acid (K_3EDTA). After 3 h incubation, the blood was immediately placed on ice and centrifuged (100×*g*) on a desk-top centrifuge. The plasma was transferred to labelled vials, frozen and stored at –20°C for HPLC analysis of RCV and (+)-FTU (see measurements of RCV and (+)-FTU in HIV-infected humans, below). Table 1 compares the percentage RCV deamination observed together with previously reported values in other mammalian species.

Pharmacokinetic studies in rats

The rat data used in this analysis were from a 28-day and a 6-month toxicity study of RCV in Sprague Dawley® rats (Harlan Inc, Indianapolis, IN, USA). Rats were randomly assigned to each study using a computer-generated randomization list of body weights according to gender (males: 267–313 g; females: 163–212 g). The 28-day study comprised three groups of three males and three females per dose group at 100, 300 or 1000 mg/kg per day of RCV. The 6-month toxicity study evaluated six males and six females per dose group at 100, 300 or 1000 mg/kg per day of RCV for 26 weeks. RCV was administered orally each day in a 0.5% solution of methylcellulose using a feeding needle connected to a syringe. Blood samples were collected (approximately 0.75 ml) by the tail vein prior to

Table 1. Stability of RCV (100 μM) in the blood of mammalian species (3 h, 37°C)

Species	% RCV converted to (+)-FTU	References
Woodchuck	46%	(Moore *et al*., 1997)
Rhesus monkey	45%	(Schinazi *et al*., 1992b)
Human	4%	new data
Dog	<1%	new data
Rat	<1%	(Frick *et al*, 1993)
Mouse	<1%	(Frick *et al*., 1994)

dosing and at 0.5, 1.0, 4.0, 8.0 and 24 h after dosing for measurement of RCV and (+)-FTU. The blood was collected in tubes containing potassium EDTA, immediately placed on ice and centrifuged. The plasma was transferred to labelled vials, frozen and stored at −20°C for HPLC analysis. Rats were tattooed on their tails for identification and were individually housed in stainless steel cages suspended over flush pans on mesh floors. The fluorescent-lighted facility (12 h on and 12 h off) was maintained between 15–29°C and 30–70% relative humidity. The rats' diet consisted of PMI® Certified Rodent Diet (Richmond, IN, USA) and tested tap water from a deep well was offered *ad libitum*.

Pharmacokinetic studies in pregnant rabbits

Twenty young adult New Zealand white rabbits (3.5–4.9 kg) (Myrtle's Rabbitry, Thompson Station, TN, USA) were bred at the supplier and the day of gestation was noted as gestation day 0. Pregnant rabbits were randomly assigned to four groups of five, using a computer-generated randomization list and treated with RCV on days 6–19. Each group received single daily doses of RCV at 100, 300 or 1000 mg/kg doses in 0.5% methylcellulose suspension by oral gastric intubation, using an appropriately sized syringe and 3/16″ tygon tubing, for 18 days. The dosing suspensions were prepared just prior to dosing by adding the vehicle to the appropriate RCV container and the contents were mixed until the suspensions looked homogenous (at least 2 min) using a polytron, followed by an additional 15–20 min stirring of the homogenate using a magnetic stirrer. The suspensions were stirred continuously during dosing. Blood samples were collected (approximately 0.75 ml) from a marginal ear vein prior to dosing and at 0.5, 1.0, 4.0, 8.0 and 24 h after dosing for measurement of RCV and (+)-FTU on days 6 and 18. The blood was collected in tubes containing potassium EDTA, immediately placed on ice and centrifuged. The plasma was transferred to labelled vials, frozen and stored at −20°C for HPLC analysis. Rabbits were housed individually in stainless steel cages suspended over flush pans. The fluorescent-lighted facility (12 h on and 12 h off) was maintained between 18–23°C and 45–65% relative humidity. The rabbits' diet consisted of approximately 200 g of PMI® Certified Rabbit Diet per day. High quality alfalfa hay cubes and tested tap water were offered *ad libitum*.

Pharmacokinetic studies in beagle dogs

The beagle dogs used in this analysis were from a 28-day and a 1-year study. Young beagle dogs (Marshall Farms, North Rose, NY, USA) were randomly assigned to each study using a computer-generated randomization list of body weights according to gender (males: 8.5–10.9 kg; females: 6.6–8.5 kg). The 28-day study comprised three groups of four males and four females per dose group at 100, 300 or 750 mg/kg per day of oral RCV administered in gelatin capsules. The 1-year toxicokinetics study was made up of six males and six females per dose group at 50, 100 or 300 mg/kg per day. Blood samples were collected on days 1 and 29 for the 1-month study and days 89, 180 and 270 for the 1-year study. The sampling times were 0, 0.5, 1, 4, 8 and 24 h after the previous dose. Blood was collected in tubes containing potassium EDTA, immediately placed on ice and centrifuged. The plasma was transferred to labelled vials, frozen and stored at −20°C for HPLC analysis of RCV (1-month study) or RCV and (+)-FTU (1-year study). Dogs were housed in individual concrete runs under fluorescent lighting (approximately 12 h on, 12 h off) at room temperature (16–24°C) and 30–54% relative humidity with hardwood shavings on the floor and a resting board. Fresh wood shavings were supplied daily and all the shavings were replaced when the runs were washed down weekly. The dogs' diet consisted of PMI® Certified Canine Diet and tested tap water from a deep well was offered *ad libitum*. Dog runs were marked with an identification card colour-coded by dose group and inscribed with study number, dose group and animal number. Dogs were identified by their USDA tattoos.

Pharmacokinetic studies in rhesus monkeys

The single dose oral pharmacokinetics of RCV in three male rhesus monkeys given an oral 33.3 mg/kg dose were previously reported (Schinazi *et al.*, 1992b). Briefly, three young adult monkeys (*Macaca mullatta*) weighing 4.4–6.4 kg, were used for the pharmacokinetic studies. The animals were maintained at the Yerkes National Primate Research Center of Emory University, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care, in accordance with guidelines established by the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* from the US National Institutes of Health (NIH).

Three monkeys were given an oral dose of 33.3 mg/kg RCV by gastric intubation and blood samples were

collected prior to and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 h after administration. Monkeys were maintained on their backs on a heated pad, covered with a blanket and kept under ketamine anaesthesia for the first 4 h after dosing.

Pharmacokinetic study in HIV-infected human volunteers

The human pharmacokinetic study was part of randomized, double-blind, dose-escalation study exploring the safety, tolerability, pharmacokinetics and virological effect of RCV dosed for 14 days at 200, 400 or 600 mg in combination with stavudine (Zerit®, d4T) and efavirenz (Sustiva®, EFV) in HIV-infected males compared with 3TC in combination with d4T and EFV (Otto MJ *et al.* Single and multiple dose pharmacokinetics and safety of the nucleoside Racivir® in male volunteers. *Frontiers in Drug Development for Antiretroviral Therapies,* Naples, FL, USA, 12–16 December 2002, Abstract 044; Otto MJ *et al.*, Sustained anti-HIV-1 effect of Racivir combined with D4T and following a 14-day treatment of infected volunteers. *10th Conference on Retroviruses & Opportunistic Infections,* Boston, MA, USA, 10–14 February 2003, Abstract 577*;* Herzmann *et al.*, in review). Briefly, three cohorts of six subjects were given a single oral dose of either 100, 200 or 600 mg of RCV in combination with d4T and EFV. Blood samples were collected in tubes containing potassium EDTA at 0, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h following oral administration, immediately placed on ice and centrifuged. The plasma was transferred to labelled vials, frozen and stored at −20°C for HPLC analysis of RCV and (+)-FTU (below).

Measurement of RCV and (+)-FTU in rats, rabbits and dogs

Plasma concentrations of RCV and (+)-FTU were measured using a validated HPLC/MS/MS assay at BAS Analytics (West Lafayette, IN, USA). The internal standards adenosine and AZdU (50 µl of 200 µg/ml) were added to K_3EDTA plasma samples (100 µl) and the nucleoside agents extracted by protein precipitation using acetonitrile. The supernatant was evaporated under a flow of nitrogen and reconstituted in 200 µl mobile phase A (below). Samples (6–15 µl) were injected onto an HPLC system using a Betasil C_{18} column (5 µm, 10×2.0 mm, Keystone Scientific, Bellefonte, PA, USA) with an ammonium acetate/acetonitrile mobile phase for 13 min. The LC/MS/MS system was equipped with a BMS PM-92 isocratic pump with LC-26A online vacuum degasser, SIL-10AD*VP* autosampler (Shimadzu, Kyoto, Japan), a Tomtec Quadra96® Model 320 with Quadra96 software and a Micromass Quattro LC tandem quadrupole mass spectrometer with electrospray ionization source and MassLynx control software. Mobile phase A contained 5% acetonitrile in 10 mM ammonium acetate buffer (pH 5.2) and was used at a flow rate of 0.3 ml/min. The column was flushed for 1.5 min between runs using mobile phase B containing 90% acetronitrile in 10 mM ammonium acetate buffer with a flow rate of 0.54 ml/min. RCV and the adenosine internal standards were detected in a positive ion mode from 2.8–5.7 min maintaining a capillary voltage of 1.0 kV, while (+)-FTU and the AzDU internal standard were detected in a negative ion mode from 5.7–13 min, maintaining a capillary voltage of 1.3 kV. The retention times for adenosine, RCV, (+)-FTU and AZdU were 3.90, 4.71, 6.66 and 11.34 min, respectively. The nebulizer and drying gas flow rates were 94 and 2 080 l/h, for positive and negative modes, respectively. The desolvation and source-block temperatures were 400 and 150°C, respectively. Argon was used as the CID gas at 1.0 mBar. The extractor and RF voltages were 2 and 0.2 V, respectively. The respective LM and HM resolution settings were 15 and 11, for positive and negative modes, respectively. A solvent delay of 2.9 min and a divert valve was used to divert flow to and from the MS system. Calibration curves for RCV and (+)-FTU were fitted in the range 100–10 000 ng/ml using six levels in duplicate and 1/concentration^2 quadratic regression. The percentage recovery was from 83–112% in this concentration range for RCV, (+)-FTU and for the internal standards adenosine and AZdU. The intra-day and inter-day variance for RCV and (+)-FTU were <7% and 11%, and <6 and 7%, respectively.

Measurement of RCV and (+)-FTU in rhesus monkeys

RCV and FTU concentrations in blood were extracted from plasma by perchloric acid precipitation of plasma proteins followed by micro-centrifugation. RCV and FTU were then measured using HPLC on a reverse phase column using d4T (50 µl of 20 µg/ml) as an internal standard. The retention times for RCV, FTU and d4T were 5.5, 8.3 and 9.5 min, respectively. The assay for RCV was linear in the range of 0.1–100 µg/ml and the lower limit of quantitation was 0.1 µg/ml (10 ng). The assay for FTU was linear in the range of 0.05–100 µg/ml, and the lower limit of quantitation was 0.05 µg/ml (5 ng). Extraction recoveries of RCV, FTU and d4T were 87, 93 and 96%, respectively (Schinazi *et al.*, 1992b).

Measurement of RCV and (+)-FTU in HIV-infected humans

RCV and the (+)-FTU metabolite concentrations were measured in human plasma at Zentrum Biopharm, GmbH Berlin, Germany, using a validated HPLC method. Briefly, 100 µl serum samples were added to 50 µl AZdU (20 µg/ml) as an internal standard and 50 µl of 2 M perchloric acid as a protein precipitant were added to

400 μl polypropylene microcentrifuge tubes, vortexed and centrifuged at 10 000×*g* for 5 min. The supernatant was neutralized using 50 μl of 2 M KOH, mixed well and centrifuged at 10 000×*g* for 5 min. Previous studies had indicated that the stability of the nucleotides was not affected by the extraction procedure. Supernatant (15–200 μl) was injected into the HPLC column. RCV was separated on a Phenomenex Hypersil ODS (C-18) reverse phase column (Torrance, CA, USA) with an isocratic mobile phase of 50 mM phosphate buffer (pH 2.40), methanol and diethylamine (93; 7; 0.1 V/V/V) with a flow rate of 0.5 ml/min at 35°C. The run time was 20 min and was followed with an additional 3.0 min wash with 25% methanol in water. Compounds were detected at a wavelength of 279 nm with an attenuation of 5. The retention times for RCV, FTU and AZdU were 6.15–6.2, 6.8 and 9.1–9.2 min, respectively. RCV and FTU standards ranged from 0.02–5.0 μg/ml. The standard curve slopes and intercepts were generated using (1/y) least squares regression. The assays for RCV and FTU were linear in the concentration range studied (r>0.999). Samples that exceeded the concentration range of standards were diluted and re-assayed. The limits of detection for RCV and FTU were 0.02 μg/ml. Extraction recoveries of RCV, FTU and AzDU were >80%. The inter- and intra-day coefficients of variation for the assay were <12.89% for both compounds at all drug concentrations. The Schimadzu HPLC hardware (Tokyo, Japan) comprised a solvent delivery system (LC-10AC/10AD), an automatic sample injector (SIL-10A), a UV detector (SPD-10A), a column thermostat (CTO-10A) and an interface (CBM-10A), and was driven by Shimadzu CLASS LC 10 chromatography software (v1.64A).

Pharmacokinetic analysis

Pharmacokinetic parameters for RCV and (+)-FTU were generated by non-compartmental analysis assuming extravascular input (model 200, WinNonlin v4.0.1, 2002; Pharsight, Mountain View, CA, USA). This program utilizes a Lagrange polynomial interpolation technique and was used to calculate the area under the plasma concentration–time curve (AUC) and the area under the first moment curve (AUMC) from time zero to the last determined sample time (Yeh *et al.*, 1978; Rocci *et al.*, 1983), with extrapolation to time infinity by use of the terminal slop (λ_z) generated by weighted (1/y) nonlinear least squares regression (Metzler, 1987). C_{max} was the observed maximal concentration in the plasma and t_{max} was the time that maximal plasma concentration was observed. The mean residence time (MRT) was calculated from AUMC/AUC and the apparent terminal half-life ($t_{1/2}$) was calculated by ln(2)/λ. The systemic clearance/F (Cl/F) was calculated as dose per kg/AUC. The fraction of drug absorbed (F) was not determined since only oral data were available. The Cl/F value could not be determined for (+)-FTU since it is a metabolite and was not administered directly. The parameter FTU% equal to AUC of plasma FTU/(AUC (+)-FTU + AUC of non-metabolized RCV) multiplied by 100 was calculated to indicate the relative plasma exposures to the (+)-FTU metabolite.

Statistics

Linear regression analysis was performed on C_{max} and AUC values of each individual species versus dose, with and without stratification by gender (Table 2). Paired 2-sample *t* tests were performed on the AUC and C_{max} for RCV and on $FTU_{\%}$ in each species versus days of treatment. Two sample *t* tests assuming equal and unequal variance were performed on AUC and C_{max} values and the $FTU_{\%}$ values versus gender and versus days of treatment. Linear regression was performed on C_{max} and AUC values versus RCV dose per kg, and r^2 values were computed for each species with and without stratification by gender, to determine whether the pharmacokinetics of RCV remained in the linear range. C_{max} observations obtained for all species at all doses and all dosing intervals where pharmacokinetic measurements were performed, were divided by dose per kg [C_{max}/(dose per kg)] and plotted versus species body weight (Figure 2). Since dogs gave the closest C_{max}/(dose per kg) value compared with humans, their statistical differences were assessed using 2-sample *t* tests (with the assumptions of equal and unequal variance). All statistical tests were performed using the data analysis module on a Microsoft Excel spreadsheet (Excel 2000, Microsoft Corp, Redmond, WA, USA). A *P* value <0.05 was taken as being statistically significant for all *t* tests.

Allometric scaling

The values of Cl/F per kg and V_β/F per kg (mean ±SD), were obtained from the non-compartment pharamacokinetic analysis for the each species, dose and treatment duration cohort (above). The corresponding parameter Cl_{abs}/F V_β/F values were then calculated by multiplying these parameters by the average body weight of animals in that cohort. The allometric equations Cl_{abs}/F or $V_\beta/F=aW^b$ (Modenti, 1986; Ings, 1990) were fitted using (1/y) weighted least squares nonlinear regression using WinNonlin (v4.01, Pharsight), using a Nelder–Mead convergence algorithm. In this equation W=body weight (kg), b=exponent to which W is raised and a=the predicted Cl_{abs}/F or V_β/F value of a theoretical animal weighing 1 kg. Allometric curves were fitted firstly for all species (including humans) and then for combinations of animal species (excluding humans) to assess which combinations of species are suitable for predicting Cl/F and V_β/F for a 74 kg human. Adequacy of the predictions were assessed by

Table 2. RCV pharmacokinetic parameters from various mammals after oral administration

Parameter (unit)	Rats (mean ±SD)		Dogs (mean ±SD)		Rabbits (mean ±SD)	Monkeys* (mean ±SD)	Humans (mean ±SD)
	females	males	females	males	pregnant	males	males
Cl/F/kg [l/kg/h]	1.40 ±0.88	2.09 ±0.93	0.39 ±0.08	0.41 ±0.10	0.99 ±0.62	2.07 ±0.45	0.68 ±0.23
V_β/F/kg [l/kg]	3.86 ±1.77	6.34 ±4.35	1.50 ±0.53	1.64 ±1.15	19.83 ±13.71	4.24 ±1.63	5.58 ±3.03
$t_{1/2}$, β [h]	1.94 ±0.47	2.06 ±0.72	3.31 ±0.77	3.26 ±0.78	14.57 ±5.38	1.38 ±0.27	6.47 ±4.36
MRT [h]	3.02 ±1.55	3.21 ±1.02	3.34 ±0.89	3.40 ±0.92	12.36 ±4.54	2.54 ±0.61	6.00 ±3.31
C_{max}/(dose/kg) [ng/ml/(mg/kg)]	220 ±80	160 ±63	721 ±176	656 ±174	227 ±80	157 ±8	495 ±161
T_{max} [h]	1.04 ±0.55	1.13 ±0.53	1.29 ±0.68	1.41 ±0.53	0.90 ±0.33	1.0	0.75 ±0.39
$FTU_{\%}$*	nd*	nd*	1.77 ±0.45	2.33 ±2.30	14.79 ±4.01	48.57 ±4.63	39.37 ±7.90
Weight (kg)	0.235 ±0.013	0.368 ±0.022	7.68 ±2.93	10.01 ±0.64	4.28 ±0.31	3.80 ±0.40	73.84 ±10.14

F, fraction of administered dose absorbed via oral route; Cl, systemic clearance; V_β, volume of distribution extrapolated from the terminal elimination phase; $t_{1/2\beta}$, half-life of RCV calculated from the terminal elimination phase; MRT, mean residence time; C_{max}, the observed maximum plasma concentration that was observed at time=T_{max}; nd, not done.
Differences were observed in the pharmacokinetic parameters in male and female rats treated at the various doses (please refer to text). However, the means ±SD values of the various parameters in male and female rats are presented Table 1 for completeness.
*The pharmacokinetics in rhesus monkeys were adapted from Schinazi *et al.*, 1992b. $FTU_{\%}=AUC_{(+)\text{-}FTU}/(AUC_{(+)\text{-}FTU} + AUC_{RCV}\times 100$ to relates the relative exposures to (+)-FTU and RCV. $FTU_{\%}$ were not determined for rats since the rate of formation of FTU was too slow and, therefore, the concentration of FTU in plasma had not equilibrated within 8 h dose interval and AUC_{FTU} could not be determined.

calculating the percentage of the mean human average observation [prediction/(observed mean)×100] and the number of SD units away each prediction was from the observed mean human value [magnitude of (observed−predicted)/SD].

Results

The percentage of RCV deamination to (+)-FTU in the whole blood of humans, rhesus monkeys, woodchucks, rats and mice is shown in Table 1. The blood of rhesus monkeys and woodchucks deaminated the greatest percentage of (+)-FTU (>45%), while dog, rat and mouse blood deaminated less than 1% of the RCV. Of the RCV incubated in human blood, 4% was deaminated to (+)-FTU.

The non-compartmental pharmacokinetic parameters fitted for RCV, percent plasma exposure to the (+)-FTU metabolite and the average body weights of the various species are presented in Table 2. Linear regression analysis of C_{max} and AUC values versus RCV dose per kg, with and without stratification by gender, indicated linear pharmacokinetics within each species in the dose ranges studied (r^2>0.81, Table 3). Therefore, the pharmacokinetic parameters measured using data from the high-dose safety study should be reflective of the lower doses used in the clinic. The species with the smallest average relative Cl/F was the dog (females: 0.39 and males: 0.41 ml/kg/h), while monkeys had the largest Cl/F value (2.07 ml/kg/h). The species with the lowest average mean residence time for RCV was the monkey (2.54 h) and that with the highest was the rabbit (12.36 h). Two-sample *t* tests (assuming both equal and unequal variances) failed to detect consistent significant differences in Cl/F values versus days of exposure to RCV in all species (P>0.05). Significant differences were observed in the Cl/F values of female rats at 100 mg/kg/day (1.61 ±0.22 ml/kg/h; mean ±SD) versus 300 mg/kg/day (2.15 ±0.23) and 1000 mg/kg/day (2.04 ±0.23) in the 28-day study. The Cl/F values of female rats in the 6-month study differed at the 100 (1.05 ±0.15) versus 1000 (1.27 ±0.27) and the 300 (1.07 ±0.13) versus 1000 mg/kg/day doses in the 6-month rat study. For male rats in the 6-month study, the Cl/F values differed at the 100 mg/kg/day dose (1.33 ±0.13) versus 1000 mg/kg/day (2.01 ±0.38), and for the 300 mg/kg/day dose (1.30 ±0.16) versus the 1000 mg/kg/day dose. Cl/F/kg values also differed significantly for male rats on day 1 compared with day 29 and on day 90 compared with day 180 at the 1000 mg/kg dose, but not when comparing dose durations. Differences between Cl/F/kg were not detected against dose durations for other animals, as noted by the overlap of symbols representing the Cl/F values determined after different durations of RCV treatment (Figure 1). Dogs had the smallest average relative V_β values (females: 1.50, males: 1.64 l/kg), while pregnant females had the highest (19.83 l/kg). There was no significant trend in C_{max}/(dose per kg) values versus body weight (Figure 2, r^2=0.055). However, the C_{max} /(dose per kg) values for dogs were the most similar of the species to humans (Figure 2), but were statistically different (dog: 709 ±181, 146 observations versus man: 495 ±161 ng/ml/(mg per kg), 34 observations, mean

Table 3. Linear regression analysis of C_{max} and AUC versus RCV dose (mg/kg) by species and gender

	C_{max}, ng/ml				AUC, ng·h/ml			
Species, gender	a*	b†	r^2	*n*	c*	d†	r^2	*n*
Humans, m	461.48	51	0.98	6	1318.09	1428.16	0.88	6
Dogs, f	410.55	48 350	0.86	15	2209.67	78 814.09	0.96	15
Dogs, m	515.96	20 146	0.98	15	2877.57	–41 692.73	0.99	15
Dogs, all	463.26	34 248	0.92	30	2543.62	18 560.68	0.96	30
Rabbits, f	125.13	25 253	0.90	6	1449.07	–42 953.92	0.97	6
Rats, f	106.94	23 983	0.89	12	637.57	18 732.70	0.85	12
Rats, m	78.29	17 835	0.86	12	434.56	30 581.23	0.89	12
Rats, all	92.61	20 909	0.81	24	536.07	24 656.97	0.81	24
Combined‡	146.82	49 766.35	0.29	67	948.14	139 275.85	0.40	67

*a and c are the fitted slopes of C_{max} and AUC, respectively, versus Reverset dose (mg/kg); †b and d are the fitted intercepts of C_{max} and AUC, respectively, versus Reverset dose (mg/kg). Monkeys were not included in this analysis, since only one dose (33.3 mg) was used; *n*=number of cohorts used in the analysis. ‡Regression analysis performed using all data (not stratified by species or gender).

±SD, $P<10^{-8}$). Monkeys and humans had the highest relative plasma exposures to (+)-FTU after receiving RCV ($FTU_{\%}$=48.57 and 39.37%, respectively, Table 2). Rabbits and humans had the longest average $t_{1/2,\ \beta}$ values (14.6 and 6.5 h, respectively), while all other species had $t_{1/2,\ \beta}$ values between 1.94 and 3.3 h.

The allometric relationships of Cl/F and V_β/F are represented graphically in Figures 1A and 1B, respectively. The observed average Cl/F value in all humans tested was 50.67 ±20.24 l/h (mean ±SD). The fitted curve relating Cl/F and body weight for all data from all species including man was $Cl/F=0.926(\text{kg wt})^{0.895}$ (r^2 of observed versus predicted=0.88, Figure 1A, curve 1). Data from species other than man were also fitted to the same model to determine which species could be used to predict Cl/F in a 75 kg human. A model fitted through all data excluding man ($Cl/F=1.268(\text{kg wt})^{0.567}$, r^2=0.90, Figure 1A, curve 2), produced a poor prediction of Cl/F value in humans of 14.49 l/h (1.79 SD from mean). The closest prediction for Cl/F in humans was derived from a fit using rat, rabbit and monkey data ($Cl/F=1.268(\text{kg wt})^{0.789}$, r^2=0.70, Figure 1A, curve 3), which predicted a Cl/F value in humans of 45.79 l/h (within 0.24 SD of observed mean). However, a reasonable prediction was also obtained using rats and pregnant rabbits ($Cl/F=1.386(\text{kg wt})^{0.743}$, predicted a Cl/F in man of 34.25 l/h, 0.81 SD of mean observed Cl/F, r^2=0.70, Figure 1A curve 4). The mean of the observed value for V_β/F value in humans was 409 ±220 l (mean ±SD). The fitted curve relating V_β/F to body weight for all data from all species including man was $V_\beta/F=6.234(\text{kg wt})^{0.976}$ (r^2= 0.81, Figure 1B, curve 1), and the species combination that produced the closest estimate of V_β/F was derived using the rat and monkey data ($V_\beta/F=4.84(\text{kg wt})^{0.923}$, r^2=0.95, Figure 1B, curve 2), which predicted a value of V_β/F in man of 261 (within 0.67 SD of mean value in humans).

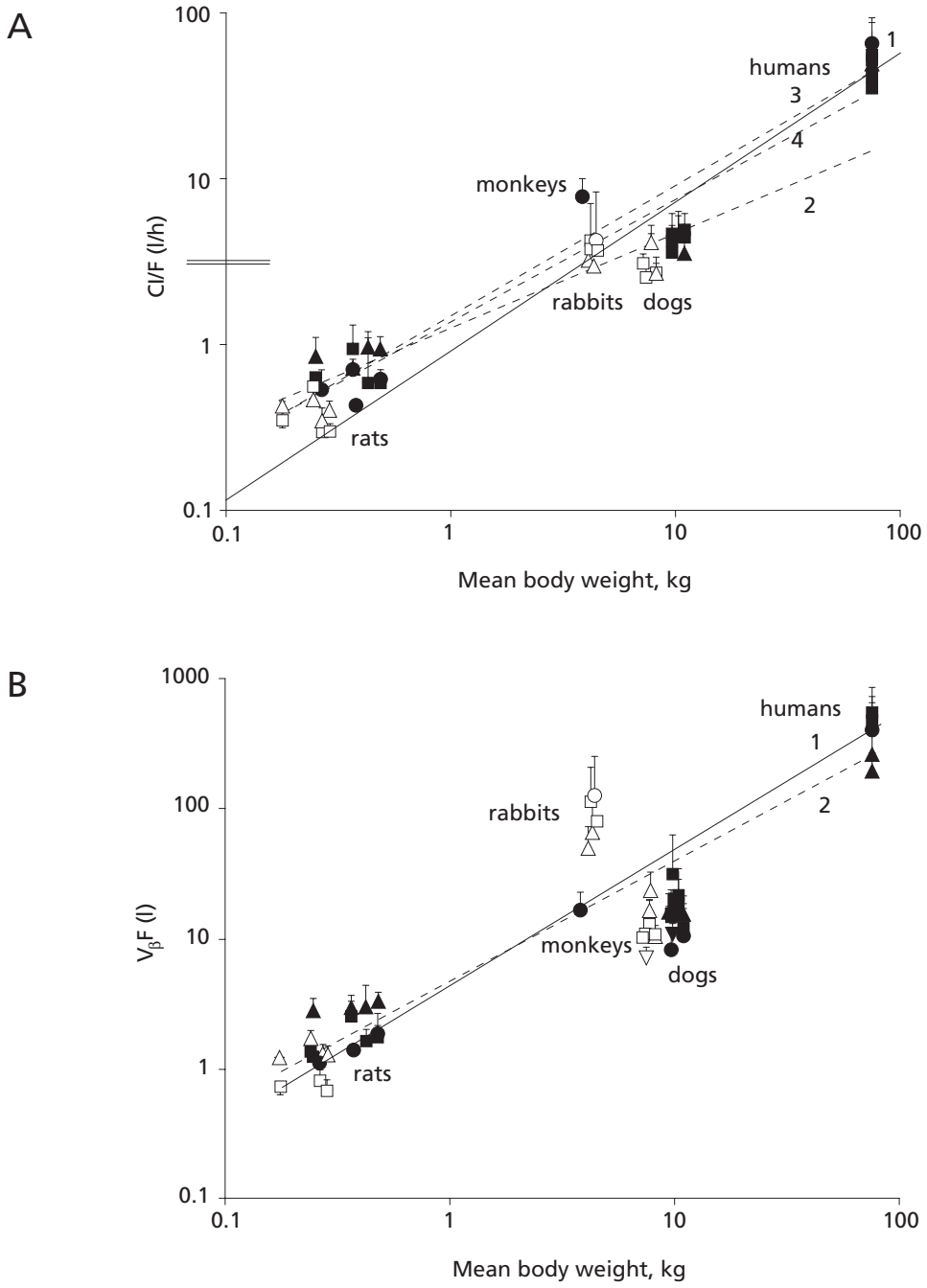
Discussion

The varying degrees of deamination of RCV in the whole blood of the various mammalian species (Table 1) suggests that interspecies differences in pharmacokinetics may result in part from differences in drug metabolism, in addition to possible differences in drug distribution.

The relationship between Cl/F and C_{max} versus RCV dose per kg remained linear for each animal species, despite the high doses selected for the toxicology study. Therefore, the pharmacokinetic parameters were considered indicative of the lower therapeutic doses. Parameters varied considerably between species (Table 2) and no individual animal mimicked the pharmacokinetics in humans. These data were therefore considered for interspecies (allometric) scaling of Cl/F and V_β/F. Allometric equations are commonly used to relate pharmacokinetic parameters including clearance values and volumes of distribution to species body weight (Modenti, 1986; Ings, 1990). Furthermore, allometric equations fitted using rat, monkey and rabbit data have previously been used to help guide dose-finding studies of the antiretroviral nucleoside agents AZT, 2′,3′dideokycytidine and 3TC (Patel *et al.*, 1990; Ibrahim & Boudinot, 1989; Hussey *et al.*, 1994). However, the nucleoside analogues previously scaled by this method were not substrates for mammalian cytidine deaminase, the enzyme responsible for the *in vivo* conversion of (+)-FTC, but not (–)-FTC to (+)-FTU. Therefore, it was of interest to determine whether this approach using these species may be used to guide early-dosing studies of RCV in humans.

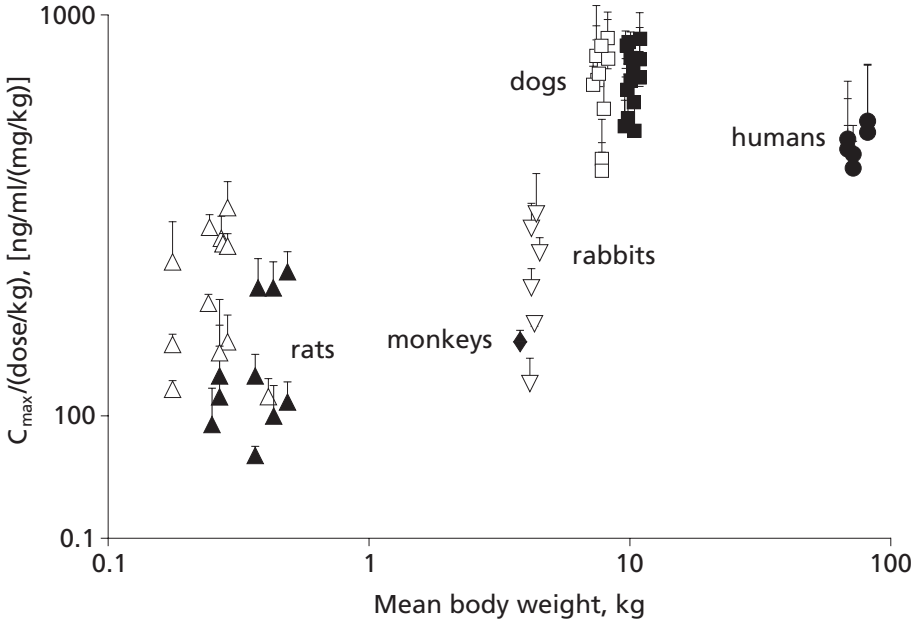
Allometric equations are usually fitted using pharmacokinetic parameters derived from intravenous drug studies in which the fraction of the drug reaching the systemic circulation is known (F=1), and from which clearance and volume parameters may be computed. However, only oral

Figure 1. Interspecies scaling for **(A)** RCV Cl/F and **(B)** V_β/F



The parameters Cl/F (Cl/F=dose/AUC; mean+SD) and V_β/F values were derived using non-compartmental analysis and plotted versus the average body weight (W, kg) of each group of animals enrolled in the various dosing protocols. The equations parameter=AxW^B were then fitted for the parameters Cl/F and V_β/F, respectively. **(A)** The value of the parameter predicted for an 'average' animal weighing 1 kg; **(B)** the exponent to which W is raised. Open symbols, females; filled symbols, males. The doses for rats and rabbits were: 100 (circles), 300 (squares) and 1000 mg/kg of RCV (triangles). The doses for dogs were 50 (inverted triangles), 100 (circles), 300 (squares) and 750 mg/kg (triangles). The monkey dose was 33.3 mg/kg (hexagon), and the human doses were 200 (circle), 400 (square) and 600 (triangle) mg, respectively. Males are represented by solid and females by closed symbols. (A) For Cl/F versus species body weight, the solid line (curve 1) is the fitted curve through all human and animal data, while curve 2 resulted from a fit using all non-human data. The fitted curve that produced the best estimates of the human Cl/F were fitted using rat, rabbit and monkey data (curve 3) and then rat and rabbit data only (curve 4). (B) The solid line (curve 1) V_βF is the fitted curve through all human and animal data. The regression curve that resulted in the best estimates of the human V_β/F was that fitted using the rat and monkey data (curve 2). Please refer to text.

Figure 2. Interspecies scaling for RCV C_{max}/dose



The parameter C_{max} was derived from actual observations of maximal plasma concentration versus time for each cohort. Since absorption rates are not known, theoretical C_{max} values were not calculated. Since species received different doses, C_{max} values were normalized by dividing by dose (mg/kg). Symbols: triangles, rats; squares, dogs; inverted triangles, rabbits; diamonds, monkeys; circles, humans. Males are represented by solid symbols and females by closed symbols.

doses of RCV were used in this study. Therefore, allometric scaling was performed using the hybrid parameters Cl/F and V_β/F, which are influenced by Cl, V_β and F, each of which may vary independently of each other across species. However, Cl/F relates AUC to oral dose (Cl/F=oral dose/observed AUC), provided the pharmacokinetics are linear and RCV is administered as an oral dose.

A reasonable fit of the allometric model for Cl/F versus species body weight was observed for all species including humans in Figure 1A, curve 1 (r^2 observed versus predicted=0.88). However, this curve did not fit all species adequately. Monkeys, the species with the highest cytidine deaminase activity for RCV, produced Cl/F values above the line of prediction (Figure 1A, curve 1), while dogs, which demonstrate a low cytidine deaminase activity, produced Cl/F values below the line of prediction. Previously published allometric studies also indicate that while allometric equations predict the expected value of a typical mammal of a given size, they are generally imprecise for specific animals that demonstrate atypical drug metabolism (Ings, 1990). The model fitted through all animals excluding humans produced a poor estimate of human Cl/F (14.49 l/h, which is within 1.79 multiples of SD from the observed mean value), even though the overall fit was reasonable (r^2=0.90, Figure 1A, curve 2). The model fitted using rats, pregnant rabbits and monkeys gave the closest prediction of Cl/F in humans at 45.79 l/h (0.24 SD from observed mean value, r^2 =0.70, Figure 1A, curve 3, followed by a model fitted using rat and rabbit (Cl/F=34.25 l/h, 0.81 SD of mean, r^2=0.70, Figure 1A, curve 4).

The allometric models for V_β/F versus species body weight are summarized in Figure 1B. A reasonable fit was obtained for the model for V_β/F versus species body weight fitted using all species including humans (r^2=0.81, Figure 1B, curve 1). However, this curve also did not fit all species adequately, since the average V_β/F value of pregnant rabbits was approximately fivefold higher, and that of dogs was approximately fivefold lower than the line of prediction for V_β/F (Figure 1B, curve 1). The correlation was insufficient to predict V_β/F using all data excluding humans (r^2<0.02). The combination of animals that resulted in the closest estimate of V_β/F was that fitted using rat and monkey data (r^2=0.95, Figure 1B, curve 2), which predicted V_β/F in man=261 l (within 0.67 SD of the mean observed value in humans).

C_{max} values following oral doses are dependent on a combination of pharmacokinetic parameters including dose, volume of the plasma compartment and the rate and extent of oral absorption (Gibaldi & Perrier, 1982). The number of plasma concentrations sampled in the regions of the t_{max} and C_{max} observations were limited to 0.5, 1 and 4 h for rats, rabbits and dogs, 0.5, 1 and 1.5 h for monkeys and 0.5, 1 and 2 h for humans. Therefore, the values of t_{max} and

C_{max} shown in Table 2 should be considered approximate. A plot of observed C_{max}/(dose per kg) versus species weight (Figure 2) demonstrated that dogs produced a C_{max} /(dose per kg) most similar to humans. However, C_{max} were statistically different for the two species ($P<10^{-8}$).

It was surprising that the $FTU_{\%}$ values for rabbits were much higher than those of the rat (Table 2), since both species are from closely related phylogenetic orders. This could be true for all rabbits, or be a function of them being pregnant. However, since only pregnant rabbits were included in this toxicity study to determine the safety of RCV in pregnant animals, data were not available from non-pregnant rabbits. The V_{β}/F values of pregnant rabbits gave the greatest positive deviation from the allometric scaling curve relative to other species (Figure 2). A higher relative V_{β}/F estimate may also result in pregnant animals, because amniotic fluid and the body composition of the foetuses would be included in the parameter V_{β}/F, provided that RCV crosses the placenta (Lederman & Rosso, 1981). No RCV measurements were performed on the amniotic fluid or rabbit foetuses.

The pharmacokinetic parameter that may be most useful for the interspecies scaling of nucleosides is probably Cl/F. Cl/F permits calculation of single dose AUC values for any given oral dose, provided the pharmacokinetics remain linear. Since single dose AUC values are equivalent to the AUC values between each dose interval once steady state has been reached, it permits calculation of average steady-state values ($Cp_{average}$) versus dose interval ($Cp_{average}$= AUC/dose interval) (Gibaldi & Perrier, 1982). Once the active nucleotides are formed in cells they are degraded slowly. Therefore, peak and trough levels in plasma may not be indicative of cellular nucleoside triphosphate levels in cells during steady-state dosing. Since the accumulation of RCV triphosphate activated in $CD4^{+}$ lymphocytes (the target of HIV) is linear in the dose range of RCV used in the clinic, $Cp_{average}$ should be proportional to the accumulation of the active nucleoside triphosphate. We have previously incorporated $Cp_{average}$ values as a component of a pharmacodynamic–virus dynamic model to predict HIV loads during nucleoside therapy. That model linked Cp_{median} with rates of cellular accumulation and dephosphorylation of active nucleoside triphosphate inhibitors of reverse transcriptase and a biological model of HIV infection dynamics, using appropriate differential equations (Hurwitz & Schinazi, 2002). Therefore, interspecies scaled values of Cp_{median} could be used as a first step for the prediction of efficacy before the detailed pharmacokinetics in humans are known. However, prediction of C_{max} values may also be helpful for some nucleoside antiretroviral agents such as AZT, where high C_{max} values may be correlated with patient toxicity, while the average efficacy may be related to patient AUC (Barry *et al.*, 1996).

In summary, this study highlights the importance of selecting animal species that demonstrate similar cytidine deaminase activity to humans when performing preclinical dosing studies on RCV as well as other analogues that are substrates for mammalian deaminases. The animal species that showed pharmacokinetic parameters for RCV most similar to humans were the rhesus monkey and pregnant rabbit. However, monkeys tended to overestimate Cl/F and pregnant rabbits tended to overestimate V_{β}/F. Although no individual animal produced a pharmacokinetic profile identical to humans, the average Cl/F and V_{β}/F regression curves using the appropriate animal species outlined above produced a reasonable approximation of these pharmacokinetic parameters in humans.

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