Species Dependent Esterase Activities for Hydrolysis of an Anti-HIV Prodrug Glycovir and Bioavailability of Active SC-48334

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Purpose. The in vitro fate of an ester prodrug, glycovir, was studied to determine if the species differences in the bioavailability of pharmacologically active SC-48334 observed after glycovir administration and not observed after SC-48334 administration is due to species differences in ester hydrolysis rate or species differences in absorption of the prodrug itself, and to determine the site(s) of ester hydrolysis which contributes most to species differences in the bioavailability of SC-48334 if any. ***Methods.*** Glycovir was incubated with small intestinal mucosa, liver S9 fractions, whole blood, red blood cells (RBC) and plasma of the rat, dog, monkey (cynomolgus and rhesus) and man, and glycovir concentrations were determined by HPLC. ***Results.*** The relative bioavailabilities of SC-48334 after prodrug administration to the rat, dog, monkey and man were 99, 15, 42 and 37%, respectively. After SC-48334 administration, SC-48334 was rapidly and similarly well absorbed in all species. The hydrolysis rate in the small intestinal mucosa was well correlated with the relative bioavailability of SC-48334 after prodrug administration. Among different species the hydrolysis rate of glycovir in liver S9 fractions, blood, RBC and plasma did not parallel those in the mucosa of the small intestine. ***Conclusions.*** The species differences in bioavailability of SC-48334 with the prodrug were due to species differences in hydrolysis rates of the prodrug in small intestinal mucosa. The monkey was a good animal model for prediction of esterase activity in human small intestine and relative bioavailability in man.

KEY WORDS: glycovir; SC-48334; esterase activities; species difference; in vitro-in vivo correlation.

INTRODUCTION

Glycovir, SC-49483 (Fig 1), is a perbutyrylated ester prodrug of an α-glucosidase 1 inhibitor, SC-48334. SC-48334 reduces replication of the human immune deficiency virus (HIV) at approximately 0.01 mg/mL (0.045 mM) in an in vitro system established by Abraham Karpas et al (1). The pharmacologically active SC-48334 is freely water soluble (>1 g/mL) with log D of −0.9 where D is a distribution coefficient between octanol and pH 7 buffer. SC-48334 was rapidly and well absorbed with little metabolism in the rat, dog, monkey and man after oral administration resulting in high bioavailability. However, inhibition of intestinal disaccharidases by high concentrations of the aminosugar derivative SC-48334 in the gastrointestinal tract led to diarrhea in animals and humans. Therefore, poorly water-soluble (<0.3 μg/mL) glycovir (log D of 4.5), which does not inhibit intestinal disaccharidases in vitro, has been designed as a prodrug to minimize residence time of diarrhetic SC-48334 in the gastrointestinal tract at high concentrations by slow conversion of the non-diarrhetic prodrug to SC-48334 and by rapid absorption of SC-48334 from the intestinal tract. Glycovir is devoid of local diarrheal effects associated with direct oral administration of SC-48334. This slow-release concept of a well absorbed drug from a poorly water soluble ester prodrug is a novel contrast to other ester-prodrugs which are designed to improve the bioavailability of poorly absorbed drugs (2,3). However, unlike pharmacologically active SC-48334, the bioavailability of SC-48334 from the prodrug was remarkably species dependent. In the dog, bioavailability of SC-48334 after prodrug administration was substantially lower compared to that in the rat, monkey and man. The bioavailability in the dog was also highly dose-dependent with lower bioavailabilities being observed at the higher doses.

Ester-type drugs and prodrugs are hydrolyzed by esterases present in the intestinal mucosa, tissues (e.g. liver, kidney, eye) and blood, and the pharmacological activity and toxicity of the esters are markedly affected by the degree of hydrolysis (4,5). Esterase activities in intestinal mucosa (6), liver (7,8) and blood (9) are known to be different among species. Thus, the present study was conducted to determine if the species differences in the bioavailability of SC-48334 observed with glycovir and not with SC-48334 is due to species differences in ester hydrolysis rate or species differences in absorption of the prodrug itself. The study was also conducted to determine the site(s) of ester hydrolysis (e.g., intestine mucosa, liver, RBC, plasma) which contributes most to species differences in the bioavailability of SC-48334 after prodrug administration if the differences in the bioavailability are due to species differences in ester hydrolysis rates.

MATERIAL AND METHODS

Materials

The following compounds were supplied from G.D. Searle & Co. (Skokie, IL): [^{14}C]glycovir (lot No. GDS2081-127A, specific activity of 0.122 mCi/mmole; lot No. GDS-2281-175A, specific activity of 19.1 μCi/mg), glycovir, [^{3}H]SC-48334 (lot No. MRC-627-56, MRC-627-239 and MRC-627-269 with specific activity of 1.30, 1.67, and 4.59 mCi/mmole, respectively), SC-48334 and internal standard SC-49270 (1,4-(butylimino)-1,4-dideoxy-L-arabinitol). All other chemicals were commercially available.

Stability of [^{14}C]Glycovir

[^{14}C]glycovir was incubated in 0.1 M phosphate buffer (pH 8.0) at a concentration of 50 μg/mL at 37°C for 2 hours. Concentrations of [^{14}C]glycovir remaining after incubation were analyzed using an HPLC procedure.

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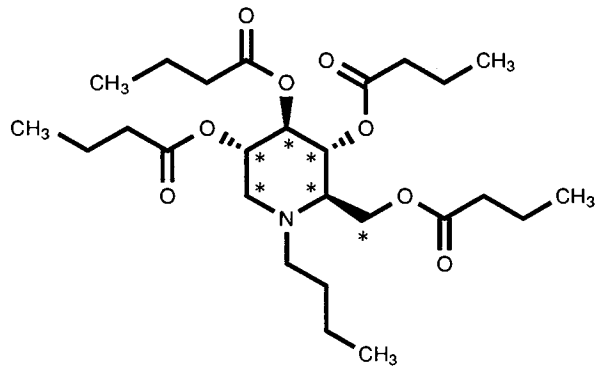

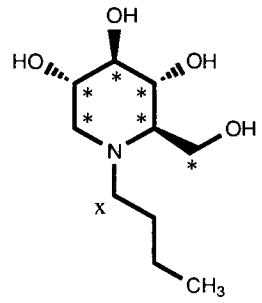

Fig. 1. Chemical structures of glycovir and SC-48334. Asterisks indicate the position of labeled carbon and X indicates the position of tritium.

In Vitro Metabolism

Gastrointestinal Mucosa. The gastrointestinal (GI) mucosa of the 3 rats, 2 dogs and 3 cynomolgus monkeys were prepared in phosphate buffer (pH 8.0) with duodenum, jejunum, ileum and colon (proximal and distal) while the GI mucosa of the 3 rhesus monkeys and 2 men were prepared with duodenum, jejunum and ileum using the method described previously (10). The human GI tract was obtained from Keystone Skin Bank, International Institute for the Advancement of Medicine (Extow, PA). The intestine was blotted to dryness and weighed. Intestinal mucosa were scrapped off with a glass slide by placing the exerted intestine on a flat surface. The mucosa was added to 1 volume (1 g in 1 mL) of saline and homogenized at 4°C. The whole mucosal homogenate was suspended in physiological saline to achieve a final concentration of 0.67 mg mucosa/mL. An aliquot (24 μL) of [^{14}C]glycovir stock solution was added to the mucosa suspension at a final concentration of 50 μg/mL of homogenate, and the mixture was incubated at 37°C for 0, 5, 10, 30, 60 and 120 min.

Liver S9 Fraction. Liver S9 fraction was prepared using the previously reported method (11). [^{14}C]glycovir was incubated with liver S9 fraction (1 mg protein/mL of incubation mixture) without an NADPH generating system at a drug concentration of 50 μg/mL at 37°C for 0, 5, 10, 30 and 60 min.

Whole Blood, RBC and Plasma. Fresh blood was obtained in heparinized tubes from the rat, dog, monkey (cynomolgus and rhesus) and healthy human volunteers on the day of the experiment. [^{14}C]glycovir was added to the fresh blood, RBC or plasma to give a final concentration of 20 μg/mL and incubated at 37°C for 0, 30 and 60 min.

In all in vitro studies, an esterase inhibitor, diethyl-p-nitrophenyl phosphate (30 μL of 10% ethanolic solution), was added to 1 mL of 0 min samples prior to the addition of [^{14}C]glycovir.

In Vivo Bioavailability

Rat. Male rats (Charles River) weighing 250 to 350 g were fasted overnight prior to drug administration. [^{3}H]SC-48334 was administered orally as an aqueous solution at a dose of 60 mg/kg. [^{14}C]glycovir was administered orally to male rats as a suspension at a dose of 137 mg/kg (60 mg SC-48334 equiv/kg). The suspension was prepared in a mixture of 0.5% methylcellulose and 0.1% polysorbate in water. Peripheral blood samples were collected at specified times from the tail vein into heparinized vacutainer tubes containing the esterase inhibitor, diethyl-p-nitrophenyl phosphate (30 μL of 10% ethanolic solution). The plasma samples were analyzed for nonvolatile tritium (obtained after removal of volatile $^{3}H_2O$) and total carbon-14 radioactivity for the [^{3}H]SC-48334 and [^{14}C]glycovir studies, respectively. Urine samples were collected every 24 h for 48 h. Metabolic profiles of plasma (selected time points) and urinary radioactivity were obtained using an HPLC procedure. The plasma concentrations of total radioactivity are expected to reflect the concentrations of pharmacologically active SC-48334 since greater than 95% of plasma radioactivity is due to SC-48334 regardless of the compounds administered.

In a separate study, [^{14}C]glycovir was administered orally at a dose of 136 mg/kg. Portal and peripheral blood, urine and fecal samples were collected. In addition, gastric and small intestinal fluids were collected by rinsing dissected stomach and small intestine with saline. All samples except fecal samples were treated with the esterase inhibitor immediately after collection.

Dog. Six male and six female beagle dogs weighing 7 to 12 kg were fasted overnight prior to dosing. SC-48334 or Glycovir was administered orally to each group of three male and three female dogs at a dose of 80 mg SC-48334 equiv/kg. Both compounds were given as neat chemicals in gelatin capsules after confirming that the bioavailabilities of SC-48334 after SC-48334 administration as a solution and after glycovir administration as a suspension were similar to those after administration of the respective compound in capsules. Blood samples were collected at specified times into heparinized vacutainer tubes containing the esterase inhibitor, diethyl-p-nitrophenyl phosphate. Plasma concentrations of SC-48334 were determined using an HPLC procedure.

[^{14}C]Glycovir and [^{14}C]SC-48334 were also administered to each group of two dogs at a dose of 80 mg SC-48334 equiv/kg. Urine samples were collected every 24 h for 48 h, and the percentages of the dose excreted in urine were determined.

Monkey. Six female rhesus monkeys weighing between 4.2 kg and 5.7 kg were fasted overnight prior to dosing. [^{3}H]SC-48334 was administered orally to one group of three monkeys as an aqueous solution at a dose of 64 mg/kg. Unlabeled Glycovir was administered orally to the other group of three monkeys as a suspension (0.5% methylcellulose and 0.1% polysorbate in water) at a dose of 64 mg SC-48334 equiv/kg. Blood samples were collected at specified times into heparinized vacutainer tubes containing the esterase inhibitor diethyl-p-nitrophenyl phosphate (30 μL of 10% ethanolic solution). Following the [^{3}H]SC-48334 dose, plasma samples were analyzed for nonvolatile tritium. Metabolic profiles of plasma (selected time points) and urinary radioactivity were obtained using an HPLC procedure. Since greater than 85% of nonvolatile plasma radioactivity was due to SC-48334, nonvolatile tritium concentrations were used as the parent drug concentration. Following the unlabeled glycovir dose, plasma concentrations of SC-48334 were determined using the HPLC procedure.

[^{14}C]Glycovir and [^{14}C]SC-48334 were also administered to each group of two rhesus monkeys at a dose of 55 mg SC-48334 equiv/kg. Urine samples were collected every 24 h for 48 h, and the percentages of the dose excreted in urine were determined.

Man. Twelve HIV antibody positive, asymptomatic male volunteers, 18 years of age or older, participated in the study. A CD4+ cell count of $\geq$300/mm^3 and a negative hepatitis B surface antigen test results obtained within 14 days prior to admission to the study were also required. Glycovir in soft gelatin capsules (lot #RCT 9295) containing 0.25 or 1.0 g glycovir along with gelatin, glycerin and titanium dioxide and SC-48334 in capsules (lot #RCT9296) containing 0.5 g SC-48334 were provided by G.D. Searle & Co. Six subjects received glycovir at a dose of 2.5 g (1.0 g SC-48334 equiv)/person (2 × 0.25 g capsules and 2 × 1 g capsules), and the other six subjects received SC-48334 at a dose of 1 g/person (2 × 0.5 g capsules). Blood samples (10 mL) were drawn into heparinized vacutainer tubes injected with the esterase inhibitor diethyl-p-nitrophenyl phosphate at the following time intervals: 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, 72, 96, 120 and 144 h. Plasma concentrations of SC-48334 were determined using the HPLC procedure.

Sample Analysis

In Vitro. An aliquot (250 μL) of incubation mixture was removed at each time point, immediately mixed with 10 mL of a solvent mixture of acetone and methanol (1:1, by vol.) and centrifuged at 16000 × g. The supernatant was separated and dried under a stream of nitrogen. The dried residue was reconstituted with a solvent mixture of 52.5% methanol in 0.02 M heptane sulfonic acid in water (pH 2.5) and analyzed using a high performance liquid radiochromatographic (HPLRC) procedure.

In Vivo. Plasma concentrations of nonvolatile tritium or carbon-14 were determined by liquid scintillation counting (LSC) after the addition of a 50-500 μL aliquot to a mixture of 2 mL of water and 5 mL of scintillant (Amersham Co., Arlington Heights, IL).

To determine SC-48334 concentrations, plasma samples were extracted using a Bond Elut procedure as follows: To an aliquot (30 μL) of each plasma sample, an internal standard, (SC-49270) and 0.6 mL of 0.01 N HCl solution were added. Each sample mixture was applied to a 100 mg SCX Bond Elut column previously activated with water. The retained SC-48334 and the internal standard were eluted with 2 × 1 mL of 1% NH_4OH in methanol. The eluent was evaporated, and the residue was resolubilized in a 0.2 mL mixture of methanol and 0.02 M heptane sulfonic acid plus 0.01 M sodium phosphate (pH 4.5) in water (28:72, v/v). An aliquot of the sample was injected onto the HPLC.

LSC. All radioactivity determinations were carried out using a liquid scintillation spectrometer (Mark III, Traco Analytic, Inc., Elk Grove, IL). Chemical quenching was corrected by the external standard channel ratio method.

HPLC and HPLRC. HPLC was performed with a Waters M600 solvent delivery system, Waters WISP autoinjector, a Sphei-10 RP-18 HPLC cartridge and holder (30 mm × 4.6 mm, 10 μ, Brownlee Labs, Inc., Santa Clara, CA) and a Supelcosil LC-8-DB (15 cm × 4.6 mm, 10 μ, Supelco Inc., Bellefonte, PA) analytical column. An aliquot (30 μL) of each sample was injected onto the HPLC with an isocratic solvent system of 28% methanol/72% 0.02 M heptane sulfonic acid and 0.01 M sodium phosphate (pH 4.5) in water at a flow rate of 1.0 mL/min. SC-48334 and the internal standard SC-49270 were detected using a Coulochem Electrochemical detector (ESA Model 5100A, ESA Inc., Bedford, MA) with an analytical cell (ESA Model 5010). The electrochemical detector was set at +0.75 volts.

HPLRC was performed on an HPLC system composed of a Hewlett Packard (HP) pump, an autosampler (HP 1050) and a Supelcosil LC-8-DB column (15 cm × 4.6 mm ID, 5 micron particle size). A linear gradient system was employed from 10% methanol in 20 mM heptane sulfonic acid in water (pH 2.5) to 95% methanol in 20 mM heptane sulfonic acid in water (pH 2.5) over a 75 minute period. The flow rate was 1.0 mL/min.

The eluent from the HPLC column was mixed with Flo-Scint III™ (Packard Instrument Co., Inc., Meriden, CT) at a ratio of 1:4 (by vol). The radioactivity was detected by a FLO-ONE radioactive detector (FLO-ONE/Beta Model IC, Packard Instrument Co.).

Glycovir has four butyryl ester groups which appear to be hydrolyzed in a random manner. However, the HPLC method was specific for SC-48334, and the HPLRC method was specific for both SC-48334 and glycovir.

RESULTS

Stability in Buffer

When [^{14}C]glycovir was incubated in 0.1 M phosphate buffer (pH 8.0) at 37°C for 2 hours, there was no substantial hydrolysis of the prodrug. These results indicate that [^{14}C]SC-49483 was chemically stable in the buffer, and the non-enzymatic hydrolysis of [^{14}C]glycovir was minimal during the incubation of the prodrug with GI mucosa, liver S9 and whole blood.

In Vitro Metabolism

The mean (±SEM) percentages of [^{14}C]glycovir remaining after incubation with whole mucosal suspension from

various sections of small intestine are shown in Fig. 2. The in vitro hydrolysis rate of [^{14}C]glycovir in duodenum and jejunum mucosa was fastest in the rat, and the mean % of [^{14}C]-glycovir after a 30 min incubation was less than 1%. In the cynomolgus monkey, rhesus monkey and man, the hydrolysis rate was moderate and the mean % of [^{14}C]glycovir after a 30 min incubation varied between 86% and 87%, 76% and 87%, and 83% and 91%, respectively. In dog mucosa, there was no substantial hydrolysis of the prodrug even after 2 h incubation.

In ileum mucosa, the hydrolysis rates of [^{14}C]glycovir were also fastest in the rat. The mean % of [^{14}C]glycovir in the 30 min incubation mixture was 67 ± 6%. The values for the cynomolgus monkey, rhesus monkey and man were 88 ± 3%, 78 ± 1% and 76% (N = 2), respectively. As in duodenum and jejunum mucosa, there was no substantial hydrolysis observed in dog ileum. In both proximal and distal colon mucosa, the hydrolysis of the prodrug was minimal in the rat, dog and monkey.

The mean percentages of [^{14}C]glycovir remaining after incubation of the prodrug with liver S9 fractions are shown in Fig. 3. With liver S9 fractions, the hydrolysis rates of the prodrug in the cynomolgus monkey and man were similar, and the mean % of [^{14}C]glycovir after a 10 min incubation were 22 ± 0.4% and 30 ± 4%, respectively. In the rat and dog

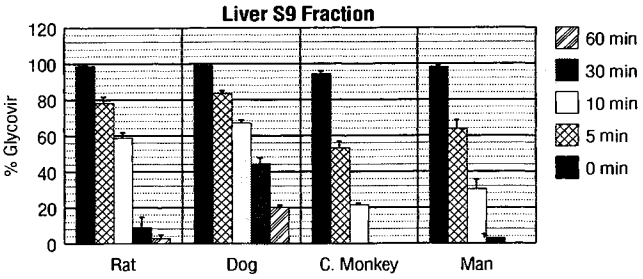

Fig. 3. The mean (±SEM) percentages of [^{14}C]glycovir remaining after incubation of the compound in liver S9 fractions.

S9 fractions, the hydrolysis rates were slower, and the mean % of [^{14}C]glycovir remaining after a 10 min incubation were 59 ± 3% and 67 ± 2%, respectively.

The hydrolysis rate of the prodrug in whole blood was fastest in the rat, and the mean % of [^{14}C]glycovir remaining after a 30 min incubation was 28 ± 4%. The mean % of [^{14}C]glycovir remaining after a 30 min incubation in dog, cynomolgus monkey, rhesus monkey and human blood were 60 ± 1%, 50 ± 4%, 45 ± 5% and 59 ± 4%, respectively (Fig 4).

When the hydrolysis rate of [^{14}C]glycovir was determined in RBC suspensions and plasma separately, more

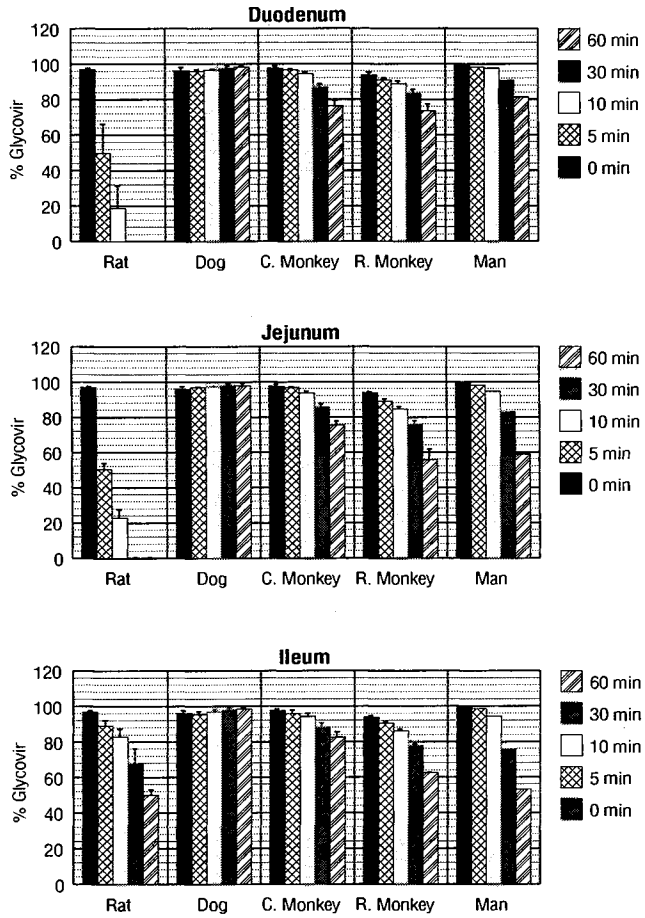

Fig. 2. The mean (±SEM) percentages of [^{14}C]glycovir remaining after incubation of the compound in duodenum (top panel), jejunum (middle panel) and ileum (bottom panel) mucosa.

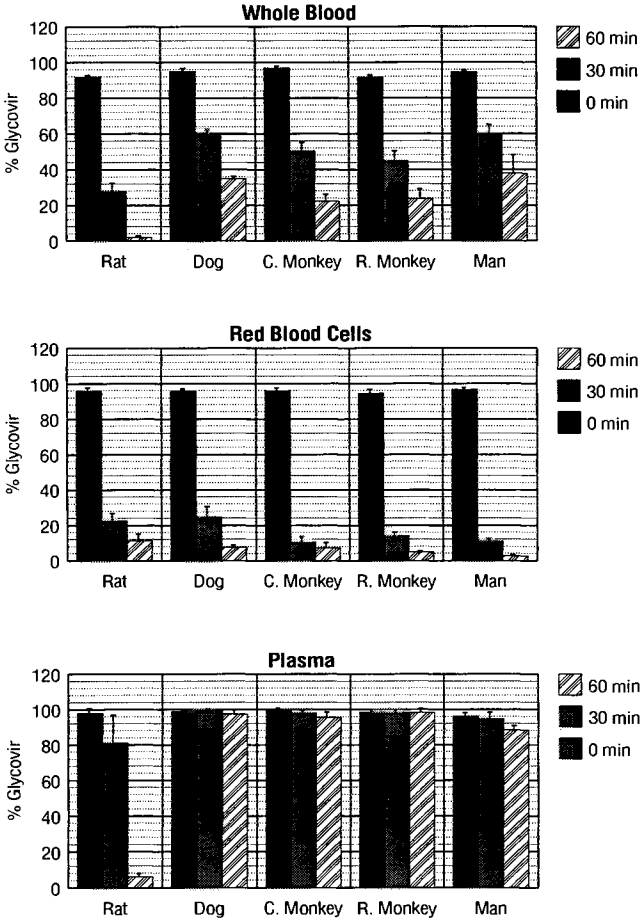

Fig. 4. The mean (±SEM) percentages of [^{14}C]glycovir remaining after incubation of the compound in whole blood (top panel), RBC (middle panel) and plasma (bottom panel).

rapid hydrolysis of [^{14}C]glycovir was observed with RBC suspensions than with plasma in all species examined (Fig 4). Except for the rat, glycovir was slowly metabolized in plasma, and more than 85% of the initial concentration remained in plasma as the prodrug after incubation for 1 hour. Thus, hydrolysis of glycovir observed in dog, monkey and human blood was primarily due to hydrolysis of the prodrug in RBC.

In Vivo Studies

Table I gives model independent pharmacokinetic parameters of SC-48334 after oral administration of SC-48334 or glycovir. Although the pharmacokinetic parameters were obtained from total radioactivity concentrations in the rat and monkey, the vast majority of plasma radioactivity was due to the pharmacologically active SC-48334 (>95% in the rat and ≥85% in the monkey). Thus these parameters are expected to reflect the approximate values of SC-48334. The bioavailability of SC-48334 in the rat after the prodrug administration was as high as after SC-48334 administration. However, in the dog the bioavailability of SC-48334 after prodrug administration was approx. 15% of the bioavailability of SC-48334 after SC-48334 administration. Furthermore, the increase in oral doses of glycovir did not substantially increase the systemic exposure of SC-48334 (unpublished data). The bioavailabilities of SC-48334 in the monkey and man after glycovir administration were moderate and approx. 42 and 37% of those after SC-48334 administration, respectively.

The times to reach peak plasma concentration (T_{max}) of SC-48334 were higher after prodrug administration compared to those after SC-48334 administration regardless of species (Table I). The delay of the Tmax with prodrug appeared to be the result of slow dissolution of glycovir in the GI tracts as well as slow conversion of the prodrug to SC-48334, resulting in an apparent slower absorption rate of SC-48334 after glycovir administration than after SC-48334 administration.

After oral administration of [^{14}C]glycovir to the rat, the majority of portal plasma radioactivity was due to pharmacologically active [^{14}C]SC-48334, and there was no substantial amount of the prodrug or other metabolites observed. In contrast, the vast majority of radioactivity in gastric fluids,

Table I. Pharmacokinetic Parameters of SC-48334 After Oral Administration of SC-48334 or Glycovir

Species	Parameters	Compound administered	
		SC-48334	Glycovir[a]
Rat	Dose (mg/kg)	60	60
	C_{max} (μg/mL)	3.88 ± 0.57[b]	5.97 ± 0.52[c]
	T_{max} (hr)	0.6 ± 0.1	1.0 ± 0.0
	AUC_{0-8h} (μg · h/mL)	16.6 ± 1.0[b]	16.4 ± 2.1[c]
	Relative BA[d] (%)	100	99
	^{14}C urinary excretion (%)	75.2 ± 2.6[e]	74.0 ± 2.9
Dog	Dose (mg/kg)	80	80
	C_{max} (μg/mL)	66.7 ± 10.0	1.43 ± 0.03
	T_{max} (hr)	1.3 ± 0.3	2.4 ± 0.8
	AUC_{0-8h} (μg · h/mL)	272 ± 23	35.5 ± 7.9
	Relative BA[d] (%)	100	15
	^{14}C urinary excretion (%)	86.4[f]	26.7[f]
Monkey	Dose (mg/kg)	64	64
	C_{max} (μg/mL)	31.5 ± 4.7[b]	6.05 ± 0.22
	T_{max} (hr)	1.3 ± 0.3	2.3 ± 0.9
	AUC_{0-24h} (μg · h/mL)	178 ± 31[b]	75.0 ± 0.1
	Relative BA[d] (%)	100	42
	^{14}C urinary excretion (%)	73.9[f]	41.4[f]
Man	Dose (g)	1.0	1.0
	C_{max} (μg/mL)	9.90 ± 1.10	1.48 ± 0.26
	T_{max} (hr)	3.3 ± 0.2	12.2 ± 3.9
	AUC_{0-72h} (μg · h/mL)	83.7 ± 7.6	33.9 ± 2.0
	Relative BA[d] (%)	100	37

[a] Dose for Glycovir are SC-48334 mg equiv/kg, and C_{max} and AUC values are SC-48334 μg equivalents.
[b] Values were obtained from nonvolatile tritium concentrations.
[c] Values are obtained from total C-14 concentrations.
[d] Relative bioavailability was calculated dividing AUC of SC-48334 after glycovir administration by AUC of SC-48334 after SC-48334 administration at molar equivalent dose.
[e] The value is % of dose excreted as total tritium after oral administration of [^{3}H]SC-48334 at a dose of 160 mg/kg.
[f] Mean of N = 2 values. Dose was 55 mg SC-48334 equiv/kg.

small intestinal fluids and feces was due to the prodrug and there was no substantial amount of [^{14}C]SC-48334. These results suggest that absorption of the prodrug itself was minimal in the rat whereas SC-48334 was rapidly and well absorbed.

DISCUSSION

Highly water soluble, aminosugar derivative, SC-48334 was rapidly and well absorbed in the rat, dog, monkey and man without substantial differences in its bioavailability among the species. However, the relative bioavailability of SC-48334 after oral administration of the poorly water soluble ester prodrug was remarkably species dependent. When the in vitro hydrolysis rates of [^{14}C]glycovir were determined in the various sections of small intestine and colon mucosa, liver S9 and blood, the hydrolysis rate in small intestinal mucosa was best correlated with the relative bioavailability of SC-48334 in the in vivo studies. The in vitro hydrolysis rate in small intestinal mucosa was highest in the rat, moderate in the monkey and man, and lowest in the dog, which is in the order of decreasing relative bioavailability.

The comparative radioactive study with [^{14}C]glycovir and [^{14}C]SC-48334 demonstrated that absorption of total radioactivity in the dog was much lower with [^{14}C]glycovir than with [^{14}C]SC-48334 as evidenced by low urinary excretion of total radioactivity. These results together with the in vitro data suggest that the low relative bioavailability of [^{14}C]SC-48334 after prodrug administration to the dog was not due to low conversion of [^{14}C]glycovir to [^{14}C]SC-48334 after good absorption of the prodrug. Therefore, it may be concluded that SC-48334 was absorbed much better than the prodrug itself, and thus rapid hydrolysis of the prodrug to SC-48334 in small intestine resulted in high relative bioavailability in the rat while slow hydrolysis of the prodrug resulted in poor bioavailability of SC-48334 in the dog. This conclusion is further supported by the fact that there was no prodrug found in portal plasma after oral administration to the rat, whereas there was no substantial amount of SC-48334 detected in GI fluids and feces. There was no evidence that the prodrug itself was substantially absorbed without being hydrolyzed in the gastrointestinal tract in the rat. Since highly lipophilic compounds with log D greater than 4 are not generally expected to be well absorbed (2,3), it is conceivable that poorly water soluble glycovir with log D of 4.5 is minimally absorbed in the GI tract.

The in vitro hydrolysis rate of glycovir in the monkey small intestinal mucosa was moderate and similar to that in man. In addition, the relative bioavailability of active SC-48334 in the monkey after prodrug administration was moderate and similar to that in man. These results indicate that the monkey is a good animal model for the prediction of the hydrolysis rate of glycovir in the human small intestine and subsequently for the prediction of the relative bioavailability of SC-48334 after glycovir administration to man.

With liver S9 fractions, the hydrolysis rate of glycovir was also slow in the dog as observed in the small intestinal mucosa. However, the relative hydrolysis rates of glycovir in liver S9 fractions among different species did not parallel those in mucosa of the small intestine. In contrast to the small intestinal mucosa and liver S9, the hydrolysis rate in dog whole blood was similar to that in man although it was slower than those found in the rat and monkey. Therefore, hydrolysis rates of glycovir in blood and liver S9 fractions were not predictive of the hydrolysis rate in small intestine. This may be due to the fact that the esterases in small intestine, liver and blood are not the same isozymes within each species and that the esterases from intestinal mucosa are characteristically different from hepatic and blood esterases (6,12-14). Cholinesterases are primarily involved in drug hydrolysis in the plasma, arylesterases in plasma and red blood cells, and carboxylesterases in the liver, intestine and other tissues (15). An additional possibility for the lack of correlation in esterase activities in various tissues among different species is that the proportion of esterase distribution in various tissues may be species dependent.

In all animal species examined [^{14}C]glycovir was more rapidly metabolized in small intestinal mucosa than in colonic mucosa. These results are consistent with previous findings with other esters (2).

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REFERENCES

1. A. Karpas, G.W.J. Fleet, R.A. Dwek, S. Petursson, S.K. Namgoong, N.G. Ramsden, G.S. Jacob and T.W. Rademacker. Aminosugar Derivatives as Potential Anti-Human Immunodeficiency Virus Agents. *Proc. Ntl. Acad. Sci.* 85:9229-9233 (1988).
2. M. Narawane, S.K. Podder, H. Bundgaard, and V.H.L. Lee. Segmental Differences in Drug Permeability, Esterase Activity and Ketone Reductase Activity in the Albino Rabbit Intestine. *J. Drug Target.* 1:29-39 (1993).
3. M. Miyauch, T. Hirota, K. Fujimoto, and J. Ide, Studies on Orally Active Cephalosporin Esters. IV. Effect of the C-3 Substituent of Cephalosporin on the Gastrointestinal Absorption in Mice. *Chem. Pharm. Bull.* 37:3272-3276 (1989).
4. A.A. Sinhura, and S.H. Yalkowsky. Rationale for design of biologically reversible drug derivatives; Prodrugs. *J. Pharm. Sci.* 64:181-210 (1975).
5. M. Rowland, S. Riegelman, P.A. Harris and S.D. Sholkoff. Absorption of aspirin kinetics in man following oral administration of an aqueous solution. *J. Pharm. Sci.* 61:379-385 (1972).
6. M. Inoue, M. Morikawa, M. Tsuboi and M. Sugiura. Species difference and characterization of intestinal esterase on the hydrolyzing activity of ester-type drugs. *Jpn. J. Pharmacol.* 29:9-16 (1979).
7. W.E. Luttrell and M.C. Castle. Species difference in the hydrolysis of meperidine and its inhibition by organophosphate compounds. *Fundam. Appl. Toxicol.* 11:323-332 (1988).
8. M. Morikawa, M. Inoue and M. Tsuboi. Substrate specificity of carboxylesterase from several animals. *Chem. Pharm. Bull.* 24:1661-1664 (1976).
9. C.Y. Quon, K. Mai, G. Patil, and H.F. Stampfli. Species differences in the stereoselective hydrolysis of esmol by blood esterases. *Drug Metab. Dispos.* 16:425-428 (1988).
10. S.K. Yang, Y. Hsieh, W. Chang and J. Huang. Enantioselectivity of microsomal and cytosolic esterases in rat intestinal mucosa. *Drug Metab. Dispos.* 20:719-725 (1992).
11. C.S. Cook, C. Hauswald, J.A. Oppermann and G.L. Schoen-

hard. Involvement of Cytochrome P-450IIIA in Metabolism of Potassium Canrenoate to an Epoxide: Mechanism of inhibition of the epoxide formation by spironolactone and its sulfur-containing metabolite. *J. Pharmacol. Exp. Ther.* **266**:1-7 (1993).
12. W.N. Aldridge. Serum esterase. I. Two types of esterases (A and B) hydrolyzing p-nitrophenyl acetate, propionate and butyrate, and a method for determination. *Biochem. J.* 53:110-117 (1953).
13. W.N. Aldridge. A-esterase and B-esterase in perspective. In E. Reiner, W.N. Aldridge and E.C.G. Hoskin (eds.), *Enzymes Hydrolyzing Organophosphorus Compounds,* Ellis Horwood, Chichester, 1989, pp. 246-253.
14. A.H. Conney. Pharmacological implication of microsomal enzyme induction. *Pharmacol. Rev.* 19:317-366 (1967).
15. F.M. Williams. Clinical significance of esterases in man. *Clin. Pharmacokinet.* 10:392-403 (1985).