The Bioavailability and Nonlinear Clearance of (−)-Carbovir in the Rat[1]

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The pharmacokinetics and bioavailability of (±)-carbovir, a carbocyclic nucleoside active against human immunodeficiency virus, have been described previously. To determine the bioavailability of (−)-carbovir, the biologically active enantiomer, four male Sprague–Dawley rats received 18 mg/kg of (−)-carbovir through the jugular vein and 54 mg/kg orally. Following the pilot studies, five rats were randomly assigned to receive (−)-carbovir in a three-way crossover design as either a single 18-mg/kg iv bolus, a single 54-mg/kg oral dose, or a single iv infusion of 18 mg/kg to achieve a target steady-state concentration (C_{ss}) of 1 μg/ml, the peak concentration after an oral dose. Blood and urine samples were analyzed by an improved ion-paired reversed-phase HPLC method with fluorescence detection. Blood concentrations of (−)-carbovir declined in a biphasic manner after the iv bolus dose. The terminal half-life was 116 and 106 min after the iv bolus and oral dose, respectively. The blood/plasma distribution ratio was approximately 1.0 in the range of 1 to 10 μg/ml of (−)-carbovir in blood. The free fraction in serum was concentration dependent. Significant differences in the renal, nonrenal, and total-body clearances after the iv bolus and iv infusion suggested nonlinear elimination of (−)-carbovir. The oral bioavailabilities derived from blood data were significantly different when the iv bolus was used as a reference rather than the iv infusion. However, the bioavailabilities were not significantly different when the total urinary excretion of unchanged (−)-carbovir after iv bolus or infusion was used as a reference. Concomitant saturation of renal and nonrenal clearances might explain these findings. The oral bioavailability was about 20% at concentrations approximating 1 μg/ml in blood.

KEY WORDS: (−)-carbovir; bioavailability; nonlinear clearance; Sprague–Dawley rats.

INTRODUCTION

Carbovir (carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine; NCS-614864) is a novel carbocyclic guanosine derivative which has been found to be a potent agent against human immunodeficiency virus (HIV) *in vitro* (1). The therapeutic index of carbovir ranged from 200 to 400 in the human lymphoblastoid cells lines, ATH8, MT-2, and CEM, with an effective concentration, (EC_{50}) of 0.15 to 0.19 μg/ml (1). In addition to the wide therapeutic index, carbovir is highly specific for the inhibition of HIV reverse transcriptase. As compared to AZT-triphosphate, the inhibition of human DNA polymerases α, β, γ, and DNA primase by carbovir-triphosphate was either nondetectable or very low (2). These two characteristics, potency and selectivity, make carbovir an excellent candidate for the treatment of AIDS.

The therapeutic range and dose of new compounds in humans may be suggested by upscaling the pharmacokinetic parameters obtained in animals. The pharmacokinetics of racemic carbovir in rats have been studied and presented previously (3). However, since (−)-carbovir (Fig. 1) is the biologically active form of carbovir (4), experiments were designed to investigate the bioavailability and pharmacokinetics of (−)-carbovir after single-dose administration of (−)-carbovir to the Sprague–Dawley rat. The present study suggests that for the determination of an absolute oral bioavailability in the presence of nonlinear clearance, the reference intravenous administration should generate concentrations of drug in the blood that are similar to those obtained with the oral dose.

MATERIALS AND METHODS

Chemicals

(−)-Carbovir and the internal standard used for the HPLC assay were synthesized as described previously (5). Triethylamine was obtained from Aldrich Chemical Co., Inc. (Milwaukee, Wis.). Hexanesulfonic acid sodium salt was purchased from Eastman Kodak Co., Inc. (Rochester, N.Y.), and sodium dodecyl sulfate was purchased from Fluka Chemical Corp. (Ronkonkoma, N.Y.). All other chemicals were the same as reported previously (3).

Animals

Nine male Sprague–Dawley rats (Bio-Labs, St. Paul, Minn.) weighing 270 ± 40 g were cannulated in both the jugular and the femoral veins under pentobarbital (55 mg/kg ip) anesthesia. The rats were rested for 24 hr after surgery. (−)-Carbovir was dissolved to a concentration of 8 mg/ml in normal saline containing approximately 1 molar equivalent of hydrochloric acid. Four rats were randomly assigned to receive (−)-carbovir following a two-way crossover design as either a single iv bolus dose of 18 mg/kg through the jugular vein or a single oral dose of 54 mg/kg. Following the pilot studies five additional rats were randomly assigned to receive (−)-carbovir following a three-way crossover design as either a single iv bolus dose of 18 mg/kg, a single oral dose of 54 mg/kg, or a single iv infusion of 18 mg/kg infused over 5–9 hr into the jugular vein. The washout period between each treatment was 48 hr. Blood samples of 250 μl were taken from the femoral vein prior to the dose and at 2.5, 5, 15, 30, 60, 90, 150, 240, 360, and 480 min after the iv bolus and oral doses. Blood samples were taken predose, then five samples were taken at approximately equal intervals during the iv infusion and at 15, 30, 45, 60, and 120 min postinfusion. Urine was collected for all treatments over the intervals of 0–2, 2–6, 6–12, 12–24, 24–36, and 36–48 hr after doses.

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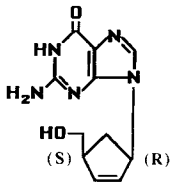

(-)-Carbovir

Fig. 1. Structure of (−)-carbovir.

Assay

The preparation of blood samples by solid-phase extraction has been described previously (3). Urine samples were diluted with mobile phase and washed by an anhydrous diethyl ether extraction, a procedure that removed interfering endogenous materials. The aqueous phase was centrifuged and then injected onto the HPLC directly. The analytical procedure and assay validation for the quantitation of (−)-carbovir in blood and urine by a reversed-phase HPLC method with an ion-pairing, acidic mobile phase was recently described by Remmel *et al.* (6). (−)-Carbovir and the internal standard were detected with a Shimadzu RF-530 fluorescence HPLC monitor (Columbus, MD; λ_{ex} = 275 nm, λ_{em} = 345 nm) (6,7).

Blood/Plasma Distribution and Protein Binding

The procedures were similar to those reported previously for (±)-carbovir (3). The concentrations of (−)-carbovir in blood and plasma were measured by the fluorometric HPLC procedure after the addition of known amounts of (−)-carbovir to blank whole blood. The blood/plasma distribution was measured by dividing the (−)-carbovir concentration in blood by its concentration in plasma.

Because anticoagulants have been reported to affect the *in vitro* protein binding of some drugs, serum was used rather than plasma for the protein binding study (8). The membrane ultrafiltration method that was used for these studies has been described previously (3). The free fraction was calculated by dividing the concentration of (−)-carbovir in the serum filtrate by its concentration in serum. Assuming that the free fraction in plasma and serum are equal *in vivo*, the fraction of unbound (−)-carbovir in blood was determined by dividing the free fraction of (−)-carbovir in serum by the blood/plasma ratio. The hematocrit (HCT) was approximately 0.42. The erythrocyte/plasma distribution of (−)-carbovir was also calculated (9).

Pharmacokinetic Analysis

Pharmacokinetic analysis of the concentration–time data in blood was carried out by noncompartmental methods (9). The oral bioavailability (F) was determined by dividing the dose-corrected area under the (−)-carbovir concentration-time profile in blood (AUC^{∞}_{po}) after an oral dose by the AUC^{∞} after an iv bolus injection (AUC^{∞}_{iv}) or iv infusion (AUC^{∞}_{inf}). Alternatively the bioavailability was determined by dividing the total amount of the unchanged drug excreted in the urine (X_u^{∞}) after an oral dose by the X_u^{∞} after an iv bolus injection or iv infusion, corrected for the dose. The time-averaged total-body clearance (CL) was calculated by dividing the dose by the AUC^{∞}. The total-body clearance at steady state was calculated by dividing the infusion rate by the steady-state concentration of (−)-carbovir achieved during the iv infusion. The time-averaged renal clearance (CL_R) was calculated by dividing the X_u^{∞} by the AUC^{∞}. Nonrenal clearance (CL_{NR}) was determined by subtracting the renal clearance from the total-body clearance. The clearances were then normalized by the body weight. The ratio of renal clearance to total-body clearance was the fraction of the (−)-carbovir dose excreted unchanged (f_e). The maximum concentration after the oral dose (C_{max}) and the time to reach C_{max} (t_{max}) were obtained by observation of the blood concentration–time data. The elimination rate constant was estimated by the least-squares linear regression of the terminal phase of the concentration-versus-time profile. The terminal half-life was calculated by dividing 0.693 by the elimination rate constant. For the oral and infusion studies, standard noncompartmental methods were also used to determine the steady-state volume of distribution (V_{ss}), mean residence time (MRT), and mean absorption time (MAT). MAT was calculated by subtracting the MRT from the mean residence time after oral dose. The MRT was obtained by subtracting one-half of the infusion interval (τ) from the mean residence time after iv infusion (9).

In order to combine the results from the pilot study (a two-way crossover) and the three-way crossover study, unpaired t tests were carried out on the elimination rate constants, nonrenal and total-body clearances, bioavailability estimates, and f_e values. Renal clearances after the three administrations were compared for all rats by ANOVA with Scheffé's procedure (10). Because of a concern about the statistical propriety of combining the two designs, paired t tests and ANOVA with paired data were also carried out on the data from the rats in the three-way study only. The statistical results of the paired t tests and ANOVA with paired data were no different from the results from the unpaired analysis, indicating small interanimal variability. Therefore the results reported in the tables and text are those obtained from all nine animals, analyzed by the unpaired design.

Free fractions of (−)-carbovir in blood and serum and blood/plasma and erythrocyte/plasma ratios were compared with respect to the effects of concentration by ANOVA with Scheffé's procedure. In all statistical comparisons a P value of less than 0.05 was considered to be significant.

RESULTS

The mean blood concentration–time profile is shown in Fig. 2 for the iv bolus and oral doses. (−)-Carbovir has a biexponential profile after the iv bolus dose, with an elimination half-life of 116 ± 19 min. Table I represents the pharmacokinetic parameters obtained from blood data after the administration of (−)-carbovir. The time-averaged total body clearance was calculated to be 27.3 ± 8.8 ml/min/kg. The terminal half-life of (−)-carbovir after the oral dose was 106 ± 12 min. The terminal phases after the iv bolus and oral doses appear to be parallel and the elimination rate constant after the oral dose was not significantly different from that

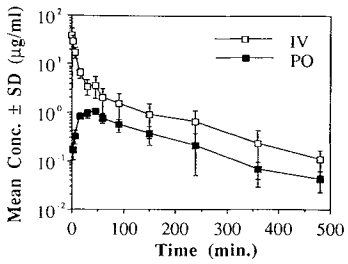

Fig. 2. Mean concentration–time profiles in the nine rats after iv bolus (18 mg/kg) and oral administration (54 mg/kg) of (−)-carbovir.

after the iv bolus dose. The maximum concentration (C_{max}) of (−)-carbovir and time to reach C_{max} (t_{max}) after an oral dose were 1.00 ± 0.16 μg/ml and 38 ± 11 min, respectively. The bioavailability was calculated to be 7.5 ± 2.0% by comparing the dose-corrected AUC^{∞} values after the oral and iv bolus doses. The MAT was 99 ± 24 min.

Renal clearances were derived from the blood and urine data and are shown in Table II. The renal clearance after the iv bolus dose was 15.1 ± 6.9 ml/min/kg, whereas the renal clearance after the oral dose was 41.0 ± 6.8 ml/min/kg, a significant difference. The nonrenal clearance and fraction excreted unchanged were 12.2 ± 5.1 ml/min/kg and 0.55 ± 0.12 after the iv bolus injection, respectively. The bioavailability calculated from the urine data was 20.6 ± 5.1%, about three times higher than that obtained from the blood data with the bolus as a reference. This inconsistency suggested there was a nonlinearity in the pharmacokinetic characteristics of (−)-carbovir, and the difference in renal clearance after oral and iv bolus dosing supported this contention. The discrepancy in the bioavailability and renal clearance was already apparent during the pilot study with the first four rats. The subsequent study with the addition of the iv infusion dose was designed to further delineate the source of the nonlinearity.

Five rats were randomly assigned to receive the iv bolus, iv infusion, and oral doses. The iv infusion dose was the same as the iv bolus dose, approximately 18 mg/kg, and the infusion rate was calculated to obtain a steady-state concentration of (−)-carbovir of approximately 1 μg/ml, the peak concentration after the oral dose. Figure 3 shows the concentration-versus-time profile after the three routes of administration in a rat. The oral bioavailability calculated from the blood data with the use of the iv infusion as a reference was 19.2 ± 7.2% (Table I), which was significantly higher than that calculated with the iv bolus data (7.5 ± 2.0%). The half-life of the postinfusion phase (15.0 ± 2.1 min) was much shorter than the terminal half-life after the iv bolus dose but most likely represented a distribution phase, with the true terminal phase being lower than the sensitivity of the assay. However, this did not appear to cause a significant underestimation of the AUC^{∞} after the infusion dose, because the time-averaged clearance determined from the total AUC^{∞} for the infusion (72.2 ± 8.6 ml/min/kg) was not significantly different from the total-body clearance at steady-state (72.6 ± 6.0 ml/min/kg; Table I). Table II shows the parameters obtained from the urine data after the iv infusion of (−)-carbovir. The oral bioavailability was calculated to be 15.5 ± 4.9%, with the use of urine data after the iv infusion as a reference. The total-body, renal, and nonrenal clearances were significantly different between the iv bolus injection and the iv infusion, but the f_e values were not significantly different. The renal clearances after the oral and iv infusion administration were not different.

Table I. Noncompartmentally Derived Pharmacokinetic Data from Blood of Rats Receiving (−)-Carbovir

Parameter	iv bolus	iv infusion	Oral
n	9	5	9
Dose (mg/kg)	17.7 ± 1.7[a]	17.5 ± 1.9	54.3 ± 7.4
CL (ml/min/kg)	27.3 ± 8.8	72.6 ± 6.0[b,*]	
$t_{1/2}$ (min)	116 ± 19	15.0 ± 2.1	106 ± 12
V_{ss} (L/kg)		2.6 ± 1.1	
C_{ss} (μg/ml)[c]		0.66 ± 0.20	
MRT (min)[d]		35.8 ± 15.5	
τ (min)		387 ± 88	
C_{max} (μg/ml)			1.00 ± 0.16
t_{max} (min)			38 ± 11
MAT (min)[e]			99 ± 24
F (%)[f]			7.5 ± 2.0
F (%)[g]			19.2 ± 7.2**

[a] Mean ± SD.
[b] Mean total-body clearance at steady state.
[c] (−)-Carbovir concentration in blood at steady state.
[d] Mean residence time.
[e] Mean absorption time.
[f] F calculated by $(AUC^{\infty}_{po} \cdot \text{iv bolus dose})/(AUC^{\infty}_{iv} \cdot \text{po dose})$.
[g] F calculated by $(AUC^{\infty}_{po} \cdot \text{iv infusion dose})/(AUC^{\infty}_{inf} \cdot \text{po dose})$.
* Significantly different from iv bolus ($P < 0.05$), unpaired t test.
** Significantly different from the value of F obtained with the use of the iv bolus dose as a reference ($P < 0.05$), unpaired t test.

Table II. Pharmacokinetic Data Obtained from the Urine of Rats Receiving (−)-Carbovir

Parameter	iv bolus	iv infusion	Oral
n	9	5	9
CL_R (ml/min/kg)	15.1 ± 6.9	48.7 ± 9.4*	41.0 ± 6.8*
CL_{NR} (ml/min/kg)	12.2 ± 5.1	23.8 ± 8.1**	
f_e	0.55 ± 0.12	0.67 ± 0.11	
F (%)[a]			20.6 ± 5.1
F (%)[b]			15.5 ± 4.9***

[a] F calculated by $(X_u^{\infty}{}_{po} \cdot \text{iv bolus dose})/(X_u^{\infty}{}_{iv} \cdot \text{po dose})$.
[b] F calculated by $(X_u^{\infty}{}_{po} \cdot \text{iv infusion dose})/(X_u^{\infty}{}_{inf} \cdot \text{po dose})$.
* Significantly different from iv bolus ($P < 0.05$), ANOVA with Scheffé's procedure.
** Significantly different from iv bolus ($P < 0.05$), unpaired t test.
*** Significantly different from the value of F obtained from blood data after an iv infusion (19.2 ± 7.2 in Table I; $P < 0.05$), paired t test.

The blood/plasma and erythrocyte/plasma distribution ratios and protein binding are shown in Table III. The blood/plasma distribution ratio was approximately 1 in the range of 1 to 10 μg/ml (−)-carbovir in blood. The fraction of unbound (−)-carbovir in serum appeared to be concentration dependent and increased from 0.821 ± 0.025 at 1 μg/ml to 0.874 ± 0.027 at 10 μg/ml.

DISCUSSION

In previous work with racemic carbovir the half-life of the terminal phase after the oral administration appeared to be longer than that after the iv bolus injection, suggesting "flip-flop" kinetics (3). In the present work, the terminal half-lives after the iv bolus and oral administration are similar. The difference is most likely due to the sensitivity limitations of the HPLC assay with UV detection that was used in the previous study. The HPLC fluorescence assay (6) is approximately four times more sensitive than the HPLC-UV method and (−)-carbovir concentrations were measurable for up to 480 min after the iv bolus and oral administrations.

The renal and nonrenal clearances after the iv infusion of (−)-carbovir were higher and significantly different from those obtained after the iv bolus injection, suggesting that both renal and nonrenal clearance pathways were saturated when high concentrations of (−)-carbovir were present. If only renal clearance was saturated, then the fraction excreted unchanged would have been decreased after the bolus dose because a greater percentage of the drug would have been shunted through the nonrenal pathway. The data suggest that both renal and nonrenal pathways were saturated to the same extent because the fraction of (−)-carbovir excreted unchanged was not significantly different between the iv bolus and the iv infusion doses. Saturation of (−)-carbovir clearance in rats has been suggested previously in a preliminary report (11).

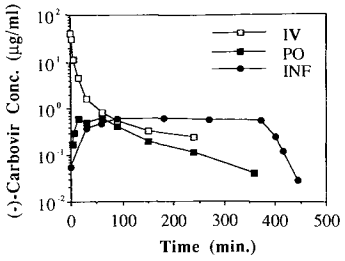

Fig. 3. Concentration–time profile in a rat after iv bolus (18 mg/kg), iv infusion (18 mg/kg), and oral dosing (54 mg/kg) of (−)-carbovir.

The time-averaged renal clearance of 48.7 ± 9.4 ml/min/kg after iv infusion greatly exceeded the glomerular filtration rate for rats (12), indicating significant renal tubular secretion. Saturation of active tubular secretion may be the source of the nonlinear renal clearance. At (−)-carbovir concentrations of 0.66 μg/ml in blood, the nonrenal clearance was 23.8 ± 8.1 ml/min/kg. Assuming that all nonrenal clearance occurred in the liver, the hepatic extraction ratio of (−)-carbovir was estimated by dividing the nonrenal clearance by the hepatic blood flow in rats (60–80 ml/min/kg) (13). The estimated extraction ratio of 0.3–0.4 suggests that (−)-carbovir is not a high extraction ratio drug. Although changes in blood binding might affect the clearance of a highly bound, low- to intermediate-extraction ratio drug, the free fraction in blood was as high as 0.85 and was not significantly different in the concentration range of 1 to 10 μg/ml. Therefore, the change in nonrenal clearance was likely due to a decrease in the intrinsic clearance of free drug. The free fraction of (−)-carbovir in serum did increase significantly with concentration, and consequently the concentration-dependent binding of (−)-carbovir should be considered when the pharmacokinetics are investigated with the serum or plasma as a reference fluid rather than whole blood.

Under linear pharmacokinetic conditions the oral bioavailability should be identical when calculated either with the AUC^{∞} from bolus or infusion administration or with X_u^{∞} as a reference. If clearance was saturated at the high concentrations of (−)-carbovir generated by the iv bolus dose, then the apparent bioavailability calculated with the AUC^{∞} from this reference dose would be decreased. To avoid saturation, a reference iv bolus dose that was much smaller could have been administered to achieve an initial concentration of approximately 1 μg/ml, the peak concentration after the oral dose. However, calculation of an accurate AUC after a low iv bolus dose would have been problematic because of limits in assay sensitivity. Therefore an infusion was used in order to prevent the saturation of clearance and the steady-state concentration was targeted to be no higher than 1 μg/ml. A concentration of 1 μg/ml is approximately five times higher than the *in vitro* EC_{50} (1) and, thus, would be at the lower end of a desired therapeutic range *in vivo*. At a dose of 17.7 mg/kg, the oral bioavailability calculated from the AUC^{∞} data after iv infusion was approximately threefold greater than that calculated with a 17.5-mg/kg iv bolus dose as a reference. As the elimination pathways were unlikely to be saturated during the infusion, the estimate of oral bioavailability based on (−)-carbovir AUC^{∞} in blood was considered to be more indicative of the true bioavailability.

If both renal and nonrenal elimination pathways were saturated to the same extent at all concentrations, the bioavailability calculated from X_u^{∞} would not be affected by the nonlinearity. When the oral bioavailabilities were calculated from the amount of (−)-carbovir excreted unchanged in the

Table III. Blood and Erythrocyte (RBC) to Plasma Ratios and Protein Binding of (−)-Carbovir[a]

Blood conc. (μg/ml)	Blood/ plasma ratio	f_u[b]	$f_{u,b}$[c]	RBC/plasma ratio
1.0	1.02 ± 0.05	0.821 ± 0.025	0.802 ± 0.048	1.06 ± 0.11
5.0	0.99 ± 0.06	0.835 ± 0.017	0.846 ± 0.045	0.97 ± 0.13
10.0	1.04 ± 0.09	0.874 ± 0.027*	0.844 ± 0.076	1.10 ± 0.20

[a] $n = 4$, mean ± SD.
[b] Fraction unbound in serum.
[c] Fraction unbound in blood.
* Significantly different from 1 μg/ml ($P < 0.05$), ANOVA with Scheffé's procedure.

urine, no difference was observed between the use of an iv bolus injection and the use of an iv infusion as a reference (Table II). Concomitant saturation of renal and nonrenal clearances may account for the discrepancy in the apparent bioavailabilities calculated from the urinary excretion data and the AUC^{∞} after the iv bolus injection.

The present study indicates that oral bioavailability studies are most appropriately designed with reference iv doses that generate concentrations of the drug in blood which are similar to those observed after the oral dose.

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