Intestinal Absorption of (−)-Carbovir in the Rat[1]

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(−)-Carbovir (CBV) is a carbocyclic nucleoside analogue with *in vitro* activity against the human immunodeficiency virus. The sites and mechanism of absorption of (−)-CBV from the rat small intestine were studied in the anesthetized male Sprague–Dawley rat. (−)-CBV was perfused through either duodenal, jejunal, or ileal segments at three concentration levels ranging from 1 to 500 μg/mL. The fraction remaining to be absorbed at steady-state and the absorptive clearance were calculated for each experiment. The effect of solvent drag on the absorptive clearance was also investigated. Two-way ANOVA for the absorptive clearance per unit length was not significant for either (−)-CBV concentration or site of perfusion. The fraction remaining to be absorbed at steady-state was found to be 0.804 ± 0.091 (n = 30). A strong correlation was found between the absorptive clearance and the net water absorptive flux. The mechanism of (−)-CBV absorption across the rat small intestine apparently consists of both passive diffusion and convection.

KEY WORDS: (−)-carbovir, intestinal absorption, solvent drag, absorptive clearance.

INTRODUCTION

Carbovir (carbocyclic 2′,3′-dideoxy-2′,3′-didehydroguanosine; CBV) (Fig. 1) is a potent *in vitro* inhibitor of the infectivity and replication of the human immunodeficiency virus (HIV) in human T cells at noncytotoxic concentrations (1). CBV exhibits a high therapeutic index and selectively inhibits the viral reverse transcriptase with little effect on the host cell DNA polymerases (2). In addition, the *in vitro* combination of CBV and zidovudine (AZT) is strongly synergistic (3). Its apparent lack of toxicity, its potency and selectivity against HIV, and its potent synergism with AZT make CBV an important candidate for anti-HIV therapy.

The oral bioavailability of (−)-CBV, the active enantiomer, was shown to be approximately 20% in the rat (4). Results from *in situ* liver perfusion and intestinal vascular perfusion experiments showed that the low oral bioavailability was not due to a significant first-pass effect (5). Therefore the low bioavailability was most likely due to poor intestinal absorption. The objectives of the present work were to investigate the possibility of specific absorption sites and the saturability of the absorption process and to determine the mechanism(s) of (−)-CBV transport in the rat small intestine.

MATERIALS AND METHODS

Surgery

Male Sprague–Dawley rats weighing 250–300 g (Bio-Labs, St. Paul, MN) were fasted for 15–20 hr before the experiment, with water available *ad libitum*. The rats were initially anesthetized with a 60 mg/kg intraperitoneal injection of pentobarbital (Nembutal sodium solution, Abbott Laboratories) and were maintained under anesthesia with intraperitoneal doses of pentobarbital as needed. The rectal temperature was monitored during the surgery and the perfusion experiment with a digital thermometer (Curtin Matheson Scientific Inc., Eden Prairie, MN) and was maintained at approximately 37°C with the use of a heating pad and lamp. The pyloric and femoral veins were cannulated to obtain blood samples during the intestinal perfusion experiments. The results of the blood analysis are described elsewhere (6). For perfusion of the duodenal segment, the inflow cannula was inserted below the pylorus and the outflow cannula was placed after the ligament of Treitz, approximately 10 cm below the inflow. For the jejunal and ileal segments, the pylorus was ligated to limit secretions from the stomach during the experiment. The jejunal segment was cannulated from the ligament of Treitz to approximately 15 cm below it. The outflow of the ileal segment was located close to the cecum and the inflow placed approximately 15 cm above.

The intestinal segment was washed with 10 mL of Krebs–Henseleit bicarbonate buffer (0.154 *M* NaCl, 0.154 *M* KCl, 0.11 *M* $CaCl_2$, 0.154 *M* KH_2PO_4, 0.154 *M* $MgSO_4$ $7H_2O$, 0.154 *M* $NaHCO_3$, pH 7.4) warmed to 37°C, followed by a slow injection of 10 mL of air. The abdominal cavity was then covered with plastic wrapping film (Handi-Wrap II, Dow Consumer Products, Inc., Indianapolis, IN) and the perfusion was started. When the perfusion experiment was completed, the inflow cannula was disconnected from the pump and the segment was flushed with air. The intestine was then weighed.

Intestinal Perfusion of (−)-CBV

A solution of (−)-CBV (provided by Glaxo Inc.) at a concentration of approximately 1, 5, 50, or 500 μg/mL was prepared in Krebs–Henseleit bicarbonate buffer on the day of the experiment. The solution was perfused through the cannulated segment with a microliter syringe pump (Model 2274, Harvard Apparatus) at a flow rate of approximately 0.055 mL/min. There was a lag between the time the perfusion solution entered the perfused segment and the time it appeared at the outflow. The end of this lag time was considered to be time 0 for the perfusion experiment. The perfusate was collected as 10-min fractions into preweighed 20-mL scintillation vials. At the end of the 2-hr experiment, the rat was sacrificed with an iv overdose of pentobarbital.

A total of 30 intestinal perfusion experiments was carried out. The experimental design and conditions are presented in Table I.

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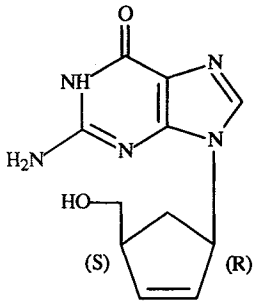

Fig. 1. Structure of (−)-CBV, with designation of absolute configuration at chiral centers.

Analytical Methods

The outflow samples from the intestinal perfusion were transferred to microcentrifuge tubes (Eppendorf, Brinkmann) and centrifuged at 13,600*g* (Micro-Centrifuge, Model 235B, Fisher Scientific, Pittsburgh, PA) for 6 min. The supernatant of the samples as well as the (−)-CBV solution remaining in the syringe was diluted with mobile phase. Analysis of the perfusate remaining in the syringe was done in triplicate to determine the inflow concentration accurately. Equal volumes of these samples together with (−)-CBV standards in mobile phase were injected into the HPLC system. The HPLC system has been described previously (4,7). Quantitation of (−)-CBV was carried out by external standardization.

Data Analysis

Absorptive Clearance

The flow rate was estimated for each perfusion experiment from the linear regression of the volume remaining in the perfusion syringe versus time. The volume of the outflow samples was estimated gravimetrically. From the difference in flow rate entering and leaving the intestinal segment, the (−)-CBV outflow concentration was corrected for net water absorption or secretion.

The ratio between the corrected (−)-CBV concentration at the outflow (C_1) and that at the inflow (C_o) was calculated for each perfusate sample collected. The average of the outflow-to-inflow concentration ratios for the fractions collected from 40 to 120 min was taken as the steady-state ratio. This ratio at steady-state is given by (8)

$$\frac{C_1}{C_0} = e^{-(2\pi rl\mathrm{Pe}/Q)} \tag{1}$$

where C_1 is the corrected concentration of (−)-CBV leaving the intestinal segment, C_o is the (−)-CBV concentration entering the segment, C_1/C_o is the fraction of (−)-CBV remaining to be absorbed at steady-state, r is the effective radius of the intestinal lumen (cm), l is the length of the perfused segment (cm), Pe is the apparent permeability coefficient (cm/min), and Q is the bulk perfusate flow rate (mL/min).

Rearrangement of Eq. (1) allows the permeability–area product (APe) to be calculated. The APe can be considered to be the absorptive clearance (mL/min) (9):

$$A\mathrm{Pe} = 2\pi rl\mathrm{Pe} = -Q \ln\left(\frac{C_1}{C_0}\right) \tag{2}$$

where A is surface area (cm^2). Two-way ANOVA was used to compare the absorptive clearance normalized to the segment length for the different drug concentrations used and intestinal regions perfused.

The amount of drug which disappeared during a collection period was calculated as the difference between the amount of drug entering and leaving the segment for the 10-min period. The cumulative amount disappearing was calculated by successively adding the amounts which disappeared over consecutive periods.

Solvent Drag Effect on Intestinal Absorptive Clearance

The influence of water flux on the absorption of (−)-CBV across the rat small intestine was studied by plotting the absorptive clearance versus the net water flux. The net amount of drug absorbed per unit time can be described as the sum of two terms: the diffusive contribution and the convective contribution corresponding to the solvent drag effect (10,11). The net amount of drug absorbed per unit time is then given by (10)

$$\frac{\Delta\mathrm{Amnt}}{\Delta t} = \frac{DK}{\Delta x} A\,(C_\mathrm{L} - C_\mathrm{P}) + \phi\, J_\mathrm{w} C_\mathrm{L} \tag{3}$$

where ΔAmnt is the net amount of drug (μg) absorbed in a time interval Δt (min), D is the diffusion coefficient (cm^2/min), K is the cell membrane–perfusate partition coefficient, A is the surface area (cm^2), Δx is the path length (cm), C_L is the drug concentration in the lumen (μg/mL), C_P is the drug concentration in the plasma (μg/mL), ϕ is the sieving coefficient (defined as the ratio of drug concentration in the convective stream to that in the lumen), J_w is the absorptive clearance of water (sometimes referred to as flux; mL/min), $DK/\Delta x$ is the permeability (cm/min), and $DKA/\Delta x$ is the absorptive clearance due to diffusion (mL/min). Equation (3) is valid when the net water transport is positive or zero. In the case of net water secretion into the lumen, C_L would be replaced by C_P in the second term.

In the present study C_P was negligible compared to C_L. When the steady-state absorption rate is divided by the lu-

Table I. Experimental Design and Conditions

Intestinal segment	Segment length (cm)	Lag time (min)	(−)-CBV concentration in perfusate (μg/mL)
Duodenum	9.4 ± 1.0[a] (4)[b]	8.7 ± 3.2 (4)	47.4 ± 2.2 (4)
Jejunum	13.0 ± 2.0 (12)	19.8 ± 5.0 (10)	4.96 ± 0.17 (3)
			45.6 ± 1.7 (6)
			441.8 ± 6.3 (3)
Ileum	13.4 ± 1.5 (14)	19.7 ± 5.4 (14)	1.17 ± 0.21 (5)
			44.9 ± 3.2 (6)
			493.0 ± 5.2 (3)

[a] Mean ± SD.
[b] *n* given in parentheses.

menal steady-state concentration of drug (9), Eq. (3) becomes

$$\frac{(\Delta Am \times t/\Delta t)_{ss}}{C_{L_{ss}}} = \frac{DK}{\Delta x} A + \phi J_w = PeA \quad (4)$$

where $(\Delta Amnt/\Delta t)_{ss}$ is the absorption rate at steady-state (μg/min) and $C_{L_{ss}}$ the drug lumenal concentration at steady-state (μg/ml), estimated as a logarithmic average (8):

$$C_{L_{ss}} = (C_o - C_l)/\ln(C_o/C_l) \quad (5)$$

$(\Delta Amnt/\Delta t)_{ss}/C_{L_{ss}}$ represents the overall absorptive clearance (mL/min), which can be estimated as the permeability–area product as in Eq. (2). When the permeability–area product is plotted against the net volumetric water absorption rate (absorptive clearance) J_w, the slope of the simple regression line gives an estimate of the sieving coefficient and the intercept gives an estimate of the absorptive clearance due to diffusion.

Statistics

Two-way ANOVA was used for comparison of parameters among three segment locations and three inlet concentrations. The unpaired *t* test was used for comparison between two segments. A *P* value of less than 0.05 was considered to be significant.

RESULTS

Absorptive Clearance

(−)-CBV was poorly absorbed at all three locations of the rat small intestine. The statistical analysis was performed on the permeability–area product normalized per unit length (Table II). The two-way ANOVA indicated that neither the concentration, nor the location (jejunum and ileum) factors, nor the interaction between the factors were significant. When the results of the duodenal segment experiments, performed with the medium (−)-CBV concentration (50 μg/mL), were included in the two-factor factorial analysis (with two missing cells), the location factor was not found to be significant. Therefore, the absorptive clearance did not seem to be dependent on either the location in the small intestine or the (−)-CBV concentration used in the perfusate. The overall absorptive clearance per unit length (Pe*A*/*l*) was 9.75 × 10^{-4} ± 5.45n 10^{-4} mL/min-cm (*n* = 30). The overall fraction remaining to be absorbed at steady state (C_l/C_o) was 0.804 ± 0.091 (*n* = 30). A plot of the fraction remaining to be absorbed versus the time at the end of the collection interval is presented in Fig. 2. Alternatively, the fraction of dose remaining to be absorbed could be calculated as the difference between the total dose administered minus the cumulative amount disappearing over the perfusion time. Normalized for the dose, this was calculated to be 0.798 ± 0.082 (*n* = 29).

Table II. Absorptive Clearance per Unit Length in the Rat Small Intestine as a Function of (−) CBV Concentration

	Pe*A*/*l* × 10^4 (mL/min-cm)		
Concentration[a]	Duodenum	Jejunum	Ileum
Low		8.64 ± 2.93[b] (*n* = 3)	6.70 ± 4.64 (*n* = 5)
Medium	7.36 ± 2.99 (*n* = 4)	7.40 ± 2.97 (*n* = 6)	16.14 ± 7.72 (*n* = 6)
High		11.15 ± 3.38 (*n* = 3)	10.49 ± 1.69 (*n* = 3)

[a] Low, approximately 5 μg/mL in jejunum and 1 μg/mL in ileum. Medium, approximately 50 μg/mL; high, approximately 500 μg/mL.
[b] Mean ± SD.

Solvent Drag Effect on Absorptive Clearance

When the permeability–area product (absorptive clearance) was plotted against the water flux (net volume absorbed from the lumen per unit time) during a collection period, a positive linear correlation was observed for each experiment (*n* = 30). The absorptive clearance data were pooled from all the experiments and plotted against the water flux; this plot is presented in Fig. 3. Since Eq. (3) is valid only for positive or zero values of net water transport, the cases with net water secretion are omitted in Fig. 3. The line in this plot represents the simple linear regression line; its equation slope and intercept are given in Table III. This relationship was also determined in each intestinal location. The sieving coefficients in the duodenum and jejunum were not significantly different from 1, while the sieving coefficient in the ileum was significantly different from 1 (Table III).

The average absorptive clearance (Pe*A*) in the collection periods in which net water absorptive flux occurred (20.3 × 10^{-3} ± 1.9 × 10^{-3} mL/min; data in Fig. 3) was significantly different (*t* test) from that during periods of net water secretive flux (11.9 × 10^{-3} ± 9.6 × 10^{-3}; data in Fig. 4). The slope of the regression line in Fig. 4 was not significantly different from zero, indicating the lack of a relationship between absorptive clearance and water secretion.

DISCUSSION

(−)-CBV was poorly absorbed from the rat small intestine. These results are consistent with previous pharmacokinetic studies of (−)-CBV in the rat, where the oral bioavailability was found to be only 20% (4). The absorptive clearance normalized for the segment length was not dependent on the (−)-CBV concentration over a 500-fold range. In

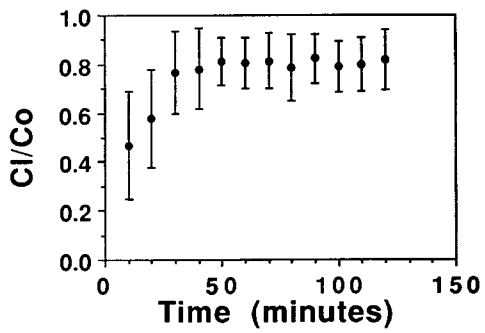

Fig. 2. Fraction remaining to be absorbed–time profile of (−)-CBV in the rat small intestine (*n* = 30). Data reported as mean ± SD.

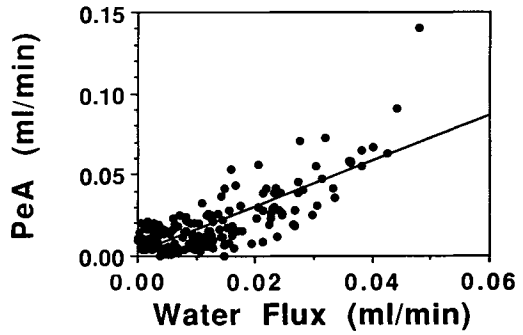

Fig. 3. Effect of net water absorptive clearance (flux) on (−)-CBV absorptive clearance (entire small intestine). $y = 2.2212 \times 10^{-3} + 1.4020x$; $R^2 = 0.612$.

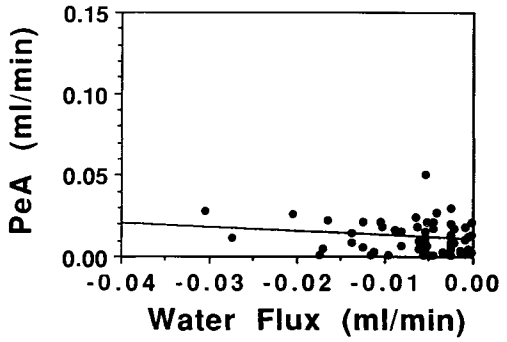

Fig. 4. Effect of net water secretion on absorptive clearance (entire small intestine). $y = 1.0349 \times 10^{-2} - 0.23836x$; $R^2 = 0.025$.

addition, the disappearance rate of (−)-CBV increased linearly with the average intralumenal concentration (data not shown). Therefore, the absorption of (−)-CBV most likely involves a passive diffusion mechanism, as reported for other purine nucleosides (16) and analogues, including theophylline (17), allopurinol (18), and acyclovir (19). This contrasts with pyrimidine transport across the rat small intestine, which occurs by a sodium-dependent active transport mechanism (12–15).

The importance of the solvent drag on the absorption of numerous drugs through the paracellular pathway has been well established (20–26). Although the rat jejunum and colon contain pores which are available to molecules at least as large as inulin (20), the importance of the paracellular pathway is dependent on the lipophilicity of the molecule (9). (−)-CBV is a very polar compound and solvent drag appears to influence its absorptive clearance. The absorptive clearance of (−)-CBV increased with increasing values of net water absorptive flux (Fig. 3). Sieving coefficients of 1 were found for (−)-CBV (Table III), at the duodenum, the jejunum, and the entire small intestine (pooled data from all three locations). A sieving coefficient of 1 is equivalent to a reflection coefficient of 0, indicating that (−)-CBV permeates through the paracellular route as easily as water does. A sieving coefficient of 1.85 (significantly different from 1) was found at the ileum. There are two possible explanations for a sieving coefficient larger than 1. First, sieving coefficients higher than 1 have been obtained for drugs such as benzoic acid and salicylic acid (22) and for antipyrine (24). The explanation given in these studies was the existence of an interaction between the drug molecules and water in the lipid part of the membrane (24). Alternatively, with the use of D_2O, Karino *et al.* (26) demonstrated the importance of the back flux of water on the determination of the solvent drag effect. The sieving coefficients of benzoic acid, salicylic acid, and antipyrine, taking water back flux into account, were not significantly different from one (26). The back flux of drug from blood to intestinal lumen can be neglected (26); in fact, net water secretion into the intestinal lumen had no effect on the absorptive clearance of (−)-CBV (Fig. 4). The present experimental conditions allowed the measurement of net water flux throughout a collection period but not a quantitation of the actual water absorption or secretion occurring. Therefore, the values of net water absorptive flux plotted in Fig. 3 may be an underestimation of the actual absorptive flux of water occurring over a collection period, leading to an overestimation of the slope (ϕ). Since (−)-CBV has a somewhat higher molecular weight (MW 247) than the compounds studied by Karino *et al.* (26), the true sieving coefficient of (−)-CBV is likely to be somewhat less than 1. However, this does not alter the conclusion that water flux contributes greatly to the absorption of (−)-CBV.

The relative contributions of the diffusive ($DKA/\Delta x$) and convective (ϕJ_w) clearances to the overall absorptive clearance [Eq. (4)] were investigated as follows. The average of the overall absorptive clearance ($DKA/\Delta x + \phi J_w$) in the situations of net water absorption is $20.3 \times 10^{-3} \pm 1.9 \times 10^{-3}$ mL/min. The mean sieving coefficient for the three locations (1.42) multiplied by the average net water absorptive flux (12.87×10^{-3} mL/min) gives 18.3×10^{-3} mL/min,

Table III. Absorptive Clearance (PeA) Versus Water Absorptive Clearance (J_w): Linear Regression-Parameters

	PeA = $(DK/\Delta x)A + \phi J_w$			
	Pooled data (n = 30)	Duodenum (n = 4)	Jejunum (n = 12)	Ileum (n = 14)
Slope (ϕ)	1.4[a]	1.28[a]	1.14[a]	1.85[a,b]
95% confidence interval for ϕ	1.23, 1.57	1.00, 1.56	0.96, 1.33	1.58, 2.12
Intercept ($DKA/\Delta x$) (mL/min)	0.0022	−0.0024	0.0022	0.0048
Coefficient of determination (R^2)	0.612	0.754	0.661	0.753
Number of data points	233	34	87	112

[a] Significantly different from 0.
[b] Significantly different from 1.

an estimate of the absorptive clearance due to convection (ϕJ_w). This constitutes approximately 90% of the overall absorptive clearance. This suggests that the major contribution to the absorptive process is due to convective transport through the paracellular route. The intercept of the plot of absorptive clearance versus water flux [Eq. (4)] gives an estimate of 2.2×10^{-3} mL/min for the diffusive absorptive clearance ($DKA/\Delta x$), approximately 10% of the overall absorptive clearance.

In summary, (−)-CBV is poorly absorbed across the rat small intestine, with a fraction remaining to be absorbed at steady-state of 0.804 and an absorptive clearance per unit length of 9.75×10^{-4} mL/min-cm. The absorptive clearance of (−)-CBV per unit length in the rat small intestine was not significantly different among the three anatomical locations used and appeared to be linear over a 500-fold concentration range. (−)-CBV is absorbed across the rat small intestine through both the transcellular route by passive diffusion and the paracellular route by convection, the latter process accounting for the major proportion of transport. Therefore one means of improving the oral absorption of (−)-CBV might be to maximize the effects of solvent drag, by administering (−)-CBV with meals that stimulate water absorption from the lumen (27).

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