Intestinal Absorption of (−)-Carbovir in the Rat

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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 ± 0.001 (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′-dideoxyhydroganoanosine; 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 synergy 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 CaCl₂, 0.154 M KH₂PO₄, 0.154 M MgSO₄ 7H₂O, 0.154 M NaHCO₃, 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.
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 13,600g (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) and that at the inflow (C0) 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)

$$\frac{C}{C_0} = e^{-\left(\frac{2\pi r f Pe}{Q}\right)}$$

(1)

where C is the corrected concentration of (-)-CBV leaving the intestinal segment, C0 is the (-)-CBV concentration entering the segment, C/C0 is the fraction of (-)-CBV remaining to be absorbed at steady-state; r is the effective radius of the intestinal lumen (cm), f 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):

$$A Pe = 2\pi rf Pe = -Q \ln \left(\frac{C}{C_0}\right)$$

(2)

where A is surface area (cm²). 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)

$$\frac{\Delta A \text{mmt}}{\Delta t} = \frac{DK}{\Delta x} A (C_L - C_P) + \phi J_o C_L$$

(3)

where ΔAmnt is the net amount of drug (µg) absorbed in a time interval Δt (min), D is the diffusion coefficient (cm²/min), K is the cell membrane–perfusate partition coefficient, A is the surface area (cm²), 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_o is the absorptive clearance of water (sometimes referred to as flux; mL/min), DK/Δx is the permeability (cm/min), and DKA/Δx 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-

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a Mean ± SD.

b n given in parentheses.