FIRST-PASS DISPOSITION OF (−)-6-AMINOCARBOVIR IN RATS. I. PRODRUG ACTIVATION MAY BE LIMITED BY ACCESS TO ENZYME

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ABSTRACT:
Several in vitro and in situ approaches were used to determine the dominant presystemic activation site for (−)-6-aminocarbovir, (−)-carbocyclic 2′,3′-didehydro-2′,3′-dideoxy-6-deoxy-6-aminoguanosine (6AC) conversion in rats. In vitro disappearance half-lives (mean ± S.D.) in the cytosolic fractions obtained from homogenates of the intestine, liver, and intestinal contents were 0.4 ± 0.1 (n = 3), 12.2 ± 1.1 (n = 3), and 15.5 (n = 1) min, respectively. An in situ vascularly perfused intestine-liver (IPIL) study was then carried out (n = 6) to determine the relative contribution of each presystemic organ to the overall first-pass extraction of 6AC. The 6AC extraction ratios in the intestine and liver in the IPIL were found to be 0.08 ± 0.02 and 0.11 ± 0.03, respectively. The intestinal extraction ratio was in dramatic contrast to the in vitro results. It was postulated that vascularly delivered 6AC had limited access to the metabolic site in the intestine. A theoretical analysis suggested that the extent of intestinal wall extraction of 6AC would be underestimated by the IPIL and should be determined after oral dosing. To compare intestinal extraction ratio in the IPIL with that after an oral administration, in situ intestinal lumen perfusions (n = 4) and intraportal infusions (n = 3) of 6AC were conducted in two groups of rats. The lumenerally administered 6AC was extracted to a much greater extent by the intestine as compared with the IPIL, which presents 6AC to the intestine by the vascular route. The extraction ratio was found to be 0.54 ± 0.06, which was significantly larger than that obtained in the IPIL.

Materials and Methods
6AC and CBV were synthesized at the University of Minnesota (Beers et al., 1990; Vince and Hua, 1990) and received as gifts from Dr. Robert Vince. Trichloroacetic acid was purchased from Aldrich Chemical Company (Milwaukee, WI), Dextran T-40 was purchased from Pharmacia (Piscataway, NJ), and glucose was purchased from LyphoMed Inc. (Rosemont, IL; dextrose injection, USP, 50%). All other chemicals were reagent grade or better.

In Vitro Incubation of 6AC with Intestine, Liver, and Intestinal Contents.
Initial studies were carried out with the cytosolic fraction from the liver and intestine of rats. Three incubations each were conducted with the intestine and liver. One incubation with intestinal contents was conducted. For each incubation, the liver, intestine, and intestinal contents from three male Sprague-Dawley rats (Bio-Labs, St. Paul, MN) were harvested. The intestine was rinsed with 10 ml of ice-cold phosphate-buffered saline (PBS) (pH 7.4) and the intestinal contents were collected. The tissues were pooled and homogenized in PBS with a Brinkmann Polytron (Westbury, NY). The organ weight to buffer volume ratios were 1:10 for the intestine and 1:5 for the liver. The homogenates were centrifuged at 10,000g at 4°C for 60 min. The supernatant was then centrifuged at 100,000g at 4°C for 60 min. Four milliliters of the final supernatant, which was the cytosolic fraction, were preincubated for 5 min at 37°C, and 100 µg 6AC (400 µg/ml) was then added to start the incubation. Serial samples of 100 µl each were taken in triplicate at specified intervals up to 30, 120, and 360 min for the intestine, intestinal contents, and deaminase (ADA) (Vince and Brownell, 1990), the intestine may be the principal organ in the first-pass conversion of 6AC, as would be consistent with the tissue distribution of this enzyme (Ho et al., 1980; Chinsky et al., 1990; Winston et al., 1992).

The objectives of the present studies were to investigate the relative contributions of the liver and intestine to the first-pass conversion of 6AC to CBV.

(−)-Carbovir, ((−)-carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine, CBV)2 is a potent and selective anti-HIV compound (Vince et al., 1988) with a low oral bioavailability and limited brain delivery (Huang et al., 1991; Wen et al., 1995). When (−)-6-aminocarbovir ((−)-carbocyclic 2′,3′-didehydro-2′,3′-dideoxy-6-deoxy-6-aminoguanosine, 6AC) was evaluated as a prodrug of CBV in rats, it exhibited superiority to the parent drug in increasing systemic and central nervous system exposure to CBV (Zimmerman et al., 1992; Wen et al., 1995). Much higher CBV femoral blood concentrations were observed after an oral dose of 6AC as compared with i.v. administration of 6AC (Zimmerman et al., 1992), indicating that 6AC was substantially converted to CBV in the first-pass organs after dosing. Both the liver and intestine probably contributed to the first-pass conversion of 6AC to CBV, but their relative contributions to the first-pass activation of 6AC could not be determined in these studies. Because 6AC is most likely metabolized to CBV by adenosine deaminase (ADA) (Vince and Brownell, 1990), the intestine may be the principal organ in the first-pass conversion of 6AC, as would be consistent with the tissue distribution of this enzyme (Ho et al., 1980; Chinsky et al., 1990; Winston et al., 1992).

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2 Abbreviations used are CBV, (−)-carbovir, (−)-carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine; 6AC, (−)-6-aminocarbovir, (−)-carbocyclic 2′,3′-didehydro-2′,3′-dideoxy-6-deoxy-6-aminoguanosine; ADA, adenosine deaminase; IPIL, in situ vascularly perfused intestine-liver; DCF, 2′-deoxycoformycin, pentostatin; CR, concentration ratio.

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the experimental setup is presented in Fig. 1. Perfusate samples of 300 μl of perfusate was prepared for each experiment. A schematic representation of different concentrations, six treatment sequences were used. A total of 1600 ml there were three concentrations to be examined and each rat was to receive two
verted from 6AC, were not carried over from period I to period III. Because (70 –120 min), the organs were perfused with a perfusate containing 6AC at a
divided into three periods. In period I (0 –50 min), the organs were perfused
were collected from the superior mesenteric artery, portal vein, and hepatic
itor that was added to prevent the in vitro conversion of 6AC to CBV. After
were inserted into the bile duct and secured in place by ligature. The celiac artery and
The perfusate was a Krebs-Henseleit buffer (Pang, 1984) containing 300 mg/100 ml (v/v) glucose, 1% (v/v) bovine serum albumin (25% solution in Tyrode’s buffer; Sigma Chemical Co., St. Louis, MO), 3% (v/v) Dextran T-40, and 20% (v/v) washed human red blood cells (American Red Cross, St. Paul, MN). A perfusion apparatus (Perfuser Two/Ten; MX International Inc., Aurora, CO) equipped with a peristaltic pump and an oxygenator was used for the study. The viability of the perfused organs was monitored by taking continuous pressure readings at the superior mesenteric artery, by monitoring oxygen consumption in the portal and hepatic veins, by monitoring t-ascpartate 2-oxoglutarate aminotransferase and lactate dehydrogenase in the hepatic vein and portal vein perfusate, respectively, by monitoring the bile flow, and by observing the gross appearance of the organs (Pang, 1984; Wen, 1995).

The organ extraction of 6AC in this preparation was examined at three concentrations (0.4, 3.5, and 20 μg/ml). Each perfusion experiment was divided into three periods. In period I (0–50 min), the organs were perfused with a perfusate containing 6AC at one concentration. Period I was followed by a washout period (period II, 50–70 min) with blank perfusate. In period III (70–120 min), the organs were perfused with a perfusate containing 6AC at a different concentration. Preliminary studies showed that 6AC and CBV, converted from 6AC, were not carried over from period I to period III. Because there were three concentrations to be examined and each rat was to receive two different concentrations, six treatment sequences were used. A total of 1600 ml of perfusate was prepared for each experiment. A schematic representation of the experimental setup is presented in Fig. 1. Perfusate samples of 300 μl each were collected from the superior mesenteric artery, portal vein, and hepatic vein at 7-min intervals from 20 to 48 min (period I) and from 90 to 118 min (period III). Aliquots of exactly 200 μl of sample were pipetted into plastic microcentrifuge vials containing 800 μl of internal standard solution (0.8 μg/ml) and 20 μl of 2-deoxyoxymoforcin (DCF) (pentostatin; Parke-Davis Pharmaceuticals, Ann Arbor, MI) solution (1 mg/ml). DCF is an ADA inhibitor that was added to prevent the in vitro conversion of 6AC to CBV. After mixing, samples were placed on dry ice for the remainder of the experiment and then frozen at –70°C until assay.

Extraction of 6AC in Intestine during Intestinal Lumen Perfusion Theory. The IPIL studies described above showed that the intestine and liver had similar abilities to extract 6AC. This was in marked contrast to the data generated from the in vitro homogenate incubations in which the intestinal tissue was much more active in converting 6AC than was the liver tissue (see Results). This suggested that the intestinal extraction of 6AC might not be a perfusion rate-limited process. Movement of 6AC from the blood to the enzyme site may play an important role in the extraction process, especially because there is evidence that ADA, the enzyme catalyzing the conversion, is located near the brush border membrane of the intestine (Holt et al., 1985; Chinsky et al., 1990). Assuming that the blood flow is in rapid equilibrium with the “serosal” space, and the ADA is located distally in a “mucosal” space, a typical 6AC molecule would have a different probability of being converted to CBV when it was supplied by the blood stream perfusing the intestine than when it was supplied to the apical side of the intestinal mucosa (Fig. 2). Therefore, the extraction ratio of 6AC (E) obtained from the IPIL preparation might not be an accurate representation of the extraction ratio (Epo) when 6AC was administered to the intestinal lumen. A theoretical analysis based on the model described in Fig. 2 was performed to elucidate the relationship between E and Epo. Because ADA is localized near the mucosal side of the cell, the model in Fig. 2 shows no conversion of 6AC to CBV in the serosal space of the intestinal wall.

The following equations were obtained (see Appendix I for the detailed derivation):

\[ E = \frac{CL_{gw,dif} + CL_{gw,inf}}{CL_{gw,dif}} \]  
\[ E_{po} = \frac{CL_{gw,dif} + CL_{gw,inf} + CL_{gw,inf}Q_{pv}}{CL_{gw,dif} + CL_{gw,inf} + CL_{gw,inf}Q_{pv}} \]  
\[ E_{po} = \frac{CL_{gw,dif} + CL_{gw,inf} + CL_{gw,inf}Q_{pv}}{CL_{gw,dif} + CL_{gw,inf} + CL_{gw,inf}Q_{pv}} \]  

where: \( Q_{pv} = \) portal vein blood flow, \( CL_{gw,dif} \) = intrinsic formation clearance of 6AC to CBV in the mucosal compartment of the intestinal wall, and \( CL_{gw,inf} \) = diffusional clearance of 6AC in the intestinal wall (assumed to be a passive process and equal in both directions).

From equations 1 and 2, the relation of E to \( E_{po} \) can be obtained:

\[ E = \frac{CL_{gw,dif} + CL_{gw,inf}}{CL_{gw,dif} + CL_{gw,inf}} \]  

Equation 3 indicates that the extraction ratio obtained from IPIL (E) would be an underestimate of the extraction ratio of a drug administered orally (\( E_{po} \)) if its metabolism was rate limited by its access to the metabolic site. The rate process for the movement to the metabolic site is designated \( CL_{gw,dif,rew} \). Based on the discrepancy in the data generated from the homogenate and IPIL studies, it was hypothesized that 6AC delivered to the intestine by the vasculature was limited in its access to ADA. The following experiment was designed to provide in vivo evidence for this hypothesis.

Modified Multiple Site of Administration Technique. A total of seven male Sprague-Dawley rats (264 ± 15 g; Bio-Labs) was divided into two groups. The experimental setup is described in Fig. 3. Four rats received a luminal perfusion of 6AC through an intestinal segment. Three rats received an infusion of 6AC via the portal vein. The rats were under pentobarbital

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**FIG. 1.** Schematic diagram of the intestine-liver vascular perfusion experimental setup.
DCF solution (1 mg/ml) had previously been added. After vortexing, blood vials, to which 400 μl of 100 min at a rate of approximately 28 ml/min with a Harvard microliter syringe pump. The volumetric flow rate was 80 ml/min for easier comparison with the intestinal incubations. Incubations with the liver homogenate was actually conducted over 360 min, but 6AC and CBV profiles are only shown for the first 120 min of incubation with the liver homogenate. For the liver, C_in was the concentration of 6AC entering the superior mesenteric artery and C_out was the concentration of 6AC in the hepatic portal vein. For the liver, C_in was the concentration of 6AC in the portal vein and C_out was the concentration of 6AC in the hepatic vein.

**Intestine and Liver Extraction Ratio of 6AC in the IPIL.** The instantaneous organ extraction ratio (E) of 6AC was calculated using eq. 4:

$$E = \frac{C_{in} - C_{out}}{C_{in}} \quad (4)$$

where C_in and C_out are the 6AC concentrations in the influent and effluent blood flows, respectively. For the intestine, C_in was the concentration of 6AC entering the superior mesenteric artery and C_out was the concentration of 6AC in the hepatic portal vein. For the liver, C_in was the concentration of 6AC in the portal vein and C_out was the concentration of 6AC in the hepatic vein.

**Intestine and Liver Extraction Ratio of 6AC in the IPIL.**

$$E_{po} = 1 - F_{gw}^{6AC}(CR_{perf})$$

where $F_{gw}$ is the fraction of 6AC surviving the intestinal wall metabolism, is calculated as a function of steady-state concentration ratios (CR) of CBV to 6AC after oral (CR_perf) and after portal infusion (CR_inf):

$$F_{gw}^{6AC} = \frac{F_{m,sys}^{CBV} [CR_{perf} - 1]}{1 + F_{m,sys}^{CBV} [CR_{perf} - 1]} \quad (5)$$

$E_{po}$ is then obtained from eq. 6:

$$E_{po} = 1 - F_{gw}^{6AC} \quad (6)$$

Figure 3 describes the experimental system.

In these anesthetized animals, 6AC exhibited a decrease in systemic clearance compared with a previous in vivo study (Zimmerman et al., 1992) in awake animals. Because CBV systemic clearance was not significantly different in this study, $F_{m,sys}$ was calculated via eq. A19 by assuming that anesthesia reduced the CBV systemic clearance by the same percentage as it did the 6AC systemic clearance.

**Analytical Methods.** Both 6AC and CBV in all matrices were analyzed by validated solid-phase extraction procedures and high-performance liquid chromatography assays (Zimmerman et al., 1992; Wen, 1995). Blood perfusate and whole-blood samples underwent two cycles of freezing and thawing to lyse the red blood cells. They were then centrifuged and the supernatant was applied to C18 solid-phase extraction columns. The homogenates and intestinal content samples were centrifuged and the supernatant applied to the solid-phase extraction column in preparation for quantitation by high-performance liquid chromatography.

**Results**

**In Vitro Tissue Homogenate Incubations.** Figure 4 shows the 6AC and CBV concentration–time profiles in incubations with the intestinal homogenate, liver homogenate, and intestinal contents. The incubation with the liver homogenate was actually conducted over 360 min, but 6AC and CBV profiles are only shown for the first 120 min for easier comparison with the intestinal incubations. Incubations in all three media achieved complete conversion of 6AC to CBV, judging by mass balance.

Table 1 summarizes the 6AC disappearance half-lives, corrected for the dilution before homogenization. The intestine appears to be the most active tissue in converting 6AC to CBV. 6AC disappears from...
the intestinal homogenate approximately 30 times as fast as from the liver homogenate.

**In Situ IPIL.** Figure 5 shows representative 6AC and CBV concentration–time profiles from an IPIL experiment. In this case, the initial 6AC concentration (20 \( \mu \text{g/ml} \)) was later switched to a lower concentration (3.5 \( \mu \text{g/ml} \)). No carryover of either 6AC or CBV was observed in preliminary studies because of the appropriate wash-out period. The shallow slope of 6AC profiles in the perfusate (superior mesenteric artery) indicates that there was a slow conversion of 6AC to CBV in the perfusate itself. This conversion proved to be insignificant relative to the overall conversion of 6AC taking place in the perfused organs. Nevertheless, the instantaneous extraction ratio of 6AC in an organ was calculated at each time point using eq. 4.

Table 2 contains the average of the instantaneous 6AC extraction ratios in the intestine and liver of each rat in the IPIL study. There was no significant effect of 6AC concentration, perfusion period, or treatment order on the extraction ratio in either organ (analysis of variance). The mean extraction ratios of 6AC in the intestine and liver were approximately 0.08 ± 0.02 and 0.11 ± 0.03, respectively. The results are in marked contrast to the incubation studies that indicated that the intestine should have been much more active in converting 6AC to CBV.

Viability of the perfused organs was monitored frequently by a variety of measurements as described in Materials and Methods. Except for the blood pressure at the superior mesenteric artery, which increased gradually as the experiment went on, other viability measurements such as L-aspartate 2-oxoglutarate aminotransferase and lactate dehydrogenase levels, oxygen consumption, and bile flow rate all appeared to be normal throughout the time of perfusion (Wen, 1995).

**Intestinal Lumen Perfusion and Intraportal Infusion.** Figure 6 shows the mean 6AC and CBV concentration–time profiles after intraportal infusion and intestinal lumen perfusion of 6AC, respectively. Steady state for both 6AC and CBV was apparently achieved quickly. The steady-state concentration used in the parameter computation was the average of those concentrations considered to be at steady state by observation. The ratio of CBV concentration to 6AC concentration was much greater after intestinal lumen perfusion than after intraportal infusion. This indicates a more substantial conversion of 6AC to CBV after intestinal lumen perfusion of 6AC.

Table 3 summarizes the results from this study. The extraction ratio (\( E_{po} \)) was calculated to be 0.54 ± 0.06, significantly larger than the extraction ratio (E) of 0.08 ± 0.02 in the IPIL study.
Discussion

The carbocyclic nucleosides, represented by the prototype molecule, CBV, are novel reverse transcriptase inhibitors with significant activity against HIV (Vince et al., 1988). An analog of CBV, abacavir, is currently in clinical trials (Faletto et al., 1997).

The present work continued the preclinical investigations of another CBV analog, 6AC, and the mechanism of its enhanced systemic delivery of CBV after oral dosing. ADA, the enzyme responsible for the conversion of 6AC to CBV, is localized in the presystemic organs, with the intestine having significantly greater activity than the liver (Ho et al., 1980; Chinsky et al., 1990). For 6AC, the intestine should be the primary organ where most of the first-pass effect takes place after an oral dose. Indeed, the disappearance half-lives of 6AC in the in vitro incubation studies were in accord with the relative tissue distribution of ADA. Homogenate incubations are often used as a means for in vitro prediction of in vivo metabolism. Obviously, tissue homogenates differ from the tissue itself by the lack of intact cellular membranes. Less obviously, the compartmentalization of the enzymatic environment in the living tissue is lost in the homogenate. Nevertheless, good in vitro–in vivo correlations have been found for a large number of compounds (Houston, 1994).

However, the extraction of 6AC in the intestine in the IPIL was drastically different from what was predicted from the in vitro incubation results. The intestinal homogenate was much more active in converting 6AC to CBV in vitro than was the liver homogenate. In contrast, in the IPIL the intestine and liver were about equal in their apparent ability to extract 6AC. This suggested that the intestinal extraction of vascularity delivered 6AC was limited for some reason. One plausible explanation was that vascularity delivered 6AC might have had restricted access to the drug-metabolizing enzymes. To test this hypothesis, the extraction ratio of orally administered 6AC (Epo) was determined with the use of the lumenal perfusion technique, and a significantly higher value was obtained, indicating that the IPIL underestimated the intestinal extraction of 6AC. In this calculation, it was assumed that both 6AC and CBV clearances decreased to a similar extent as a result of anesthesia. Ideally, simultaneous estimation of CBV and 6AC clearance should have been obtained. However, this was not done for a variety of reasons, including the lack of availability of radiolabeled CBV. On the other hand, FMsys calculated with this approach (Table 3) approximated the previous value of 0.48 ± 0.14 reported in conscious rats (Zimmerman et al., 1992).

A model was then developed to illustrate the relationship between the extraction ratio of vascularity delivered 6AC in the IPIL (E) and the extraction ratio of orally absorbed 6AC (Epo). It is clear from eq. 3 that the interrelationship of E and Epo is determined by the access of 6AC molecules to the enzyme site (CLgw,dif) and the portal blood flow (Qpv). When the diffusional process is much faster than the convective portal blood flow, the value of E will approach Epo. The intestinal wall extraction determined in an IPIL experiment would then be an accurate estimate of the extraction ratio after an oral dose.

The ability to diffuse across a membrane or through the cytoplasm to the enzyme site will depend on the physicochemical characteristics of the drug as well as the organ distribution of the enzyme. If the diffusional process for 6AC is slow compared with the convective perfusate flow, many prodrug molecules will be carried through the extracting organ by the perfusate flow without having had the opportunity to diffuse to the metabolic site. Although there may be large amounts of metabolizing enzyme in the intestine, as suggested by the incubation results, there is a localization of enzyme activity that limits its ability to activate vascularity delivered 6AC. In the present case, CLgw,dif was estimated for the IPIL experiment with eq. 3 and the data in Tables 2 and 3. The CLgw,dif was calculated to be 1.74 ml/min, considerably lower than the Qpv used in the IPIL preparation (10 ml/min). This indicates that the intestinal wall extraction of a compound such as 6AC would be underestimated by the IPIL, and an accurate estimation of the extent of extraction could only be determined by oral dosing. Additional validation of this model could be done by carrying out perfusion studies at flow rates closer to the estimated CLgw,dif.

The process of serumal 6AC moving to the enzyme site has been described here as a diffusional clearance, a concept long-recognized in the organ distribution of certain drugs and metabolites (Dedrick et al., 1975; Sato et al., 1986; Brouwer and Jones, 1990; Pang et al., 1984; Gwilt et al., 1988; Schwab et al., 1990). This, however, implies a rate-limiting membrane, which may be unnecessarily restrictive. Lack of access may also be caused by the compartmentalization of the metabolizing enzymes (Sato et al., 1986), i.e., the intestinal cell is not “well-stirred” (Rowland et al., 1973). If the enzyme is located near the mucosal side of the cell, as is the case for ADA (Holt et al., 1985; Chinsky et al., 1990), 6AC molecules being absorbed from the lumen will have greater contact time in the drug-metabolizing compartment than will 6AC molecules being swept through the gut wall by the blood flow.

An alternative interpretation of the present findings is that the superior mesenteric arterial flow is actually fractionated into flows separately perfusing the metabolically active mucosa and other metabolically inactive subregions of the intestine (Klippert and Noord- hoek, 1983). Drug delivered by the oral route would by necessity be carried into the portal venous flow by the mucosal blood. Drug
delivered systemically would be exposed to the metabolically active mucosa blood in only a fraction of the total mesenteric blood flow. This fractionation of blood flow could thus lead to the described discrepancy in the intestinal extraction ratio of drug delivered by the two routes. This explanation also supports the contention that the intestine is not a well-stirred organ.

Conclusions. Several approaches, both theoretical and experimental, have been used to determine the dominant presystemic site for 6AC conversion. The in vitro homogenate incubations suggested that the intestine was the most active organ in converting 6AC to CBV. However, the in situ perfused intestine-liver appeared to be limited in its ability to predict the intestinal wall extraction of 6AC. A theoretical analysis pointed out that the extent of intestinal wall extraction of 6AC should be determined after an oral dose. Indeed, after intestinal lumen perfusion, 6AC was extracted to a much greater extent in the intestine as compared with the IPIL. In other words, the intestine cannot be considered to be well-stirred with respect to the metabolism of 6AC.

The positive aspect of a substantial first-pass conversion of prodrugs is that the systemic exposure to the active drug is increased, which is probably the goal for most oral prodrugs. On the other hand, if subsequent delivery to a targeted tissue and/or organ is desired, e.g., the brain, then such a first-pass effect might not be viewed favorably. In the case of 6AC, because the first-pass conversion of 6AC primarily takes place in the intestine, quenching of the intestinal activation of 6AC by orally administering ADA inhibitors may result in an increase in 6AC bioavailability, which may in turn improve brain exposure to CBV (Wen et al., 1995). Results of such inhibition studies will be presented in the second article of this series.

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Appendix 1. Derivation of Interrelation of E and \(E_{po}\)

According to the model depicted in Fig. 2, the rate of change of the amounts of CBV and 6AC is as follows, after intestinal lumen perfusions of 6AC:

CBV in systemic compartment:

\[
V_{\text{sys}} \frac{dC_{\text{sys}, \text{CBV}}}{dt} = Q_{\text{pv}} C_{gwn, \text{ex}, \text{CBV}} + \left( CL_{\text{sys}, \text{CBV}} + Q_{\text{gs}} \right) C_{\text{sys}, \text{CBV}} \tag{A1}
\]

CBV in the serosal compartment of the intestinal wall (in equilibrium with perfused vasculature):

\[
V_{\text{gw,ex}} \frac{dC_{\text{gw,ex}, \text{CBV}}}{dt} = Q_{\text{pv}} C_{\text{sys}, \text{CBV}} + CL_{\text{gw,def}} C_{ \text{gw,ex}, \text{CBV}} - Q_{\text{gs}} C_{gws, \text{ex}, \text{CBV}} - CL_{\text{gw,def}} C_{\text{gw,ex}, \text{CBV}} \tag{A2}
\]

CBV in the mucosal compartment of the intestinal wall:

\[
V_{\text{gw,in}} \frac{dC_{\text{gw,in}, \text{CBV}}}{dt} = CL_{\text{gw,def}} C_{gws, \text{ex}, \text{CBV}} - CL_{\text{gw,def}} C_{gwn, \text{in}, \text{CBV}} \tag{A3}
\]

6AC in the systemic compartment:

\[
V_{\text{sys}} \frac{dC_{\text{sys}, \text{6AC}}}{dt} = R_{\text{abs}} \text{6AC} + Q_{\text{pv}} C_{\text{gw,ex}, \text{6AC}} - \left( CL_{\text{sys}, \text{6AC}} + Q_{\text{gs}} \right) C_{\text{sys}, \text{6AC}} \tag{A4}
\]

6AC in the serosal compartment of the intestinal wall:

\[
V_{\text{gw,ex}} \frac{dC_{\text{gw,ex}, \text{6AC}}}{dt} = Q_{\text{pv}} C_{\text{sys}, \text{6AC}} + \left( CL_{\text{gw,def}} + Q_{\text{gs}} \right) C_{\text{sys}, \text{6AC}} - Q_{\text{gs}} C_{\text{gw,ex}, \text{6AC}} \tag{A5}
\]

6AC in the mucosal compartment of the intestinal wall:

\[
V_{\text{gw,in}} \frac{dC_{\text{gw,in}, \text{6AC}}}{dt} = CL_{\text{gw,def}} C_{\text{gw,ex}, \text{6AC}} - Q_{\text{gs}} C_{\text{gw,in}, \text{6AC}} \tag{A6}
\]

Similarly, according to the model depicted in Fig. 2, the rate of change of the amounts of CBV and 6AC is as follows, after intestinal lumen perfusions of 6AC:

CBV in systemic compartment:

\[
V_{\text{sys}} \frac{dC_{\text{sys}, \text{CBV}}}{dt} = Q_{\text{pv}} C_{\text{gw,ex}, \text{CBV}} + CL_{\text{sys}, \text{CBV}} C_{\text{sys}, \text{CBV}} \tag{A7}
\]

CBV in the serosal compartment of the intestinal wall (in equilibrium with perfused vasculature):

\[
V_{\text{gw,ex}} \frac{dC_{\text{gw,ex}, \text{CBV}}}{dt} = Q_{\text{pv}} C_{\text{sys}, \text{CBV}} + CL_{\text{gw,def}} C_{\text{gw,ex}, \text{CBV}} - Q_{\text{gs}} C_{\text{gw,ex}, \text{CBV}} - CL_{\text{gw,def}} C_{\text{gw,ex}, \text{CBV}} \tag{A8}
\]

CBV in the mucosal compartment of the intestinal wall:

\[
V_{\text{gw,in}} \frac{dC_{\text{gw,in}, \text{CBV}}}{dt} = CL_{\text{gw,def}} C_{\text{gw,ex}, \text{CBV}} \tag{A9}
\]

6AC in the systemic compartment:

\[
V_{\text{sys}} \frac{dC_{\text{sys}, \text{6AC}}}{dt} = Q_{\text{pv}} C_{gws, \text{ex}, \text{6AC}} - \left( CL_{\text{sys}, \text{6AC}} + Q_{\text{gs}} \right) C_{\text{sys}, \text{6AC}} \tag{A10}
\]

6AC in the serosal compartment of the intestinal wall:

\[
V_{\text{gw,ex}} \frac{dC_{\text{gw,ex}, \text{6AC}}}{dt} = Q_{\text{pv}} C_{\text{sys}, \text{6AC}} + CL_{\text{gw,def}} C_{\text{gw,ex}, \text{6AC}} - Q_{\text{gs}} C_{\text{gw,ex}, \text{6AC}} \tag{A11}
\]

6AC in the mucosal compartment of the intestinal wall:

\[
V_{\text{gw,in}} \frac{dC_{\text{gw,in}, \text{6AC}}}{dt} = R_{\text{abs}} \text{6AC} + CL_{\text{gw,def}} C_{\text{gw,ex}, \text{6AC}} - Q_{\text{gs}} C_{\text{gw,in}, \text{6AC}} \tag{A12}
\]
where:

\[ C_{\text{sys},i} \]  
= CBV concentration in the systemic circulation after infusion,

\[ C_{\text{gw,ex},i} \]  
= CBV concentration in the serosal space of the gut wall after infusion,

\[ C_{\text{gw,in},i} \]  
= CBV concentration in the mucosal space of the gut wall after infusion,

\[ C_{\text{sys},p} \]  
= 6AC concentration in the systemic circulation after infusion,

\[ C_{\text{gw,ex},i} \]  
= 6AC concentration in the serosal space of the gut wall after infusion,

\[ C_{\text{gw,in},i} \]  
= 6AC concentration in the mucosal space of the gut wall after infusion,

\[ C_{\text{sys},CBV} \]  
= CBV concentration in the systemic circulation after perfusion,

\[ C_{\text{gw,ex},p} \]  
= CBV concentration in the serosal space of the gut wall after perfusion,

\[ C_{\text{gw,in},p} \]  
= CBV concentration in the mucosal space of the gut wall after perfusion,

\[ R_{\text{inf}} \]  
= 6AC systemic volume of distribution excluding the formation of CBV,

\[ Q_{pv} C_{\text{sys},i} \]  
= portal blood flow,

\[ R_{\text{abs}} \]  
= intraportal infusion rate of 6AC,

\[ V_{\text{sys,CBV}} \]  
= CBV systemic volume of distribution excluding the gut wall,

\[ V_{\text{gw,ex}} \]  
= CBV volume of distribution in the serosal space of the gut wall,

\[ V_{\text{gw,in}} \]  
= CBV volume of distribution in the mucosal space of the gut wall,

\[ V_{\text{sys}} \]  
= 6AC systemic volume of distribution excluding the gut wall,

\[ V_{\text{gw,ex}} \]  
= 6AC volume of distribution in the serosal space of the gut wall,

\[ V_{\text{gw,in}} \]  
= 6AC volume of distribution in the mucosal space of the gut wall.

At steady state, for the infusion administration, the rate equations become:

**CBV in systemic compartment:**

\[ Q_{pv} C_{\text{gw,ex},p} + C_{\text{sys},i} V_{\text{sys,CBV}} - (CL_{\text{sys}} f + CL_o 6AC + Q_{pv}) C_{\text{sys},p} = 0 \]  
(A1')

**CBV in the serosal compartment of the intestinal wall (in equilibrium with perfused vasculature):**

\[ Q_{pv} C_{\text{sys},i} + CL_{\text{gw,def}} C_{\text{gw,in},i} - Q_{pv} C_{\text{gw,ex},i} = 0 \]  
(A2')

**CBV in the mucosal compartment of the intestinal wall:**

\[ CL_{\text{gw}} C_{\text{gw,in},i} + CL_{\text{gw,def}} C_{\text{gw,ex},i} - CL_{\text{gw,def}} C_{\text{gw,in},i} = 0 \]  
(A3')

6AC in the systemic compartment:

\[ R_{\text{inf}} 6AC + Q_{pv} C_{\text{gw,ex},i} 6AC - (CL_{\text{sys}} f + CL_o 6AC + Q_{pv}) C_{\text{sys},i} 6AC = 0 \]  
(A4')

6AC in the serosal compartment of the intestinal wall:

\[ Q_{pv} C_{\text{sys},i} 6AC + CL_{\text{gw,def}} 6AC C_{\text{gw,in},i} 6AC - (CL_{\text{gw,def}} 6AC C_{\text{gw,in},i} 6AC) = 0 \]  
(A5')

6AC in mucosal compartment of the intestinal wall:

\[ CL_{\text{gw,def}} 6AC C_{\text{gw,ex},i} 6AC - CL_{\text{gw,def}} 6AC C_{\text{gw,in},i} 6AC = 0 \]  
(A6')

At steady state, for the perfusion administration, the rate equations become:

**CBV in systemic compartment:**

\[ Q_{pv} C_{\text{gw,ex},p} CBV + C_{\text{sys},p} V_{\text{sys,CBV}} - (CL_{\text{sys}} CBV + Q_{pv}) C_{\text{sys},p} CBV = 0 \]  
(A7')

**CBV in the serosal compartment of the intestinal wall (in equilibrium with perfused vasculature):**

\[ Q_{pv} C_{\text{sys},i} CBV + CL_{\text{gw,def}} CBV C_{\text{gw,in},p} CBV - Q_{pv} C_{\text{gw,ex},i} CBV = 0 \]  
(A8')

**CBV in the mucosal compartment of the intestinal wall:**

\[ CL_{\text{gw,def}} CBV C_{\text{gw,in},i} 6AC + CL_{\text{gw,def}} CBV C_{\text{gw,ex},i} 6AC - CL_{\text{gw,def}} CBV C_{\text{gw,in},p} CBV = 0 \]  
(A9')

6AC in the systemic compartment:

\[ Q_{pv} C_{\text{gw,ex},i} 6AC - (CL_{\text{sys}} f + CL_o 6AC + Q_{pv}) C_{\text{sys},i} 6AC = 0 \]  
(A10')

6AC in the serosal compartment of the intestinal wall:

\[ Q_{pv} C_{\text{sys},i} 6AC + CL_{\text{gw,def}} 6AC C_{\text{gw,in},i} 6AC - CL_{\text{gw,def}} 6AC C_{\text{gw,ex},i} 6AC = 0 \]  
(A11')

6AC in the mucosal compartment of the intestinal wall:

\[ R_{\text{abs}} 6AC + CL_{\text{gw,def}} 6AC C_{\text{gw,ex},i} 6AC - CL_{\text{gw,def}} 6AC C_{\text{gw,in},p} 6AC = 0 \]  
(A12')

Equations A1’ to A12’ were solved simultaneously for the concentrations of CBV and 6AC in each compartment using Mathematica (student version 22.2, Wolfram Research, Champaign, IL). The four relevant expressions for 6AC are listed below:

\[ C_{\text{sys},i} 6AC = (CL_{\text{gw}} f CL_{\text{gw,def}} 6AC + CL_{\text{gw}} Q_{pv} + CL_{\text{gw,def}} 6AC Q_{pv}) R_{\text{inf}} 6AC \]  
\[ (CL_{\text{gw}} f CL_{\text{sys}} CL_{\text{gw,def}} 6AC + CL_{\text{gw}} Q_{pv} CL_{\text{gw,def}} 6AC CL_o 6AC) \]  
\[ + CL_{\text{gw}} f CL_{\text{sys}} Q_{pv} + CL_{\text{gw}} f CL_{\text{gw,def}} 6AC Q_{pv} \]  
\[ + CL_{\text{sys}} CL_{\text{gw,def}} 6AC Q_{pv} + CL_{\text{gw}} f CL_o 6AC Q_{pv}) \]  
(A13)
The extraction ratios of 6AC after intraportal infusions (E) and intestinal perfusions are as follows:

\[
E = 1 - \frac{Q_{gw,ex,i}^{6AC}}{Q_{sys,i}^{6AC}} = 1 - \frac{Q_{gw,ex,i}^{6AC}}{Q_{sys,i}^{6AC}} \tag{A17}
\]

\[
E_{po} = 1 - \frac{Q_{gw,ex,p}^{6AC} - (1 - E)Q_{gw,i}^{6AC}}{R_{abs}^{6AC}} \tag{A18}
\]

Substituting eqs. A13 to A16 into eqs. A17 and A18 and simplifying, the following expressions are obtained:

\[
E = \frac{CL_{gw}^{6AC}CL_{gw,df}^{6AC}}{CL_{gw}^{6AC}CL_{gw,df}^{6AC} + CL_{sys}^{6AC}Q_{gw}^{6AC}} + CL_{gw}^{6AC}Q_{gw}^{6AC} + CL_{sys}^{6AC}Q_{gw}^{6AC} + CL_{gw,df}^{6AC}CL_{gw}^{6AC} \tag{A19}
\]

\[
E_{po} = \frac{CL_{gw}^{6AC}CL_{gw,df}^{6AC} + CL_{gw}^{6AC}Q_{gw}^{6AC}}{CL_{gw}^{6AC}CL_{gw,df}^{6AC} + CL_{sys}^{6AC}Q_{gw}^{6AC}} \tag{A20}
\]

**Appendix 2. Derivation of an Expression for \( E_{po} \) with CBV/6AC CRs**

At steady state during an intraportal infusion, the mass balance equations are as follows:

- rate of formation of CBV from 6AC = rate of elimination of CBV
  \[ R_{inf}^{6AC} = \frac{CL_{sys}^{CBV}C_{sys,inf}^{6AC}}{CL_{gw}^{6AC}CL_{gw,df}^{6AC} + CL_{sys}^{6AC}Q_{gw}^{6AC}} \tag{A19} \]

- rate of input of 6AC = rate of elimination of 6AC
  \[ R_{inf}^{6AC} = \frac{CL_{sys}^{6AC}C_{sys,inf}^{6AC}}{CL_{gw}^{6AC}CL_{gw,df}^{6AC} + CL_{sys}^{6AC}Q_{gw}^{6AC}} \tag{A20} \]

where:

- \( R_{inf}^{6AC} \) = intraportal infusion rate of 6AC,
- \( F_{gw}^{6AC} \) = fraction of 6AC metabolized to CBV after an intraportal administration,
- \( C_{sys,inf}^{6AC} \) = clearance of CBV after an intraportal administration,
- \( C_{sys,inf}^{CBV} \) = steady-state systemic concentration of CBV, and
- \( C_{sys,inf}^{6AC} \) = steady-state systemic concentration of 6AC.

By the same principle, mass balance equations for 6AC and CBV are obtained during steady state after an intestinal luminal perfusion. These are slightly more complex because of the first-pass intestinal absorption of 6AC to CBV.

The mass balance for CBV after an intestinal luminal perfusion is:

\[
R_{inf}^{6AC}F_{gw}^{6AC}F_{m}^{sys} + R_{inf}^{6AC}(1 - F_{gw}^{6AC}) = CL_{sys}^{CBV}C_{sys,perf}^{CBV} \tag{A21}
\]

The mass balance for 6AC after an intestinal luminal perfusion is:

\[
R_{inf}^{6AC}F_{gw}^{6AC} = CL_{sys}^{6AC}C_{sys,perf}^{6AC} \tag{A22}
\]

where:

- \( R_{inf}^{6AC} \) = absorption rate of 6AC from the intestinal lumen, and
- \( F_{gw}^{6AC} \) = fraction of 6AC surviving the intestinal wall metabolism,
- \( C_{sys,perf}^{CBV} \) = steady-state systemic concentration of CBV after an intestinal luminal perfusion, and
- \( C_{sys,perf}^{6AC} \) = steady-state systemic concentration of 6AC after an intestinal luminal perfusion.

The absorption of lumenally generated CBV is absent in eq. A21 because only moderate luminal conversion of 6AC to CBV was observed. The intestinal absorption of CBV itself is poor (Soria and Zimmerman, 1994), so absorption of lumenally generated CBV was considered to be insignificant.

\[ F_{gw}^{6AC} \] is obtained by simultaneously solving eqs. A19 to A22 for the CRs of CBV/6AC after oral (CR\(_{perf}\)) and after portal infusion (CR\(_{int}\)).
Huang S, Remmel RP and Zimmerman CL (1991) The bioavailability and nonlinear clearance of
Klippert PJM and Noordhoek J (1983) Influence of administration route and blood sampling site
on the area under the curve. Assessment of gut wall, liver, and lung metabolism from a
Pang KS (1984) Liver perfusion studies in drug metabolism and drug toxicity, in *Drug Metab-
olism and Drug Toxicity* (Mitchell JR and Horning MG eds) pp 331–352, Raven Press, New
York.
metabolite, enalaprilat, in a perfused rat liver preparation. Presence of a diffusional barrier for
Rowland M, Benet LZ and Graham GG (1973) Clearance concepts in pharmacokinetics.
on the effect of a uniform diffusional barrier across hepatocytes on drug metabolism by evenly
or unevenly distributed uni-enzyme in the liver. *J Pharm Sci* 75:3–8.
Vince R and Brownell J (1990) Resolution of racemic carbovir and selective inhibition of human
Vince R and Hua M (1990) Synthesis and anti-HIV activity of carbocyclic 2’,3’-dideoxy-2,3’-
Vince R, Hua M, Brownell J, Daluge S, Lee F, Shannon WM, Lavelle GC, Qualls J, Weislow
OS, Kiser K, Canonico PG, Schultz RH, Narayanan VL, Mayo JG, Shoemaker RH and Boyd
614846) against human immunodeficiency virus *in vitro*. *Biochem Biophys Res Commun* 
156:1046–1053.
Wen Y. Brain uptake and presystemic disposition of 6-aminocarbovir as a prodrug of (−)-
to (−)-carbovir after (−)-carbovir or (−)-6-aminocarbovir intravenous infusion in rats. *Pharm
Winston JH, Hanten GR, Overbeek PA and Kellems RE (1992) 5’ Flanking sequences of the
murine adenosine deaminase gene direct expression of a reporter gene to specific prenatal and
evaluation of (−)-6-aminocarbovir as a prodrug for (−)-carbovir in rats. *Drug Metab Dispos*
20:47–51.