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

YANDONG WEN,1 RORY P. REMMEL, AND CHERYL L. ZIMMERMAN

Departments of Pharmaceutics and Medicinal Chemistry, University of Minnesota, College of Pharmacy, Minneapolis, Minnesota

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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-amino-guanosine (6AC) conversion in rats. In the 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.

(−)-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 prodrg 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).

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.

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 carried out 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 µl 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...
liver, respectively. Samples were transferred into ice-cooled microcentrifuge vials containing 400 μl of internal standard solution (0.8 μg/ml, carbocyclic 2',3'-dideoxyguanosine) and 10 μl of trichloroacetic acid (60%). Samples were immediately mixed on a vortex mixer for 30 s. After centrifuging at 13,000g for 6 min in a Fisher (Pittsburgh, PA) model 255B microcentrifuge, 25 μl of saturated NaHCO₃ was added to 400 μl of the supernatant to neutralize the sample. The samples were stored at −20°C until assay. 6AC was also incubated with PBS as a control.

Extraction of 6AC in the In Situ Vascularly Perfused Intestine-Liver (IPIL). Six male Sprague-Dawley rats (271.5 ± 26.2 g) were used. A previously described surgical procedure (Hirayama et al., 1989; Xu et al., 1989) was modified for use in the current study. The rats were anesthetized with 50 mg/kg pentobarbital i.p. (Abbott Labs, North Chicago, IL). A V-shaped incision was then made to expose the abdominal cavity. The gastric artery, gastroduodenal artery, and splenic blood vessels perfusing the stomach and spleen were ligated. A cannula of Intramedic PE-20 polyethylene tubing (Clay Adams, Division of Becton Dickinson and Co., Parsippany, NJ) with a beveled tip was inserted into the bile duct and secured in place by ligature. The celiac artery and the pyloric vein were ligated. A 20-gauge i.v. catheter placement unit (Criticon Inc., Tampa, FL) was placed into the portal vein for portal blood sampling and secured with Superglue. Immediately after the aorta was tied off near the superior mesenteric artery, a cut was made in the superior mesenteric artery. A blunted 19-gauge × 1½ inch needle (Monoject, St. Louis, MO) was inserted into the artery. The needle was secured with 3-0 surgicalilk (Look Inc., Norwell, MA). Perfusion into the superior mesenteric artery with blank perfusate was immediately initiated at a flow rate of 2 to 3 ml/min. The thoracic cavity was opened and a cut was made in the right atrium of the heart. The Teflon catheter from an i.v. placement unit was inserted into the opening of the right atrium and secured in place. The catheter was connected to Tygon tubing allowing the exit of perfusate from the liver. The perfusion flow rate was then adjusted to approximately 10 ml/min.

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% (w/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-aspartate 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. Perfusion 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'-deoxyoxycarmycin (DCF) (pentosanit; 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 perfunct 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):

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

where:

\[ CL_{gw} = CL_{gw,dif} + CL_{gw,po} \]

\[ CL_{gw,po} \]

\[ CL_{gw,diff} = CL_{gw,po} + CL_{gw,po,CL_{gw,diff}} \]

Equation 3 indicates that the extraction ratio obtained from IPIL (E) would be an underestimate of the extraction ratio of a drug administered orally (Epo) 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 CLgw,diff. 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. Three rats received an infusion of 6AC via the portal vein. The rats were under pentobarbital

![FIG. 1. Schematic diagram of the intestine-liver vascular perfusion experimental setup.](Image)
DCF solution (1 mg/ml) had previously been added. After vortexing, blood vials, to which 400 μl each were withdrawn from the rats starting approximately 40 min after the initiation of the perfusion or infusion. To obtain the extraction ratio of 6AC in the intestine after an intestinal lumen administration of 6AC, an intraportal administration of 6AC was also required. The extraction ratio could then be derived based on the data generated by experiments. To obtain the instantaneous organ extraction ratio (E) of 6AC was calculated using eq. 4:

\[ E = \frac{C_{in} - C_{out}}{C_{in}} \]

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.** The instantaneous organ extraction ratio (E) of 6AC was calculated as a function of steady-state concentration ratios (CR) of CBV to 6AC after oral (CR_{perf}) and after portal infusion (CR_{inf}):

\[ E_{po} = \frac{1 - F_{gw} 6AC}{1 + F_{sys} CR_{inf} - CR_{perf} / CR_{inf}} \]

\[ E_{po} = 1 - F_{gw} 6AC \]

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 separately determined in this study, \( F_{sys} \) 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 C_{18} 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 samples were placed on dry ice for the remainder of the experiment and then kept at ~70°C until assay.

**Data Analysis.**

**Disappearance Half-Life of 6AC in Tissue Incubations.** The disappearance rate constant (k) of 6AC in each tissue incubation was obtained by linear regression of the logarithmic concentration–time data. The disappearance half-life (T^{1/2}_{1/2}) was calculated from this first-order rate constant.

\[ T^{1/2}_{1/2} = \frac{\ln(2)}{k} \]

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

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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 mg/ml) was later switched to a lower concentration (3.5 mg/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 in the intestine after intestinal lumen perfusion of 6AC.

Table 3 summarizes the results from this study. The extraction ratio (Epo) was calculated to be 0.54 ± 0.06, significantly larger than the extraction ratio (E) of 0.08 ± 0.02 in the IPIL study.
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 have greater contact time in the drug-metabolizing compartment, and indeed, the extraction ratio of orally administered 6AC (Epo) was determined to be 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 vascularly 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 diffusion process for 6AC is slow compared with the convective perfusate flow, many prodrug molecules will be carried into the portal venous 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 vascularly delivered 6AC. In the present case, 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 serosal 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 Noordhoek, 1983). Drug delivered by the oral route would by necessity be carried into the portal venous flow by the mucosal blood.

**TABLE 3**

<table>
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<tr>
<th>Parameters</th>
<th>CLgw,dif (ml·min⁻¹·kg⁻¹)</th>
<th>CRperf</th>
<th>CRinf</th>
<th>Fm</th>
<th>Sys</th>
<th>Epo</th>
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<td>0.11</td>
<td>0.07</td>
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**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).

![Blood concentration profiles (mean ± S.D.) after intraportal infusions (A, n = 3) and intestinal lumen perfusions (B, n = 4).](image_url)
delivered systemically would be exposed to the metabolically active mucosal 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 intraportal infusions of 6AC:

**CBV in systemic compartment:**

\[
V_{sys} \frac{dC_{sys, CBV}}{dt} = Q_{ps} C_{gw, ex, CBV} + CL_{sys} f_{CBV} C_{sys, CBV}
\]

(A1)

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

\[
V_{gw, ex} \frac{dC_{gw, ex, CBV}}{dt} = Q_{ps} C_{gw, ex, CBV} + CL_{gw, dif} C_{gw, in, CBV} - Q_{ps} C_{gw, ex, CBV} - CL_{gw, dif} C_{gw, ex, CBV}
\]

(A2)

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

\[
V_{gw, in} \frac{dC_{gw, in, CBV}}{dt} = CL_{gw} f_{CBV} C_{gw, in, CBV} + CL_{gw, dif} C_{gw, in, CBV} - CL_{gw, dif} C_{gw, in, CBV}
\]

(A3)

6AC in the systemic compartment:

\[
V_{sys} \frac{dC_{sys, 6AC}}{dt} = R_{abs} 6AC + Q_{ps} C_{gw, ex, 6AC} - \left( CL_{sys} f + CL_{o} 6AC + Q_{ps} \right) C_{sys, 6AC}
\]

(A4)

6AC in the serosal compartment of the intestinal wall:

\[
V_{gw, ex} \frac{dC_{gw, ex, 6AC}}{dt} = Q_{ps} C_{sys, 6AC} + CL_{gw, dif} C_{gw, in, 6AC} - CL_{gw, dif} C_{gw, ex, 6AC}
\]

(A5)

6AC in the mucosal compartment of the intestinal wall:

\[
V_{gw, in} \frac{dC_{gw, in, 6AC}}{dt} = CL_{gw, dif} C_{gw, in, 6AC} - CL_{gw, dif} C_{gw, in, 6AC}
\]

(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:**

\[
V_{sys} \frac{dC_{sys, CBV}}{dt} = Q_{ps} C_{gw, ex, CBV} + CL_{sys} f_{CBV} C_{sys, CBV}
\]

(A7)

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

\[
V_{gw, ex} \frac{dC_{gw, ex, CBV}}{dt} = Q_{ps} C_{sys, CBV} + CL_{gw, dif} C_{gw, in, CBV} - Q_{ps} C_{gw, ex, CBV} - CL_{gw, dif} C_{gw, ex, CBV}
\]

(A8)

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

\[
V_{gw, in} \frac{dC_{gw, in, CBV}}{dt} = CL_{gw} f_{CBV} C_{gw, in, CBV} + CL_{gw, dif} C_{gw, in, CBV} - CL_{gw, dif} C_{gw, in, CBV}
\]

(A9)

6AC in the systemic compartment:

\[
V_{sys} \frac{dC_{sys, 6AC}}{dt} = Q_{ps} C_{gw, ex, 6AC} - \left( CL_{sys} f + CL_{o} 6AC + Q_{ps} \right) C_{sys, 6AC}
\]

(A10)

6AC in the serosal compartment of the intestinal wall:

\[
V_{gw, ex} \frac{dC_{gw, ex, 6AC}}{dt} = Q_{ps} C_{sys, 6AC} + CL_{gw, dif} C_{gw, in, 6AC} - CL_{gw, dif} C_{gw, ex, 6AC}
\]

(A11)

6AC in the mucosal compartment of the intestinal wall:

\[
V_{gw, in} \frac{dC_{gw, in, 6AC}}{dt} = R_{abs} 6AC + CL_{gw, dif} C_{gw, ex, 6AC} - CL_{gw, dif} C_{gw, in, 6AC}
\]

(A12)
where:

\[ C_{\text{sys},i}^{\text{CBV}} = \text{CBV concentration in the systemic circulation after infusion}, \]
\[ C_{\text{gw,ex},i}^{\text{CBV}} = \text{CBV concentration in the serosal space of the gut wall after infusion}, \]
\[ C_{\text{gw,in},i}^{\text{CBV}} = \text{CBV concentration in the mucosal space of the gut wall after infusion}, \]
\[ C_{\text{sys},i}^{6\text{AC}} = \text{6AC concentration in the systemic circulation after infusion}, \]
\[ C_{\text{gw,ex},i}^{6\text{AC}} = \text{6AC concentration in the serosal space of the gut wall after infusion}, \]
\[ C_{\text{gw,in},i}^{6\text{AC}} = \text{6AC concentration in the mucosal space of the gut wall after infusion}, \]
\[ C_{\text{sys},p}^{\text{CBV}} = \text{CBV concentration in the systemic circulation after perfusion}, \]
\[ C_{\text{gw,ex},p}^{\text{CBV}} = \text{CBV concentration in the serosal space of the gut wall after perfusion}, \]
\[ C_{\text{gw,in},p}^{\text{CBV}} = \text{CBV concentration in the mucosal space of the gut wall after perfusion}, \]
\[ C_{\text{sys},p}^{6\text{AC}} = \text{6AC concentration in the systemic circulation after perfusion}, \]
\[ C_{\text{gw,ex},p}^{6\text{AC}} = \text{6AC concentration in the serosal space of the gut wall after perfusion}, \]
\[ C_{\text{gw,in},p}^{6\text{AC}} = \text{6AC concentration in the mucosal space of the gut wall after perfusion}, \]
\[ CL_{\text{sys},i}^{f} = \text{CBV formation clearance in the systemic circulation}, \]
\[ CL_{\text{CBV}} = \text{CBV total body clearance}, \]
\[ CL_{\text{gw,df}}^{\text{CBV}} = \text{CBV diffusional clearance in the gut wall}, \]
\[ CL_{\text{gw},i}^{6\text{AC}} = \text{6AC clearance excluding the formation of CBV}, \]
\[ CL_{\text{gw,df}}^{6\text{AC}} = \text{6AC diffusional clearance in the gut wall}, \]
\[ Q_{pv}^{6\text{AC}} = \text{portal blood flow}, \]
\[ R_{\text{inf}}^{6\text{AC}} = \text{intraportal infusion rate of 6AC}, \]
\[ V_{\text{sys}}^{\text{CBV}} = \text{CBV systemic volume of distribution excluding the gut wall}, \]
\[ V_{\text{gw,ex}}^{\text{CBV}} = \text{CBV volume of distribution in the serosal space of the gut wall}, \]
\[ V_{\text{gw,in}}^{\text{CBV}} = \text{CBV volume of distribution in the mucosal space of the gut wall}, \]
\[ V_{\text{sys}}^{6\text{AC}} = \text{6AC systemic volume of distribution excluding the gut wall}, \]
\[ V_{\text{gw,ex}}^{6\text{AC}} = \text{6AC volume of distribution in the serosal space of the gut wall}, \]
\[ V_{\text{gw,in}}^{6\text{AC}} = \text{6AC volume of distribution in the mucosal space of the gut wall}. \]

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

**CBV in systemic compartment:**

\[ Q_{pv}^{6\text{AC}} C_{\text{gw,ex},i}^{\text{CBV}} + CL_{\text{gw,df}}^{\text{CBV}} C_{\text{gw,in},i}^{\text{CBV}} - Q_{pv}^{6\text{AC}} C_{\text{gw,ex},i}^{\text{CBV}} - CL_{\text{gw,df}}^{6\text{AC}} C_{\text{gw,in},i}^{6\text{AC}} = 0 \quad (A1') \]

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

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

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

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

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

**CBV in systemic compartment:**

\[ Q_{pv}^{6\text{AC}} C_{\text{gw,ex},p}^{\text{CBV}} + CL_{\text{sys}}^{f} C_{\text{sys},p}^{\text{CBV}} - (CL_{\text{sys}}^{f} + Q_{pv}^{6\text{AC}}) C_{\text{sys},p}^{6\text{AC}} = 0 \quad (A7') \]

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

\[ Q_{pv}^{6\text{AC}} C_{\text{gw,ex},p}^{\text{CBV}} - CL_{\text{gw,df}}^{6\text{AC}} C_{\text{gw,in},p}^{\text{CBV}} - CL_{\text{gw,df}}^{6\text{AC}} C_{\text{gw,in},p}^{6\text{AC}} = 0 \quad (A9') \]

**6AC in the systemic compartment:**

\[ R_{\text{inf}}^{6\text{AC}} + Q_{pv}^{6\text{AC}} C_{\text{gw,ex},p}^{6\text{AC}} - (CL_{\text{gw,df}}^{6\text{AC}} + CL_{\text{gw,df}}^{6\text{AC}}) C_{\text{gw,ex},p}^{6\text{AC}} = 0 \quad (A10') \]

**6AC in the serosal compartment of the intestinal wall:**

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

**6AC in the mucosal compartment of the intestinal wall:**

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

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:

\[ C_{\text{sys},i}^{6\text{AC}} = (CL_{\text{gw,df}}^{6\text{AC}} + CL_{\text{gw,df}}^{6\text{AC}}) R_{\text{inf}}^{6\text{AC}} \]

\[ (CL_{\text{pq}}^{6\text{AC}} CL_{\text{pq}}^{6\text{AC}} + CL_{\text{pq}}^{6\text{AC}} CL_{\text{pq}}^{6\text{AC}})^{CL_{\text{gw,df}}^{6\text{AC}}} \]

\[ + CL_{\text{gw}}^{6\text{AC}} Q_{pv}^{6\text{AC}} + CL_{\text{gw}}^{6\text{AC}} Q_{pv}^{6\text{AC}} \]

\[ + CL_{\text{gw}}^{6\text{AC}} Q_{pv}^{6\text{AC}} \]
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 activation of 6AC to CBV.

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

rate of formation of CBV from 6AC = rate of elimination of CBV
rate of systemic formation of CBV from = rate of elimination of CBV
rate of first-pass formation of CBV from 6AC

\[
R_{abs}^{6AC} F_{gw}^{6AC} F_{m,s}^{sys} + R_{abs}^{6AC} (1 - F_{gw}^{6AC}) = C_{sys}^{CBV} \text{CR}_{perf}^{CBV}
\]

(A21)

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

rate of input of 6AC = rate of elimination of 6AC

\[
R_{abs}^{6AC} F_{gw}^{6AC} = C_{sys}^{6AC} \text{CR}_{perf}^{6AC}
\]

(A22)

where:

\[
R_{abs}^{6AC} = \text{absorption rate of 6AC from the intestinal lumen,}
\]

\[
F_{gw}^{6AC} = \text{fraction of 6AC surviving the intestinal wall metabolism,}
\]

\[
C_{sys,perf}^{CBV} = \text{steady-state systemic concentration of CBV after an intestinal luminal perfusion, and}
\]

\[
C_{sys,perf}^{6AC} = \text{steady-state systemic concentration of 6AC after an intestinal luminal perfusion.}
\]

The absorption of luminally 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 luminally generated CBV was considered to be insignificant.

\[
F_{gw}^{6AC} \text{is obtained by simultaneously solving eqs. A19 to A22 for the CRs of CBV/6AC after oral (CR}_{perf}^{CBV}) and after portal infusion (CR}_{perf}^{6AC}).
\]

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

\[
R_{inf}^{6AC} F_{m,s}^{sys} = C_{sys}^{CBV} \text{CR}_{inf}^{CBV}
\]

(A19)

rate of input of 6AC = rate of elimination of 6AC

\[
R_{abs}^{6AC} F_{gw}^{6AC} = C_{sys}^{6AC} \text{CR}_{inf}^{6AC}
\]

where:

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

\[
F_{m,s}^{sys} = \text{fraction of 6AC metabolized to CBV after an intraportal administration,}
\]

\[
C_{sys}^{CBV} = \text{clearance of CBV after an intraportal administration,}
\]

\[
C_{sys,inf}^{CBV} = \text{steady-state systemic concentration of CBV, and}
\]

\[
C_{sys,inf}^{6AC} = \text{steady-state systemic concentration of 6AC.}
\]

References


