FIRST-PASS DISPOSITION OF (−)-6-AMINOCARBOVIR IN RATS: II. INHIBITION OF INTESTINAL FIRST-PASS METABOLISM

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ABSTRACT:

A CBV [(−)-carbovir, (−)-carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine] prodrug, 6AC [(−)-6-aminocarbovir, (−)-carbocyclic 2′,3′-didehydro-2′,3′-dideoxy-6-deoxy-6-aminoguanosine], was previously evaluated in rats, and it exhibited superiority to the parent drug in increasing systemic and central nervous system exposure to CBV. The gut wall was determined to be the dominant site of the first-pass activation of 6AC after lumenal administration. If subsequent delivery to the brain is desired, then such a first-pass effect might not be viewed favorably. Because the first-pass conversion of 6AC primarily takes place in the intestine by adenosine deaminase (ADA), quenching of the intestinal activation of 6AC by oral administration of ADA inhibitors may result in an increased 6AC bioavailability, and thus an improved brain exposure to CBV. The objectives of the study were to determine whether the ADA inhibitors 2′-deoxycoformycin and erythro-9-(2-hydroxy-3-nonyl)adenine were capable of achieving a substantial and selective inhibition of gut wall activation of 6AC, and to determine whether the systemic concentrations of 6AC would be thus increased. Thirty-nine male Sprague-Dawley rats were divided into two groups. One group received 6AC by either the portal vein or intraluminally with the coadministration of intraluminal 2′-dideoxycoformycin. Similarly, the other group received 6AC with coadministration of erythro-9-(2-hydroxy-3-nonyl)adenine. Substantial suppression of the first-pass conversion of 6AC was achieved with both inhibitors. This inhibition appeared to be relatively selective, allowing the choice of dose of inhibitor that would sufficiently inhibit the first-pass metabolism while leaving the activation capacity in the systemic circulation unralted. The systemic level of 6AC increased with the escalating dose of inhibitors, thus increasing the driving force for passive uptake into the brain.

CBV [(−)-carbovir, (−)-carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine] is a carbocyclic nucleoside with potent and selective anti-HIV activity (Vince et al., 1988), a low oral bioavailability, and limited brain delivery (Huang et al., 1991; Wen et al., 1995). A CBV prodrug, 6AC [(−)-6-aminocarbovir, (−)-carbocyclic 2′,3′-didehydro-2′,3′-dideoxy-6-deoxy-6-aminoguanosine], was evaluated in rats, and 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). The gut wall was determined to be the dominant site of the first-pass activation of 6AC after lumenal administration. However, the in situ perfused intestine-liver (in which the vasculature of the intestine is perfused) appeared to be limited in its ability to predict the intestinal wall extraction of 6AC. After intestinal lumen perfusion, 6AC was extracted to a much greater extent by the intestine. In other words, the intestine could not be considered to be “well-stirred” with respect to the metabolism of 6AC (Wen et al., 1999).

The positive aspect of a substantial first-pass conversion of prodrugs is that the systemic exposure to the active drug is increased, which probably is the goal for most oral prodrugs. If subsequent delivery to a targeted tissue/organ is desired, e.g., the brain, then such a first-pass effect might not be viewed favorably. It has been shown that 6AC is more favorably partitioned into the brain than is CBV. Once there, 6AC is converted to the active CBV (Wen et al., 1995). Because the first-pass conversion of 6AC primarily takes place in the intestine by adenosine deaminase (ADA; EC 3.5.4.4), decreasing the intestinal activation of 6AC by oral coadministration of ADA inhibitors may result in an increase in 6AC bioavailability, which may in turn improve brain exposure to CBV. Similar ideas have been applied to improve the therapeutic efficacy of several agents, most notably the combination use of levodopa/carbidopa in Parkinson’s disease. Carbidopa alters the kinetics of levodopa favorably by increasing its bioavailability (Nutt et al., 1985; Cedarbaum et al., 1986), elevating its blood levels (Cedarbaum et al., 1986; Rose et al., 1988; Deleu et al., 1993), prolonging its duration in the peripheral circulation (Nutt et al., 1985; Deleu et al., 1993), and ultimately providing an increased driving force for levodopa brain uptake. The effects of carbidopa on levodopa have been attributed to its inhibition of gastrointestinal aromatic amino acid decarboxylase (Nutt et al., 1985).
The inhibitors of ADA chosen for this study were pentostatin (2'-deoxycoformycin, DCF) and \textit{erythro}-9-(2-hydroxy-3-nonyl)adenine (EHNA) (Fig. 1). DCF is a tight-binding inhibitor of ADA (Agarwal, 1982), with a $K_i$ value of 0.25 to 1.5 $\times$ 10^{-11} M (mouse intestinal, brain, and human erythrocytic ADA). EHNA is classified as a semitight-binding inhibitor of ADA (Agarwal, 1982) with a $K_i$ value of 1.6 to 6.5 $\times$ 10^{-9} M (calf intestinal ADA). Both EHNA and DCF are reported to be competitive inhibitors of ADA (Agarwal, 1982; Blick et al., 1990).

The objectives of the study were 3-fold: 1) to determine whether ADA inhibitors were capable of achieving a substantial inhibition of gut wall activation of 6AC; 2) to determine whether the inhibition of gut wall activation of 6AC was site-selective with little change in the systemic pharmacokinetics of 6AC; and 3) to investigate whether the systemic concentrations of 6AC were increased by coadministration of ADA inhibitors.

**Experimental Procedures**

**Materials.** 6AC and CBV (Fig. 1) were synthesized at the University of Minnesota (Beers et al., 1990; Vince and Brownell, 1990; Vince and Hua, 1990), and received as gifts from Dr. Robert Vince (University of Minnesota). DCF was supplied by Parke-Davis Pharmaceuticals (Ann Arbor, MI). EHNA was obtained from Burroughs Wellcome (Research Triangle Park, NC). All other chemicals were reagent grade or better.

**In Situ Inhibition Experimental Design.** Thirty-nine male Sprague-Dawley rats (255 ± 15 g) were used in this study. The rats were divided into two groups. One group (21 rats) received 6AC by either the portal vein or intralumenally with the coadministration of DCF intralumenally. Similarly, the other group (18 rats) received 6AC with coadministration of EHNA. A previous publication reported the results of similar studies with a control group of rats ($n = 7, 264 ± 15$ g), which received 6AC by the two routes, but without inhibitor (Wen et al., 1999). The experiments in the control group and in the inhibitor groups were carried out during the same time period, but the results are reported separately for clarity of presentation.

The experimental procedure is described in Fig. 2. The rats were under pentobarbital anesthesia for the entire duration of the perfusion or infusion. For the rats in the perfusion group, a 40-cm segment of the small intestine starting from the ligament of Treitz was isolated with the vasculature kept intact. Both ends of the segment were connected to custom-made glass cannulas. The contents in the intestinal segment were initially flushed with warmed normal saline. The duodenal end of the segment was connected to a 50-ml plastic syringe. The segment was perfused with 6AC in HEPES (10 mM, pH 6.5) buffer for 100 min at a rate of approximately 80 $\mu$g/min with a Harvard microcrite syringe pump. The volumetric flow rate was 0.21 ml/min. Perfusion was collected from the distal cannula in 10-min fractions. In the rats receiving the portal vein infusion, the portal vein was catheterized with an i.v. catheter placement unit, which was connected to a 12-ml plastic syringe. 6AC in normal saline was infused into the portal vein for 100 min at a rate of approximately 28 $\mu$g/min with a Harvard microcrite syringe pump. A femoral vein catheter (silastic tubing connected to PE-50 tubing) was implanted for blood sampling for both routes of administration. Four to five blood samples of 150 $\mu$l each were withdrawn from the rats starting approximately 40 min after the initiation of the perfusion or infusion. Blood samples were first placed into heparinized Vacutainers (Becton Dickinson, Franklin Lakes, NJ), and the tubes were gently inverted five times. Aliquots of 100 $\mu$l of the blood samples were pipetted into microcentrifuge vials, to which 400 $\mu$l of internal standard solution (0.8 $\mu$g/ml) and 10 $\mu$l of DCF solution (1 mg/ml) had been added previously. After mixing on a vortex-mixer, blood samples were placed on dry ice for the remainder of the experiment and then kept at −70°C until assay.

To reiterate, the rats receiving intraportal infusions of 6AC were also receiving intestinal lumen perfusates of inhibitors. In the rats receiving intestinal lumen perfusates of 6AC, inhibitors were administered along with 6AC in the perfusion medium. The effects of three levels of each inhibitor were examined. DCF was examined at luminal concentrations of 0.02, 0.1, and 0.5 mM, corresponding to total doses of approximately 0.44 ± 0.03, 2.20 ± 0.11, and 11.25 ± 0.55 mg/kg, respectively. EHNA was examined at luminal concentrations of 0.005, 0.015, and 0.05 mM, corresponding to total doses of 0.14 ± 0.01, 0.40 ± 0.03, and 1.40 ± 0.06 mg/kg, respectively. Both DCF and EHNA were freely soluble in the perfusion medium.

**Analytical Methods.** Perfusate samples and blood samples were extracted and analyzed for 6AC and CBV, as described previously (Zimmerman et al., 1992; Wen et al., 1995).

**Data Analysis.** The extraction ratio of 6AC in the gut wall ($E_{gw}$) during luminal perfusion in the presence of DCF or EHNA was calculated as a function of steady-state concentration ratios (CR) of CBV to 6AC after luminal perfusion ($CR_{inf}$) and after portal infusion ($CR_{sys}$) with the analysis published previously (Wen et al., 1999):

$$E_{gw} = \frac{F_{m,sys} \left[ CR_{inf} - 1 \right]}{1 + F_{m,sys} \left[ CR_{inf} - 1 \right]}$$

where $F_{m,sys}$ is the fraction of 6AC metabolized to CBV systemically. This analysis assumes that because the hepatic extraction ratio of 6AC was so low (Wen et al., 1999) the liver can be included in the “systemic” compartment, and that portal administration will allow an estimate of systemic pharmacokinetic parameters. A determination of $E_{gw}$ during intestinal perfusion requires that 6AC be administered by portal infusion in a separate set of animals to determine the underlying systemic pharmacokinetic parameters.

As reported previously (Wen et al., 1999), $F_{m,sys}$ was calculated with the use of the intraportal clearance of CBV ($CL_{sys,inf}$), the steady-state concentration of CBV during the portal infusion ($C_{sys,inf}$), and the portal infusion rate of 6AC ($R_{inf}$):

$$F_{m,sys} = CL_{sys,inf}CBV/R_{inf}6AC$$

Because 70% of CBV is excreted unchanged in urine (Huang et al., 1991), it was assumed that $CL_{sys}$ CBV would not be affected by the deaminase inhibitors. The systemic clearance of 6AC after intraportal infusions ($CL_{6AC,sys}$) was given by:

$$CL_{sys}6AC = R_{inf}6AC/C_{sys,inf}6AC$$

**Fig. 1. Chemical structures of CBV, 6AC, and two inhibitors of ADA.**
where $C_{\text{sys,inf}}^{6\text{AC}}$ is the steady-state concentration of 6AC during the portal infusion. The average values of $F_{m,\text{sys}}$ and $CR_{\text{inf}}$ were then used to calculate the $E_{\text{gw}}$ (eq. 1) for the individual rats receiving the lumenal perfusion.

For the lumenal perfusions, the steady-state rate of disappearance of 6AC ($R_{\text{diss}}^{6\text{AC}}$) from the perfusate was calculated as the difference between the rate entering the intestine segment ($C_{\text{in}}^{6\text{AC}} \times Q$) and the rate exiting the segment ($C_{\text{out}}^{6\text{AC}} \times Q$):

$$R_{\text{diss}}^{6\text{AC}} = Q(C_{\text{in}}^{6\text{AC}} - C_{\text{out}}^{6\text{AC}})$$  (4)

where $Q$ was the perfusion flow rate (milliliters per minute).

The steady-state rate of formation of CBV ($R_{\text{form}}^{\text{CBV}}$) in the lumen was obtained from the steady-state effluent concentration of CBV ($C_{\text{out}}^{\text{CBV}}$) and the flow rate ($Q$):

$$R_{\text{form}}^{\text{CBV}} = QC_{\text{out}}^{\text{CBV}}$$  (5)

The difference between the rate of disappearance of 6AC and the rate of formation of CBV in the lumen was the rate of absorption of 6AC ($R_{\text{abs}}^{6\text{AC}}$), assuming that 6AC was converted only to CBV and that there was no sequential metabolism or subsequent absorption of the CBV generated in the lumen (Soria, 1992; Wen, 1995):

$$R_{\text{abs}}^{6\text{AC}} = R_{\text{diss}}^{6\text{AC}} - R_{\text{form}}^{\text{CBV}}$$  (6)

Additionally, because of its poor permeability across the enterocyte membrane (Soria, 1992), any CBV formed in the gut wall was assumed to be unable to cross into the lumen. The fraction of 6AC absorbed intact ($F_{\text{abs}}^{6\text{AC}}$) and the fraction of 6AC metabolized to CBV ($F_{m,\text{lumen}}$) in the intestinal lumen were calculated as follows:

$$F_{\text{abs}}^{6\text{AC}} = \frac{R_{\text{abs}}^{6\text{AC}}}{Q_{pv}} = \frac{C_{\text{in}}^{6\text{AC}} - C_{\text{out}}^{6\text{AC}} - R_{\text{CBV}}^{\text{CBV}}}{C_{\text{in}}^{6\text{AC}} - R_{\text{CBV}}^{\text{CBV}}}$$  (7)

$$F_{m,\text{lumen}} = \frac{R_{\text{form}}^{\text{CBV}}}{Q_{pv}} = \frac{C_{\text{out}}^{\text{CBV}}}{C_{\text{in}}^{6\text{AC}} - R_{\text{CBV}}^{\text{CBV}}}$$  (8)

To characterize the inhibition of the gut wall metabolism, the expression for the apparent intrinsic clearance of 6AC in the gut wall ($CL_{l,\text{gw}}$) was assumed to take the Michaelis-Menten form:

$$CL_{l,\text{gw}} = \frac{V_{\text{max}}}{1 + C_{\text{i}}/K_{\text{i}}}$$  (9)

where $V_{\text{max}}$ was the maximal rate of conversion of 6AC to CBV in the gut wall, $K_{\text{m}}$ was the Michaelis-Menten constant, and $C_{6\text{AC}}$ was the concentration of 6AC at the enzyme site.

The inhibition of the gut wall metabolism by both inhibitors was assumed to be competitive in nature (Agarwal, 1982; Blick et al., 1990). In the presence of inhibitors, $CL_{l,\text{gw}}$ becomes:

$$CL_{l,\text{gw}} = \frac{V_{\text{max}}}{K_{\text{m}}(1 + C_{\text{i}}/K_{\text{i}}) + C_{6\text{AC}}}$$  (10)

Assuming that the intestinal wall is well-stirred, the extraction ratio in the gut wall ($E_{\text{gw}}$) is:

$$E_{\text{gw}} = \frac{CL_{l,\text{gw}}}{Q_{pv} + CL_{l,\text{gw}}} = \frac{V_{\text{max}}}{K_{g}(1 + C_{i}/K_{i})}$$  (11)

where $Q_{pv}$ is the portal vein blood flow in rats under anesthesia, previously determined as 7 ml/min (Soria, 1992).

At each concentration of inhibitor ($C_{i}$), $E_{\text{gw}}$ was calculated from eq. 1. Nonlinear regression was carried out with KaleidaGraph (version 3.08d; Synergy Software, Reading, PA) on the $E_{\text{gw}}$ and $C_{i}$ data to obtain the estimates of $V_{\text{max}}/K_{m}$ and $K_{i}$ from eq. 12.

**Statistical Analysis.** One-way ANOVA was carried out on each parameter with the inhibitor dose as the main effect. Post hoc analysis was carried out with the use of Fisher’s post hoc least significant difference, and a $P < .05$ was
The effects of ADA inhibition were evaluated both at the intestinal level and the systemic level. The fraction of 6AC metabolized to CBV in the intestinal lumen ($F_{m,\text{lumen}}$) was drastically reduced in the presence of even the lowest dose of EHNA (Fig. 3). Similarly, $F_{m,\text{lumen}}$ was significantly decreased in the presence of DCF. EHNA appears to be a more potent inhibitor than DCF, because the same inhibitory effect on $F_{m,\text{lumen}}$ was achieved with a much lower dose of EHNA.

The absorption of intact 6AC ($F_{\text{abs}}$) was not significantly different in the presence of EHNA. However, significantly more 6AC was absorbed intact from the intestinal lumen in the presence of DCF. The extent of absorption was not significantly different among the three inhibitor doses used (Fig. 3).

The extraction ratios of 6AC in the gut wall ($E_{gw}$) significantly decreased as the inhibitor dose increased. The high dose of EHNA completely abolished the activity of gut wall metabolism (Fig. 4).

The effects of gut wall inhibition on the blood levels of 6AC and CBV are illustrated in Fig. 5. Data are reported as CBV/6AC concentration ratio as a function of inhibitor concentrations for intraportal infusions and intestinal lumen perfusions. As a general observation, the concentration ratios are higher after intestinal lumen perfusions than those after intraportal infusions, indicating significant intestinal metabolism to CBV. Both the ratios after infusions and those after perfusions decrease with increasing doses of inhibitors. However, the effect of inhibition appears to be much more significant for the luminal perfusions. The ratios after perfusions approach the ratios after infusions, suggesting that the activity of gut wall metabolism significantly decreased as the inhibitor dose escalated. Because there is less effect of inhibitors on the concentration ratios after intraportal infusions than after the intestinal lumen perfusions, the systemic pharmacokinetics appeared to be altered less than the gut wall metabolism.

A moderate effect of the inhibitors on the fraction of 6AC metabolized to CBV systemically was observed (Table 2). Only the highest doses of either EHNA or DCF had a statistically significant effect on $F_{m,\text{sys}}$. The inhibitors had no effect on the systemic clearance of 6AC (Table 2).

The concentration of 6AC in blood after intraportal infusions did not change with the inhibitor treatment. The concentration of 6AC after intestinal lumen perfusions appeared to gradually increase with the escalating doses of inhibitors, (Fig. 6), but only the highest doses of EHNA and DCF resulted in a statistically significant increase in the steady-state concentration of 6AC. With 0.05 M EHNA, the Css of 6AC increased almost 3-fold over the control perfusion.

**Discussion**

CBV represents a prototypical carbocyclic nucleoside, and an analog, abacavir, has recently been approved for the treatment of HIV. In this study, the conversion of 6AC to CBV in the intestinal lumen and gut wall was substantially reduced as a function of ADA inhibitor dose. At the highest doses of inhibitors, the steady-state ratio of systemic CBV to 6AC during luminal perfusions approached the ratio observed during intraportal infusions. The results supported the notion that inhibitors of ADA administered orally would be effective in protecting 6AC from first-pass metabolism to CBV.

Although the ADA in the intestine appeared to be effectively inhibited, a more important question was whether this effect was specifically localized to the first-pass organs. Because one of the objectives of ADA inhibition is to increase the driving force for brain uptake (that is, to increase the systemic levels of 6AC), the ability of the brain to convert 6AC to CBV must be retained. It seems clear that oral administration of an ADA inhibitor is the preferred route. First of all, in the rat and in humans, ADA is present in the greatest abundance in the intestine, second only to the spleen (Ho et al., 1980). In mice, ADA levels are significantly more abundant in the gastrointestinal tract than in other organs (Ho et al., 1980; Chinsky et al., 1990). The distribution within the intestine appears to be lumenally oriented (Chinsky et al., 1990) and in greater abundance toward the tips of the villi (Holt et al., 1985). Furthermore, the gut wall has been shown to be the dominant site among the first-pass organs for the conversion of 6AC (Wen et al., 1999). An additional advantage is that the orally
administered inhibitors may themselves be removed by first-pass metabolism. For example, EHNA is rapidly metabolized in the liver (Agarwal, 1982). Studies by others have indicated that parenteral administration of EHNA or DCF is accompanied by prolonged inhibition of brain ADA (Mendelson et al., 1983; Padua et al., 1992), which would be disadvantageous in this case.

The apparent $K_i$ values for EHNA and DCF were 1.2 and 26 $\mu$M, respectively. These values should be interpreted with caution. The $K_i$ value for EHNA for human erythrocytic ADA has been reported to be as low as 1.6 nM. The $K_i$ values for DCF inhibition of ADA from mouse jejunum, human erythrocytes, and semipurified from mouse brain were in the range of 0.0025 to 0.015 nM (Agarwal, 1982). The inhibition of ADA by DCF in intact cells requires 100- to 1000-fold of the concentration needed for inhibition of partially purified ADA or ADA in a cell-free system (Agarwal, 1982). In this study, the $K_i$ value was estimated on the basis of the inhibitor concentration in the intestinal lumen. It would not be surprising if the concentration of the inhibitor in the lumen and the concentration of the inhibitor at the enzyme site were quite different.

Nonetheless, the apparent $K_i$ values determined in this experiment allow some insight into the relative potency of the two inhibitors in vivo. Based on in vitro studies, DCF was at least 100-fold more potent as an ADA inhibitor than EHNA (Agarwal, 1982). However, in this study, EHNA was a more effective in vivo ADA inhibitor, with a $K_i$ value that was 1/20 of DCF. It may be speculated that EHNA is more readily absorbed from the lumen into the enterocyte due to its
higher lipophilicity. In addition, EHNA may be more chemically stable at pH ranges where DCF is degraded (Al-Razzak et al., 1990). The in vivo $K_i$ values represent both the intrinsic inhibitory potency and the ability of the inhibitor to reach the enzyme.

The inhibition of gut wall ADA by both inhibitors substantially reduced the gut wall extraction ratio ($E_{gw}$) at even the lowest doses of each inhibitor. EHNA was able to completely abolish the extraction of 6AC at its highest lumenal dose. The expression for $E_{gw}$ (eq. 12) assumed that the intestine was well stirred. In a previous article it was shown that there may be a diffusional barrier to the conversion of 6AC to CBV by the gut wall. However, that situation occurred when 6AC was presented to the gut wall from the mucosal side, and were immediately entering the metabolically active “compartment” of the intestine.

Although the inhibitors clearly had an effect on the intestinal conversion of 6AC, their effect on the systemic pharmacokinetics of 6AC was much less marked. Neither EHNA nor DCF affected the systemic clearance of 6AC, as indicated by the lack of effect on the steady-state level of 6AC from intraportal infusions. Under control conditions, the systemic clearance of 6AC is approximately 72% nonrenal (primarily through conversion to CBV) and 28% renal (Zimmerman et al., 1992). Given that EHNA is also subject to high

![Fig. 5. Effects of ADA inhibitors, EHNA (top) and DCF (bottom), on the concentration ratios, $C_{CBV}/C_{6AC}$, after intestinal lumen perfusions (filled columns) and portal vein infusions (hatched columns).](image)

*significantly different from control, $P < .05$; **significantly different from control and from 0.005 mM EHNA, $P < .05$; + significantly different from control and from 0.02 mM DCF, $P < .05$; ***significantly different from all other groups, $P < .05$.
first-pass metabolism, it is not surprising that little effect on 6AC systemic clearance was observed. The fraction of 6AC converted to CBV in the systemic circulation \( F_{m,sys} \) was affected only by the highest dose of EHNA or DCF. It is interesting that at the high inhibitor doses a decrease in \( F_{m,sys} \) did not lead to a decrease in the 6AC systemic clearance. It may be that these inhibitor doses are decreasing the systemic clearance of CBV, leading to an underestimation of \( F_{m,sys} \) by eq. 2. This, in turn, would lead to an underestimation of \( E_{gw} \) at the high inhibitor doses. However, this underestimation would not significantly affect the conclusions of this study.

With the systemic clearance unchanged, and an increased fraction of 6AC absorbed intact (\( F_{abs} \)), one would expect a higher steady-state level of 6AC after lumenal perfusion of 6AC with the inhibitors. Indeed, this was the case after administration of both EHNA and DCF, with statistical significance reached at the highest dose of inhibitors.

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<th>TABLE 2</th>
<th>Influence of ADA inhibitors on systemic pharmacokinetics of 6AC</th>
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<tr>
<td></td>
<td>( F_{m,sys} )</td>
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<tr>
<td></td>
<td>ml/min/kg</td>
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<tr>
<td>EHNA</td>
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<tr>
<td>0 mM (control)</td>
<td>0.457 ± 0.071</td>
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<td>0.005 mM</td>
<td>0.410 ± 0.053</td>
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<td>0.015 mM</td>
<td>0.230 ± 0.026</td>
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<tr>
<td>0.05 mM</td>
<td>0.100 ± 0.010(^a)</td>
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<tr>
<td>DCF</td>
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<tr>
<td>0 mM (control)</td>
<td>0.457 ± 0.071</td>
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<td>0.02 mM</td>
<td>0.410 ± 0.089</td>
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<tr>
<td>0.1 mM</td>
<td>0.306 ± 0.076(^b)</td>
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<td>0.5 mM</td>
<td>0.21 ± 0.035(^c)</td>
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\(^a\) Significantly different from control and 0.005 mM EHNA (ANOVA, \( P < .05 \)).

\(^b\) Significantly different from control (ANOVA, \( P < .05 \)).

\(^c\) Significantly different from 0.02 mM DCF (ANOVA, \( P < .05 \)).

**FIG. 6.** Steady-state concentrations of 6AC after intraportal infusions (hatched columns) and intestinal lumen perfusions (filled columns) in the presence of EHNA (top) and DCF (bottom).

Statistical comparisons were done within the perfusion or infusion group. *different from control, \( P < .05 \); ** different from control and different from 0.1 mM DCF, \( P < .05 \).
One would expect that the higher systemic concentrations of 6AC would in turn lead to greater exposure of the central nervous system to CBV. Previous studies have demonstrated that an infusion of 6AC achieves higher brain concentrations of CBV than does an equimolar infusion of CBV (Wen et al., 1995). Consequently, coadministration of ADA inhibitors orally could be expected to increase the brain exposure to CBV.

Conclusions

Both EHNA and DCF proved to be very effective inhibitors of ADA in vivo, although EHNA was found to be more potent. Substantial suppression of the first-pass conversion of 6AC was achieved with both inhibitors. This inhibition appeared to be relatively selective, allowing selection of an inhibitor dose that would sufficiently inhibit the first-pass metabolism while leaving the activation capacity in the systemic circulation unaltered. The systemic level of 6AC increased with the escalating dose of inhibitors, thus increasing the driving force for penetration into the brain.

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