Transport of Levovirin Prodrugs in the Human Intestinal Caco-2 Cell Line

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ABSTRACT: The transport of 10 amino acid ester prodrugs of levovirin (LVV) was investigated in the human intestinal Caco-2 cell line in order to overcome the poor oral bioavailability of LVV, an investigational drug for the treatment of hepatitis C infection. The prodrugs were designed to improve the permeability of LVV across the intestinal epithelium by targeting the di/tri-peptide carrier, PepT1. Caco-2 cell monolayers were employed to study the transport and hydrolysis properties of the prodrugs. Among all mono amino acid ester prodrugs studied, the LVV-5′-(L)-valine prodrug (R1518) exhibited the maximum increase (48-fold) in permeability with nearly complete conversion to LVV within 1 h. Di-amino acid esters did not offer significant enhancement in permeability comparing with mono amino acid esters and exhibited slower conversion to LVV in Caco2 cell monolayers. Pharmacokinetic screening studies of the prodrugs in rats yielded the highest fold increase (6.9-fold) of AUC with R1518 and in general displayed a similar trend to that observed in increases of permeability in Caco-2 cells. Mechanisms involved in the Caco-2 cell transport of R1518 were also investigated. Results of bi-directional transport studies support the involvement of carrier-mediated transport mechanisms for R1518, but not for the LVV-5′-(D)-valine prodrug or LVV. Moreover, the permeability of R1518 was found to be proton dependent. PepT1-mediated transport of R1518 was supported by results of competitive transport studies of R1518 with the PepT1 substrates enalapril, Gly-Sar, valganciclovir, and cephalexin. R1518 was also found to inhibit the permeability of valganciclovir and cephalexin. These results suggest that R1518 is a PepT1 substrate as well as an inhibitor. J Pharm Sci 95:1318–1325, 2006

Keywords: levovirin; prodrugs; hepatitis C; Caco-2 cells; transport; permeability; peptide transporters

INTRODUCTION

Levovirin (1-β-L-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a guanosine nucleoside analog and the L-enantiomer of ribavirin. Levovirin (LVV) is an investigational drug for the treatment of hepatitis C virus-mediated diseases. The combination of ribavirin and interferon is the current first line therapy for the treatment of chronic hepatitis C.[1] LVV has similar immunomodulatory potency to ribavirin *in vitro* without accumulating in red blood cells or causing hemolytic anemia, a known side effect of ribavirin.[2]

LVV exhibits poor oral bioavailability in rat and monkey (15% and 17%, respectively)[3] and in

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human clinical studies (estimated apparent oral bioavailability of ~10%).[4] It has been suggested that the low oral bioavailability of LVV is due to its limited permeability across the intestinal epithelium. Although ribavirin was reported to be actively transported by the human intestinal N1 sodium-dependent concentrative nucleoside transporters,[5] LVV is not likely to be recognized by these nucleoside transporters since it contains the nonnatural L-ribose moiety. Caco-2 cell monolayers have been found to lack the concentrative NT1 and NT2 nucleoside transporters.[6]

Prodrug strategies have been employed to optimize physicochemical properties of poorly absorbed compounds for improving drug delivery.[7–9] One successful prodrug approach for improved intestinal absorption exploits active transport systems to move the prodrug across the intestinal membrane. The promoiety portion of the molecule is designed to confer recognition by the active transport system and is cleaved after transport is complete to yield the active compound. The intestinal di/tri-peptide transporter, PepT1 has been investigated as a target for improving the bioavailability of poorly-absorbed drugs due to its broad substrate specificity.[10–12] Valine esters of acyclovir (valacyclovir) and ganciclovir (valganciclovir) have been shown to exhibit improved absorption, which is attributed to the uptake *via* a peptide transporter even though there is no peptide bond in the structures.[13–16] For example, valganciclovir exhibited a 10-fold increase in plasma ganciclovir concentrations compared to oral formulations of ganciclovir.[17] Valganciclovir was found to be recognized as a substrate by the intestinal transporter PepT1.[16]

In the present investigation, the permeability of 10 amino acid ester prodrugs of LVV (see Fig. 1) was determined in human intestinal Caco-2 cells. The transport mechanisms of the lead prodrug, R1518 were also studied. Furthermore, the inhibitory effects of known PepT1 inhibitors on the permeability of R1518 and the reverse inhibitory effect of R1518 on PepT1 substrates were investigated. Pharmacokinetic screening studies in rats were carried out for LVV and the prodrugs.

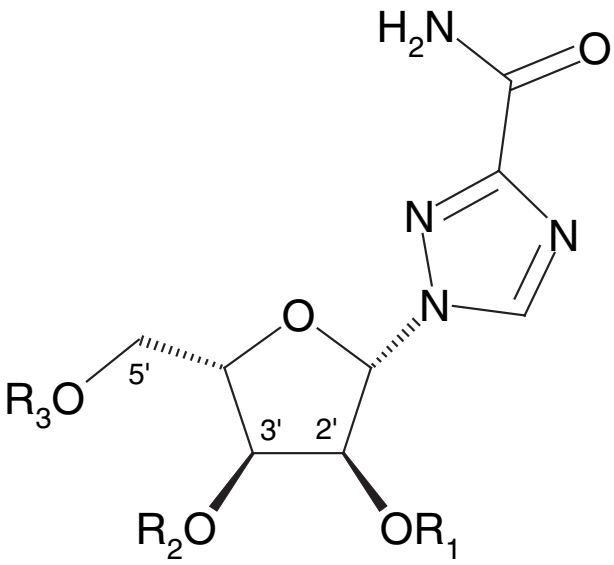

Figure 1. Levovirin (R_1, R_2, $R_3 = H$) and amino acid ester prodrugs: 5′-(L)-valinate [R_1, $R_2 = H$, $R_3 =$ (L)-Val]; 5′-(D)-valinate [R_1, $R_2 = H$, $R_3 =$ (D)-Val]; 5′-(L)-isoleucinate [R_1, $R_2 = H$, $R_3 =$ (L)-Ile]; 5′-(L)-alaninate [R_1, $R_2 = H$, $R_3 =$ (L)-Ala]; 5′-(L)-leucinate [R_1, $R_2 = H$, $R_3 =$ (L)-Leu]; 5′-(L)-sarcosinate [R_1, $R_2 = H$, $R_3 =$ (L)-Sar]; 5′-(L)-phenylalaninate [R_1, $R_2 = H$, $R_3 =$ (L)-Phe]; 2′,3′-(L)-bis-valinate [R_1, $R_2 =$ (L)-Val, $R_3 = H$]; 5′-(L)-valinylprolinate [R_1, $R_2 = H$, $R_3 =$ (L)-Val-Pro]; 5′-(L)-prolinylvalinate [R_1, $R_2 = H$, $R_3 =$ (L)-Pro-Val].

MATERIALS AND METHODS

Materials

Caco-2 cells (passage 108–120) were obtained from Roche Basel. 12-well Transwell® inserts (diameter: 12 mm) with collagen-coated polytetrafluoroethylene membrane (0.4 μm pores) and T225 cm^2 cell culture flask (tissue culture treated) were purchased from Corning Incorporated (Corning, NY). Dulbecco's Modified Eagle Media with high glucose and L-glutamine (DMEM), L-glutamine, penicillin-streptomycin, nonessential amino acids and fetal bovine serum were obtained from Gibco/Life Technologies (Gaithersburg, MD). Krebs–Henseleit bicarbonate buffer mix, calcium chloride dihydrate, enalapril, glycylsarcosine (Gly-Sar), and cephalexin were purchased from Sigma (St. Louis, MO). Sodium bicarbonate was obtained from Mallinckrodt Chemicals (Phillipsburg, NJ). Nanopure water was used for the buffer preparation.

Amino acid ester prodrugs of LVV were generally prepared *via* a three or more step synthesis from LVV. The 5′-monoester syntheses utilized either a 2′,3′-LVV cyclopentylidene or isopropylidene ketal intermediate which allowed selective esterification with the *N*-butoxycarbonyl (*N*-Boc) protected amino acids valine, isoleucine, alanine, leucine, sacrosine, and phenylalanine. The 2′, 3′-bis-valinate prodrug was synthesized using the