Factors that Restrict Intestinal Cell Permeation of Cyclic Prodrugs of an Opioid Peptide (DADLE): Part II. Role of Metabolic Enzymes in the Intestinal Mucosa

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ABSTRACT: The objective of this study was to determine the relative importance of metabolism by cytochrome P450 (CYP) enzymes versus efflux by P-glycoprotein (P-gp) in restricting the intestinal mucosal permeation of cyclic prodrugs (AOA-DADLE, CA-DADLE, OMCA-DADLE) of the opioid peptide DADLE (H-Tyr-D-Ala-Gly-Phe-D-Leu-OH). AOA-DADLE, CA-DADLE, and OMCA-DADLE were shown to be rapidly metabolized by rat liver microsomes and human CYP-3A4 and to a lesser extent by esterases. Using an *in situ* perfused rat ileum model, ketoconazole, a CYP 3A inhibitor, was shown to have no effect (AOA-DADLE) or a slight enhancing effect (OMCA-DADLE, twofold; CA-DADLE, threefold) on their intestinal mucosal permeation. In contrast, inclusion of PSC-833, a P-gp inhibitor, in the perfusate significantly enhanced (7–16-fold) the permeation of the three cyclic prodrugs. Since PSC-833 was found to be a weak inhibitor of CYP 3A4 and to have no inhibitory effects on esterases, phenol sulfotransferases, and glucuronyltransferases, it is suggested PSC-833 enhances intestinal mucosal permeation of these cyclic prodrugs by inhibiting their polarized efflux and not by inhibiting their metabolism. Furthermore, efflux transporters (e.g., P-gp), not metabolic enzymes (e.g., CYP 3A, esterases), restrict the permeation of peptide prodrugs across the rat intestinal mucosa.
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INTRODUCTION

Cyclic peptide prodrugs have been designed and synthesized in our laboratory in an attempt to improve the oral bioavailability and blood–brain barrier (BBB) permeation of DADLE (H-Tyr-D-Ala-Gly-Phe-D-Leu-OH), an analog of the opioid peptide [Leu5]- enkephalin.[1–3] The resulting cyclic prodrugs, AOA-DADLE, CA-DADLE, and OMCA-DADLE, are uncharged, lipophilic and exist in unique solution structures that yield intramolecular hydrogen bonds, which suggest that they should be more able to permeate across cell membranes by transcellular pathway.[4,5] However, *in vitro* cell culture studies (e.g., Caco-2, MDCK-MDR1 and

Abbreviations: AOA, acyloxyalkoxy; AP, apical; APMSF, (*p*-amidinophenyl) methyl sulfonyl floride; BBB, blood–brain barrier; BL, basolateral; BCRP, breast cancer resistant protein; CA, coumarinic acid; CyA, cyclosporin A; CYP, cytochrome P450; DADLE, H-Tyr-D-Ala-Gly-Phe-D-Leu-OH; MRP, multidrug resistant associated protein; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NADP, nicotinamide adenine dinucleotide phosphate sodium salt; OMCA, oxymethyl-modified coumarinic acid; PAPS, 5′-phosphoadenosine 3′-phosphosulfate; P_B, mesenteric permeability coefficient; P-gp, P-glycoprotein; PNPB, *p*-nitrophenyl butyrate.

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MDCK-MRP2 cells) have shown that the permeation of all three cyclic prodrugs of DADLE across cell monolayers is restricted by their substrate activity for apically polarized efflux transporters e.g., P-glycoprotein (P-gp) and multidrug resistant associated protein-2 (MRP2)].[4–9]

Preliminary *in vivo* pharmacokinetic studies indicated that AOA-DADLE, CA-DADLE, and OMCA-DADLE are not well absorbed after oral administration to rats (unpublished data). Recent mechanistic biopharmaceutical studies, using an *in situ* perfused rat ileum model of the intestinal mucosa, showed that PSC-833, a known inhibitor of P-gp, increased the mesenteric blood permeability coefficients (P_B) of AOA-DADLE, CA-DADLE, and OMCA-DADLE by 7.6-, 13.4-, and 15.7-fold, respectively.[10] Surprisingly, CyA and GF-12918, also known P-gp inhibitors, were either inactive or substantially less active than PSC-833 in increasing the P_B values of these cyclic prodrugs in this *in situ* perfused rat ileum model.[10] In contrast, despite the fact that PSC-833 was slightly more potent than GF-120918 and CyA in Caco-2 cells, an *in vitro* model of the intestinal mucosa, as inhibitors of the polarized efflux of these cyclic prodrugs measured by $P_{app,\ BL\text{-}to\text{-}AP}/P_{app,\ AP\text{-}to\text{-}BL}$ ratio, the difference in the inhibition of efflux transporters in Caco-2 cells were much less significant than in perfused rat ileum model.[10] Based on these observations, several hypotheses were put forward to explain the different effects that P-gp inhibitors have on the permeation of the cyclic prodrugs in the Caco-2 cell model versus their effects in the perfused rat ileum model of the intestinal mucosa. One hypothesis was that AOA-DADLE, CA-DADLE, and OMCA-DADLE are substrates of metabolic enzymes [e.g., cytochrome P-450s (CYP), esterases, phenol sulfotransferases, glucuronyltransferases] that play important roles in limiting the intestinal mucosal absorption of these cyclic prodrugs and that these enzymes are differentially inhibited by PSC-833, CyA, and GF-120918.[10] This hypothesis is based in part on the following information: (i) Caco-2 cells are generally thought to express variable and often different levels of metabolic enzymes found in the intestinal mucosa;[11] and (ii) differences exist in the activity and/or substrate specificity of esterases expressed in human and rat tissues.[12] Therefore, the experiments described in this manuscript were designed specifically to prove or disprove the hypothesis stated above.

It has been well established that enzymes expressed in the intestinal mucosa can be a major factor responsible for reducing the oral bioavailability of some drugs.[13,14] CYP 3A is the most important member of the CYP enzyme family and is the most abundant CYP isozyme expressed in the small intestine[13,15] (CYP 3A1/2 are expressed in rat and CYP3A4/5 are expressed in human). Because CYP 3A and P-gp exhibit significant overlap in their substrate specificities,[16] are coinducible,[17,18] and are colocalized in small intestinal enterocytes,[19] it has been suggested that they work synergistically in reducing the oral bioavailabilities of their substrates.[16,20,21]

In addition to CYP 3A, esterases,[22] UDP-glucuronyltransferases,[14] and phenol sulfotransferases,[14] are enzymes that could potentially metabolize these cyclic prodrugs in the intestinal mucosa. For example, the ester bonds of the cyclic prodrugs are known to be susceptible to esterase-catalyzed hydrolysis.[12] The phenolic functionality on the tyrosine residue of DADLE might be susceptible to phase II conjugation metabolism by UDP-glucuronyltransferases and/or phenol sulfotransferases. Since DADLE is relatively stable to rat intestinal peptidases with a half-life over 120 min,[23] we were not concerned about peptidase metabolism in our studies.

The objective of the present study is to investigate the influence of efflux and enzyme catalyzed degradation on the apparent permeability of DADLE and its prodrugs (AOA-DADLE, CA-DADLE, and OMCA-DADLE) across rat small intestine. Strategies were designed to determine apparent permeabilities by intestinal perfusion studies in rats. Influence of efflux and enzyme catalyzed degradation on apparent permeability is investigated by respectively coadministering specific efflux- and enzyme inhibitors. To further elucidate possible influence of enzyme catalyzed degradation of the prodrugs on apparent permeability, prodrug degradation was investigated in biological media with relevant enzymes, that is, CYP3A and esterases catalyzed degradation was investigated in rat liver microsomes. The potential effects of P-gp inhibitors on UDP-glucuronyltransferase and phenol sulfotransferase were investigated in rat intestinal microsomes and rat intestinal cytosolic media.

EXPERIMENTAL

Materials

DADLE, [Leu5]-enkephalin, paraoxon, *p*-nitrophenyl butyrate (PNPB), ketoconazole, reduced