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Intestinal absorption and activation of decitabine amino acid ester prodrugs mediated by peptide transporter PEPT1 and enterocyte enzymes

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ABSTRACT

Decitabine (DAC), a potent DNA methyltransferase (DNMT) inhibitor, has a limited oral bioavailability. Its 5′-amino acid ester prodrugs could improve its oral delivery but the specific absorption mechanism is not yet fully understood. The aim of this present study was to investigate the *in vivo* absorption and activation mechanism of these prodrugs using *in situ* intestinal perfusion and pharmacokinetics studies in rats. Although PEPT1 transporter is pH dependent, there appeared to be no proton cotransport in the perfusion experiment with a preferable transport at pH 7.4 rather than pH 6.5. This suggested that the transport was mostly dependent on the dissociated state of the prodrugs and the proton gradient might play only a limited role. In pH 7.4 HEPES buffer, an increase in P_{eff} was observed for L-val-DAC, D-val-DAC, L-phe-DAC and L-trp-DAC (2.89-fold, 1.2-fold, 2.73-fold, and 1.90-fold, respectively), compared with the parent drug. When co-perfusing the prodrug with Glysar, a known substrate of PEPT1, the permeabilities of the prodrugs were significantly inhibited compared with the control. To further investigate the absorption of the prodrugs, L-val-DAC was selected and found to be concentration-dependent and saturable, suggesting a carrier-mediated process (intrinsic K_m: 7.80 ± 2.61 mM) along with passive transport. Determination of drug in intestinal homogenate after perfusion further confirmed that the metabolic activation mainly involved an intestinal first-pass effect. In a pharmacokinetic evaluation, the oral bioavailability of L-val-DAC, L-phe-DAC and L-trp-DAC were nearly 1.74-fold, 1.69-fold and 1.49-fold greater than that of DAC. The differences in membrane permeability and oral bioavailability might be due to the different stability in the intestinal lumen and the distinct PEPT1 affinity which is mainly caused by the stereochemistry, hydrophobicity and steric hindrance of the side chains. In summary, the detailed investigation of the absorption mechanism by *in vivo* intestinal perfusion and pharmacokinetic studies showed that the prodrugs of DAC exhibited excellent permeability and oral bioavailability, which might be attributed to a hybrid (partly PEPT1-mediated and partly passive) transport mode and a rapid activation process in enterocytes.

1. Introduction

Oral administration of nucleoside analogues in the treatment of viral infections and cancer is often limited by poor intestinal absorption. For example, the low oral bioavailability decitabine (DAC) is due to its poor membrane permeability. Strategies involving targeting receptors/transporters have been used to design prodrugs of these poorly permeable drugs. Researchers have attempted to improve the oral absorption mainly by forming ester or amide bonds to an amino acid promoiety for targeting the oligopeptide transporter, SLC15A1 (peptide transporter-1, PEPT1) (Sun et al., 2010b). Encouraged by the success of valacyclovir and valganciclovir (Fig. 1a), Lilly research laboratories found that oral administration of LY544344, an amino acid amide-based prodrug of a metabotropic glutamate receptor 2 agonist, exhibited up to an 8-fold increase in drug absorption in rats (Eriksson et al., 2010a). This prodrug strategy has also been applied to cytarabine, a first-line drug used for the treatment of acute myelogenous leukemia (AML). Our team has found that the absolute bioavailability of cytarabine following rat oral administration of cytarabine and 5′-valyl-cytarabine were 21.8% and 60.0%, respectively. Due to the prodrug-based significant improvement in oral availability, 5′-valyl-cytarabine has received clinical approval from the China Food and Drug

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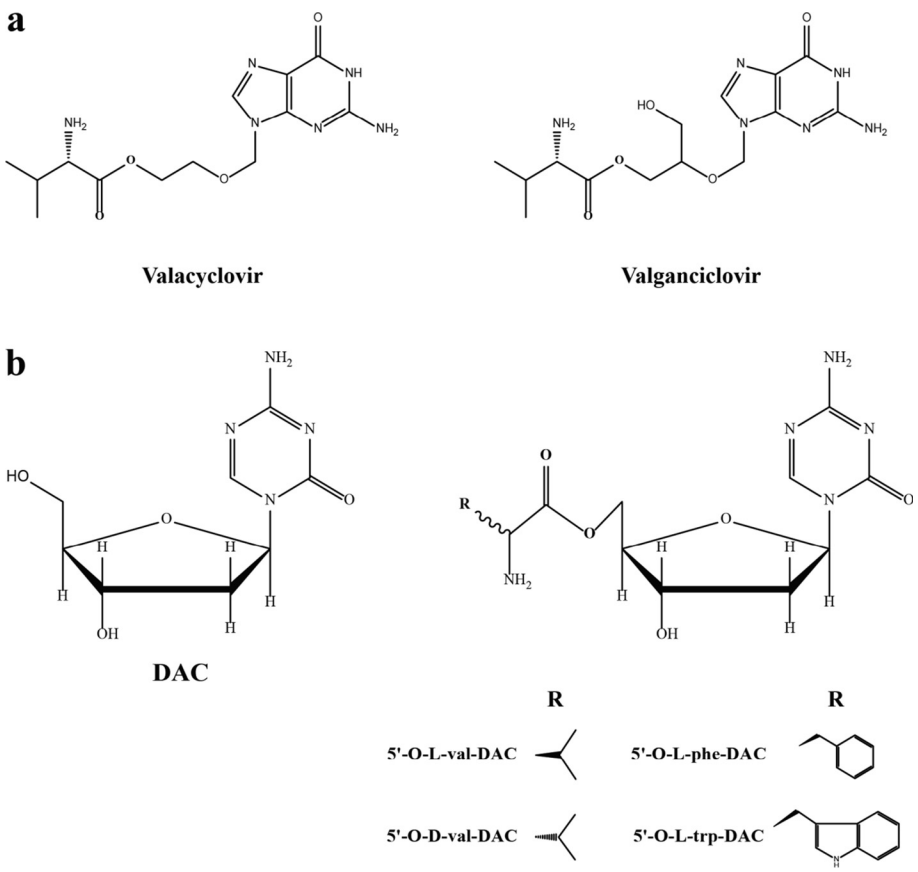

Fig. 1. The chemical structures of (a) Classic nucleotide prodrugs, valacyclovir and valganciclovir and (b) DAC and its 5′-amino acid ester prodrugs.

Administration in 2017 (Sun et al., 2009). In addition, the apparent bioavailability of ethylene glycol-linked L-val diester prodrugs of oleanolic acid (OA) for PEPT1-mediated transport was 2.21 times higher than that of OA in rats, suggesting that it is an effective PEPT1-targeted prodrug strategy for improving the oral absorption of OA (BCS class IV drug) (Cao et al., 2012).

PEPT1 transport is influenced by the configuration, charge, hydrophobicity, size, and side chain flexibility of its amino acid ester substrate (Bonella et al., 2014; Wilce et al., 1995). In addition, the PEPT1 gene sequences for rat is 82% identical to the human gene and the *in vivo* transport of PEPT1 substrates between rats and humans have demonstrated functional similarity (Herrera-Ruiz and Knipp, 2003). As reported in the current study, the highest PEPT1 protein expression and greater PEPT1 activity is found in the jejunum compared with the rest of the intestine (Jappar et al., 2010; Ogihara et al., 1996). Due to its high capacity and broad substrate specificity, PEPT1 could be particularly useful as a molecular target for nucleoside prodrug transport. In our preliminary report (Zhang et al., 2013), we designed and synthesized L-valine, D-valine, L-phenylalanine and L-tryptophan esters of a model parent compound [5-aza-2′-deoxycytidine] decitabine (DAC, Fig. 1b). Although good permeability and transmembrane passage of these amino acid ester prodrugs were confirmed across Caco-2 cell monolayers, the mode of transport and specific absorption mechanism were not fully understood. Currently, the single-pass intestinal perfusion (SPIP) technique in rats is used to improve the prediction of *in vivo* absorption in humans due to the more realistic physiological state of drug absorption and metabolism in the intestinal lumen (Lennernaäs, 1998; Ohura et al., 2012). Using the SPIP study, we explored the underlying mechanisms of drug intestinal absorption including intestinal absorption in segments, carrier-mediated transport or passive diffusion, and enterocyte metabolism (Cao et al., 2006).

The aim of this study was to characterize the intestinal absorption and activation mechanism of prodrugs by performing *in situ* jejunal perfusion and pharmacokinetics studies in rats. Due to the H^+ gradient-driven active transport of PEPT1, two pH perfusion conditions (weak acidic pH 6.5 and classic neutral pH 7.4) within the pH range of the intestine were chosen to investigate whether the transport of prodrugs was proton-dependent or not (Fagerholm, 2007; Lu et al., 2017). A transport inhibition experiment with Glysar and a concentration-dependent transport study were carried out to determine the transport mode of the prodrugs. In addition, an intestinal tissue accumulation experiment was also performed to investigate the activation pattern of the prodrugs. The bioavailability of DAC and prodrugs was determined after their oral administration to rats in the pharmacokinetics study. Finally, a molecular docking study was also carried out to explore the affinity of the prodrugs for PEPT1 transporter.

2. Materials and methods

2.1. Materials

Decitabine (98% pure) was obtained from Lianyungang JARI Pharmaceutical Co., Ltd. (Jiangsu, China). HEPES was supplied by Dalian Meilun Biotech Co., Ltd. (Liaoning, China) and MES was obtained from Nanjing SenBeiJia Biological Technology Co., Ltd. (Jiangsu, China). Glysar was purchased from Aladdin (Shanghai, China). Tetrahydrouridine (THU), the deaminase inhibitor, was obtained from Calbiochem (CA, USA). Diazepam (purity > 99.0%) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). 5′-O-L-valyl -decitabine (L-val-DAC), 5′-O-D-valyl-decitabine (D-val-DAC), 5′-O-L-phenylalanyl-decitabine (L-phe-DAC), and 5′-O-L-tryptophyl-decitabine (L-trp-DAC) (98% pure) were synthesized in Shenyang Pharmaceutical University (Shenyang, China).