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L-type amino acid transporter 1 utilizing prodrugs: How to achieve effective brain delivery and low systemic exposure of drugs

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ABSTRACT

L-type amino acid transporter 1 (LAT1) is selectively expressed in the blood-brain barrier (BBB) and brain parenchyma. This transporter can facilitate brain delivery of neuroprotective agents and additionally give opportunity to minimize systemic exposure. Here, we investigated structure-pharmacokinetics relationship of five newly synthesized LAT1-utilizing prodrugs of the cyclooxygenase inhibitor, ketoprofen, in order to identify beneficial structural features of prodrugs to achieve both targeted brain delivery and low peripheral distribution of the parent drug. Besides, we studied whether pharmacokinetics and bioconversion of LAT1-utilizing prodrugs in vivo can be predicted in early stage experiments. To achieve these goals, we compared the in vitro brain uptake mechanism of prodrugs, rate of BBB permeation of compounds using in situ perfusion technique, their systemic pharmacokinetics and release of parent drug in brain, plasma and liver of mice. The results revealed that both excellent LAT1-binding ability and transporter utilization in vitro can be achieved by conjugating the parent drug to aromatic amino acids such as phenylalanine in comparison to prodrugs with an aliphatic promoiety. The presence of an aromatic promoiety directly conjugated in meta- or para-position to ketoprofen led to LAT1-utilizing prodrugs capable of delivering the parent drug into the brain with higher unbound brain to plasma ratio and reduced liver exposure than with ketoprofen itself. In contrast, the prodrugs with aliphatic promoieties and with an additional carbon attached between the parent drug and phenylalanine aromatic ring did not enhance brain delivery of ketoprofen. Furthermore, we have devised a screening strategy to pinpoint successful candidates at an early stage of development of LAT1-utilizing prodrugs. The screening approach demonstrated that early stage experiments could not replace pharmacokinetic studies in vivo due to the lack of prediction of the intra-brain/systemic distribution of the prodrugs as well as the release of the parent drug. Overall, this study provides essential knowledge required for improvement of targeted brain delivery and reduction of systemic exposure of drugs via the LAT1-mediated prodrug approach.

1. Introduction

The development of new central nervous system (CNS) drugs remains a high-risk process with only small progress in ongoing research [1]. The major challenge is the presence of the complex and dynamic blood–brain barrier (BBB) which regulates the passage of molecules into and out of the brain [2]. Several promising strategies have been developed to overcome the obstacles to CNS drug delivery [3]. These approaches involve the direct drug delivery into the brain via different invasive methods, intranasal delivery, opening the BBB, modification of drug molecule to facilitate its BBB permeation, carrier-mediated transport, transcytosis based receptor-mediated approaches and use of nanocarriers [4]. Certainly, all these strategies have strong advantages and disadvantages limiting their use for the successful CNS treatment due to methodological or safety issues. The ideal delivery strategy has to combine both targeted transport of the drug into the brain without altering its pharmacological properties and low systemic distribution to avoid possible adverse effects. In this respect, the prodrug approach utilizing specific transporters highly expressed at the BBB is considered as a feasible way to enhance delivery of small molecules into the brain selectively [3]. The strategy is based on the transfer of an active drug via a prodrug designed as a BBB influx transporter substrate. Subsequently the prodrug will be bioconverted at the target site.

Although there are several endogenous transporters expressed at the BBB, the L-type amino acid transporter 1 (LAT1) possesses several features conferring the possibility for successful transporter-mediated prodrug delivery into the brain [5–9]. First, unlike many other tissues, LAT1 is selectively expressed on both luminal and abluminal capillary membranes of the endothelial cells of the BBB and the membrane of cells within the brain parenchyma [10,11]. This enables LAT1-utilizing

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prodrugs to cross not only the BBB but also to penetrate specific cell membranes in the brain. The importance of the brain intracellular delivery is attributable to the fact that some CNS agents, for example cyclooxygenase (COX)-inhibitors, have intracellular targets [12]. It has been shown that after crossing the BBB, a LAT1-utilizing prodrug of ketoprofen rapidly gained access to the brain cells from extracellular fluid (ECF), while ketoprofen itself remained mainly distributed in the ECF [5]. Another important benefit of the brain-targeted delivery via LAT1 is the possibility to avoid extensive systemic exposure and as a result to minimize peripheral adverse effects.

The following LAT1 substrate structural properties have been recognized in the design of LAT1-utilizing prodrugs: a negatively charged carboxylic acid group, a positively charged amino group and hydrophobic side chain [13–15]. Based on these features, it has been demonstrated that several prodrugs have an ability to bind to LAT1 and exploit this transporter for BBB permeation or/and cell uptake [5–9,16]. However, there is limited information about systemic and brain pharmacokinetics of these prodrugs including the distribution of the prodrugs and their parent drugs in the liver and their first pass metabolism [5,8,16,17].

In the present study, we designed and synthesized five LAT1-utilizing prodrugs of the COX-inhibitor, ketoprofen, based on previous reports [5,6,15]. The structure-pharmacokinetic relationship for the LAT1-utilizing prodrugs, including the brain uptake mechanism, systemic pharmacokinetics and the ability of compounds to deliver unbound ketoprofen into the brain was studied. The purpose of the study was to determine which structural properties of LAT1-utilizing prodrugs facilitate the highest brain delivery and selective release of the active parent drug in the brain and low peripheral exposure. In addition, we examined whether the data from early stage experiments (in vitro LAT1 binding, nonspecific tissue binding and in situ brain perfusion) can be used for prediction of brain delivery and pharmacokinetics of LAT1-utilizing prodrugs in vivo.

2. Methods

2.1. Synthesis of prodrugs

All reactions were performed with reagents obtained from Sigma-Aldrich (St. Louis, MO, USA), Acros Organics (Waltham, MA, USA) or Merck (Darmstadt, Germany). Reactions were monitored by thin-layer chromatography using aluminium sheets coated with silica gel 60 F_{245} (0.24 mm) with suitable visualization. Purifications by flash chromatography were performed on silica gel 60 (0.063–0.200 mm mesh). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 spectrometer (Bruker Biospin, Fällanden, Switzerland) operating at 500.13 MHz and 125.75, respectively, using tetramethylsilane as an internal standard. pH-dependent NH-protons of the compounds were not observed. ESI-MS spectra were recorded by a Finnigan LCQ quadrupole ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA) equipped with an electrospray ionization source. Over 95% purities were obtained for the final products by elemental analysis (C, H, N) with a Perkin Elmer 2400 Series II CHNS/O organic elemental analyzer (Perkin Elmer Inc., Waltham, MA, USA) and by HPLC-UV method (Agilent Zorbax SB-C18 analytical column (4.6 mm × 150 mm, 5 μm) eluting with acetonitrile and 0.1% formic acid buffer (pH ca. 3.0) with a ratio of 55:45 (v/v) at flow rate 1.0 mL/min).

(2*R*,*S*)-2-Amino-3-(3-(2-(3-benzoylphenyl)propanamido)phenyl) propanoic acid, **1**. Prodrug **1** was prepared according to the literature procedure [9]. Ketoprofen ((*R*,*S*)2-(3-benzoylphenyl)-propionic acid) (0.27 g, 1.07 mmol) in anhydrous CH_2Cl_2 (10 mL) was refluxed with $SOCl_2$ (100 μL, 1.43 mmol) under Ar-atm overnight. The reaction mixture was evaporated and the residue was redissolved in CH_2Cl_2 (10 mL) and reacted with *t*-Boc-3-amino-L-phenylalanine [9] (0.20 g, 0.71 mmol) in the presence of powdered NaOH (80 mg, 1.43 mmol) at RT under Ar-atm overnight. The solvent was removed and the residue was purified by flash column chromatography eluting with 1–30% MeOH/CH_2Cl_2 to yield (2*R*,*S*)-3-(3-(2-(3-benzoylphenyl)propanamido) phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid as yellowish solid, 0.31 g (84%).

(2*R*,*S*)-3-(3-(2-(3-benzoylphenyl)propanamido)phenyl)-2-((*tert*-butoxycarbonyl)amino)propanoic acid (0.31 g, 0.60 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) and reacted with trifluoroacetic acid (1.38 mL, 17.98 mmol) by stirring the reaction mixture at RT overnight. The solvents were removed and the residue was redissolved in THF and stirred with 1 M HCl (0.50 mL) at RT for 30 min. The mixture was evaporated and the residue was purified by flash column chromatography eluting with 1–50% MeOH/CH_2Cl_2 to yield off white solid, 0.20 g (83%). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ ppm 10.16 (s, 1H), 7.82 (s, 1H), 7.76–7.67 (m, 4H), 7.60 (d, J = 7.6 Hz, 1H), 7.58–7.52 (m, 3H), 7.50–7.46 (m, 2H), 7.21 (t, J = 7.8 Hz, 1H), 6.94 (d, J = 7.4 Hz, 1H), 3.97 (q, J = 7.0 Hz, 1H), 3.68–3.60 (m, 1H), 3.11–3.05 (m, 1H), 2.90–2.83 (m, 1H), 1.45 (d, J = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ ppm 195.66, 171.77, 169.66, 142.33, 139.98, 138.71, 136.94, 136.93, 132.72, 131.62, 129.61 (2C), 128.74, 128.66, 128.58 (2C), 128.50, 128.35, 124.28, 120.09, 117.73, 56.00, 45.65, 36.66, 18.54. MS (ESI^+) for $C_{25}H_{25}N_2O_4$ $(M + H)^+$: Calcd 417.48, Found 417.12. Anal. Calcd for ($C_{25}H_{24}N_2O_4 * 0.80CH_2Cl_2$): C, 63.97; H, 5.16; N, 5.78; Found: C, 63.88; H, 5.59; N, 5.69. HPLC-UV purity: 97.16%.

(2*R*,*S*)-2-Amino-3-(4-(2-(3-benzoylphenyl)propanamido)phenyl) propanoic acid, **2**. Prodrug **2** was prepared as above according to the literature procedure [9] to yield off white solid, 0.11 g (55% over 2 steps). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ ppm 10.12 (s, 1H), 7.81 (s, 1H), 7.75–7.67 (m, 4H), 7.60 (d, J = 7.6 Hz, 1H), 7.58–7.51 (m, 3H), 7.48 (d, J = 8.2 Hz, 2H), 7.15 (d, J = 8.2 Hz, 2H), 3.95 (q, J = 7.0 Hz, 1H), 3.43–3.36 (m, 1H), 3.10–3.04 (m, 1H), 2.84–2.76 (m, 1H), 1.44 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ ppm 195.65, 171.64, 169.51, 142.42, 142.39, 137.61, 136.93, 133.80, 132.71, 131.57, 129.59 (2C), 129.57, 129.53 (2C), 128.65, 128.57 (2C), 128.43, 119.17 (2C), 54.52, 45.65, 37.10, 18.52. MS (ESI^+) for $C_{25}H_{25}N_2O_4$ $(M + H)^+$: Calcd 417.48, Found 417.13. Anal. Calcd for ($C_{25}H_{24}N_2O_4 * 0.80CH_2Cl_2$): C, 63.97; H, 5.16; N, 5.78; Found: C, 63.88; H, 5.59; N, 5.69. HPLC-UV purity: 97.13%.

(2*R*,*S*)-2-Amino-3-(3-((2-(3-benzoylphenyl)propanamido)methyl) phenyl)propanoic acid, **3**. Prodrug **3** was prepared as above according to the literature procedure [9] to yield off white solid, 72 mg (23% over 2 steps). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ ppm 8.56–8.51 (m, 1H), 7.77–7.71 (m, 3H), 7.70–7.64 (m, 2H), 7.61–7.49 (m, 4H), 7.17 (t, J = 7.4 Hz, 1H), 7.12 (d, J = 7.5 Hz, 1H), 7.09 (s, 1H), 6.99 (d, J = 7.3 Hz, 1H), 4.28–4.17 (m, 2H), 3.81 (q, J = 7.0 Hz, 1H), 3.43–3.37 (m, 1H), 3.14–3.07 (m, 1H), 2.83–2.75 (m, 1H), 1.40 (d, J = 7.1 Hz, 3H). ^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ ppm 195.70, 172.85, 169.16, 142.67, 140.00, 138.61, 137.90, 136.36, 135.90, 131.60, 129.01, 128.53, 128.46, 128.08 (2C), 127.90 (2C), 126.86, 126.66, 125.24, 123.69, 65.61, 43.59, 41.67 39.05, 18.47. MS (ESI^+) for $C_{26}H_{27}N_2O_4$ $(M + H)^+$: Calcd 431.51, Found 431.30. Anal. Calcd for ($C_{26}H_{26}N_2O_4 * 0.40CH_2Cl_2 * 0.20MeOH$): C, 67.85; H, 5.82; N, 5.95; Found: C, 67.76; H, 6.22; N, 5.63. HPLC-UV purity: 98.32%.

N^6-((*R*,*S*)-2-(3-Benzoylphenyl)propanoyl)-L-lysine, **4**. Prodrug **4** was prepared as above according to the literature procedure [9] to yield off white solid, 330 mg (50% over 2 steps). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ ppm 8.22–8.17 (m, 1H), 7.76–7.71 (m, 3H), 7.69 (t, J = 7.5 Hz, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.60–7.54 (m, 3H), 7.49 (t, J = 7.7 Hz, 1H), 3.71 (q, J = 6.8 Hz, 1H), 3.66–3.60 (m, 1H), 3.09–2.91 (m, 2H), 1.80–1.64 (m, 2H), 1.34 (d, J = 6.7 Hz, 3H), 1.41–1.20 (m, 4H)·^{13}C NMR (125 MHz, $(CD_3)_2SO$): δ ppm 195.73, 172.73, 170.78, 142.81, 137.05, 136.81, 132.67, 131.64, 129.58 (2C), 128.56 (2C), 128.48, 128.38, 128.04, 56.00, 44.82, 38.29, 29.84, 28.52, 21.93, 18.54. MS (ESI^+) for $C_{22}H_{27}N_2O_4$ $(M + H)^+$: Calcd 383.46, Found 383.17. Anal. Calcd for ($C_{22}H_{26}N_2O_4 * 0.65H_2O * 0.65MeOH$): C, 56.78; H, 6.29; N, 5.85; Found: C, 57.21; H, 6.71; N, 5.40. HPLC-UV purity: 96.13%.