LAT1-mediated prodrug uptake: a way to breach the blood–brain barrier?

"*Designing high-affinity prodrugs capable of replacing endogenous substrates for LAT1 at the blood–brain barrier can indeed result in an improved prodrug delivery to the brain.*"

Keywords: blood–brain barrier ■ carrier-mediated drug delivery ■ CNS ■ LAT1 ■ prodrug

The blood–brain barrier (BBB) is a continuous layer of endothelial cells that form a thin but very effective barrier preventing the delivery of potential neurotherapeutics from blood into brain parenchyma. It has been estimated that more than 98% of small-molecule drugs and nearly all novel protein and peptide pharmaceuticals developed for CNS disorders do not reach therapeutic concentrations in the brain, mainly because of the BBB [1]. The high-resistance tight junction is one contributor to the barrier function of the BBB; this effectively seals off the aqueous paracellular channels between brain endothelial cells, forcing most drugs and solutes to cross the BBB through transcellular passive diffusion. Therefore, if a drug is to readily enter into the brain from the circulation via passive diffusion, it should have the appropriate drug-like properties, in terms of lipophilicity, ionic charge, hydrogen bonding potential and molecular size [2]. On the other hand, a drug may be transported across the BBB by one of the 20 or more active or facilitated carrier systems. These carriers are expressed at high levels in brain endothelial cells ensuring the supply of essential water-soluble nutrients [3]. In fact, there is increasing evidence that the role of transporters in drug distribution, including brain penetration, has been substantially underestimated [4].

LAT1

LAT1 or SLC7A5 is a major nutrient transporter protein that is responsible for the transport of large neutral, aromatic or branched amino acids from extracellular fluids into the cells. These include many essential amino acids, such as phenyl-alanine, leucine, isoleucine, valine, tryptophan, histidine and methionine [5]. LAT1 was first cloned in 1998, and it requires another cell surface glycoprotein, 4F2hc, with which it forms a functional heterodimeric transporter complex, to allow it to become expressed on the cell surface [5,6]. There are other members of the L-system transporter family, for example, LAT2, LAT3 and LAT4; of these, LAT2 displays 50% amino acid sequence homology with LAT1 [7]. LAT2 displays a broader substrate range, but generally has lower affinities for individual amino acids than LAT1 [8]. Human LAT1 transports large neutral amino acids at high affinity, but can also bind to glutamine and asparagine with lower affinity [9]. LAT1 is expressed on both the luminal and abluminal membranes of the capillary endothelial cells, and it transports its substrates in a sodium-independent and stereospecific manner favoring L-forms. LAT1 transporters are most abundant in the brain, tumors and placenta, and their tissue localization indicates that LAT1 is essential for the supply of amino acids into growing cells and for the transport of these essential amino acids across some epithelial/endothelial barriers.

Prodrug strategies for improved brain drug delivery of small molecules

An intriguing medicinal chemistry-based strategy to improve the brain uptake of small-molecule drugs is to create prodrugs that require biotransformation, either enzymatic or chemical, prior to their therapeutic activity [10,11]. Since lipophilicity is a factor favoring good BBB penetration, most of the early prodrug examples focused on modifying a drug to make it more lipophilic by masking its polar and/or ionizable groups. This approach has been encouraged by the successful examples of methylated and diacetylated forms of morphine, codeine and heroin [12]. Since both prodrugs are more lipophilic than morphine, they cross the BBB quickly; approximately ten-times faster in the case of codeine and 100-times faster for heroin. Some of the other lipophilic CNS prodrugs having modest success

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are those that readily enter the brain and are rapidly converted within brain tissue to more hydrophilic, often charged, intermediates [13,14]. Depending on the rate of regeneration of active drug from the intermediate that has become trapped in the brain, it is possible to achieve sustained pharmacological activity.

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Although improved lipophilicity may result in enhanced BBB penetration, it can also be associated with problems including rapid metabolic clearance, enhanced binding to plasma and tissue proteins, elevated volume of distribution, and increased affinity for efflux transporters. Therefore, improving the lipophilicity may not achieve any increase in the drug distribution into the brain. In some cases, it may actually lead to substantial reductions in total brain concentrations, especially in the unbound brain concentration. Therefore, the remainder of this article will focus on newer prodrug strategies with the aim of improving CNS drug delivery utilizing carrier-mediated systems at the BBB.

Carrier-mediated brain drug delivery via LAT1

Recent CNS prodrug strategies have attempted to exploit the endogenous influx transporters present at the BBB. Over 20 transporters have been identified in the cerebral capillaries of the BBB. These include transporters for glucose, amino acids, choline, vitamins, low density lipoproteins and nucleosides [1].

The LAT1 displays several properties that make it well suited to serve as a drug carrier into the brain. First, LAT1 has both a large maximal transport capacity (V_{max} ≈ 40–60 nmol/min/g) and appreciable binding affinity (K_m ≈ 10–200 μM) [15] resulting in rapid rates of BBB exchange (K_{in} > 10–3 ml/s/g) with half-times (half of a time required to reach equilibrium of drug concentration between the brain and systemic circulation) of less than 15 min for high-affinity substrates. Importantly, it is possible to design prodrugs with even higher affinities for LAT1 than the endogenous substrates [16]. Second, the structural requirements for substrate binding to LAT1 are not very stringent, and therefore, it accepts a wide variety of amino acids and their structural analogs as substrates [17,18]. Similar to the natural amino acids L-phenyl-alanine and L-leucine, a potential substrate of LAT1 should have both a positively charged amino group and a negatively charged carboxyl group, as well as an aromatic function next to a hydrogen bond acceptor that is preferred over a hydrophobic moiety in the center core of LAT1 substrates [18]. Lastly, a transient disruption in the brain supply of essential amino acids will not evoke any irreversible brain damage. This is a potential risk that may limit the use of GLUT1 as a drug/prodrug carrier, due to the critical need for a continuous supply of D-glucose for energy metabolism within the brain.

The most well-known prodrug that enters the brain predominantly via LAT1-mediated transport is L-DOPA. The neurotransmitter dopamine exhibits negligible brain uptake because of its hydrophilic nature and its susceptibility to enzymatic breakdown in the epithelial cells. The α-amino acid precursor of dopamine, L-DOPA, is ferried across the BBB by LAT1 [19] and is subsequently decarboxylated into dopamine in the brain tissue by aromatic amino acid decarboxylase, and therefore, enabling successful drug therapy.

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Another example is L-melphalan, the para-nitrogen mustard analog of L-phenylalanine. While L-phenylalanine is an excellent substrate for LAT1 (K_m = 11 ± 1 μM and V_{max} = 41 ± 2 nmol/min/g) [15], melphalan possesses tenfold lower affinity and eightfold lower V_{max} than L-phenylalanine, resulting in a BBB permeability that is only 1% of that of L-phenylalanine [20]. Instead, a tetralin analog of melphalan, DL-2-NAM-7, has 50-fold greater affinity compared with that of melphalan and consequently a BBB permeability perhaps only two–three-fold lower than that of L-phenyl-alanine [16]. Both melphalan and DL-2-NAM-7 are prodrugs converted sequentially into azridinium and carbonium ions from the nitrogen mustard and these ions can alkylate nucleic acids and proteins.

These earlier cases have proved conclusively the efficacy of LAT1 to transport

drugs/prodrugs across the BBB for the treatment of CNS disorders. In these cases, converting drugs into LAT1 substrate prodrugs required a structural modification where no clear cleavable promoiety was introduced. Another way to utilize LAT1 is to conjugate a small-molecule drug with a LAT1 substrate, which is typically an actual or an analog of an amino acid. The amino acid L-tyrosine is a LAT1 substrate (K_m = 64 µM and V_{max} = 96 nmol/min/g) [15] that has a phenolic hydroxyl group amenable to conjugation with various structurally different drugs providing a biodegradable linkage. In our studies, the L-tyrosine prodrug of ketoprofen demonstrated significant reversible inhibition of brain uptake of the radiotracer [^{14}C] L-leucine in an *in situ* rat brain perfusion model [21]. More importantly, the prodrug exhibited both concentration-dependent (K_m = 22 ± 9 µM and V_{max} = 1.4 ± 0.15 nmol/min/g) and saturable brain uptake, which was significantly decreased by the nonspecific LAT1 inhibitor, BCH.

In further studies, brain uptake of the amino acid prodrugs of other hydrophilic drugs, dopamine [22] and valproic acid [23], was found to be concentration-dependent in rats, and both BCH and cold conditions (+4°C perfusate) significantly decreased their brain uptake, thus demonstrating that the brain transport was carrier mediated [21–24]. These studies also demonstrated that amino acids or amino acid analogs that are not substrates for LAT1 as such can function as LAT1-targeted promoieties for a certain type of parent drugs. For example, a lysine prodrug was shown to utilize LAT1, having an affinity of 231.6 ± 60.4 µM and V_{max} of 1.50 ± 0.20 pmol/mg/min, although it is known that lysine itself is not a LAT1 substrate [24]. The latest studies also indicated that regiospecific positioning on the bond between the parent drug and promoiety is very important for optimal affinity for LAT1 [23].

Future perspective

The endogenous transport systems at the BBB offer an attractive approach for ferrying drugs into the brain in a carrier-mediated manner. At present, only a fraction of the BBB transporters are well characterized, and research is needed to accelerate the discovery of novel carrier mediated transport systems at the BBB. This can be assisted by the application of BBB genomics or BBB proteomics technologies, which will create new opportunities for the future discovery of neurotherapeutics.

With respect to the currently known transporters, at least carriers for the large amino acids (i.e., LAT1) and glucose (i.e., GLUT1) have a sufficiently high transport capacity to hold promise for significant drug delivery into brain. However, because the GLUT1 demands both a relatively strict substrate size and structural requirements and, because, disruption of glucose brain uptake may cause brain damage, it may be difficult to achieve clinical success with this approach. On the other hand, LAT1 accepts a wide variety of compounds, not only amino acids but also their analogs as substrates, and a transient disruption in the supply of essential amino acids to the brain does not cause any irreversible brain damage. These properties make it an attractive target for drugs aimed at improving brain delivery. The potential utilization of LAT1 may be hampered by competition for LAT binding by high levels of endogenous amino acids, and by the relatively low affinities of drugs for the LAT proteins. However, endogenous substrates can be replaced by high-affinity compounds, such as prodrugs, as has been demonstrated in several experimental studies.

> *"The exploitation of yet unknown brain-specific metabolic enzymes may provide a suitable means for achieving site-selective prodrug bioconversion in the future."*

In order to enable brain uptake via carrier-mediated transport systems, drugs need to be engineered to resemble endogenous substrates but still maintain their key functionalities that are responsible for therapeutic effects. While this may prove very challenging, an alternative approach is to conjugate drugs with substrates in a bioreversible manner to form prodrugs capable of carrier-mediated brain uptake.

This article is finally tackling the question presented in the title: whether the amino acid transporter can be one way to breach the BBB. Designing high-affinity prodrugs capable of replacing endogenous substrates for LAT1 at the BBB can indeed result in an improved prodrug delivery to the brain. However, because the prodrug strategy relies on bioconversion to release active drug molecules, premature prodrug metabolism during passage through the endothelial cell or while the drug is still

in the systemic circulation can, in fact, compromise the usage of prodrugs. Therefore, this prodrug approach may be best suited for intravenous administration where presystemic metabolism is bypassed and utilized primarily for drugs with negligible brain penetration properties. The exploitation of yet unknown brain-specific metabolic enzymes may provide a suitable means for achieving site-selective prodrug bioconversion in the future.

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