Scientific Meeting
AuPS | ASB
HOBART · 2022

Abstract: 169P

What makes a good B^0AT1 (SLC6A19) inhibitor?

Yashan Jiang [1], Aditya Yadav [1], Junyang Xu [2], Malcolm D. McLeod [2], Stefan Bröer [1]

[1]Research School of Biology and [2]Research School of Chemistry, Australian National University, Canberra, ACT

An imbalance of amino acid homeostasis occurs in several diseases. Elevated plasma levels of branched-chain amino acids, for instance, are a strong predictor of future Type II diabetes (T2D). High levels of phenylalanine occur in phenylketonuria (PKU), where patients lack functional phenylalanine hydroxylase (PAH), the first enzyme involved in the breakdown of phenylalanine. Therefore, restoring amino acid homeostasis could be a potential treatment strategy for these diseases.

The apical broad range of neutral amino acid transporter B^0AT1 (SLC6A19) is expressed in enterocytes of the small intestine and kidney proximal tubule epithelial cells. B^0AT1 mediates the transport of one sodium ion together with all neutral amino acids, including phenylalanine but prefers branched-chain amino acids such as leucine and isoleucine. B^0AT1 is a heteromeric membrane transporter, requiring the co-expression of angiotensin-converting enzyme 2 (ACE2) or collectrin in the small intestine and kidney, respectively, for trafficking, surface localisation, and catalytic function. Due to its role in amino acid homeostasis, B^0AT1 is a potential target to treat T2D and PKU because blocking its transport reduces the absorption of neutral amino acids in the intestine and causes spill over of neutral amino acids into the urine. In support of this notion, B^0AT1-knockout (B^0AT1-KO) mice showed improved insulin sensitivity. Moreover, ablation of B^0AT1 normalised plasma phenylalanine concentration in mice lacking PAH and improves physiological and neurological impairment observed in PKU. These results suggested that inhibiting B^0AT1 can benefit T2D and PKU patients by normalising amino acid homeostasis.

A Chinese hamster ovary cell line stably expressing human B^0AT1 and collectrin (CHO-BC) was used to identify inhibitor candidates by high-throughput screening. The potency of these inhibitors was improved by medicinal chemistry.

To compare the compounds, the radioactive uptake assay in CHO-BC cells was improved by inhibiting endogenous transporters to isolate B^0AT1 activity. JPH203 (3 µM) was introduced to block LAT1 activity and L-γ-glutamyl-*p*-nitroanilide (3 mM) was used to block ASCT2.

Using the optimised assay, the structure-activity relationship of inhibitor analogues of initial hit E4 were analysed. Eleven analogues of lead compound E4 inhibited B^0AT1, of which seven showed improved potency and one showed $IC_{50} < 1$ µM.