Review Article:

Involvement of ACE2 in the intestinal transport of amino acids: Possible health and nutritional consequences in altered expression

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Abstract:

Background:Absorption of neutral amino acids across the intestinal cells is mediated by the broad neutral amino acid transporter B^0AT1. Intestinal expression of B^0AT1 depends on the co-existence with the membrane-attached angiotensin-converting enzyme 2 (ACE2) which binds to the B^0AT1 or the sodium-dependent imino-acid transporter 1 (SIT1) in the intestinal epithelial cells. ***Objectives:***The objective of this article is to provide an overview of the involvement of ACE2 in the transport of amino acids from the intestinal lumen and possible harmful effects on health and nutrition due to altered ACE2 expression. ***Methods:*** PubMed, Scopus, and Google Scholar were searched using the keywords ACE2 paired with intestinal and gut B^0AT1, SIT1, amino acid transport or absorption, health, and nutrition. Expression of B^0AT1 in the intestine was completely absent in ACE2 knockout mice. Lack of ACE2 and B^0AT1 expression lead to low plasma and muscle tryptophan, niacin deficiency, decreased intestinal antimicrobial peptide, and increased susceptibility to inflammatory bowel disease in animal models. ***Results:*** Expression of ACE2 in the small intestine and its association with amino acid transporters play a crucial role in the absorption of amino acids, maintenance of structural and functional gut integrity and overall health and nutrition status of an individual.

Keywords: ACE2, B^0AT1; SIT1; intestine; gut, amino acid transport; amino acid absorption; nutrition

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Introduction:

Angiotensin Converting Enzyme 2 (ACE2) is a membranous protein that possesses extracellular carboxypeptidase activity[1]. It is distributed in almost all body tissues with maximum expression at the brush border membrane of small intestinal cells[2]. In the small intestine, ACE2 mRNA expression is reported to increase with the advancement of the age of an individual[3]. ACE2 protein coexists with the neutral amino acid transporter broad neutral amino acid transporter 1 (B^0AT1) or the sodium-dependent Imino acid transporter 1 (SIT1) in the small intestine. The coexistence of these molecules is required for the proper expression of these transport proteins which is essential for the binding of amino acids to the transporters for subsequent absorption[4]. However, studies of ACE2 have mostly focused on its role in the renin-angiotensin system and not on its association or role in the B^0AT1 protein.

B^0AT1 mutation causes Hartnup disorder, a condition characterized by a high level of neutral amino acid tryptophan and subsequent aminoaciduria and, in several circumstances, pellagra-like symptoms, such as skin rash, diarrhea, and dementia. Lack of ACE2

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and the concurrent absence of B^0AT1 expression in the small intestine causes decreased tryptophan absorption and subsequent niacin deficiency, decreased intestinal antimicrobial peptide synthesis, and increased susceptibility to inflammatory bowel disease (IBD) in mice[5]. Recently, angiotensin-converting enzyme 2 (ACE2) has been identified as the cellular binding site for the novel coronavirus 2, the causative agent of COVID-19[6]. Binding and internalization of ACE2 by the coronavirus might lead to altered ACE2 expression and thus less B0AT1 and SITI stabilization and functions which might lead to various effects on the health and nutrition status of an individual.

Materials and Methods:

PubMed, Scopus, and Google Scholar were searched using the keywords ACE2 paired with intestinal and gut B^0AT1, SIT1, amino acid transport, amino acid absorption, health, and nutrition. We searched the related articles without limiting the years of publication.

Ethical clearance: Not applicable

Results and Discussion:

After ingestion of food, dietary proteins are broken down into small oligopeptides and amino acids in the gastrointestinal lumen. In the small intestine, they are transported across the enterocytes into the systemic circulation and then into different organs of the body. Various transporters are available for the intestinal absorption of nutrients across the membranes. Most neutral amino acids are transported across the apical brush-border membrane of the small intestine and the renal proximal tubules by the luminal broad neutral amino acid transporter B^0AT1, which was first identified in mice in 2004[7,8,9,10].

Jando J has reported the expression of B^0AT1 throughout the small intestine of male Wistar rats. They found that B^0AT1 expression was regulated by the presence of angiotensin-converting enzyme 2 (ACE2). B^0AT1 and ACE2 were observed to co-exist on the luminal membrane of small intestinal villi. Immunofluorescence and western blot analysis revealed that their protein expression was higher in the distal part as compared to the proximal part of the small intestine [11].

Localization and distribution of intestinal amino acid transporters in human beings were reported by the Vuille-dit-Bille RN research team. Analysis of tissues from intestinal biopsies collected during routine endoscopy of the gastrointestinal tract (n=46) revealed the presence of B^0AT1 and SIT1 with ACE2 in the brush-border membrane of small intestinal epithelial cells. Real-time PCR and immunofluorescence further confirmed the significant expression of the corresponding genes in all the samples. Among the tissues collected by them, 9 samples were from patients treated with ACE inhibitors (ACEI). Patients who were treated with ACEIs expressed higher intestinal mRNA levels of ACE2, and amino acid transporters B^0AT1 as compared to the untreated controls[12].

Recently, the cryo–electron microscopic structure of ACE2 in the intestine of human beings was described by Yan et al. ACE2 in these samples was found to be associated with the B^0AT1. ACE2-B^0AT1 was gathered as a dimer in the structure of the whole complex[13]. In their study, B^0AT1 has also been shown to interact with the receptor binding domain (RBD) of the spike glycoprotein (S protein) of SARS-CoV-2 and also one more coronavirus receptor, aminopeptidase N[11]. These findings suggest that in addition to its role in amino acid transport, B^0AT1 may play an important role in the intestinal infections by the above coronaviruses which might hamper the absorption of neutral amino acids, and might explain the gastrointestinal and other health problems in coronavirus infection.

Back in 2012, Hashimoto et al conducted a very elaborate study and confirmed the concurrent presence of ACE2 and B^0AT1 in the intestinal epithelial cells. None of the small or large intestines of *ACE2* knockout mice had abnormal morphological or ultra-structural changes but the challenge with chemicals such as dextran sodium sulfate (DSS) and trinitrobenzene sulphonic acid (TNBS) in knock-out mice produced higher inflammatory reactions and severe colitis accompanied by the presence of inflammatory cells, shortening of the gut length, bleeding, crypt damage, severe diarrhea and weight loss [14].

Researchers have found that recombinant soluble ACE2 (rsACE2) preserved ACE2 functions of the RAS system *in the in vivo* models[15]. However, rsACE2 lacks the membranous domain that is responsible to stabilize the location of B^0AT1. Thus, it is presumed that the soluble ACE2 is unable to stabilize the neutral amino acid transporter in the intestine[4]. Hashimoto et al reported that treatment of mice with rsACE2 did not improve the condition of

the DSS-induced colitis in *ACE2* mutant mice[14].

A higher level of angiotensin II was found in the DSS-induced colitis model of *ACE2* mutant mice, which returned back to a normal level after treatment with rsACE2. But the deletion of angiotensinogen receptor 1 did not prevent DSS-induced colitis which indicates that although ACE2 is essential for its function in the RAS, it has no role in DSS-induced intestinal inflammation, these effects are independent of the classical RAS system[16].

Lack of intestinal B^0AT1 protein expression in the small intestine of *ACE2* mutant mice caused very low levels of the neutral amino acids valine, threonine, tyrosine and essential amino acid tryptophan in the blood. Inadequate distribution of *ACE2* resulted in an impairment of intestinal tryptophan homeostasis which has the potential to increase the susceptibility of intestinal inflammation[17]. B^0AT1 mutations in patients with Hartnup's disease also caused a significant reduction of serum tryptophan level[18,19].

Expression of several antimicrobial peptides was markedly reduced in the epithelial cells of the ileum in *ACE2* mutant mice. Dietary tryptophan is mainly absorbed via the B^0AT1/ACE2 transport pathway. After absorption, it activates the mammalian target of rapamycin (mTOR). mTOR regulates the expression of antimicrobial peptides, which has a favorable role in the composition of gut microbiota. Supplementation with tryptophan and a nicotinamide-rich diet induced the secretion of antimicrobial peptides in *ACE2* mutant mice[13]. Singer et al also reported that mice devoid of *ACE2* have an increased susceptibility to intestinal inflammation and damage[20].

The absence of intestinal B^0AT1 in mice *devoid of ACE2* caused reduced amino acid transport and adequate weight gain in newborns. In addition to that, the transport of proline most probably using SIT1 was also hampered, the researchers could not confirm protein expression of SIT1 due to the lack of specific antibodies specific for mouse SIT1 back then. But glucose uptake via sodium-glucose transporter 1 was not affected in these mice. Less absorption of several amino acids one hour after an oral intake of an amino acid mixture was associated with a severalfold increase of tryptophan and of other neutral amino acids in the lumen of the ileum. Decreased absorption of these amino acids from the gut lumen caused significantly low blood and muscle level of tryptophan, glycine and other neutral amino acids in mice devoid of *ACE2*[20]. But a low-protein/low-niacin diet for a prolonged period of nearly 3 months changed plasma amino acid levels in both ACE2-positive and in ACE2- negative mice, but only *ACE2-negative* mice stopped weight gain. Despite this deficient diet, contrary to the previous findings, plasma niacin levels which is synthesized from tryptophan remained normal in *ACE2*-negative mice and they did not develop any pellagra symptoms such as skin rash and diarrhea[20]. Unfortunately, the authors did not explore the underlying mechanisms for their findings, doing so might have explored some alternate pathways to maintain normal niacin levels in conditions with altered ACE2 status.

Table 1. Normal Expression of B^0AT1 in the intestine and consequences in altered expression

Subject	Findings	Authors
Expression of B^0AT1 in rodent intestine	Expression of B^0AT1 in rodent intestine was supported by the presence of angiotensin-converting enzyme 2 (ACE2).	Jando J 2017
Expression of B^0AT1 in the human intestine	The simultaneous presence of $B^0AT1$and SIT1 gene with ACE2 was observed in the brush-border membrane of human small intestinal cells.	Vuille-dit-Bille RN 2015
Cryo-electron microscopy of human intestinal ACE2-B^0AT1 complex	The ACE2-B^0AT1 complex is assembled as a dimer with ACE2.	Yan R 2020
Intestinal B^0AT1 in ACE2 null mice	B^0AT1 substrates in everted intestinal tissues were deficient. Body weight gain was poor, and uptake of proline, was absent. Plasma and muscle levels of glycine and tryptophan were significantly low.	Singer D 2012
Intestinal B^0AT1 expression and serum amino acid levels *in* $Ace2^{-/-}$ mice	No expression of B^0AT1 protein was found. Serum levels of the neutral amino acids valine, threonine, tyrosine and tryptophan were markedly reduced.	Hashimoto T 2012

Conclusion:

Adequate expression of ACE2 in the small intestine and its association with amino acid transporters appears to play a crucial role in the digestion of protein and the absorption of amino acids and thereby the structural and functional integrity of gut. The binding of the coronavirus and decreased availability of ACE2 in the intestine may have subsequent effects on the amino acid transport and absorption from the intestinal lumen. Amino acids are the building blocks of the body and are required for the synthesis of important biomolecules in our body. Thus, a lack of ACE2 might have several harmful health and nutritional consequences for the body.

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Authors' contribution:

Idea owner of this study: Jesmine Khan

Study design: All authors contributed equally

Data gathering: All authors contributed equally

Writing and submitting the manuscript: Jesmine Khan

Editing and approval of final draft: All authors contributed equally

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