



COVID-19 and Hartnup disease: an affair of intestinal amino acid malabsorption

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Received: 13 June 2020 / Accepted: 10 July 2020
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Abstract

Since the outbreak of COVID-19, clinicians have tried every effort to fight the disease, and multiple drugs have been proposed. However, no proven effective therapies currently exist, and different clinical phenotypes complicate the situation. In clinical practice, many severe or critically ill COVID-19 patients developed gastrointestinal (GI) disturbances, including vomiting, diarrhoea, or abdominal pain, even in the absence of cough and dyspnea. Understanding the mechanism of GI disturbances is warranted for exploring better clinical care for COVID-19 patients. With evidence collected from clinical studies on COVID-19 and basic research on a rare genetic disease (i.e., Hartnup disorder), we put forward a novel hypothesis to elaborate an effective nutritional therapy. We hypothesize that SARS-CoV-2 spike protein, binding to intestinal angiotensin-converting enzyme 2, negatively regulates the absorption of neutral amino acids, and this could explain not only the GI, but also systemic disturbances in COVID-19. Amino acid supplements could be recommended.

Level of evidence No level of evidence: Hypothesis article.

Keywords Amino acids · ACE2 · B⁰AT1 · COVID-19 · Gastrointestinal disturbances · Hartnup disease

Introduction

The recent outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the resulting coronavirus disease 2019 (COVID-19) has had an enormous impact worldwide. By June 5, 2020, the number of confirmed cases had increased to over 7.2 million globally, and deaths surpassed 413,000. In clinical practice, the typical manifestations of COVID-19 are fever, cough, and dyspnea. Various gastrointestinal (GI) symptoms may also occur at disease onset. A recent meta-analysis found that 29 out of 35 studies reported GI symptoms (most commonly nausea, vomiting,

diarrhoea, or loss of appetite) in COVID-19 patients at diagnosis, with a pooled prevalence of 15% [1]. The pooled analysis did not show statistically significant relations between GI symptoms and clinical outcomes in terms of rates of death [1]. Notably, however, the subgroup analysis showed that: (1) patients with severe COVID-19 had higher rates of GI symptoms compared with those with non-severe disease, and (2) patients with GI involvement had a higher prevalence of severe COVID-19 course, with acute respiratory distress syndrome or complications [1]. Thus, understanding the mechanism of GI disturbance in SARS-CoV-2 infection is necessary to explore the optimal clinical care for COVID-19 patients.

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Ileal absorptive enterocytes express the entry machinery used by SARS-CoV-2 to infect cells

Angiotensin-converting enzyme 2 (ACE2) was identified as the receptor for SARS-CoV during the SARS epidemic in 2003 [2]. Virus entry requires viral spike (S) protein binding to ACE2 and priming by host cellular proteases. Recent reports show that SARS-CoV-2 engages ACE2 as

entry receptor and employs the serine protease TMPRSS2 for S protein activation on target cells [3, 4]. In humans, ACE2 is expressed in airway epithelia, kidney cells, small intestine, lung parenchyma, vascular endothelia, as well as in neurons and glial cells [5]. A high number of ACE2 and TMPRSS2 co-expressing cells reside in the small intestine, principally within the ileum, jejunum, and, to a lesser extent, the liver and colon; notably, ileal ACE2⁺ TMPRSS2⁺ cells are absorptive enterocytes [6].

ACE2 is a central component of the renin-angiotensin system. While its homologue ACE cleaves angiotensin I to generate angiotensin II (which drives pulmonary oedema and severe acute lung failure), ACE2 inactivates angiotensin II acting as a negative regulator of the system, thus exerting protective functions against lung pathology [7]. Upon binding of SARS-CoV, the ACE2 extracellular domain is cleaved, while its transmembrane domain is internalized and transferred into early endosomes. Notably, SARS-CoV infection or in vivo treatment with recombinant SARS-CoV S protein downregulate ACE2 protein expression in mice lungs [8]. As a consequence, reduced ACE2 expression during SARS-CoV infection contributes to the pathogenesis of severe acute lung failure in SARS.

SARS-CoV-2 RNA has been detected in fecal specimens from pediatric and adult COVID-19 patients, persisting even after negative nasopharyngeal tests [9], leading to speculate that viral targeting of ileal ACE2⁺ TMPRSS2⁺ enterocytes cells might be the cause of GI symptoms (Fig. 1). Further work is needed to clarify the effects of SARS-CoV-2 infection on enterocyte ACE2 expression and the possible consequences on local and systemic disease progression.

ACE2, intestinal amino acid transport, and Hartnup disorder

ACE2 is a chimeric protein displaying homology with ACE in the extracellular catalytic domain and with collectrin (Tmem27) in the membrane-anchoring domain [10]. Collectrin acts as a binding partner of the solute carrier family 6 (SLC6) amino acid transporter B⁰AT1 in kidney proximal tubule, regulating its trafficking on the luminal cell surface and its neutral amino acid transporter activity [11]. Collectrin is nearly absent in small intestine, the other primary site of B⁰AT1 expression. On the contrary, ACE2 does not bind to amino acid transporters in kidneys, but it is highly expressed in the intestine, where it acts as a specific binding partner of the luminal B⁰AT1 [12]. In ACE2 knockout mice, luminal expression of B⁰AT1 in the intestine is completely lost [12], demonstrating its fundamental role for the intestinal absorption of neutral amino acids (i.e., tryptophan, alanine, asparagine, glutamine, histidine, isoleucine, leucine, phenylalanine, serine, threonine, tyrosine, and valine) [12].

Interestingly, therapy with ACE inhibitors was found to increase intestinal expression of both ACE2 and B⁰AT1 [13].

Hartnup disorder is a rare inherited condition early described in 1956. Mutations in the SLC6A19 gene encoding B⁰AT1 have been identified as a cause of the disease, resulting in defective amino acid transport in the kidney and small intestine [14, 15]. Besides the characteristic neutral aminoaciduria, patients may develop pellagra-like skin rash, variable neurologic manifestations (e.g., cerebellar ataxia, dysarthria, seizures, headache, dizziness), or psychiatric symptoms (e.g., anxiety, rapid mood changes, delirium), as well as diarrhoea. Symptoms may begin in childhood or early adulthood, mostly under stress conditions like malnutrition or infections, and seem mainly related to the reduced tryptophan absorption, which in turn causes improperly low production of nicotinamide (also known as niacin), necessary for the biosynthesis of nicotinamide adenine dinucleotide (NAD⁺). Notably, the neurological and psychiatric symptoms of Hartnup patients evoke the most common neuropsychiatric symptoms of COVID-19 [5].

The mutations that interfere with ACE2 and B⁰AT1 interaction cause altered amino acid absorption. Of note, recent work has elucidated 47 potential functional missense variants from genomic databases within ACE2/SLC6A19/TMPRSS2, warranting genomic enrichment analyses in SARS-CoV-2 patients, useful for the ongoing pandemic [16]. Experimental studies demonstrated that ACE2 knockout mice display highly increased susceptibility to intestinal inflammation and diarrhoea, which were reverted by dietary nicotinamide [17]. Altered composition of the gut microbiota (as a consequence of impaired amino acid transport and reduced secretion of antimicrobial peptides by Paneth cells in the small intestine) and changes in innate immunity contribute to the colitis phenotype observed in ACE2 knockout mice [17]. Together, these facts may explain diarrhoea and intestinal inflammation observed in Hartnup disorder, pellagra, or under conditions of severe malnutrition [17]. Similarly, diarrhoea and the other GI symptoms observed in COVID-19 can be explained by the SARS-CoV-2-sequestered ACE2 in intestinal absorptive enterocytes (Fig. 1). Notably, under chronic amino acid-supplemented diet, B⁰AT1 protein is increased in the proximal small intestine, while ACE2 protein levels raise in the distal one [18], suggesting that nutrition may play an important therapeutic role in both Hartnup and COVID-19 disorder.

Hypothesis

Based on clinical observations and basic research, we hypothesise that, in response to the SARS-CoV-2 binding to intestinal ACE2, the absorption of neutral amino acids is negatively regulated in COVID-19 patients. Upon

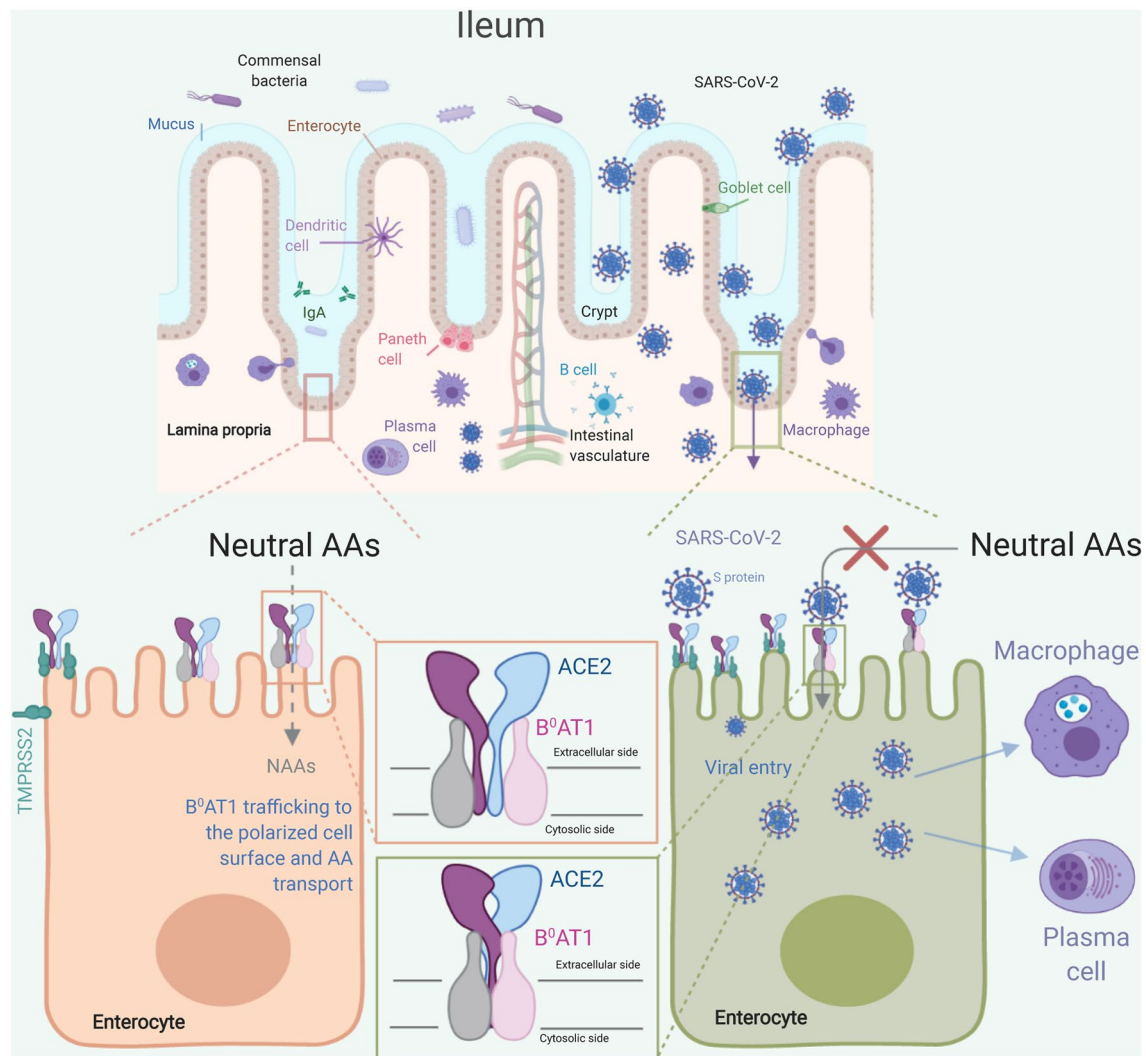


Fig. 1 SARS-CoV-2 binding to ACE2 in the small intestine, principally ileum and jejunum, may cause functional changes of enterocytes and start the innate immune response. If host defense mechanisms are defective, massive viral replication may occur, leading to hyperinflammation and severe systemic complications. ACE2 also functions as the chaperone for surface expression of the amino acid transporter B⁰AT1, which mediates the uptake of neutral amino acids into ileal enterocytes. Mutations in B⁰AT1 cause Hartnup disorder, an inherited defect of amino acid transport, whose symptoms are reminiscent of those of COVID-19. Cryo-electron microscopy structure

of the full-length human ACE2-B⁰AT1 complex has been recently described [23]. The ACE2 forms homodimers which are sandwiched by B⁰AT1, resulting in ACE2-B⁰AT1 complex. Structural analysis suggests that two SARS-CoV-2 spike (S) protein trimers simultaneously bind to an ACE2 homodimer. While the ACE2-B⁰AT1 complex exists in the open or closed state, the SARS-CoV-2-ACE2-B⁰AT1 ternary complex only displays the closed conformation. AA amino acids. This figure was created by an author (E.N.) using the website <https://app.biorender.com>

this change, neutral amino acids (and probably small proteins) accumulate in the intestinal lumen, promoting diarrhoea with protein malabsorption, microbiota changes, and immune defects. These pathological events may be causes of the multiple symptoms observed in COVID-19 patients, both in early and late phases of the disease. Immune response to SARS-CoV-2 was suggested—especially in pulmonary parenchyma—to be markedly affected by inflammation processes started by resident macrophages or epithelial cells and followed by the production of proinflammatory

cytokines and chemokines and recruitment of circulating monocytes. Further, the reduction and dysfunction of lymphocytes impedes the effective onset of the adaptive immune response. Uncontrolled virus infection leads to a vicious cycle and further worsening of lung injury and multiorgan dysfunction [19]. Mounting the innate and adaptive immune response is a highly energy-consuming process. To timely undertake the required cellular tasks, immune cells undergo a dramatic reprogramming of their cellular metabolism, which enables the production of ATP and the synthesis

of new biomolecules. Thus, the flow of nutrient substrates (glucose, amino acids, and fatty acids) is redirected towards immunity and away from storage or other non-immune organismal processes. In malnourished patients or conditions of intestinal amino acid malabsorption, as in the COVID-19 or Hartnup patients, the adaptive immune response cannot be effectively initiated because the absorption of essential energy substrates is impaired by SARS-CoV-2 binding to ACE2. Meanwhile, the diversion of energy substrates to the maintenance of the immune system, in addition to reduced NAD⁺ synthesis, causes insufficient protein synthesis and energy production in skeletal muscle and brain. Similarly, the reduced serotonin synthesis may affect brain function and explain some neurological and psychiatric symptoms in COVID-19.

A high-protein diet can overcome the deficient transport of neutral amino acids in most Hartnup patients. Thus, we propose to treat COVID-19 patients, both acutely (in the early phase of the disease) and chronically (at least for subsequent 3–6 months after dismissal), with specific amino acid supplements and niacin. Very recently, we and others have proved the ability of designer amino acid-supplemented diets to promote energy production, amino acid absorption, protein synthesis, healthy gut microbiota, and immune response [20, 21]. This approach may be crucial to prevent long-term physical, neurologic, and psychiatric sequelae in COVID-19 patients after recovery.

Future research is needed to explore whether SARS-CoV-2 directly attacks absorptive enterocytes—as suggested recently [22], and to examine the effect of SARS-CoV-2 on intestinal and renal absorption of neutral amino acids. Efforts are also necessary to confirm whether and how SARS-CoV-2 influences the intestinal adaptive immune response. Preclinical and clinical studies should investigate the impact of amino acid supplements, with or without niacin, on the outcome of SARS-CoV-2 infection. Based on the ability to increase intestinal expression of both ACE2 and B⁰AT1 [13], ACE inhibitors could potentiate the efficacy of the dietary supplements. Randomised clinical trials are mandatory to assess the effectiveness of nutritional therapies on physical and psychological resilience in COVID-19 patients.

What is already known on this subject?

Many severe COVID-19 patients developed gastrointestinal (GI) disturbances, even in the absence of cough and dyspnea. Understanding the mechanism of GI disturbances is warranted for exploring better clinical care for COVID-19 patients. The Hartnup disease is a rare inherited disease, in which the ACE2-dependent trafficking of B⁰AT1—a transporter of neutral amino acids—is genetically defective. Notably, Hartnup disease includes cutaneous alterations, neurologic manifestations, or psychiatric symptoms,

frequently described in severe COVID-19 cases, together with the GI disturbances. Thus, we hypothesise that COVID-19 may share a pathophysiological mechanism with the Hartnup disease.

What does this study add?

As a consequence of our hypothesis, we propose to treat COVID-19 patients as Hartnup patients. High-protein diets indeed have been found to overcome the deficient transport of neutral amino acids in most Hartnup patients. This approach may be crucial to prevent long-term physical, neurologic, and psychiatric sequelae in COVID-19 patients after recovery.

Acknowledgements The research work in the laboratories of E.N. and A.V. is partly supported by Cariplo Foundation (Grant 1006-2016) and Professional Dietetics (Milan, Italy).

Author contributions EN and AV did the literature search, put forward the hypothesis, and drafted the manuscript. EN, SC and AV revised the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

Informed consent For this type of study (Hypothesis study) formal consent is not required.

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