Could SARS-CoV-2 Invade the Brain?

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Abstract

COVID-19 caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is highly contagious. Patients with COVID-19 usually have respiratory symptoms such as fever, cough and difficulty in breathing, but also exhibit symptoms such as anosmia/hyposmia, ageusia/dysgeusia, unilateral or bilateral weakness of limbs, altered mentation, seizures etc. owing to complicated or concomitant onset of neurological disorders such as stroke, encephalopathy, meningitis, encephalitis, Guillain-Barré syndrome or Miller-Fisher syndrome. The route by which SARS-CoV-2 enters the brain and infects neurons remains to be explored, but it starts from binding to a functional receptor of angiotensin-converting enzyme 2 before the virus enters into host cells. In this article we summarize current knowledge from the literature on coronavirus related to SARS-CoV-2 and postulate the possible pathways for SARS-CoV-2 to enter into the brain.

Keywords

Brain, Corona virus, COVID-19, Severe acute respiratory syndrome, SARS-CoV-2

Introduction

COVID-19 caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is a newly recognized pandemic disorder. Its typical symptoms usually include fever, cough and difficulty in breathing [1]. Chest imaging shows ground-glass opacities [1]. COVID-19 was initially considered a primary respiratory system disease but eventually it infected multiple systems including cardiovascular [2], gastrointestinal [3, 4], renal [5, 6], hematologic [7-9], as well as the central (CNS) and peripheral (PNS) nervous systems [10-14].

Neurologists have moved to the frontlines [15]. Many patients present with neurological disorders may be infected with SARS-CoV-2 with or without typical COVID-19 classic respiratory symptoms. Current understanding is limited relative to the underlying pathogenesis for SARS-CoV-2 to cause structural and functional abnormalities in the nervous system, which complicates neurologists in efficiently managing neurologic patients with COVID-19. We are facing many clinical challenges with unanswered questions. As neurologists on the frontlines, we are eager to know how SARS-CoV-2 attacks neurons and neural tissues. What evidence do we have to understand how the virus routes into the brain? Why do some, particularly in young, patients with COVID-19 develop strokes [10]? What are the underlying pathophysiologies that would cause COVID-19 patients to die unexpectedly? To help understand SARS-CoV-2 pathogenesis associated with
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neurologic disorders and its possible transmissibility routing into the brain and causing COVID-19 neuropathology, we summarize current understanding from laboratory research on coronavirus relevant to SARS-CoV-2 and postulate its possible pathways routing into the brain.

**CoVs Overview**

There are four subgroups of CoVs, namely, alpha, beta, gamma and delta CoVs. The alpha-CoVs and beta-CoVs infect only mammals causing respiratory illness and gastroenteritis. The gamma-CoVs and delta-CoVs infect birds, but some of them can also infect mammals. All CoVs share similar features in the organization and expression of their genome [16, 17]. Beta-CoVs are highly contagious and include SARS-CoV (or SARS-CoV-1, causing Severe Acute Respiratory Syndrome, or SARS epidemic in Asia and China in 2003), MERS-CoV (causing Middle East Respiratory Syndrome, or camel flu, in 2012) and SARS-CoV-2 (causing COVID-19 pandemic). SARS-CoV-1 and SARS-CoV-2 infect humans sharing the same mechanism initially binding to Angiotensin-Converting Enzyme 2 (ACE2) as a receptor to enter target cells [17, 18], whereas MERS-CoV infects human cells by binding to a different receptor, dipeptidyl peptidase 4 (DPP4, also called CD26) [19].

Our knowledge relative to the transmissibility of SARS-CoV-2 is limited. Since most CoVs have similar structural and infection properties, both SARS-CoV-1 and SARS-CoV-2 share approximately 79.6% identity in the genomes and use the same host receptor ACE2 for cell entry [17, 18], the infection mechanisms previously understood for SARS-CoV-1 may be applicable to SARS-CoV-2. In fact, SARS-CoV-1 and SARS-CoV-2 are similar in many respects comparable clinically in causing symptoms of respiratory diseases as SARS and COVID-19, respectively. However, SARS-CoV-2 has markedly increased infectivity (please see below) [1, 20].

The coronaviral genome encodes four major structural proteins: the spike (S), nucleocapsid, membrane and the envelope proteins [21]. The spike protein is essential for CoVs to enter into the target cells. Sequence analysis of spike protein genome showed 75% identical between the SARS-CoV-2 and SARS-CoV-1 [17]. The spike protein of CoVs is the binding site to ACE2 [16, 21] and comprises of subunits S1 and S2 for receptor binding and membrane fusion, which have important implications in vaccine development.

**ACE2 vs. ACE**

Both ACE and ACE2 are type 1 integral transmembrane glycoprotein located at the outer cell membrane. ACE gene is localized on the chromosome Xp22 [22]. ACE was originally identified in testis, heart and kidney. ACE2 is wide spreading in many organs and cells including lungs, arteries, heart, kidney and intestines [23, 24]. Presence of ACE2 protein and mRNA has been demonstrated predominantly in neurons in animal studies, but also has been identified in human postmortem tissue. The highest level of ACE2 has been observed in kidney, lungs and heart [23]. The primary function of ACE2 is to act as a counter balance to ACE, which cleaves angiotensin (Ang) I into the vasoconstricting Ang II which is linked to hypertension. ACE2 in turn cleaves Ang II into the vasodilator Ang 1-7 to lower blood pressure (Figure 1) [25]. Although there are approximately 40% homology between ACE and ACE2, the latter does not convert Ang I to Ang II nor is it blocked by ACE inhibitors [23]. Notably, ACE has two active sites whereas ACE2 has only one. ACE acts as a carboxy-dipeptidase removing two amino acids from C-terminal substrates whereas ACE2 acts as a carboxypeptidase removing a single amino acid. The major substrate for ACE2 is Ang II [23] and ACE2 has a much higher catalytic efficiency for hydrolysis of Ang II (400-fold) compared with Ang I. Ang II interacts with the autonomic nervous system regulating the central and peripheral sensory information.

**ACE2 and SARS-CoVs**

ACE2 acts as a functional receptor for SARS-CoVs and serves as the main entry point into cells for SARS-CoV-1 [26, 27] and SARS-CoV-2 [28]. Structurally, ACE2 has a short intracellular tail, a transmembrane anchor, and a large ectodomain that constitutes the receptor binding subunit for S1 and membrane-fusing subunit for S2 (Figure 2) [16]. The receptor binding domain of ACE2 has high affinity to the spike S1 protein of the SARS-CoVs [16, 20] and directly binds to the peptidase domain of ACE2 [16], whereas S2 is responsible for membrane fusion [29]. When the spike S1 protein binds to the host receptor of ACE2, another cleavage site is exposed and the S1 is cleaved by host proteases to allow the virus to enter the host cell by membrane fusion, a process that is critical for viral infection [19]. The binding of the spike S1 protein of SARS-CoVs to the enzymatic domain of ACE2 on the surface of cells results in endocytosis and translocation of both the virus and the enzyme into endosomes intracellularly [29]. ACE2 also serves as an entry point into cells for other coronaviruses [27]. TMPRSS2 is a host transmembrane serine protease and is required for SARS-CoV-2 to enter the host cell by cleaving the spike protein and allowing the viral and cellular membranes to fuse [28, 30]. Inhibition of TMPRSS2
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ACE2 is found in a wide variety of cells including enterocytes of the small intestine, endothelial cells of arteries and veins, arterial smooth muscle cells, epithelial cells of tongue, renal tubular epithelium and Leydig cells in the testes [20, 24, 33]. In the CNS, ACE2 mRNA expression has been demonstrated in the cerebral cortex, striatum, hypothalamus and brainstem [34] and coexists with ACE [35]. The presentation of ACE2 protein in various human organs (oral and nasal mucosa, nasopharynx, lung, stomach, small intestine, colon, skin, lymph nodes, thymus, bone marrow, spleen, liver, kidney and brain) indicates many organs and cells may become targets and victims of SARS-CoV-2. Thus, COVID-19 may be a disorder of multitude, rather than a single respiratory system.

Roads for SARS-CoVs to Invade the Nervous System

Previous studies showed that SARS-CoV-1 is able to infect the CNS, as the virus has been detected in the brains of infected patients, almost exclusively in neurons [36-38]. As aforementioned, human pathogenic coronaviruses SARS-CoV-1 and SARS-CoV-2 invade their target cells through ACE2 [18]. Presence of ACE2 protein and mRNA has been demonstrated predominantly in neurons in animal studies [39], which is widespread throughout the brain and brainstem [40] including the nuclei involving central regulation of cardiovascular function, such as the cardiorespiratory neurons of the brainstem, as well as in non-cardiovascular areas such as the motor cortex and raphe nuclei which have robust functions in the brain including modulating pain, mood, vigilance and levels of consciousness [38, 39].

SARS-CoV-1 may enter the brain via the nose through the olfactory epithelium, as it has been reported in humans and confirmed in animals [40]. This approach of infection may be applicable to SARS-CoV-2 in causing anosmia/hyposmia [41] and attacking brain neurons [11, 12].

An alternative pathway for SARS-CoV-2 to enter the CNS may be hematogenous via the areas of the brain that lack blood-brain barrier such as subfornical organ, the roof of the third and fourth ventricles, capillaries in the pial gland on the roof of the diencephalon and pineal gland [42], involving transcytosis by leukocytes. Although clinically rare, previous observation in a SARS-patient disclosed that SARS-CoV-1 could be identified in the CSF [41].

Trans-synaptic transfer of SARS-CoV-2 from PNS to CNS may also be possible. Previous animal studies demonstrated that CoVs may first invade peripheral nerve terminals and then gain access to the CNS via a synapse-connected route [43, 44]. The trans-synaptic transfer has been well documented for other CoVs, such as HEV67 [45] and avian bronchitis virus [44, 46]. Following oronasal inoculation to nasal mucosa, CoVs can first be detected in the tonsil, lung and small intestine, and then delivered retrogradely via peripheral nerves to the medullary neurons in animals [44] causing neural infection, in addition to bronchitis or pneumonia, pertinent to critical illness-related conditions [44, 46].

Clinically, patients with neurologic disorders are often elderly who also are vulnerable to COVID-19 with poor outcome due, at least partially, to their comorbidities of aging-related chronic medical conditions [1]. Because of widespread distribution of ACE2 in the brain, neurons are potential targets of SARS-CoV-2. The expression of ACE2 is substantially increased in patients with type 1 or type 2 diabetes and hypertension, which may facilitate infection with SARS-CoV-2 and predispose the elderly at an increased risk for developing severe and fatal COVID-19 infection [47, 48]. Neurological symptoms seen in patients with COVID-19 range from fairly specific neurologic symptoms such as anosmia/hyposmia (impaired smell) and ageusia/dysgeusia (impaired tasting) [49], myopathy or peripheral nerve injury (e.g. Guillain–Barré syndrome, Miller–Fisher syndrome) [50-52], altered mental status, encephalopathy [14, 53], meningitis [12], encephalitis [14], seizures [1, 13] and stroke [10, 11, 14]; to nonspecific symptoms such as headache, dizziness and fatigue [1, 13].
However, the exact route by which SARS-CoV-2 enters the CNS remains to be explored. Experimental animal studies revealed that SARS-CoV-1 [48] and MERS-CoV [54] could enter the brain, when given intranasally, possibly via the olfactory nerves and thereafter rapidly spread to some specific brain areas including the thalamus and brainstem. In the mice infected with low inoculum doses of MERS-CoV the virus was detected only in the brain, not in the lung, indicating that the infection in the CNS was more important for the high mortality in the infected mice [54]. Among the involved brain areas, the brainstem has been demonstrated to be heavily infected by SARS-CoV-1 [48, 55] or MERS-CoV [54]. Autopsies from patients died of SARS have provided convincing evidence of CNS infection resulting in the detection of SARS-CoV-1 in neurons by electron microscopy, immunohistochemistry, and real-time reverse transcription–polymerase chain reaction [48]. Notably, patients with SARS have been demonstrated the presence of SARS-CoV-1 particles in the brain, where they were located almost exclusively in neurons, no viral particles was detected in nonneuronal cells in the infected human brains [36–38]. In this respect, SARS-CoV-2 may possess a similar mechanism as SARS-CoV-1 to infect neurons via several routes to enter CNS. The pathological findings of the brain caused by SARS-CoV-2 in COVID-19 are urgently needed [11, 12].

Conclusion
In summary, clinical observations and animal studies have shown CoVs infectivity. It may be reasonable to postulate that, because of the similarity in structure and subcellular morphology shared by CoVs, the routes to the brain of other CoVs may be applicable to the recently identified SARS-CoV-2 [55], pending clinical and laboratory verification and confirmation. In this context, understanding transmissibility to the brain would assist healthcare providers better manage patients with COVID-19.

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