Yanchao Li ORCID iD: 0000-0002-2884-9829

The neuroinvasive **potential of SARS-CoV2 may be at least partially responsible for the respiratory failure of COVID-19 patients**

Yan-Chao Li[1]*, Wan-Zhu Bai[2], Tsutomu Hashikawa[3]

1Norman Bethune College of Medicine, Jilin University, Changchun, Jilin Province, China 130021

2Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Science, Beijing, China 100700

3Advanced Technology Development Group, RIKEN Brain Science Institute, Saitama, Japan 351-0198

Running title: T**he neuroinvasive potential of the SARS-CoV-2**

Page number: 10; Figures: 0; Words: 1650

*CORRESPONDENCE TO:

Yan-Chao Li, Norman Bethune College of Medicine, Jilin University, Changchun, Jilin Province, China, 130021. E-mail: liyanchao@jlu.edu.cn

Abstract

Following the severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), another highly pathogenic coronavirus named SARS-CoV-2 (previously known as 2019-nCoV)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jmv.25728.

emerged in December 2019 in Wuhan, China, and rapidly spreads around the world. This virus shares highly homological sequence with SARS-CoV, and causes acute, highly lethal pneumonia (COVID-19) with clinical symptoms similar to those reported for SARS-CoV and MERS-CoV. The most characteristic symptom of COVID-19 patients is respiratory distress, and most of the patients admitted to the intensive care could not breathe spontaneously. Additionally, some COVID-19 patients also showed neurologic signs such as headache, nausea and vomiting. Increasing evidence shows that coronavriruses are not always confined to the respiratory tract and that they may also invade the central nervous system inducing neurological diseases. The infection of SARS-CoV has been reported in the brains from both patients and experimental animals, where the brainstem was heavily infected. Furthermore, some coronaviruses have been demonstrated able to spread via a synapse-connected route to the medullary cardiorespiratory center from the mechano- and chemoreceptors in the lung and lower respiratory airways. In light of the high similarity between SARS-CoV and SARS-CoV2, it is quite likely that the potential invasion of SARS-CoV2 is partially responsible for the acute respiratory failure of COVID-19 patients. Awareness of this will have important guiding significance for the prevention and treatment of the SARS-CoV-2-induced respiratory failure. (229 words)

Keywords

Coronavirus; Cell susceptibility; Dissemination; Nervous system

Coronaviruses (CoVs), which are large enveloped non-segmented positive-sense RNA viruses, generally cause enteric and respiratory diseases in animals and humans [1]. Most human CoVs, such as hCoV-229E, OC43, NL63, and HKU1, cause mild respiratory diseases, but the worldwide spread of two previously unrecognized coronaviruses, the severe acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV), has called global attention to the lethal potential of human CoVs [2]. While MERS-CoV is still not eliminated from the world, another highly pathogenic CoV, currently named SARS-CoV-2 (previously known as 2019-nCoV), emerged in December 2019 in Wuhan, China. This novel CoV has caused a national outbreak of severe pneumonia (COVID-19) in China, and rapidly spreads around the world.

Genomic analysis shows that SARS-CoV-2 is in the same Betacoronavirus clade as MERS-CoV and SARS-CoV, and shares highly homological sequence with SARS-CoV [3]. The public evidence shows that COVID-19 shares similar pathogenesis with the pneumonia induced by SARS-CoV or MERS-CoV[4]. Moreover, the entry of SARS-CoV-2 into human host cells has been identified to use the same receptor as SARS-CoV [5, 6].

Most CoVs share a similar viral structure and infection pathway[7, 8], and therefore the infection mechanisms previously found for other CoVs may also be applicable for SARS-CoV-2. A growing body of evidence shows that neurotropism is one common feature of CoVs [1, 9-12]. Therefore, it is urgent to make clear whether SARS-CoV-2 can gain access to the central nervous system (CNS) and induce neuronal injury leading to the acute respiratory distress.

The clinical features of SARS-CoV-2 infection

SARS-CoV-2 causes acute, highly lethal pneumonia with clinical symptoms similar to those reported for SARS-CoV and MERS-CoV [2, 13]. Imaging examination revealed that most patients with fever, dry cough, and dyspnoea showed bilateral ground-glass opacities on chest CT scans [14]. However, different from SARS-CoV, SARS-CoV-2-infected patients rarely showed prominent upper respiratory tract signs and symptoms, indicating that the target cells of SARS-CoV-2 may be located in the lower airway [2].

Based upon the first-hand evidence from Wuhan local hospitals [2, 14-15], the common symptoms of COVID-19 were fever (83% ~ 99%) and dry cough (59.4% ~ 82%) at the onset of illness. However, the most characteristic symptom of patients is respiratory distress (~55%). Among the patients with dyspnoea, more than half needed intensive care. About 46% ~ 65% of the patients in the intensive care worsened in a short period of time and died due to respiratory failure. Among the 36 cases in the intensive care reported by Wang et al.[15], 11.1% received high-flow oxygen therapy, 41.7% received noninvasive ventilation, and 47.2% received invasive ventilation. These data suggest that most (about 89%) of the patients in need of intensive care could not breathe spontaneously.

It is now known that CoVs are not always confined to the respiratory tract and that they may also invade the CNS inducing neurological diseases. Such neuroinvasive propensity of CoVs has been documented almost for all the betacoronaviruses, including SARS-CoV [1], MERS-CoV [11], HCoV-229E [16], HCoV-OC43 [12], mouse hepatitis virus (MHV) [17], and porcine hemagglutinating encephalomyelitis coronavirus (HEV) [9-10, 18-19].

In light of the high similarity between SARS-CoV and SARS-CoV2, it is quite likely that the potential neuroinvason of SARS-CoV-2 plays an important role in the acute respiratory failure of COVID-19 patients. According to the complaints of a survivor, the medical graduate student (24 years old) from Wuhan University, she must stay awake and breathe consciously and actively during the intensive care. She said that if she fell asleep, she might die because she had lost her natural breath.

The neuroinvasive potential of SARS-CoV-2

It is believed that the tissue distributions of host receptors are generally consistent with the tropisms of viruses [20-22]. The entry of SARS-CoV into human host cells is mediated mainly by a cellular receptor angiotensin-converting enzyme 2 (ACE2), which is expressed in human airway epithelia, lung parenchyma, vascular endothelia, kidney cells, and small intestine cells [23-25.] Different from SARS-CoV, MERS-CoV enters human host cells mainly via dipeptidyl peptidase 4 (DPP4), which is present in the lower respiratory tract, kidney, small intestine, liver, and the cells of the immune system [26-27].

However, the presence of ACE2 or DPP4 solely is not sufficient to make host cells susceptible to infection. For example, some ACE2-expressing endothelial cells and human intestinal cell lines failed to be infected by SARS-CoV [28-29], while some cells without a detectable expression level of ACE2 such as hepatocytes could also be infected by SARS-CoV [20]. Likewise, the infection of SARS-CoV or MERS-CoV was also reported in the CNS, where the expression level of ACE2 [30] or DDP4 [31] is very low under normal conditions.

Early in 2002 and 2003, studies on the samples from SARS patients have demonstrated the presence of SARS-CoV particles in the brain, where they were

located almost exclusively in the neurons [32-34]. Experimental studies using transgenic mice further revealed that either SARS-CoV [30] or MERS-COV [11], when given intranasally, could enter the brain, possibly via the olfactory nerves, and thereafter rapidly spread to some specific brain areas including thalamus and brainstem. It is noteworthy that in the mice infected with low inoculum doses of MERS-CoV virus particles were detected only in the brain, but not in the lung, which indicates that the infection in the CNS was more important for the high mortality observed in the infected mice [11]. Among the involved brain areas, the brainstem has been demonstrated to be heavily infected by SARS-CoV [30, 35] or MERS-CoV [11].

The exact route by which SARS-CoV or MERS-COV enters the CNS is still not reported. However, hematogenous or lymphatic route seems impossible, especially in the early stage of infection, since almost no virus particle was detected in the non-neuronal cells in the infected brain areas [32-34]. On the other hand, increasing evidence shows that CoVs may first invade peripheral nerve terminals, and then gain access to the CNS via a synapse-connected route [9-10, 19, 36]. The trans-synaptic transfer has been well documented for other coronaviruses, such as HEV67 [9-10, 18-19] and avian bronchitis virus [36-37].

HEV 67N is the first coronavirus found to invade the porcine brain, and it shares >91% homology with HCoV-OC43 [38-39]. HEV first oronasally infects the nasal mucosa, tonsil, lung, and small intestine in suckling piglets, and then is delivered retrogradely via peripheral nerves to the medullary neurons in charge of peristaltic function of the digestive tract, resulting in the so-called vomiting diseases [18-19]. The transfer of HEV67N between neurons has been demonstrated by our previous ultrastructural studies to use the clathrin-coating-mediated endo-/exocytotic pathway [10].

Similarly, the trans-synaptic transfer has been reported for avian bronchitis virus [36-37]. Intranasal inoculation in mice with avian influenza virus was reported to cause neural infection besides bronchitis or pneumonia [36]. Of interest, viral antigens have been detected in the brainstem, where the infected regions included the nucleus of the solitary tract and nucleus ambiguus. The nucleus of the solitary tract receives sensory information from the mechano- and chemoreceptors in the lung and respiratory tracts [40-42], while the efferent fibers from the nucleus ambiguus and the nucleus of the

solitary tract provide innervation to airway smooth muscle, glands, and blood vessels. Such neuroanatomic interconnections indicate that the death of infected animals or patients may be due to the dysfunction of the cardiorespiratory center in the brainstem [11, 30, 36].

Taken together, the neuroinvasive propensity has been demonstrated as a common feature of CoVs. In light of the high similarity between SARS-CoV and SARS-CoV2, it is quite likely that SARS-CoV-2 also possesses a similar potential. Based on an epidemiological survey on COVID-19, the median time from the first symptom to dyspnea was 5.0 days, to hospital admission was 7.0 days, and to the intensive care was 8.0 days [15]. Therefore, the latency period is enough for the virus to enter and destroy the medullary neurons. As a matter of fact, it has been reported that some patients infected with SARS-CoV-2 did show neurologic signs such as headache (about 8%), nausea and vomiting (1%).

The implications of the potential neuroinvasion of SARS-CoV-2

As an emerging virus, no effective treatment has been developed for the disease resulting from SARS-CoV-2. Therefore, awareness of the possible entry of SARS-CoV-2 into the CNS will have important guiding significance for the prevention and treatment.

If the neuroinvasion of SARS-CoV-2 does take a part in the development of respiratory failure in COVID-19 patients, the precaution with masks will absolutely be the most effective measure to protect against the possible entry of the virus into the CNS. It may also be expected that the symptoms of the patients infected via facal-oral or conjunctival route will be lighter than those infected intranasally. The possible neuroinvasion of SARS-CoV-2 may also partially explain why some patients developed respiratory failure, while others not. It is very possible that most of the persons in Wuhan, who were the first exposed to this previously unknown virus, did not have any protective measure, so that the critical patients is much more in Wuhan than in other cities in China.

Considering the potential neuroinvasion of SARS-CoV-2, antiviral therapy should be carried out as early as possible to block its entry into the CNS. Airway

inhalation of antiviral agents will be the first choice at the early stage of infection, which will inhibit the replication SARS-CoV-2 in the respiratory tracts and lung and prevent from its subsequent neuroinvasion. It is also urgent to find effective antiviral drugs that can cross the blood-brain barrier. Moreover, corticosteroids, which are used frequently for severe patients, may have no treatment effect, but rather accelerate the replication of the virus within the neurons.

Since SARS-CoV2 may conceal itself in the neurons from the immune recognition, complete clearance of the virus may not be guaranteed even the patients have recovered from the acute infection. In support of this, there is evidence that SARS-CoV-2 is still detectable in some patients during the convalescent period [43]. Therefore, given the probable neuroinvasion the risk of SARS-CoV-2 infection may be currently underestimated.

References

1Glass WG, Subbarao K, Murphy B, Murphy PM. Mechanisms of host defense following severe acute respiratory syndrome-coronavirus (SARS-CoV) pulmonary infection of mice. J Immunol 2004;173:4030-4039.

2Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.

3Yu F, Du L, Ojcius DM, Pan C, Jiang S. Measures for diagnosing and treating infections by a novel coronavirus responsible for a pneumonia outbreak originating in Wuhan, China. Microbes Infect 2020; pii: S1286-4579(20)30025-3.

4Song Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han Y, Qin C. From SARS to MERS, Thrusting Coronaviruses into the Spotlight. Viruses 2019; pii: E59. doi: 10.3390/v11010059.

5Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng

Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020; In Press. pii: S0140-6736(20)30251-8.

6Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. J Virol 2020; pii: JVI.00127-20.

7Yuan Y, Cao D, Zhang Y, Ma J, Qi J, Wang Q, Lu G, Wu Y, Yan J, Shi Y, Zhang X, Gao GF. Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. Nat Commun 2017; 8:15092.

8Hulswit RJ, de Haan CA, Bosch BJ. Coronavirus Spike Protein and Tropism Changes. Adv Virus Res 2016;96:29-57.

9Li YC, Bai WZ, Hirano N, Hayashida T, Hashikawa T. Coronavirus infection of rat dorsal root ganglia: ultrastructural characterization of viral replication, transfer, and the early response of satellite cells. Virus Res 2012;16:628-35.

10Li YC, Bai WZ, Hirano N, Hayashida T, Taniguchi T, Sugita Y, Tohyama K, Hashikawa T. Neurotropic virus tracing suggests a membranous-coating-mediated mechanism for transsynaptic communication. J Comp Neurol 2013;521:203-212.

11Li K, Wohlford-Lenane C, Perlman S, Zhao J, Jewell AK, Reznikov LR, Gibson-Corley KN, Meyerholz DK, McCray PB Jr. Middle East Respiratory Syndrome Coronavirus Causes Multiple Organ Damage and Lethal Disease in Mice Transgenic for Human Dipeptidyl Peptidase 4. J Infect Dis 2016;213:712-722.

12Dubé M, Le Coupanec A, Wong AHM, Rini JM, Desforges M, Talbot PJ. Axonal Transport Enables Neuron-to-Neuron Propagation of Human Coronavirus OC43. J Virol 2018;92: pii: e00404-18.

13Khan S, Ali A, Siddique R, Nabi G. Novel coronavirus is putting the whole world on alert. J Hosp Infect. 2020; pii: S0195-6701(20)30043-8.

14Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395(10223):507-513.

15Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA 2020; In Press. doi: 10.1001/jama.2020.1585.

16Talbot PJ, Ekandé S, Cashman NR, Mounir S, Stewart JN. Neurotropism of human coronavirus 229E. Adv Exp Med Biol 1993;342:339-346.

17Zhou X, Huang F, Xu L, Lin Z, de Vrij FMS, Ayo-Martin AC, van der Kroeg M, Zhao M, Yin Y, Wang W, Cao W, Wang Y, Kushner SA, Marie Peron J, Alric L, de Man RA, Jacobs BC, van Eijk JJ, Aronica EMA, Sprengers D, Metselaar HJ, de Zeeuw CI, Dalton HR, Kamar N, Peppelenbosch MP, Pan Q. Hepatitis E Virus Infects Neuronsand Brains. J Infect Dis 2017;215(8):1197-1206.

18Mengeling WL, Boothe AD, Ritchie AE. Characteristics of a coronavirus (strain 67N) of pigs. Am J Vet Res 1972;33(2):297-308.

19Andries K, Pensaert MB. Immunofluorescence studies on the pathogenesis of hemagglutinating encephalomyelitis virus infection in pigs after oronasal inoculation. Am J Vet Res 1980;41(9):1372-1378.

20To KF, Lo AW. Exploring the pathogenesis of severe acute respiratory syndrome (SARS): the tissue distribution of the coronavirus (SARS-CoV) and its putative receptor, angiotensin-converting enzyme 2 (ACE2). J Pathol 2004;203:740-743.

21Tang JW, To KF, Lo AW, Sung JJ, Ng HK, Chan PK. Quantitative temporal-spatial distribution of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) in post-mortem tissues. J Med Virol 2007;79:1245-1253.

22Kam YW, Okumura Y, Kido H, Ng LF, Bruzzone R, Altmeyer R. Cleavage of the SARS coronavirus spike glycoprotein by airway proteases enhances virus entry into human bronchial epithelial cells in vitro. PLoS One 2009;4(11):e7870.

23Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circ Res 2000;87(5):E1-E9.

24Harmer D, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett 2002;532:107-110.

25Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus A first step in understanding SARS pathogenesis. J Pathol 2004;203(2):631-637.

26Mattern T, Scholz W, Feller AC, Flad HD, Ulmer AJ. Expression of CD26 (dipeptidyl peptidase IV) on resting and activated human T-lymphocytes. Scand J Immunol 1991;33:737-48.

27Boonacker E, Van Noorden CJ. The multifunctional or moonlighting protein CD26/DPPIV. Eur J Cell Biol 2003;82:53-73.

28Chan PK, To KF, Lo AW, Cheung JL, Chu I, Au FW, Tong JH, Tam JS, Sung JJ, Ng HK. Persistent infection of SARS coronavirus in colonic cells in vitro. J Med Virol 2004;74:1-7.

29Ding Y, Wang H, Shen H, Li Z, Geng J, Han H, Cai J, Li X, Kang W, Weng D, Lu Y, Wu D, He L, Yao K. The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. J Pathol 2003;200:282-289.

30Netland J, Meyerholz DK, Moore S, Cassell M, Perlman S. Severe acute respiratory syndrome coronavirus infection causes neuronal death in the absence of encephalitis in mice transgenic for human ACE2. J Virol 2008;82:7264-7275.

31Bernstein HG, Dobrowolny H, Keilhoff G, Steiner J. Dipeptidyl peptidase IV, which probably plays important roles in Alzheimer disease (AD) pathology, is upregulated in AD brain neurons and associates with amyloid plaques. NeurochemInt 2018;114:55-57.

32Ding Y, He L, Zhang Q, Huang Z, Che X, Hou J, Wang H, Shen H, Qiu L, Li Z, Geng J, Cai J, Han H, Li X, Kang W, Weng D, Liang P, Jiang S. Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS-CoV) in SARS patients: implications for pathogenesis and virus transmission pathways. J Pathol 2004;203:622-630.

33Gu J, Gong E, Zhang B, Zheng J, Gao Z, Zhong Y, Zou W, Zhan J, Wang S, Xie Z, Zhuang H, Wu B, Zhong H, Shao H, Fang W, Gao D, Pei F, Li X, He Z, Xu D, Shi X, Anderson VM, Leong AS. Multiple organ infection and the pathogenesis of SARS. J Exp Med 2005;202(3):415-424.

34Xu J, Zhong S, Liu J, Li L, Li Y, Wu X, Li Z, Deng P, Zhang J, Zhong N, Ding Y, Jiang Y. Detection of severe acute respiratory syndrome coronavirus in the brain: potential role of the chemokine mig in pathogenesis. Clin Infect Dis 2005;41:1089-1096.

35McCray PB Jr, Pewe L, Wohlford-Lenane C, Hickey M, Manzel L, Shi L, Netland J, Jia HP, Halabi C, Sigmund CD, Meyerholz DK, Kirby P, Look DC, Perlman S. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. J Virol 2007;81:813-821.

36Matsuda K, Park CH, Sunden Y, Kimura T, Ochiai K, Kida H, Umemura T. The vagus nerve is one route of transneural invasion for intranasally inoculated influenza a virus in mice. Vet Pathol 2004;41:101-107.

37Chasey D, Alexander DJ. Morphogenesis of avian infectious bronchitis virus in primary chick kidney cells. Arch Virol 1976;52:101-111.

38González JM, Gomez-Puertas P, Cavanagh D, Gorbalenya AE, Enjuanes L. A comparative sequence analysis to revise the current taxonomy of the family Coronaviridae. Arch Virol 2003;148:2207-2235.

39Li Z, He W, Lan Y, Zhao K, Lv X, Lu H, Ding N, Zhang J, Shi J, Shan C, Gao F. The evidence of porcine hemagglutinating encephalomyelitis virus induced nonsuppurative encephalitis as the cause of death in piglets. Peer J 2016;4:e2443.

40Kalia M, Mesulam MM. Brain stem projections of sensory and motor components of the vagus complex in the cat: II. Laryngeal, tracheobronchial, pulmonary, cardiac, and gastrointestinal branches. J Comp Neurol 1980;193:467-508.

41Hadziefendic S, Haxhiu MA. CNS innervation of vagal preganglionic neurons controlling peripheral airways: a transneuronal labeling study using pseudorabies virus. J Auton Nerv Syst 1999;76:135-145.

42Raux H, Flamand A, Blondel D. Interaction of the rabies virus P protein with the LC8 dynein light chain. J Virol 2000;74:10212-10216.

43 Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T, Thiel V, Janke C, Guggemos W, Seilmaier M, Drosten C, Vollmar P, Zwirglmaier K, Zange S, Wölfel R, Hoelscher M. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. N Engl J Med. 2020; In Press. doi:10.1056/NEJMc2001468.