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Strong ACE-2 expression in the choroidal vessels: do high choroid plexuses serve as a gateway for SARS-CoV-2 infection on the human brain?

M. PIRAS[1], F. CAU[1], M. MANCHIA[2], P. PARIBELLO[2], L. SABA[3], J.S. SURI[4], G. FAA[1,8], G. PICHIRI[1], G. CERRONE[1], A. SCANO[5], G. ORRÙ[5], G. LA NASA[6], F. COGHE[7], M. CASTAGNOLA[8], D. FANNI[1,9], C. GEROSA[1,9]

[1]Department of Medical Sciences and Public Health, University of Cagliari, Electron Microscopy Laboratory, Division Pathological Anatomy, Cagliari, Italy
[2]Department of Medical Sciences and Public Health, Section of Psychiatry, University of Cagliari, Cagliari, Italy
[3]Department of Radiology, Azienda Ospedaliero Universitaria (AOU) di Cagliari, Monserrato, Cagliari, Italy
[4]Stroke Monitoring and Diagnostic Division, AtheroPoint™, Roseville, CA, USA
[5]Department of Surgical Sciences, Molecular Biology Service (MBS), University of Cagliari, Cagliari, Italy
[6]Hematology Unit, Department of Medical Sciences, University of Cagliari, Cagliari, Italy
[7]Division of Clinical Chemistry and Microbiology, Azienda Ospedaliero Universitaria di Cagliari, Monserrato, Cagliari, Italy
[8]Laboratorio di Proteomica, Centro Europeo di Ricerca sul Cervello, IRCCS Fondazione Santa Lucia, Rome, Italy
[9]Department of Biology, College of Science and Technology, Temple University, Philadelphia (PA), USA

Daniela Fanni and Clara Gerosa contributed equally to this work

Abstract. – OBJECTIVE: Previous studies have confirmed the key mechanism by which SARS-CoV-2 enters human cells. It is well established that ACE2 is the receptor that can mark the beginning of the infection. In light of this, the organs that express higher levels of ACE2 are generally considered at higher risk, while those with lower levels should be somehow more protected. This – if related to the scarcity of ace2-expressing cells in the brain – seems to contrast with the presence of a variety of neurological symptoms that follow infection with ace2. The aim of this work was to analyze ACE2 expression in the human brain, focusing on the choroid plexuses.

PATIENTS AND METHODS: Twenty brain samples were obtained at autopsy from ten human fetuses and from ten adult subjects. All samples were selected to contain the choroid plexus. Specimens were fixed in 10% formalin, routinely processed and paraffin embedded. 5-micron sections were stained with Hematoxylin and Eosin (H&E) and immunostained with a commercial anti-human ACE2 rabbit monoclonal antibody at 1:100 dilution.

RESULTS: We analyzed 20 samples by immunohistochemistry, and we noted that, as far as fetal samples are concerned, a strong reactivity for ACE2 was detected in the myxoid stroma of the choroid plexuses and in the endothelial cells in fetuses. The complete absence of the ACE2 marker was detected in epithelial cells, neurons and glial cells of the cerebral cortex, both in fetuses and in adults. Whereas a strong but selective reactivity for ACE2 was also detected in adult choroid plexuses, mainly localized in the endothelial cells of the choroid capillaries.

CONCLUSIONS: Our study shows a strong expression of ACE in the fetal and adult brain choroid plexuses. This new histopathological finding may clarify the susceptibility of the human brain to SARS-COV-2 infection. Our data indicate the choroid plexus as the entry gate of virus for in the human brain; therefore, the entrance of SARS-CoV-2 into the cerebrospinal fluid through the choroid plexuses might represent the mechanism utilized by this coronavirus to cause direct injury to brain cells.

Key Words:
COVID-19, SARS-CoV-2, Immunohistochemistry, Brain, CNS, ACE2, Choroid plexus.

Corresponding Author: Monica Piras, MD; e-mail: monica.piras@unica.it

Introduction

It has been shown[1] that SARS-CoV-2 uses the angiotensin converting enzyme-2 (ACE2 receptor to enter human cells through binding of the spike protein. Over the past decade, studies[2] concerning the possible linkage between ACE2 polymorphism and the risk of developing several diseases have received considerable attention. Organs with cells expressing elevated levels of ACE2 – including type 2 pneumocytes, enterocytes and cardiomyocytes – are considered as higher risk targets for SARS-CoV-2 infection, when compared to cells of other body districts[3-5]. The brain, and the Central Nervous System (CNS) at large, should be included among the non-vulnerable organs, given the scarcity of ACE2-expressing cells found in the gray and white matter[6]. This could be contrary to clinical observations showing that patients infected by SARS-CoV-2 manifest a variety of neurological symptoms, including dysgeusia (loss of taste), anosmia (loss of smell)[7], as well as neuralgia, sphincter disturbances, headaches, and fatigue[8]. Moreover, recent evidence suggests that SARS-CoV-2 may trigger multiple molecular pathways in different organs, including the brain. As a consequence, patients with COVID-19 have a disproportionately higher incidence of cerebral ischemic stroke[8-11]. Of interest, a prominent bilateral frontotemporal hypo-perfusion has been observed in patients who underwent perfusion imaging[12]. Among other potential mechanisms, these findings might be due to the damage caused by SARS-CoV-2 on the endothelial cells. Indeed, subjects infected with SARS-CoV-2 have an increased risk of conversion from asymptomatic, subclinical, atherosclerotic disease to an unstable state of vulnerable plaques in the carotid arteries, with potential consequences at CNS level[12]. Other pathways leading to neurological disturbances in COVID-19 carriers, with implications also for psychopathology, include hypoxia and the exaggerated inflammatory response known as the "cytokine storm"[9,13].

Microscopic examination of brain tissues from patients deceased for COVID-19 showed acute hypoxic injury in the brain and cerebellum, with loss of neurons in the cerebral cortex, hippocampus, and in the cerebellar Purkinje cell layer[14]. The same authors did not find, however, cytoplasmic viral staining of SARS-CoV-2 on immunohistochemical analysis of brain samples[15].

This work reveals for the first time, ACE2 expression in the choroid plexuses, which appear as a potential modulator of CNS damage caused by SARS-CoV-2.

Patients and Methods

Twenty brain samples were obtained at autopsy from ten human fetuses, of gestational age ranging from 20 up to 38 weeks of gestation, and from 10 adult subjects, ranging from 62 up to 80 years of age. All samples were selected to contain the choroid plexus. Specimens were fixed in 10% formalin, routinely processed and paraffin embedded. 5-micron sections were stained with Hematoxylin and Eosin (H&E) and immunostained with a commercial anti-ACE-2 antibody. In brief, sections to be incubated with the anti-ACE2 antibody were first automatically dewaxed and rehydrated with EZ Prep 1X (Ref. 950-102) and pre-treated at 95°C for 36 minutes with heat-induced epitope retrieval in Ultra CC1 (Ref. 950-224), following Dealer's instructions. Slides were then incubated for 20 minutes at room temperature with anti-human ACE2 rabbit monoclonal antibody [clone EPR4435(2) – Ref. ab108252 – Abcam – Discovery Drive, Cambridge Biomedical Campus, Cambridge CB2 0AX, UK] at 1:100 dilution. Counterstaining was performed incubating sections for 8 minutes with Hematoxylin II (Ref. 790-2208) and for 4 minutes with Bluing Reagent (Ref. 760-2037). All immunostaining procedures were performed using the UltraView Universal DAB Detection Kit (Ref. 760-5000) on the BenchMark Ultra (Ventana Medical Systems Inc. 1910 E. Innovation Park Drive Tucson, AZ, USA) instrument, according to the manufacturer's instructions. All experimental procedures were approved by the Ethics Experimentation Committee, University of Cagliari (Prot. PG/2021/1531). In this context, all protocols have been carried out in full conformity with the rules and guidelines of the Helsinki Declaration.

Results

We analyzed immunoreactivity for the ACE2 receptor in the fetal and adult human brain using a commercial anti-ACE2 antibody. A strong expression of ACE2 was detected in all the choroid plexus samples analyzed in this study. In the fetal brain, ACE2 was expressed in endothelial cells

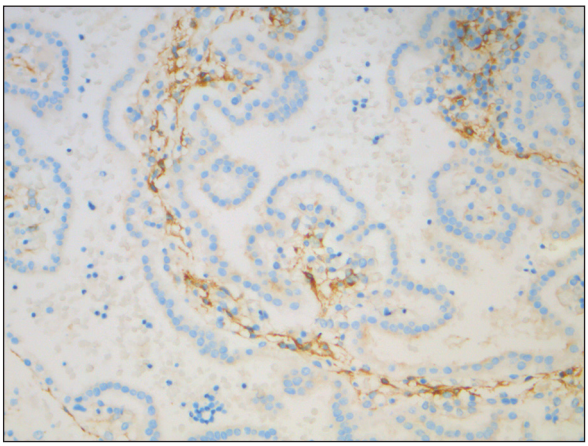

Figure 1. Immunoreactivity for ACE2 in endothelial and stromal cells in the choroid plexus of a 20-week-old fetus. (Original magnification x20).

of choroidal capillaries and in the surrounding stromal cells embedded in the myxoid stroma of the choroid plexus (Figure 1). No reactivity for ACE2 was detected in the epithelial cells. In the adult brain, reactivity for ACE2 was strong, but it was restricted to endothelial cells. ACE2, in the adult choroid plexus, was not expressed by stromal cells nor by epithelial cells (Figure 2). No reactivity for ACE2 was detected in epithelial cells, even in the adult choroid plexus. ACE2 was not expressed both in neuronal and glial cells of the white and the gray matter fetuses and adults.

Discussion

The choroid plexus plays a key role in monitoring the composition and circulation of cerebrospinal fluid, participating in the development of the central nervous system and in immune surveillance[16]. Its structure is characterized by a network of specialized blood vessels embedded within a loose stroma, surrounded by epithelial cells, secured by tight junctions. Endothelial cells have a cytoplasm with large fenestrae that allow inflammatory cells to enter the central nervous system from the blood[15]. Here we propose a new function for choroid plexuses, regarding brain involvement in subjects infected by SARS-CoV-2. Our findings suggest that SARS-CoV-2 can reach the cerebrovascular fluid and the CNS through ACE2 receptors, strongly expressed in the choroidal vessels, which putatively might represent the gateway for invasion of the central nervous system by SARS-CoV-2 (Figure 3). Although there are only few reports on SARS-CoV-2 induced encephalitis[17,18], the expression of ACE2 in several brain areas suggests a possible trophism of this virus for the CNS[19]. Our findings induced us to hypothesize that the choroid plexus might serve as a gateway for the infection of the CNS by SARS-CoV-2 (Figure 3). According with our hypothesis, the coronavirus interaction with ACE2 in the endothelium of the choroid plexus could result in the involvement of the brain stem, crucial for the control of respiratory function, where ACE2 is highly expressed[19]. New imaging cubosome strategies[20] and the potential relationship between Mg homeostasis, Covid infection and endothelial cells status open new prospective of treatment that need to be investigate[21,22].

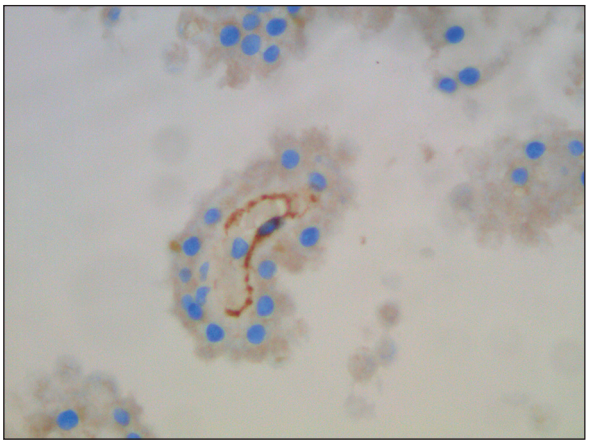

Figure 2. ACE2-expressing endothelium in the chorioid plexus of a 70-year-old subject. (Original magnification x40).

Conclusions

Analyzing the clinical observations of COVID-19 positive patients, a strong variety of neurological symptoms are present, despite the brain is included among those organs that express less ACE2. Starting from the assumption that whatever happens affects neighboring things, every phenomenon can lead to a complete overview. In this work, we confirm the absence of ACE2 expression in neurons and glial cells of the brain cortex. Moreover, we demonstrated, the strong presence of ACE2 in the endothelial cells of the choroid plexus, both in the fetal and in the adult brain. In the light of these results, we present a new possible function of the choroid plexus: a pathway for SARS-CoV-2 infection of the Central Nervous System (CNS).

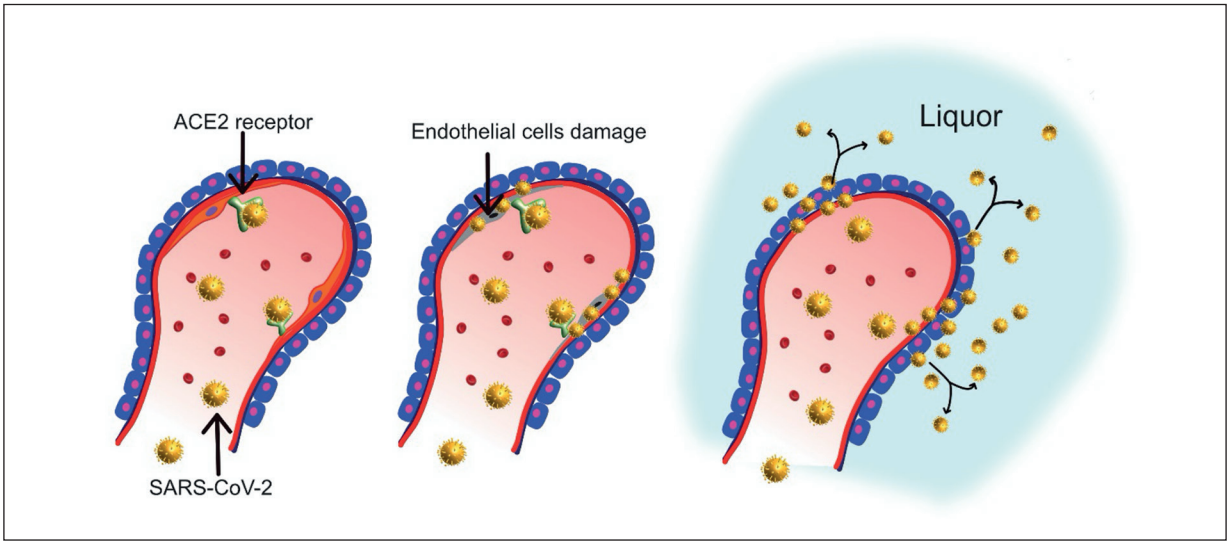

Figure 3. Schematic representation of the interaction between SARS-CoV-2 and its receptor ACE2 expressed by endothelial cells of choroidal vessels, followed by viral translocation into the liquor

Conflict of Interest

The Authors declare that they have no conflict of interests.

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