Review or Mini-review

Dual function of sialic acid in gastrointestinal SARS-CoV-2 infection

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ABSTRACT

Recent analysis concerning the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)- angiotensin converting enzyme (ACE) receptor interaction in enterocytes, the definition of gut-lung axis, as well as the molecular basis of sialic acid-related dual recognition concept in gastrointestinal SARS-CoV-2 infection, have brought a new perspective to potential therapeutic targets. In this review evolving research and clinical data on gastrointestinal SARS-CoV-2 infection are discussed in the context of viral fusion and entry mechanisms, focusing on the different triggers used by coronaviruses. Furthermore, it is emphasized that the viral spike protein is prevented from binding gangliosides, which are composed of a glycosphingolipid with one or more sialic acids, in the presence of chloroquine or hydroxychloroquine. In gastrointestinal SARS-CoV-2 infection the efficiency of these repositioned drugs is debated.

1. Introduction

Human coronaviruses (hCoVs) are a large family of pathogenic enveloped viruses that carry a single strand of RNA. Infection with these viruses generally results in mild to moderate respiratory system disorders, which can also be fatal in some vulnerable individuals. (Graham et al., 2013). To date, six hCoV strains have been identified and classified into four groups. Among these, one of the group B β-CoVs, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the microorganism responsible for the 2019 pandemic coronavirus disease (COVID-19) (Huang et al., 2015). Although the transmission via respiratory tract is the principal exposure route for this disease, the faecal-oral route of transmission should not be ignored. Many hypotheses have been proposed regarding why COVID-19 causes significant gastrointestinal symptoms. Firstly, recent analysis revealed that angiotensin-converting enzyme-2 (ACE2) despite being highly expressed in alveolar cells in the lung, it is also expressed on a large scale in the glandular epithelial cells of gastrointestinal system. Thus, it is accepted that interaction between SARS-CoV-2 and ACE2 may mediate gastrointestinal symptoms (Liang et al., 2020; Zhang et al., 2020a). Secondly, SARS-CoV-2 indirectly damages the gastrointestinal epithelial cells via initiating a cascade of inflammatory reactions. The bacterial abundance of the gastrointestinal system reciprocally influences the respiratory tract through the "gut-lung axis" (Budden et al., 2017; Pan et al., 2020). The third option is that dual recognition of gangliosides and ACE2 by the spike (S) protein of SARS-CoV-2 leads to malabsorption, unstable gut secretion and hyperactive enteric motility (Matrosovich et al., 2015; Zhang et al., 2020a). Recently, the presence of mutual recognition systems by the use of sialic acid (SiA) and sugar chains, between viruses and their host cells has focused interest on this research area leading to the accumulation of knowledge in the field of "sialoglycovirology." (Sriwilaijaroen and Suzuki, 2020). In this context, receptor-related approaches for determination of the binding sites on host and virus proteins will clarify the perspective in potentially drug repurposing and developing new drug candidates for COVID-19.

2. Gastrointestinal SARS-CoV-2 infection

In a meta-analysis reviewing 60 different investigations, a total of 4243 cases had digestive system symptoms and this distribution calculated as 17.6 % in average. In this series of patients, 48 % of the stool specimens were RNA virus positive, although 70 % of them were taken after the disappearance of virus from respiratory tract (Cheung et al., 2020; Grassia et al., 2020). Nationwide data from China showed that 8.7 % of 1099 SARS-CoV-2 confirmed patients had gastrointestinal symptoms (Guan et al., 2020). In another study, among the 651 cases with COVID-19, the proportion of patients with gastrointestinal symptoms was found to be 11.4 %. Increased tendency to suffer from gastrointestinal symptoms among the patients with COVID-19 enhances the risk of contamination in healthcare workers who treat the suspected

COVID-19 patients with no presentation of either respiratory symptoms or fever. Furthermore, the frequency of chronic hepatic disease was also found to be elevated in COVID-19 patients who had digestive system complaints (Jin et al., 2020). Patients who do not suffer from digestive system symptoms were shown to have better prognosis and shorter hospital stay than the cases with gastrointestinal symptoms (60 % vs. 34.3 %). This result arises from either the viral replication in the gastrointestinal system that promotes disease severity or the lack of the characteristic respiratory symptoms at the early phases of the disease (Pan et al., 2020). In patients identified as being positive for SARS-CoV-2 RNA in faeces samples, the severity of illness was not found to be related to the presence of the gastrointestinal complaints. Among these patients, approximately 64 % were still positive for viral RNA in the stool even after no viral RNA could be detected in the pharyngeal swabs (Chen et al., 2020). Similarly, SARS-CoV-2 RNA was detected in faeces of half of the patients (53.4 %). In these patients, virus was negative in respiratory tract samples, -although 23 % of stool specimens -tested as virus positive. These data demonstrate the threat posed by the presence of infection in the gastrointestinal system, even after it has gone from respiratory tract, due to the potential of the faecal-oral transmission (Cipriano et al., 2020). However, the level of gastrointestinal symptoms in severe -COVID-19 cases is greater compared to the non-severe cases. In these cases, ACE2 and virus nucleocapsid protein and other viral material are found in gastrointestinal epithelial cells, and can be isolated from the stool samples (Fig. 1). Approximately in one third to half of all patients, faecal PCR becomes positive, after 2–5 days than sputum positivity and faecal virus excretion may persist after sputum excretion for up to 11 days (Tian et al., 2020). Furthermore, Holshue et al. demonstrated the SARS-CoV-2 nucleic acid in stool samples of a patient (Holshue et al., 2020). Consequently, the digestive system can also be

confirmed as a potential entry track for the virus infection by single cell analysis (Zhang et al., 2020c). The viral mRNA amount expands by approximately 10^4-fold in human duodenal enterocytes during the first day of the infection due to SARS-CoV-2 S-dependent cell tropism and entry. Additionally, viral RNA and protein levels increase more than 10^3-fold within the intestinal epithelium at the end of the first day after contamination (Zang et al., 2020). Since SARS-CoV-2 has a significantly large capacity to provoke vigorous infection and rapidly replicate in human enterocytes, the high amounts of viral RNA pass through the gastrointestinal tract mean that intestinal content is a marker of its ability to raise infection (Zang et al., 2020). However, presence of SARS-CoV-2 in faeces is the evidence of the transportation and invasion of the infectious virions to the gastrointestinal system. For this reason, it is strongly recommended that real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) analysis to investigate the SARS-CoV-2 in stool should be done routinely in patients with SARS-CoV-2. Transmission-based measures for SARS-CoV-2 patients in hospitals should be strictly followed in the case of positive rRT-PCR results of the stool samples (Xiao et al., 2020).

On the other hand, bile acids and salts that function like detergents in gut cause glycosylation of virus S protein. Glycan coating of virus provides reasonable stability and protection from enzymatic damage and bile salt solubilization (Zhang et al., 2020e). In addition, it is well known that ACE2 is extensively expressed in bile duct cells. However, the latest investigations demonstrated that the levels of circulating liver enzymes representing the impairment of hepatocytes, were increased in half of COVID-19 cases, while only in some patients elevated alkaline phosphatase levels were measured. Thus, 2–11 % of cases who suffer from COVID-19 have hepatic comorbidities (Musa, 2020; Zhang et al., 2020a). In this context, SARS-CoV-2 binds to the ACE2 receptors of bile
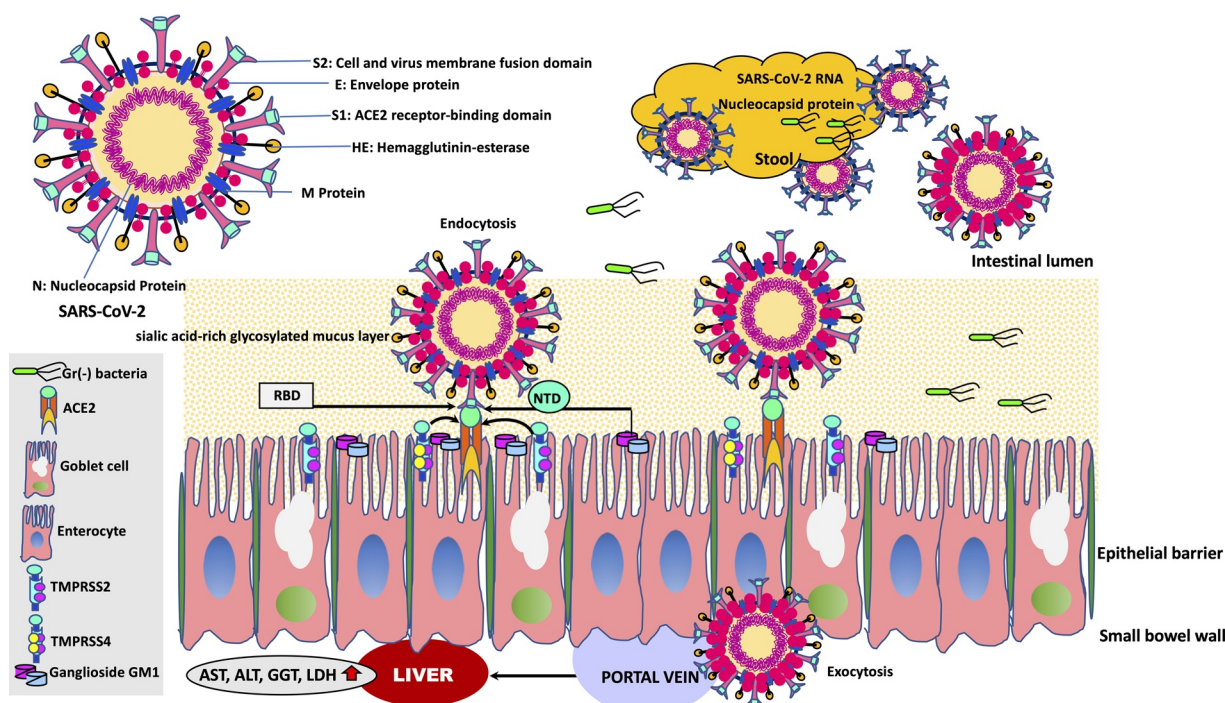

Fig. 1. To initiate infections, many CoVs use SiAs, either as receptor determinants or as attachment factors helping the virus find its receptor underneath the heavily glycosylated mucus layer. Binding to SiAs is required for the enteropathogenicity of CoVs. Interaction with sialoglycoconjugates may help the virus to pass through the SiA-rich mucus layer that covers the viral target cells in the epithelium of the small intestine. SiAs exist predominantly as sialylglycoconjugates on N- and O-linked glycoproteins as well as gangliosides in the cell plasma membrane. The concept of a dual recognition of gangliosides and ACE2 by SARS-CoV-2 S protein: While the RBD binds to the ACE2 receptor, the NTD binds to the ganglioside-rich domain of the plasma membrane. Binding to MGP may allow the virus to stay longer in the intestine *(Abbreviations*. ACE2: angiotensin converting enzyme 2; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CoVs: coronaviruses; GGT: gamma-glutamyl transferase; LDH: lactate dehydrogenase; MGP: mucin-type glycoprotein; NTD: N-terminal domain; RBD: receptor-binding domain; S: spike protein; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SiAs: sialic acids; TMPRSS2: transmembrane protease, serine 2; TMPRSS4: transmembrane protease, serine 4).*

duct and might directly cause liver damage (Hoffmann et al., 2020a). Indeed, findings of the two independent cohorts showed a significant increase in ACE2 expression in bile duct epithelium (59.7 %) in comparison to hepatocytes (2.6 %). SARS-CoV-2 can bind directly to ACE2-positive bile duct epithelium and it can impair hepatocellular functions. (Fig. 1) (Chai et al., 2020). Since in some patients with COVID-19, the gastrointestinal symptoms precede the respiratory illness (D'Amico et al., 2020; Lin et al., 2020), the small intestinal proteases may enhance viral infection by triggering intestinal epithelial cells fusion. Consequently, gut epithelium might permit the virus to spread to other systemic organs. For these reasons, it is expected that the pharmacokinetic properties of the drugs intended to be used in gastrointestinal COVID-19 should be compatible with the mechanism of the disease.

3. Coronavirus receptors

CoVs, in order to readily penetrate into the cells, recognize diverse and specific cellular receptors and coreceptors, consisting of proteins and sugar moieties. Currently, four main protein receptors, aminopeptidase N (APN), ACE2, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), and dipeptidyl peptidase 4 (DPP4) are defined for CoVs. While almost all members of α-CoVs interact with APN as the receptor for infecting host cells, SARS-CoVs, which belong to β-CoVs group, as mentioned earlier, use ACE2 receptors. In fact, ACE2 is a type I integral membrane glycoprotein with an N-terminal extracellular domain, which contains a catalytic site with a coordinated zinc ion (Delmas et al., 1992; Li et al., 2003; Masters, 2006). SARS-CoV-2 uses ACE2 as a viral receptor to enter host cells (Wan et al., 2020a, 2020b; Zhou et al., 2020b), and ACE2 is one of the important regulators of intestinal inflammation (Hashimoto et al., 2012). Because its cell entry receptor pathway is - extensively expressed in gastrointestinal epithelial cells, high levels of SARS-CoV-2 RNA have been identified in faecal samples of infected patients (Wong et al., 2020). By analyzing single-cell RNA sequencing data, Liang et al. detected that ACE2 was highly expressed in the small intestine particularly in proximal and distal enterocytes (Liang et al., 2020) and indeed, live SARS-CoV-2 were isolated from stools of patients. Furthermore, in 23 % of patients, their stools were found virus positive, despite their respiratory samples were negative (Cipriano et al., 2020). In addition to live virus in stool, intense staining of viral nucleocapsid protein of SARS-CoV-2 RNA in the enterocytes confirm the faecal-oral transmission is an another important route that should not be ignored for viral spread (Wang et al., 2020; Xiao et al., 2020). Thus, infection of the gastrointestinal tract with SARS-CoV-2 results in shedding of virus in the environment and potentiates the human-to-human transmission (Ding and Liang, 2020). Virus detection in the stool lasts during the course of disease, as long as 12 days even after the respiratory symptoms disappear. During the SARs outbreak of the early 2000′s, around 27 % of the patients at the time suffered from diarrhea and the full-length genome sequence homology of the COVID-19 infection SARS-CoV-2 is within 79 % of SARS-CoV. Both share the identical membrane receptor, ACE2 as their cellular entry point (Donnelly et al., 2003). It is likely therefore, that the proportion of the cases presenting gastrointestinal symptoms will be much greater in patients suffering from SARS-CoV-2 infection (de Wit et al., 2016).

Similar to respiratory mucosa, both ACE2 receptor and plasma membrane-associated type II transmembrane serine proteases facilitate viral entry into the enterocytes (Hoffmann et al., 2020b; Matthai et al., 2020). Thereby, expression of two mucosa-specific serine proteases, transmembrane protease, serine 2 (TMPRSS2) and TMPRSS4, boost SARS-CoV-2 S protein fusogenic activity and assist virus penetration into enterocytes (Zang et al., 2020). Consequently, the successful SARS-CoV-2 entry into the cell primarily depends on the availability of ACE2, in addition to the TMPRSS2, which cleaves the S protein of hCoV on the cell membrane. Both proteases are crucial for fusion and are indispensable to deliver the viral contents into the enterocytes (Hoffmann

et al., 2020b; Zhang et al., 2020b). In brief, TMPRSS2 and homologous proteases facilitate SARS-CoV breaking into the cell by two distinct mechanisms. These are ACE2 dissociation that may support the viral uptake, and SARS-S dissociation, which activates the S protein for membrane fusion (Fig. 1) (Heurich et al., 2014). In this respect, antiviral activities of the serine protease inhibitors could be taken into account for the treatment of SARS-CoV-2-infected patients (Hoffmann et al., 2020b; Yamamoto et al., 2016).

Entire typical coronaviral genes are identified among the 14 potential open reading frames (ORFs) that encode the four structural proteins. These are named as the S glycoprotein, the membrane (M) protein, the envelope (E) protein, and the nucleocapsid (N) protein (Oostra et al., 2006).The E protein of SARS-CoV interacts at least with five proteins of the host. Moreover, SARS-CoV E protein carries a post-synaptic density protein-95/discs Large/zonula occludens-1 (PDZ) domain-binding motif (PBM), that is an essential element of virulence. SARS-CoVs that do not have E protein PBM are characterized by a diminished expression of inflammatory cytokines, and resultant viral attenuation (Jimenez-Guardeño et al., 2014; Teoh et al., 2010). Therefore, determination of proteins and units that interact with CoV E would ensure a better understanding for the development of more precise targeted therapeutic approach. Following the binding to the receptor, SARS-CoVs fuse their envelope with the membrane of the enterocytes to release their nucleocapsid into the host cell. All of the CoV S proteins carry the same S domains. S1, N-terminal domain (NTD) is responsible for receptor binding, and a S2, C-terminal domain (CTD) is responsible for fusion (Belouzard et al., 2012). The aminopeptidase N (APN) is a type II transmembrane protein expressed on the apical domain of epithelial cells of both respiratory, as well as enteric systems (Belouzard et al., 2012). APN is a Zn^{2+} dependent protease, and degrades peptides or proteins with a N-terminal neutral amino acid. Because of the capability of their S proteins to recognize the specific amino acid variations in APN, CoVs demonstrate tropism differences (Tusell et al., 2007). Fusion is activated by sequential dissociation of the S protein at two distinct sites. The initial dissociation at the S1-S2 boundary facilitates the second dissociation that is essential for the final fusion activation (Belouzard et al., 2012; Watanabe et al., 2008). The second dissociation eventuates directly at the N-terminal of the fusion peptide. Thus, SARS-CoV can be integrated directly to the cell surface. It is thought that this mode of virus entry is 10^2 to 10^3 times more efficient than the endosomal pathway (Matsuyama et al., 2005). TMPRSS2 and TMPRSS4 are associated with ACE2 receptor. The presence of these proteases in the extracellular medium is the essential factor of SARS-CoV tropism (Shulla et al., 2011). Thereby, TMPRSS2 presumably acts as the main factor in the initial infection and spread of the virus. The fine balance between the two antagonistic effects on SARS-CoV infection, the shedding of the receptor and fusion activation, underlines the significance of this protease (Guillén et al., 2008, 2005). Furthermore, the SARS-CoV S protein binds to human ACE2 with a robust affinity. As stated earlier, the general high sequence homology between SARS-CoV-2 and SARS-CoV is of course also seen with their S proteins (Zhang et al., 2020d). Thus, both SARS-CoVs use the same receptor while entering into the enterocytes (Wan et al., 2020a). In view of these findings, it is probable that one course for the passage of SARS-CoV-2 from respiratory system into the enterocytes of gastrointestinal tract may be the lung to gut axis (Zhang et al., 2020a).

4. SARS-CoV-2 and sialic acid

SiA is the first defined virus receptor. BetaCoVs recognize O-acetylated SiAs and comprise an acetylesterase which acts on the receptors to destroy them (Matrosovich et al., 2015). The receptor-eliminating enzyme, acetylesterase of the β-CoVs allows virus to be liberated from the infected cell, facilitating penetration into the mucus layer, and preventing aggregate formation (Fig. 1) (Storz et al., 1992; Vlasak et al., 1988). Besides binding to their specific receptors, α-, β-, and γ-CoVs

have evolved variant SiA binding activities (Hoffmann et al., 2013). SiA modifications of the carbon backbone at the C5 position yields four core molecules: Neu (C5-NH$_2$), Neu5Ac (C5-N-acetyl), Neu5Gc (C5-N-gly-colyl), and KDN (ketodeoxynonulosonic, C5-hydroxyl). SiAs pre-dominantly appear as sialylglycoconjugates on N- and O-linked glyco-proteins, in addition to gangliosides in the cellular plasma membrane (Varki, 1992). According to the concept of a dual recognition of gangliosides and ACE2 by SARS-CoV-2 S protein, while the receptor-binding domain (RBD) binds to the ACE2 receptor, the NTD binds to the ganglioside-rich domain of the cellular plasma membrane. Interest-ingly, when two hydroxychloroquine (HCLQ) molecules are bound to a ganglioside, binding of a SARS-Cov-2 S protein to the same ganglioside is completely blocked (Fantini et al., 2020b). Following the binding of RBD in S1 subunit of S protein on the virion to the ACE2 receptor on the host cell, the heptad repeat1 (HR1) interacts with HR2 domains in S2 subunit of S protein. Thereby, a six-helix bundle fusion core forms. Thus, virus and host membranes are drawn into close proximity for fusion and subsequent infection (Bosch et al., 2004). Indeed, the CoV S2 protein seems to be an ideal target for organizing and designing more universal CoV inhibitors. Potent SARS-CoV HR2 peptide inhibitors bind to the C-terminal residues of HR2 which are significantly crucial for the stability of the six-helix bundle (Aydin et al., 2014). As mentioned above, the S protein has a substantial role in virus entry into the cell. This protein assists the binding of virions to the host cell. Thereby, the subsequent fusion of the viral and the target cell membranes actualizes. The S protein of these viruses is a hemagglutinin-esterase protein and hence is the main SiA binding protein (Gagneten et al., 1995; Storz et al., 1992). The initial step of the viral replication cycle is the adhe-sion of the S viral protein to the cell surface. Besides their protein membrane receptor, hCoVs bind SiA-containing glycoproteins and gangliosides that function as the principal binding elements in gastro-intestinal tract (Matrosovich et al., 2015). Chloroquine (CLQ) is a po-tential blocker of the SARS-CoV-2 NTD and S-ganglioside interaction that becomes the initial step of the viral replication cycle (Fantini et al., 2020b). The attachment to SiA-containing cell surface structures eventuates via the receptor-binding domains on the viral S protein. This binding is achieved by the S glycoprotein in the CoVs (Hoffmann et al., 2020b; Yan et al., 2020). The interactions of the host ACE2 protein with SARS-CoV and SARS-CoV-2 are the key events of the contagiousness of viruses (Li et al., 2003). Dual recognition of SiA-containing gangliosides and ACE2 by SARS-CoV-2 S protein occurs via two diverse domains that are readily accessible for distinct types of interactions. Thus, CoVs are dependent upon gangliosides, sialylated membrane integral elements that act as attachment cofactors within lipid raft membrane (Li et al., 2017; Matrosovich et al., 2015; Park et al., 2019). Lipid rafts that are membrane domains enriched in gangliosides and cholesterol, constitute a favorable interface for efficiently positioning the viral S protein at the initiation of the infection (Fantini et al., 2020b). The RBD binds to the ACE2 receptor, and the NTD binds to the ganglioside-rich domain of the cellular membrane. Lipid raft deterioration by cholesterol exhaustion results in a significant decrease in the human SARS-CoV infection (Glende et al., 2008). CLQ is an interesting chemical structure con-structed with the integration of cationic nitrogen atoms and aromatic rings. The properties of both of these components were demonstrated to function as key factors for the identification of SiAs and gangliosides by proteins during SARS-CoV-2 infection (Fantini and Yahi, 2010; Yahi and Fantini, 2014). The World Health Organization (WHO) re-commended CLQ for malaria chemotherapy either 300 mg for max-imum 5.5 years/week or 100 mg for 3 years/day of continuous intake (WHO, 1990). The encouraging outcomes of CLQ in the treatment of COVID-19 and the low frequency of side effects of this drug in long-term use offer an application potential for CLQ (100 mg/day) or HCLQ (300 mg/week) in the mass prophylaxis of people who are likely to encounter with SARS-CoV-2 (Gendrot et al., 2020; Zhou et al., 2020a). Zinc enhances intracellular uptake of CLQ (Xue et al., 2014). Un-fortunately, CLQ did not show efficacy in inhibiting viral replication in

a rodent SARS-CoV model (Barnard et al., 2006). Nevertheless, by considering its anti-inflammatory properties, CLQ/HCLQ may have some effect on SARS-CoV, by inhibiting the production of proin-flammatory cytokines, tumor necrosis factor (TNF)-α, interleukin (IL)-6 and interferon (IFN)-γ. Consequently, CLQ/HCLQ may alleviate the inflammatory reaction raised towards the SARS-CoV-2 that is translated into the potentially deadly cytokine storm (Barnard et al., 2006; Engin et al., 2020; Mégarbane and Scherrmann, 2020; Savarino et al., 2003). Both these drugs orally effective and almost completely absorbed from the gastrointestinal tract with around 75 % bioavailability (McLachlan et al., 1994). Therefore, maximum drug levels are achieved at around 4–12 h following the oral intake. CLQ is extensively distributed through all the body (Müller et al., 2011), with significant intracellular se-questration and a long functional half-life (van den Borne et al., 1997). Both drugs may be effective choices in gastrointestinal SARS-CoV-2 infection. The presence of prolonged faecal shedding even after viral clearance in the respiratory tract of the COVID-19 patients makes the use of these two drugs potentially effective for the treatment of patients and antiviral prophylaxis of the medical personnel dealing with these patients (Ng and Tilg, 2020). On the other hand, clinical studies also demonstrated the capability of azithromycin (ATM) to diminish the viral load. At the same time, ATM interacts with the ganglioside-binding domain of SARS-CoV-2 S protein. The binding domain is also shared by ATM and ganglioside monosialotetrahexosylganglioside (GM1). HCLQ fully occupies the virus binding regions on gangliosides in the neighborhood of the main CoV receptor, ACE2. In combination therapy, while ATM is directed against the virus, HCLQ is oriented to the cellular attachment receptor (Fantini et al., 2020a). CLQ has two diverse binding domains which are bound to isolated SiAs. The first one is positioned at the edge of the saccharide moiety of the ganglioside. The carboxylated group of the SiA of GM1 is directed towards the ca-tionic groups of CLQ. The chlorine of CLQ binds to the second site at the ceramide-sugar junction axis (Fantini et al., 2020a). In fact, CLQ and HCLQ are well suited for SiAs interaction, whether the SiAs are isolated or bound to gangliosides. The ACE2 receptor is the ganglioside-binding region on the other cell-attainable domain of the S glycoprotein (Fantini et al., 2020b). In this case, CLQ inhibits quinone reductase-2, a struc-tural neighbour of uridine diphosphate (UDP)-N-acetylglucosami-ne-2-epimerases, involved in SiA biosynthesis (Mégarbane and Scherrmann, 2020). Whereas, HCLQ inhibits ACE2 glycosylation and SiA biosynthesis, thus, hinders SARS-CoV-2 interaction with its target cell receptor and subsequent virus/host cell membrane fusion (Savarino et al., 2006). Consequently, CLQ or HCLQ inhibits internalization of the virus by reducing ligand recognition and preventing the fusion of SARS-CoV-2 with the host cell. In this process, inhibition of the addition of SiA moiety to ACE2 and neutralization of the acidic pH of the endosome is effective (Savarino et al., 2006; Vincent et al., 2005). Although there is extremely limited evidence of a possible synergy between ATM and HCLQ, the HCLQ-ATM combination can be recommended at least me-chanistically as anti–COVID-19 drug, if administered early enough in the disease time course (Gbinigie and Frie, 2020; Mégarbane and Scherrmann, 2020). Nevertheless, the equilibrium problem between the anticipated synergistic impact and possible risks of cardiotoxicity con-stitutes a significant safety concern. Despite the well- described me-chanisms of action of these drugs, some of non-randomized studies have claimed that the high doses of HCLQ-macrolide combination treatment have a high mortality rate due to its adverse effects in COVID-19. Further investigations on combination therapy are recommended in these studies where dose-response analysis and severity grading of SARS-CoV-2 infection are ignored (Retraction in: Lancet. 2020 June 5 -Mehra et al., 2020).

Other CoVs have also SiA binding activity. For the members of group 2a CoVs, SiA moieties on glycoproteins are crucial receptor de-terminants for the infection (Huang et al., 2015). Like SARS-CoV-2, the enteric pathogenicity of transmissible gastroenteritis virus, which are typical virus of the genus α-CoV, arises by binding to SiAs under

unfavorable environmental conditions (Schwegmann-Wessels et al., 2011). By interacting with sialo-glycoconjugates, the virus can migrate through the SiA-rich mucus layer that coats the enterocytes (Schwegmann-Wessels et al., 2011; Schwegmann-Wessels and Herrler, 2006). Whereas, non-enteropathogenic virus that is lacking a SiA binding activity cannot bind to a mucin-type glycoprotein in brush border membranes of enterocytes (Schwegmann-Wessels et al., 2003). Ample amount of SiA in mucins facilitates the penetration of virus to the mucus layer and subsequently binding to APN on the surface of the intestinal epithelial cells. Attachment of sialylated macromolecules to the virions surface may enhance the structural conservation of transmissible gastroenteritis virus (Krempl et al., 1998). It is thought that this attachment may also protect the virus against the destructive effects of gut emulsifiers (Krempl et al., 2000).

The β-CoV group contains receptor-destroying enzyme, hemagglutinin esterase (HE), however, the precise role of HE in the course of coronavirus entry to enterocytes is still vague (Belouzard et al., 2012). Furthermore, SARS-CoV S2 is durable in a broad range of pH, enabling it to function efficiently, and fuse into the plasma membrane, and also into the endosome (Aydin et al., 2014). The CoV S protein-receptor pairing is a decisive factor of tropism. Binding to mucin-type glycoprotein (MGP) may ensure the virus remains in prolonged contact with the intestine and facilitates encountering the APN receptors for initiating intestinal infection (Schwegmann-Wessels et al., 2003). As noted, the SiA binding activity is also present in that fragment of the S protein. The availability of a hemagglutinating activity in enteric CoVs and lack of this ability in respiratory CoVs, exhibits the probability that the SiA binding activity contributes to the enterotropism of CoV (Schultze et al., 1996).

In the respiratory system, SiAs are generally fragments of glycoproteins and gangliosides. However, the SiA binding activity resides in the N-terminal portion of the S1 subunit that is related with the enteropathogenicity of virus (Schultze et al., 1996). The S protein has a trimeric structure and this maintains its SiA binding activity in soluble forms of the protein (Schwegmann-Wessels and Herrler, 2008; Shahwan et al., 2013). The S protein launches the infection by attaching to the host cell surface; it further mediates the consecutive fusion between the viral and target cell membranes. The S protein presents two binding activities. Binding to aminopeptidase N is essential for virus to trigger the infection in the host cells (Delmas et al., 1992).

5. Conclusion

The CoV S protein-receptor interaction and pairing is the key determinant of tropism. SiAs have dual functions in gastrointestinal SARS-CoV-2 infection. By interacting with sialo-glycoconjugates, the SARS-CoV-2 can move along the intestinal lumen and migrate through the SiA-rich mucus layer that coats and protects the enterocytes from viral attack. The RBD binds to the ACE2 receptor, and the NTD interacts and attaches to the ganglioside-rich domain of the plasma cell membrane. SiA moieties on glycoproteins are crucial, as well as essential receptor determinants for the infection. Binding to MGP may permit the virus to accommodate extended periods in the intestine and can be spread with stool. In this context, human waste from hospitals is thus currently one of the main viral contamination threats to the environment in municipal wastewater, which has not to date been fully appreciated as a contributor to the persistence of the COVID-19 infection in our local communities.

Transparency document

The Transparency document associated with this article can be found in the online version.

Declaration of Competing Interest

The authors declare no conflict of interest.

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