Exploring SK/S1P/S1PR pathway as a target for antiviral drug development

Sharada Prasanna Swain,*(a) Chandra Sekhara Mahanta,*(a) Madan Maurya,(b) Debabrata Mandal,(b) V. Ravichandiran,(a),(b) Vipan Parihar(b)

(a) National Institute of Pharmaceutical education and Research-Kolkata, 168, Chunilal Bhawan, Maniktala Main Road, Kolkata 700054, India.
Email: spswain2013@gmail.com; spswain@niperkolka.edu.in

(b) National Institute of Pharmaceutical education and Research-Hajipur, Export Promotion Industrial Park (EPIP) Zandaha Road, NH322, Hajipur, Bihar 844102, India.

ABSTRACT
Sphingosine-1-phosphate (S1P) is a signal transmitter. The lipid sphingosine is converted to S1P by catalysis of Sphingosine kinase enzymes (SK1 or SK2). S1P acts extracellularly as a receptor ligand. The SK1/S1P axis plays important roles in cell signalling, the cell death/survival, the production of a pro-inflammatory response, and maintaining vascular integrity. There are five subtypes of S1P receptor known as S1P1, S1P2, S1P3, SP4, and S1P5. Glycosphingolipids promote viral entry. The drug molecules targeting the SK1/S1P axis such as fingolimod, ozanimod, and ponesimod are used for treatment of multiple sclerosis. S1P receptor modulator drug fingolimod shows antiviral activity against human immunodeficiency virus. Currently, the clinical trials of ozanimod (a sphingosine receptor modulator), and opaganib (a SK2 inhibitor) are being conducted for treatment of COVID-19. It is worth to target SK/S1P pathway to develop antiviral drugs, by repurposing existing inhibitors/modulators, and designing new specific inhibitors of SK1, SK2, and SP receptor.

Key words: Sphingosine-1-phosphate, sphingosine kinase, virus, cytokine storm.

1. Introduction
Sphingolipids are essential components of all eukaryotic membranes, which contain a sphingoid base, and a fatty amino alcohol called sphingosine. De novo synthesis of the sphingoid base starts with serine palmitoyl transferase catalysed condensation of palmitate and serine, generating dihydrosphingosine. Then (dihydro)ceramide synthase enzymes facilitate amino-acylation with a chain of 14-32 carbons to form various dihydroceramide.1 Dihydroceramides are desaturated to give ceramides and complex sphingolipids, like glycosphingolipids and sphingomyelin. Sphingomyelin is hydrolysed by sphingomyelinases during catabolism to regenerate ceramide. Ceramide is a bioactive lipid which is deacylated by ceramidases to form sphingosine. Sphingosine is phosphorylated by sphingosine kinase 1 (SK1) and (SK2) to sphingosine-1-phosphate (S1P). S1P is a potent signalling molecule, which can be irreversibly degraded by S1P lyase (SPL) or dephosphorylated to sphingosine, which can then be re-acylated back to ceramide [1]. It is the quick compartment-specific interconversion of these three metabolites, with distinct effects on cell fate that forms the biochemical basis of “sphingolipid rheostat”, as proposed in the year 1996 (Figure 1).2
S1P and ceramide play major roles in regulation of cell growth and survival by modulation of opposing signalling pathways.\textsuperscript{[2-4]} The elevation of ceramide level induces cell growth arrest and apoptosis,\textsuperscript{[4]} whereas S1P production is essential for optimal cell proliferation, induced by growth factors\textsuperscript{[5]} and suppresses ceramide-promoted apoptosis.\textsuperscript{[2]} The interconversion between S1P and ceramide in sphingolipid rheostat is a sensing mechanism for cells to regulate their fate.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{sphingolipid_rheostat.png}
\caption{Sphingosine rheostat. After de novo synthesis in the endoplasmic reticulum (ER), ceramide is transported to the Golgi apparatus. Ceramide is the starting material for the synthesis of sphingolipids (SL) like sphingomyelin (SM) and glycosphingolipids (GSL). SL are transported to the plasma membrane and SM is converted to Ceramide by sphingomyelinase. Ceramide is degraded to sphingosine by ceramidase. Sphingosine phosphate (S1P) is synthesized by phosphorylation of sphingosine by sphingosine kinase enzymes (SK1 or SK2).}
\end{figure}

S1P can play role as a signalling molecule intracellularly and extracellularly as a receptor ligand. The sphingolipid rheostat plays vital roles in viral replication, activation/modulation of the immune response, and maintaining vasculature integrity. The S1P receptors are key factors in vascular protection against endotheliosis.\textsuperscript{[6]} The SK1/S1P axis has significant roles in the production of a pro-inflammatory response, immunomodulation, and control of vascular integrity. The SK1/S1P axis going to be an attractive target for
development of drug candidates for treatment of cancer and inflammatory disorders. The pathology of infectious disease is associated with control of cell death/survival and proinflammatory immune responses. Sphingosine-1-phosphate is also involved in other vital biological processes like Ca\(^{++}\) mobilization, cell growth, motility, and cytoskeleton organisation, which is mediated by phospholipase D.

2. Role of Sphingolipids in viral entry to host cell and infection

As the replication of viruses is strictly intracellular, the interaction of viruses with cellular membranes is one of the key components of their life cycle. They also need most prominent membrane lipids such as sphingolipids (SL). The role of steady state SL metabolism and regulation by pathogens in their respective host cells has been extensively studied.\(^{[9]}\) The topological accumulation of SLs in cellular compartments in the context of viral infection is studied by high resolution quantitative analysis and microscopy.\(^{[10]}\) Whole cell lipidomic analyses have demonstrated that viruses modulate the SL pool in their host cells. The role of SLs for specific steps within the viral replication process has been studied at a cellular level. The use of novel genetically modified cell, tissue and animal models have helped in more detailed understanding of the role of SL metabolism in viral pathogenesis. This kind of studies help in understanding both viral replication, host responses, especially composition and function of the immune compartment. These research data will definitely be indispensable to pinpoint and optimize SL targets for therapeutic purposes.

2.1. Attachment and entry of virus

Strong attachment to the host cell plasma membrane initiate, then facilitate viral replication and therefore, the role of SLs in these processes has been studied. The role of membrane microdomains enriched for specific SLs and the impact of their accumulation, metabolism there has been investigated with regard to receptor segregation, membrane biophysical alterations, and initiation of signalling cascades associated with uptake and trafficking.\(^{[9,10]}\)
Fig. 2. Role of sphingosine metabolism in interaction with virus. Membrane domains contain GSLs, which are involved in cellular signal initiation, and facilitate viral host cell entry. In HIV, the GSL globo-triarylceramide interact with the V3 loop within HIV gp120 envelope protein subunit, and promote virus entry. Glucosylceramide (GC) levels are very essential for endosomal uptake of influenza A virus (IAV), and Ebola virus. There uptake is sensitive to depletion of both anabolic and catabolic GC enzymes. The interaction of membrane-bound Gb4Cer (globo-tetraosyl-ceramide) causes rearrangement of the viral parvovirus B19 capsid, which is essential for subsequent steps of internalization in cells.\cite{6}

The complex sphingolipids are transported from the golgi apparatus to the plasma membrane, and then, conversion of sphingomyelin (SM) to ceramide by sphingomyelinase (SMase) happens there. The conversion of glycosphingolipids (GSL) takes place by the stepwise catalysis, facilitated by specific hydrolases. Ceramide is degraded by ceramidase (CDase) to sphingosine. Phosphorylation of sphingosine by sphingosine kinase (SK1/SK2) to S1P occurs. S1P degrades by S1P phosphatase (SPP) to sphingosine or by S1P lyase (SPL) to hexadecenal and phospho-ethanolamine. Interaction with host cell membranous compartments is highly essential for replication of viruses and is very much dependent on SL metabolism.\cite{6}

2.2. Glycosphingolipids (GSL) facilitating Human Immunodeficiency Virus (HIV) entry

Membrane domains enriched in GSLs are recognised as functional entities associated with cellular signal initiation, and may directly promote viral host cell entry. The GSL Gb3 (globo-triaryl-ceramide) and galactosyl-ceramide (Gal-Cer), interact with the V3 loop within HIV gp120 or the HIV gp41 envelope protein subunit, respectively. This initiates their interaction with chemokine receptors and promote HIV uptake into
CD4-negative cells like mucosal epithelial cells. Then transmission HIV to CD4+ T cells or DCs occurs. The evidence of role of GSLs in HIV entry is further proved by its sensitivity to compounds affecting GSL biosynthesis like D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), which inhibits glucosyltransferase activity, and to variations in cellular GSL content. In case of Fabry disease patients, the high level accumulation of Gb3 can also act as a resistance factor for HIV infection. It is predicted that the differential effects of Gb3 on HIV entry rely on its turnover, and compartmentalization to membrane microdomains might be dependent on the respective host cell. Bosque and co-workers investigated whether targeting S1P pathway would inhibit HIV-1 infection and generate the latent reservoir in primary CD4 T cells. Fingolimod (Gilenya), an FDA-approved from Novartis, an antagonist of S1P receptors, blocks cell-free, cell-to-cell transmission of HIV and thereby reduces detectable latent virus. Fingolimod interferes the HIV-1 life cycle at two levels: (i) reduces the surface density of CD4, and inhibits viral binding and fusion; (ii) decreases the phosphorylation of the innate HIV restriction factor SAMHD1, which is associated with reduced levels of total and integrated HIV, and reduced expression of Cyclin D3.

2.3. Connecting S1P/S1PR1 with influenza viral infections

SK, S1P, and S1P receptors as key modulators of pulmonary diseases has been reported. S1P/S1PR signalling involvement in pulmonary inflammation caused by viral infections, such as the influenza virus is demonstrated. The mortality of patients with the H5N1 strain of influenza was linked to high pharyngeal virus loads and hypercytokinemia. S1PR1 is expressed on the endothelial cells and lymphocytes in lung. In infectious diseases, the tracking of innate immune cells, macrophage polarisation, and plasmacytoid dendritic cell functions are influenced by S1P1. The binding of extracellular S1P binding to S1P1 plays an important role in inflammation by the activation of intracellular inflammatory signalling pathways during viral infections. During influenza (H1N1) viral infection, S1PR1 agonists protected mice and ferrets against acute immunopathologic damage in influenza infections. Using an endothelial cell-specific inducible S1P1 knockout mouse line (S1PR1-ECKO), S1PR1 gene ablation resulted in an increased inflammation, lung injury, severe exudation, and oedematous with vascular haemorrhaging and increase in inflammatory cell infiltration, in response to H1N1 influenza virus challenge.

The role of endothelial S1PR1 in virus pulmonary injury was studied using the endothelial cell-specific inducible S1PR1 knockout mouse line (S1PR1 ECKO). The floxed S1PR1 allele with a Tek-CreERT2 transgene expresses a TM-inducible Cre recombinase under the control of the Tek promoter. S1PR1loxP/loxP Tek-CreERT2 mice were injected with TM, thus deleting S1PR1 in a several tissues. The S1PR1 protein level was remarkably decreased in most of the organs such as lung, heart, liver, kidney and spleen. The study was monitored by immunofluorescence staining with almost complete loss of S1PR1 immunofluorescence in the pulmonary endothelium. Mice with endothelial S1PR1 deletion possessed reduced survival relative to similarly infected control mice. It is also investigated that whether S1PR1 gene ablation led to an increased degree of virus infection-induced inflammatory response. After 5 days of infection,
histological examination of S1PR1 ECKO, mouse lung damage with severe consolidation of pulmonary tissue, vascular haemorrhage, excessive exudation in alveolar air sacs, hyaline membrane formation, and significant inflammatory cell infiltration were observed. The levels of IFN-alpha, TNF-alpha, IFN-γ, CCL2, CCL3, CCL5, CXCL2, CXCL10, and IL-6 were significantly high in ECKO S1PR1 mice compared to control mice.\textsuperscript{[22,23]}

2.4. Role of sphingosine kinase enzyme in Dengue virus (DENV) and Chikungunya virus (CHIKV) infections

Dengue virus is a mosquito-borne human pathogen which causes mild to severe dengue haemorrhagic fever and also dengue shock syndrome. Severe dengue haemorrhagic fever proinflammatory, vasoactive cytokines and chemokines levels are elevated.\textsuperscript{[24,25]} In late stage of infection, DENV-infected cells show decreased SK1 activity and is associated with increased TNF-α-stimulated death.\textsuperscript{[26]} This could be a natural DENV-infected cell clearance process that would not be supposed to be associated with pathogenic levels of pro-inflammatory mediators seen in severe DENV diseased by analogy and in combination with known function of sphk1 in promoting the development of proinflammatory mediators. The SK1 inhibitors may have dual benefits in severe DENV disease by decreasing DENV-induced production of inflammatory mediators and facilitating clearance of DENV-infected cells. However, macrophages are a target for DENV replication and DENV can enter into these cells by phagocytosis and antibody-dependent uptake through Fc receptors.\textsuperscript{[27]} These DENV entry pathways are parallel to SK1-dependent phagocytosis found with \textit{C. neoformans} and Mycobacterium that involve SK1 for microbial killing.\textsuperscript{[28,29]} SK1/S1P plays important role in the control of vascular leakage.\textsuperscript{[30-32]} Hence, decrease in late SK1 activity in DENV-infected cells, especially if this happens in ECs, where \textit{in vivo} DENV infection has been observed,\textsuperscript{[33,34]} can play a crucial role in DENV-induced haemorrhage. In this case, intracellular DENV replication and vascular leakage would be aggravated by therapeutic inhibition of SK1. In CHIKV infection, the higher levels of pro-inflammatory mediators such as TNF-α, IFN-γ, monocyte chemotactic protein1, and macrophage migration inhibitory factor are found, similar to DENV.\textsuperscript{[35-38]} Inflammation is observed locally in joint and muscle tissue and is accompanied with immune cell infiltration in CHIKV. In vivo experiments have proved that macrophages play a major role in the development of inflammatory mediators in CHIKV disease via the development of inflammatory mediators, and this can be enhanced by treatment with agents that inhibit the synthesis of monocyte chemotactic protein 1.\textsuperscript{[38,39]} The long-term joint disease observed in CHIKV infections is due to the persistence of viral replication in affected joints.\textsuperscript{[40]} Both CHIKV and RRV can cause disease pathologies that are similar to rheumatoid arthritis (RA) pathogenesis,\textsuperscript{[41]} where SK1 and S1P are significant mediators. S1P levels are higher in synovial fluids in patients with RA,\textsuperscript{[42]} there is elevated expression of SK1, S1P1 in the synovium,\textsuperscript{[43,44]} and synovial fibroblasts express higher levels of SK2.\textsuperscript{[45]} In a mouse model of collagen induced arthritis, inhibition of SK1 activity decreased S1P level and joint pathology.\textsuperscript{[46]} Whereas, in a mouse model of TNF-α-induced arthritis, inhibition of SK1 decreased inflammation and bone erosion.\textsuperscript{[47]} S1P lyase inhibitor reduced peripheral circulating...
lymphocytes in mice model and remarkably delayed the start of arthritis in a rat model.\textsuperscript{[48]} Whereas, this same arthritis model emerges with normal occurrence and incidence in SphK1 knock out mice.\textsuperscript{[49]} S1P lyase inhibitor administration can also be expected to raise the level of S1P in the joints, which is associated with deteriorating joint pathology. However, some tissue selectivity was seen in the increased S1P levels in the reaction to this inhibitor of S1P lyase.\textsuperscript{[48]} This suggests a strong need for more detailed study to clarify the pathways that control localized S1P homeostasis in joint inflammatory pathology. However, these results indicate that systemically increasing S1P, or reducing SphK1 activity and S1P locally in joints, may have beneficial effects in RA, and S1P lyase inhibitors are under study for RA.\textsuperscript{[48]} While there is currently no data showing changes in SphK1/S1P in CHIKV and infections, parallels in the joint pathogenesis of RA indicate that SphK1/S1P may play a role in these viral diseases. The available \textit{in vivo} models of RRV\textsuperscript{[50]} would provide useful systems to assess the effectiveness of systemic administration of S1P lyase inhibitors or the local function of SphK1 and S1P in the joints for viral infection pathology, viral infection, immune infiltrates, and the long-term persistence of viral joint tissue.

2.5. \textit{SK/S1P/S1PR role in cardio protection in SARS CoV-2 infection}

There is possibility of long-term or permanent heart damage in extreme cases of COVID-19 infection. S1P signalling possess cardio protection, by maintaining cardiac cell survival and function.\textsuperscript{[51]} Receptors for S1P are available in cardiac vascular endothelial and smooth muscle cells, and also in cardiac fibroblasts.\textsuperscript{[51]} The S1P receptors are associated with the remodelling, differentiation, and proliferation of cardiac fibroblasts (mainly S1PR3).\textsuperscript{[51a]} S1PR2 and S1PR3 mediated cardio protection from ischaemia/reperfusion injury is reported (S1PR knockout studies in mice).\textsuperscript{[51a]} During post myocardial infarction (MI) associated with the proinflammatory response, increase in cardiac S1P, SK1 and S1PR1 were observed.\textsuperscript{[51b]} SARS-CoV-2 infection causes endothelial damage, resulting in vasculitis and systemic inflammatory vascular diseases. S1P potentially regulates the function of endothelial cell barrier, and inhibits the SARS-CoV-2 to infect the endothelial cells. These results indicate that S1P has significant role in controlling damages caused by COVID-19 infection.\textsuperscript{[52]}

2.6. \textit{S1P/S1PR pathway and vascular integrity}

The S1P-S1PR signalling pathway plays a major role in maintaining vascular endothelial integrity. The post mortem reports of COVID-19 patients reveal that catastrophic vascular endothelial failure is a distinctive symptom of severe SARS-CoV-2 infection.\textsuperscript{[53,54]} Loss of vascular endothelial integrity is one of the major causes of severe COVID-19 symptoms like hyperinflammation, oedema, and tissue ischaemia. Abnormalities in this signalling network led to serious consequences like morbidity and death. The roles of S1P signalling in the vasculature are studied and proved by using \textit{in vivo} mouse models and \textit{in vitro} primary human umbilical vein endothelial cells (HUVEC).\textsuperscript{[55-59]} As per the study report, the premature death of SK1 and SK2 deficient mice from haemorrhages occurred. This could be a dysfunction in vascular development.\textsuperscript{[60]} S1P is essential
for angiogenesis and vasculogenesis, blood pressure homeostasis, barrier protection and integrity, vascular tone by modulating SIPR1-3, in the migration and differentiation of endothelial cells lining the inside wall of the blood vessels.[61-63] Pathophysiology of the endothelial cells is closely associated with lung disorders, cardiovascular diseases, heart failures, has great impact on morbidity and death.[64]

2.7. *Sphingosine pathway and neurodegeneration in viral infection*

Thromboembolic events including cerebral venous system thrombosis are being frequently observed in COVID-19 patients.[65,66] SK/S1P regulates all the different brain cell populations, neural development and survival. The role of SK/S1P is termed to be a “double-edged sword” in the brain.[67] SK/S1P mediate signalling pathways involved in the infiltration of peripheral immune cells in the CNS during neuroinflammation. However, the protective immune response can change into chronic neuroinflammation, which may lead to neurodegeneration, and synaptic losses.[68,69] S1PR1 and S1PR2 are expressed in the blood vessels of the brain and regulate distinguishable cellular responses during an acute ischaemic event. The activation of S1PR1 protects blood–brain-barrier (BBB) function and reduces inflammation. Whereas, S1PR2 facilitates BBB loss of integrity and bring out proinflammatory phenotype.[70] Therefore, selective S1PR1 ligands, especially at the cerebrovascular level, are gaining attention of neuroscientists as effective modulators of stroke pathogenesis, as it preserves BBB integrity and prevents the development of vascular inflammation.[71]

3. *Emergence of novel corona virus (COVID-19)*

Coronavirus disease 19 (COVID-19) is caused by the new beta coronavirus (2019-nCoV), which is also called as pneumonia-associated respiratory syndrome (PARS), and sudden acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[72-74] As the outbreak of 21st century, SARS-CoV-2 is very quickly evolved. The number of COVID-19 cases has exceeded 330 million as of January 2022, and more than five million confirmed deaths worldwide.[75] Although about 80% of all cases are either symptomatic or have moderate signs with no documented chronic health conditions, 20% of patients experience extreme acute respiratory distress syndrome (ARDS), dyspnoea, reduced oxygen saturation, and pulmonary infiltrates.[76] The harsh symptoms of COVID-19 infection are hyperinflammation, disastrous damage to the vascular endothelium, thrombotic problems, brain damage, acute neurological and psychiatric complications. The hyperinflammatory leads to as “cytokine storm syndrome”, blood clotting, followed by multiorgan dysfunction, and these are major causes of death in SARS CoV-2 infection. The human body will show an inflammatory response to inhibit the excess manufacture of the virus particles. This hyperinflammation will dysregulate the immune response and produce excess cytokines and chemokines (cytokine storm), which will eventually lead to the attack on infected cells, and normal cells as well.[77] In cases where COVID-19 patients develop critically low blood-oxygen readings, blood clots in the lungs of patients may restrict oxygenated blood flowing through the lungs. The study showed that CD8+ T cells and NK cells infiltrated the lungs of
SARS CoV infected mice at the late stage of infection. Generally, CD4+ T cells are required for viral clearance instead of CD8+ T cells, whereas CD8+ T cells were associated with lung pathology during viral infection.\(^{[78]}\) S1P analogs can block the infiltration of immune cells with inflammatory phenotype, particularly CD8+ T cells, which secrete TNF-a or IFN-g, which may prevent acute lung injury during COVID-19 infection.

The sphingolipid rheostat plays a major role in regulating viral replication, hyperinflammatory immune response, and maintaining vascular endothelial integrity.\(^{[79]}\) Therefore, sphingosine kinase (SK), sphingosine-1-phosphate (S1P), and the S1P cognate receptors (G-protein-couple receptors) are attractive targets for developing drugs or therapies to control viral replication, hyperinflammation, and help in the maintenance of vascular endothelial integrity. The SK/S1P/S1PR pathway could be considered for developing therapeutic techniques to provide symptomatic relief from, and battle the serious unprecedented effect of COVID-19.

3.1. Immuno-pathology in Corona virus (CoV) infection

Prior to the emergence of novel SARS-CoV-2, MERS-CoV, and SARS-CoV were considered to be extremely pathogenic. These two CoVs causes acute lung pathology due to cytokine storm in infected patients, which becomes lethal if left untreated.\(^{[80]}\) Earlier research in cynomolgus macaques monkeys with SARS-CoV infection, led to increased expression of IFN-a, IFN-b, and IFN-g, IFN-1 at mRNA level in the lungs of infected macaques.\(^{[81]}\) Similarly, SARS-CoV infection showed increased pro-inflammatory cytokine expression in BALB/c mice, leading to fatal acute lung injury in mice and elevated TNF-a at mRNA level in the lungs of infected mice.\(^{[82]}\) As per in vitro infection studies, both SARS-CoV and MERS-CoV upregulated the expression of TNF-a, IL-6, and IL-12 at mRNA level in monocytes derived macrophages.\(^{[83]}\) SARS-CoV-2 infection is showing a similar trend in cytokine profile as with SARS-CoV and MERS-CoV.\(^{[77,84,85]}\) In Serious situation of COVID-19 patients, increased levels of serum cytokines IL-2, TNF- a, IL-1b, IFN-g, MCP-1, MIP1A, and IL-6 were found.\(^{[84,86]}\) The cytokine storm may lead to life-threatening complication called ARDS, and may occur more commonly in elderly COVID-19 patients.\(^{[85,87,88]}\) It is also found that patients with ARDS have decreased serum S1P levels compared to healthy controls.\(^{[89a]}\) Serum S1P level detection in patients with COVID-19 encourages virologists for further investigation, because it acts as an ARDS biomarker. In COVID-19 patients, acute lung injury/acute respiratory distress syndrome (ALI/ARDS) is triggered by a cytokine storm. Studies using tocilizumab, an IL-6 receptor inhibitors have shown promising results, which supports this hypothesis.\(^{[89b]}\) Hence, it is worthy to consider the use of drugs or combination of drugs with broader ability to inhibit these cytokine receptors.\(^{[89b]}\)

3.2. Sphingolipid pathway involvement in corona virus infection

Enveloped RNA viruses like SARS-CoVs are highly dependent on the host’s lipid biosynthesis. There are several reports confirming the vital role of sphingolipid pathway and SK/S1P intracellular signalling in viral permissiveness and replication.\(^{[90-95]}\) Sphingosine and ceramide were found to interfere with the uptake of virus particles of COVID-19 into epithelial cell lines, especially primary human nasal cells in culture.\(^{[96]}\)
Ceramide facilitates the SARS CoV-2 viral entry, whereas sphingosine blocks. Acid sphingomyelinase (ASM) is essential for the generation of ceramide. The SARS-CoV-2 entry into the epithelial cells was reduced by functional inhibition of ASM using drugs like amitriptyline. The stimulation of S1PR1 might also provide relief from the hyper-inflammatory conditions associated with SARS-CoV-2 infection. Sphingosine was found to prevent and also eliminate bacterial infections of the respiratory tract. Edwards et al. studied whether sphingosine can also prevent viral infections.\(^9\) To prove this hypothesis, they tried to understand whether sphingosine regulates the infection of cultured and freshly isolated ex vivo human epithelial cells with pseudoviral particles expressing SARS–CoV-2 spike (pp-VSV–SARS–CoV-2 spike). They observed that exogenously applied sphingosine suspended in 0.9% NaCl prohibits cellular infection with pp-SARS–CoV-2 spike. Pretreatment of freshly isolated human nasal epithelial cells with sphingosine blocked adhesion of and infection with pp-VSV–SARS–CoV-2 spike.\(^9\) Sphingosine phosphate level is reduced in patients with COVID-19 compared with a healthy human.\(^9\) High density lipoprotein (HDL) is the major carrier for S1P in the plasma, and hence, this decrease in S1P could be a consequence of the decrease in HDL levels in patients with COVID-19, as observed in patients with sepsis\(^9\). There are also reports of viral exploitation of SK/S1P pathway by influenza,\(^1\) measles,\(^2\) hepatitis B,\(^3\) dengue virus,\(^4\) the respiratory syncytial virus (RSV), hepatitis B virus (HBV), and hepatitis C viruses (HCV).\(^5\)

3.3. Sphingosine and binding of SARS CoV-2 with ACE2 receptor

Sphingosine possess antibacterial activities\(^6\) and antiviral activities.\(^7\) SARS-CoV-2 enter through the upper respiratory track, and it raises the question whether sphingosine could have a role in the virus infection. Recent studies in a model system of epithelial cell cultures and infection with pseudoviral particles containing the spike protein of SARS-CoV-2 showed that sphingosine can intermeddle with the binding of the SARS-CoV-2 to ACE2 receptor.\(^8\) Sphingosine is an amphiphilic molecule, bearing charges at one end of a saturated fatty acid and the hydrophobic, hydrophilic interactions with ACE2 are likely to facilitate the binding.

3.4. Effect of COVID-19 on neurological system

Coronaviruses are found to be neurotropic.\(^9\) The majority of COVID-19 patients show mild neurological symptoms like headaches, loss of smell and other neurological symptoms, which indicates that coronavirus is affecting the brain.\(^10\) Other serious neurological symptoms experienced by few COVID-19 patients are encephalitis, encephalopathies, acute disseminated encephalomyelitis and myelitis, cerebrovascular disease, seizures, ischaemic strokes, intracerebral haemorrhages, and altered mental health status.\(^11\) Recently, there is an unusual increase in cerebral venous thrombosis in COVID-19 patients creating a high risk of acute ischaemic strokes.\(^12\) The cerebrovascular endothelium plays a major role in maintaining blood vessel integrity and cerebrovascular homeostasis. Disruption of the vascular endothelial barrier and ensuing hyperinflammation are key symptoms of COVID-19 infection. Inflammation and loss of
endothelial integrity led to brain oedema and poststroke neuronal injury. SARS-CoV direct invade the central nervous system (CNS), as reported in the brains of patients, in animal models, and spread of the virus through the synapse-connected route to the medullary cardiorespiratory centre.\textsuperscript{[84]} The ACE2 receptor which is essential for SARS-CoV-2 cell entry, is present in brain vascular endothelium, and may promote direct viral entry into the host brain cells.\textsuperscript{[113]} The SARS-CoV-2 may cause direct damage to host brain through the unprotected olfactory bulb, carriage across the blood–brain barrier (BBB) following viraemia, or by infected leukocytes. Alternatively, hyperinflammation due to the body’s immune response (innate and adaptive) may cause brain damage.\textsuperscript{[113]} Damage of olfactory cell and smell loss are found in patients after SARS-COV-2 infection and this could be due to an impaired neurogenesis. S1P is functions as a pro-survival factor and a regulator of stem cell proliferation. Hence, S1P production and its transport outside of the olfactory cells, might be essential for basal cell proliferation. Therefore, there is possibility of effect of SARS-CoV-2 on S1P generation and release in the microenvironment of olfactory epithelium cells.\textsuperscript{[115]} Further experimental evidences are required. Recently, a possible case of Parkinson’s disease after SARS-CoV-2 infection has been reported.\textsuperscript{[116a]} This has raised serious concerns that there is possibility of COVID-19 patients developing Parkinson’s disease. Hence, there is a need for monitoring of all COVID-19 patients for stroke\textsuperscript{[117]} and potentially Parkinson’s disease, as well.\textsuperscript{[116b]} SARS-CoV-2 antigens have been found in sustentacular cells and Bowman glands in post mortem tissue of COVID-19 infected patients.\textsuperscript{[118a,118b]} The same was also confirmed in experimentally inoculated hamsters, mice, and ferrets.\textsuperscript{[118c-e]} SARS-CoV-2 can infect choroidal epithelial cells, as studied in choroid plexus organoids.\textsuperscript{[119]}

3.5. Treatment of COVID-19 infection—targeting vasculature failure

Vasculature failure is one of the major causes of deaths in COVID-19 infection.\textsuperscript{[54,120]} About 40% of COVID-19-related deaths are due to cardiovascular complications, multiorgan inflammation, such as lungs, ear, kidneys, liver, bowel, and severe blood clots. These data indicates that COVID-19 infection also causes vascular infection in preference to a purely respiratory problem. Hence, the conventional antiviral therapies may not be sufficient and a drug that stabilises the vascular endothelium could be used along with antiviral therapy for COVID-19 patients. However, treatments with cardiovascular drug statins for COVID-19 patients come with a disadvantage like they upregulate the ACE2 receptor (the receptor for SARS-CoV-2).\textsuperscript{[121,122]} Therefore, anti-inflammatory agents, immunosuppressive drugs, along with antiviral drugs, oxygen therapy, intubation, and protective mechanical ventilator support, has been suggested to manage symptoms for severely affected COVID-19 patients.

3.6. SK/S1P/S1PR and treatment for Relief of COVID-19 Symptoms

There was no drug specific for remedy of COVID-19, till molnupiravir was approved by FDA. Systematic approach is needed for this multifaceted complex disease to block viral replication and also get relief from the acute and chronic symptoms of COVID-19 disease. Sphingolipid signalling plays a very
important role in viral replication, immune response activation, and in preserving the integrity of the vasculature. Modulation of sphingolipid signalling has shown several positive effects, which includes reducing inflammation, specific vascular barrier defence, managing blood clot, cardio protection, and neuroprotection, which are symptoms of COVID-19 patients. Hence, targeting of SK-S1P-S1PRs to relieve the acute and chronic symptoms of COVID-19 (SARS-COV-2) infection is worth considering. FDA-approved sphingolipid-based drugs are already existing for immune-based diseases, and repurposing of these drugs to treat COVID-19 disease is being considered and few of them currently in clinical trials.

3.7. S1P receptor modulators as an adjunctive therapy in COVID-19 treatment

The sphingosine receptor modulators as drugs for treatment of multiple sclerosis (MS) are Fingolimod, Siponimod, Ozanimod and Ponesimod, as described in figure 3. Fingolimod (Gilenya) developed by Novartis, is an US FDA drug for treatment of MS. It is the first-in-class sphingosine 1-phosphate (S1P) receptor (S1PR) modulating agent approved for the treatment of the relapsing forms of multiple sclerosis (MS). There are 5 subtypes of S1PR called S1P1, S1P2, S1P3, S1P4, S1P5. Fingolimod is not specific to any subtypes of S1PR. Whereas, Siponimod, Ozanimod and Ponesimod are specific for S1PR1 and S1PR5. Ozanimod was approved by the US FDA and Health Canada for the treatment of relapsing multiple sclerosis in the year 2020. Ozanimod targets S1P1 and S1P5 receptors with high affinity. S1P1 ligands plays major role in controlling vascular leakage in the airways. The ligands like ozanimod which do not modulate S1P3 receptor, possess an excellent safety profile. Though S1P1 ligands have mild impact, but do not compromise viral clearance. They reduced lung injury in preclinical studies, when used without any combination of antivirals and exhibited a synergistic effect when associated to antiviral agents. Ozanimod does not possess cardiac conduction abnormalities, fibrosis, hypertension in clinical studies. Hence, it could be considered safer in comparison to fingolimod. The phase-II clinical trial of ozanimod for treatment of COVID-19 patients is under progress. The virologists hypothesize that this immune modulator can mitigate the morbidity and mortality of COVID-1. The clinical trial of opaganib, a SK2 inhibitor is under progress for treatment of COVID-19 Pneumonia. A phase 2/3 clinical trial in adult subjects hospitalized with severe SARS-CoV-2 positive pneumonia is being studied. The potential of opaganib to improve and/or stabilize the clinical status of the patient are being monitored. Opaganib is an SK2 inhibitor, which shows antiviral and anti-inflammatory activities. Hence, opaganib was considered to have beneficial effect for moderate to severe COVID-19 pneumonia. The clinical trial studies of opaginib on COVID-19 pneumonia hospitalized patients was conducted to evaluate the safety and effect on supplemental oxygen requirements and time to hospital discharge. Forty-two enrolled patients were enrolled and received opaganib (n = 23) or placebo (n = 19). The patients were subjected to standard of care for up to 14 days. Then, they were followed up for 28 days after receiving their last dose of opaganib/placebo. No safety concerns were observed during the study. The incidence of ≥ Grade 3 treatment-emergent adverse events was 17.4% in the opaganib, whereas there was
33.3% in case of placebo groups. Three deaths happened in each group. The patients receiving oral opaganib required less supplementary oxygen and also had earlier hospital discharge, in comparison to placebo.

![Chemical structures of drugs](image)

**Fig. 3.** Drugs targeting sphingosine receptors (S1PR) and SK2.

4. Treatment of other viral infections

4.1. Possibility of therapeutic application of S1P analogs for treatment of H1N1 infection

S1P pathway as a possible target for providing therapeutic benefits in pulmonary disease has been studied. When mice challenged with H1N1 infection was treated with intra-tracheal AAL-R [(R)-2-amino-4-(4-heptyloxyphenyl)-2-methylbutanol], a S1P analogue, it showed better survival relative in comparison to traditional antiviral therapy. Walsh et al. has reported that intra-tracheal administration of AAL-R in H1N1 infected mice, resulted in decreased lung tissue damage. The proinflammatory cytokines such as IFN-a, IL-6, and IFN-g, and chemokines like CCL2, CCL3, CCL5, CXCL2, and CXCL10, were also reduced in the bronchoalveolar lavage (BAL) fluid of these mice. However, treatment with AAL-R does not clear the viral load, but it does not hinder the host's capacity to clear the viral load. Treatment of mice infected with influenza virus with RP-002, an agonist of S1PR1, decreased the mortality by reducing cytokines/chemokines (CCL2, IL-6, IFN-a, and IFN-g). RP-002 also increased survival of paramyxovirus PMV infection in mice, reduced inflammation in lungs with normal morphology of alveolar sacs of infected mice. Secretion of IFN-g, TNF-a, CCL2, CCL5, CXCL10, IL-1a, and IL-6 was also reduced in BAL fluid, and CD8+ T, Natural Killer (NK) cells in the lung infiltrate of infected mice were decreased, while treated with RP-002.

Zhao et al. studied therapeutic benefits of CYM5542, a S1PR1 agonist, in the H1N1 infected mice. The intra-tracheal delivery of CYM5542 resulted in a decline in lung injury and improved therapeutic efficacy when used along with oseltamivir, an antiviral drug. Role of CYM5542 in mitogen-activated protein kinase (MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) has been investigated. CYM5542 decreased ERK1/2, JNK1/2, p65 subunits of NFkB, leading to inactivation of MAPK, and
production of cytokines.\textsuperscript{[22,129]} CYM5542 also facilitated the degradation of interferon alpha receptor1 (IFNAR1), deactivation of signal transducer and activator of transcription 1 (STAT1), and thereby reduced the IFN-α response.\textsuperscript{[130]}

4.2. Targeting SK enzyme and S1P receptor for treating cytokine storm

Significant efforts have been devoted by medicinal chemists to design inhibitors, modulators targeting Sphingolipid-S1P-SK pathway. Potential anti-cancer agents, specific inhibitors of SK have been developed. At present, Fingolimod (Gilenya) and AAL-R, which are sphingosine analog pro-drugs that can be phosphorylated by SK2 to generate S1P receptor agonists. The Fingolimod and AAL-R may act as high-affinity agonists for all S1P receptors, except S1P2. However, stimulation often leads to downregulation of the S1P1 receptor, so these agents can also be considered as functional S1P1 antagonists too.\textsuperscript{[131]} The advantages of sphingosine analogs and S1P receptor modulators in viral infections have been studied in animal models. High-level proinflammatory responses in the lung are found in influenza virus infection, which are responsible for lung injury. Teijaro et al. have reported that endothelial cells in the lung are essential to the cytokine storm and innate cell recruitment during influenza virus infection, and these two processes are dependent on S1P/S1P1.\textsuperscript{[20,132]} Therefore, S1P receptor is worth considering as a suitable target for the influenza-induced cytokine storm.\textsuperscript{[17,133]}

4.3 Limitations of S1P analog therapy in viral infection

The above mentioned studies predict that S1P analogues could be a potential therapy against COVID-19. The possible risks or limitations associated with S1P analogues should also be discussed. The cytokine storm leads to acute lung injury that results in ARDS, in severe cases of SARS-CoV-2 infection. Therefore, it is highly essential to maintain cytokine homeostasis in response to pulmonary infection by targeting pro-inflammmatory immune cells or activating anti-inflammatory pathways.\textsuperscript{[134]} However, targeting pro-inflammatory immune cells may limit the capacity of the host to clear the infection. Therefore, careful use of S1P analogs along with anti-viral therapy is suggested to ensure clearance of infection without undermining the host defence. The activation of S1P signalling during \textit{M. tuberculosis} infection has dual action of host protection or disease progression, depending on stage of \textit{M. tuberculosis} infection. S1P treatment during early infection reduces infection and disease associated histopathology. Whereas, S1P treatment during acute \textit{M. tuberculosis} infection aggravate the disease.\textsuperscript{[135]} S1P analogues has been proven to be safe and approved by FDA for treatment of neurological disorder like Multiple Sclerosis, and studies for Crohn’s disease are under progress.\textsuperscript{[136]}
5. Conclusion and future directions

The SK1/S1P axis, sphingosine rheostat is a well-known central component of cell death/survival, inflammation, immune function, and vascular integrity. There are FDA approved therapeutics and many agents are under development stage, for S1P-receptor-specific targeting and inhibition of SK1, SK2 activities to get selective control of SK1/S1P functions. Effective inflammatory immune responses are crucial in curtailing virus infection. When the virus invades the host cell, the human body’s immune system shows a hyperinflammatory response (cytokine storm), as observed in the case of many COVID-19 patients. A common COVID-19 symptoms are loss of vascular integrity, leading to the failure of major organs of the body, such as lungs, heart, and brain. Specific targeting of the SK/S1P/S1PRs signalling pathway can manage many of the severe complications of COVID-19, like reduction of the hyper inflammation, with preserving vascular endothelial integrity and preventing multiorgan failure (pulmonary, neurological and cardiovascular symptoms). It may also help in reduction/prevention of blood clot formation, and stopping rapid clinical patient deterioration. Currently, clinical trial of S1P analogs ozanimod, and opaganib are being conducted for COVID-19 treatment. Phosphorylation of fingolimod (Gilenya) by SK2 provides active fingolimod-phosphate, and it inhibits S1P receptor signalling, and thereby, reduce inflammation. Fingolimod has been reported as a promising therapy approach for treatment and prevention of HIV. It prevents viral spread in human CD4+ T cells by reducing surface density of this receptor. Fingolimod decreases the phosphorylation of the SAMHD1, an HIV restriction factor, which is associated with decreased levels of total and integrated HIV, and also reduces the expression of Cyclin D3. Targeting the SK/S1P/SPR pathway with specific SK inhibitors and SPR modulators could be a novel strategy to inhibit HIV, COVID-19, dengue and chikungunya virus replication.

The phase II clinical trial data of opaganib on COVID-19 patients shows early discharge and had less supplementary oxygen requirement. Severe COVID-19 patients have low lymphocyte count. When patients were treated with opaganib, there was significant increase in lymphocyte count.\[137]\ The faster increase in lymphocyte count might indicate better prognosis for the treatment group. Hence, drug molecules targeting SP/SK/SP receptor have potential to be considered as an effective therapy for treatment of COVID-19 patients.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References


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