Neutralizing the free radicals could alleviate the disease severity following an infection by positive strand RNA viruses

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Abstract
Free radical release due to oxidative stress is gaining importance in the field of viral pathogenesis. Recent studies suggest the involvement of oxidative stress and ROS levels in regulating disease virulence during RNA virus infection. Most of the RNA virus infections lead to vascular dysfunction and disease severity. However, the biology of free radicals in maintaining vascular endothelium integrity is not completely understood. In the present review, we discuss some of the common features in positive-strand RNA virus infections such as dengue and SARS-CoV-2 and suggest that anti-oxidant therapy could pave the way to develop therapeutic strategies in combating emerging and re-emerging RNA viruses.

Keywords RNA virus · ROS · Anti-oxidant therapy · COVID-19 · Endothelial activation

Abbreviations
ssRNA Single strand RNA
ECs Endothelial cells
ROS Reactive oxygen species
ACE2 Angiotensin converting enzyme 2
DENV Dengue virus
SARS-CoV-2 Severe acute respiratory syndrome coronavirus–2
Nrf 2 Nuclear factor erythroid 2-related factor 2
COVID-19 Coronavirus disease 2019
Nox2 NADPH oxidase 2

One-third of the virus genera are made up of positive single-strand RNA (+ssRNA) viruses. This includes clinically important and deadly pathogens like severe acute respiratory syndrome coronavirus–2 (SARS-CoV-2), dengue virus (DENV), and hepatovirus A. The viruses utilize host factors for initial access, cell invasion, and replication processes. Perhaps most importantly, studies have suggested that these RNA viruses, upon entry into the host, make use of oxidative stress–induced ambience for their genome capping and replication, thereby contributing towards disease severity (Reshi et al. 2014). In addition, +ssRNA can also alter the gene expression or reprogram the function of host-cell defense mechanisms by co-opting host factors (Nagy and Pogany 2011). Thus, anti-oxidant therapy might be a potential therapeutic approach in combatting RNA viruses.

The endothelium is a continuous monolayer of endothelial cells (ECs) aligned along the direction of blood flow and plays an important role in regulating vascular integrity within the blood vessel wall (Shin et al. 2017). Upon stimulation, the endothelium undergoes a specific alteration in its phenotype referred to as “endothelial activation” which is characterized by enhanced expression of endothelial selectins, increased endothelial-leukocyte interaction, and permeability. Evidence suggests that ROS-mediated modulation of signal transduction pathways (activation of transcription factor AP-1 and activation of NFκB and p38 MAPK pathways) on ECs are key signaling mechanisms for endothelial activation (Alom-Ruiz et al. 2008). Oxidative stress–induced activation of ECs and platelets is presumed to contribute towards disease virulence during virus infection. The endothelium could dynamically elicit responses that may
contribute to the hyper-inflammation and altered vascular permeability during ssRNA viral infection, especially in the case of DENV infection (Dalrymple and Mackow 2012b, a). Thus, a proper understanding of the participation of ECs in stabilizing fluid barrier functions of the endothelium may lead to developing a therapeutic approach for reducing vascular leakage in the severe form of the disease (Dalrymple and Mackow 2012a). Though there are several factors like cellular interactions, cell–cell aggregation, and direct binding of the viral proteins that are presumed to be the causes for endothelial dysfunction, the actual mechanism remains obscure. Most importantly, the activation of ECs during the critical phase of infection when there is no virus in circulation makes us assume that there exist alternative means of host responsive factors that regulate endothelial permeability.

An imbalance in the production of reactive oxygen species (ROS) and the inability of the host to detoxify ROS results in oxidative stress. The resulting oxidative stress is associated with pro-inflammatory cytokine release, as was reported for severe cases of dengue fever (Soundravally et al. 2014). Recently, we have reviewed the generation of ROS by endoplasmic reticulum (ER) and mitochondria that leads to the hyper-inflammatory response and disease severity in ssRNA viral infections like dengue, HIV, HBV, and HCV (Pillai et al. 2019). On one hand, some of the ssRNA infections alter the status of the mitochondrial chaperone prohibitin, resulting in dysregulation of the mitochondrial respiratory chain leading to the cause of ROS overproduction (Dang et al. 2011). On the other hand, virus replication is directly associated with protein oxidation in the ER and enhances ROS production and oxidative stress (Paracha et al. 2013). This leads to oxidative stress–induced cellular damage and disease severity (Reshi et al. 2014). A recent study has shown that the envelope (E) protein of SARS-CoV-2 binds to the extracellular iron or haem. This E-protein-haem bounded complex in turn produces oxygen and water and then converts them to $O_2^-$, $H_2O_2$, and hydroxyl radicals leading to ROS attack (Wenzhong and Hualan 2021). In addition, excessive production of ROS disrupts the lysosomal membrane and releases hydrolases. This results in autophagy in phagocytes and causes subsequent cell death followed by a strong cytokine storm and organ failure (Wenzhong and Hualan 2021).

Interestingly, ROS is identified as an essential component of the host response to viral infections. For instance, recent experimental findings have suggested that DENV infection induces intracellular ROS levels that regulate the activation of innate antiviral immune responses and stimulate apoptosis. Thus, a further understanding of the molecular details underlying the biological targets of ROS during DENV infection may facilitate the identification of novel treatment strategies for dengue-associated diseases. Parallel activation of antioxidant pathways regulated by Nrf2 also contributes to the regulatory control of antiviral and apoptotic responses by maintaining redox homeostasis. However, excess ROS can hamper this equilibrium and thus ROS was identified as an essential component of the host response to DENV infection (Olagnier et al. 2014; Pillai et al. 2019).

Another important downstream pathway of ROS is the MAPK signaling pathway (Son et al. 2011). Recently, it has been demonstrated that the NS1 protein of DENV activates the p38 MAPK pathway, thereby contributing to the hyper-permeability of ECs in vitro (Barbachano-Guerrero et al. 2020). Similarly, the p38 MAPK pathway was reported to be upregulated in SARS-CoV-2 (Ma et al. 2020), SARS-CoV (Kopecky-Bromberg et al. 2006), and other respiratory viral infections (Börgeling et al. 2014). In this view, Grimes and Grimes et al. suggested MAPK inhibitors could be an effective therapeutic and promising approach for the management of coronavirus disease 2019 (COVID-19) (Grimes and Grimes 2020). An overview of the role of ROS, various associated signaling pathways, and disease severity during RNA virus infection is depicted in Fig. 1. Potentially useful inhibitors targeting some of the signaling pathways during ssRNA virus infection are listed in Table S1.

### Endothelial dysfunction and SARS-CoV-2

In the case of SARS-CoV-2 infection, the participation of EC and EC injury in the disease pathogenesis has been described by Varga et al. (2020). In this line, biopsy of SARS-CoV-2-infected lungs exhibited mononuclear and polymorphonuclear aggregation accompanied by apoptotic ECs (Varga et al. 2020). Pulmonary endothelium serves as a selective barrier between the plasma and interstitium. Any drastic change in the endothelium will have an effect on the barrier function that leads to lung injury and pulmonary edema. In the case of COVID-19 pathogenesis, the molecular mechanism of endothelial dysfunction/dysregulation is not completely understood.

From the available literature on the disease mechanism exhibited by SARS-CoV-2, it shares some of the below-mentioned properties with dengue viral infection in terms of disease pathogenesis.

(i) Activation of ECs and vascular dysfunction in severe COVID-19 cases — the vascular tone which is regulated by the endothelium is affected by the infection of the ECs by the virus and the presence of viral inclusion structures in ECs (Varga et al. 2020); expression of ACE2 receptors by ECs favors the virus to infect ECs and leads to cell death (Beyerstedt et al. 2021).
(ii) Activation of the coagulation pathway with disseminated intravascular coagulation in severe cases (Zhou et al. 2021).

(iii) Patients with severe disease had thrombocytopenia (low platelet count) as compared to those of non-severe cases (Lippi et al. 2020).

(iv) Cellular interactions and adhesions of platelet-leukocyte and ECs during the infection (Canzano et al. 2021; Mariappan et al. 2021; Balakrishna Pillai et al. 2022).

(v) The coagulopathy observed in severe cases may be due to the results of inflammatory responses and endothelial activation or damage (Zhou et al. 2021).

(vi) Elevated fibrin degradation products (D-dimer) and fibrinogen in severe cases (Poudel et al. 2021).

(vii) Excessive production of ferritin, macrophage activation, and ROS production leads to the release of free radicals, which convert Fe (II) to Fe (III) resulting in cellular apoptosis and coagulation. Elevated oxidative stress is associated with pro-inflammatory cytokines and cytokine storms (Perricone et al. 2020).

(viii) ROS is also capable of activating calcium and NF-κB signaling to induce adhesion molecules and pro-inflammatory cytokines, which can increase vascular permeability and promote leukocyte adhesion. A recent study suggests that oxidative stress caused by Nox2 activation contributes to COVID-19 pathogenesis and is associated with thrombotic events in COVID-19 patients (Violi et al. 2020). Therefore, the beneficial effect of antioxidant drugs on endothelial function should be considered for the treatment of COVID-19 in the future.

**Could neutralizing free radicals stop cytokine storm and disease severity in COVID?**

Though there are many studies on the association of oxidative stress response and viral infection, it is currently not known how the free radicals released by mitochondria and ER could trigger the hyper-inflammatory response. To the same degree, how the excess production or decreased scavenging of ROS and the inflammatory signal could activate macrophages, mast cells, leukocytes, platelets, and ECs and dysfunction is not clear. For instance, during inflammation, chemical substances released by macrophages and
subsequently by mast cells activate EC signaling pathways, which target structural elements such as actin and myosin that regulate vascular permeability. Evidence indicates that SARS-CoV-2 infection could affect capillary endothelium by inducing endothelial inflammation and contribute to COVID-19 severity (Jin et al. 2020). When vascular endothelium is exposed to various blood-borne pathogens or agents, the activated neutrophils could release a large amount of ROS into the circulation via membrane-bound NADPH oxidase during the neutrophil respiratory burst, causing host tissue injury and endothelial barrier dysfunction. In addition to this, vascular endothelium is the primary target for oxidants released by the activated blood cells at the site of injury or inflammation. These cellular events lead to reduced nitric oxide bioavailability, impairment of vascular tone, and alteration in endothelial phenotype (upregulation of adhesion molecules, MMP activity, formation of intercellular gaps, hyper-permeability, and leukocyte transmigration) (Alom-Ruiz et al. 2008; Incalza et al. 2018). Thus, in excess conditions, ROS might play a crucial role in the activation of ECs, resulting in vascular leakage as observed in dengue and COVID-19 patients. In this context, serotonin inhibitors, a class of anti-depressants with potential anti-viral, immune-modulatory, and anti-oxidant properties, are proposed to alleviate SARS-CoV-2 disease virulence (Hamed and Hagag 2020). In addition, treatment and/or supplementation with antioxidant drugs or compounds has shown to reduce the expression of various endothelial cell proteins (MMP-9, VEGF, PECAM-1, ET-1, and Syndecan-1) thereby protecting the vascular endothelial membrane (Reiter et al. 2010; Fang et al. 2013; Lee et al. 2014; Almatroodi et al. 2020). More research should be done on the antioxidant-oxidant status that is specifically altered in their expression during the course of viral infection, for developing modalities based on anti-oxidant-based COVID-19 and dengue management. Some of the potential antioxidants like curcumin, N-acetyl-L-cysteine, zinc, resveratrol, catechin, vitamin C, and vitamin D are currently under various phases of clinical trials in controlling respiratory virus severity (Delić 2021; Sherkawy 2021; Lai-Becker 2022). Most importantly, a patient suffering from a severe form of COVID-19 along with pneumonia cannot be amenable to any anti-oxidant therapy. Anti-oxidants should be administered in the early course of infection, before the development of pneumonia, which helps to prevent excessive ROS release (Lapenna 2021) and hyper-inflammatory responses, thus alleviating the disease severity caused by RNA viruses (Soto et al. 2020). In the above context, a list of antioxidant drugs and herbal extracts undergoing pre-clinical evaluation against RNA viral disease is mentioned in Table S2.

Thus, anti-oxidant therapy could help the supportive strategies, thereby improving the outcomes in patients with COVID-19 and other diseases caused by RNA viruses. Developing strategies for supplementing antioxidant therapy may augment the current disease management of debilitating positive-strand RNA virus diseases.

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