



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



ELSEVIER



REVIEW ARTICLE

Oxidative Stress as Key Player in Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Infection

Livan Delgado-Roche^a and Fernando Mesta^b

^aGerencia de Investigación Clínica, Dirección Médica, Laboratorios Liomont S.A. de C.V., Ciudad de México, México

^bEscuela Nacional de Medicina y Homeopatía, Instituto Politécnico Nacional, Guillermo Massieu Helguera 239, La Escalera, Gustavo A. Madero 07320, Ciudad de México, México

Received for publication April 12, 2020; accepted April 24, 2020 (ARCME_2020_477).

The emergence of viral respiratory pathogens with pandemic potential, such as severe acute respiratory syndrome coronavirus (SARS-CoV), the pathogenic agent of Covid-19, represent a serious health problem worldwide. Respiratory viral infections are, in general, associated with cytokine production, inflammation, cell death, and other pathophysiological processes, which could be link with a redox imbalance or oxidative stress. These phenomena are substantially increased during aging. Actually, severity and mortality risk of SARS-CoV-2 infection or Covid-19 disease have been associated with the age. The aim of the present work was to contribute with the understanding of the possible link between oxidative stress and the pathogenesis, severity and mortality risk in patients affected by SARS-CoV infection. © 2020 IMSS. Published by Elsevier Inc.

Key Words: SARS-CoV, Virus infection, Oxidative stress, Covid-19.

Introduction

Respiratory viral infections represent a group of diseases that affect millions of people worldwide, mainly kids and elderly people. Respiratory viruses, which comprises influenza (IV), human respiratory syncytial (HRSV), human rhinovirus (HRV), human metapneumovirus (HMPV), parainfluenza, and adenovirus and coronavirus (CoV), can infect the upper and/or lower respiratory tract in humans. Many of them cause common clinical signs and symptoms, including nasal congestion, cough, sore throat, and fever, or more specific and severe manifestations, such as bronchiolitis, pneumonia and severe acute respiratory syndrome (1). Respiratory viral infections are, in general, associated with cytokine production, inflammation, cell death, and other pathophysiological processes, which could be link with a redox imbalance or oxidative stress (OS). It is known that an overproduction of reactive oxygen species (ROS) and antioxidant mechanisms deprivation are crucial for viral replication and the subsequent virus-associated disease (2).

Severe acute respiratory syndrome coronavirus (SARS-CoV) first emerged in China's Guangdong Province in November 2002 (3). CoVs are a large family of single-stranded RNA viruses, which can infect animals and humans, causing respiratory, gastrointestinal, hepatic, and neurologic diseases (4). Nowadays, this pathogen has become the center of global attention, due to the recent spread of a new strain, named SARS-CoV-2 (previously 2019-nCoV), pathogenic agent of Covid-19 disease. SARS-CoV-2 has expanded from Wuhan province throughout China and is being exported to a growing number of countries, some of which have seen onward transmission (5). To date (April 7th), SARS-CoV-2 has affected to 1.430.453 persons and caused 82,133 deaths in the world (6). Covid-19 infection is characterized by fever, severe pneumonia, RNAemia, combined with the incidence of ground-glass opacities, and acute cardiac injury. Of note, a significant high blood levels of cytokines and chemokines have been observed in patients with Covid-19 infection. The “cytokine storm” trigger a proinflammatory environment which is strongly associated with a severe tissue damage, contributing with the fatal outcomes of Covid-19 patients (7). It is well established the link between inflammation and OS (8). Thus, the aim of the present work was to review the role of OS in the pathogenesis and progression of respiratory diseases caused by SARS-CoVs.

Address reprint requests to: Livan Delgado-Roche, Dr, PhD, Gerencia de Investigación Clínica, Dirección Médica, Laboratorios Liomont S.A. de C.V., Carretera México Toluca 5420, Colonia El Yaqui, Alcaldía Cuajimalpa de Morelos, 05320, Ciudad de México, México. Phone: (+52) (55) 5814-1510; FAX: (+52) (55) 5814-1200; E-mail: ldelgadoroche@gmail.com

Oxidative Stress Physiology and Pathology

OS has been defined as a disturbance in the prooxidant-antioxidant balance in favor of the prooxidants, leading to cell damage (9). However, the current knowledge on the redox signaling pathways has been reconceptualized OS, including two main mechanisms: (1) macromolecular damage, and (2) disruption of thiol redox circuits, which leads to aberrant cell signaling and dysfunctional redox control (10,11). The disruption of redox circuits caused by specific reaction with the redox-sensitive thiol elements, altered pathways of electron transfer, or interruption of the gating mechanisms (11) play important roles in physiology and physiopathology.

High levels of ROS generation due to pollutants, toxins, as well as viral infections in airways, are associated with OS causing cellular damage. Several respiratory viruses induce a dysregulated ROS formation, as a result of increased inflammatory cell recruitment at the site of infection. Also, viral infections disrupt antioxidant mechanisms, leading to unbalanced oxidative-antioxidant status and subsequent oxidative cell damage. While exposure to several pro-oxidants usually leads to nuclear factor Nrf2 activation and upregulation of antioxidant response elements expression, respiratory viral infections have been associated with inhibition of Nrf2 pathways and/or NF- κ B signaling activation, leading to inflammation and oxidative damage (12).

Oxidative Stress and SARS-CoV Infection

While there is a clear correlation between OS markers and the severity of many viral diseases such as hepatitis C (HCV), for SARS-CoV clinical data is limited. However, in the preclinical setting, many lines of evidence suggest that overproduction of ROS and a deprived antioxidant system plays a major role in the pathogenesis of SARS-CoV infection, as well as in the progression and severity of the respiratory disease. Experimental animal models of severe acute respiratory syndrome have shown an enhanced ROS levels and disturbance of antioxidant defense during SARS-CoV infection (13). Some authors suggest that the onset of severe lung injury in SARS-CoV infected patients depends on activation of the oxidative stress machinery that is coupled with innate immunity and activates transcription factors, such as NF- κ B, resulting in an exacerbated proinflammatory host response (14). Lin and coworkers (15), showed that SARS-CoV 3CL^{pro} (a viral protease) caused a significant increase in ROS production in HL-CZ cells, which in turn, is involved in 3CL^{pro}-induced cell apoptosis. In this study, the authors demonstrated that SARS-CoV 3CL^{pro} activates NF- κ B-dependent reporter gene, which was correlated with the increase of ROS levels in HL-CZ cells. Consensus NF- κ B sites exist in the promoters of apoptosis related genes and proinflammatory genes. Therefore, the authors suggest that ROS-activated NF- κ B signal transduction pathway, induced by SARS-CoV 3CL^{pro}, might be considered a key player in SARS-CoV pathophysiology.

In addition, other SARS-CoV's protease, the 3a protein, has been associated with the activation of mitochondrial cell death pathways (intrinsic and extrinsic signaling). The proposed mechanism involves Bax oligomerization and higher levels of p53 in 3a protein-expressing Huh7 cells, which depended on the p38 MAPK activation in these cells (16). Mitogen-activated protein kinase (MAPK) are a family of serine/threonine that are activated in response of environmentally stresses including oxidative stress, DNA damage, carcinogenic stimuli and viral infections. Activated (phosphorylated) forms of all MAPK members has been detected in cells infected with SARS-CoV (2).

Apoptosis induced by human CoV (HCoV) infection has been studied by immunopathologic techniques, in which hallmarks of apoptosis were observed in SARS-CoV-infected lung, spleen, and thyroid tissues. Also, activation of apoptosis induced by SARS-CoV, in particular HCoVs, was described in previous *in vitro* studies and animal models (17). Apoptosis can be induced by multiple mechanisms in HCoV-infected cells. CoV was shown to induce caspase-dependent apoptosis, needed for viral replication (18). Literature reports suggested that SARS-CoV replicates in Vero-E6 cells inducing a weak Akt signaling pathway activation, which cannot prevent apoptosis induced by SARS-CoV infection (19). Activation of the PI3K/Akt signaling pathway by a variety of viruses is thought to be involved in the establishment of latent and chronic infections by allowing virus-infected cells to escape from apoptosis. Activation of the PI3K/Akt signaling pathway may lead to a delay in apoptosis of host cells, and the virus life cycle might be completed before apoptosis of host cells takes place. Thus, cellular apoptosis facilitates the spread of the virus in the organism (20). A recent study demonstrated experimentally that rhein, an anthraquinone natural compound, suppresses influenza A virus (IAV)-induced acute lung injury *in vitro* and *in vivo* by arresting inflammation, IAV-mediated oxidative stress and IAV-induced activation of TLR4, Akt, p38, JNK, and NF- κ B signal pathways (21).

Oxidative stress - NF- κ B - toll-like receptor (mainly TL4) signaling pathways, triggered by viral pathogens like SARS-CoV, may further amplify the host inflammatory response, ultimately leading to acute lung injury. TLR4-TRIF-TRAF6 signaling was identified as a pathogenic pathway that can mediate the severity of acute lung injury. The oxidized phospholipid, generated by lung macrophages, was able to induce cytokine overproduction and lung injury via TLR4-TRIF. Oxidized phospholipids have been previously identified in human and animal lungs infected with SARS virus. In *in vivo* models, loss of TLR4 or TRIF expression protected mice from H5N1-induced acute lung injury (ALI). In addition, deletion of ncf1, which can regulate ROS generation, improves the severity of IV-induced ALI. Thus, these authors suggest that oxidative stress and innate immunity play key roles in the severity of ALI caused by respiratory viruses (22).

In the clinical setting, Shao and coworkers (23) observed an upregulation of mitochondrial genes and genes responding to oxidative stress in peripheral blood mononuclear cells (PBMC) of convalescent SARS-CoV patients. Some of these genes, including PRDX1, FTH1, and FOS are sensitive to oxidative stress, showed a remarkable elevation. In addition, stress response protein DNAJB1, differentiation-associated gene IFRD1, cytokine IL-1B, and other genes were overexpressed in PBMC of SARS-CoV infected patients. These results support the association between oxidative stress, inflammation and the pathogenesis of SARS-CoV infection (23).

Oxidative Stress and Ageing in SARS-CoV Infection

The severity and mortality risk of SARS-CoV-2 infection or Covid-19 disease have been associated with the age (24). Based on current data, the mean case fatality rate for adults aged under 60 is estimated to be less than 0.2%, compared with 9.3% in those aged over 80. Furthermore, comorbidities such as diabetes, obesity, and hypertension increased mortality risk by five times, however the risk seems to be lower for younger patients than older subjects (25). In a large study analyzed 4021 confirmed cases of CoV pneumonia, the results showed that 1 052 (26.2%) patients were aged at 60 years or older. The observed mortality rate of patients aged 60 years and over (5.3%) was significantly higher than that of patients under 60 years (1.4%) (26). On the other hand, a retrospective study of patients with Covid-19, who were hospitalized in Hainan Provincial People's Hospital during January–February 2020, showed that elderly patients (32.14%) were more likely to progress to severe disease than youngsters (27).

It is well established that antioxidant mechanisms deprivation together with oxidative damage accumulation occur during ageing process (28,29). It has been observed that in aged mice there are more severe SARS-CoV-induced lung lesions than in young-adult mice. The transcription profile in aged mice generally indicated a stronger proinflammatory environment than in young animals. It is suggested that age related accumulated oxidative damage and a weakened antioxidant defense system cause a disturbance in the redox balance, resulting in increased reacting oxygen species. Therefore, ageing is not only associated with alterations in the adaptive immune response, but also with a proinflammatory state in the host. In macaques, a stronger host response to virus infection than young adult macaques, with an increase in differential expression of genes associated with inflammation, with NF- κ B as central player, has been documented (30).

Conclusions

In conclusion, literature evidence suggests that oxidative stress and chronic low-grade inflammation in the lungs

are associated with aging and may contribute to age-related immune dysfunction and mortality risk in aged patients affected by respiratory virus infections, such as SARS-CoV-2.

Acknowledgments

The authors would like to acknowledge all the healthcare professionals which are in the first line against Covid-19 disease worldwide.

Conflict of Interests

The authors declare no conflict of interests.

References

1. Kuchar E, Miśkiewicz K, Nitsch-Osuch A, et al. Pathophysiology of Clinical Symptoms in Acute Viral Respiratory Tract Infections. *Adv Exp Med Biol Respir* 2015;6:25–38.
2. Khomich OA, Kochetkov SN, Bartosch B, et al. Redox biology of respiratory viral infections. *Viruses* 2018;10:E392.
3. Gu J, Korteweg C. Pathology and pathogenesis of severe acute respiratory syndrome. *Am J Pathol* 2007;170:1136–1147.
4. Weiss SR, Leibowitz JL. Coronavirus pathogenesis. 1st ed. *Advances in Virus Research*. Elsevier Inc 2011;81:85–164.
5. Lipsitch M, Swerdlow DL, Finelli L. Defining the Epidemiology of Covid-19 - Studies Needed. *N Engl J Med* 2020;382:1194–1196.
6. Johns Hopkins University. Coronavirus COVID-19 Global Cases by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU). Available from: <https://coronavirus.jhu.edu/map.html>. Accessed March 29, 2020.
7. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;395:497–506.
8. Sies H. Oxidative stress: A concept in redox biology and medicine. *Redox Biol* 2015;4:180–183.
9. Sies H. Oxidative stress: From basic research to clinical application. *Am J Med* 1991;91:S31–S38.
10. Jones DP. Redefining Oxidative Stress. *Antioxid Redox Signal* 2006;8:1865–1879.
11. Jones DP. Radical-free biology of oxidative stress. *Am J Physiol Cell Physiol* 2008;295:C849–C868.
12. Komaravelli N, Casola A. Respiratory Viral Infections and Subversion of Cellular Antioxidant Defenses. *J Pharmacogenomics Pharmacoproteomics* 2014;5.
13. van den Brand JMA, Haagmans BL, van Riel D, et al. The pathology and pathogenesis of experimental severe acute respiratory syndrome and influenza in animal models. *J Comp Pathol* 2014;151:83–112.
14. Smith JT, Willey NJ, Hancock JT. Low dose ionizing radiation produces too few reactive oxygen species to directly affect antioxidant concentrations in cells. *Biol Lett* 2012;8:594–597.
15. Lin CW, Lin KH, Hsieh TH, et al. Severe acute respiratory syndrome coronavirus 3C-like protease-induced apoptosis. *FEMS Immunol Med Microbiol* 2006;46:375–380.
16. Padhan K, Minakshi R, Towheed MAB, et al. Severe acute respiratory syndrome coronavirus 3a protein activates the mitochondrial death pathway through p38 MAP kinase activation. *J Gen Virol* 2008;89:1960–1969.
17. Krahling V, Stein DA, Spiegel M, et al. Severe Acute Respiratory Syndrome Coronavirus Triggers Apoptosis via Protein Kinase R but Is Resistant to Its Antiviral Activity. *J Virol* 2009;83:2298–2309.

18. Fung TS, Liu DX. Human Coronavirus: Host-Pathogen Interaction. *Annu Rev Microbiol* 2019;73:529–557.
19. Mizutani T, Fukushi S, Saijo M, et al. Importance of Akt signaling pathway for apoptosis in SARS-CoV-infected Vero E6 cells. *Virology* 2004;327:169–174.
20. Hirata N, Suizu F, Matsuda-Lennikov M, et al. Inhibition of Akt kinase activity suppresses entry and replication of influenza virus. *Biochem Biophys Res Commun* 2014;450:891–898.
21. Wang QW, Su Y, Sheng JT, et al. Anti-influenza virus activity of rhein through regulating oxidative stress, TLR4, Akt, MAPK, and NF- κ B signal pathways. *PLoS One* 2018;13:1–19.
22. Imai Y, Kuba K, Neely GG, et al. Identification of Oxidative Stress and Toll-like Receptor 4 Signaling as a Key Pathway of Acute Lung Injury. *Cell* 2008;133:235–249.
23. Shao H, Lan D, Duan Z, et al. Upregulation of mitochondrial gene expression in PBMC from convalescent SARS patients. *J Clin Immunol* 2006;26:546–554.
24. Fauci AS, Lane HC, Redfield RR. Covid-19 — Navigating the Uncharted. *N Engl J Med* 2020;382:1268–1269.
25. Jordan RE, Adab P, Cheng KK. Covid-19: risk factors for severe disease and death. *Bmj* 2020;368:m1198.
26. Liu Y, Gayle AA, Wilder-Smith A, et al. The reproductive number of COVID-19 is higher compared to SARS coronavirus. *J Travel Med* 2020;27.
27. Liu K, Chen Y, Lin R, et al. Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients. *J Infect*, 2020;. <https://doi.org/10.1016/j.jinf.2020.03.005>. [Epub ahead of print].
28. Gil del Valle L, Gravier Hernández R, Delgado Roche L, et al. Oxidative Stress in the Aging Process: Fundamental Aspects and New Insights. In: *ACS Symposium Series*, 2015. pp. 177–219.
29. Davies KJA. The Oxygen Paradox, oxidative stress, and ageing. *Arch Biochem Biophys* 2016;595:28–32.
30. Smits SL, De Lang A, Van Den Brand JMA, et al. Exacerbated innate host response to SARS-CoV in aged non-human primates. *PLoS Patho* 2010;6:e1000756.