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REVIEW

NITRIC OXIDE PATHWAY AS A POTENTIAL THERAPEUTIC TARGET IN COVID-19

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Abstract

The Covid-19 viral infection is linked to a severe pulmonary reaction, leading to an acute lung injury in a large percentage of affected patients. Various inflammatory pathways regulated by SARS-CoV-2 are under-investigated. Some potential therapeutic options aimed to alleviate the inflammatory response of SARS-CoV-2 infection are involving different strategies for blocking the activation of its binding receptors on host cells and immunomodulation. Given that excessive lung inflammation is likely to cause death in Covid-19 patients, using nitric oxide (NO) pathway to mitigate this risk appears to be a reasonable approach to avoid serious lung injury. Such therapy could be paired with systemic immunomodulatory therapy to combat the multiple organ damage of Covid-19. Therefore, regulating the NO pathway has a potential therapeutic strategy to minimize the mortality of SARS-CoV-2 infection.

Rezumat

Infecția virală Covid-19 determina o reacție pulmonară severă, provocând leziuni acute la un procent mare de pacienți. Căile inflamatorii activate de SARS-CoV-2 sunt incomplet cunoscute. Unele opțiuni terapeutice care vizează ameliorarea răspunsului inflamator în infecția cu SARS-CoV-2 implică strategii diferite la nivelul legării acestuia de celulele gazdă precum și un efect imunomodulator. Având în vedere că inflamația pulmonară excesivă poate provoca moartea pacienților cu Covid-19, utilizarea căii oxidului nitric (NO) pentru a atenua acest risc pare a fi o abordare rezonabilă pentru a evita leziunile pulmonare grave. O astfel de terapie ar putea fi asociată cu terapia imunomodulatoare sistemică pentru a combate leziunile multiple ale organelor afectate de Covid-19. Prin urmare, reglarea căii NO este o strategie terapeutică potențială pentru a reduce mortalitatea infecției cu SARS-CoV-2.

Keywords: nitric oxide, SARS-CoV-2, Covid-19, thymoquinone

Introduction

A novel coronavirus reported in late 2019 as a cluster of Pneumonia cases in Wuhan city in China. It spreads dramatically around the world and accompanied by a staggering number of cases in many countries. The World Health Organization (WHO) named the disease "Covid-19" which caused by coronavirus2 (SARS-CoV-2). The understanding of Covid-19 is evolving, and WHO and the United States Centers for Disease Control and Prevention (CDC) have issued interim guidance for the disease [1, 2]. Certain investigational drugs such as hydroxychloroquine, azithromycin, tocilizumab, lopinavir-ritonavir and favipiravir have been used as treatment options. It is imperative to acknowledge however that there are no evidence-based data supporting the use of such therapies and their efficacy in Covid-19 is largely undefined. Other interventions of interest but with limited clinical data include interferon beta and convalescent plasma. More recently, the US Food and Drug Administration (FDA) has approved the experimental anti-viral drug remdesivir for emergency use in treatment of Covid-19 (www.fda.gov). Therefore, there is a clear priority to develop new strategies to combat this pandemic by better understanding the pathogenesis of the infection.

Nitric oxide and virus infections

Nitric oxide (NO) is an essential molecule for many physiological and disease-based processes. Indeed, NO has several biological effects such as muscle relaxation, in neurotransmission and also plays a vital role in body homeostasis [3-7]. NO is synthesized by the enzyme nitric oxide synthase (NOS), which comprises three known isoforms: endothelial (e), inducible (i), and neuronal (n) [3]. Both eNOS and nNOS) are constitutively expressed and regulated by transcription and post-transcription processes [8]. On the contrary, iNOS is released *de novo* in response to inflammation. Interestingly, the enzyme iNOS is expressed in leukocytes including neutrophils and macrophages [9-11]. In the defence against virus, iNOS is connected to phagocytosis following the activation by IFN-γ 12-14]. For example, in influenza-induced pneumonia, NO release is greatly reduced in IFN-γ deficient mice [3]. Moreover, the iNOS-inducing

capacity in broncho-alveolar fluid of pneumonia caused by influenza virus is mostly due to the effect of IFN-γ as indicated by an immuno-adsorption investigation using an anti-IFN-γ antibody [15]. These results emphasize the role of IFN-γ in iNOS upregulation and overproduction of NO in viral infections [15-19]. Since macrophages are the leading producers of NO during viral infections, this can significantly contribute to organ injury (Figure 1).

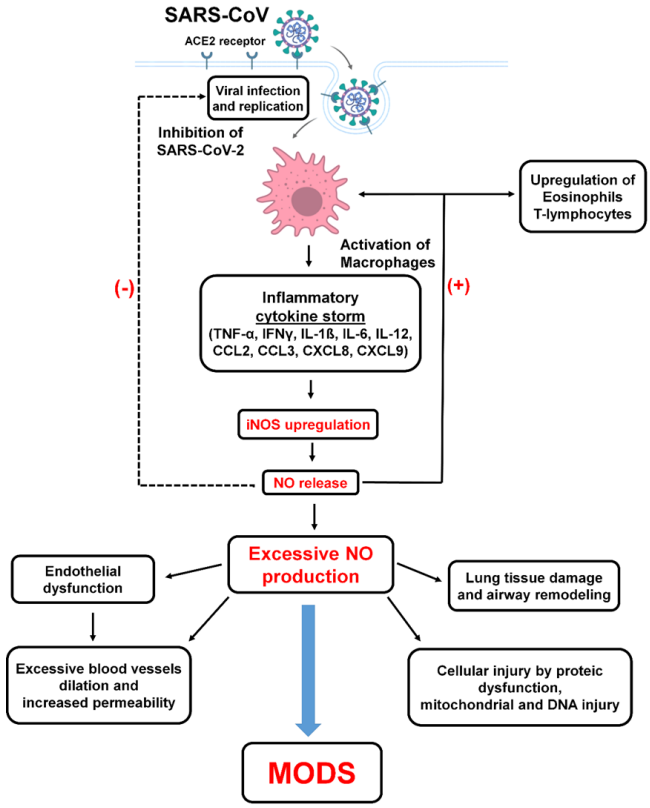

Figure 1.

The dual effects of nitric oxide (NO) in pathological conditions

NO can have both beneficial and detrimental effects. The production of NO during infections inhibits viral replication and mediates smooth muscle relaxation leading to bronchodilation and vasodilation. Large quantities of NO produced by iNOS in severe infections induce excessive immune cell responses, particularly by macrophages, eosinophils and T-lymphocytes. NO along with increased oxidative stress mechanisms can prime an uncontrolled cellular dysfunction, DNA injury, lung tissue damage, and endothelial dysfunction. Persistent effect of NO induces airway remodelling and mucus production by controlling the arginase pathway. (+) activation; (-) inhibition.

The cytokine storm, a lethal unregulated systemic inflammatory reaction resulted from the secretion of large quantities of pro-inflammatory cytokines, is one of the main causes of acute respiratory distress syndrome (ARDS) in many critically ill patients. These include the release of various cytokines (e.g., IFN-α, IFN-γ, IL-1ß, IL-6, IL-12, TNF-α, TGF-ß) and chemokines (e.g., CCL2, CCL3, CCL5, CXCL9 and CXCL10) by immune cells in SARS-CoV infection [20, 21]. The cytokine storm consequently prompts the release of significant amounts of NO. NO is generally advantageous both in normal conditions and during infection, allowing improved immune adaptation and immune cells migration through vascular epithelium [22]. However, NO has a mutagenic impact on the virus genome when presents in elevated concentrations as in the case with viral pneumonia [14]. In effect, high levels of NO found in the lungs of 1918 pandemic H1N1 and H5N1 mouse models were attributed to neutrophils and macrophages [23]. This upregulated activity of the immune cells in the lung can lead to a massive release of inflammatory mediators and NO, which causes parenchymal tissues degradation, loss of usable alveolar surface and insufficient flow of gases, reduced respiration and eventually death. Respectively, the multiple organ dysfunction syndrome (MODS) may occur, which increases mortality in patients affected [24].

Currently, there are no approved methods to therapeutically modulate NO level under pathological processes. Potential pharmacological inhibition of NO is achieved *via* inhibition of NOS, inhibition of downstream signalling pathways and NO inhibition/ scavenging [8]. Two endogenous NOS inhibitors: asymmetric dimethylarginine (ADMA) is a potent, non-competitive NOS inhibitor while its NG-mono-

methyl-L-arginine acetate (L-NMMA, "Tilarginine") congener is a less potent, competitive inhibitor of NOS [22, 25]. Dose-dependent L-NMMA increases the blood pressure by inducing human arterial vaso-constriction [25] and restore vasomotor tone balance in patients with septic shock, reducing concomitant norepinephrine treatment requirements [26].

The present evidence indicates that NO may interrupt the early stage of corona viral replication and deter its dissemination, facilitating clearance and host recovery [27]. Studies have, however, revealed that relative to the regular H1N1 isolates, pathogenic H5N1 and 1918 pandemic viruses can trigger more NO release *in vivo* and also by neutrophils derived from lung tissues subjected to 1918 virus *ex vivo* [15]. Interestingly, treatment with L-NMMA resulted in a substantial increase in the rate of survival of animals inoculated with H2N2 virus [15]. These findings were further reinforced by the observations that inhibition of NOS with L-NMMA has an advantageous impact on disease outcomes. By blocking NO production using selective NOS inhibitors or in NOS2-/- mice, a reduction in lung cytokine production was observed. In addition, there was a reduction in morbidity and death rates compared with controls [28]. Collectively, a significant function does exist for NO in the end result of H5N1 and 1918 pandemic infections in animal models.

A strategy based on using anti-inflammatory drugs has been advocated as a clinical alternative for patients with coronavirus infection [29]. Along with NO modulators, such treatment strategies may be successful in minimizing acute lung injury and MODS that are associated with Covid-19. However, the regulation of NO production should be gauged to maintain its positive antiviral effect while minimizing organ damage. Interestingly, natural compounds that demonstrated a prospective use, as inhibitors of NO, are under exploration. Notably, (-)-epigallocatechin-3-gallate (EGCG) inhibits mammary cancer cell migration, whereas proanthocyanidins inhibit non-small cell lung cancer cell migration by inhibiting NOS and guanylate cyclase (GC) [30]. Another compound which revealed imperative biological and pharmacological activities is thymoquinone [31]. This compound has shown remarkable anti-inflammatory and immuno-modulatory properties, making it a potential candidate for further development for SARS-CoV-2 infection [32-34]. Further, thymoquinone exhibits a strong antioxidant action through a redox cycling mechanism and it modulates NO production. These properties are likely responsible for the protective effect of this compound against organ failure and mortality in severe sepsis [32, 33, 35]. Thymoquinone may also inhibit and interfere with SARS-CoV-2 binding to ACE2 receptors, which can prevent virus entry and replication inside the host cell [36, 37]. Hence, targeting excessive NO production in SARS-CoV-2 infection constitutes an important area for future research in the quest for finding a cure for Covid-19.

Conclusions

In pathological conditions, regulating NO production by utilizing NO modulators/inhibitors to decrease the output of cytokines, reduced morbidity and delay in death should be encouraged. At present, there is no information concerning the levels of NO in COVID-19 patients. Thus, the consequence of NO modulation in SARS-CoV-2 infections using promising compounds like thymoquinone in animal models and patients constitute important areas for future studies.

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Conflict of interest

The authors declare no conflict of interest.

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