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Review

NLRP3 inflammasome activation and its inhibitory drugs in connection with COVID-19 infection

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Abstract

SARS-CoV-2 virus belongs to the beta coronavirus family that cause the inflammatory condition, acute pneumonia, and acute respiratory distress syndrome (ARDS). ARDS is the most important reason of mortality in patients, characterized as a highly increased levels of pro-inflammatory cytokine secretion. Inflammasome is a complex, which has an essential role in inflammatory situation, and NOD, LRR- and pyrin domain-containing protein 3 (NLRP3) is the most studied inflammasome that is considered to play vital roles in the virus infection and its pathogenesis. Our search language was limited to English and the search was performed in Web of Science, PubMed and Embase. Based on published articles, our current narrative review first explains the structure of the SARS-Cov2 virus and then describes the function of the NLRP3 inflammasome in relation to COVID-19 and drugs effective in controlling it. The NLRP3 inflammasome activation related to the initiation of inflammatory cascade including important cytokines production and releases such as IL-6, TNF-α and IL-1β. Thus, targeting the NLRP3 as a member of the innate immune system may be helpful for the reduction of ARDS clinical symptoms in COVID-19 patients.

Keywords

COVID-19, NLRP3, inflammasome, inflammation

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is belongs to family of beta coronavirus that cause the inflammatory condition, acute pneumonia, and acute respiratory distress syndrome (ARDS). Infection is followed by inflammatory reaction that is believed to promote tissues damage. Reports have stated that NOD, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome stimulation could play a dominant function in this extreme inflammatory condition. The level of NLRP3 activation associate with disease severity. ARDS is a main reason of mortality in patients and is a result of cytokine excessive release that is defined as extremely increased levels of pro-inflammatory cytokines. Inflammasome is a complex, which has essential roles in inflammatory situation, and NLRP3 is the most studied inflammasome that is considered to play vital roles in the virus infection and its pathogenesis.[1,2]

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Literature search strategy

Based on our mentioned keywords: COVID-19, NLRP3, Inflammasome, Inflammation; online databanks and search engines such as SCOPUS, Google Scholar, ISI Web of Knowledge and PubMed were used. The articles were limited only to those in the English language.

SARS-CoV2, structure and immunopathogenesis

Nowadays, SARS-Cov-2 is a serious problem for public health that caused significant harm to social development and human health.[3] For the first time, this virus was seen in Wuhan, China,[4] but rapidly disseminated and caused a global pandemic confirmed by the World Health Organization (WHO).[3] It is reported that the disease origin is a zoonotic transmission started through a seafood marketplace in Wuhan, but the main reason for wide dissemination in the human population is person-to-person transmission.[5]

COVID-19 infection is caused by the virus that is associated to the beta coronavirus genus of the Coronaviridae family.[3] It is an enveloped single-stranded positive-sense RNA virus[6] with a length of 30 kb[7] and encodes necessary structural proteins, such as spike or S protein, membrane or M protein, envelope or E protein, and nucleocapsid or N protein,[8] and accessory proteins (3a, 6, 7a, 7b, 8b, and 9).[7]

Most Covid-19 patients may only experience a slight fever and cough or an asymptomatic period -as carriers;[4] however, the general clinical symptoms are dry cough, fever, shortness of breath, fatigue, diarrhea, body aches and many other conditions. Few patients may experience ARDS, septic shock, metabolic acidosis, clotting dysfunction and even death.[3] In later stages of COVID-19, patients may struggle with organ damages such as type I respiratory failure, acute respiratory distress syndrome, acute cardiac injury, sepsis, acute kidney injury, heart failure, shock, hypoxic encephalopathy, acute liver injury and circulated intravascular coagulation.[9] ARDS is the main cause of mortality in most human populations and is a result of cytokine release syndrome (CRS).[6] This situation defined as highly increased levels of many inflammatory mediators, for example TNF-α, IL-2, IFNα, IL-6, IFNγ, IL-1β and IL-18 secretion.[10] Overview of respiratory infection by COVID-19 shown in Figure 1.

The virus cell entrance depends on the S protein[8] and the angiotensin-converting enzyme (ACE) on target cells, like as macrophages, alveolar and endothelial cells.[11] S protein is made of two subunits, S1 that binds to ACE2 and helps the S2 for fusion and cell entrance.[6] There are also other molecules on the surface of the cells that can help the virus entry by binding to S1 subunit, like dipeptidyl peptidase-4 (DPP4), aminopeptidase N (APN) and CD147. CD147 as a

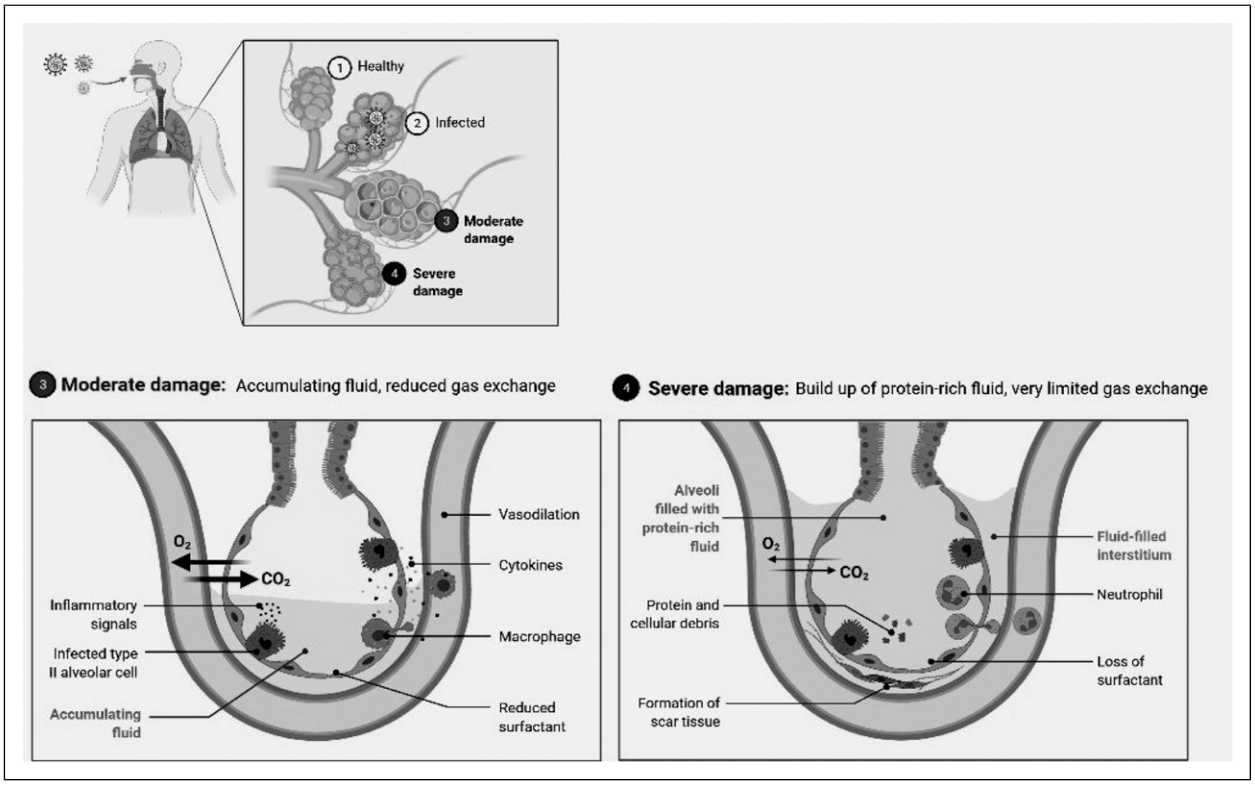

Figure 1. COVID-19 respiratory infection.

member of the immunoglobulin family is expressed on the surface of the neuronal, epithelial, myeloid and lymphoid cells and also known as a receptor for plasmodium falciparum.[12] Due to these interactions, virus could access to its targeted cells.

After virus infection, the innate immune system recognizes the virus entry due to the pathogen associated molecular patterns (PAMPs) such as virus dsRNA and damage associated molecular patterns (DAMPs) with pattern recognition receptors (PRRs), like RIG-I-like receptors (RLRs) and Toll-like receptors (TLRs).[4] Innate immune system mediator cells in the airways are epithelial cells, alveolar cells and dendritic cells (DCs), and among them, DCs and macrophages maintain protection from pathogens until adaptive immunity starts its function.[5] T cells are adaptive immune system managers, and their function is mediated by DCs and macrophages as antigen presenting cells (APCs).[5] $CD8^+$ T cells are killer cells destroying virus infected cells, and $CD4^+$ T (T helper) cells support B cells to perform their function, which is producing antibodies against the virus.[5]

Inflammasome is essential for the inflammatory cytokines such as IL-18 and IL-1β synthesis and secretion. This complex as an intracellular macromolecular inflammatory signaling structure[11,13] is a member of NOD-like receptors (NLR), a subgroup of PRRs, starts a pathway leading to maturation and release of interleukin-1β (IL-1β) and interleukin-18 (IL-18).[3,4,14] Based on different sensing proteins, it was divided into four types: NLRP1, NLRP3, AIM2 and NLRC4.[3] NLRP3 is the most studied form of inflammasome and is regarded to play an important function in the virus pathogenesis and infection.[4,10]

NLRP3 activation and its function

As an essential element of the innate immune system, NLRP3 inflammasome extends systemic and destructive inflammation due to the IL-1β, IL-18, and gasdermin D (GSDMD) production. Extremely cytokine production started in this manner could be a factor responsible for the patients mortality.[15]

NLRP3 signaling pathway turned on due to DAMPs, PAMPs, lysosomal damage, ionic flux, mitochondrial dysfunction, microbial agents, extracellular ATP, Reactive oxygen species (ROS) production, and K^+ through purine sensing receptors like P2X7.[8,13] NLRP3 activation is started over two signaling pathways. The first pathway is called priming, which is activated by cytokines or signals coming from DAMPs and TLRs that sensing a microbial particle,[8,16] and is followed by a transcription factor nuclear factor kappa B (NF-κB); leads to pro-inflammatory cytokines gene translation.[17,18] The second pathway is activated by PAMPs and DAMPs followed by pro-caspase-1 and the apoptosis associated speck-like protein containing a CARD (ASC) proteins.[8,19,20] Pro-caspase-1 and ASC already exist in the cytosol in inactivated form.[10]

Activated caspase-1 splits pro-inflammatory cytokines like pro IL-1β and pro IL-18 to mature IL-1β and IL-18. IL-1β as a proinflammatory cytokine, stimulates the neutrophil chemokine production in the lungs, attracts neutrophil toward the lungs, inspires the alveolar cell death, and increases the platelet-derived growth factor (PDGF) releases as well as the transforming growth factor β (TGF-β) that one of its functions is to increase the collagen production via resident fibroblasts. IL-18 is important to interferon γ (IFN-γ) production and NK cell and T cells activation.[13,21,22] In addition, caspase-1 cleaves the GSDMD to make pore channels in cell membrane; this is a phenomenon known as pyroptosis.[23,24] Pyroptosis (as a form of necrosis) activation leads to the interruption of pathogen replication process.[23] Figure 2 depicts the primary and secondary signaling pathways for NLRP3 activation.

Both apoptosis and pyroptosis are defined as a form of cell death, which need specific Caspase activity. Despite of apoptosis, pyroptosis happens afterward Caspase-1 stimulation.[25,26]

Studies on COVID-19 patients revealed that in different cells and tissues, such as circulating myeloid cells, PBMCs and leukocytes of lungs in patients with lethal COVID-19 pneumonia, there is over-stimulation of NLRP3 inflammasome.[27,28]

It is now discovered that COVID-19 can activate NLRP3 in the following ways: E22 and Open reading frame 3a (ORF3a), (an ion channel of potassium) proteins of COVID-19 virus stimulate the NLRP3 inflammasome by changing the K^+ charge through the cell membrane and by increasing the levels of mitochondrial Oxygen Reactive Species (ORS). ORF3a can form ion channels in the cell membrane to release sodium and potassium and replace calcium, and thus trigger further NLRP3 inflammasome stimulation.[7] Furthermore, it has been demonstrated that the N protein of the virus is able to stimulate the inflammasome activation and accelerate the lung damage and cell death in mouse models with acute SARS-CoV-2 infection.[3,29–31] The activation pathway for NLRP3 during COVID-19 infection is shown in Figure 3.

Three ion channel proteins, E protein, ORF3a and ORF8a encodes by SARS-CoV, oligomerize together to form pores that interrupt homeostasis in the host cells and involved to the viral pathogenicity. E and ORF3a proteins were described to be required for ideal viral replication and virulence.[29,32]

It was reported that E protein has essential functions in some signaling pathways such as NF-κB and p38 mitogen-activated protein kinase (MAPK) that finally results in inflammatory responses during viral infection. It is revealed that E protein creates a channel for calcium ion in the

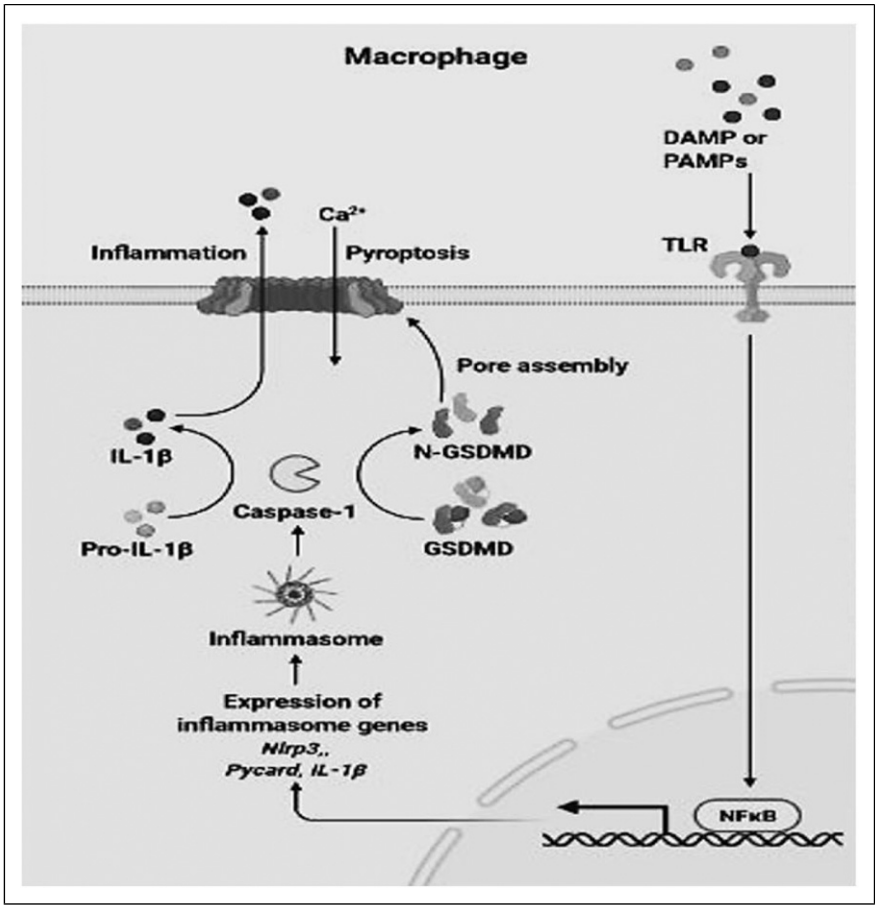

Figure 2. Pathways for activation of NLRP3.

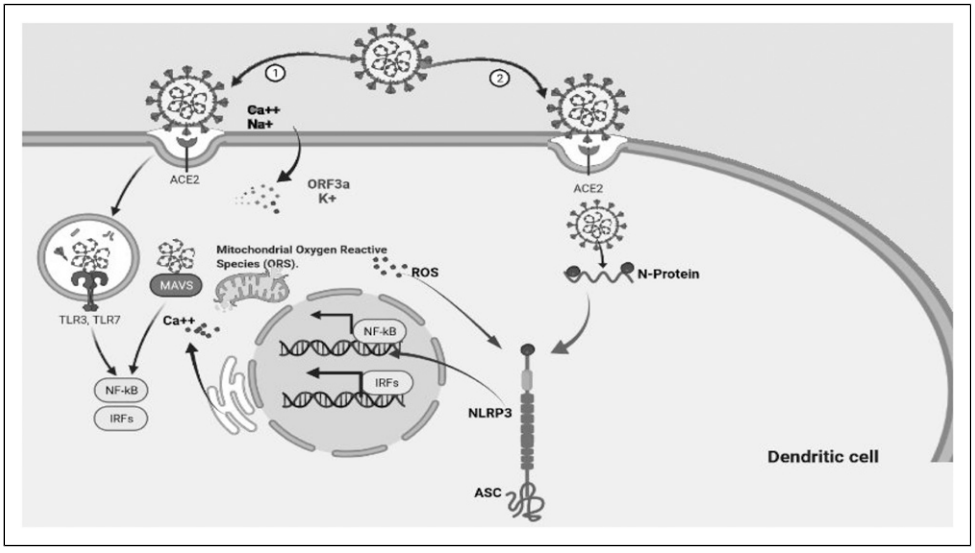

Figure 3. NLRP3 activation due to the COVID-19 infection.

Endoplasmic-Reticulum–Golgi Intermediate Compartment/Golgi membranes and the changes in the cytoplasmic level of calcium starts the innate immune system signaling receptors activation, specifically NLRP3.[8,33] Interestingly E protein deletion of reduces the virus growth in vivo and in vitro which means that virus becomes completely none-infectious.[34] SARS ORF3a protein damages lysosomes or interrupts the lysosomal function, promotes the TNF receptor-associated factor 3 (TRAF3) facilitated ubiquitination of ASC, and induces pro IL-1β transcription by activating NF-κB factor.[13] Hence, it is believed that SARS-CoV ORF3a stimulate the NLRP3 inflammasome that leads to initiation of inflammatory cascade includes important cytokines release such as IL-1β, TNF-α and IL-6 as part of the host inflammatory reactions to infection of SARS-CoV2.[29] It has been shown that SARS-CoV 3a protein also stimulates NLRP3 complex in lipopolysaccharide-stimulated macrophages.[7]

ORF8a and b are products of expression of a 27.894–28.259 bp sequence in SARS-CoV genome.[35] ORF8b, which is the other accessory protein, induces the NLRP3 activation by endoplasmic reticulum (ER) stress and lysosomal injury through stimulation of transcription factor EB (TFEB), and starts autophagy and lysosome machinery.[13] ORF8b is responsible for inducing the virus envelope proteins expression in cells and ORF8a and ORF9b induce cellular apoptosis.[35]

Autophagy is an essential phenomenon for the cell to block pathogen replication, but some viruses, such as coronavirus, use this tactic to replicate themselves.[36]

Along the routes we outlined, SARS-CoV unique domain (SUD), which consists of three domains, named C, N and M, activates NLRP3 in lung epithelial cells.[11] Activated NLRP3 in alveolar macrophages induces pyroptosis, which is followed by releasing the cytosolic contents, such as ATP, ROS and viral particles into extracellular spaces.[18,23] Increased levels of ATP can active the inflammasome by stimulating P2X7, are suspicious for development of neuroinvasion in patients, and have short-term and long-term effects on the patients' central nervous system (CNS).[10] The activation of inflammasomes, particularly NLRP3, is the reason of ARDS in the patients, leading to increased levels of inflammatory cytokines, release of Renin-Angiotensin Aldosterone System (RAAS) mediators, elevation of angiotensin II levels and the innate immune system hyper activation by complement cascade (ComC) stimulation.[10] Decreased respiratory capacity occurs by ARDS through damaging the alveoli–capillary membrane and elevates the pulmonary edema fluid into the airways.[10]

In associated with E protein ion channel activity, it is described that this protein stimulate accumulation of fluid, inflammation of lung and alveolar epithelial cells damage.[18,33]

Drugs targeting NLRP3

Given the important functions, we have mentioned for NLRP3, this complex can be an important target for drugs that inhibits or activate its function.

Colchicine. Colchicine is an alkaloid extracted from a plant named autumn crocus of the genus colchicum and its mechanism of action is not completely clear yet. As an anti-inflammatory mediator, colchicine has improved survival in COVID-19 infection by inhibiting the pyrin gene expression, caspase-1 activation and maturing IL-1β and P2X7 receptor-induced pore formation.[23,24] In addition, NLRP3 oligomerization could be prevented by colchicine, leads to the inhibition of IL-1β and IL-18 production and releases. In this manner, reduction in the process of inflammatory situation and its related clinical manifestation could be desired. Treatment with colchicine can reduce the hospitalization rate of Covid-19 patients and their needs for oxygen supplementation.[37] Colchicine also impairs tubulins by suppressing the MEFV (MEFV Innate Immunity Regulator, Pyrin) gene expression and microtubule polymerization; it is a non-selective NLRP3 inhibition of colchicine.[38–40] This drug inhibits the mitochondria transportation and prevents the approach of ASC to NLRP3 to create an ideal site for NLRP3 activation.[38] Behind this data, prof. Jean-Claude Tardif reported that in their study on Covid-19 patients (COLCORNA trial, 2021), colchicine effects on COVID-19 associated clinical symptoms was not statistically significant between the drug receivers and the placebo receivers. In this trial, between PCR-confirmed COVID-19 patients, it is stated that colchicine led to a lower degree of death or hospitalization than placebo.[41] Due to this data, more study on colchicine effects in Covid-19 patients is recommended.

Acalabrutinib. Acalabrutinib is a selective bruton tyrosine kinase (BTK) enzyme suppressor; it has a reactive butynamide group which bonds to the Cystein-481 residue in BTK.[42] Acalabrutinib is also used in COVID-19 infection and has significant effects by inhibiting the BTK enzyme, which is a NLRP3 regulator.[24]

Hydroxychloroquine. Hydroxychloroquine inhibits the NLRP3 inflammasome assembly in COVID-19 infection.[24] Hydroxychloroquine originally is an antimalarial agent and its functions is through changing the pH of the intracellular vacuoles which results to decreasing the immune system stimulation.[43]

Necrosulfonamide and disulfiram. Necrosulfonamide and Disulfiram both prevent the pyroptosis by inhibiting GSDMD effects on NLRP3 activation.[24] Necrosulfonamide is used in Alzheimer's disease (AD) and prevents the

Table 1. Drugs and their targets in inhibition of NLRP3.

Drug or modulator	Mechanism of action	Administration in Covid-19 patients
Colchicine	Inhibiting the pyrin gene expression and caspase-1, inhibition of maturing IL-1β and P2X7 receptor-induced pore formation	Phase 2, 3 clinical trial (NCT04322682)
Acalabrutinib	Inhibiting the BTK enzyme	Phase 1, 2 clinical trial (NCT04346199 and NCT04497948)
Hydroxychloroquine	Inhibition of NLRP3 inflammasome assembly	Phase 2, 3 clinical trial (NCT04353336)
Necrosulfonamide	Inhibition of GSDMD	Not studied in Covid-19
Disulfiram	Inhibition of GSDMD	Phase 2 clinical trial (NCT04485130)
Hexamethylene amiloride	Inhibition of E protein functions	Not studied in Covid-19
Tranilast	Inhibition of IL-33 secretion	Phase 3 clinical trial (IRCT20200419047128N1)
Azithromycin	Reduces the NLAP3 mRNA stability and NF-κB activity	Phase 3 clinical trial (NCT04332107)
Parthenolide	Caspase 1 inhibition	Pre-clinical phase
MNS	Inhibition of NLRP3-induced ASC development	Pre-clinical phase
O3FA	Inhibition of NLRP3-inflammasome-mediated inflammatory response	Pre-clinical phase
Curcumin	K+ outflow inhibition	Pre-clinical phase
EPA	Direct inhibition of NLRP3	Pre-clinical phase
Artigenin	Inhibition of IL-1β activation	Pre-clinical phase
Sinomenine	Direct inhibition of NLRP3	Pre-clinical phase

MNS: 3,4-methylenedioxy-β-nitro styrene; O3FA: omega-3 fatty acids; EPA: eicosapentaenoic acids.

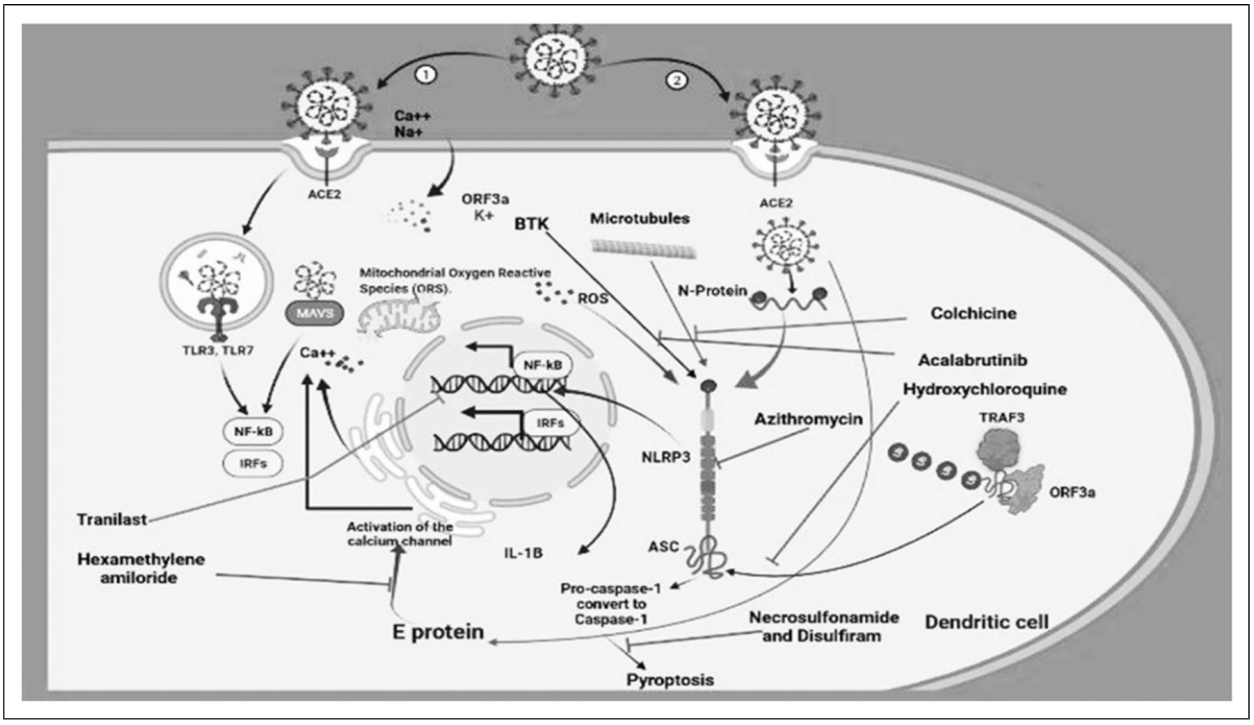

Figure 4. Inhibitory action of drugs that target the NLRP3 activation.

brain tissue cells death which is caused by proinflammatory agents like TNF-α.[44] Disulfiram is used to block the transcription factors such as NF-κB in cancers and metastatic disease.[45]

Hexamethylene amiloride. It was detected that Hexamethylene amiloride as the inhibitor of the HIV virus Vpu protein, similarly limited the replication of coronavirus and restricted the E protein functions in human coronavirus and mouse hepatitis virus.[46]

Tranilast. In association with this point that cytokines contribute to the immune system regulation, these molecules can be considered as a targets of therapeutic drugs. As one of the IL-1 family, IL-33 increases ROS, activate the NLRP3 inflammasome, and directly related to the pulmonary inflammation. Tranilast is an anti-allergic drug that able to disrupt the expression of cytokine pattern. The secretion of IL-33 from lipopolysaccharide-stimulated macrophages inhibited by Tranilast. Consequently, it can protect patients from the happening of acute pulmonary inflammation.[47,48]

Azithromycin. This drug is a macrolide which reduces the NLAP3 (coding NLRP3 gene transcript) mRNA stability, NF-κB activity and enhances NLRP3 transcript decay, results to decreased IL-1β cytokine levels.[49]

Parthenolide, MNS, O3FA, curcumin, EPA, artigenin and sinomenine. Parthenolide, 3,4-methylenedioxy-β-nitro styrene (MNS), omega-3 fatty acids (O3FA), Curcumin, eicosapentaenoic acids (EPA), Artigenin and Sinomenine are in a pre-clinical phase that used in ischemic stroke situation and stopped or suppressed NLRP3 activation due to the different pathways such as direct inhibition of NLRP3 (Sinomenine and EPA), caspase 1 inhibition (Parthenolide), Restriction of NLRP3-induced ASC development (MNS), K+ outflow inhibition (Curcumin), Inhibition of NLRP3-inflammasome-mediated inflammatory response (O3FA) and IL-1β activation inhibition (Artigenin).[50]

The inhibitory mechanism of these drugs is summarized in Table 1 and is shown in Figure 4.

Conclusion

Between drugs and modulators that mentioned in this review article, Colchicine, Acalabrutinib, Hydroxychloroquine, Necrosulfonamide, Disulfiram, Hexamethylene amiloride, Tranilast and Azithromycin are in phase 1 or 2 or 3 of clinical trials. Parthenolide, MNS, O3FA, Curcumin, EPA, Artigenin and Sinomenine are in pre-clinical phase. Some of these drugs directly inhibit NLRP3 activation (Hydroxychloroquine, Azithromycin, MNS, O3FA, EPA and Sinomenine) and some of them indirectly prevents the activation of NLRP3 and its functions (Colchicine, Acalabrutinib, Necrosulfonamide, Disulfiram, Hexamethylene amiloride, Tranilast, Parthenolide, Curcumin and Artigenin). In the case of our research, it is possible that a drug or modulator was investigated but we did not have access to its article, and this should be cited as the main limitation of our study. The NLRP3 inflammasome have essential roles in the process of inflammation. The relation between its activation and function in the pathogenesis of COVID-19 has been proven in various studies. Therefore, controlling the function of this supra-molecular complex and its associated mediators in relation to the inflammatory conditions in COVID-19 disease can play effective role in decreasing patient mortality and improving their living conditions.

Author contributions

All authors have been involved in data acquisition and writing of the manuscript. All the images used in this article were designed by Javad Poursamimi and permission to use these images was obtained from him.

Declaration of conflicting interests

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Data availability

The data presented in this review article are available from the corresponding author after well-reasoned request.

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