

Xanthine oxidase inhibition in SARS-CoV-2 infection: the mechanism and potency of allopurinol and febuxostat in COVID-19 management

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ABSTRACT

The number of coronavirus disease 2019 (COVID-19) infection cases has been increasing globally, including in Indonesia. Definitive therapy for COVID-19 has not yet been found; hence, repurposed drugs for COVID-19 have been considered and have been practiced by several researchers in the world. This literature review investigates the action of xanthine oxidase as a component of the biomolecular pathway against severe acute respiratory syndrome-related coronavirus-2, the cause of COVID-19, and describes the mechanism and potential of uric acid drugs (allopurinol and febuxostat) as prophylaxis and curative therapy for COVID-19.

KEYWORDS COVID-19, free radicals, uric acid, xanthine oxidase

The year 2019 ended with an epidemic in Wuhan, China, caused by a virus that was later known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); the outbreak spread worldwide, evolving into a pandemic.¹ The number of detected cases and daily mortality caused by the coronavirus disease 2019 (COVID-19) continue to increase due to the unavailability of definite drugs.^{2,3} Clinicians and researchers worldwide have attempted various treatment methods while waiting for a definite drug to be invented. A few available antiviral drugs have been used as an option in COVID-19 treatment. The US Center for Disease Control and Prevention, for instance, recommended the use of remdesivir⁴; however, it has not yielded any consistent results and was often accompanied by clinical symptom deterioration. The clinical symptoms observed in COVID-19 cases included chronic obstructive pulmonary disease (COPD),⁵ acute respiratory distress syndrome (ARDS), sepsis, acute kidney

failure, acute heart failure, multiorgan failure, and even death.⁶ Therefore, antiviral drugs should be administered with adjuvants such as corticosteroid, although this combined therapy is not recommended in COVID-19 patients.⁷

Reactive oxygen species (ROS) is believed to play a role in the pathogenesis of COVID-19, starting from xanthine oxidase (XO) activity to produce superoxide radicals to cell necrosis⁸ and from damaging the lung epithelial cells to cytokine interleukin (IL)-6 production induced by hydroxyl radicals (OH)⁹ formed from Fenton reaction as a continuation of free radical reaction in the cells.¹⁰ Cytokine IL-6 is a proinflammatory cytokine identified as a cytokine storm syndrome marker in the acute phase of COVID-19 patients (ARDS).¹¹

Uric acid drugs, particularly allopurinol and febuxostat, are known to induce antiviral effects and a significant clinical safety level compared to antiviral drugs. These drugs inhibit

XO production, a biomolecular component involved in free radical formation.¹² In contrast, clinical symptom deterioration in COVID-19 occurs as a result of cytokine storm caused by the uncontrollable production of free radicals by the body's immune system.¹³ This review describes the role of XO in the pathogenesis of COVID-19 and the role of allopurinol as an XO inhibitor and its potential in COVID-19 treatment.

The possible role of XO in SARS-CoV-2 infection pathogenesis

The study of Xu et al¹⁴ revealed that the presence of SARS-CoV-2 in the immune system causes a histopathological change in the lung tissue similar to that in ARDS. This results from the virus exposure in the respiratory tract, causing a series of immune system responses in the form of an inflammation intermediated by macrophage cells, epithelial cells of the respiratory tract, and neutrophils.¹⁵ The inflammation pathogenesis of the respiratory virus involves free radical reaction initiated by XO as explained in the following paragraphs.

A virus is recognized and phagocytized by the macrophage, where the virus-containing phagosome is subsequently recognized by virus ligands such as the toll-like receptors-3, 7, 8, and 9. This then results in a series of intracellular stress signal activation inducing the activity of pro-free radical enzymes, such as nicotinamide adenine dinucleotide phosphate oxidase and XO.¹⁰ The activity of these enzymes affects the production of free radicals such as ROS and reactive nitrogen species, which are intended to destruct the virus trapped in the phagosome that further forms into a phagolysosome for phagocytosis to occur (Figure 1).¹⁶ The free radical activity, however, needs a catalyst for inflammation not to occur in healthy tissues. Akaike¹⁷ showed that the administration of an inhibitor to one of the ROSs, i.e., superoxide anion radical (O₂⁻), improves pathological lung condition, and pneumonia survival rate from the virus. In addition, XO action in the excessive formation of O₂⁻ may damage lymphocyte T cells,⁸ especially through necrosis.¹⁸ This potentially explains the reduced number of T cells in 452 COVID-19-positive patients in Wuhan, China.¹⁹

The overactivity of XO may increase ROS, triggering cell death, and further activation of macrophage. Moreover, an increase in ROS activity may subsequently increase cytokine production, the so-called cytokine storm, and may result in epithelial cell damage.

Additionally, proinflammatory cytokines are produced by the infected cells through the activation of the nuclear factor- κ B pathway that will urge transcription of related genes and through the activation of the nod-like receptor protein-3 inflammasome that further induces cytokine secretion (Figure 2). Inflammatory cytokines affect epithelial cells, developing body vulnerability into invasive microorganism attacks, such as viruses and bacteria.¹⁰

Lung tissue damage is marked by increased cytokine IL-6,⁹ which is also noted in ARDS.¹¹ ARDS patients show increased IL-6 and IL-8 levels on days 1–7 from the day of infection. IL-6 and IL-8 levels return to normal with a good prognosis.¹¹ IL-6 increase was consistently observed in COVID-19 cases in Wuhan, China.¹³ Increase in IL-6 level indicates a simultaneous increase in XO activity^{20,21}; this is confirmed by increased plasma uric acid levels in patients with ARDS.^{22,23}

An experimental study on mice by Fonseca et al²⁴ showed that respiratory syncytial virus (RSV) infection increased XO expression on days 2–8 post-infection. Uric acid obtained from bronchoalveolar lavages was also observed to have significantly increased in days 2–8 following infection. The threshold value of serum uric acid level of 8.4 ml/dl was found to predict patients' mortality with ARDS, with 89% sensitivity and 80% specificity.²² Cheng et al²⁵ revealed a significant association between COVID-19 and blood uric acid level. Furthermore, Huang et al²⁶ reported that severe COVID-19 patients with ARDS have high mortality as 15%, as short as 2 days. Therefore, serum uric acid level in patients with ARDS can be used as a prognostic marker,²³ particularly in COVID-19 patients with a high risk of ARDS.^{25,26}

Allopurinol and febuxostat as XO inhibitor

Allopurinol as an antiviral may occur through the neutrophil inhibition to release interferon- γ and IL-2, which play a role in cytokine storm in viral infections; however, this study has not yet been further developed.²⁷ Akaike¹⁷ showed that rats infected with influenza virus indicated XO enzyme activity resulting in ROS production, i.e., O₂⁻, potential to be OH that are considered damaging to cells. This finding demonstrated that the pathology of influenza virus infection in rats is associated with XO activity, and this will lead to eliminating free radicals by XO inhibitors, such as allopurinol.²⁸

Allopurinol at 100 mg twice daily is known to significantly reduce C-reactive protein levels, a cytokine

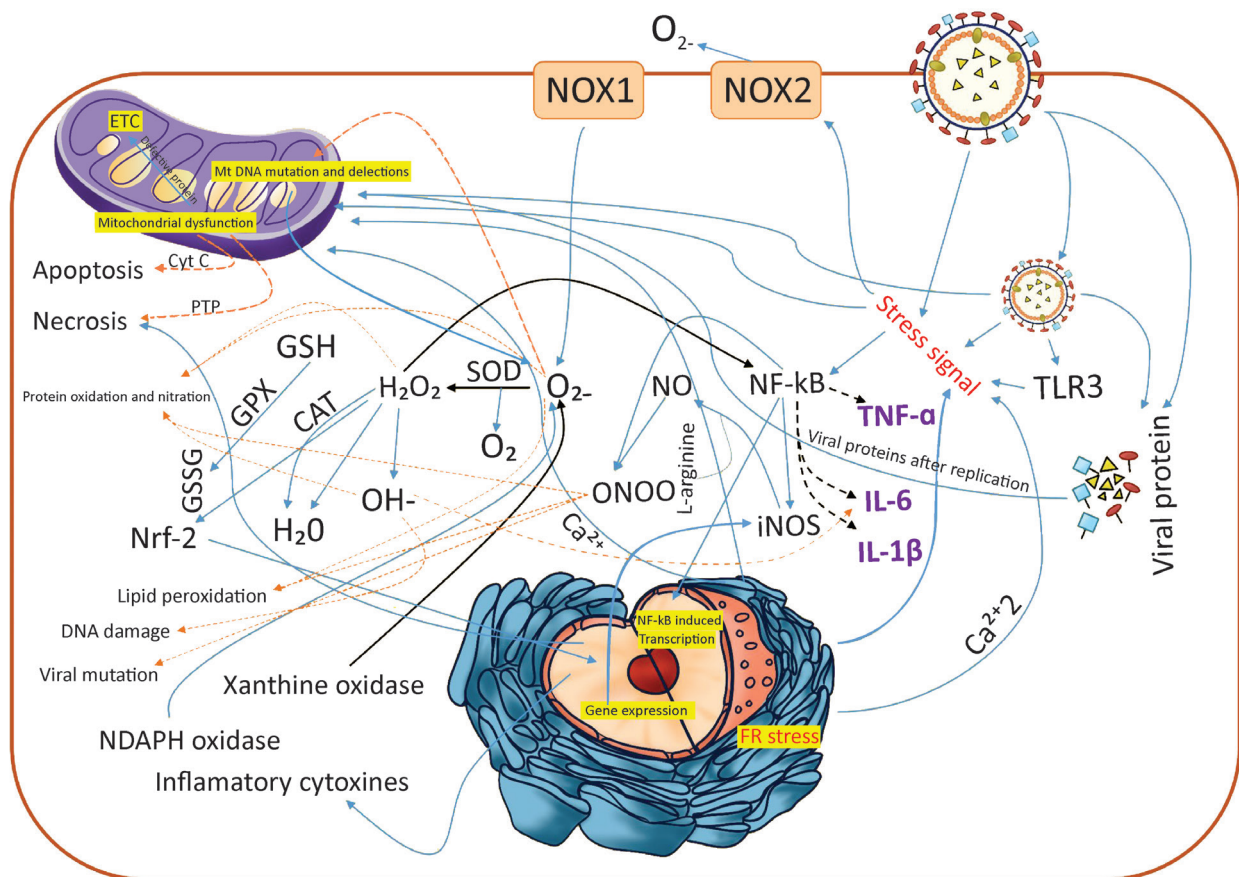


Figure 1. The basic mechanism of viral cross-talk by a cellular pathway in inducing reactive oxygen species (ROS) and causing oxidative damage of cellular components. Adapted from: Rehman ZU, Meng C, Sun Y, Safdar A, Pasha RH, Munir M, et al. Oxidative stress in poultry: lessons from the viral infections. *Oxid Med Cell Longev.* 2018;2018:5123147. Under the Creative Commons Attribution License (CC BY 4.0) <https://creativecommons.org/licenses/by/4.0/>

storm marker, in hyperuricemia patients²⁹ and inhibit IL-6.³⁰⁻³² Allopurinol has been approved for RSV infection management in pediatric patients, inhibiting IL-6.³³ IL-6 level reduction by XO inhibition using allopurinol is expected to reduce the risk of ARDS in COVID-19 patients, improving prognosis. Allopurinol administered during RSV infection in mice was found to reduce IL-1b expression,²⁴ which is also a proinflammatory marker of COVID-19. Clinically, allopurinol has been shown to improve exercise capacity and clinical symptoms of COPD patients.³⁴ In addition, the side effects of allopurinol should be of concern, with the most common being indigestion, hypersensitivity reaction, skin rash vasculitis, eosinophilia, and decreased kidney function such as interstitial nephritis.³² Hypersensitivity reaction may appear after months or years of treatment, and adverse drug effects generally occur in individuals with decreased kidney function who also use allopurinol without reduced dose.³⁵ However, another study by Goicoechea et al³⁶ demonstrated that

allopurinol reduced the risk of disease progression in patients with chronic renal disease. Furthermore, it has been noted that allopurinol administration reduced cardiovascular and hospitalization risk of patients with hyperuricemia. In Table 1, we summarize allopurinol as xanthine oxidase inhibitors in viral infections.

Another alternative that may be used for uric acid therapy is febuxostat. Febuxostat is a selective, non-purine inhibitor of XO, and has a mechanism similar to allopurinol, i.e., inhibition through access competition to the enzyme active site of molybdenum-pterin.³⁷ Febuxostat's superiority over allopurinol is still unclear and considered as not clinically relevant; however, the risk of adverse events is lower in patients who received febuxostat than those administered with allopurinol.³⁸ However, febuxostat was not recommended as a first-line urate-lowering therapy (ULT) owing to an increased mortality risk in gout and cardiovascular morbidity. Thus, allopurinol dose escalation is more recommended for ULT.³⁹

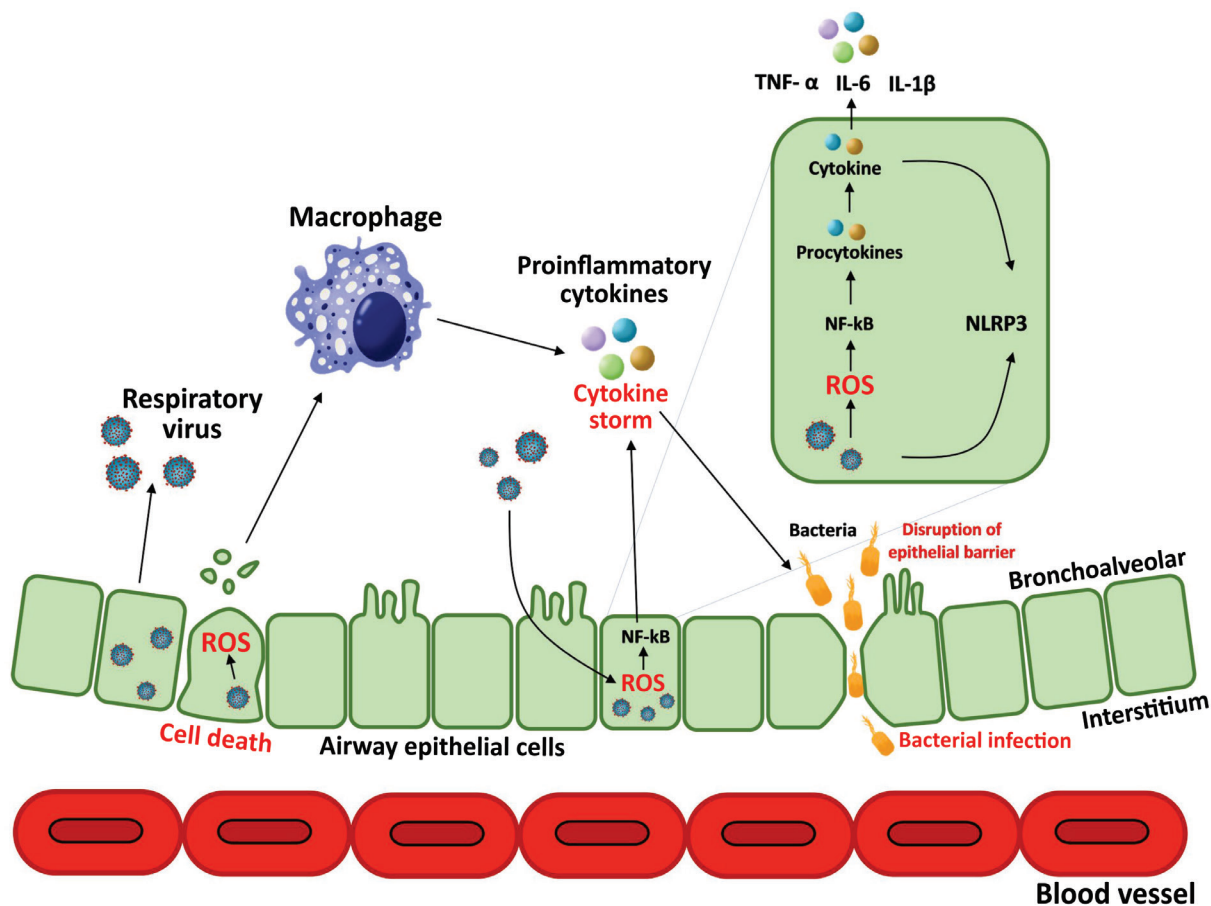


Figure 2. Production mechanism of cytokine (cytokine storm) and epithelial barrier disruption by respiratory virus. Adapted from: Khomich OA, Kochetkov SN, Bartosch B, Ivanov AV. Redox biology of respiratory viral infections. *Viruses*. 2018;10(8):392. Under the Creative Commons Attribution License (CC BY 4.0) <https://creativecommons.org/licenses/by/4.0/>

As the abovementioned demonstrate the association between viral infection, XO activity, and the benefit for inhibiting its activity, for future applications, allopurinol and other XO inhibitors can be used as prophylactic and therapeutic options in COVID-19 patients. This is because allopurinol has the potential of inhibiting XO activity and controlling lymphopenia and the release of cytokines that cause hyper inflammation which leads to ARDS. Improving the patient’s clinical condition is essential for the body to have time to develop specific antibodies for fighting

viruses. Managing proinflammatory formations that cause inflammations is a key factor for the therapy of diseases caused by respiratory viral infections, including SARS-CoV-2. Therefore, the clinical trial of XO inhibitor is recommended for COVID19 management.

In conclusion, XO plays a role in inflammation caused by SARS-CoV-2 by producing free radicals triggering cytokine storm. One of the cytokines released during this process is IL-6, which is involved in ARDS. Serum uric acid is associated with the XO level and indirectly reflects the IL-6 level; therefore, uric acid

Table 1. Summary of xanthine oxidation in respiratory viral infection models

Drugs	Mechanism	Models	References
Allopurinol	Neutrophil inhibition, to release IFN-γ and IL-2	Rats infected with influenza, increase of ROS production	17, 27
Allopurinol	Reduce CRP, inhibits IL-6	Patients at dosage of 100 mg twice daily	29, 30
Allopurinol	IL-6 reduction	RSV in mice models	24

IFN=interferon; IL=interleukin; ROS=reactive oxygen species; CRP=C-reactive protein; RSV=respiratory syncytial virus

level is a potential prognostic marker in COVID-19 cases at risk for developing ARDS. Moreover, XO inhibitors allopurinol and febuxostat can be used as prophylactic and therapeutic therapies in COVID-19 patients.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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