

NSAIDs Immunomodulation in COVID-19 Might Inhibit SARS CoV-2 ORF Proteins  
Induced Caspase Activation, Necroptosis and Endoplasmic Reticulum Stress.

Mina T. Kelleni, MD, PhD

Pharmacology Department, College of Medicine, Minia University, Egypt

[mina.kelleni@mu.edu.eg](mailto:mina.kelleni@mu.edu.eg); [drthabetpharm@yahoo.com](mailto:drthabetpharm@yahoo.com)

Mobile: +201200382422

<https://orcid.org/0000-0001-6290-6025>

Abstract

We have previously postulated potential numerous immunomodulatory and anti-inflammatory benefits when we used non-steroidal anti-inflammatory drugs (NSAIDs) safely and effectively to manage the hyperinflammatory COVID-19 and in this short communication, we add other potential benefits related to SARS CoV-2 ORF proteins dependent activation of caspases with their subsequent mitochondrial dysfunction, endoplasmic reticulum stress and necroptosis that were described with complicated COVID-19 and we pharmacologically confirm the fact that NSAIDs are known to be caspase inhibitors. Moreover, NSAIDs might independently inhibit other caspases related COVID-19 associated downstream pathological signaling mechanisms. We also postulate that CARD-14, a caspase recruitment domain-containing protein, polymorphisms might play a role in development of severe and critical COVID-19. Finally, it is very unfortunate that for more than one year of relentless struggle, our recommendation to adopt NSAIDs as first choice COVID-19 therapy has not adopted while precious lives are succumbed every single day of delay.

Keywords

COVID-19, NSAIDs, Caspases, Endoplasmic reticulum stress, CARD-14 polymorphisms.

To date, May 25, 2021, COVID-19 has globally succumbed almost three million and five hundred thousand lives and the search for the underlying pathophysiological mechanisms induced by SARS CoV-2 infection, especially in severe and critical cases, is still evolving including searching genetic polymorphisms which have been postulated to influence the course and complications of COVID-19[1,2].

Caspases are a family of enzymes associated with apoptosis, pyroptosis and their dysregulated activation was suggested to share in the pathogenesis of tumors, autoimmune, autoinflammatory, inflammatory cytokine secretion including IL-1 $\beta$  from viable monocytes as well as infectious disorders[3]. Interestingly, SARS-CoV-2 infection was reported to activate caspase-8 triggering pro-inflammatory cytokines, including IL-1 $\beta$ , TNF- $\alpha$ , IL-7, IL-8, apoptosis, necroptosis and activation of the NF $\kappa$ B pathway in lung epithelial cells which were suggested to share in COVID-19 induced downstream immune pathogenesis causing lung damage[4]. Moreover, open reading frames (ORF) 3a protein of SARS-CoV-2 was shown to significantly induce cellular apoptosis which was shown experimentally to be significantly inhibited by either a caspase 8 or caspase 9 inhibitor[5] to be noted that several ORF SARS CoV proteins were previously shown to induce apoptosis and ORF-6 protein overexpression was shown to induce caspase-3 mediated c-Jun N-terminal kinase (JNK)-dependent apoptosis that was blocked by a specific caspase 3 inhibitor or JNK inhibitor[6].

Importantly, several caspases were reported to modulate B and T cell proliferation and altered transcriptome levels of caspase genes were reported in natural killer cells and neutrophils. Moreover, uncontrolled caspase response in COVID-19 was suggested to share the immune pathological processes as well as in the inflammatory microvascular thrombi found in multiple organs leading to severe outcomes. Interestingly, caspase-1 in CD4+ T cells was shown to be upregulated in hospitalized COVID-19 patients and caspase-3 levels were reported to be significantly upregulated, compared to controls, in circulating red blood cells from COVID-19 patients as well as in tissue macrophages in postmortem analysis and were also demonstrated to be suppressed ex vivo by a pan caspase inhibitor [7]. Thus, unsurprisingly, suppression of apoptosis was suggested to prevent viral pathogenesis in some diseases including SARS and targeting virus-induced apoptosis was

implied as a promising strategy in COVID-19 management[8]. Similarly, inhibition of the necroptosis signaling pathway, a subsequent outcome of caspase-8 activation, was suggested to possess a potential to protect against COVID-19 complications[9].

Taken together, we would like to pharmacologically confirm that NSAIDs are well known, at physiologic in vivo concentrations, caspase inhibitors [10] and notably, NSAIDs were relentlessly suggested, and daily used, by the author to safely and effectively manage COVID-19 through their potent anti-inflammatory and immunomodulatory effects[11-15] that might prevent or restore the immune dysregulation that is well described in COVID-19 and other diseases [16].

Furthermore, endoplasmic reticulum stress (ERS) was suggested to play an important role in the development of COVID-19[17] and ERS markers were shown to be significantly increased in SARS CoV-2 infection and COVID-19 pneumonia [18] and interestingly, several NSAIDs including diclofenac, indomethacin, ibuprofen, aspirin, and ketoprofen were shown to suppress the ERS induced human neuroblastoma SH-SY5Y cell death[19]. Similarly, oxicam-derived NSAIDs have been demonstrated to possess neuroprotective effects potentially through suppressed activation of caspase-3 and cell death as well as amelioration of ERS and/or mitochondrial dysfunction signaling pathways[20] and thus, NSAIDs potential direct and indirect positive immunomodulatory effects in COVID-19 are further amplified through their potential anti-ERS effects.

Finally, we postulate that CARD 14, a caspase recruitment domain-containing protein of the membrane-associated guanylate kinases family <https://www.ncbi.nlm.nih.gov/gene/79092>, mutations might play a crucial role in COVID-19 pathogenesis and complications especially in severe and critical patients and we further suggest that the quest to design and develop novel caspase inhibitors and/or modulators might evolve to be corner stone in management of several immune-inflammatory diseases, yet until this goal is fulfilled, NSAIDs are being acknowledged as safe tools to manage COVID-19 and we suggest that economic, safe and effective FDA approved immunomodulators are available to help us to win our current COVID-19 battle [14].

87 **Conflict of interest**

88 The author has no conflict of interest to declare.

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