

**NSAIDs Immunomodulation in COVID-19 Might include Inhibition of COX-1  
and/or COX-2, SARS CoV-2 ORF Proteins Induced Caspases,  
Necroptosis and Endoplasmic Reticulum Stress.**

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Highlights

Both COX-1 and COX-2 inhibition by NSAIDs might benefit COVID-19 patients

Low dose aspirin is least likely to improve COVID-19 mortality through anticoagulation

SARS CoV-2 and its ORF proteins induce caspases, necroptosis and ERS

NSAIDs are known caspases inhibitors and might also inhibit necroptosis and ERS

CARD-14 polymorphisms might share in development of severe COVID-19

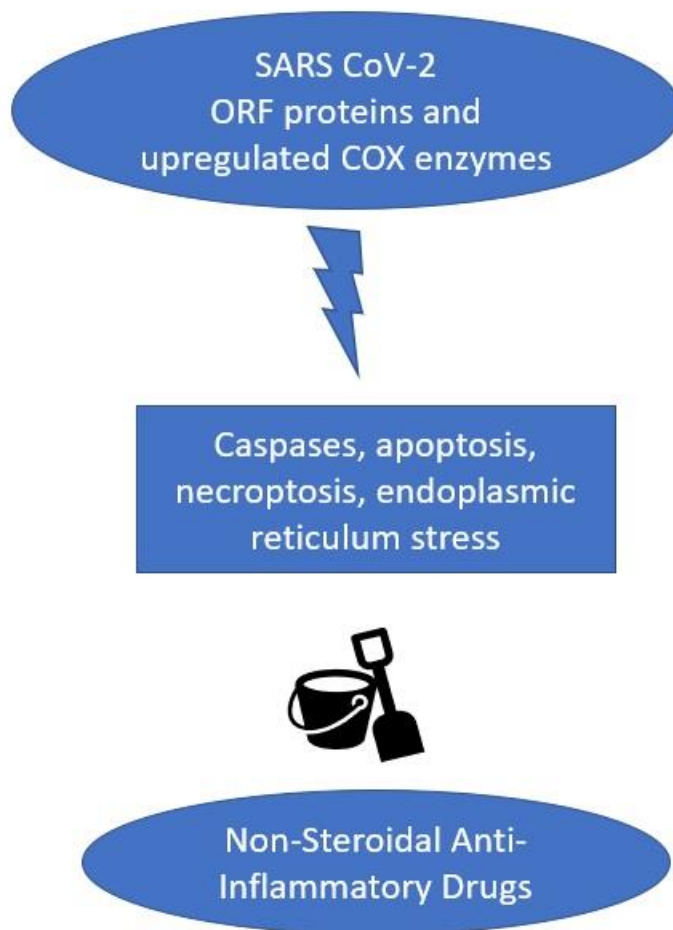
Abstract

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 COVID-19. However, after aspirin has been suggested to be independently associated with reduced risk of mechanical ventilation, ICU admission and in-hospital mortality of COVID-19, we claim that the molecular interpretation of the results that led to this suggestion was not scientifically accurate, and we provide our academic interpretation. Moreover, we add other potential NSAIDs benefits related to aspirin triggered lipoxins and resolvins, SARS CoV-2 ORF proteins dependent activation of caspases and their subsequent mitochondrial dysfunction, endoplasmic reticulum stress and necroptosis that were described with complicated COVID-19. We pharmacologically confirm the fact that NSAIDs are known to be caspase inhibitors and they might

independently inhibit other caspases related COVID-19 associated downstream pathological signaling mechanisms. Finally, we postulate that CARD-14, a caspase recruitment domain-containing protein, polymorphisms might play a role in development of severe and critical COVID-19.

**Keywords:** COVID-19, COX-1, COX-2, NSAIDs, aspirin triggered lipoxins, aspirin triggered resolvins, Caspases, Endoplasmic reticulum stress, CARD-14 polymorphisms.

### Graphical Abstract



## **Introduction**

To date, COVID-19 has globally succumbed over four million 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] and without thorough understanding COVID-19 pathophysiology, insightful pharmacotherapy cannot be achievable.

Interestingly, an interesting study demonstrated that aspirin use was independently associated with reduced risk of mechanical ventilation, ICU admission and in-hospital mortality while there were no differences in major bleeding or overt thrombosis between aspirin and non-aspirin users[3]. However, we suggest that this study has some major flaws in its interpretation and should be properly interpreted from a pathophysiologic and pharmacologic point of view for the best interests of the prospective medical research and more importantly the welfare of our precious COVID-19 patients.

Thus, we would discuss some insights about the use of low dose aspirin and more importantly the potential crucial role that NSAIDs might play in management of COVID-19 through aspirin triggered lipoxins and resolvins, inhibition of cyclooxygenases and SARS CoV-2 ORF proteins and induced caspase activation, necroptosis and endoplasmic reticulum stress aiming at further exploration of COVID-19 pathophysiology that might guide us in our vigorous quest for a highly anticipated cure.

## **Low dose aspirin and COVID-19 coagulopathy**

Chow et al. have cited numerous references that correlated SARS CoV-2 induced hypercoagulable state and subsequent development of platelet rich thrombi with severe COVID-19 and mortality and they have cited a study performed by Paranjpe et al.[4] which has suggested that systemic treatment-dose anticoagulation may be associated with improved outcomes among hospitalized COVID-19 patients hospitalized with COVID-19 to suggest that their reported aspirin beneficial outcomes might be due to its well-known antithrombotic properties. However, Paranjpe et al. have clearly enumerated numerous limitations of their study, the effect of the prophylactic low dose aspirin tested by Chow et

al. might differ from that of the systemic treatment dose anticoagulants studied by Paranjpe et al. to be also noted that a large observational study has demonstrated no significant association between ongoing use of direct oral anticoagulants and severe COVID-19 and wisely suggested that therapies should be better directed against thrombogenic inflammation, the cause, rather than against hypercoagulability; the symptom[5]. Importantly, Chow et al. have not found a difference in incidence of overt thrombosis between aspirin and non-aspirin users and thus, we would like to discuss some sub-overt mechanisms that might be attributed to reason for the potential aspirin beneficial effects in COVID-19 as expressed by Chow et al.

#### **Low dose aspirin and COVID-19 inflammation**

Chow et al. have stated that aspirin, as a cyclooxygenase-1 (COX-1) inhibitor, modifies both inflammatory and coagulation responses and they cited a review written by Warner et al.[6] However, in that cited reference, no mention to a link between COX-1 inhibition and inflammation was found and it was clearly stated, at that reference as elsewhere, that COX-1 is the constitutive form of the enzyme which is also exclusively or dominantly expressed in the anucleated platelets and that COX-2 is the inducible one associated with inflammation. Moreover, Chow et al. have cited a resourceful review and meta-analysis written by Panka et al. [7] to reason for the aspirin's anti-inflammatory mechanisms including lipoxin formation. However, in that reference these mechanisms were evident in murine or in in-vitro preclinical models, which in some used aspirin by local administration and in all of these models aspirin was used, as also stated, in high doses in contrast to the low doses used in clinical studies including that of Chow et al. and thus the evidence cited from Panka et al. does not reason for Chow et al. aspirin's anti-inflammatory properties. Additionally, Panka et al. discussed some contradictory results found in sheep and murine models and chow et al. have also wisely confirmed that aspirin showed mixed results when tested for acute respiratory distress syndrome and cited few studies though only thoroughly discussed the positive ones.

Similarly, Chow et al. have also cited a study performed by Ikonomidis et al. [8] in which 300 mg daily aspirin was administered for six weeks and decreased IL-6 and CRP to reflect on their 81 mg aspirin dose and this is also not scientifically justified as low dose aspirin

cannot inhibit the inflammatory COX-2, as stated by Chow et al., and inhibits COX-1 almost selectively. Moreover, Ikonomidis et al. have also mentioned that aspirin exhibits anti-inflammatory action in a dose dependent manner and its greatest effects occur at doses as high as 2 g.

#### **Potential benefits of low dose aspirin in COVID-19**

In our opinion, the results presented by Chow et al. should be interpreted and built upon by researching potential aspirin's non COX dependent anti-inflammatory effects through modulation of the immune and inflammatory function of platelets[9] as well as its peculiar ability to trigger induction of the beneficial anti-inflammatory and immunomodulatory lipoxins and resolvins which are synthesized through acetylated COX-2[10].

Notably, while COX-2 acetylation, and the subsequent formation of lipoxins and resolvins, is not achievable by low dose aspirin, induction of COX-1 upregulation in COVID-19 might be considered for further research as it has been previously described, with potential benefits of its inhibition under certain conditions, in some neuroinflammatory and neurodegenerative diseases. Additionally, COX-1 and/or COX-2 potential role in SARS CoV-2 replication should be assessed and NSAIDs were also suggested, in a preprint, to directly affect SARS CoV-2 replication[11].

#### **NSAIDs potential modulation of SARS CoV-2 induced activation of caspases, apoptosis, and necroptosis.**

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[12]. 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[13]. 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[14]

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[15].

Another important aspect is that 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 [16]. 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[17]. 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[18].

Thus, 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 [19].

More importantly, we would like to pharmacologically confirm that NSAIDs are well known, at physiologic in vivo concentrations, caspase inhibitors [20] 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[19,21-23] that might prevent or restore the immune dysregulation that is well described in COVID-19 and other diseases [24].

#### **NSAIDs potential modulation of SARS CoV-2 induced endoplasmic reticulum stress**

Furthermore, endoplasmic reticulum stress (ERS) was suggested to play an important role in the development of COVID-19[25] and ERS markers were shown to be significantly increased in SARS CoV-2 infection and COVID-19 pneumonia [26] and interestingly, several NSAIDs including diclofenac, indomethacin, ibuprofen, aspirin, and ketoprofen were shown to suppress the ERS induced human neuroblastoma SH-SY5Y cell death[27]. 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[28] and thus, NSAIDs potential direct and indirect positive immunomodulatory effects in COVID-19 are further amplified through their potential anti-ERS effects.

#### **Potential therapeutic role of NSAIDs in COVID-19**

Taken together, we recommend adoption of large clinical trials that adopt therapeutic doses of NSAIDs in management of COVID-19 and we have previously postulated prevention of complications and significant reduction of mortality [22,23] and we have explained the potential molecular immunomodulatory mechanisms upon which their COVID-19 efficacy might be reasoned[21]. Moreover, we updated our real-life clinical protocol that adopts NSAIDs as integral part of COVID-19 management[19] to be noted that we recommend against the concomitant use of prophylactic low dose aspirin and NSAIDs, or at least some of them[29] while conducting the anticipated clinical trials, while obviously opting for NSAIDs over low dose aspirin.

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None

#### **Conflicts of interest**

The author declares that there is no conflict of interest.

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