

1 **Perspective**

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

4 Mina T. Kelleni, MD, PhD

5 Pharmacology Department, College of Medicine, Minia University, Egypt

6 mina.kelleni@mu.edu.eg; drthabetpharm@yahoo.com

7 Mobile: +201200382422

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

9 **Abstract**

10 We have previously postulated potential numerous immunomodulatory and anti-
11 inflammatory benefits when we used non-steroidal anti-inflammatory drugs (NSAIDs)
12 safely and effectively to manage COVID-19. However, after aspirin has been suggested to
13 be independently associated with reduced risk of mechanical ventilation, ICU admission
14 and in-hospital mortality of COVID-19, we claim that the molecular interpretation of the
15 results that led to this suggestion was not scientifically accurate, and we provide our
16 academic interpretation. Moreover, we add other potential NSAIDs benefits related to
17 aspirin triggered lipoxins and resolvins, SARS CoV-2 ORF proteins dependent activation
18 of caspases and their subsequent mitochondrial dysfunction, endoplasmic reticulum stress
19 and necroptosis that were described with complicated COVID-19. We pharmacologically
20 confirm the fact that NSAIDs are known to be caspase inhibitors and they might
21 independently inhibit other caspases related COVID-19 associated downstream
22 pathological signaling mechanisms. Finally, we postulate that CARD-14, a caspase
23 recruitment domain-containing protein, polymorphisms might play a role in development
24 of severe and critical COVID-19.

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

27

Introduction

To date, July 02, 2021, COVID-19 has globally succumbed almost 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(Kelleni, 2021a; Pairo-Castineira et al., 2020) 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(Chow et al., 2021). 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.(Paranjpe et al., 2020) 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(Flam, Wintzell, Ludvigsson, Mårtensson & Pasternak, 2021). 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.(Warner, Nylander & Whatling, 2011) 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. (Panka, de Grooth, Spoelstra-de Man, Looney & Tuinman, 2017) 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. (Ikonomidis, Andreotti, Economou, Stefanadis, Toutouzas & Nihoyannopoulos, 1999) 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(Sonmez & Sonmez, 2017) 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(Serhan, Chiang & Van Dyke, 2008).

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(Chen et al., 2020).

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 β from viable monocytes as well as infectious disorders(Van Opdenbosch & Lamkanfi, 2019). SARS-CoV-2 infection was reported to activate caspase-8 triggering pro-inflammatory cytokines,

including IL-1 β , TNF- α , IL-7, IL-8, apoptosis, necroptosis and activation of the NF κ B pathway in lung epithelial cells which were suggested to share in COVID-19 induced downstream immune pathogenesis causing lung damage(Li et al., 2020). 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(Ren et al., 2020) 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(Ye, Wong, Li & Xie, 2008).

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 (Plassmeyer et al., 2020). 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(Donia & Bokhari, 2021). 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(Cao & Mu, 2021).

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 (Kelleni, 2021c).

More importantly, we would like to pharmacologically confirm that NSAIDs are well known, at physiologic in vivo concentrations, caspase inhibitors (Smith, Soti, Jones, Nakagawa, Xue & Yin, 2017) 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(Kelleni, 2021c; Kelleni, 2020; Kelleni MT, 2021a; Kelleni MT, 2021b) that might prevent or restore the immune dysregulation that is well described in COVID-19 and other diseases (Kelleni, 2021b).

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(Banerjee, Czinn, Reiter & Blanchard, 2020) and ERS markers were shown to be significantly increased in SARS CoV-2 infection and COVID-19 pneumonia (KÖSeler, Sabirli, GÖRen, TÜrkÇÜEr & Kurt, 2020) and interestingly, several NSAIDs including diclofenac, indomethacin, ibuprofen, aspirin, and ketoprofen were shown to suppress the ERS induced human neuroblastoma SH-SY5Y cell death(Yamazaki et al., 2006). 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(Omura et al., 2018) 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 (Kelleni, 2020; Kelleni MT, 2021a)

and we have explained the potential molecular immunomodulatory mechanisms upon which their COVID-19 efficacy might be reasoned(Kelleni MT, 2021b). Moreover, we updated our real-life clinical protocol that adopts NSAIDs as integral part of COVID-19 management(Kelleni, 2021c) to be noted that we recommend against the concomitant use of prophylactic low dose aspirin and NSAIDs, or at least some of them(Gurbel, Tantry & Weisman, 2019) while conducting the anticipated clinical trials, while obviously opting for NSAIDs over low dose aspirin.

Funding

None

Conflicts of interest

The author declares that there is no conflict of interest.

Data Availability

N/A

References

- Banerjee A, Czinn SJ, Reiter RJ, & Blanchard TG (2020). Crosstalk between endoplasmic reticulum stress and anti-viral activities: A novel therapeutic target for COVID-19. *Life Sci* 255: 117842.
- Cao L, & Mu W (2021). Necrostatin-1 and necroptosis inhibition: Pathophysiology and therapeutic implications. *Pharmacological research* 163: 105297-105297.
- Chen JS, Alfajaro MM, Wei J, Chow RD, Filler RB, Eisenbarth SC, *et al.* (2020). Cyclooxygenase-2 is induced by SARS-CoV-2 infection but does not affect viral entry or replication. *bioRxiv : the preprint server for biology*: 2020.2009.2024.312769.
- Chow JH, Khanna AK, Kethireddy S, Yamane D, Levine A, Jackson AM, *et al.* (2021). Aspirin Use Is Associated With Decreased Mechanical Ventilation, Intensive Care Unit Admission, and In-Hospital Mortality in Hospitalized Patients With Coronavirus Disease 2019. *Anesthesia & Analgesia* 132.
- Donia A, & Bokhari H (2021). Apoptosis induced by SARS-CoV-2: can we target it? *Apoptosis* 26: 7-8.

207
208 Flam B, Wintzell V, Ludvigsson JF, Mårtensson J, & Pasternak B (2021). Direct oral anticoagulant
209 use and risk of severe COVID-19. *Journal of Internal Medicine* 289: 411-419.

210
211 Gurbel P, Tantry U, & Weisman S (2019). A narrative review of the cardiovascular risks
212 associated with concomitant aspirin and NSAID use. *Journal of Thrombosis and Thrombolysis* 47:
213 16-30.

214
215 Ikonomidis I, Andreotti F, Economou E, Stefanadis C, Toutouzas P, & Nihoyannopoulos P (1999).
216 Increased Proinflammatory Cytokines in Patients With Chronic Stable Angina and Their
217 Reduction By Aspirin. *Circulation* 100: 793-798.

218
219 Kelleni M (2021a). ACE2 Polymorphisms Reflected on the Immune and Apelinergic Peptide
220 Systems: Potential COVID-19 Tools for Risk Stratification and Therapy. *Authorea (Preprint)* 2021
221 DOI: 1022541/au16212667006196092/v2.

222
223 Kelleni M (2021b). COVID-19, Ebola Virus Disease and Nipah Virus Infection Reclassification as
224 Novel Acute Immune Dysrhythmia Syndrome (n-AIDS): Potential Curative Role for
225 Immunomodulators. . *Authorea (Preprint)* 2021 DOI: 1022541/au16212670110777816/v2.

226
227 Kelleni M (2021c). NSAIDs/Nitazoxanide/Azithromycin Immunomodulatory Protocol Used in
228 Adults, Geriatric, Pediatric, Pregnant, and Immunocompromised COVID-19 Patients: A
229 Prospective Observational Study and Case-Series. *Authorea (Preprint)* 2021 DOI:
230 1022541/au16212660115715282/v4.

231
232 Kelleni MT (2020). ACEIs, ARBs, ibuprofen originally linked to COVID-19: the other side of the
233 mirror. *Inflammopharmacology* 28: 1477-1480.

234
235 Kelleni MT (2021a). Early use of non-steroidal anti-inflammatory drugs in COVID-19 might
236 reverse pathogenesis, prevent complications and improve clinical outcomes. *Biomed*
237 *Pharmacother* 133: 110982.

238
239 Kelleni MT (2021b). NSAIDs/Nitazoxanide/Azithromycin Repurposed for COVID-19: Potential
240 Mitigation of the Cytokine Storm Interleukin-6 Amplifier via Immunomodulatory Effects. *Expert*
241 *Review of Anti-infective Therapy* 2021;DOI: 101080/1478721020211939683.

242
243 KÖSeler A, Sabirli R, GÖRen T, TÜRKÇÜEr İ, & Kurt Ö (2020). Endoplasmic Reticulum Stress
244 Markers in SARS-COV-2 Infection and Pneumonia: Case-Control Study. *In Vivo* 34: 1645.

245
246 Li S, Zhang Y, Guan Z, Li H, Ye M, Chen X, *et al.* (2020). SARS-CoV-2 triggers inflammatory
247 responses and cell death through caspase-8 activation. *Signal Transduction and Targeted*
248 *Therapy* 5: 235.

249
250 Omura T, Sasaoka M, Hashimoto G, Imai S, Yamamoto J, Sato Y, *et al.* (2018). Oxidant-derived
251 non-steroidal anti-inflammatory drugs suppress 1-methyl-4-phenyl pyridinium-induced cell
252 death via repression of endoplasmic reticulum stress response and mitochondrial dysfunction in
253 SH-SY5Y cells. *Biochemical and Biophysical Research Communications* 503: 2963-2969.

254
255 Pairo-Castineira E, Clohisy S, Klaric L, Bretherick AD, Rawlik K, Pasko D, *et al.* (2020). Genetic
256 mechanisms of critical illness in Covid-19. *Nature*.

257
258 Panka BA, de Grooth H-J, Spoelstra-de Man AME, Looney MR, & Tuinman P-R (2017). Prevention
259 or Treatment of Ards With Aspirin: A Review of Preclinical Models and Meta-Analysis of Clinical
260 Studies. *Shock* 47: 13-21.

261
262 Paranjpe I, Fuster V, Lala A, Russak AJ, Glicksberg BS, Levin MA, *et al.* (2020). Association of
263 Treatment Dose Anticoagulation With In-Hospital Survival Among Hospitalized Patients With
264 COVID-19. *Journal of the American College of Cardiology* 76: 122-124.

265
266 Plassmeyer M, Alpan O, Corley MJ, Lillard K, Coatney P, Vaziri T, *et al.* (2020). Caspases in COVID-
267 19 Disease and Sequela and the Therapeutic Potential of Caspase Inhibitors. *medRxiv*:
268 2020.2011.2002.20223636.

269
270 Ren Y, Shu T, Wu D, Mu J, Wang C, Huang M, *et al.* (2020). The ORF3a protein of SARS-CoV-2
271 induces apoptosis in cells. *Cellular & Molecular Immunology* 17: 881-883.

272
273 Serhan CN, Chiang N, & Van Dyke TE (2008). Resolving inflammation: dual anti-inflammatory and
274 pro-resolution lipid mediators. *Nature Reviews Immunology* 8: 349-361.

275
276 Smith CE, Soti S, Jones TA, Nakagawa A, Xue D, & Yin H (2017). Non-steroidal Anti-inflammatory
277 Drugs Are Caspase Inhibitors. *Cell Chem Biol* 24: 281-292.

278
279 Sonmez O, & Sonmez M (2017). Role of platelets in immune system and inflammation. *Porto*
280 *Biomedical Journal* 2: 311-314.

281
282 Van Opdenbosch N, & Lamkanfi M (2019). Caspases in Cell Death, Inflammation, and Disease.
283 *Immunity* 50: 1352-1364.

284
285 Warner TD, Nylander S, & Whatling C (2011). Anti-platelet therapy: cyclo-oxygenase inhibition
286 and the use of aspirin with particular regard to dual anti-platelet therapy. *British Journal of*
287 *Clinical Pharmacology* 72: 619-633.

288

289 Yamazaki T, Muramoto M, Oe T, Morikawa N, Okitsu O, Nagashima T, *et al.* (2006). Diclofenac, a
290 non-steroidal anti-inflammatory drug, suppresses apoptosis induced by endoplasmic reticulum
291 stresses by inhibiting caspase signaling. *Neuropharmacology* 50: 558-567.

292

293 Ye Z, Wong CK, Li P, & Xie Y (2008). A SARS-CoV protein, ORF-6, induces caspase-3 mediated, ER
294 stress and JNK-dependent apoptosis. *Biochim Biophys Acta* 1780: 1383-1387.

295

296