Targeting cyclooxygenase enzyme for the adjuvant COVID-19 therapy

Parteek Prasher1,3 | Mousmee Sharma1,2 | Ravi Gunupuru3

1UGC Sponsored Centre for Advanced Studies, Department of Chemistry, Guru Nanak Dev University, Amritsar, India
2Department of Chemistry, Uttaranchal University, Dehradun, India
3Department of Chemistry, University of Petroleum & Energy Studies, Dehradun, India

Correspondence
Parteek Prasher, UGC Sponsored Centre for Advanced Studies, Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, India.
Email: parteekchemistry@gmail.com

Abstract
Despite vigorous efforts, the COVID-19 pandemic continues to take a toll on the global health. The contemporary therapeutic regime focused on the viral spike proteins, viral 3CL protease enzyme, immunomodulation, inhibition of viral replication, and providing a symptomatic relief encouraged the repurposing of drugs to meet the urgency of treatment. Similarly, the representative drugs that proved beneficial to alleviate SARS-CoV-1, MERS-CoV, HIV, ZIKV, H1N1, and malarial infection in the past presented a sturdy candidature for ameliorating the COVID-19 therapeutic doctrine. However, most of the deliberations for developing effective pharmaceuticals proved inconsequential, thereby encouraging the identification of new pathways, and novel pharmaceuticals for capping the COVID-19 infection. The COVID-19 contagion encompasses a burst release of the cytokines that increase the severity of the infection mainly due to heightened immunopathogenicity. The pro-inflammatory metabolites, COX-2, cPLA2, and 5-LOX enzymes involved in their generation, and the substrates that instigate the origination of the innate inflammatory response therefore play an important role in intensifying and worsening of the tissue morbidity related to the coronavirus infection. The deployment of representative drugs for inhibiting these overexpressed immunogenic pathways in the tissues invaded by coronaviruses has been a matter of debate since the inception of the pandemic. The effectiveness of NSAIDs such as Aspirin, Indomethacin, Diclofenac, and Celecoxib in COVID-19 coagulopathy, discouraging the SARS viral replication, the inflammasome deactivation, and synergistic inhibition of H5N1 viral infection with representative antiviral drugs respectively, have provided a silver lining in adjuvant COVID-19 therapy. Since the anti-inflammatory NSAIDs and COXIBs mainly function by reversing the COX-2 overexpression to modulate the overproduction of pro-inflammatory cytokines and chemokines, these drugs present a robust treatment option for COVID-19 infection. This commentary succinctly highlights the various claims that support the status of immunomodulatory NSAIDs, and COXIBs in the adjuvant COVID-19 therapy.

KEYWORDS
COVID-19, COX-2, immunomodulation
Typically, the inception of severe COVID-19 contagion results from a dysregulated inflammatory immune response causing elevated levels of inflammatory chemokines and cytokines, especially Interleukin-6 (IL-6) in the infected patients (Ulhaq et al., 2020). The crucial role played by the cyclooxygenase enzyme, and the metabolites biosynthesized by its catalytic activity on the membrane bound phospholipids contribute to the development and progression of this heightened immune response that manifests chronic inflammation and related ailments, homeostatic dysregulation, and organ dysfunction that proves hazardous. The severity of incursion by the invading stimuli elicits the innate immune response to create a cytokine storm, which onsets the pathogenesis of these perilous conditions (Prasher et al., 2019). As such, the arachidonic acid pathway, associated cyclooxygenase enzymes, and the resultant metabolites serve as mainstay in the manifestation of a chronic immune response towards an external physical, chemical, or biological stimulus, which cause the release of the polyunsaturated fatty acid substrates from the membrane-bound phospholipids (Hoxha, 2020). Principally, the inducible COX-2 isoform belonging to the prostaglandin-endoperoxide synthase (PTGS) 'cyclooxygenase' family of enzymes overexpresses in response to an adverse physicochemical background, or invasion by pathogenic viruses thereby executing the production of pro-inflammatory cytokines that directly influence the physiological homeostasis of the affected/infected tissues (Capuano et al., 2020). The profusely produced COX-2 metabolites in response to a microbial invasion further result in the manifestation of coagulopathy, pleurisy, and sepsis that further intensify the infection. Presently, the urgency of an effective treatment regime for managing the COVID-19 infection has labeled several biochemical and metabolic pathways under clinical investigation that however, managed a trivial output. While some managed to progress, majority of the repurposed drugs aimed at ameliorating COVID-19 infection including remdesivir, and favipiravir provided inconclusive results in the clinical trials for curbing the pandemic, which further raises an alarming situation, while looking at the successive deadly waves of COVID-19 contagion (Mullard 2020) that continue to claim a significant global morbidity and mortality. In this commentary, we propose the relevance of the inhibitors of cyclooxygenase enzyme as latent therapeutics in adjuvant COVID-19 therapy.

Reportedly, the SARS-associated coronaviruses require spike (S) protein for identifying the receptors, and enduring the cell membrane fusion processes that reportedly activate the expression of COX-2 isozyme in a physiological setting, thereby supporting the prospect of causing inflammation by the former (Liu et al., 2006). The spike protein mediated activation of COX-2 in SARS-CoV infection manifests pulmonary inflammation and immune hyperactivity that further aggravate the pathogenesis of the infection caused by coronaviruses (Huang et al., 2020). Owing to the association between the COVID-19 contagion, and the role of cyclooxygenase enzyme in amplifying the immunogenic response in response to the former, the immunomodulator drugs such as NSAIDs and COXIBs that moderate the expression of cyclooxygenases to sustain tissue homeostasis present a robust candidature in managing the immunopathogenicity of COVID-19 in the form of an adjuvant therapy.

Amici et al. (2006) reported an important aspect of COX inhibitor NSAID 'Indomethacin' that directly influenced the viral replication machinery and viral RNA synthesis at physiologically tolerable doses, instead of the anticipated symptomatic relief in SARS by cyclooxygenase inhibition by a typical NSAIDs. Moreover, the drug exhibited a trivial effect on the binding and entry of coronavirus to the host cells, while presenting a substantial (>1000 fold) reduction in the viral load. Li et al. (2014) presented similar observations for selective COX-2 inhibitor 'Celecoxib' that offered a synergistic inhibition of the CK1 and H5N1 viral infection in combination with the antiviral drug ‘Zanamivir’. The combination therapy successfully ameliorated the acute lung inflammation by lowering the viral load, and by improving the survival rate of the infected mouse models (Hong et al., 2020). On the contrary, the mechanism of virus suppression by the COX inhibitor NSAID ‘Diclofenac’ occurs by blocking the activity of acid-sensing ion channels essential for the stimulation of pro-inflammatory NLRP3 inflamasome. Essentially, the transmembrane pore forming viral protein ‘Viroplon 3a’ of the SARS coronaviruses induce the activation of inflamasome, to which the diclofenac poses a considerable inhibitory effect (Chen et al., 2019). Diclofenac reportedly inhibits the production of PPAR-γ, and Phospholipase A2 G2D, whose overexpression in alveolar macrophages in response to SARS-CoV infection leads to the production of inflammatory cytokines (Gan et al., 2010). These observations lay a strong foundation for the possible use of COXIBs and NSAIDs as impending therapeutics in overcoming the COVID-19 infection.

Despite the benefits of NSAIDs and COXIBs in managing SARS coronavirus, the World Health Organization discouraged the repurposing of COX inhibitor ‘Ibuprofen’ in COVID-19 therapy, owing to its role in causing the overexpression of angiotensin-converting enzyme (ACE)-2 receptor, which the SARS coronaviruses utilize for entry to the host cells (Moore et al., 2020). The tissues that display high expression of ACE2 become highly vulnerable to the injury caused by SARS coronavirus infection. Similarly, the dual pro/anti-inflammatory stature of the eicosanoid ‘Prostaglandin D2’ (PGD2) cannot be undermined while appraising the role of cyclooxygenase in COVID-19 therapy (Robb et al., 2020). The reports suggested a necessity of D-prostanoid receptor 1 (DP1) mediated PGD2 signaling for the activation of macrophages and Interferon gamma expression following coronavirus attack (Vijay et al., 2017). However, the evidence in support of these affirmations lack clinical evidence, since several reports indicated an inconsequential impact of the upregulation of ACE2 activity on the severity of COVID-19 pathogenesis (Reynolds et al., 2020). Nevertheless, the COVID-19 patients using ACE2 inhibitors displayed a lower mortality rate, which further confirm these observations (Ip et al., 2020). In addition, the upregulation of ACE2 by ibuprofen relies on a single animal experimental data obtained from the streptozotocin-induced diabetic animals with myocardial fibrosis (Qiao et al., 2015). Hence, it makes the claim of ignoring Ibuprofen in adjuvant COVID-19 therapy highly debatable.
A recent report by Ong et al. (2020) annulled the rising speculations on the adverse effects of COX inhibitor NSAIDs and COXIBs as impending therapeutics or adjuvant drugs against COVID-19 by performing large randomized controlled trials in the infected patients. The cohorts administered with the NSAID ‘Etoricoxib’ displayed a significant reduction in the levels of Interleukin-6 resulting in the non-requirement of non-invasive or invasive ventilation or transfer to the Intensive Care Units. Interestingly, the cohorts administered with NSAIDs did not develop adverse effects associated with the cicloxygenase inhibition therapy, such as gastrointestinal or cardiovascular intricacies. These observations provided impetus towards the clinical benefits of NSAIDs and selective COX-2 inhibitors as an attractive intervention in managing COVID-19 pandemic. Another report by Kelleni (2020; 2021) provided the clinical validation for the effectiveness of ibuprofen and diclofenac potassium in delivering antipyretic and analgesic profile among COVID-19 patients, who experienced an ameliorated immune response, high lymphocytic count and a rapid recovery rate when administered with the mentioned drugs.

Besides, the NSAIDs and COXIBs offer a sturdy resolute in guiding the COVID-19 coagulopathy, reversing of virus-induced pleurisy, and COVID-associated sepsis. Rojas et al. (2020) reported coagulation abnormalities in patients contacted with pneumonieae, showing pleurisy caused by COVID-19 infection. The human subjects admitted in intensive care units in spite of tolerable thrombophrophylaxis developed pulmonary embolism, and deep vein thrombosis (Lodigiani et al., 2020; Middeldorp et al., 2020). The presence of capillarostasis and microthrombi further supported the coagulopathy induced by COVID-19 infection as a biomarker for the diseases progression (Menter et al., 2020). The association of coagulopathy and COVID-19 pathogenesis provided an opportunity for investigating the anti-thrombotic effects of COX inhibitors such as ‘Aspirin’ for ameliorating the COVID-19 instigated coagulopathy. Aspirin offers antithrombotic effects by performing the regulation of platelet function and by irreversibly inhibiting the activity of COX-2 isozyme (Pillinger et al., 1998). Interestingly, the antiviral effects of aspirin include the inhibition of replication by downregulating the COX-2 mediated prostaglandin E2 production in macrophages, while upregulating the production of type I interferon. Several investigations suggested the improvement in the lung injury by aspirin, which prevents the agglomeration of neutrophils and platelets at the injured tissues (Middleton et al., 2016). The administration of Aspirin for antplatelet therapy in COVID-19 patients having cardiovascular ailments proved quite safe (Little et al., 2020), while for pregnant women, the administration of prophylactic aspirin in combination with heparin proved highly beneficial (Kwiatkowsky et al., 2020; Hamulyak et al., 2020). In addition, the clinical investigations by Vivas et al. (2020) recommended COX inhibitor aspirin as antithrombotic drug of choice for the patients having stable angina, while a combination of aspirin and clopidogrel proved better as antplatelet therapy in patients recommended for percutaneous revascularization. These claims rightfully validate the application of COX inhibitor ‘Aspirin’ for the prophylaxis of COVID-19 triggered coagulopathy (Hussein et al., 2020).

Notably, the COVID-19 patients with critical illness developed viral sepsis symptomized by clinical manifestations of shock, weak peripheral pulses, cold extremities, dysfunctional microcirculation, and organ dysfunction in extreme scenarios. The COVID-19 induced sepsis and the treatment options thereof provided a robust foundation for relieving the viral infection (Li et al., 2020). The prostaglandins biosynthesized by COX-2 isoenzyme play a critical role in determining the severity of septic shock that causes progressive organ failure, and hemodynamic breakdown. The clinical trials on COX-2 inhibitor ‘Aspirin’ and NSAIDs such as ibuprofen, diclofenac, and indomethacin in septic patients indicated a lower in-hospital mortality rate, where low-dose aspirin exhibited a more remarkable effect (Sossdorf et al. 2013). Besides, the NSAIDs and COX-2 inhibitors play an important role in reversing of COVID-19 manifested pleurisy, which serves as the initial symptoms in the virus infection. The case studies conducted by Oleynick 2020, reported the onset of pleuritic chest pain in hypertensive and type 2 diabetes mellitus patients, who eventually developed hypoxemia followed by the confirmation of COVID-19 infections. The symptoms however improved with time thereby indicating the pleurisy as a preliminary biomarker for the onset of COVID-19 infection. The translational investigations by Gilroy et al. (1999) revealed the efficacy of COX inhibitor ‘Indomethacin’ in the inhibition of carrageenan-induced pleurisy. Notably, the administration of selective COX-2 inhibitor NS-398 offered a more pronounced effect. The pre-clinical investigations on H7N9 virus infected animal models on treatment with selective COX-2 inhibitor ‘Celecoxib’ co-administered with Zanamivir displayed a remarkable improvement in the mortality rate and lung pathology (Li et al., 2014).

The application of immunomodulating NSAIDs in attenuating the coronavirus infection suffered setback in the beginning, however the claims in the latter half of 2020 rightfully supported the accommodation of NSAIDs and COXIBs in the concurrent adjuvant COVID-19 therapy. In addition to providing a symptomatic relief, several NSAIDs disrupt the development and annihilate the propagation machinery of SARS coronaviruses, which however further require a robust scientific justification and clinical trials to appraise the hullabaloo centered on their application of in COVID-19 therapy. It necessitates a rapid intensification of detailed investigations in the first quarter of the year 2020, focused on the immunomodulatory drugs based COVID-19 therapy for the development of impending COVID-19 chemotherapeutics.

ACKNOWLEDGMENT
The authors duly acknowledge SEED support by the R&D, UPES.

CONFLICT OF INTEREST
The authors have no conflict of interest.

DATA AVAILABILITY STATEMENT
Data sharing not applicable – no new data generated

ORCID
Parteek Prasher https://orcid.org/0000-0002-9412-9424
signaling. *Proceedings of the National Academy of Sciences*, 114, E5444–E5453.

Vivas, D., Roldan, V., Pastor, M. A. E., Roldan, I., Montoliu, A. T., Nodar, J. M. R., Sales, J. C., Gamez, J. M., Consuegra, L., Ferreiro, J. L., & Marin, F. (2020). Recommendations on antithrombotic treatment during the COVID-19 pandemic. Position statement of the working group on cardiovascular thrombosis of the Spanish Society of Cardiology. *Revista Española de Cardiología*, 73, 749–757.