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REVIEW

Indomethacin: an exploratory study of antiviral mechanism and host-pathogen interaction in COVID-19

Nishant Shekhar, Harpinder Kaur, Phulen Sarma, Ajay Prakash and Bikash Medhi

Department of Pharmacology, Postgraduate Institute of Medical Education and Research, Chandigarh, INDIA

ABSTRACT

Introduction: COVID-19, a dreadful pandemic that has impacted human life like no other pathogenic invasion, has claimed the lives of over 100 million people. The need for effective treatment strategies is still a subject of intense research considering the rapidly evolving genome and continental diversity. Indomethacin is administered mostly as co-treatment for affected patients as a non-steroidal anti-inflammatory drug (NSAID). However, the underlying mechanism of action is unresolved. This study explores the basal mechanism of indomethacin and potency in alleviating the damage caused by SARS-CoV-2 and discusses the experimental and clinical efficacy in recent studies.

Areas covered: The literature search and system biology-based network formation were employed to describe the potent effects and risks associated with indomethacin in in-vitro, in-vivo, and clinical studies. This study also highlights the plausible mechanism of antiviral action of indomethacin with its apparent viral protein targets. The SARS-CoV-2 protein, the interacting host proteins, and the effect of indomethacin on this interactome as a standalone treatment or as part of a co-therapy strategy are particularly emphasized using network modeling.

Expert opinion: Indomethacin has demonstrated excellent clinical endpoint characteristics in several studies, and we recommend that it be utilized in the treatment of mild-to-moderate COVID patients.

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COVID-19; indomethacin; NSAID; anti-viral; genetic interaction network; protein–protein interaction; mechanism of action

1. Introduction

In the current pandemic, medical history has seen the array of trials for the repurposed application of several drugs, where Indomethacin, a non-steroidal anti-inflammatory drug (NSAID) lies as one of the common drugs being used in symptomatic treatments. NSAIDs are inhibitors of the enzyme cyclooxygenase (COX) which plays a role in converting arachidonic acid into prostaglandins, thromboxane, and prostacyclins. COX-1 is expressed in the body constitutively and participates in various homeostatic functions, whereas COX-2 is expressed at the time of inflammatory response and is involved in the production of prostanoids. NSAIDs can be either nonselective (inhibit both COX-1 and −2) or selective (inhibit COX-2). Indomethacin is a nonselective reversible COX inhibitor used in the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, bursitis, gouty arthritis, and for the closure of patent ductus arteriosus [1]. It has also been demonstrated to have potential antitumor activity and to enhance the action of neoplastic medicines. Further, it has been shown to have antiviral activity against hepatitis B virus, rhabdovirus vesicular stomatitis virus, and coronavirus [2]. It is a well-established, cost-effective, easily available, and efficacious drug with expectable toxicity profiles [3,4].

Hanly et al. (1987) reported that indomethacin significantly improved mean arterial oxygen tension in 10 hypoxemic respiratory failure patients; however, the response was variable among patients [5]. Moreover, Steinberg et al. also reported that oxygenation was improved by indomethacin in acute respiratory distress syndrome (ARDS) patients [6]. Further, Sacerdote et al. (1995) and Bour et al. (2000) reported that indomethacin considerably decreases the levels of the pro-inflammatory cytokine interleukin-6 (IL-6) [7,8]. Amici et al. reported direct antiviral activity of indomethacin by inhibition of viral RNA synthesis against SARS-CoV and canine CoV. The authors suggested that this effect was not dependent on the COX inhibition effect of indomethacin. Additionally, indomethacin has shown remarkable efficacy in pro-inflammatory actions of unusually high bradykinin levels and is effective in angiotensin-converting enzyme inhibitors induced dry cough. Therefore, Alkotaji et al. proposed that indomethacin can also be used as a potential therapy for dry cough induced by COVID-19 which can be possible because of bradykinin accumulation. Looking at this manifold of responsive efficacy of indomethacin, it is important to explore the underlying mechanism and look for relevant clinical trials data for COVID-19 treatment.

However, several studies (mainly *in vitro* and preclinical) have reported that NSAIDs do not always exhibit protective effects. Bancos et al. (2006) suggested that NSAIDs may reduce host defense after an infection or vaccination. According to their study, NSAIDs reduced the synthesis of IgM and IgG in stimulated human peripheral blood mononuclear cells (PBMCs) [9]. The relationship between NSAIDs use and complication risk in pneumonia patients has also been reported, and Voirot et al. (2019) proposed that this may be because of a delay in detection of pneumonia, resulting in a more invasive

CONTACT Bikash Medhi drbikashus@yahoo.com Department of Pharmacology, Pgimer, Chandigarh

Article highlights

- Independently of its COX-inhibitor activity, indomethacin, an NSAID has been found to display antiviral activity, albeit its action mechanism is not particularly apparent.
- This work underlines how indomethacin's potential viral protein targets are feasible antiviral action mechanisms.
- With our original host-virus interactome investigation, we summarize the pre-clinical and clinical results of indomethacin treatment in COVID-19 cases.

We believe that thorough investigations that elaborate on the pharmacology of indomethacin in COVID-19 should be emphasized. This can be achieved by comprehensive studies like proteomics, genetic interaction and expression profile studies of the drug.

illness and a reduction in recruitment of innate immune cells [10]. As of 2 June 2021, three clinical trials of indomethacin to test its efficacy in COVID-19 have been registered in the public domain (Table 1).

2. Antiviral activity of Indomethacin

It has been reported that indomethacin exhibits antiviral activity independent of its COX-inhibitory activity, although the mechanism of action is not very clear. Moreover, it is also known to have anti-cancer properties. Brunelli et al. (2012) showed that double-stranded RNA (dsRNA)-dependent protein kinase PKR is selectively activated by indomethacin, which causes quick phosphorylation of eIFα (eukaryotic translation initiation factor 2's α-subunit) and blocks protein synthesis in cancer cells [11]. PKR plays a crucial part in the cellular defense response against infection by the virus too. Amici et al. (2015) studied indomethacin's effect on the activity of PKR on infection with prototype rhabdovirus vesicular stomatitis virus. Indomethacin activated PKR, resulting in eIF2α phosphorylation, and in turn shutting of translation of viral protein and inhibiting replication of the virus (IC50 = 2 M) and thus protecting the host cells from damage caused by the virus. The authors suggested that PKR is an important target for indomethacin's antiviral activity and phosphorylation of eIF2α could be an important element in its broad-spectrum antiviral activity [12]. NSAIDs have also been reported to play a role during the inflammatory process via alteration of adherence, degranulation, phagocytosis, and production of ROS by polymorphonuclear neutrophils (PMN). They can decrease PMN recruitment and alter their intrinsic roles. Further, in the acute pleural effusion model, treatment with indomethacin and other NSAIDs considerably decreases the exudate volume and leukocyte migration. NSAIDs inhibit NF-κB transcriptional activity induced by TNFα and thus contribute to a decrease in the local release of pro-inflammatory cytokines [13–15].

3. Evidence for use of Indomethacin in COVID-19

3.1. *In vitro*

Kiani et al. (2021) evaluated the in vitro antiviral activity of naproxen, ketotifen, and indomethacin alone and in combination. The effectiveness of the drugs to reduce replication of SARS-CoV-2 and their cytotoxicity was assessed. The results revealed that ketotifen when combined with indomethacin or naproxen reduced the viral yield. They suggested an additive/synergistic effect for these combinations of drugs. Ketotifen alone showed 60% inhibition of SARS-CoV-2 and this percentage was increased to 79%, 83%, and 93% when ketotifen was administered in combination with 25, 50, and 100 μM indomethacin, respectively. In comparison to ketotifen alone, the percentage inhibition of SARS-CoV-2 increased to 68%, 68%, and 92% when it was administered in combination with 25, 50, and 100 μM naproxen, respectively. Cytotoxic effects were not seen for naproxen, ketotifen, and indomethacin's administered dosages [16].

3.2. *In vivo* (pre-clinical)

In vivo efficacy of indomethacin was ascertained by assessing the time of recovery in dogs infected with canine-CoV in a study conducted by Xu et al. (2020). Significantly rapid improvement was observed in infected dogs treated symptomatically and with indomethacin (1 mg/kg) as compared to symptomatic treatment and ribavirin, however, there was no significant difference from that with symptomatic treatment + canine hemoglobin + anti-canine coronavirus serum + canine blood immunoglobulin + interferon treatments [17,18].

Gomeni et al. (2020) also suggested that indomethacin could prove to be a potential candidate for treating SARS-CoV-2 infection. They developed a multi-stage model-based

Table 1. Clinical trials of indomethacin.

	Trial ID	Phase/ Status	Intervention	Study design	Number enrolled/ Location
1	NCT04344457	Phase 1| Phase 2 Recruiting	Hydroxychloroquine Indomethacin Zithromax Oral Product	Single Group Assignment Masking: None	80 US
2	NCT04410536	Not Applicable Recruiting	Symptomatic drugs (oral triptan or 50–100 mg suppository of indomethacin, on maximum 3 days/10 days, in case of extremely severe headache only- and to use metoclopramide) Bridge therapy Mindfulness program	Single Group Assignment Masking: None	25 Italy
3	IRCT20200427047215N1	Phase 3 Recruitment complete	Slow release of Indomethacin tablets, 75 mg daily for five days A placebo similar to indomethacin tablets is given daily for five days	Randomized single blind	60 Iran

method to describe both percent recovery as well as the viral load in dogs infected with C-CoV, evaluate the pharmacokinetics of indomethacin in dogs as well as in humans by utilizing the data that has been published on the administration of immediate-release (IR) and sustained-release (SR) formulations, and evaluate estimated antiviral action as a function of various suppositions on effective exposure in humans. According to the results of this study, the dosage regimes that performed best among other doses were 50 mg thrice a day for IR formulation and 75 mg twice a day for SR formulation and treatment using this dose of SR formulation is believed to accomplish a full response in 3 days for management of COVID-19 patients [19].

3.3. Clinical

Several studies have shown the efficacy of indomethacin in COVID-19. Rajan et al. suggested that to decrease the severity of nonspecific symptoms such as cough, fever, and musculoskeletal pain, treatment with indomethacin's low dose can be initiated as early as possible in the progression of COVID-19. Moreover, according to the author, indomethacin can also decrease the requirement for hospitalization as well as the risk of spreading the infection. The authors treated 17 individuals (14 RT-PCR positives and 3 having close contact with positive COVID-19 patients) with 25 mg indomethacin, two times a day. The nonspecific symptoms were resolved after two indomethacin doses in 14 patients, one required dose escalation to 75 mg, and 2 developed hypoxemia [20].

Kanakaraj et al. (2020) also treated 12 mild COVID-19 patients who were kidney transplant recipients with 25 mg IR indomethacin two times a day. The condition of four patients worsened and required hospital admission, but all the patients made a good recovery after 4 weeks. The authors also suggested that in addition to symptomatic treatment, indomethacin also helped in preventing more hospitalizations by either attenuating or preventing a cytokine storm [21].

Further, Ravichandran et al. (2020) conducted a study in two groups. In group one, mild and moderate patients were included and the endpoint was the development of hypoxia. In this group, treatments based on indomethacin and paracetamol in addition to the standard protocol for the treatment of COVID-19 patients were compared. In group two, severe patients were included and the endpoint was need for mechanical ventilation or ICU admission. In this group, patientswho had hypoxia were given indomethacin and remdesivir. According to their observations, with indomethacin treatment there was a decrease in the number of days to become afebrile, lessening of myalgia and cough by half in comparison to treatment with paracetamol. 1/ 72 patients treated with indomethacin required oxygenation, whereas 28/72 patients treated with paracetamol required oxygenation. No patients in the second group required mechanical ventilation, and no adverse effects were seen with indomethacin administration. Hence, the authors suggested that indomethacin added to standard care offers more rapid symptomatic relief and prevents progression of pneumonia and therefore, replacement of paracetamol with indomethacin should be taken into consideration [22].

4. Network-based mechanistic interaction of Indomethacin

This section of the study aims to employ network modelling to explore the possible molecular targets and pathways that indomethacin interacts with and modulates. Network modelling is a system biology approach that facilitates the exploration of biological systems and interactions between different biological systems as a whole and as components. Several published experimental studies on SARS-CoV-2-human protein–protein interactions (PPI) have also been illustrated as network clusters in order to make convenient remarks. Gordon et al. (2020) used affinity-purification mass spectrometry (AP-MS) to study interactions of human proteins and 26 expressed SARS-CoV-2 proteins [23]. The authors also identified 66 druggable human targets in the study. More recently, Oh et al. (2021) published an article focusing on the network formation that defines the direct interaction of NSAIDs with human proteins in order to discover druggability [24]. The authors also tried to unmask the endogenous pathways that are altogether affected by COVID-19 infection. These studies are the foundation of this section in that we have made a curated network from publicly available databases by merging them with common attributes. We have obtained a human-SARS-CoV-2 PPI network from Gordon & Jang et al. (2020), a chemical–gene interaction network from CTD (comparative toxicogenomics database), a drug–gene interaction database (DGIdb) , and a SARS-CoV-2 infected human cell transcriptomics consensome network from signaling pathways database [25–28]. Cytoscape 3.8.2 was used to access and create the resulting drug → human target gene → viral protein network [29]. The contrariety in the outcome of literature-based evidence, i.e. strong and adequate shreds of evidence, led us to address this in two subsections.

4.1. Indomethacin as a strong lead

DGIdb drug-gene network, human-SARS-CoV-2 PPI network, and SARS-CoV-2 infected human cell transcriptomics consensome network were combined to form an integrated network with the matching affected human genes/gene products as overlapping nodes. The edge between indomethacin and PTGES2 (prostaglandin-endoperoxide synthase) & ABCC1 (ATP Binding Cassette Subfamily C Member 1 or Multidrug resistance-associated protein 1) indicates the drug–gene interaction and its width signifies their interaction score (Figure 1). Furthermore, the edges between SARS-CoV-2 proteins Nsp7 and Orf9c with PTGES2 and ABCC1 respectively denote PPI where its width is attributed to the average spectral count (AvgSpec), which indicates the abundance of bait (CoV proteins) and human proteins complex formation. To reduce the false-positive selection of affected human genes from SARS-CoV-2 infection, we calibrated the node selection in a consensome network by selecting the nodes with a p-value < 0.05.

Indomethacin actively inhibits the expression of PTGES2 hence inhibiting the prostaglandin synthesis and reducing pain and inflammation significantly [30–32]. Gordon & Jang et al. (2020) have also highlighted indomethacin as a PTGES2 inhibitor in their work. The

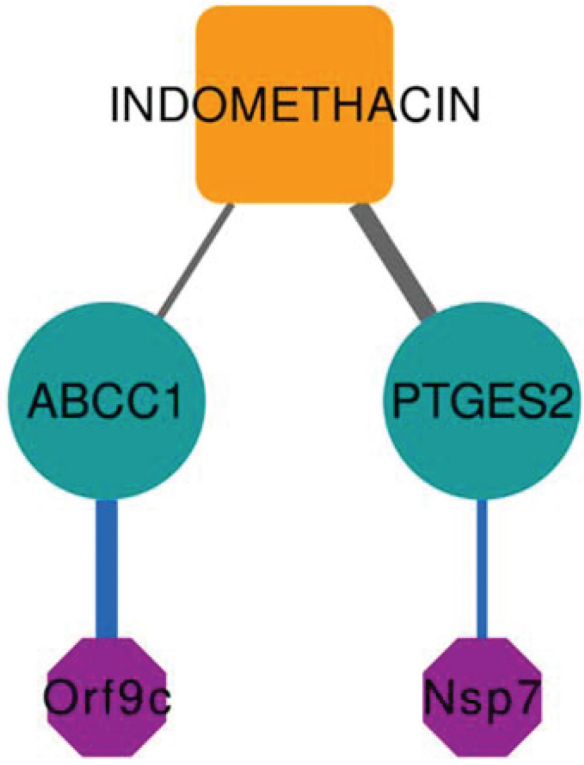

Figure 1. High confidence drug-protein-protein (human-virus) interaction with 5 nodes and 4 edges depicting the action of indomethacin on SARS-CoV-2 infection perturbed human genes (teal colour nodes). The width of grey edges is proportional to drug–gene interaction strength. Similarly width blue edges represents the strength of human-SARS-CoV-2 protein interaction.

inhibition of ABCC1 transporter by indomethacin treatment alone is supported by in vitro studies like Matsunaga et al. (2006) and Leite et al. (2007), which report significant reversal of ABCC1 activity leading to reduced multi-drug resistance in the target cells [33,34]. The SARS-CoV -2 infection perturbed-consensome network suggests a 1.25 times geometric fold change ($p = 0.0089$) in ABCC1 expression in the human Calu-3 lung epithelial cells infected with SARS-CoV-2 with a multiplication of infection (MOI) equal to 5 [28,35]. This interaction clearly suggests that SARS-CoV-2 infection hampers the host's drug efflux machinery. Marginally responsive action of indomethacin in clinical administration might be attributed to the increased susceptibility of the infected cell to the antiviral treatment due to ABCC1 inhibition. Furthermore, we studied old CoVs-human protein interactions and found that there was no interaction or effect of SARS and MERS protein with ABCC1 or any ABC transporter in general, suggesting SARS and MERS COVs to be comparatively more susceptible to antiviral treatment than SARS-CoV-2 [36]. A recent study from Ong EZ et al. (2021) also identifies indomethacin targeting PTGES2 and ABCC1 explaining the causative genes for respiratory dysfunction by the number of gene clusters indomethacin interacts with, which was 3 in their case [37].

4.2. Indomethacin as adequate lead

We utilized CTD chemical–gene interaction network, the human-SARS-CoV-2 PPI network, and the SARS-CoV-2 infected transcriptomics consensome to form the resulting network. The selection of perturbed human genes ($p < 0.05$) was done to reduce the false-positive selection. No filter was applied to AvgSpec labeled edges of SARS-CoV-2-human PPIs (outer nodes). The resulting candidates in Figure 2 were studied individually for their significance in SARS-CoV-2 infection and modulation by indomethacin. The ones with significant evidence are discussed below:

Heme-oxygenase-1 (HMOX1) is the inducible isoform of heme-oxygenases (HO) that causes oxidative heme cleavage to biliverdine, carbon monoxide, and ferrous iron discharge, It has a regulatory role in intravascular inflammation activity [38]. Datillo (2020) using various studies, including Renieris (2020) explained that fatal SARS-CoV-2 infection leads to a very low level of serum H_2S level in contrast to recovered cases [39,40]. In his model, Datillo proposed that SARS-CoV-2 infection downregulates HO-1 which leads to reduced heme breakdown, low CO concentration, which leads to low serum H_2S concentration (HO-1/CO/H_2S axis). Indomethacin is reported to consequently upregulate HO-1 levels in gastric mucosal cells of rats, according to Aburaya et al. (2006) [41]. In another gastric mucosal cell line study of rats, indomethacin was observed to reduce the indomethacin-induced mitochondrial oxidative stress (MOS) due to upregulation of HO-1 and prevention of MOS proinflammation [42]. These results point out the protective mechanism of HO-1 in response to oxidative stress and indomethacin-induced injury rather than directly modulating the HO-1 expression. The interaction of Orf3a with the HMOX1 gene might be associated with inhibition of HO-1 expression in SARS-CoV-2 infection since there is stated downregulation of HO-1 in SARS-CoV-2 affected individuals according to Datillo (2020). However, the effect of indomethacin in inducing HO-1 expression in SARS-CoV-2 infected patients remains undetermined.

Centrosome-associated protein CEP250 is involved in the synthesis of core centrosomal protein required for centriole–centriole cohesion, during interphase. Its activity can be rendered crucial for centriole dynamics and cell cycle regulation. In a recent study by Shigdel et al. (2020), the authors discuss the human-SARS-CoV-2 interactome network from Gordon & Jang et al. (2020), where CEP250 interacts with the CoV protein Nsp13 [43]. The AvgSpec value of CEP250-Nsp13 complex detection was 151.0, which was the 2nd most strongest interaction in the human-SARS-CoV-2 interactome in addition to a 1.43-fold increase in expression level upon SAR-CoV-2 infection. Shigdel et al. (2020) report from their study that the small molecule of their interest WDB002 which directly targets CEP250 can lead to SARS-CoV-2 inhibition. According to the CTD database, co-treatment of Indomethacin with Insulin, dexamethasone, 1-Methyl-3-isobutylxanthine, and bisphenol F decreases. Similar CEP250-Nsp13 interaction was also found in SARS & MERS virus-human protein interactome. The strong interaction of Nsp13 helicase with CEP250 protein raises questions on the comprehensive explanation about its function and how it influences host cell viability.

SARS-CoV-2 Nucleocapsid (N) protein enters the host cell along with viral RNA coating it from the phosphate backbone side to facilitate replication, process assembly, and release of the virus particle in the host cell. The La-related protein 1 (**LARP1**) and Poly-adenylate binding protein 1 (**PABPC1**) are

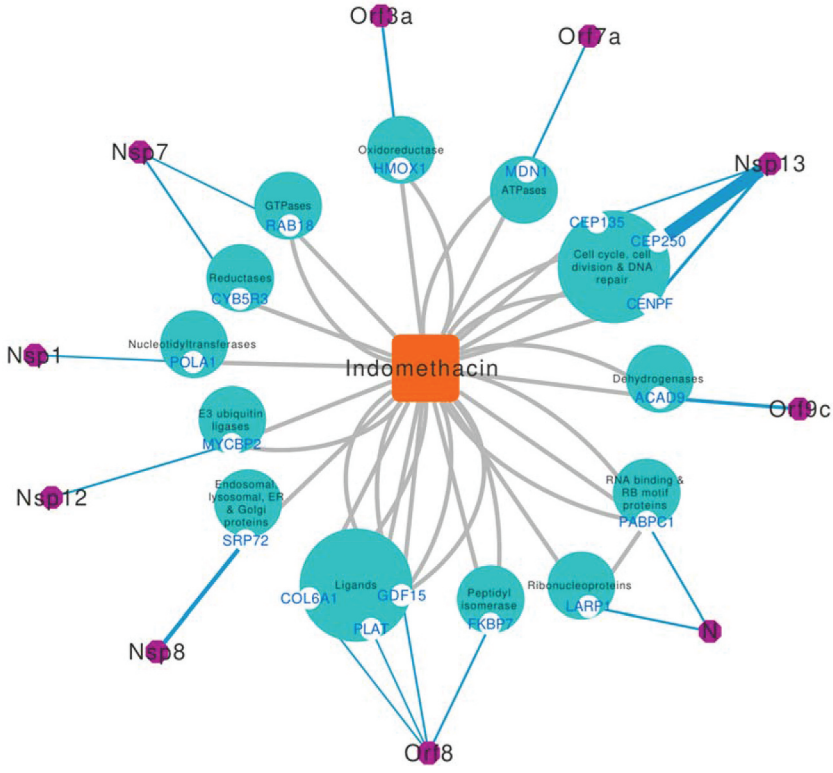

Figure 2. Drug–protein–protein (human-virus) interaction with 41 nodes and 48 edges depicting mere interaction of indomethacin with SARS-CoV-2 infection perturbed human genes, where teal nodes represent the classification of human genes/gene products (white nodes) interacting with viral proteins (purple nodes). Grey edges show drug-human gene interaction with no mapping filter and blue edges represent human-viral protein interaction strength as their width.

RNA-binding proteins (RBP) that are involved in the regulation of translation of particular target mRNA transcripts downstream of the mTORC1 cascade. An exploratory study on the roles of LARP1 and PABP1 in the pathogenesis of Dengue virus (DENV) represents the pre-translation complex formation, where LARP1 and PABPC1 interact with the host eukaryotic translation initiation factors eIF4G and eIF4E forming a loop [44]. In this study, LARP1 was reported to positively regulate the DENV genome translation. Very recently, Lee et al. (2021) in their PPI interactome resource article identified LARP1 as an antiviral RBP binding to SARS-CoV-2 RNA molecule [45]. SARS-CoV-2-infected human Calu-3 lung epithelial cells consensome network reports that geometric fold change in LARP1 and PABPC1 expression was 1.15 and 1.21, respectively [35]. There was evidence of the interaction of PABPC1 and LARP1 with SARS & MERS proteins in virus-host interactome study as well [36]. Moreover, the CTD database reports that indomethacin co-treated with insulin, dexamethasone, 1-methyl-3-isobutylxanthine, and bisphenol F decreases the expression of PABPC1 and LARP1. However, no evidence of direct action of indomethacin was found for LARP1 and PABPC1.

Lastly, **Growth and differentiation factor 15 (GDF15)**, a.k. a NSAID-activated gene 1 (NAG-1) is an inflammation-induced cellular mediator of tissue tolerance, as entitled by Luan et al. (2019) [46]. GDF15 or NAG-1 is upregulated in cases of tissue injury or inflammation in the liver, kidney, and lungs and is often associated with regulation of apoptosis, cell repair, and viability [47,48]. Interestingly, an observational pilot study published by Notz et al. (2020) states that GDF15 levels are consistently elevated in SARS-CoV-2 infected patients suffering from ARDS, which was also consistently elevated for recovered patients who showed tissue resilience during treatment. Contrastingly, an *in vivo* study conducted by Wu et al. (2018) on human rhinovirus (HRV) infection reports that increased levels of human GDF15 in transgenic mice lead to an exaggerated inflammatory response and increased infectious particle release, also attributed to lung epithelial cell inflammation [49]. The foggy understanding of the roles and associated effects of GDF15 is a challenge especially when it comes to viral diseases [50]. In an experimental study conducted by Barezi et al. (2010), authors report upregulation of NAG-1 in the fibrosarcoma cell line HT1080 in the presence of indomethacin, hence inducing cell viability in NAG-1 expressing cells [51]. Authors suggest that NAG-1 or GDF15 can be a crucial biomarker for chemotherapy and tumor progression. Moreover, Ackerstaff et al. (2007) have clearly demonstrated upregulation of GDF15 upon indomethacin treatment in breast cancer cell line, which was also proven in another *in vitro* study conducted by Glunde et al. (2006) [52,53]. However, indomethacin co-treated with insulin, dexamethasone, and 1-methyl-3-isobutylxanthine results in decreased expression of GDF15, but the direct action of indomethacin on GDF15 expression levels in SARS-CoV-2 patients remains unexplored. But also, it appears from the above literature that GDF15 is an important biomarker of tissue inflammation, also in the case of SARS-CoV-2 infection and the geometric fold

increase of 1.68 in SARS-CoV-2 infected Calu-3 cells in addition to positive interaction with orf8 protein, suggests a possible mediator-like property of GDF15.

These interactions become important in deciphering the mechanism of drug action and druggability of a particular target protein, but narrowing it down to a probable candidate molecule is a challenging and tedious task. From the evidence stated above, it is thus insinuated that ABCC1 is associated with multi-drug resistance reversal in SARS-CoV-2 infected cells. The interaction of Orf9c with ABCC1 might also be associated with increased drug efflux action and the evidently reduced ABCC1 activity upon indomethacin treatment can be attributed to its efficacy. Among the above-discussed human targets, ABCC1, HO-1, and CEP 250 can be directly targeted for structure-based drug discoveries, given that their crystal structures are publicly available with defined ligand-binding sites on PDB. Approaches like these can aid in the identification of small molecules that can be used in symptomatic treatments.

5. Discussion

This study focuses on the endogenous mechanism of action of indomethacin in having antiviral activities other than its role as an NSAID. Many *in vitro* as well as *in vivo* studies have shown that indomethacin has variable degrees of antiviral activity. It has been reported to interrupt the life cycle of several herpes viruses and may fade out latent infections via inhibition of synthesis of prostaglandin. It was also reported that COX-2 accumulation is induced by cytomegalovirus and indomethacin reduced cell-to-cell spread of the virus *in vitro*. Further, indomethacin was shown to eradicate DNA of hepatitis B virus in seven human patients and reduce the infection of rotavirus in human intestinal Caco-2 cells via inhibition of viral protein synthesis (50). Moreover, Hoxha conducted a literature search of several databases and revealed that prostaglandins (PGs), in particular PGE2, have pro-inflammatory action in the pathophysiology of COVID-19 [54]. Arachidonic acid plays a role as an endogenous antiviral compound and its deficiency can make individuals more susceptible to COVID-19. Indomethacin was clearly seen reducing cytokine stor m in a lone treatment network, significantly by downregulating PTGES2 (51). Due to lack of direct interaction, indomethacin cannot be said to directly affect the viral particle's replication and proliferation. However, its evident action on PTGES2 can be attributed to its major role in COVID-19 due to its anti-inflammatory response. Nonetheless, its antiviral activity cannot be limited to its anti-inflammatory action alone, hence we identified the ABCC1 transporter as another target that indomethacin regulates significantly. ABCC1's activity can be crucial in cotreatments with antiviral drugs. It can increase the susceptibility of the infected host cell to the antiviral drugs. HO-1, CEP250, LARP1, and PABPC1 also showed positive involvement in SARS-CoV-2 infection, but the action of indomethacin was not completely determined for all.

However, as discussed earlier, a few studies have reported that NSAIDs do not always manifest a protective effect in patients with infections. Indomethacin can lead to gastritis, renal, and platelet dysfunction that can damage severely infected patients, particularly multi-organ dysfunctions due to cytokine storm. Furthermore, NSAIDs have been reported to reduce levels of IL-6 in human fluids, which leads to the probability that indomethacin can reduce levels of IL-6 in nasopharyngeal-respiratory tract secretions. Marinella suggested a clinical strategy to monitor the levels of IL-6 or C-reactive protein in non-critical patients and administer indomethacin when their levels start to increase and monitor them regularly as timely administration of anti-inflammatory agents has been suggested to decrease systemic inflammation before a cytokine storm develops. Therefore, indomethacin can be proposed to be used either alone or as an add-on therapy to other antivirals in mild-moderate COVID-19 patients [4].

6. Expert opinion

The lack of information and resources that elucidate the mechanism of drug action is one of the most significant challenges that we face in drug discovery and repositioning studies. This can be overcome with high-throughput genetic interaction and expression profile studies, accompanied with appropriate interpretation. This study led us to look out for the in vitro, in vivo, and clinical outlook of indomethacin as a treatment in COVID-19 patients. We came across multiple pieces of evidence of the direct alleviating action of indomethacin on SARS-CoV-2 infected cells, but the underlying uncertain mechanism needs to be unravelled for a thorough understanding of an NSAID's therapeutic action as an antiviral treatment.

The experimental observation of the change in expression levels of these proteins upon indomethacin treatment might reveal interesting outcomes that might provide more evidence of the plausible action of indomethacin other than an anti-inflammatory response. We encourage conducting clinical trials extensively examining the effect of indomethacin principally on PTGES2, ABCC1, HO-1, CEP250, and GDF15 in order to understand the underlying mechanism that resembles antiviral activity. Such studies might provide information on the druggability of the above-mentioned human protein targets. Moreover, such studies will add weight to an effective exploration of what we would call as "cross-pathway interaction." This will refine our strategies in discovering druggable targets with high confidence in pre-clinical research. Moreover, based on the above-mentioned clinical records of indomethacin, especially considering the favorable endpoint attributes like treatment of nonspecific symptoms, need for hospitalization, and need for mechanical ventilation, we propose indomethacin to be used in the treatment of mild-to-moderate patients infected with SARS-CoV-2.

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Author contributions

N Shekhar: Study design, writing, formatting, data retrieval. H Kaur: Study design, writing, data retrieval. P Sarma: Supervisor, study design, revision.

A Prakash: Supervisor, study design, revision. B Medhi: Supervisor, correspondence.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

ORCID

Phulen Sarma http://orcid.org/0000-0003-1926-7605
Ajay Prakash http://orcid.org/0000-0002-3487-8482
Bikash Medhi http://orcid.org/0000-0002-4017-641X

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