

# Use of Mefenamic Acid as a Supportive Treatment of COVID-19: A Repurposing Drug

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**Abstract:** COVID-19 (coronavirus disease 2019) is a Pandemic of concern, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). As of now, there is no known effective pharmaceutical or other medicine available for cure or prevention of COVID-19. In this review, based on the current understanding of the disease and the structure of novel Coronavirus SARS-CoV-2, SARS-CoV and other human viruses, I have identified Mefenamic Acid, an anti-inflammatory medicine, of having some role as an anti-viral medicine also. It can be used along with different anti-viral drugs being tried for the treatment of COVID-19. Since it is also an anti-pyretic, its main role may be of bringing down the fever but its action as an anti-viral, as a supportive medicine can be very useful. Clinical trials with this concept in mind can be started immediately at the Centres dedicated for treatment of COVID-19.

**Keywords:** COVID-19; SARS-CoV-2; Mefenamic acid, serine protease inhibitor, NLRP3 inflammasome

## 1. Introduction

The Pandemic, COVID-19 has engulfed the whole world and the whole medical fraternity is fighting to save lives. Researchers all around the world are trying to find out a Drug specific to this disease. Various combinations of anti-viral drugs and antibiotic are being worked upon. Other than the drug acting on the virus, various other measures have to be taken to support the patient and treat other symptoms.

The supportive treatment is one of the most important part of the patients suffering from COVID-19 infection the world over.

Most of the patients do require a drug to control the fever. Paracetamol has been a commonly used medicine for it. Other non-steroidal anti-inflammatory drugs (NSAIDs) have been used in some places, when fever is not controlled by Paracetamol. Ibuprofen has been used in the past episodes of viral fevers but some adverse effects have restricted its use.

## 2. Review of Literature

### *Antipyretic, anti-inflammatory and anti-viral action of Mefenamic Acid*

Mefenamic acid is also an NSAID and it has been used as an antipyretic, especially in paediatric cases. Other benefits of Mefenamic Acid may be of considerable value in the present pandemic caused by Corona virus.

Paracetamol (PCM) does not inhibit the synthesis of prostaglandins in the periphery; it does not possess any anti-inflammatory action. Besides its beneficial effects PCM also has potential side effects and may cause severe hypersensitivity reactions<sup>1,2</sup>

Mefenamic acid is a potent inhibitor of cyclooxygenase. It has a central as well as peripheral analgesic action. The drug is commonly used in patients with injuries, osteoarthritis, rheumatoid arthritis and dysmenorrhea.<sup>3,4</sup>

The results were in accord with S. Keininen., *et al* which also states Mefenamic acid to be more potent and powerful antipyretic drug.<sup>5</sup>

Mefenamic acid was found to be more effective and equally tolerable than paracetamol as an antipyretic in paediatric patients with febrile illness and can be the best alternative to paracetamol.<sup>6</sup>

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of anti-inflammatory drugs, inhibiting cyclooxygenase (COX) enzymes in the synthesis of prostaglandins and other mediators, and widely used for the treatment of pain and inflammation.

Studies have shown that, unlike other NSAIDs, fenamates (mefenamic acid, flufenamic acid) selectively inhibit the NLRP3 inflammasome and IL-1b release via inhibiting the membrane volume regulated anion (Cl<sup>-</sup>) channel (VRAC), independent of its cyclooxygenase-1 (COX-1) mediated anti-inflammatory activity<sup>7</sup> In agreement with these findings, fenamates (mefenamic acid and meclofenamic acid) were observed to have considerable activity against viral replication, and a combination of ribavirin together with mefenamic acid was shown to be effective in reducing viral yield in cells infected with a positive-sense RNA genome chikungunya virus<sup>8</sup>.

NSAIDs are the best drug candidates to alleviate viral infection symptoms, such as musculoskeletal disorders, inflammation and pain. It was hypothesized that NSAIDs could be drugs with dual effects and not only cause symptom alleviation but also suppress Chikungunya virus (CHIKV) replication, especially in combination with common antiviral drugs, such as Ribavirin (RIBA). It was confirmed that Mefenamic acid, a primary compound in the NSAID group, has potential antiviral activity in vitro and in vivo, and this activity is better achieved when delivered in combination with the common antiviral drug, RIBA.<sup>8</sup>

It was observed that there is a crucial role of NLRP3 inflammasome activation in the pathogenesis of diseases

caused by SARS-CoVs, there is a role of inhibitors of the NLRP3 inflammasome in the context of inflammatory diseases and attention was drawn toward potential role of these (and similar agents) inhibitors in the treatment of SARS-CoV-2 (COVID-19). The evaluation of these reported (and other similar known or novel agents) inhibitors of the NLRP3 inflammasome in pre-clinical and/or clinical studies might offer new alternatives, especially in the form of potential repurposing of approved drugs for the treatment of COVID-19. Considering the clinical use of several NLRP3 inhibitor drugs for the treatment of other inflammatory diseases, further studies may determine potential usefulness of these agents in the treatment of COVID-19.<sup>9</sup>

Two cyclooxygenase (COX) isoforms, COX-1 and COX-2, catalyze the first two steps of prostaglandin biosynthesis from arachidonic acid (AA) and are the pharmacological targets of non-steroidal anti-inflammatory drugs (NSAIDs). Mefenamic acid is one of several NSAIDs that have shown selective inhibition of 2-arachidonylglycerol (2-AG) oxygenation by COX-2<sup>10</sup>. The structural determination of murine COX-2 structures complexed with several fenamates and biochemical investigation of the role of individual residues in their inhibition has been reported. The crystal structures for the first time revealed that the fenamates are bound in the cyclooxygenase channel in an inverted binding mode similar to those of propionic acid derivatives such as diclofenac and lumiracoxib. The side chains of Tyr-385 and Ser-530 form critical H-bonds with the carboxylic group of the fenamates. The aniline ring substituent of mefenamic acid, tolfenamic acid and flufenamic acid exhibit a preference for the space beneath Leu-531 as opposed to the side pocket off the active site channel. In contrast, when there is an additional chloride substituent, as in the case of meclofenamic acid, this extra substituent orients toward the side pocket. These observations provide a structural basis for the fact that meclofenamic acid is a slow, tight-binding inhibitor of COX-2 with a low IC<sub>50</sub> (31 nM) whereas mefenamic acid, flufenamic acid, niflumic acid, and tolfenamic acid are weak, rapidly reversible inhibitors with IC<sub>50</sub>'s greater than 10 μM for AA oxygenation. Strikingly, when 2-AG is used as the substrate, these weak, rapidly reversible inhibitors become potent inhibitors with IC<sub>50</sub>'s ranging from 20 to 50 nM.<sup>11,12</sup>

A review article by Bharat Bhushan Subudhi, et al., studies the effect of the drugs taking note of the increasing incidence of Chikungunya (CHIKV). Increasing incidences of Chikungunya virus (CHIKV) infection and co-infections with Dengue/Zika virus have highlighted the urgency for CHIKV management. Failure in developing effective vaccines or specific antivirals has fuelled further research. Possibilities of repurposing existing drugs based on their in vitro findings have also been elucidated by the authors. Probable modes of interference of these compounds at various stages of infection, including entry and replication, have been highlighted. While most of the earlier antivirals were effective in the early phases of the CHIKV life cycle, this review also focuses on drug candidates that are effective at multiple stages of its life cycle.<sup>13</sup>

Non-steroidal, anti-inflammatory drugs (NSAIDs) are being administered to manage the arthritic symptoms of CHIKV

infection. It was noted in a recent study, the acidic class of NSAIDs consisting of anthranilic acid derivatives, including mefenamic acid, meclofenamic acid, flufenamic acid, and tolfenamic acid, showed direct evidence of antiviral action against CHIKV. Mefenamic acid and meclofenamic acid exhibited an EC<sub>50</sub> of 13 M and 18 M, respectively, with CC<sub>50</sub> more than 100 M in Vero cells.<sup>14</sup>

To determine whether these NSAIDs can complement the CHIKV replication inhibitory capacity, combinations (1:1) of mefenamic acid and meclofenamic acid with ribavirin were investigated, which showed higher antiviral potency with EC<sub>50</sub> of 3 M and 5 M, respectively. This in vitro synergistic antiviral action of ribavirin with mefenamic acid (1:1) was further validated in vivo, which showed a 6.5-fold reduction in CHIKV titer<sup>14</sup> additionally, pathological signs were significantly reduced by this combination, which was ascribed to a combination of the antiviral and anti-inflammatory effects of mefenamic acid<sup>14</sup>.

In this respect, its benefits can be compared to those of chloroquine, as both drugs antagonize CHIKV entry. Nonetheless, it can be administered in combination with antivirals such as ribavirin, which act at post-entry levels of CHIKV. While the synergistic antiviral effects of drug combinations are desirable, other pharmacodynamic consequences must be studied in detail before further clinical trials. Considering these findings, mefenamic acid can be considered as a useful drug to repurpose against CHIKV. The Novel Corona virus, COVID-19, can also be included as a candidate for vaccine research and also other repurposing effects of drugs like Mefenamic acid, which may be useful in combination to treat fever and inflammation.

The study points towards the role of Mefenamic acid as having an anti-viral effect in synergy with the original anti-viral drugs and can be repurposed for COVID-19 treatment along with its action as an antipyretic.

#### ***Serine Protease Inhibition by Mefenamic Acid***

Since no vaccine will be available for large populations until the end of 2020, it is mandatory to identify approved off-label and experimental drugs against SARS-CoV-2 infection and COVID-19 disease. Such drugs may constitute inhibitors of TMPRSS2 and ACE2 and others. Camostatmesilate is one such drug. It is Serine protease inhibitor and targets TMPRSS2.<sup>15</sup>

There exists a number of candidate drugs that may inhibit infection with and replication of SARS-CoV-2. Such drugs comprise inhibitors of TMPRSS2 serine protease and inhibitors of angiotensin-converting enzyme 2 (ACE2). Blockade of ACE2, the host cell receptor for the S protein of SARS-CoV-2 and inhibition of TMPRSS2, which is required for S protein priming may prevent cell entry of SARS-CoV-2.<sup>16</sup>

Based on the experiences with infections by other viruses, such as HIV and influenza virus, post-exposure prophylaxis with candidate drugs against SARS-CoV-2 infection may be effective in preventing disease after potential exposure to the

virus or positive test results, and in reducing the risk of secondary virus spread and COVID-19 disease.<sup>17,18</sup>

The type II transmembrane protease TMPRSS2 activates the spike (S) protein of severe acute respiratory syndrome coronavirus (SARS-CoV) on the cell surface following receptor binding during viral entry into cells. In the absence of TMPRSS2, SARS-CoV achieves cell entry via an endosomal pathway in which cathepsin L may play an important role, i.e., the activation of spike protein fusogenicity.<sup>19</sup>

It was seen that a commercial serine protease inhibitor (camostat) partially blocked infection by SARS-CoV and human coronavirus NL63 (HCoV-NL63) in HeLa cells expressing the receptor angiotensin-converting enzyme 2 (ACE2) and TMPRSS2. Cathepsin L/B (CTSL/CTSB) plays an important role in a second pathway, the endosomal pathway.<sup>15</sup>

In order to gain entry into cells, diverse viruses, including Ebola virus, SARS-coronavirus and the emerging MERS-coronavirus, depend on activation of their envelope glycoproteins by host cell proteases. The respective enzymes are thus excellent targets for antiviral intervention. In cell culture, activation of Ebola virus, as well as SARS- and MERS-coronavirus can be accomplished by the endosomal cysteine proteases, cathepsin L (CTSL) and cathepsin B (CTSB). In addition, SARS- and MERS-coronavirus can use serine proteases localized at the cell surface, for their activation.

Employing a pathogenic animal model of SARS-CoV infection, it was demonstrated that viral spread and pathogenesis of SARS-CoV is driven by serine rather than cysteine proteases and can be effectively prevented by camostat. Camostat has been clinically used to treat chronic pancreatitis, and thus represents an exciting potential therapeutic for respiratory coronavirus infections.<sup>20</sup>

Boceprevir, a protease inhibitor, is under clinical development for the treatment of human hepatitis C virus infections. In human liver microsomes, formation of oxidative metabolites after incubations with boceprevir was catalyzed by CYP3A4 and CYP3A5. Attempts were made to identify the enzymes responsible for the formation of carbonyl-reduced metabolites. Human liver S9 and cytosol converted ~ 28 and ~ 68% of boceprevir to M28, respectively, in the presence of an NADPH-generating system. Screening of boceprevir with recombinant human aldo-keto reductases (AKRs) revealed that AKR1C2 and AKR1C3 exhibited catalytic activity with respect to the formation of M+2 metabolites (M28 and M31).<sup>21</sup>

The formation of M28 was inhibited by 100  $\mu$ M flufenamic acid (80.3%), 200  $\mu$ M mefenamic acid (83.7%), and 100  $\mu$ M phenolphthalein (86.1%), known inhibitors of AKRs, suggesting its formation through carbonyl reduction pathway. These data demonstrated that CYP3A4 and CYP3A5 are primarily responsible for the formation of oxidative metabolites and the formation of M28 and M31, the keto-reduced metabolites, are most likely mediated by AKR1C2 and AKR1C3.<sup>17,21</sup>

The inhibition of protease inhibition has found to be an important part of treatment in the studies conducted using Camostat. The formation of M28 was found to be inhibited by mefenamic acid. It is thus possible for it to be a protease inhibitor.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to angiotensin II receptor in different tissues of the body, especially in the mouth cavity and the tongue and the skin. Therefore, it needs the serine protease, transmembrane serine protease 2 (TMPRSS2), for cell entry. This work focused on serine protease inhibitor Camostat, which partially blocks infection by SARS-CoV-2.<sup>22</sup>

Simultaneous treatment of the cells with camostat and a cathepsin inhibitor has efficiently prevented both cell access and multi-step growth of SARS-CoV-2 in human Calu-3 weather pathway epithelial cells.<sup>23</sup>

This efficient inhibition could be attributed to the double blockade of access from the cell surface and through the endosomal pathway. These observations suggest camostat as a candidate antiviral drug to prevent or suppress TMPRSS2-dependent infection by the SARS-CoV-2.<sup>24</sup>

To date, many different treatment options are used “off-label” in severe cases or are under clinical trial. Further investigations are necessary. A new therapy option in COVID-19 patients is based on the combination of camostat (serine protease TMPRSS2 inhibitor) and a cathepsin inhibitor. First clinical off-label trials should be performed as soon as possible in COVID-19 patients.<sup>15</sup>

Nafamostatmesilate, a synthetic serine protease inhibitor, is also being studied for treatment of COVID-19. A group of scientists from the University of Tokyo in Japan and Leibniz Institute for Primate Research in Germany recently demonstrated that Nafamostat at very low concentrations suppresses a protein (TMPRSS2) that the COVID-19 virus uses to enter human lung cells.<sup>25</sup>

Hussin, A.R et al. conducted a study to evaluate the anti-dengue properties of some compounds that were used as antibiotics (tetracycline derivatives) and anti-inflammatory agents (mefenamic acid derivatives). It was found that mefenamic acid and doxycycline exhibited the highest inhibitory effect against dengue NS2B fused to NS3pro via 9 amino acids (G4-T-G4). The results confirmed the higher inhibition potential of mefenamic acid compared to doxycycline towards viral replication in vitro.<sup>26</sup>

Previous studies have reported the anti-viral activity of mefenamic acid and doxycycline. The inhibitory effect of mefenamic acid against RNA viruses has been estimated as 90% at a concentration of 30  $\mu$ M.<sup>27</sup>

Virus polyprotein is cleaved by viral NS2B-NS3 serine protease and cellular proteases to 10 structural and non-structural proteins.<sup>28</sup> Disruption of viral NS2B-NS3pro would lead to inhibition of viral replication in host cells.<sup>29</sup>

A variety of different flaviviruses protease inhibitors have been previously reported.<sup>30</sup>

It has been observed that mefenamic acid and doxycycline showed significant anti-dengue activity through their ability to inhibit viral protease activity.<sup>26</sup>

Flaviviridae and Coronaviridae are both single stranded Positive-sense RNA viruses with Genetic structure being + ssRNA and linear. There is a possibility of similar response to Mefenamic acid along with anti-viral drugs. Mefenamic acid can thus be considered a drug having protease inhibitory action in combination with some anti-viral drugs or even on its own. Further experimental and clinical studies need to be carried out to investigate this for potential utilization for the attenuation of the clinical symptoms of COVID-19 infection.

### 3. Conclusion

These studies show that a combination of ribavirin together with mefenamic acid was shown to be effective in reducing viral yield in cells infected with a positive-sense RNA genome chikungunya virus<sup>8</sup>.

It was confirmed that Mefenamic acid, a primary compound in the NSAID group, has potential antiviral activity in vitro and in vivo, and this activity is better achieved when delivered in combination with the common antiviral drug, RIBA.<sup>8</sup> Pathological signs were significantly reduced, which was ascribed to a combination of the antiviral and anti-inflammatory effects of mefenamic acid<sup>14</sup>.

It has been observed that there is a crucial role of NLRP3 inflammasome activation in the pathogenesis of diseases caused by SARS-CoVs, there is a role of inhibitors of the NLRP3 inflammasome in the context of inflammatory diseases and attention be drawn toward potential role of these (and similar agents) inhibitors in the treatment of SARS-CoV-2 (COVID-19).

Considering the clinical use of several NLRP3 inhibitor drugs for the treatment of other inflammatory diseases, further studies may determine potential usefulness of these agents in the treatment of COVID-19.<sup>9</sup>

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The synergistic antiviral effects of drug combinations are desirable, other pharmacodynamic consequences must be studied in detail before further clinical trials. Mefenamic acid can be used as an anti-antipyretic in patients of COVID-19 with an additional benefit of it being also having the possibility of an anti-viral activity. Studies are required to validate this opinion so as to repurpose the use of Mefenamic acid in viral infections, such as of SARS-CoV-2.

### Authors' Contribution

Conceived the idea and showed an interest in there purposing of an anti-inflammatory and antipyretic drug, Mefenamic Acid as an anti-viral medicine also when used along with other drugs. Done a review of literature to study the use of Mefenamic Acid along with anti-viral medicines and the mechanism of action of these medicines. Generating an interest in a common drug for its uncommon use i.e. an anti-inflammatory having an anti-viral action. Writing the manuscript.

### 4. Conflicts of interest

NIL

### 5. Acknowledgements

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