In Search of Feasible Interventions for the Prevention and Cure of Novel Coronavirus Disease 2019

Dr. Sunil Kumar Verma

Principal Scientist, CSIR-Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India. Email: sunilverma@ccmb.res.in

Running Title: In search of feasible interventions for COVID-19 pandemic

Summary

COVID-19 (coronavirus disease 2019) is a public health emergency of international concern caused by *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2). As of this time, there is no known effective pharmaceutical, phytopharmaceutical or traditional medicine for cure or prevention of COVID-19, although it is urgently needed. In this review, based on the current understanding of the disease molecular mechanisms of novel Coronavirus SARS-CoV-2 and its closest relative SARS-CoV and other human Coronaviruses, I have identified some naturally occurring plant based substances and Ayurvedic medicinal herbs that could feasibly be tested as a matter of urgency for prevention as well as therapeutic option for COVID-19 in India and other parts of the world. I conclude that dried rhizome of *Curcuma longa* L. i.e. turmeric, and its active ingredient curcumin may be effective in preventing as well as cure the COVID-19 pandemic due to its proven antiviral activities, this however need to be tested by appropriate clinical trials as research priority.

Keywords: COVID-19; SARS-CoV-2; Severe Acute Respiratory Syndrome Coronavirus-2; Curcumin

Introduction

Brief Introduction of SARS-CoV-2

The Coronavirus (CoVs) have been classified into four genera : α-CoVs, β-CoVs, γ-CoVs, and δ-CoVs, among which α- and β-CoVs are able to infect mammals, whereas the other two genera can infect birds and could also infect mammals [1]. So far, seven coronaviruses have been found to infect humans and cause respiratory diseases. These CoVs are – HCoV-229E (known since 1960s), HCoV-OC43 (known since 1960s), SARS-CoV (known since 2002), HCoV-NL63 (known since 2004), HCoV-HKU1 (known since 2005), MERS-CoV (known since 2012), and most recently the SARS-CoV-2. The SARS-CoV-2 was isolated from human airway epithelial cells and was identified to be a new member of *β*-coronavirus with a genome sequence similarity of 96.2% to a bat CoV RaTG13, and 79.5% to SARS-CoV [2]. Similar to other *β*-coronaviruses - SARS-CoV and MERS-CoV, the SARS-CoV-2 also infects the lower respiratory tract and cause a respiratory syndrome. The clinical symptoms of COVID-19 patients include fever, cough, fatigue and a small population of patients appeared gastrointestinal infection symptoms. Since the emergence of COVID-19 in the Wuhan city of China in December 2019 until 23 March 2020 10:00CET, there were a total of 332,935 confirmed cases of this disease from 190 countries and 14,510 deaths. A total of 415 confirmed positive cases have also been reported from India by WHO [3] leading to declaration of emergency or emergency like situation in many countries.

SARS-CoV-2 Pathogenesis

Receptor recognition by viruses is the first and essential step of viral infections of host cells. Knowledge about the receptor recognition mechanisms of Coronaviruses is critical for understanding of their pathogenesis and for developing novel prevention or cure for the disease caused by them. It has been known that the receptor binding domain (RBD) of Coronaviruses recognize a variety of host protein receptors including – angiotensin converting enzyme 2 (ACE2), aminopeptidase N (APN) (also known as CD13), dipeptidyl peptidase 4 (DPP4), carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) [4] and cellular serine protease TMPRSS2 [5]. It was also described previously that SARS-CoV *β*-Coronavirus indeed utilizes ACE2 and TMPRSS2 to enter

into the host cells [5]. In the light of the fact that the novel SARS-CoV-2 genome has high similarity with SARS-CoV β-Coronavirus, and that the most amino acid residues essential for ACE2 binding by SARS-CoV RBD were conserved in SARS-CoV-2 RBD [6], it was logical to predict that ACE2 and TMPRSS2 could also facilitate the entry of novel SARS-CoV-2 into the host cells. This prediction was indeed found true [6] ; and this so far has been a major breakthrough to understand, develop and establish options for prevention and treatment of COVID-19.

Nevertheless, for the Coronaviruses, it has been found that highly similar Coronavirus RBD within the same genus can recognize different protein receptors, and that very different Coronavirus RBD from different genera can recognize the same protein receptor [4]. This gives a clue that other protein receptors known to facilitate the entry of human Coronavirus in host cells (i.e. APN / CD13, DPP4 and CEACAM1) may also play role in the pathogenesis of SARS-CoV-2 in human, this however need to be tested further. Various plant-based inhibitors of these receptors are known for these receptors. In this review, I summarize some of these naturally occurring plant based substances and Ayurvedic medicinal herbs that are known to inhibit these coronavirus receptors. I propose that some of these herbs and their active ingredient may feasibly be tested for their preventive and probably curative effects against the SARS-CoV-2 as research priority.

Establishing options for prevention and treatment of COVID-19

ACE2 and its inhibitors

Identifying the route of infection through functional receptor(s) has major implications for understanding the pathogenesis, treatment and cure of SARS-CoV-2. Angiotensin converting enzyme 2 (ACE2) has been proved to be a functional receptor of SARS-CoV-2 [6] ; therefore, the next logical step was to correlate its expression and organ distribution with COVID-19 pathogenesis. ACE2 is expressed on lung alveolar epithelial cells and enterocytes of the small intestine as well as arterial and venous endothelial cells and arterial smooth muscle cells of various organs [7]. This epithelial expression, together with the presence of ACE2 in vascular endothelium perfectly correlates with the pathogenesis of the COVID-19 manifestations [8], thus suggesting that ACE2 inhibitors

might be effective for the prevention of COVID-19 infection. Hoffmann et al. (2020) [6] indeed found that BHK-21 cells transfected to express ACE2 receptor, but not the parental BHK-21 cells, were heavily infected with both with SARS-CoV as well as SARS-CoV-2; and that anti-serum raised against human ACE2 blocked SARS-CoV as well as SARS-CoV-2 entry into these cells. These researchers further confirmed that the entry of both SARS-CoV as well as SARS-CoV-2 in lung cells is primed at Cellular Serine Protease TMPRSS2; and that clinically proven serine protease inhibitor camostat mesylate [9], which is active against TMPRSS2 was also able to block SARS-CoV-2 Infection of Lung Cells [6].

These studies indicated that the inhibition of ACE2 receptor on the entry point of SARS-CoV-2 might positively help to prevent the SARS-CoV-2 infection and deserve attention.

Based on a PubMed search on 18 March, 2020, I did not find any plant based active ingredient or herbal formulation that has been reported to be associated with downregulation of ACE2 expression. Yet, as a matter of priority, the research must be directed to identify the effective pharmaceuticals, phytopharmaceuticals or herbal formulations that could reduce the ACE2 expression on the epithelial cells to discover novel preventive measure for SARS-CoV-2.

It is worth mentioning that a correspondence published in 'The Lancet' recently suggested that the anti-inflammatory drugs such as ibuprofen may worsen the COVID-19 outcome as ibuprofen leads to enhanced expression of ACE2 receptor making the person more vulnerable to SARS-CoV-2 infection [10]. This claim was however debunked by various profound researchers in social and mainstream media, by stating that there was no evidential data suggesting the enhanced expression of ACE2 following the ibuprofen treatment [11]. WHO first released the public advisory on 18th March 2020 to not consume ibuprofen; but retracted it after one day on 19th March 2020 [12]. My independent analysis of the implication of Ibuprofen in COVID-19 prognosis supports the claims of 'The Lancet' paper, as there are few evidences suggesting that treatment with ibuprofen could indeed enhance the ACE2 expression in diabetic rats [13]. This suggest that the regulating authorities must again notify to public that the use of ibuprofen must be avoided during SARS-CoV-2 pandemic.

Further, It is notable that elevated ACE2 expression appears to occur at the initial stage of several pathologic cardiovascular and renal conditions [14]; therefore the people with these diseases may be treated as vulnerable for COVID-19 until more extensive research are done on understanding of this specific link.

APN / CD13 and its inhibitors

Aminopeptidase N (EC 3.4.11.2, APN / CD13) is a widely expressed (endothelial, epithelial, fibroblast and leukocyte) membrane-bound zinc metalloprotease [15] ; and its expression is dysregulated in inflammatory diseases as well as in cancers (solid and hematologic tumors) [4,15]. CD13 is known to serve as receptor for human Coronavirus HCoV-229E, and also other Coronaviruses including porcine respiratory CoV, porcine transmissible gastroenteritis virus (TGEV), feline infectious peritonitis virus (FIPV), feline enteric CoV (FECoV) and canine CoV (CCoV) [16]. It was recently shown that CD13 deficient pigs are resistant to Coronavirus infection [17]. Coronavirus recognition of APN is species-specific, and specificity is associated with N-linked glycosylations in the APN protein [18] . The defined role of CD13 in the pathogenesis of SARS-CoV-2 is not yet fully explored; however, blockage of CD13 could serve as prophylaxis for COVID-19 as suggested previously [19]. Various synthetic and naturally occurring inhibitors of CD13 are known [20] . For the purpose of this article, I have chosen to focus primarily on the natural / plant based inhibitors of CD13 that could feasibly be tested and used as a preventive and curative measure for COVID-19 as described below.

Extract of *Macleaya cordata*

Macleaya cordata, the five-seeded plume-poppy, is a species of flowering plant in the poppy family Papaveraceae, and is used ornamentally. The Quaternary benzo[c]phenanthridine alkaloids (QBA) extract from this plant has been shown as a potent inhibitor of CD13 [21].

Curcuma longa L. (Turmeric)

The dried rhizome of *Curcuma longa* L. is the source of the spice turmeric, which is widely employed in food and has a long tradition of use in Ayurveda. Curcumin (E,E-1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione),   a   yellow   natural   phenolic

compound isolated from turmeric has been found to irreversibly inhibit Aminopeptidase N / CD13 [22], indicating that curcumin (and turmeric) could indeed be effective in preventing the Coronavirus infection by inhibiting its cell binding via CD13.

The turmeric- derived polyphenol curcumin has also been shown as a potential anti-HIV agent due to its HIV-1 or HIV-2 protease inhibitor [23] and HIV-1 integrase inhibitor activities [24]. Various commercially available antiviral drugs have been shown to be effective against SARS-CoV [25] and SARS-CoV-2 [26]. Since the curcumin has also been proved as a promising natural compound against many viruses [27–31], I propose that *Curcuma longa* L. (Turmeric) could be tested for its preventive as well as therapeutic potential against SARS-CoV-2.

Betulinic Acid

3b-hydroxylup-20(29)-en-28-oic acid), a pentacyclic compound, is widely present in the plan kingdom and has been shown to have the CD13 inhibition activity in a dose-dependent manner [32]. Betulinic Acid has been extracted from various plants and vegetal species such as *Betula sp.* (birch trees), *Ancistrocladus sp.*, *Arbutus sp.*, *Diospyros sp.*, *Paeonia sp.*, *Picramnia sp.*, *Syzygium sp.*, *Tetracera sp.*, *Tryphillum sp.*, *Zizyphus sp.* and the bark of plane trees (*Platanus acerifolia*). Exploring the preventive and therapeutic potential of these plants for COVID-19 is a priority research area.

DPP4 and its inhibitors

Dipeptidyl-peptidase (DPP) 4, also known as CD26, is a ubiquitously expressed glycoprotein of 110 kDa [33] and implicated in a variety of disease states including inflammation, cancer, obesity, and diabetes [34]. Currently, there are seven DPP-4 inhibitors that have already been approved for clinical use, namely sitagliptin, vildagliptin, saxagliptin, linagliptin, and alogliptin [34]; however, these may have serious side effects on various vital organs in human [35]. While searching safer, natural and more effective inhibitors of DPP-4, I have found that the extract of three plants namely *Pterocarpus marsupium* Roxb. (Leguminosae), *Eugenia jambolana* Lam. (Myrtaceae) and *Gymnema sylvestre* (Retz.) R. Br. ex Schult. (Asclepiadaceae) have the potential concentration-dependent anti-DPP-4 activity [36]. Since DPP-4 inhibition may act as

prophylaxis for several coronaviruses, the potential of these plants for the prevention of COVID-19 must be investigated as a research priority.

CEACAM1 and its inhibitors

The homophilic cell adhesion molecule CEACAM1 (C-CAM, BGP, CD66a) are cell surface glycoproteins involved in intercellular binding and belong to the immunoglobulin superfamily [37]. They are implicated in various cellular functions governing growth and differentiation and have an important role in insulin homeostasis, vascular neogenesis and immune modulation [38]. Searching the literature for a plant based natural inhibitor of CEACAM1 did not yield any result as of 18 March 2020. Taking into consideration that CEACAM1 has been described as a reception for various Coronavirus, its potential as a measure to develop novel Coronavirus therapeutics may be explored in coming times.

Conclusion

COVID-19 (coronavirus disease 2019), first detected in Wuhan, China on 31 December 2019 has now become a Public Health Emergency of International Concern and worldwide pandemic. The scientists from all over the world are restlessly working to understand the SARS-CoV-2 pathogenesis for the development of its preventive and treatment options; however, there has been no success until today. Based on the current understanding of Coronavirus pathogenesis and SARS-CoV-2 disease mechanisms known so far, I have identified various naturally occurring plant substances and Ayurvedic medicinal herbs that could feasibly be tested as preventive and therapeutic option for COVID-19 patients as a research priority.

 I further highlight that the dried rhizome of *Curcuma longa* L. i.e. turmeric, and its active ingredient curcumin, which has previously been shown effective in inhibiting the virus replication in human cells due to its anti-viral activities as demonstrated for HIV and AIDS [27–29], Zika and chikungunya [30] and herpes simplex virus [31], may also be explored for its possible preventive and treatment option for the COVID-19 pandemic. The research efforts may be guided towards finding whether (i) Cucumin or turmeric inhibits SARS-CoV-2 replication in human cells as demonstrated for HIV and AIDS [27–29], Zika and chikungunya [30] and herpes simplex virus [31] (ii) whether cucumin or turmeric has a

role in controlling the SARS-CoV-2 infection by inhibiting its entry into the host cell. It has also been shown that Zika and chikungunya virus incubated with curcumin loses its infectivity [30], this property of curcumin may also be explored for the possible inactivation of SARS-CoV-2.

It is to note that the plant based preventive and curative options unearthed in this systematic review are only the suggestions on immediate research priorities in search of plant-based common and novel interventions to control the current COVID-19 pandemic; and that it is not yet a medical advice or guideline for the public - until proved and confirmed to be effective in an authorized laboratory following specific clinical trials.

This research review has also been submitted to the COVID 19 Solution Challenge initiated by Government of India [39] for in depth review, consideration, further testing, and adoption if found suitable for the control of COVID-19 pandemic.

Acknowledgement

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