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REVIEW ON DRUG REPURPOSING OF CAMOSTAT MESYLATE FOR TREATMENT OF COVID

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ABSTRACT

This review includes the proper aspects of designing an antiviral drug, from an existing drug with different indication can be used for inhibition of the viral genome entry and replication of the gene in the host cell, *repurposing of drug*. The viral genomic entry of SARS-CoV2 relies on the activation of S protein by the cleavage of s1/s2 done by the action of protease TMPRSS2, after the attachment of itself to the ACE2 receptors present in the endothelium of the lungs. This action eventually leads to the viral genetic material entering the host cell for its replication. Therefore, the usage of a serine protease inhibitor *Camostat mesylate* which is primarily used for the chronic pancreatitis condition, it can also inhibit the action of TMPRSS2. This review has a potential effect over the formulation of antiviral drugs, its market gaps as well as the concern of patient care.

INTRODUCTION

COVID-19; the global pandemic was a huge threat to the entire mankind; which led to many financial crises, breakdowns, human losses, and to add cherry to the cake, isolation of the people for society and family, leading to an unbothered generation which disturbed the minds of the youth.

The factors discussed above were only based on the macroscopic level of modification this pandemic has caused to mankind, but when we look at the microscopical level the causative agent was found to be SARS-CoV2 (*Severe Acute Respiratory Syndrome CoV2*).

SARS-CoV2 is an enveloped positive-sense single-stranded RNA virus of β -coronavirus, which shares 79% of its nucleotides with the SARS-CoV the causative agent for the 2002-2004 epidemic.[1][2]

The genus of the SARS-CoV2 includes:

- Human coronavirus:
- HCoV-OC43
- HCoV-HKU1
- MERS-CoV- Middle East Respiratory Syndrome- CoV

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The binding of SARS-CoV to the ACE2 receptor dominantly located in the regions of brain, eyes, lungs, liver, kidney, stomach and intestine. This ACE2 receptor (Angiotensin II Converting Enzyme 2 receptor) are also the receptors for the α -coronavirus HCoV-NL63 and HCoV-229E; β - coronavirus HCoV-OC43 and HCoV-HKU1; which are known to be the causative agents of mild upper respiratory tract infection.[3][4][5][6]

STRUCTURAL INTERPRETATION OF SARS-CoV2:

Structural interpretation of SARS-CoV2 virus involves the tight packaging of the long RNA polymer into the center and surrounded by a protective capsid, which is a lattice of reported protein molecules referred to as coat or capsid protein. In coronavirus these proteins are called *Nucleocapsid* (N). For further protection, there is *Envelop* (E) surrounding the outer membrane of the core particle of coronavirus made up of lipid particles and inert proteins. These membranes are modified to contain specific viral protein, including the *Spike* (S), *Membrane* (M) and *Envelope* (E) protein. [7]

MECHANISM OF COVID-19

The mechanism of any viral disease involves the synthesis of viral protein which having the capacity to hijack the human cells and take the control over the human cells. For this mechanism these are Nuclear capsid or N-Protein which binds the virus single positive strand. The N-Protein coats the viral RNA genome which plays a vital role in its replication and transcription.

The S protein of SARS-CoV2 and MERS-CoV is cleaved into S1 and S2 subunits

during their biosynthesis in the infected cell; by proprotein convertase such as Furin in the virus- producer cells. This includes the work of TMPRSS2 (Transmembrane Protease Serine2)

The S protein on the mature virion consists of two non-covalently associated subunits: The S1 subunit binds to ACE2 and S2 subunit anchors the S protein to the membrane. The S2 subunit also includes a fusion peptide and other machinery necessary to mediate membrane fusion upon infection of a new cell.[8]

Translocation of viral structural protein and genomic RNA to the ER-Golgi Intermediate (ERGI) occurs via an unknown mechanism. Only 4 viral proteins are incorporated as virion; involving the association of Spike (S), Envelop (E), Membrane (E) and the Nucleocapsid (N) protein bound to the viral genomic RNA are packed in the virion.[8]

The S protein is arranged in the pattern of crown (*corona*), which allows the binding to the receptor. During the process of biosynthesis and maturation in the infected cell, the S protein is cleaved by Furin or Furin-like proprotein convertase in the Golgi apparatus into the S1 and S2 subunits, which remain associated.

The S protein on the virus consists of two non-covalently associated subunits with different functions: In the new target cell of the virus, the S1 subunit binds the receptor and the S2 subunit anchors the S protein to the virion membrane and mediates membrane fusion. This allows the interaction of E and M protein contributing to virus assembly and budding through the interactions with other viral proteins. These assembled buds of virus move to the plasma membrane via the ERGIC lumen, through

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the means of secretory pathway; which leads to the release of virus into the extracellular space after the fusion of Virus-Containing vesicles with plasma membrane FP, fusion peptide.[9][10]

TMPRSS2: UNLOCKING THE MYSTERY

Transmembrane Serine Protease 2 (TMPRSS2) are the surface cell protein expressed predominantly on the endothelial cells across the digestive and respiratory tracts. These are found to be the cleaving knife of the viral peptides which have the nucleophilic amino acid within the active site. But still the biological significance of this serine protease remains unknown to the biological world of science.[11]

There is evidences of some traces of TMPRSS2 elevation in the blood serum level during certain pathogenic conditions like; in the samples of patients with prostate cancer were showing elevation of TMPRSS2 in their blood plasma; for the response of androgen elevation in those patients. Specifically, TMPRSS2 fuses with ERG, an oncogenic (cancer-promoting) transcription factor.[12]

This fusion of TMPRSS2 with ERG is seen in half the population of prostate cancer involving the genes of European ancestry; but limited in the population of Asian and African men. There were also studies involving the analysis of the co-relation between the ERG regulation by the androgen elevation. But this specific type of fusion had kept an endcard to this study suggesting that regulation of ERG is controlled by androgen aggravating prostate cancer and promotes metastasis.[12]

PROOF OF TMPRSS2 REQUIREMENT IN SARS-CoV

An animal comparative study was conducted which included a mice (healthy-wild type) exposed to SARS-CoV developed symptoms of acute pneumonia and body loss up to 15%, there was also recovery being shown. Whereas in the case of TMPRSS2 kicked out mice when exposed to the SARS-CoV doesn't develop pneumonia or suffer any kind of weight loss and also shown less viral replication in the lungs. Furthermore wild-type mice had shown strong expression of antigen-positive cells in the bronchiolar epithelial which seemed to be weak in the TMPRSS2 knocked out mice; they also have shown reduced inflammatory response compared to wild-type mice. This therefore, conforms to the requirement of TMPRSS2 for the viral replication into the host body.[13]

INHIBITION OF TMPRSS2

Camostat mesylate, is an orally bioavailable synthetic serine protease inhibitor with properties including anti-inflammatory, anti-fibrotic, anti-viral and even antineoplastic properties. Upon oral administration it's metabolite 4-(4-guanidino benzoyloxyl) phenyl acetic acid (FOY251) inhibits activity of various protease including trypsin. Therefore, this is used in the treatment management of chronic pancreatitis in Japan; as the activation of trypsinogen is found to be the triggering factor of pancreatitis.

Further investigation of the particular not so unfamiliar drug, we have come to the conclusion of it having the capacity of suppressing the inflammatory responses like cytokines interleukin-1beta (IL-1b), interleukin-6 (IL-6).

Along with these properties it was also found to have a potential effect on inhibiting the TMPRSS2 *Transmembrane protease serine2*, a host cell serine protease

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helping in the entry of influenza virus and coronavirus as discussed before. The clinically approved camostat mesylate dose (600 mg/day) will result in antiviral activity.[14]

THERAPEUTIC APPROACH: PRE-CLINICAL**AMMONIUM CHLORIDE:**

Different expressions of TTSPs are expressed along with SARS-CoV2 receptor, ACE2 in poorly susceptible 11+BHK-21 cells. This set up was treated firstly with the ammonium chloride which acts as an agent blocking the cathepsin-L-dependent, auxiliary pathway and (VSV) based on pseudotypes bearing SARS-CoV2.[15]

Treatment with ammonium chloride reduced the SARS-2-S driven transduction and this effect was restored by the overexpression of TMPRSS2; i.e; the entry of SARS-CoV2 when was facilitated by the TMPRSS2 and there were also evidences of TMPRSS13, TMPRSS11D, TMPRSS11E and TMPRSS11F.

This proves the activation of SARS-CoV2 and can be done by diverse group of TTPS; i.e; had shown the result of overexpression of TMPRSS13 being particularly robust.

CAMOSTATE MESYLATE

The rescue assay performed had also shown the evident blockade of TMPRSS2 by camostat mesylate. But the entry of SARS-CoV2 was still evident due to the activation of Cathepsin-L-dependent auxiliary pathway taking over the control of cell BHK-21 cell.

CONCLUSION

In ammonium medium, the BHK-21 did show a reduced entry of SARS to some

extent due to its inactivating capacity of cathepsin-L-dependent pathway. But there was the activation of SARS via TTSP expression, which could be inhibited to some extent by administration of camostat mesylate.

In conclusion, the entry of the viral genome has to be blocked in all the possible pathway; which could be achieved by the administration of a combination therapy of camostat mesylate along with ammonium chloride which is a potent method to inhibit both the pathways of entry. [15]

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