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The updates on Middle East Respiratory Syndrome coronavirus (MERS-CoV) epidemiology, pathogenesis, viral genome and currently available drugs

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Abstract: The Middle East Respiratory Syndrome (MERS) is caused by the novel coronavirus belongs to the family *Betacoronaviridae* was first identified in Saudi Arabia during 2012. The first epidemic outbreak of the MERS-CoV has been started reporting in the South Korea and other Asian Countries. The disease was transmitted to humans to humans from the Middle East to other countries through travelling history. The MERS-CoV is responsible for the lower acute and severe respiratory disorder causes the shortness of breath along with fever and cough. The treatment for the disease is purely symptomatic and vaccination is not existed. In the present work we are tried to compile the epidemiology, pathogenesis, viral genome and currently available drugs. At the last the promising approaches for the drug design and development process has been presented.

Keywords: MERS-CoV; Interferons; PLpro; replication; inhibitors

1. Introduction

The viral respiratory syndrome in humans is caused by the novel coronavirus and is popularly known as Middle East Respiratory Syndrome coronavirus (MERS-CoV).[1,2] The causative virus MERS-CoV was isolated and identified in Saudi Arabia during 2012.[3,4] The current outbreak related to the MER-CoV is started to exist in South Korea, becoming more dangerous and proper response to disease was initiated.[5] The WHO was stated, among the total number of MER-CoV infected cases, approximately 36% of deaths due to the MER-CoV has occurred.[6] The MERS-CoV infections was originated from the zoonotic cycle, camels were suspected to be carriers and may become a source of reservoir for the virus.[7-11] The detailed mode of the transmission to humans from animals is a mystery, but infected human to human close contact and unprotected health care during treatment may responsible for the spreading of the viral disease.[12-14] The MERS-CoV infected individuals may not show the symptoms in the early days, but the delaying time leads the development of mild to severe acute respiratory syndrome and sometimes failure of respiratory system results in death.[15] The major symptoms include fever associated with cough and difficulty in breathing, pneumonia, disturbances in GIT and diarrhea.[16,17] The treatment for MERS-CoV infections is purely sympathetic, the antiviral therapy has not existed and vaccination program is under development.[18,19] The ingestion of fluids and administration of analgesics and the mechanical ventilation was suggested in case of severe breathing problems.[2,19] The patients with a history of diabetes, lung diseases, kidney disorders and immunobalanced persons were prone to high risk for MERS-CoV infections.[20] Therefore, the travel to countries with high risk of MERS-CoV is not recommended.[21,22] The MERS-CoV strains can be diagnosed by the RTPCR techniques in qualified laboratories. The chest X-ray indicates development of pneumonia, but the specificity and differentiation between SARS-CoV and MERS-CoV is becoming the major problem in the early detection of the viral disease.[23, 24]

2. Epidemiology

The two basic distinct types coronaviruses i.e, SARS-CoV and MERS-CoV has been reported for causing upper respiratory tract infections.[8] The first one SARS-CoV (coronavirus related to Severe acute respiratory syndrome) was first identified in China during an outbreak in 2012 and caused the 778 deaths among 8,098 infected cases.[25] According to a recent epidemiological survey, the wide spread of MERS-CoV infected cases have been reported in almost 20 Asian, American and European countries.[26,27] The first re-emergence of the MERS-CoV is now occurring in the South Korea and was suspected to be imported through the MER-CoV infected person with a travelling history of the Middle East.[28,29] More than 1,279 numbers of the cases in countries with MERS-CoV probability were reported among them 495 death cases were included.[5,30] (Figure 1 & 2)

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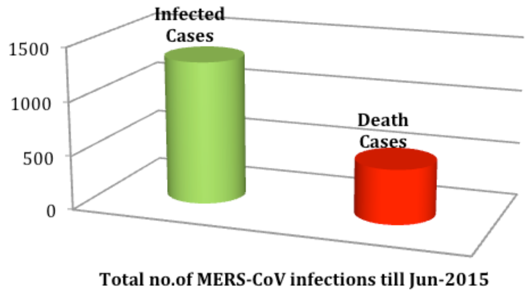

Figure 1. MERS-CoV infected and death cases

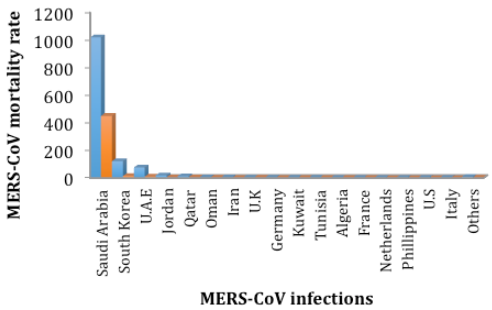

Figure 2. Countries reported with MERS-CoV infections

3. Pathophysiology of MERS-CoV

The Middle East respiratory syndrome coronavirus (MERS-CoV) is the latest emerged coronavirus causing severe respiratory disease along with renal dysfunction or failure in the immune compromised individuals.[31] The pathogenesis and mode of transmission of MERS-CoV virus are poorly understood, because no human samples are available due to the cultural and religious reasons, making it more difficult to implement intervention and preventive measures. However, very few *in vitro* experiments and animal models tried to reveal the pathogenesis of MERS virus. MERS virus enters into the body through DPP4 receptors.[32,33] DPP4 has many diverse functions in glucose homeostasis, T cell activation, neurotransmitter function, and modulation of cardiac signaling. After entry through DPP4 receptors, MERS virus can infect the epithelial cells of the respiratory tract. Recently, Doremalen *et al.*, 2014 have shown that MERS-CoV was found to replicate predominantly in type I and II pneumocytes in the lower respiratory tract of rhesus macaques.[34] During early in infection, MERS virus infected marmosets develop severe pneumonia. Neutrophil and macrophage infiltration and alveolar oedema have also been reported in infected lung tissue.[35] Thus, all these reports have indicated that MERS virus causes severe respiratory diseases, but the exact mechanism is still an open question for researchers to be explored.[36-38]

3.1 Immune system and MERS virus

How the MERS virus interacts with the immune system, very little information is known. The patients who recover from this infection may be due to induction of protective innate and adaptive immune response. The immune response against invading pathogens begins with a direct infection of airway epithelium. Normally, Toll-like receptors (TLRs) which are widely expressed

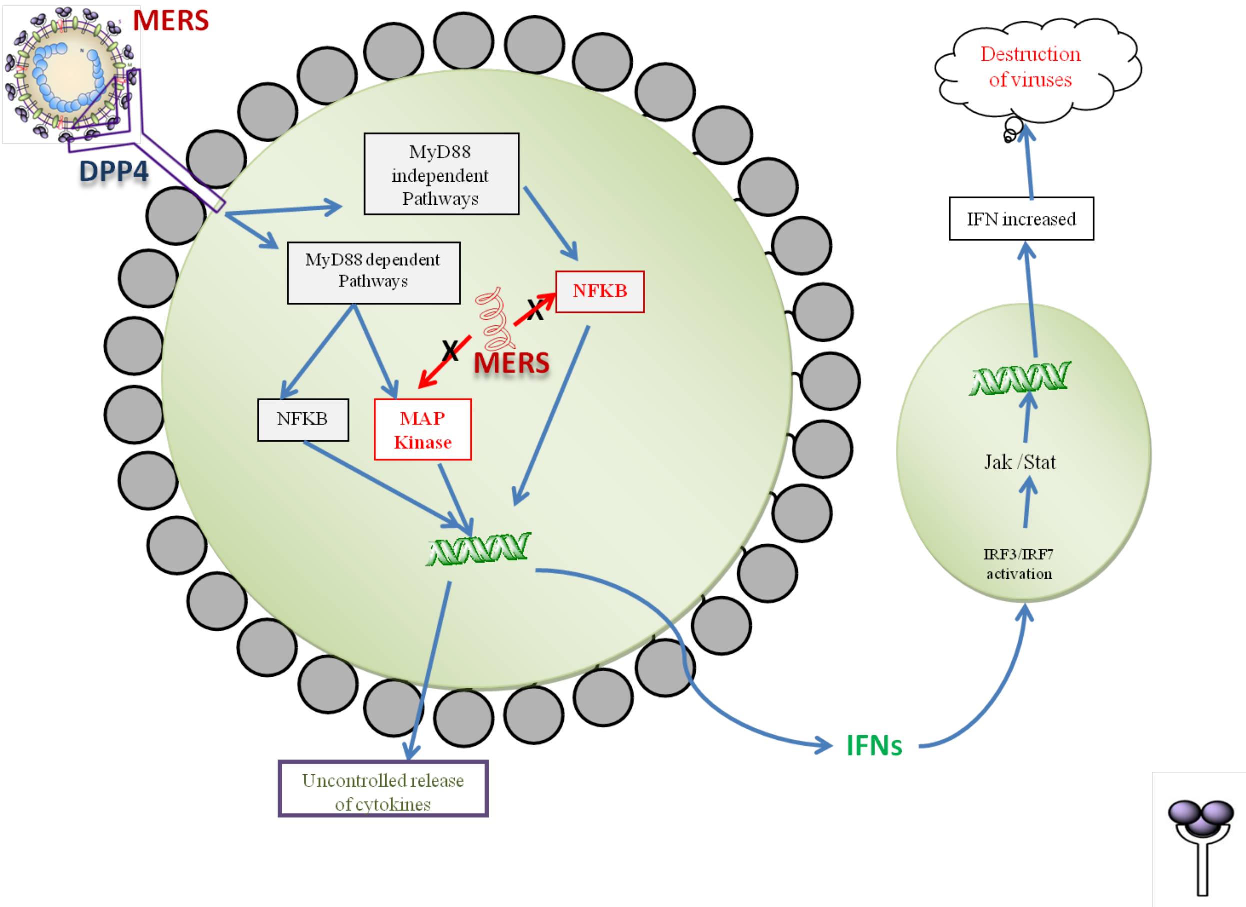

Figure 3. Normal and MERS virus altered interferon signaling pathways

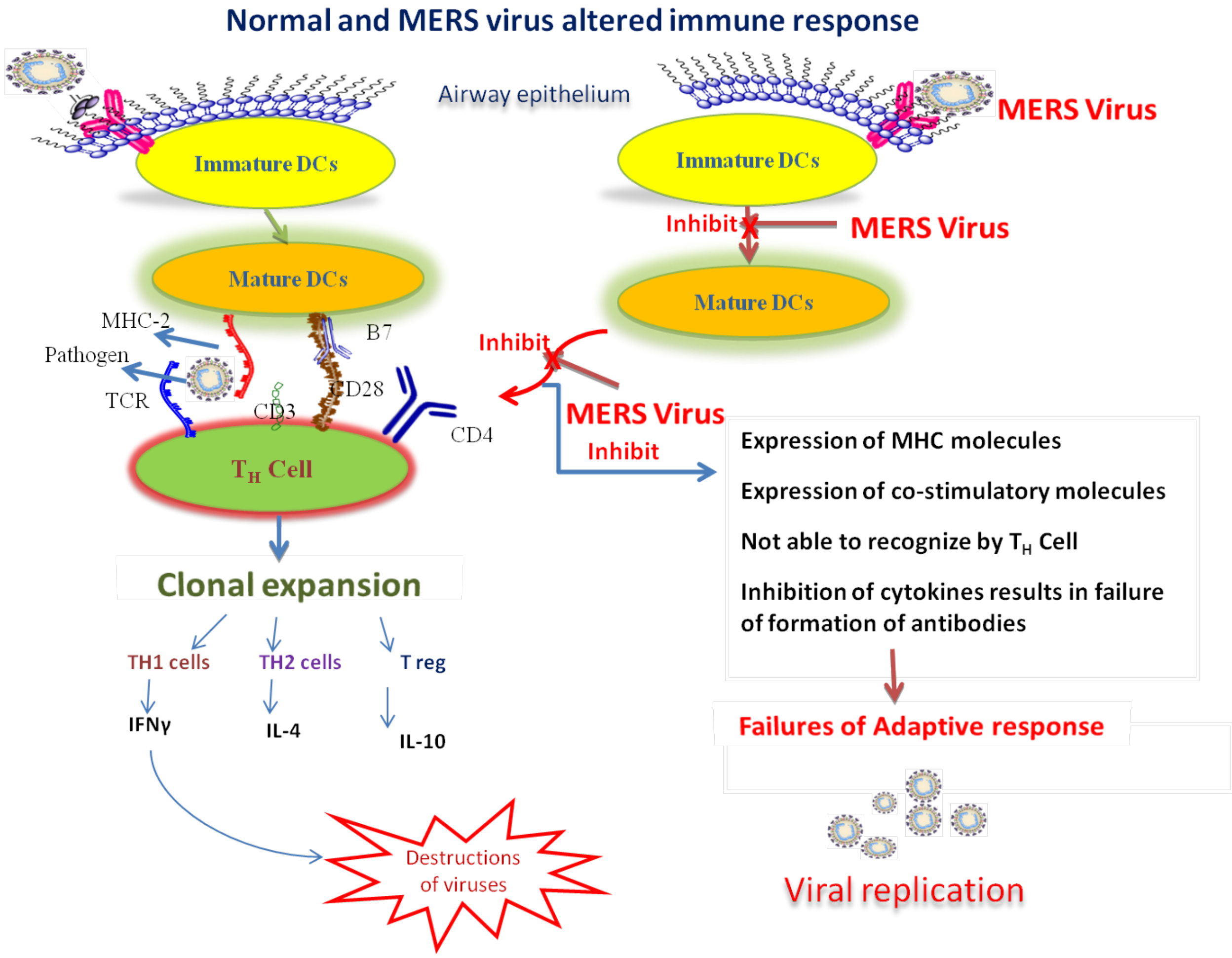

Figure 4. MERS virus altered connection between innate and adaptive immune system as compare to normal response

in many cell types such as macrophages, neutrophils and dendritic cells sense the viral product and activate MyD88-dependent and independent signaling pathways that leads to activation of transcription factors nuclear factor-κB (NF-κB), MAP Kinase, interferon regulatory factor 3/7 (IRF3/7) and/or activator protein-1 (AP-1), which collaborate to induce transcription of a large number of downstream genes and production of antiviral proteins known as interferons as shown in Figure 3.[39,40] MERS virus inhibits the MAP Kinase and NF-κB signaling pathways, which prevent these interferon-based signals which are important for destruction of viruses (Figure 3).The M, ORF 4a, ORF 4b, and ORF 5 proteins of MERS-CoV are potential interferon antagonists via the inhibition of the IFN-β promoter activity, IRF-3 and NF-κB signaling pathways.[41] Faure *et al.,* 2014 have shown that in the infected persons, a significant decrease in receptors and regulators was observed which are involved in the recognition of MERS-CoV such as RIG-1, MDA5, and IRF3-7. The decrease in IRF3 and 7 was associated with a major decrease in IFN α expression.[42] Chu *et al.,* 2014 reported that under *in vitro* and *in vivo* condition, MERS-CoV elicits attenuated innate immune responses with delayed pro-inflammatory cytokine induction, which could contribute to a dysregulated immune response.[43] Further, lung-resident respiratory dendritic cells (rDCs) become activated, process the antigen and migrate to the draining lymphnodes. In lymph nodes, rDCs present the processed antigen in the form of MHC/peptide complex to naive circulating T cells which are activated by the two signals. One signal triggered by MHC–peptide complex, and another from co-stimulatory molecules.[44] These signals lead to the proliferation of T cells and finally migrate to the site of infection for the destruction of viruses by releasing various antiviral cytokines (IFN-α, TNF-α, IL-2) as shown in Figure 4.In the literature lots of information is available regarding adaptive immune response against respiratory viruses but regarding coronavirus respiratory infections, very less is known. Josset *et al.*, 2013 demonstrated that MERS-CoV suppressed the antigen presentation pathways by down-regulating the type I and II major histocompatibility complex (MHC) genes.[45] The possibility of MERS virus altered adaptive immune response has been summarized in Figure 4.The modulation of these innate and adaptive immune response signaling pathways may explain the increased lethality of MERS-CoV.

4. Treatment

There is no current treatment available for MERS-CoV, but, with the continuation of the outbreak, identification of therapeutics is a top priority. However, various promising drugs are under preclinical and clinical phase, but till date no one reach to the market. Supportive treatment is the mainstay of management.

5. MERS-CoV genome

The MERS-CoV virus is the positive single stranded RNA belongs to the family *coronaviridea* and its genome consists of structural and nonstructural poly proteins.

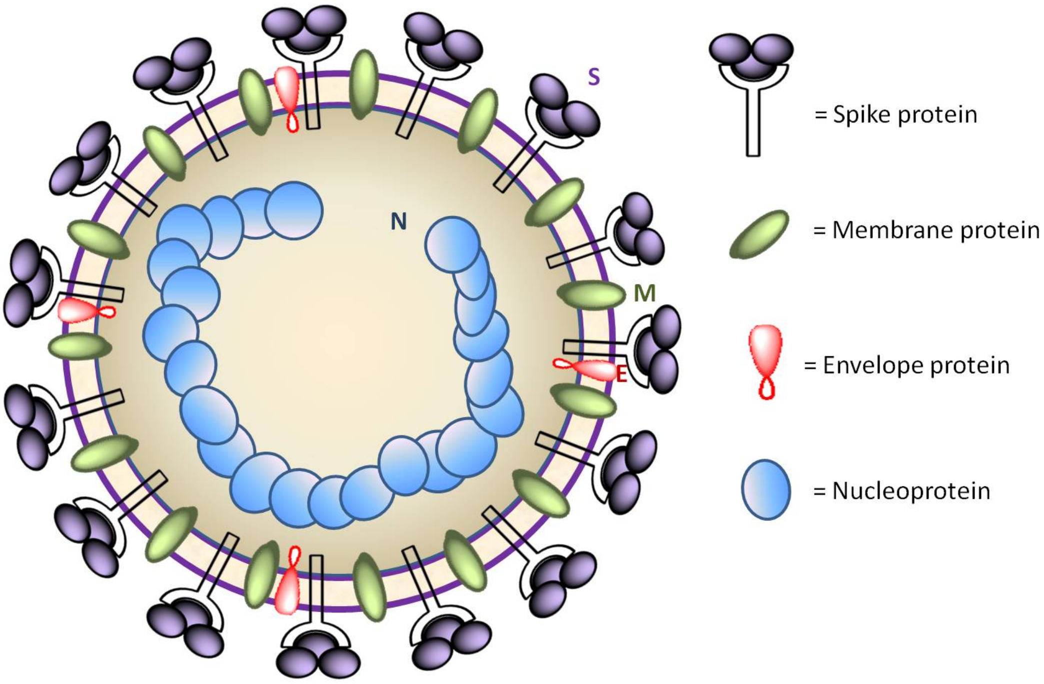

Figure 5. MERS-CoV Virus structure

The structural proteins play a vital role during viral entry into the host cell and viral assembly after the viral replication. Four major structural proteins include spike (S), envelope (E), membrane (M) and nucleoprotein (N) proteins were reported; the spike (S) protein found to be critical for host cell attachment (Figure 5).[41] The nonstructural proteins include ORF1ab or ORF1a and ORF1b. The complete ORF1ab was fragmented into 16 nonstructural proteins and its genome was completely sequenced.[46, 47] The ORF1ab region was evidenced to be exhibiting the papain like protease (PLpro) and catalytic activity, hence it was named as MERS-CoV papain like proteases (PLpro).[48, 49] The thorough investigation on MERS-CoV (PLpro) provided the its replicase function during viral genome replication in a host cell. Even though, the complete sequencing was done in the nonstructural protein region (ORF1ab), the lack of information about the role of its neighbor proteins makes the strenuous situation to understand exact functions. The detailed available genome sequences of the structural and nonstructural proteins along with their PDB codes were provided in the Table 1.[50,51]

6. Current available inhibitors

The recent research studies provided the valuable information about the MERS-CoV inhibitors, most of them found where the various classes of pharmaceuticals and marketed drugs. The huge number of clinically available drugs with different pharmaceutical class were screened against MERS-CoV and SAR-CoV yielded the fewest hits. The detailed classes of drugs with a history of MERS-CoV inhibition were presented in the Table 2.[52] The potent compounds from this study were also having the history of an antiviral profile against few others viral species include the deadly ebola virus, pox virus, chikungunya and west nile virus.[53] This type of screening studies has created the basic idea for the drug development utilizing a variety of available pharmaceutical agents focusing multiple targets.

6.1 Coronaviral replication inhibitors

The antiviral screening of the FDA approved drugs with the different pharmaceutical class against various coronaviruses were afforded the effective antiviral agents. Among them the chlorpromazine (antipsychotic), chloroquine (antimalarial), loperamide (anti-diarrheal) and lopinavir (anti-HIV) drugs were found to be having the potent efficacious property to inhibit the viral replication process in cell culture mediums. They four drugs have been investigated against MERS-CoV along with SARS-CoV and HCoV-229E-GFP viral strains. The antiviral activity (EC_{50}) of the four compounds was reported between 3.0 to 8.0 µM.[54] However, the cytotoxicity (reported between CC_{50} = 15.5 to 58.1 µM) of these drugs were playing the major role or limiting the rate of success.

Table 1. The Uniprot accession number and list of available PDB

S.No	ORF frame	Type of protein	Uniprot accession number	Available PDB
1	ORF1a	Nonstructural protein	W6A941	4WMD, 4WMF, 4WME
2	ORF1ab	Nonstructural protein	V5REQ9, M4STU1, K4L41	4REZ, 4RF0, 4RF1, 4RNA, 4PT5, 4P16
3	ORF3	Nonstructural protein (ns3a)	R9UMM5	-
4	ORF4a	Nonstructural protein (ns3b)	R9UMC1	-
5	ORF4b	Nonstructural protein (ns3c)	R9UQP3	-
6	ORF5	Nonstructural protein (ns3d)	V5RD61	-
7	ORF8b	Other protein	R9UNW8	-
8	Spike (S) protein	Structural protein	V5RDT9, R9UQP3	4NJL
9	Envelop (E) protein	Structural protein	R9UQN1	-
10	Membrane (M) protein	Structural protein	R9UNX5	-
11	Nucleoprotein (N)	Structural protein	T2B9H8	-

Table 2. Drugs having the inhibition property against MERS-CoV* and SARS-CoV (Dyall *et al*)

S.No	Drug classification	List of Drugs	Probable mode of action
1	Neurotransmitter inhibitor	Clomipramine HCl Chlorphenoxamine HCl Astemizole Promethazine HCl Fluphenazine HCl Thiothixene Fluspirilene Benztropine mesylate	Clathrin-mediated endocytosis inhibitors
2	Antibiotic agent	Anisomycin	Protein-processing inhibitor
3	Anticancer agents	Dasatinib Imatinib mesylate	Inhibition of viral replication
4	Estrogen receptor inhibitor	Toremifene citrate Tamoxifen citrate	Viral entry inhibitor
5	Cathepsin inhibitor	E-64-D	cysteine peptidase inhibitor
6	Antibacterial agent	Emetine 2HCl.H_2O Cycloheximide	Unknown Protein synthesis inhibitor
7	Antiparasitic agent/Antimalarial	Chloroquine 2H_3PO_4 Mefloquine Hydroxychloroquine SO_4 Amodiaquine 2HCl.H_2O	Inhibition of viral replication
8	Nucleoside analog	Gemcitabine HCl	DNA metabolism inhibitor
9	Antimycotic	Terconazole	Unkown
10	Sterol metabolism inhibitor	Triparanol	Unkown
11	Cytoskeleton inhibitors	Nocodazole	Microtubule deploymerization
12	Ion channel inhibitors	Monensin Salinomycin Na	Blocks the formation of virus particle and eruption

*Drugs reported with EC_{50} of less than 50 μM and cytotoxicity by less than 30%

6.2 MERS-CoV (nsp13) helicase/replication inhibitors

The corona viral helicases were crucial for the replication of the viral genome, therefore, they can be targeted for the blocking of the viral replication. The chemical agent SSYA10-001 was successfully inhibited the SARS-CoV (nsp13) helicase enzyme at 7 μM with higher selectivity index (>71). The antiviral screening of the SSYA10-001 against MERS-CoV helicase was delivered the antiviral activity (25 μM) with lowest selectivity index (>20) than SARS-CoV (nsp13) helicase. The antiviral potency may afforded due to homologous feature of MERS-CoV helicase with SARS-CoV (nsp13) helicase.[55] In addition to this, the present was also found to be effective against mouse hepatitis virus. The cytotoxicity profile of this compound is remarkably lower when compared to the class of marketed pharmaceutical drugs and it makes it advantageous. Chemically the SSYA10-001 (Figure 6) can be termed as 1,2,4 triazole analog and the similar small synthetic triazole derivatives can be screened against corona viral helicases to get an efficacious dual or specific inhibitors against both species.

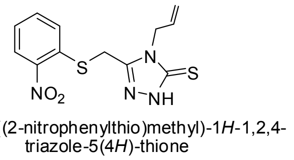

Figure 6. Structure of SSYA10-001

6.3 Natural product isolates as MERS-CoV inhibitors

Now a days the testing of natural products against a wide range of viral infections was seem common phenomenon. A few natural products like jatropane esters, trigocherin and harringtonine were exhibited potent antiviral effects on the RNA viruses like chikungunya. The similar inhibitory property of homoharringtonine (HHT) against the MERS-CoV was reported (Figure 7). The HHT is a pharmaceutical class of drug marketed as Omacetaxine mepasuccinate, a natural alkaloid ester obtained from plant *Cephalotaxus harringtonii*. The HHT has inhibited the MERS-CoV at 0.07 μM with cytotoxicity lower than 30%. With this regard it indicated that few more similar derivatives can be tested due to their promising antiviral profile.[52]

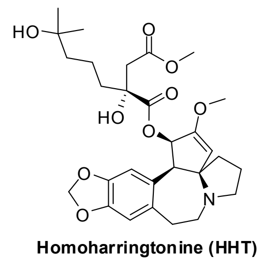

Figure 7. Structure of Homohorringtonine (HHT)

6.4 Drugs in combination with interferons as MERS-CoV inhibitors

The antiviral drug ribavarin, the most popular nucleoside analog was tested by administering to the patients along with interferon-α & β. The combination therapy of ribavarin with interferon reduced the mortality rate in MERS-CoV positives.[56] Mycophenolic acid (MPA), which as an immunosupprasant drug first was obtained from the fungus called *Byssochlamys nivea*. The MPA becomes the successful drug is being widely using for the suppressing immunity during organ transplantation. The antiviral effect of MPA at 2.87 μM was observed and effect of this drug in *in vitro* studies was reported to be better than the ribavarin therapy (Figure 8).[57, 58] Apart from these they also had the history of antiviral activity against chikungunya when they were combined with the interferons.[59]

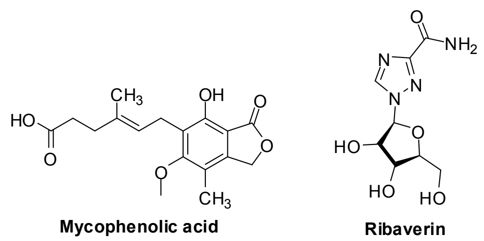

Figure 8. Structure of Mycophenolic acid and ribavirin

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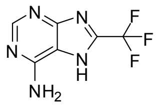
6.5 MERS-CoV papain like protease (PLpro) inhibitors

The genome of the MERS-CoV virus remains to be explored completely and the structure based design approaches were becoming successful process to identify the effective inhibitors for MERS-CoV. The MERS-CoV papain like proteases (PLpro) (PDB: 4RNA) said to be target of interest for the design of novel compounds. The high throughput screening (HTS) strategy with a library of 25,000 compounds was applied to recognize a few small synthetic MERS-CoV (PLpro) inhibitors. The HTS method afforded the purine analog having the capacity of binding with MERS-CoV (PLpro) (Figure 9). Since, the MERS-CoV (PLpro) (PDB: 4RNA) and SARS-CoV (PLpro) (PDB: 3E9S) proteases were structurally homologous. The identified purine derivative was also inhibited the SARS-CoV (PLpro) enzyme in screening. The antiviral efficacy of this compound against MERS-CoV (PLpro) was reported to be higher with a competitive mode of binding (IC_{50} = 6μM), whereas, it was slightly lower (IC_{50} = 11μM) in SARS-CoV (PLpro) with the an allosteric mode of binding.[48]



8-(trifluoromethyl)-7H-purin-6-amine

Figure 9. Purine analog

Similarly the few SARS-CoV (PLpro) inhibitors were screened against MERS-CoV (PLpro) and the results were found to be negative. The reason behind this is the difference in amino acid residues at the catalytic site despite of homologous sequence. Therefore, an additional research has to be conducted in order to develop the specific corona viral agents.

7. Druggable targets for inhibition of MERS-CoV

The structure based ligand design process for the development of promising antiviral agent against MERS-CoV infections is depends on the availability of the three dimensional protein X-ray crystal structures. In such case a very few X-ray crystallographic structure of MERS-CoV were resolved till date. The ORF1ab sequence from various MERS-CoV strains were utilized for the development of the 3D structures. This region contributes the nonstructural polyproteins, which are crucial for the viral replication phenomenon. The MERS-CoV papain like protease (PLpro) is one among them reported to be promising future druggable target. The active site of the MERS-CoV (PLpro) consists of the catalytic triad made with three catalytic residues i.e, Cys119, His278 and Asp293, which is homologous to SARS-CoV (PLpro). The few active site residues include Leu106, Asn109, Asp108 and Trp93 were also present near to the catalytic triad.[48,60] The X-ray crystal structure of envelope protein (E) is also available and can be targeted for the inhibition of the virus at an entry stage. Further, the detailed structures of the above targets were provided in Figure 10.

8. Vaccines

There is no licensed vaccine is available for use in humans against MERS-CoV, but most of promising approaches are under pre-clinical and clinical development. Human monoclonal neutralizing antibodi

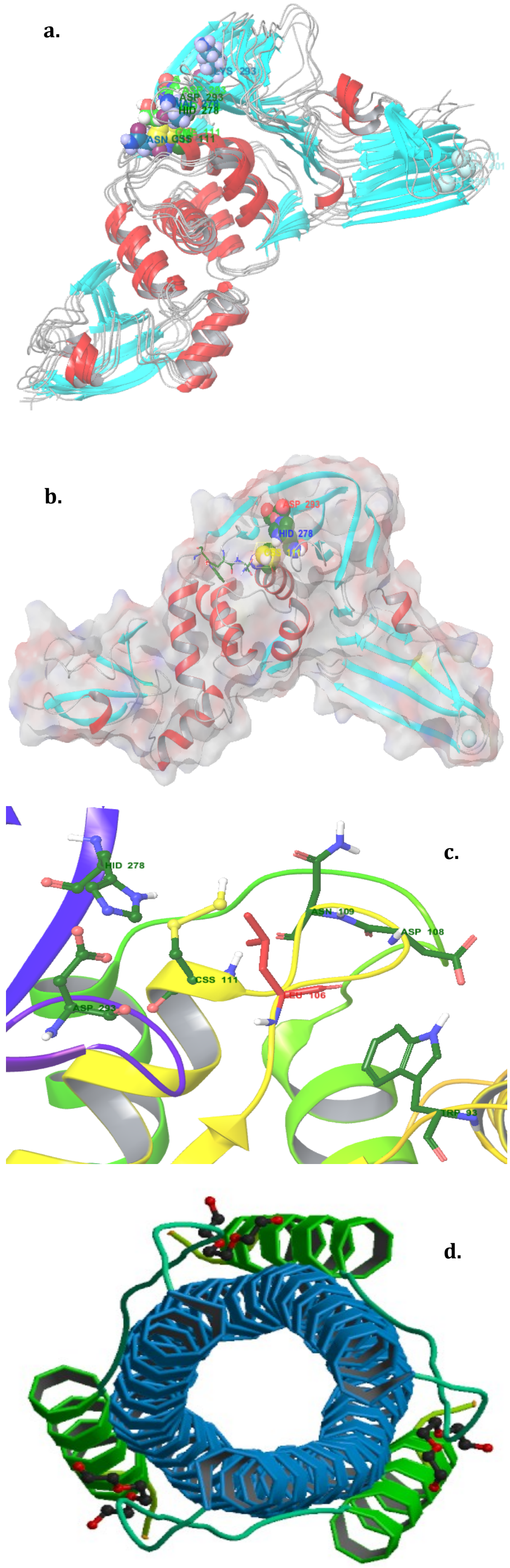


Figure 10. a. Super pose of listed MERS-CoV PLpro PDBs; **b**. Surface diagram of 4RNA with active site; **c**. The active site residues, catalytic traid was represented in ball and stick residues, other active site residues were shown sticks and red colur residue is Leu106 not present in SARS-CoV PLpro; **d**. MERS-CoV envelope protein (E)

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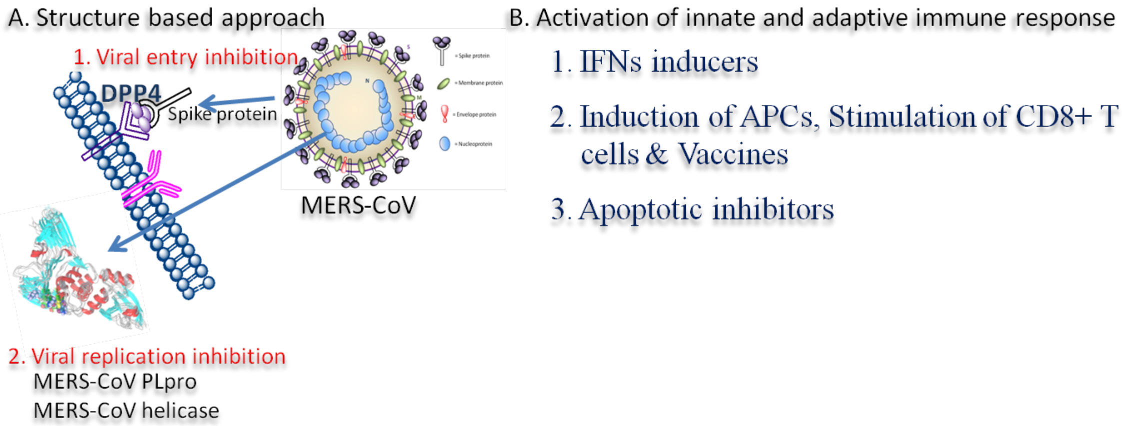

Figure 11. Promising approaches for design of MERS-CoV inhibitors and others techniques

-es and convalescent sera from recovered patients might be useful for treatment if delivered in a timely fashion. While passive immunization has value in saving lives but its protection is temporary, the antibodies given to patients do not endure. A better approach is immunization, which induces anti-viral antibodies as well as creates the immune memory but for activation it takes some time about 1-2 weeks. There are several promising vaccine candidates that have demonstrated immunogenicity and efficacy in animal models of MERS-CoV infection. Ma *et al.*, 2014 reported that the receptor binding domain (RBD) fragment encompassing spike residues 377-588 in MERS-CoV is ideal candidate for development of effective MERS vaccines in BALB/c mice.[61] Further, the researchers also using the recombinant nanoparticle technology to produce MERS vaccine. Recently, Colemam *et al.,* 2014 have used this approach and demonstrated the formation of coronavirus neutralizing antibodies by injecting virus-like particles (VLP) into the mice.[62] All these results are encouraging but there are a number of scientific hurdles left for all of these candidates before they can be used in humans with a reasonable expectation that they would be safe, immunogenic, and efficacious.

9. Promising approaches

The few optimistic ways of advancing the drug discovery process to find the MERS-CoV inhibitors can be suggested (Figure 11). The first one is the structure based drug discovery tools, may yield curious results in finding the viral inhibitors. The availability of the X-ray crystal structures of MERS-CoV Papain Like protease (MERS-CoV PLpro) and viral envelope protein (E) is the best examples for initiating the target oriented high throughput virtual screening. The very few viral replication inhibitors were reported through the HTS techniques. The identification of viral entry inhibitors has to be done. The next approach said to be an indirect way of viral inhibition through activation of immune responses. It can be achieved by the supplement of interferon inducers, vaccination and miscellaneous methods.

Conclusion

The MERS-CoV was transmitted to human s from the animals, camels are likely confirmed as reservoirs of the virus. The traveling from the South Middle East to other countries was the main reason behind the transmission of disease from humans to humans. The prevention of disease through careful supportive therapy is advised. The direct inhibitors of MERS-CoV infection is not available and the viral oriented vaccination program is still in the pipeline. However, the review on the availability of the reported drugs and viral inhibitors developed through the structure based targeted were compiled. It was also observed that the mutations or the presence of the variant active site residues in MERS-CoV PLpro makes difficult in the testing of drugs developed through its homologous SARS-CoV PLpro**.** Therefore, the present review report may provide an essential information for research work allied with the drug development process.

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