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Current treatment options and the role of peptides as potential therapeutic components for Middle East Respiratory Syndrome (MERS): A review

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ABSTRACT

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a highly pathogenic respiratory virus with mechanisms that may be driven by innate immune responses. Despite the effort of scientific studies related to this virus, Middle East Respiratory Syndrome (MERS) is still a public health concern. MERS-CoV infection has a high mortality rate, and to date, no therapeutic or vaccine has been discovered, that is effective in treating or preventing the disease. In this review, we summarize our understanding of the molecular and biological events of compounds acting as MERS-CoV inhibitors, the outcomes of existing therapeutic options and the various drugs undergoing clinical trials. Currently, several therapeutic options have been employed, such as convalescent plasma (CP), intravenous immunoglobulin (IVIG), monoclonal antibodies and repurposing of existing clinically approved drugs. However, these therapeutic options have drawbacks, thus the need for an alternative approach. The requirement for effective therapeutic treatment has brought the necessity for additional MERS treatments. We suggest that antimicrobial peptides (AMPs) may be used as alternative therapeutic agents against MERS-CoV infection. In addition, we propose the feasibility of developing effective agents by repurposing the existing and clinically approved anti-coronavirus and anti-viral peptide drugs.

Contents

Abbreviations: MERS-CoV, Middle East Respiratory Syndrome Coronavirus; CP, convalescent plasma; IVIG, intravenous immunoglobulin.
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Introduction

Middle East Respiratory Syndrome (MERS) is a respiratory disease caused by the MERS coronavirus (MERS-CoV) [1,2]. MERS-CoV was first reported in 2012 in Saudi Arabia and has spread to other countries, mostly in the Arabian Peninsula (United Arab Emirates, Qatar, Oman, Jordan, Kuwait, Yemen, and Lebanon). As of March 2017, the World Health Organization (WHO) reported 1905 confirmed MERS cases, including 677 deaths in 27 countries. MERS spreads among people causing more severe complications, which leads to death; hence, the need for the development of effective therapeutic and prophylactic agents for its prevention and treatment.

It has been shown that a hospital outbreak of MERS was due to human-to-human transmission [3]. Currently, there is no registered treatment or vaccine for the disease, but the MERS-CoV infection is rather managed by a drug treatment regime, in addition to some preventive measures for infection and re-infection [4]. Several studies have demonstrated that a variety of therapeutics can inhibit MERS-CoV replication in cell culture [5,6].

In this review, we (i) summarize the important MERS-CoV drug targets, (ii) present therapeutic options available for MERS and describe their efficacies and (iii) discuss the role of peptide research in virology and the importance of antimicrobial peptides (AMPs) as potential therapeutic options for MERS. With the rapid development of computational biology approaches, it is envisaged that novel and effective MERS therapy could be developed using AMPs.

MERS-CoV structural and non-structural proteins as drug targets

Human coronaviruses are broadly classified as either alphacoronavirus or betacoronavirus. MERS-CoV belongs to the betacoronavirus family [7]. The causal transmission pathway of MERS-CoV has not been demonstrated [8] but it may have originated from bats with dromedary camels serving as intermediate hosts for human infection [9]. MERS-CoV has been shown to modulate the innate immune response, antigen presentation, mitogen-activated protein kinase (MAPK), and apoptotic pathways [10].

The structure of MERS-CoV consists of four structural proteins as shown in Fig. 1A, including spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein. The S protein is a type I transmembrane glycoprotein, which is located at the viral envelope surface in a trimer state. It consists of S1 and S2 subunits and plays a role in viral entry, binding, and fusion. The S1 subunit has a receptor-binding domain (RBD) (Fig. 1B). The RBD is responsible for binding to the cellular receptor dipeptidyl peptidase 4 (DPP4). The S2 subunit involves two regions, namely heptad repeats 1 and 2 (HR1 and HR2) (Fig. 1C and D), which assemble into a complex called the fusion core, and represents a key membrane fusion architecture [7,11]. The E protein present mainly in the intracellular membranes of the virus [12] and plays a major role in viral assembly, budding, and intracellular trafficking [12]. In 2013, Surya et al. reported that coronavirus E proteins are 76–109 amino acids long and are predicted to have at least one α-helical transmembrane [13]. It was later found that the E protein has a total length of 82 amino acid residues [12]. The M protein is a component of the viral

envelope, which plays a role in virus morphogenesis and assembly via its interactions with other viral proteins [14]. The S, M and E proteins are inserted into the membrane of the rough endoplasmic reticulum and get transported into the vicinity of the endoplasmic reticulum-golgi area in which they interact with the N proteins to form particles [15]. This interaction will eventually interfere with the fusion of cellular and viral membranes. Thus, developing fusion peptides can be of great importance in peptide-based therapeutic options.

In addition, MERS-CoV is comprised of two large polyproteins, namely pp1a and pp1ab. These polyproteins are further cleaved into 16 non-structural proteins (nsps) [16]. Two proteases, papain-like protease (PLpro or nsp3) and 3C-like protease (3CLpro or nsp5), are responsible for processing all 16 nsps, which are required for replication and transcription [17]. PLpro is responsible for the cleavage at position 1-3 to develop three nsps, whereas 3CLpro cleaves the remaining positions 4-16. These nsps are responsible for viral RNA-dependent RNA polymerase activity (nsp12), primase activity (nsp8), RNA helicase activity (nsp13), exoribonuclease activity (nsp14), endoribonuclease activity (nsp15), and 2′-*O*-methyltransferase activity (nsp16) [18]. The 2′-*O*-methyltransferase activity is stimulated by nsp10, which acts as an allosteric activator of nsp16 [19]. Studies reveal that coronavirus nsps could be used as drug targets for developing new therapies [20–22]. Fig. 1E shows the nsp5 (3CLpro) protease bound with a designed inhibitor. Both the structural and non-structural proteins can act as therapeutic targets, in which various therapeutic options have been implemented and will be discussed in Sections V̈arious therapeutic agents against MERS-CoV infectionänd M̈echanism of action of peptides with antiviral activity against various coronaviruses.̈

Various therapeutic agents against MERS-CoV infection

There are several ways to inhibit the spread of MERS-CoV by (i) repurposing of clinically developed drugs, (ii) using convalescent plasma (CP) and whole blood therapy, (iii) intravenous immunoglobulin (IVIG), (iv), monoclonal antibodies, and (v) other therapeutics, which will be discussed in the following subsections.

Repurposing of existing clinically approved drugs

Repurposing is the method of targeting existing drug molecules to treat new diseases. During repurposing, a viable target profile is developed for the drug or receptor molecule, and the screening of compounds from different molecule libraries can occur. Repurposing has become possible at a faster pace with the latest developments in computational biology, rational design, and development of novel and approved agents with potent antiviral activity. Repurposing of existing clinically FDA approved drugs can be used to target viral entry. High throughput screening of compounds and small molecules has helped researchers to evaluate large libraries of drugs for the in vitro antiviral activity against novel targets [24,25]. The advantages of the repurposing method are i) that it saves time and ii) lowers the cost of developing a new drug [26].

Repurposed drugs may have potential antiviral effects against MERS-CoV. Examples of repurposed drugs that have shown anti-
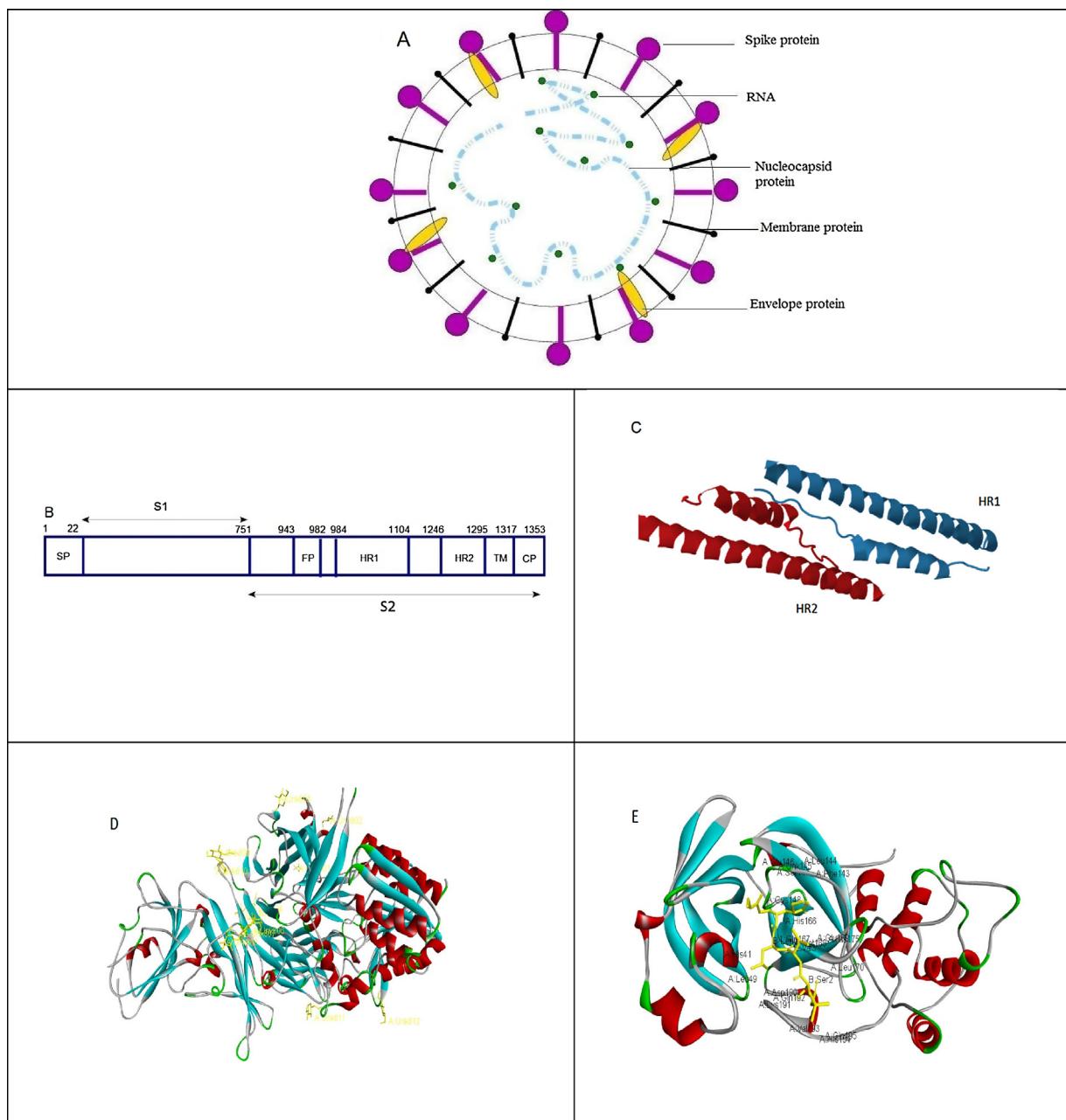
Fig. 1. A Schematic representation of the MERS-CoV structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N). B shows spike (S) protein and its subunits S1 and S2 form a fusion core, where SP is signal peptide, FP is fusion peptide, HR1 and HR2 are the heptad repeats 1 and 2, TM is transmembrane domain, and CP is cytoplasmic domain. C shows HR1 and HR2 with the linker. D shows the MERS-CoV structure complexed with human DPP4 with PDB ID: 4L72 visualised in Biovia DS Visualizer [23], and E shows structure of MERS-CoV nsp5 protease bound with a designed inhibitor, PDB ID: 4RSP visualised in Biovia DS Visualizer [23].

coronavirus validated activity include ribavirin, hexachloropene, nitazoxanide and homoharringtonine [27]. Ribavirin has been reported to have anti-MERS-CoV activity [28], but has adverse side effects with the occurrence of hemolysis [29]. Nitazoxanide has shown in vitro activity against MERS-CoV and other coronavirus infections by inhibiting expression of the viral nucleocapsid protein [30]. In vitro studies show that cyclosporine inhibits MERS-CoV in human and monkey cell lines . A recent study by Shih-Chao et al. tested the antiviral activities of resveratrol, a phytocompound in grape seeds and red wine, and showed that resveratrol could significantly inhibit MERS-CoV nucleocapsid expression [31]. Other examples of the use of small molecules as therapeutic options for MERS-CoV are given in Table 1.

Various studies have repurposed drugs by employing a hybrid of two or more drugs. For example, Wilde et al. employed alisporivir which inhibits the replication of MERS-CoV and SARS-CoV. However, they found that alisporivir in combination with ribavirin, further enhanced the antiviral efficacy of alisporivir. This was done using cell-culture based screening. [32]. Another study showed that the combination of lopinavir/ritonavir and interferon-β1b was found to have an effect on marmosets infected with MERS-CoV [33].

Interferons have been shown to be potent inhibitors of MERS-CoV replication [33]. However, the combination of interferon-alpha 2b and ribavirin (drugs routinely used to treat hepatitis C) reduced MERS-CoV replication when administered to rhesus macaques [34]. In addition, ribavirin and interferon-alfa-2a were administered to patients with severe MERS-CoV infection and their survival

Table 1
List of anti-MERS-CoV therapeutics using small molecules.

Example	Toxicity $TC_{50}/CC_{50}/SI$	EC_{50}/IC_{50}	Cell line/animal model	References
K22	CC50 ≥ 40 μM	–	Human airway epithelia (HAE)	[68]
Acyclovir	CC50 > 1000	EC50 > 1000	Huh7	[69]
Acyclovir	CC50 > 1000	EC50 > 1000	Vero	
Analogues of acyclovir 2	CC50 = 149 ± 6.8	EC50 = 27 ± 0.0	Huh7	
Analogues of acyclovir 2	CC50 = 71 ± 14	EC50 = 23 ± 0.6	Vero	
SSYA10-001	SI > 20	EC50 ~25 μM	Vero E6	[70]
Chlorpromazine hydrochloride	Low cytotoxicity (<30%)	EC50 = 9.514	Vero	[26]
Trifluupromazine hydrochloride	Low cytotoxicity (<30%)	EC50 = 5.758	E6	
Imatinib mesylate	Low cytotoxicity (<30%)	EC50 = 17.689		
Dasatinib	Low cytotoxicity (<30%)	EC50 = 5.468		
Gemcitabine hydrochloride	Low cytotoxicity (<30%)	EC50 = 1.216		
Toremifene citrate	Low cytotoxicity (<30%)	EC50 = 12.915		
Chloroquine, chlorpromazine, loperamide, and lopinavir	Viability > 75%	EC50 = 3 to 8 μM	Vero E6	[71]

improved [35]. In another case report, the lopinavir/ritonavir-based combination antiviral therapy was used for MERS-CoV infection in South Korea [36]. Tawalah et al. evaluated the virological and clinical progresses in response to lopinavir/ritonavir, by administering different treatment options in MERS patients. This study suggested that the most effective therapeutic regimen was a low dose ribavirin/pegylated interferon α combination [37]. Several studies have shown the effectiveness of ribavirin coupled with interferons [38–40]. Mycophenolic acid, which is an immunosuppressant drug, together with interferon-β1b was found to have a strong inhibitory action against MERS-CoV. The combination of mycophenolic acid and interferon-β1b lowered the EC_{50} [26,41].

Convalescent plasma and whole blood therapy

The use of convalescent plasma (CP) and whole blood therapy has been suggested to be a potential treatment for infectious diseases such as MERS, Ebola and SARS. CP is the use of whole blood or plasma collected from patients that have recovered from viral diseases and has been used as a treatment during outbreaks [42]. CP is therapeutically administered when there are no specific vaccines or drugs available for treating the diseases [43].

Arabi et al. analysed the feasibility of CP therapy as well as its safety, clinical and laboratory effects in critically ill patients with MERS-CoV infection. They suggested that CP and other neutralizing antibodies have immunotherapeutic (hyperimmune immunoglobulins and monoclonal antibodies) potential for treatment of MERS-CoV infection. However, the study noted that large-scale screening would be required because of the limited availability of eligible potential donors with sufficient levels of antibodies [42]. One drawback of CP is that it is unavailable and there is lack of evidence to prove its safety and efficacy [44].

Intravenous immunoglobulin

Intravenous immunoglobulin (IVIG) is a blood product prepared from serum, which is generally used in treating a number of autoimmune and inflammatory conditions [45]. The administration of IVIG is well studied in various diseases including heart failure, mycobacterial infection, epilepsy, Alzheimer's etc. [46]. During treatment, these antibodies are administered intravenously and may lead to renal failure or thrombosis in MERS patients [44]. However, there is no evidence that IVIG has anti-MERS activity. Luke et al. reported a study in which transchromosomic (Tc) bovines produced human polyclonal immunoglobulin G antibodies that neutralized MERS-CoV in both in vitro assays and animal model(s) [47]. The implication of this study shows that it potentially offers a method to manufacture therapeutic immunoglobulin to prevent and/or treat MERS-CoV infection and/or other new infectious diseases.

Monoclonal and polyclonal antibodies

Monoclonal antibodies (mAbs) have been used to clinically diagnose various diseases for several years, and are considered as a potential method of intervention. Monoclonal and polyclonal antibodies against DPP4 have been reported to inhibit MERS-CoV infection in primary human bronchial epithelia cells and Huh-7 cells [48]. The LCA60 antibody which was developed using cell clone technology from the immortalized B cells of a human donor recovering from MERS, is considered to be a treatment option for severe MERS-CoV infections [49,50]. A recent study has identified and characterized a set of seven human neutralizing Abs (nAbs) [51] and two monoclonal antibodies (mAbs) namely MERS-4 and MERS-27 [52]. These agents are RBD specific potent inhibitors, and they show strong neutralizing activity against MERS-CoV. Another study succeeded in developing two potential antibodies, REGN3051 and REGN3048, which have proven effective in animal models for MERS-CoV infection [53]. Other mAbs that have shown activity against MERS-CoV include (i) 1E9, IF8, 3A1, 3B12, 3C12, 3B11 and M14D3 [54], (ii) 3B11-N [55], (iii) m336, m337 and m338 [56–58], (iv) hMs-1 [59] and (v) 4C2 h [60]. Despite the utility, the production of monoclonal antibodies are time consuming and difficult [61].

Other therapeutics

Other therapeutics that block MERS-CoV S-mediated cell entry and virus cell membrane fusion include cathespin inhibitors [62], TMPRSS2 inhibitors [63], furin inhibitors [64], kinase inhibitors [65] and IFITM proteins [66]. A study in China used high flow nasal cannula (HFNC) for treating a patient with MERS [67].

In summary, the above therapeutic options, namely repurposing of clinically developed drugs, CP and whole blood therapy, IVIG and use of monoclonal antibodies have advantages and disadvantages in terms of generation, cost and overall applications. This has led to interest in peptides as an alternative therapeutic option, which will be discussed in the coming sections.

Role of peptides as potential anti-viral/anti-coronavirus components

There is an interest in peptide therapeutics and their mimetics as potential antagonists for various pathogens. Peptide research is an important aspect in pharmaceutical research and approximately 140 peptide therapeutics are currently being evaluated in clinical trials [72].

The reasons for using peptides are (i) that they inhibit protein-protein interactions (ii) they can be used as an alternative for diseases that are difficult to target, (iii) there are advance tech-

Table 2
List of anti-coronavirus peptides (* calculated by APD3 [106]: antimicrobial peptide calculator)

(A) The peptides inhibiting fusion

Peptide	Sequence	Peptide source	Virus	*Net charge	*Hydrophobic residues (%)	Toxicity (TC$_{50}$)/ selectivity index (SI)	IC$_{50}$	Cell line/animal model	References
SARS$_{WW-III}$	GYHLMSFPQAAPHGVVFLHVTW	S2 subunit of SARS-CoV	SARS-CoV	0	50	No difference in absorbance as compared to untreated cells	~2 μM	Vero E6 and L2 cells	[96]
SARS$_{WW-IV}$	GVFVFNGTSWFITQRNFFS	S2 subunit of SARS-CoV	SARS-CoV	1	47	No difference in absorbance as compared to untreated cells	~2 μM	Vero E6 and L2 cells	[96]
MHV$_{WW-IV}$	GYFVQDDGEWKFTGSSYYY	S2 subunit of MHV	MHV	−2	21	Not cytotoxic at a concentration of 30 μM on L2 cells	4 μM	Vero E6 and L2 cells	[96]
P1	LTQINTTLLDLTYEMLSLQQVVK ALNESYIDLKEL	HR2 region of MERS-CoV	MERS-CoV	−3	42	–	~3.013 μM	293T	[104]
HR2L	SIPNFGSLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELGNY	HR2 region of MERS-CoV	MERS-CoV	−3	37	–	0.5 μM	293T/EGFP + Huh-7 cells	[94]
HR2P	SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKEL	HR2 region of MERS-CoV	MERS-CoV	−3	41	SI = CC$_{50}$/IC$_{50 > 1667}$	0.97 ± 0.15 μM	293T/EGFP + Huh-7 cells	[94]
HR2P-M1	SLTQINTTLLDLEYEMRSLQQVVKALNESYIDLKEL	HR2P region of MERS-CoV	MERS-CoV	−3	38	–	0.85 ± 0.08 μM	293T/EGFP + Huh-7 cells	[94]
HR2P-M2	SLTQINTTLLDLEYEMKKLEEVVKKLEESYIDLKEL	HR2 region of MERS-CoV	MERS-CoV	−4	36	–	0.55 ± 0.04 μM	293T cells and Huh-7 cells	[105]
P9	NGAICWGPCPTAFRQIGNCGHFKVRCCKIR	Mouse β-defensin-4	SARS-CoV MERS-CoV	5	46	Low toxicity in vivo	5 μg/ml	Mice	[99]

(B) The peptides inhibiting viral replication

Peptide	Sequence	Peptide source	Virus	*Net charge	*Hydrophobic residues (%)	Toxicity (TC$_{50}$) selectivity index (SI)		IC$_{50}$	Cell lines/ animal model	Reference
K29	FGGASCCLYCRCHIDHPNPKGFCDLKGKY	nsp10 of SARS-CoV	SARS-CoV	2	37	–		160 μM	*E. coli*	[102]
K12	GGASCCLYCRCH	nsp10 of SARS-CoV	SARS-CoV	1	50	–		160 μM	*E. coli*	[102]
HR2P	SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKEL	HR2 region of MERS-CoV	MERS-CoV	−3	41	SI > 1667		~0.6 μM	Vero cells	[94]
HR2P	SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKEL	HR2 region of MERS-CoV	MERS-CoV	−3	41	SI > 1667		0.6 μM	Calu-3 cells	[94]
HR2P	SLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKEL	HR2 region of MERS-CoV	MERS-CoV	−3	41	–		13.9 Mm	HFL cells	[94]
Mutant mucroporin-M1	LFRLIKSLIKRLVSAFK	Mucropin AMP	SARS-CoV	5	58	TC$_{50}$ = 70.46	SI = 9.85	7.15 μg/ml	MDCK	[101]

(C) The peptides inhibiting virus entry (K$_D$: peptide's dissociation constant)

Peptide	Sequence	Peptide source	Virus	*Net charge	*Hydrophobic residues (%)	Toxicity (TC$_{50}$) selectivity index (SI)	IC$_{50}$	Cell lines/animal model	Reference
H	HVTTTFAPPPPR	Porcine aminopeptidase N (pAPN) of TGEV	TGEV	1	25	0.55 (490 nm)	11 μg/ml	ST	[107]
S	SVVPSKATWGFA	Papn of TGEV		1	50	0.5 (490 nm)	15 μg/ml	ST	[107]
RBD-11b	YKYRYL	RBD of SARS-CoV	SARS-CoV	2	16	No evidence for toxicity	K$_D$ = 46 μM	Vero E6	[108]
P8	PSSKRFQPFQQFGRDVSDFT	S protein of SARS-CoV	SARS-CoV	1	25	–	–	HEK293T	[109]
P9	CANLLLQYGSFCTQLNRALSGIA	S protein of SARS-CoV	SARS-CoV	1	52	–	–	HEK293T	[109]

niques to improve peptide half-life and (iv) they have a shorter market time [73].

Peptide drugs have little side effects and little drug tolerance compared with chemical drugs. Meanwhile, the specificity of treatment is high. Peptide drugs need special storage conditions otherwise the protein function becomes inactive causing low oral bioavailability and the propensity to be rapidly metabolized [74].

Many peptide inhibitors have shown activity against viruses [75–77]. For instance, the RVFV-6 peptide, which is derived from the membrane proximal stem region of the RVFV glycoprotein Gc, prevents the viral fusion in Rift Valley fever virus [78]. The RVFV-6 peptide is not only specific to the Rift Valley fever virus (and closely related variants), but also shows activity against unrelated viruses such as EBOV and VSV [78,79]. Scorpion venom peptide derivative Kn2-7 was identified as a potential anti-HIV-1 peptide which shows very low cytotoxic effects in biological assays [80].

The lack of effective treatment has encouraged the development of additional therapeutic agents with increased efficacy to overcome adverse side effects that occur with the current MERS treatment. There has been a growing interest in the field of peptide therapeutics over the last decade due to numerous adverse effects of chemical based drugs [81].

AMPs are excellent candidates as novel therapeutic agents since they have been reported to possess anti-coronavirus activity [82]. AMPs are components of the first line of defence of the immune system produced by both eukaryotic and prokaryotic species. They are small, positively charged, gene encoded peptides, which have selective toxicity towards gram-positive and gram-negative bacteria, protozoa, fungi and viruses [83–86]. Their selective toxicity is due to the fact that the microbes membrane bilayer is rich in lipopolysaccharides (LPS) and lipoteichoic acid (LPA), thus is negatively charged in contrast to the positive charge of the AMPs [85]. Antiviral AMPs have several modes of actions, including the blocking of viral entry by heparan sulphate interaction, inhibition of viral entry by fusing with specific cellular receptors, and stopping viral fusion by interacting with glycoproteins, membrane and envelope of the virus [87].

Mechanism of action of peptides with antiviral activity against various coronaviruses

There are various antiviral mechanisms of action by antimicrobial peptides, which include (i) direct interaction ("virolysis"), (ii) blockage of host cell surface receptors, (iii) inhibition of viral fusion to host cells, (iv) inhibition of viral replication and (v) activation of adaptive immune response [88]. The life cycle of the coronavirus begins by (i) fusing its spike protein into the host receptor, (ii) viral entry into the host cell, (iii) intracellular viral replication and transcription, (iv) protein production, assembly, and release of new virus particles [79]. Therefore, therapeutic strategies should focus on these four important processes. The main mechanism of action of antiviral peptides can be categorized generally into three major groups: (1) peptides that inhibit fusion (2) peptides that inhibit virus entry and (3) peptides that inhibit replication and (4) peptides that inhibit assembly and release of virus.

Peptides inhibiting fusion

Coronaviruses enter target cells by inducing fusion between the viral and cellular membranes, a process mediated by the viral spike (S) glycoprotein [89]. The S1 subunit of the S protein facilitates receptor binding [90], whereas the S2 subunit is responsible for driving viral and target cell membrane fusion [91].

There are various approaches in creating peptide-based therapeutics, including peptides that interrupt RBD interaction, peptides that block the HR1 and HR2 interaction from forming a fusion-active core, and peptides that interfere with the cleavage of the S protein [92]. Strategies have been employed to inhibit fusion of the S protein by either deriving peptides from HR1 and HR2 regions of the S, or by deriving peptides from an antimicrobial peptide. Note that peptides derived from either S protein or antimicrobial peptides, are both antiviral compounds. The only difference is that peptides derived from antimicrobial peptides can be broad spectrum, may have other roles such as immunomodulatory activity [93] and is derived from other organism other than virus. These are described as follows:

(a) Peptides derived from HR1, HR2 and RBD subunits of the spike protein

Fusion inhibiting peptides derived from the S protein are shown in Table 2 (A). For example, Lu et al. designed two synthetic peptides, HR1P and HR2P that have amino acid residues spanning 998-1038 in HR1 and 1251-1286 in HR2 domains, which form a stable six-helical bundle (6-HB) fusion core structure [94]. This 6-HB structure ensures the fusion of the viral envelope and the host cell membrane [95]. Of the two peptides, HR2P inhibits both MERS-CoV replication and its spike protein-mediated cell-cell fusion [94]. A previous study showed the derivation of five regions that span the length of the S2 subunits of SARS-CoV and murine hepatitis virus (MHV). In this study, three peptides, $SARS_{WW-III}$, $SARS_{WW-IV}$ and MHV_{WW-IV} exhibited antiviral activity greater than the other peptides studied, with inhibitory concentration (IC_{50}) values ranging from 2–4 μM [96].

(b) Peptides derived from antimicrobial peptides

Peptides can be derived from antimicrobial peptides by either truncating the fragments of the original peptide or replacing a certain residue of an AMP with another residue, for instance replacement of lysine with arginine. These derived peptides have shown activities against various micro-organisms [97,98]. For instance, the antiviral activity of 11 peptides derived from mouse β-defensin-4 was tested and one of them, P9, exhibited potent and broad spectrum antiviral effects against multiple respiratory viruses in vitro and in vivo, including influenza A virus H1N1, H3N2, H5N1, H7N7, H7N9, SARS-CoV and MERS-CoV [99].

Peptides inhibiting viral entry and replication

The entry and replication process of coronaviruses in the infected host commences by the S glycoprotein located on the surface of the coronavirus virion fusing with receptor ACE2 or DPP4 to facilitate viral entry into the host. A chain of events leads to the release of viral genomic RNA into the host cytoplasm, replication of the viral RNA, and final release of viral particles [18,100]. Several studies have been employed to inhibit viral entry and replication, which are shown in Table 2(B) and (C). These peptides can be classified into major subgroups:

(a) Peptides derived from HR1 and HR2 subunits of the spike protein

The HR2P peptide, was derived from the HR2 domain. HR2P has shown activity against MERS-CoV by inhibiting viral replication in calu-3 and HFL cells [94].

(b) Peptides derived from antimicrobial peptides

Mutant mucropin-M1 is a peptide derived from mucropin AMP. The glycine and proline residues of mucropin were substituted with

lysine and arginine respectively. Mutant mucropin-M1 has shown activity against SARS-CoV, by inhibiting viral replication [101].

(c) Peptides derived from non-structural protein (nsp)

Two peptides (K12 and K29) were derived from the non-structural protein nsp10 of SARS-CoV. These two peptides inhibit the replication of SARS-CoV with an inhibitory concentration of 160 μM [102].

Peptides inhibiting assembly and release of virus

Though much is not known about the assembly and release process or enzymes involved in assembly or release process, inhibition of assembly and release of virus, could be a good future target [103].

Other examples of anti-coronavirus peptides against SARS-CoV, TGEV and MHV are provided in an antiviral peptide database (AVPdb) [82]. This database consists of various anti-coronavirus peptides with numerous mechanisms of action against SARS-COV, TGEV and MHV and can be employed as potential therapeutic options for MERS-CoV because coronaviruses share a similar structure and mechanism of action [39].

Our understanding of peptide research suggests that peptides may have the capacity to be lead molecules as potential drugs against MERS. The advantage of peptide repurposing is that, it will lead to the identification of peptide-based therapeutics for the treatment of MERS, with potentially wider utility of therapeutics use for the treatment of infections caused by the different human coronaviruses. Furthermore, this can help to maintain the quality and reduce the cost of new medicine development.

Conclusion

There is an urgent need for developing the most effective MERS therapy and developing protocols to be used in randomized-controlled trials. Studies have shown that peptides have evolved as highly potent signal transduction agents for viral diseases. We suggest that AMPs can be a successful therapeutic option for emerging MERS pathogen. Computational biology coupled with virology, can accelerate the most advanced design by developing peptide therapeutics against MERS-CoV with greater efficacy. In addition, our study suggests that the repurposing of existing and clinically approved anti-coronavirus and anti-viral peptide drugs may be used as promising leads for the development of new anti-MERS-CoV agents.

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Conflict of interest

None declared.

Ethical approval

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