Expert Review of Anti-infective Therapy

ISSN: 1478-7210 (Print) 1744-8336 (Online) Journal homepage: http://www.tandfonline.com/loi/ierz20

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To cite this article: Jaffar A. Al-Tawfiq & Ziad A. Memish (2016): Update on therapeutic options for Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Expert Review of Anti-infective Therapy

To link to this article: http://dx.doi.org/10.1080/14787210.2017.1271712

Accepted author version posted online: 11 Dec 2016.

Publisher: Taylor & Francis

Journal: *Expert Review of Anti-infective Therapy*

DOI: 10.1080/14787210.2017.1271712

Update on therapeutic options for Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

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Abstract

Introduction: The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is an important emerging respiratory pathogen. MERS-CoV resulted in multiple hospital outbreaks within and outside the Arabian Peninsula. The disease has a high case fatality rate, with the need for a therapeutic option.

Areas covered: In this review, we provide an overview of the progress in the development of therapeutic strategies for MERS. We searched PubMed, Embase, Cochrane, Scopus, and Google Scholar, using the following terms: 'MERS', 'MERS-CoV', 'Middle East respiratory syndrome' in combination with 'treatment' or 'therapy'.

Expert commentary: There are multiple agents tried *in vitro* and *in vivo*. None of these agents were used in large clinical studies. Available clinical studies are limited to the use of the combination of interferon and other agents. These clinical studies are based solely on case reports and case series. There are no prospective or randomized trials. There is a need to have prospective and randomized clinical trials for the therapy of MERS-CoV. However, this strategy might be hampered by the sporadic cases outside the large hospital outbreaks.

Key words:

MERS-CoV, interferon, ribavirin, pegelated interferon, therapy

1. Introduction:

The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) emerged as an important virus in 2012 and since then has caused multiple outbreaks in hospitals especially in the Kingdom of Saudi Arabia and outside the Arabian Peninsula [1–3]. Since the emergence of MERS-CoV, a total of 1800 cases including 640 deaths were reported by the World Health Organization (WHO) [4]. Due to the increased morbidity and mortality of MERS-CoV infection, the attention was directed towards the development of prevention strategies and the establishment of therapeutic modalities. An earlier review was based on the SARS experience and had suggested few possible options for the treatment of MERS-CoV infection [5]. In this review, we provide an overview of the progress in the development of therapeutic strategies for MERS.

2. Search Strategy and Classification of Reviewed Articles:

We searched PubMed, Embase, Cochrane, Scopus, and Google Scholar using the following terms: 'MERS', 'MERS-CoV', 'Middle East respiratory syndrome' in combination with 'treatment' or 'therapy'. We also reviewed the references of each article to further include other studies or reports not identified by the search. We classified the studies into the following categories: *in vivo* and *in vitro* studies, animal studies and human case reports or case series. For clinical studies, we graded the level of the evidence based on the "Oxford Centre for Evidence-based Medicine"[6].

3. *In vivo* and *In vitro* Studies:

In vitro studies showed variable activity of various agents against MERS-CoV (Table 1). These agents include: interferon, ribavirin, HIV protease inhibitors (nelfinavir, ritonavir and lopinavir). Interferon is antiviral type I IFN system, a major part of the innate immune response [7,8]. *In vitro* studies showed that IFN-β has an IC_{50} of 1.37 U/ml and that IFN-β has anti-MERS-CoV activity of 16-, 41-, 83- and 117-fold higher than IFN-α2b, IFN-γ, IFN-universal type 1 and IFN-α2a, respectively [9]. *In vitro* studies showed that IFN- β has a lower IC50 for MERS-CoV compared to IFN-a2b [9].

Ribavirin is a nucleoside analog that is activated by host kinases to a nucleotide [7,10,11]. It was shown that *in vitro* doses of ribavirin required to inhibit MERS-CoV replications are too high to be achieved *in vivo* [7,10]. Nelfinavir and lopinavir inhibit MERS-CoV *in vitro* [7,12]. The mean 50% effective concentration (EC50) of lopinavir using Vero E6 and Huh7 cells was 8.0 μM [13].

Camostat and the heptad repeat 2 peptide (HR2P) are two MERS-CoV fusion inhibitors that were tested *in vitro* [14,15]. The fusion inhibitor, Camostat, inhibited viral entry into human bronchial submucosal gland-derived Calu-3 cells but not the immature lung tissue [14]. The second fusion inhibitor, HR2P, inhibits MERS-CoV replication and the spike protein-mediated cell-cell fusion [15].

Cyclosporin affects the function of many cyclophilins that act as chaperones and facilitate protein folding [16,17]. *In vitro*, cyclosporine inhibited MERS-CoV replication [16,17].

Nitazoxanide, a broad-spectrum antiviral agent, and teicoplanin, an inhibitor of Cathepsin L in the Late Endosome/Lysosome and blocker of the entry of MERS-CoV, also showed inhibitory effect of MERS-CoV *in vitro* [18,19].

4. Animal Model and the use of anti-MERS-CoV agents:

There are few studies evaluating various agents as therapy for MERS-CoV in animal models (Table 2) [12,20–23]. In the rhesus macaques model, interferon-α2b-ribavirin combination decreased viral replication within 8 hours of MERS-CoV infection [24]. In a primate model, the mortality rate at 36 hours post-inoculation was reduced from 67% in untreated to 0-33% in animals treated with a combination of interferon-β1b and either lopinavir or ritonavir [12]. Intranasal use of an HR2P analogue with improved pharmaceutical property, HR2P-M2, was protective in mice model [20]. In an animal model using MERS-CoV infected mice, the use of high titer MERS immune camel serum was effective in reducing lung injury and acceleration of virus clearance [21]. Mycophenolate has a direct and indirect antiviral activity by modulation of IFN response [25]. The use of mycophenolate in the common marmoset animal model resulted in higher mortality than untreated animals [12]. A monoclonal antibody designated as m336 is an antibody derived from a large phage-displayed antibody library from B cells of healthy donors [26]. The use of this m336 in mice showed promising results as a therapeutic and a prophylactic agent [22].

Currently, there is no animal model that completely reflects the course of MERS-CoV disease in humans and thus the data obtained from these animal models are to be interpreted cautiously. And animal models utilize therapy shortly after infection.

5. Clinical use of combination therapy of ribavirin-interferon in MERS-CoV Patients:

Based on analysis of SARS data, interferon-ribavirin combination was suggested as a possible therapeutic option for the treatment of MERS-CoV infections [5]. Limited data are available

regarding the clinical efficacy of anti-viral agents [27–36], (Table 3). The first use of the combination of ribavirin-interferon therapy was in five patients with MERS infection [27]. The therapy was started late in the course of the disease with a median time from admission to therapy of 19 days [27]. Of the included 5 patients, none responded to therapy [27].

In a subsequent retrospective cohort study, 20 MERS patients received ribavirin-interferon compared to 24 patients who did not [28]. The 14-day survival rate was better in those who received the combination therapy (70% vs. 29%, p = 0.004), however, the 28-day survival rate was not statistically different (30% vs. 17%) (table 1) [28]. In another case series, 11 MERS patients had ribavirin and peginterferon α-2a [29].

Ribavirin-IFN-α2a was compared to ribavirin-IFN-β1a in a study of 13 and 11 patients, respectively [30]. The mortality rate was not statistically different between the two groups (85% vs. 64%) [30]. In a large cohort study of 51 patients, various combinations of interferon and ribavirin were used with different outcomes (table 3) [31]. In a case series of 6 patients, 3 patients received ribavirin and interferon-alfa 2b within 1-2 days of admission and they survived compared to the other 3 patients who died as they received the therapy 12-19 days after admission [36]. Another study evaluated the use of interferon beta, interferon alpha, or ribavirin and showed survival rates of 18/23 (78.3%), 6/8 (75%), and 13/19 (68.4%), respectively (table 3) [31]. The combination therapy was also used in other case reports, (table 3) [33,34].

The role of the combination of ribavirin and IFN was also tried as a treatment and a prophylaxis [34]. The current studies of the use of ribavirin and IFN combination therapy for MERS-CoV infection rely on small number of patients but there is a trend for improvement. Thus, it was suggested that the combination of type 1 interferon and ribavirin could be used [37]. Due to the

inhomogeneous nature of available studies and the limited data that are available, a precise recommendation on therapy of MERS could not be established.

6. Other combinations (lopinavir/ritonavir, ribavirin and interferon):

The combination of lopinavir/ritonavir, ribavirin and interferon-alpha was used in one case [32]. One patient received pegylated interferon, ribavirin and lopinavir/ritonavir from day 13 of illness and the patient had continued MERS-CoV in the respiratory tract secretions until the fourth week of illness [33]. However, viremia was detected for only two days after initiation of triple therapy [33]. In a case series, eight patients received mycophenolate mofetil and all survived [31].

7. Neutralizing antibodies:

In the SARS epidemic, passive immunotherapy with neutralizing antibodies was considered as a therapeutic approach. There are multiple antibodies against MERS-CoV [38–48], (table 4). In the MERS-CoV infection, the production of large quantities of MERS-CoV neutralizing human polyclonal antibodies was possible using gamma-irradiated whole killed virion vaccine or a spike protein nanoparticle vaccine in a bovine model [49]. Utilizing one dose of these antibodies prevented infection in mice [49]. These antibodies were effective when given 12 hours before or 24 and 48 hours after MERS-CoV infection [49].

Corti et al isolated a potent MERS-CoV–neutralizing antibody (LCA60) from memory B cells of an infected individual. The LCA60 antibodies bind to a site on the spike protein and neutralize MERS-CoV infection [48]. These LCA60 antibodies were used successfully in mice model [48]. Similarly, utilizing a humanized mouse model of MERS-CoV infection, antibodies against the spike protein were efficacious as prophylaxis [39]. Antibodies obtained from the sera of MERS immune camels were supportive of the clearance of the virus, and reduction of the

severity of the disease in MERS-CoV-infected mice [21]. However, the purification and safety of these antibodies in humans has not been established yet.

The cellular dipeptidyl peptidase IV (DDP IV; known as CD26 or adenosine deaminase (ADA)-complexing protein-2) is an important receptor that mediates MERS-CoV infection through the viral spike (S) protein [46,50]. The MERS-CoV receptor binding domain (RBD), present on the surface spike protein (S), binds to the host cells receptor DPP IV [46,50,51]. In humans, DPP IV is present mainly on the lower respiratory tract area such as the bronchial epithelial and alveolar cells [52,53]. Although DDP IV is important for the viral entry into host cells, the use of DPP IV inhibitors, sitagliptin, vildagliptin and saxagliptin, does not block the infection of MERS-CoV [50]. *In vitro* use of monoclonal antibodies (MERS-4) exhibited IC50 of 0.056 μg/mL [43]. Other possible human mAb (m336, m337 and m338) neutralize pseudovirus and live virus [45]. In rhesus model of MERS-CoV infection, a human monoclonal antibody, 3B11-N, against MERS-CoV was effective in reducing the pathology of MERS-CoV [47]. The use of polyclonal antibody (pAb) against CD26 inhibits MERS-CoV infection *in vitro* [50]. Humanized anti-CD26 monoclonal antibodies (MAb) such as MAb YS110 and 2F9 significantly inhibit MERS-COV infection *in vitro* [38]. Polyclonal antibodies against the MERS-COV S1 domain neutralize the virus infection [46]. Many other MERS-CoV antibodies are being developed and tested [38].

8. Convalescent plasma:

In the SARS epidemic, convalescent plasma was thought to improve the outcome of SARS patients [5]. Previous studies suggest that convalescent plasma may be used for patients with SARS and severe influenza and may result in decreased viral load and a lower mortality rate

[54–57]. However, most of the studies were of low or very low quality, lacked control groups, and had risk of bias [58]. Two patients with MERS-CoV infection received intravenous immunoglobulin in an attempt to treat the infection, one patient was in Saudi Arabia [59]and the other was in the United States of America [60]. A protocol for the use of convalescent plasma as a therapeutic option for MERS was suggested [61]. Plasma donors were identified as those with anti-MERS-CoV indirect immunofluorescence assay (IFA) antibodies (titer of ≥1:160) with no evidence of active MERS-CoV infection [61]. In nine confirmed survivors of MERS-CoV infection, 55%, 33%, and 22% of them had positive MERS antibodies by IFA at 3, 10, and 18 months respectively [62]. The two patients who had long lasting antibodies had severe disease, however, the titre of the IFA antibodies was not measured in the study [62]. In a larger study, MERS-CoV neutralizing antibodies were produced at low levels and were short-lived [63]. Further studies of the kinetics of the MERS-CoV antibodies showed that all surviving patients and 50% of fatal cases produced IgG and neutralizing antibodies [64]. The presence of antibodies did not lead to the elimination of virus from the lower respiratory tract [64]. In a study of 12 patients from South Korea, nine patients had PRNT50 titers >1:320 by day 21 and two had titers >1:320 by day 28 [65]. In a study of 443 samples, 12 (2.7%) had reactive ELISA results, and 9 of those had reactive indirect fluorescent antibody and microneutralization assay titers [66]. Thus, the use of convalescent plasma for the treatment of MERS-CoV in a clinical trial may be challenging due to a small pool of potential donors with sufficient antibody titers [66].

9. Glucocorticoid:

Based on SARS experience, some authors suggested that steroid therapy might be beneficial for severe MERS-COV infection [67]. Corticosteroid use for patients with SARS showed that early use of corticosteroids significantly increased viral load, and 20.7-fold increase in risk of ICU ICU admission and mortality [68]. In another study of 16 non-ICU patients, the median time for SARS-CoV to become undetectable in plasma was 12 days compared to 8 days in patients who did and did not receive early corticosteroid therapy [69].

Corticosteroids were used as adjunct therapy for many patients with MERS-CoV [27,70]. In a study of 13 patients with MERS-CoV infection, one patient received steroid and intravenous immunoglobulin for thrombocytopenia [59]. Initial study of five patients, three patients received a combination of interferon and ribavirin in addition to adjunct steroid on day 0-21 and all died during hospitalization [27]. However, steroids were not evaluated systematically as therapy for MERS patients.

10. Other Therapeutic Drugs:

The host protease, furin, is an important factor to breakdown the S1-S2 region of the MERS-CoV [71]. *In vivo* studies showed activity of multiple agents against MERS-CoV and include: chloroquine, chlorpromazine, cyclosporine, and mycophenolic acid [7,72]. In addition, the US FDA approved repurposed agents with broad antiviral activity including *in vitro* activity against MERS-CoV [73]. These agents include the polymerase inhibitor BCX4430 and the helicase inhibitor SSYA10-001, spike binding (immunoadhesin (DPP4-Fc)) [73]. There are many other agents currently in pre-clinical investigation such as: fluspirilene, thiothixene, fluphenazine hydrochloride, promethazine hydrochloride, astemizole and chlorphenoxamine hydrochloride [72].

11. Expert Commentary:

The emergence and continued cases of MERS-CoV infection require the availability of MERS therapy. There is an urgent need for the development of standardized animal models and the establishment of standardized clinical therapeutic protocols. The current clincial studies are limited to case reports and case-series with no control arm. The quality of evidence these studies offer is too low to make a conclusion. It is difficult to draw conclusion in the face of these limited studies. The most used combination of therpay was interferon and ribavarin as developed initally in 2013. The devlopment of novel therapeutic agents or the repurposing old therapeutic agents against MERS-CoV are needed as alternative pathways for testing and clinical trials. It was thought that convalescent sera may provide an exciting alternative as a therapeutic agent, the present data does not support the wide adaptation of this therapy. The current MERS therapy relies on supportive care and providing circulatory and ventilation support.

12. Five-year view:

It is expected that the development and the use of repurposed drugs would allow the development of therapeutic agents for MERS-CoV. The best location to provide a randomized controlled trail was thought to be the intensive care units for the use of convalescent sera, the presence of milder disease may necessitate the development of therapeutic protocols for patients with severe and those with milder disease. Monoclonal and polyclonal antibodies may offer further therapeutic options for the disease in humans. Since the prospect for a randomized clinical trial is low due to the sporadic nature of the disease outside hospital outbreaks, it is prudent to have well conducted prospective clinical studies. The proteins involved in MERS-CoV entry and replication are attractive targets for the development of anti-viral therapeutics.

Clinical studies utilizing anti-MERS-CoV antibodies as therapeutic options would add to the prospect to develop therapeutic agents for this syndrome. Although, many drugs appear to be effective in vitro, a consideration of their availability, pharmacokinetic/pharmacodynamic properties and side effects should be taken into consideration. Of medications with an attractive use, lopinavir, interferon, and mycophenolate are among these agents. Accelerated and preferably randomized controlled trails should be conducted. Neutralizing antibodies are also promising and the use of these agents in humans. Targeting the DDP4 receptor may be of particular importance, however, it is important to keep in mind that the development of any mutation in the binding sites may limit the use of these agents [74]. Few monoclonal antibodies showed protective efficacy as a prophylaxis in animal models [39,48]. The development and testing of monoclonal antibodies are associated with high costs and lack of an undefined population for their use [75]. These monoclonal bodies had not been used in phase 1 clinical trials and that further development of these agents require time and cost.

13. Key issues:

- MERS-CoV emerged in 2012 and has caused multiple hospital outbreaks.
- Currently, there are no licensed therapeutic agents for MERS-CoV infection.
- Multiple monoclonal and polyclonal antibodies were developed and may offer therapeutic options.
- Repurposing old drugs against MERS-CoV is an interesting strategy that deserves further development and use in clinical settings.

- Current clinical data are limited to case reports and case series in the use of combination antiviral medications such as ribavirin and interferon.
- The combination of ribavirin and interferon may offer a survival advantage at 14 days but not at 28 days.
- Animal models are promising in further delineating the disease and the therapeutic options.
- Further studies should include in vitro mechanism studies, enhancing animal models of MERS-CoV infection, clinical trials, and evaluation of combination therapy.

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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* of special interest

** of outstanding interest

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Table 1: A Summary of Anti-MERS-CoV Agents Used *in vitro* and the mechanism of Action

Molecule	Mechanism of Action	Reference
Interferon (IFN)	antiviral type I IFN system, a major part of the innate immune response	[7–9],
Ribavirin	A nucleoside analog that is activated by host kinases to a nucleotide	[7,10,11]
Nelfinavir	Protease inhibitor	[7,12] [13]
Lopinavir	Protease inhibitor	[7,12] [13]
Camostat	Fusion inhibitor	[14]
Heptad repeat 2 peptide (HR2P)	Fusion inhibitor	[15]
Cyclosporine	Affects the function of many cyclophilins that act as chaperones and facilitate protein folding	[16,17]
Nitazoxanide	broad-spectrum antiviral agent	[18,19]
Teicoplanin	Inhibits Cathepsin L in the Late Endosome/Lysosome	[18,19]

	and Block the Entry of MERS-CoV	
Mycophenolate	direct and indirect antiviral activity by modulation of IFN response	[25]

Table 2: A Summary of Animal Model of Therapeutic Agents against MERS-CoV infection

Number	Animal Model	Treatment	Outcome	Reference
1	Primate	Interferon-β1b and either lopinavir or ritonavir	Mortality rate at 36 hours post-inoculation was reduced from 67% in untreated to 0-33% in animals treated with a combination of	[12]
2	Mice	HR2P analogue	>1000-fold reduction of viral titers in lung	[20]
3	Mice	High titer MERS immune camel serum	Increase the kinetics of MERS-cov clearance and decrease severity of pathological changes	[21]
4	Primate	Mycophenolate	Mortality rate was 67% (untreated and MMF-treated) at 36 hours postinoculation vs. 0-33% (lopinavir/ritonavir-treated and interferon-β1b-treated).	[12]
5	Transgenic Mice	Germline-like Neutralizing Human Monoclonal Antibody	Treated mice prior to or post lethal MERS-cov challenge were fully protected	[22]
6	Mouse	Humanized monoclonal	Single-dose completely protected transgenic mice from	[23]

model	antibody, hms-1, against RBD	lethal MERS-cov	

Table 3: Clinical Experience with Anti-Viral Therapy for MERS-CoV Infection

Number	Study Type	Treatment	time to initiation of therapy	Treatment group, n/N (% survival)	Control group	Level of Evidence	Reference
1	case series	ribavirin and interferon-alfa 2b	19 days post-admission	0/5 (0)	None	4	[27]
2	retrospective cohort study	ribavirin and interferon-alfa 2a	3 days of onset	14/20 (70 at 14 days); 6/20 (30 at 28 days)	24; survival at 14 days 29% and 17% at 28 days	4	[28]
3	case series	ribavirin and	6 days of onset	11/11 (100)	None	4	[29]

		interferon-alfa 2a					
4	case series	ribavirin and interferon-alfa 2a	1 day following diagnosis	11/13 (85)	None	4	[30]
5	case series	ribavirin and interferon-b1a	1 day following diagnosis	7/11 (64)	None	4	[30]
6	case series	Interferon beta	Not indicated	18/23 (78.3)	None	4	[31]
7	case series	Interferon alpha	Not indicated	6/8 (75)	None	4	[31]
8	case series	Ribavirin	Not indicated	13/19 (68.4)	None	4	[31]
9	case series	Mycophenolate mofetil	Not indicated	8/8 (100)	None	4	[31]
10	case report	lopinavir/ritonavir, ribavirin and interferon-α	Not indicated	none	4	4	[32]
11	case report	pegylated interferon, ribavirin and lopinavir/ritonavir	from Day 13 of illness	?	None	4	[33]

12	case report	ribavirin and interferon-alfa 2a	day 1 of admission	survived	None	4	[34]
13	case report	ribavirin and interferon-alfa 2a	day 12 from onset	died	None	4	[35]
14	case series	ribavirin and interferon-alfa 2b	1-2 days in survivals and 12-19 days in those who died	3/6 (50)	None	4	[36]

Table 4: A Summary of Generated anti-MERS-CoV Antibodies

Number	**Antibody**	**Reference**
1	Anti-CD26 monoclonal antibody	[38]
2	Anti–MERS-cov Spike Protein Antibodies	[39]
3	Recombinant receptor-binding domain of S spike	[40]
4	Receptor-binding domain in S spike protein	[41]
5	Receptor-binding domain in S spike protein	[42]
6	Receptor-binding domain in S spike protein	[43]
7	Receptor-binding domain in S spike protein	[44]
8	Receptor-binding domain in S spike protein	[45]
9	S spike protein	[46]
10	Human neutralizing antibodies	[44]
11	Human monoclonal antibodies against CD26/DPP4 binding domain	[45]
12	Human RBD-specific neutralizing monoclonal antibodies	[43]
13	A human anti-MERS monoclonal antibody 3B11-N	[47]
14	S spike protein	[48]