Future treatment strategies for novel Middle East respiratory syndrome coronavirus infection

“Given the similarity of Middle East respiratory syndrome coronavirus with severe acute respiratory syndrome coronavirus, what lessons can we learn from past work on severe acute respiratory syndrome coronavirus, or other positive strand RNA viruses?”

Keywords: cathepsin L inhibitors • coronavirus • drug resistance • inhibitors • interferons • Middle East respiratory syndrome • pseudotyped virus • ribavirin • severe acute respiratory syndrome • small molecule

More than a year ago, a novel coronavirus (CoV), the Middle East respiratory syndrome CoV (MERS-CoV), was first identified as the causative agent of a severe pneumonia in several patients in the Middle East [1]. As of 5 August, 2013, a total of 94 cases have been identified in the Middle East (Saudi Arabia, Jordan, Qatar and the United Arab Emirates), Europe (the UK, France, Italy and Germany) and North Africa (Tunisia), with a case fatality rate of 56%. Since this CoV is closely related to severe acute respiratory syndrome CoV (SARS-CoV), an epidemic that was short-lived but alarming in 2002–2003 that resulted in approximately 8000 cases and 800 deaths, several health agencies that include governmental, public health, clinical and laboratory authorities have all moved rapidly to respond to this new virus outbreak. Following identification of MERS-CoV, details about its genomic sequence and organization, species tropism and host-cell receptor (dipeptidyl peptidase 4) have been reported [2,3].

Although there have been several reports of inhibitors of SARS-CoV, to date, there are no approved drugs or vaccines for the treatment of SARS-CoV. Studies have shown that ribavirin has a very minimal effect in controlling adverse outcomes from SARS-CoV infection. Its use has attracted a lot of criticism due to its unproven and low efficacy and unwarranted side effects [4]. At nontoxic concentrations, ribavirin has no direct in vitro activity against SARS-CoV [5]. Among some of the promising agents for the control of SARS-CoV infection are interferons (IFNs). A large number of recombinant IFNs that belong to the three classes (α, β and γ) have been tested for their antiviral activities against SARS-CoV in vitro.

IFN-α and -β were shown to be active against SARS-CoV, with IFN-β being the most active of the three [6].

More recently, Falzarano et al. proposed a potential therapeutic regimen for the treatment of MERS-CoV based on cell-based experiments [7]. In this report, the authors compared the use of both IFN-α2b and ribavirin individually or in combination for the inhibition of MERS-CoV in two different cell lines, Vero and LLC-MK2. When used individually, to completely eliminate the cytopathic effect of SARS-CoV infection, each drug required concentrations as high as 1000 U/ml IFN-α2b (IFN-α2b) and 200 μg/ml ribavirin. However, when the two drugs were combined they acted synergistically, and only 125 U/ml IFN-α2b and 25 μg/ml ribavirin were required to completely eliminate the cytopathic effect. This represented an eight- and 16-fold decrease in the amount of IFN-α2b and ribavirin respectively, which is required to attain the same reduction when used individually.

While the combination therapy portends to be a promising strategy, these concentrations are still very much higher than the current doses at which these two drugs are being used for hepatitis C virus (HCV) infections in humans [8]. High doses of ribavirin have been associated with hemolytic anemia, elevated transaminase levels and bradycardia [9]. Despite the lower doses of these drugs for the treatment of HCV, many side effects accompany this combination therapy, which include depression, suicide, relapse of drug abuse/overdose, bacterial infections and many others [10]. Nonetheless, given the severity of this disease and the lack of any available treatments, this is a promising finding that should be further explored.
Given the similarity of MERS-CoV with SARS-CoV, what lessons can we learn from past work on SARS-CoV, or other positive strand RNA viruses? MERS-CoV was recently shown to use dipeptidyl peptidase 4 as its functional receptor to gain entry into cells [3]. While this virus is utilizing a receptor different than the one used by SARS and other respiratory coronaviruses, there appears to be some similarities in their mechanisms of entry. MERS-CoV has been shown to require cathepsins B and L for entry, similar to SARS-CoV [10]. Cathepsin L inhibitors have been shown to prevent SARS-CoV entry in SARS-CoV-pseudotyped assays (SARS-CoVenv/HIV core) [11]. Published cathepsin L inhibitors include oxocarbazate, MDL28170 and SSA09E1. MDL28170 and oxocarbazate inhibit SARS-CoV pseudotyped virus with EC50 values of approximately 100 and 300 nM, respectively [12,13]. These two compounds appear to be peptidomimetic in nature, thereby limiting their use only to parenteral administration if they were to be considered for therapeutic purposes. SSA09E1, a non-peptidomimetic small molecule that inhibited SARS-CoV-pseudotyped virus with an EC50 of approximately 7 µM can also inhibit live SARS-CoV in Vero cells [Adedeji AO, Sarafianos SG, Unpublished Data]. Therefore, any of these cathepsin L inhibitors would be worth testing for inhibition of MERS-CoV as well as other coronaviruses that require cathepsin L for entry.

In thinking about treatments of viral infections, one has to also consider the challenge of drug resistance, which is the result of the ability of viruses to rapidly evolve and escape treatments through multiple and diverse mechanisms of resistance. There is an extensive body of clinical data on development of resistance against antivirals that are used in the clinic for the treatment of HIV, hepatitis B virus, and HCV infections. For HIV, remarkable progress has been made with highly active antiretroviral therapies, which are based on combinations of drugs that almost exclusively target directly the virus. Highly active antiretroviral therapies regimens significantly suppress the viral load in the blood plasma of HIV patients, resulting in significant decreases in HIV-related deaths. However, prolonged treatment inevitably results in resistance to all of these available drugs [14–16]. Similar benefits have been reported in the treatment of hepatitis B virus-infected patients with antiretrovirals.

For many years, HCV has been treated with a combination therapy of Pegylated IFN and ribavirin. Clinical trials have shown sustained virological response rates of approximately 50% (efficacy), which does not even provide equivalent results in clinical practice [17]. HCV has also evolved various mechanisms to develop resistance to these therapies. For example, HCV circumvents the IFN response by blocking downstream signaling actions via STAT1, STAT2, IRF9 and JAK-STAT pathways [18,19]. Hence, MERS-CoV can also develop resistance to IFN treatment.

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Ribavirin is a nucleoside analog that after activation by host kinases to a nucleotide, it can be used by viral polymerases and incorporated into the nascent viral genomes, causing mutations by allowing base mismatches. During the amplification of HCV replicons, error frequency is known to increase upon ribavirin treatment. Hence, ribavirin’s antiviral activity is likely to result from error catastrophe caused by increased mutagenesis of viral genomes thereby reducing the fitness of the virus. Another mechanism through which ribavirin may exhibit its antiviral activity is by depletion of intracellular GTP pools (by inhibition of the cellular IMPDH by the 5’-monophosphate metabolite of ribavirin) [20]. This mechanism may be useful in explaining the drug’s general cytotoxic and anti-DNA replication effect as well as some effect on DNA viral replication.

While reports of viral resistance to ribavirin are rare, its reliance on host factors to be able to convert to its mono- and tri-phosphate active forms stands as a potential problem. Falzarano et al. showed the difference in the efficacies of ribavirin depending on the cell types, as ribavirin was more effective in LLC-MK2 cells than the Vero cells [7]. Since MERS-CoV naturally infects respiratory epithelial cells, it will be more expedient to monitor the effect of ribavirin in respiratory epithelial cells to see if comparable viral inhibition will be observed.

With limited efficacy of this therapy, coupled with multiple side effects and resistance...
In conclusion, the recent report that IFN-α2b and ribavirin have some synergistic activity in cell-based assays is indeed encouraging, as these drugs are currently used to treat hepatitis C and are therefore widely available even though they are imperfect but with known pharmacological properties. Moreover, the study highlights how past research in related viruses can provide useful insights in the treatment of emerging diseases.

**Financial & competing interests disclosure**

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