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Future Treatment Strategies for Novel Middle East Respiratory Syndrome Coronavirus Infection

Adeyemi O. Adedeji^{1,*} and Stefan G. Sarafianos²

¹Veterinary Medical Teaching Hospital, School of Veterinary Medicine University of California, Davis, CA 95616, USA

²Christopher Bond Life Sciences Center, Department of Molecular Microbiology & Immunology, Department of Biochemistry, University of Missouri School of Medicine, Columbia, MO 65211, USA

More than a year ago, a novel coronavirus, the Middle East respiratory syndrome coronavirus (MERS-CoV), was first identified as the causative agent of a severe pneumonia in several patients in the Middle East [1]. As of August 5, 2013, a total of 94 cases have been identified in the Middle East (Saudi Arabia, Jordan, Qatar, and the United Arab Emirates), Europe (United Kingdom, France, Italy, and Germany), and North Africa (Tunisia), with a case fatality rate of 56%. Since this coronavirus is closely related to severe acute respiratory syndrome coronavirus (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 non-toxic 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. A large number of recombinant interferons that belong to the three classes (α , β and γ) have been tested for their antiviral activities against SARS-CoV *in vitro*. Interferon- α and - β were shown to be active against SARS-CoV, with interferon- β being the most active of the three [6].

More recently, Falzarano et al [7] proposed a potential therapeutic regimen for the treatment of MERS-CoV based on cell-based experiments. In this report, the authors compared the use of both interferon- α 2b 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 (CPE) of SARS-CoV infection, each drug

*Correspondence: Adeyemi O. Adedeji aoadedeji@ucdavis.edu.

required concentrations as high as 1000 U/ml interferon- α 2b (IFN- α 2b) and 200 μ g/ml ribavirin. However, when the two drugs were combined they acted synergistically, and only 125 U/ml IFN- α 2b and 25 μ g/ml ribavirin were required to completely eliminate the CPE. This represented an 8- and 16-fold decrease in the amount of IFN- α 2b 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 (<http://www.hepatitis.va.gov/provider/reviews/treatment-side-effects.asp#S1X>). 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-CoV env / HIV core) [11]. Published cathepsin L inhibitors include oxocarbazate, MDL28170 and SSAA09E1. MDL28170 and oxocarbazate inhibit SARS-CoV-pseudotyped virus with EC_{50} s of \sim 100 nM and \sim 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. SSAA09E1, a non-peptidomimetic small molecule that inhibited SARS-CoV-pseudotyped virus with an EC_{50} of \sim 7 μ M can also inhibit live SARS-CoV in Vero cells (unpublished data). Therefore, any of these cathepsin L inhibitors would be worth testing for inhibition of MERS-CoV as well as of 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 Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV) infections. For HIV, remarkable progress has been made with highly active anti-retroviral therapies (HAART), which are based on combinations of drugs that almost exclusively target directly the virus. HAART 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 HBV-infected patients with antiretrovirals.

For many years, HCV has been treated with a combination therapy of pegylated interferon and ribavirin. Clinical trials have shown sustained virological response rates (SVRs) of ~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 coronavirus can also develop resistance to IFN treatment.

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 hepatitis C 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 Inosine monophosphate dehydrogenase [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. Darryl Falzarano et al [7] 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. Since, MERS-CoV naturally infects respiratory epithelial cell, 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 development, many scientists have been working on IFN-free therapy that involves development and the use of small molecule compounds that directly inhibit the viral replication proteins. The efficacies of these compounds are being tested alone or in combination with ribavirin or pegylated IFNs. These compounds are referred to as "Direct Acting Antivirals". Examples include the recently approved boceprevir and telaprevir NS3/4A protease inhibitors, as well as NS5A inhibitors and NS5B polymerase inhibitors [21] that are in clinical trials. For coronaviruses, several reports of SARS-CoV small molecule compounds that inhibit the viral replication proteins, or prevent entry of the virus into cells, are well documented [11,12,22,23]. All these compounds, with known mechanisms of action, introduce potential leads for the development of MERS and other coronaviruses therapeutics.

In conclusion, the recent report that interferon- α 2b 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.

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