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Broad-spectrum antivirals for the emerging Middle East respiratory syndrome coronavirus

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Summary *Objectives:* Middle East respiratory syndrome coronavirus (MERS-CoV) has emerged to cause fatal infections in patients in the Middle East and traveler-associated secondary cases in Europe and Africa. Person-to-person transmission is evident in outbreaks involving household and hospital contacts. Effective antivirals are urgently needed.
Methods: We used small compound-based forward chemical genetics to screen a chemical library of 1280 known drugs against influenza A virus in Biosafety Level-2 laboratory. We then assessed the anti-MERS-CoV activities of the identified compounds and of interferons, nelfinavir, and lopinavir because of their reported anti-coronavirus activities in terms of cytopathic effect inhibition, viral yield reduction, and plaque reduction assays in Biosafety Level-3 laboratory.
Results: Ten compounds were identified as primary hits in high-throughput screening. Only mycophenolic acid exhibited low EC_{50} and high selectivity index. Additionally, ribavirin and interferons also exhibited *in-vitro* anti-MERS-CoV activity. The serum concentrations achievable at therapeutic doses of mycophenolic acid and interferon-β1b were 60–300 and 3–4 times higher than the concentrations at which *in-vitro* anti-MERS-CoV activities were demonstrated, whereas

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that of ribavirin was ~2 times lower. Combination of mycophenolic acid and interferon-β1b lowered the EC_{50} of each drug by 1—3 times.
Conclusions: Interferon-β1b with mycophenolic acid should be considered in treatment trials of MERS.

Introduction

A novel lineage C betacoronavirus, previously known as human coronavirus EMC/2012 and later renamed as Middle East respiratory syndrome coronavirus (MERS-CoV), has emerged in the Arabian Peninsula since April 2012 to cause a "severe acute respiratory syndrome (SARS)-like" disease in 136 laboratory-confirmed cases with 58 fatalities in 9 countries in the Middle East, Europe, and North Africa as of 4 October 2013.[1—5] Animal-to-human transmission has been suspected in view of MERS-CoV's close phylogenetic relatedness to other lineage C betacoronaviruses found in bats in Hong Kong, Mexico, Europe, and Africa,[6—13] and its broad species tropism in various animal cell lines including those of bats, primates, pigs, civets, and rabbits.[14,15] Recently, a serological study of major livestock suggested dromedary camels to be a possible host based on the high prevalence of MERS-CoV neutralizing antibodies in dromedary camels from Oman.[16] However, targeted studies are needed to confirm this finding and its possible relevance to human cases of MERS-CoV infection as most cases did not have contact with camels and the virus has not been isolated in animals yet. The epidemic continues to evolve with recent outbreaks occurring among epidemiologically-linked household contacts in the Kingdom of Saudi Arabia, the United Kingdom, Italy, and Tunisia, and hospital contacts in the Kingdom of Saudi Arabia, Jordan, the United Kingdom, and France providing evidence for MERS-CoV's potential for person-to-person transmission.[17—23]

Unlike most other human coronavirus infections which are generally mild, most patients with MERS have suffered from rapidly progressive pneumonia with some also developing acute renal failure, hepatic dysfunction, gastrointestinal upset, pericarditis, disseminated intravascular coagulation, and/or cytopenias.[2,24] The resulting crude mortality rate of nearly 50% in documented cases far exceeded those seen in all other human coronavirus infections including SARS despite aggressive supportive treatment including extracorporeal membrane oxygenation in some of the MERS cases. While mild and asymptomatic cases have been recognized,[2,19,24] these recent case clusters signify a global health threat especially in view of the unusual clinical severity of MERS, travel of infected persons to other countries and influx of religious pilgrims to the Kingdom of Saudi Arabia, and the lack of proven effective specific antiviral treatment.

After our initial success in applying chemical genetics in probing novel targets and compounds for antiviral development,[25] we started looking for broad-spectrum antiviral compounds that may be active against both influenza A viruses and coronaviruses, the two viral pathogens responsible for causing the recent 2009 pandemic and large-scale epidemics.[9] While neuraminidase inhibitors such as oseltamivir and zanamivir remain effective against most seasonal and avian influenza A viruses,[26—30] proven antiviral therapeutic options for coronavirus infections is lacking. Given the limited time available to develop novel anti-MERS-CoV agents in this evolving epidemic, we attempted to provide an alternative solution by identifying potential broad-spectrum antiviral agents against MERS-CoV and influenza A viruses by a small compound-based forward chemical genetics approach using chemical libraries consisting of 1280 drug compounds already marketed or having reached clinical trials in the United States, Europe, or Asia (Microsource Discovery Systems, USA).[25] We then assessed the anti-MERS-CoV activities of the identified drug compounds in cell culture by cytopathic effect (CPE) inhibition, viral yield reduction, and plaque reduction assay (PRA) assays, as well as drug cytotoxicity.

Materials and methods

Viruses

A clinical isolate of MERS-CoV was kindly provided by R. Fouchier, A. Zaki, and colleagues.[3] The isolate was amplified by one additional passage in Vero cells to make working stocks of the virus (4×10^5 $TCID_{50}$/ml). All experimental protocol involving live MERS-CoV isolate followed the standard operating procedures of the approved Biosafety Level-3 facility as we previously described.[31] The influenza A/WSN/1933 (H1N1) virus was expanded in chick embryo as we previously described.[25]

Chemical reagents and high-throughput screening (HTS)

A total of 1280 pre-existing drug compounds (Microsource Discovery Systems) were screened against influenza A/WSN/1933 (H1N1) virus. High-throughput screening (HTS) was carried out in a fully automated Beckman Coulter Core System (Beckman Coulter, USA) integrated with a Kendro robotics CO_2 incubator (Thermo Fisher Scientific) at Chemical Genetics Unit, Department of Microbiology, Research Center of Infection and Immunology, Li Ka-shing Faculty of Medicine, the University of Hong Kong as we previously described with modifications.[25] Briefly, compounds were added in 96-well microtitre plates (TPP) in duplicate with a final concentration of 10 μM or 100 μM and 20,000 Madin—Darby canine kidney (MDCK) cells per well in 100 μl complete Eagle's minimal essential medium (EMEM) supplemented with 1% heat-inactivated FBS. Cells were then inoculated at an MOI of 0.01 with influenza A/WSN/1933 (H1N1) virus for detection of broad-spectrum antivirals. After infection, the plates were incubated at 37 °C with 5% CO_2 and monitored daily using a Leica DM inverted light microscope for virus-induced CPE. Drugs that