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Effect of interferon alpha and cyclosporine treatment separately and in combination on Middle East Respiratory Syndrome Coronavirus (MERS-CoV) replication in a human in-vitro and ex-vivo culture model

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ABSTRACT

Middle East Respiratory Syndrome Coronavirus (MERS-CoV) has emerged as a coronavirus infection of humans in the past 5 years. Though confined to certain geographical regions of the world, infection has been associated with a case fatality rate of 35%, and this mortality may be higher in ventilated patients. As there are few readily available animal models that accurately mimic human disease, it has been a challenge to ethically determine what optimum treatment strategies can be used for this disease. We used in-vitro and human ex-vivo explant cultures to investigate the effect of two immunomodulatory agents, interferon alpha and cyclosporine, singly and in combination, on MERS-CoV replication. In both culture systems the combined treatment was more effective than either agent used alone in reducing MERS-CoV replication. PCR SuperArray analysis showed that the reduction of virus replication was associated with a greater induction of interferon stimulated genes. As these therapeutic agents are already licensed for clinical use, it may be relevant to investigate their use for therapy of human MERS-CoV infection.

1. Introduction

The past 15 years has seen the emergence of two novel coronaviruses that have infected humans resulting in a high morbidity and mortality. Until 2003 it was thought that coronavirus infections, such as OC43 and 229E were associated with mild respiratory disease and there was little reason to develop novel therapeutic options for these coronavirus infections. The global outbreak of SARS in 2003 with a 10% fatality rate, and the recent emergence of MERS-CoV infection, as well as other recently recognized coronavirus infections such as NL63 and HKU1 have demonstrated the need for investigation into treatment options of severe coronavirus infections. MERS-CoV was first identified in June 2012 from a 60 year old patient from Middle East who developed clinical symptoms and signs similar to SARS, and who eventually died from multi organ failure (Zaki et al., 2012). A novel coronavirus was isolated, initially called HCoV-EMC but renamed as Middle East Respiratory Syndrome coronavirus (MERS-CoV) (de Groot et al., 2013). Since 2012 the virus has continued to cause severe zoonotic human disease in the Middle East, sometimes associated with outbreaks of human-to-human transmission within health care facilities (Arabi et al., 2017; Chan et al., 2014; Perlman and McCray, 2013). In May 2015 a large outbreak occurred in Korea (Korean Society of Infectious, Korean Society for Healthcare-associated Infection and Prevention, 2015), highlighting the threat to global public health security.

Previously we showed that MERS-CoV replicate in human upper and lower respiratory tract where it infected non-ciliated bronchial epithelial cells, bronchiolar epithelial cells, type I and type II alveolar pneumocytes and endothelial cells using *ex vivo* explants culture (Chan et al., 2013). Furthermore, we showed that in contrast to SARS-CoV infection, MERS-CoV infection elicited a lower pro-inflammatory cytokine response including the type I and III interferons (Chan et al., 2013). This evasion of innate immune induction and reduced interferon (IFN) response suggested that exogenous IFN may be a possible treatment options for human MERS-CoV infection. A previous study showed that pegylated IFN exhibited a more potent antiviral effect to MERS-CoV than SARS-CoV in cell culture and macaque model (de Wilde et al., 2013; Falzarano et al., 2013) and it was proposed that this was due to the lack of a MERS-CoV homolog of SARS-CoV ORF6 protein that blocks

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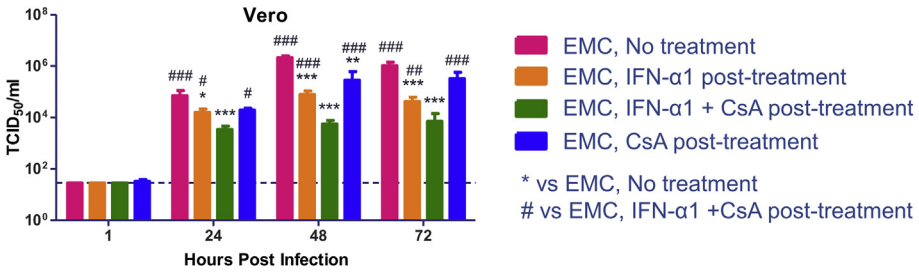

Fig. 1. Evaluation of the changes in MERS-CoV replication after the addition of IFN-α1, CsA and a combination of the two agents in Vero cells. 9 μM CsA and/or 2.4×10^4 U/ml of IFN-α1 were used. The data shown are the mean ± standard error of the mean in three representative experiments, which are analyzed by two-way ANOVA followed by Bonferroni's *post hoc* test (*/#$P < 0.05$, **/##$P < 0.01$, ***/###$P < 0.001$).

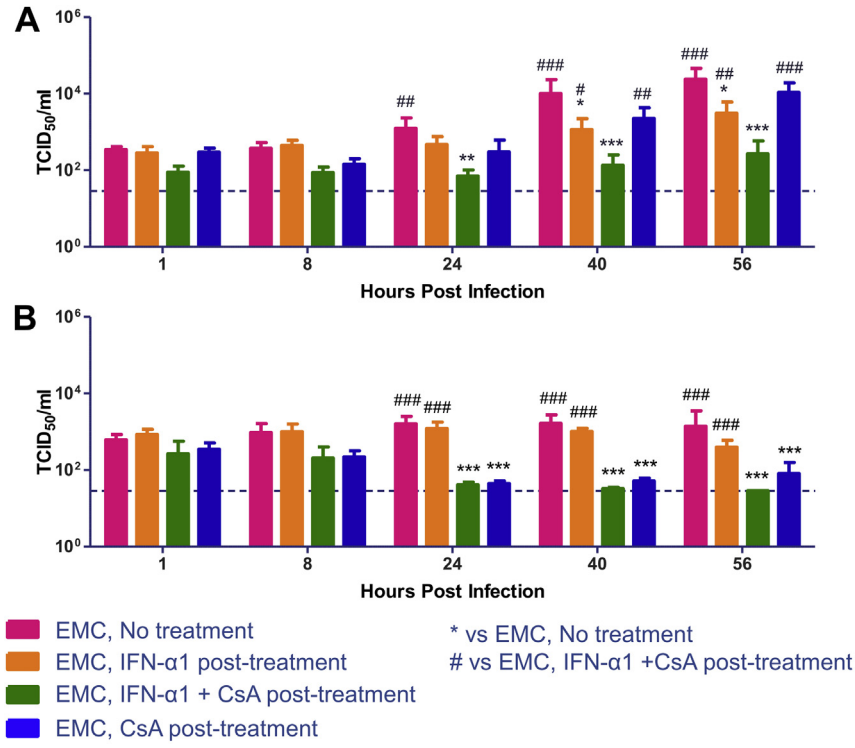

Fig. 2. Evaluation of the changes in MERS-CoV replication after the addition of IFN-α1, CsA and a combination of the two agents in (A) human bronchus and (B) human lung explant cultures. 9 μM CsA and/or 2.4×10^4 U/ml of IFN-α1 were used. The data shown are the mean ± standard error of the mean in at least three independent experiments, which are analyzed by two-way ANOVA followed by Bonferroni's *post hoc* test (*/#$P < 0.05$, **/##$P < 0.01$, ***/###$P < 0.001$).

the IFN induced translocation of STAT1 - a factor essential for signaling via the IFN receptor that leads to the induction of IFN associated antiviral genes. In the macaque model, IFN was used together with ribavirin and this led to improved clinical symptoms following MERS-CoV infection, with microarray analysis showing lower expression of inflammatory genes (Falzarano et al., 2013). Nevertheless, a number of recent clinical studies reported that the IFN and ribavirin combination did not improve long-term survival, and was not beneficial to patients who received the treatment late after infection (Al-Tawfiq et al., 2014; Omrani et al., 2014). This indicates that there is a need to consider other therapeutic combination for treating MERS-CoV infection.

Cyclosporine, such as cyclosporin A (CsA) and its derivatives has been shown to inhibit MERS-CoV replication *in vitro* (de Wilde et al., 2013). It has been demonstrated that CsA could restore type I IFN expression upon hepatitis C or rotavirus virus infection (Liu et al., 2011; Shen et al., 2013). Combined use of CsA and type I IFN was shown to inhibit hepatitis C virus replication and trigger greater virological response than IFN treatment alone (Henry et al., 2006; Inoue et al., 2003). As CsA has known immune suppressive function, non-immunosuppressive cyclophilin inhibitors have been tried in combination with ribavirin for MERS-CoV infection. Though these have an *in vitro* effect on MERS-CoV and SARS-CoV, this did not translate into a benefit in a mouse model (de Wilde et al., 2017). Here, we report the individual and combined effects of CsA and IFN-α1 on inhibiting MERS-CoV replication in an *in vitro* and human lung and bronchus *ex vivo* explant culture model. We found the combined use of CsA and IFN-α1 had inhibitory effects on MERS-CoV infection and replication, as well as on the induction of interferon stimulated genes (ISG), which sheds light on the potential use of this combination in curing MERS-CoV infection.

2. Material and methods

Ex vivo explants culture of human respiratory tract was obtained from patients undergoing surgery at Queen Mary Hospital, according to previously established criteria (Hui et al., 2017). We selected areas of morphologically normal lung, and histology was performed on a control sample. The samples were subjected to virus culture and bacterial culture. If there was intrinsic disease or infection in the resected lung specimens, they were not used for research. The project was approved by the local institutional review board (UW 14-549).

2.1. Ex vivo organ culture and infection

Fresh biopsies of human bronchi and lung were sampled from human lungs that were removed at surgery as part of clinical care, but surplus for routine diagnostic requirements. *Ex vivo* infections of human bronchus and lungs was performed as previously published (Chan et al., 2013, 2014). In brief, the bronchial mucosae were placed on a surgical sponge with their apical epithelial surface facing upwards while the lung parenchymal tissues were placed into 24 well-plates directly with 1 ml of culture medium at 37 °C. Human betacoronavirus of lineage C virus (HCoV-EMC) was provided by R. Fouchier, Erasmus MC,

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