



Early View

Editorial

Cyclosporine: an old weapon in the fight against Coronaviruses

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Cyclosporine: an old weapon in the fight against Coronaviruses.

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“ Cyclosporine inhibits the replication of coronaviruses and could potentially suppress the cytokine storm associated with coronavirus infections”

Coronaviruses have been known to cause respiratory infections in humans and intestinal infections in other mammals. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which causes COVID-19, is the 7th virus of the *Coronaviridae* family that is known to infect humans. Until 2002, four Coronaviruses infecting humans were described (HCoV- NL63, HCoV-229E, HCoV- OC43 and HKU1). These viruses caused only mild respiratory diseases in immunocompetent hosts. Since 2002, three highly pathogenic viruses from this family have been identified. SARS-CoV (also referred to as SARS-CoV-1) is an enveloped, positive-sense, single-stranded RNA virus which infects the epithelial cells within the lungs. The virus enters the host cell by binding to angiotensin-converting enzyme 2 [1]. It infects humans, bats, and palm civets [1]. It emerged in 2002-2003 with an approximate 10% mortality but limited transmissibility (approximately 8100 cases, $R_0 < 1$) [2-5]. Infected persons develop influenza-like symptoms which may progress to pneumonia, Acute Respiratory Distress Syndrome (ARDS) and death from respiratory failure and multi-organ failure. Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in 2012 in Saudi Arabia and was the second highly pathogenic virus of this family [6], yet with relatively low rate of transmission. MERS-CoV had even higher mortality than SARS-CoV (30%), but again was characterized by low transmissibility (approximately 2500 cases since 2012, $R_0 < 1$) [6]. Typical MERS symptoms include fever, cough and shortness of breath. Pneumonia is common, but not always present. Gastrointestinal symptoms, including diarrhea, have also been reported. Some laboratory-confirmed cases of MERS-CoV infection are reported as asymptomatic. Most of these asymptomatic cases have been detected following aggressive contact tracing of a laboratory-confirmed case. Dromedary Camels are a major reservoir host for this virus.

SARS-CoV-2 emerged in 2019 in Wuhan, China and is characterized by a much higher person-to-person transmissibility than SARS-CoV or MERS-CoV. The SARS-CoV-2 virus, responsible for COVID-19 disease, contains four main structural proteins. One of them, the S (spike) glycoprotein, a transmembrane protein, found in the outer portion of the virus, forms homotrimers binding with human ACE2 receptors of host cells [7]. The SARS CoV-2 S protein has significant homology with the SARS-CoV-1 equivalent protein [8], which also binds to ACE2 receptors [9]. However, the SARS CoV-2 S protein binds with higher affinity to ACE2 receptors [10] due to the presence of a furin-like cleavage site (⁶⁸²RRAR/S⁶⁸⁶) inserted in the S1/S2 protease cleavage site [11]. A considerable percentage of COVID-19 patients (5-19%) develop ARDS within 24 – 48 h after onset of symptoms, requiring mechanical ventilation with supplemental oxygen and succumb to the disease. The mortality of patients who develop ARDS is 49% [12]. Many patients with COVID-19 develop multi-organ failure (MOF). The leading causes of deaths are ARDS, septic shock with MOF, hemorrhage/coagulopathy (disseminated intravascular coagulopathy, DIC), acute heart/liver/kidney injury, and secondary bacterial infections [12]. The mean length of stay in the ICU is about 8 days for severely ill patients with considerable variation.

Most infected individuals are asymptomatic and may spread the disease by respiratory droplets carrying the viruses. Persons over 60 years old with a variety of

health ailments such as diabetes, hypertension, chronic obstructive lung disease, asthma or compromised kidney function are highly susceptible to SARS CoV-2 and develop a more severe disease when infected. It has been proposed that a common factor in all comorbidities is the presence of high levels of plasmin in the circulation which cleaves the furin site of the virus increasing its infectivity [12]. Although, a number of clinical trials are ongoing (approximately 1300 interventional trials approved worldwide when this editorial was written according to the NIH database [13]) no effective treatments are available and patients receive mainly supportive treatment.

MERS-CoV and SARS-CoV pathogenesis has been studied more extensively than SARS-CoV-2. MERS-CoV and SARS-CoV disease evolves through two different mechanisms: immunopathology and viral evasion [1]. SARS-CoV and MERS-CoV have been shown to induce a cytokine storm that results in a positive feedback loop of immune system activation that becomes independent of viral load [14-16]. Transfection of human cells with plasmids containing either the S or N proteins of SARS CoV-1 activate various isoforms of PKC and decrease the activity of the amiloride sensitive epithelial sodium channel (ENaC) [17] which may impede the clearance of alveolar edema and worsen the outcome of the disease [18]. Furthermore, SARS-CoV and MERS-CoV utilize a wide variety of mechanisms to evade host immune detection [1]. SARS-CoV and MERS-CoV replicate in vesicles that lack pattern recognition receptors (PPRs) [19, 20]. In addition SARS-CoV and MERS-CoV produce several proteins that interfere with signaling cascades downstream of PPRs [1]. Finally, there is evidence that SARS-CoV and MERS-CoV can inhibit the activation of type I IFNs through SARS-CoV PLpro and MERS-CoV PLpro respectively [20-23]. SARS-CoV-2 has been hypothesized to work in a similar manner due to its close relationship with SARS-CoV and MERS-CoV.

The paper by Sauerherring and colleagues attempts not only to provide a possible therapeutic treatment for MERS-CoV, but also provides valuable insights on the mechanism of action of their proposed treatment, namely Cyclosporin A.

Cyclosporin A (CsA) was isolated from the fungus *Tolypocladium inflatum* in 1971 and came into medical use in 1983. Currently, CsA is extensively prescribed in United States and most of the western world. In the USA, CsA is approved, by the FDA to treat and prevent graft-versus-host disease in bone marrow transplantation and to prevent rejection of kidney, heart, and liver transplants. Furthermore, CsA is approved for the treatment of rheumatoid arthritis, as well as other autoimmune related disorders.

CsA has been shown to inhibit *in vitro* the replication of several coronaviruses including SARS-CoV and MERS-CoV (Figure 1A) [24-26]. Under normal conditions after T-cell receptor activation, cyclophilin A (Cyp-A) and calcineurin form a complex that leads to the activation of the nuclear factor of activated T cell (NF-AT) [27, 28]. CsA exerts its immunosuppressive effects through the binding of Cyp-A and

calcineurin preventing the activation of NF-AT (Figure 1B) [27-29]. CsA binds Cyp-A at its hydrophobic pocket inhibiting its isomerase function, but the immunosuppressive properties of CsA are independent of this function [30, 31]. The CsA-Cyp-A complex binds calcineurin (an important downstream mediator of T-cell receptors) without activating it [32], blocking downstream signaling of the T-cell receptor. Furthermore, the CsA-Cyp-A complex has been shown to be essential to the suppression of viral replication in several viruses and Sauerherring and colleagues provide insights about the mechanism involved in this function of the complex [33]. Cyp-A has similar structure to other cyclophilins and CsA does form complexes with them and affects different mechanisms and cell functions. An important example is the binding to Cyp-D. CsA binds to Cyp-D preventing cell death under stress conditions by inhibiting the opening of the mitochondrial permeability transition pore (mPTP), a pathophysiological event triggered under stress conditions (Fig 1C) [27].

The non-structural protein 1 of SARS-CoV has been found to induce IL-2 production through NF-AT [34, 35]. CsA has been shown to significantly inhibit IFN- γ , IL-4, and IL-17 production from memory T cells *in vitro* within 24 hours of administration [36]. These compounds also inhibited T cell differentiation into the Th1, Th2, and Th17 subsets, even when used at a low concentration within a 6 day *in vitro* protocol [36].

The authors for the first time show that CsA treatment not only inhibits MERS-CoV viral replication *in vitro* but also in a murine *in vivo* model. Furthermore, the *in vivo* model utilized showed improved disease outcomes. These results by themselves are extremely important. To our knowledge this the first *in vivo* study showing inhibition of viral replication for any of the highly pathogenic coronaviruses. This finding is important as it provides experimental evidence that cyclophilin inhibitors and CsA in particular are effective not only in the isolated setting of an *in vitro* setting but also in the complicated setting of a whole animal. The successful mitigation of lung pathology presented after CsA treatment indicates the potential therapeutic usages of CsA against MERS-CoV and potentially other coronaviruses.

The authors also provided important insights on the mechanism of action of CsA. They showed that the activation of the Interferon regulatory factor 1- Interferon lamda (IRF1-IFN λ) signaling pathway is essential to the MERS-CoV antiviral effects of CsA. The IRF1-IFN λ signaling axis is an extensively studied pathway that has been shown to be involved in antiviral mechanisms most notably in influenza host defense and malfunction of this pathway is implicated in the progression of viral infections to chronicity [37, 38]. Using an appropriate combination of *in vivo* and *in vitro* experiments the authors showed that CsA upregulates the IRF1-IFN λ signaling pathway *in vivo*. *In vitro* they showed that inhibition of the IRF1-IFN λ signaling pathway blocked the antiviral properties of CsA and allowed viral replication.

Moving forward it would be interesting to investigate whether the improved *in vivo* outcomes of this study are not only due to the antiviral effects that the authors investigate thoroughly, but also to a downregulation of the cytokine storm that is evident in coronavirus infections. A possible tool in this research are the transgenic

murine models (*Ifnlr1*^{-/-} and *Ifnar1*^{-/-}) currently utilized in influenza research [37]. CsA is an appropriate countermeasure against cytokine storm in a number of diseases and in particular secondary hemophagocytic lymphohistiocytosis (SHL) [39-41]. Furthermore, determination of the timing of the administration of this agent is important: early administration may render the host susceptible to bacterial infections, which are known to enhance mortality significantly. Administration later on in the disease, may limit the onset of the cytokine storm which contributes to mortality from coronavirus infections. Namely, how early during the infection and before or during the “cytokine storm” CsA should be applied.

Before considering CsA for human clinical trials to treat COVID-19, a careful evaluation of cost/benefit should take place given the variety of side-effects including increased susceptibility to infections, nausea, vomiting and triggering of cancer development among others [42]. Furthermore, aerosolized administration of CsA has been proposed as a treatment or potential preventative measure of bronchiolitis obliterans syndrome (BOS) in lung transplant patients [43, 44]. The results of these clinical trials have provided interesting insights and have shown that CsA when administered as an aerosol has little or no systemic toxicity. Considering the acute life threatening aspects of COVID-19 from respiratory complications of the disease, a direct local application could make sense. However, this approach has a potential pitfall. The virus has several reservoirs outside the pulmonary system. A local application of CsA might not effectively combat those virus reservoirs, leading to unforeseen complications. It would also be useful to identify more specific targets and therapeutic agents targeting the IRF1-IFN λ signaling pathway. This is more possible now thanks to the very important work on the mechanism proposed by Sauerherring and colleagues.

Considering the familial relationship between MERS-CoV, SARS-CoV and SARS-CoV-2, this study is extremely relevant during the current COVID-19 pandemic. The authors provide useful insights to the mechanism of action and possible therapeutic role of CsA against coronaviruses. Although, CsA is not currently approved for use in SARS-CoV-2 cases, the results of this study warrant a more careful investigation and preclinical studies into the possible use of CsA as a therapeutic agent against COVID-19.

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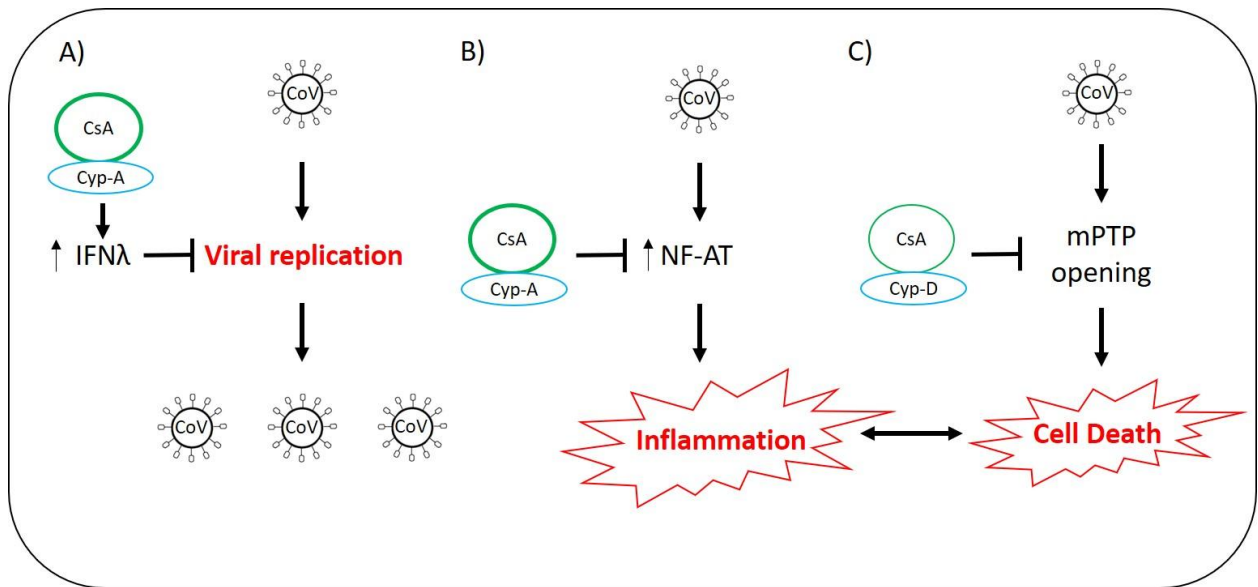


Figure 1. Schematic overview of the interactions of cyclosporine A (CsA) and coronaviruses. **A)** CsA binds to cyclophilin A (Cyp-A) and upregulates Interferon lambda ($\text{IFN}\lambda$) which blocks viral replication. **B)** Coronaviruses activate nuclear factor of activated T cell (NF-AT), which triggers the release of inflammatory cytokines and causes inflammation. The CsA-Cyp-A complex prevents the activation NF-AT reducing inflammation. **C)** Coronaviruses cause aberrant opening of the mitochondrial permeability transition pore (mPTP), which results in cell death. CsA in complex with cyclophilin-D (Cyp-D) prevents the opening of mPTP reducing cell damage and cell death.

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