Mycophenolate mofetil is active against SARS-CoV-2 in Vero E6 cells

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Abstract

Mycophenolate mofetil was reported to have broad *in vitro* activity against different viruses and had been tried in combination with IFN-β in treating MERS infection. We tested the pharmacological activity of mycophenolate mofetil using SARS-CoV-2 infected Vero cells. The half-maximal effective concentration (EC_{50}) of mycophenolate mofetil against SARS-CoV-2 was 0.47 μM while that of remdesivir was 0.77 μM. Molecular docking results of mycophenolate mofetil to potential target proteins of COVID-19 suggested that mycophenolate mofetil might inhibit SARS-CoV-2 mainly by interacting with DHODH and IMPDH2. Furthermore, mycophenolate mofetil as an immunosuppressant may be a good therapeutic option for the management of hyperinflammation in patients with severe COVID-19. Based on its high potency against SARS-CoV-2 in Vero E6 cells, its good pharmacokinetics and clinical safety profile, mycophenolate mofetil deserves further exploration as potential treatment for COVID-19.

Keywords: Mycophenolate mofetil, SARS-CoV-2, DHODH, IMPDH2

Introduction

The SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) epidemic

started in late December 2019 highlight the inadequacy of available treatments for life-threatening zoonotic CoV infections in humans. As of April 16, 2020, there are 1 991562 confirmed COVID-19 (coronavirus disease 2019) cases and 130885 deaths worldwide caused by SARS-CoV-2 infection, carrying a mortality of approximately 6.6%. [1] Although several drugs such as remdesivir, chloroquine, hydroxychloroquine and favipiravir are currently undergoing clinical studies (Fig.1), none of them has been proven to be specifically effective by double-blind randomized controlled trial. [2] Therefore, more drug candidates as potential treatments for COVID-19 are urgently required.

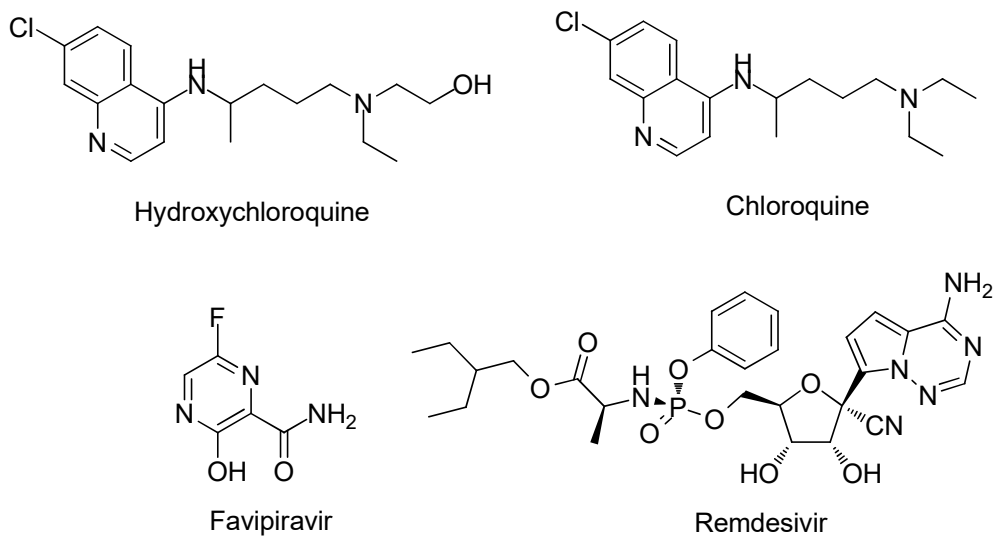

Figure 1. Representative potential drugs against COVID-2019.

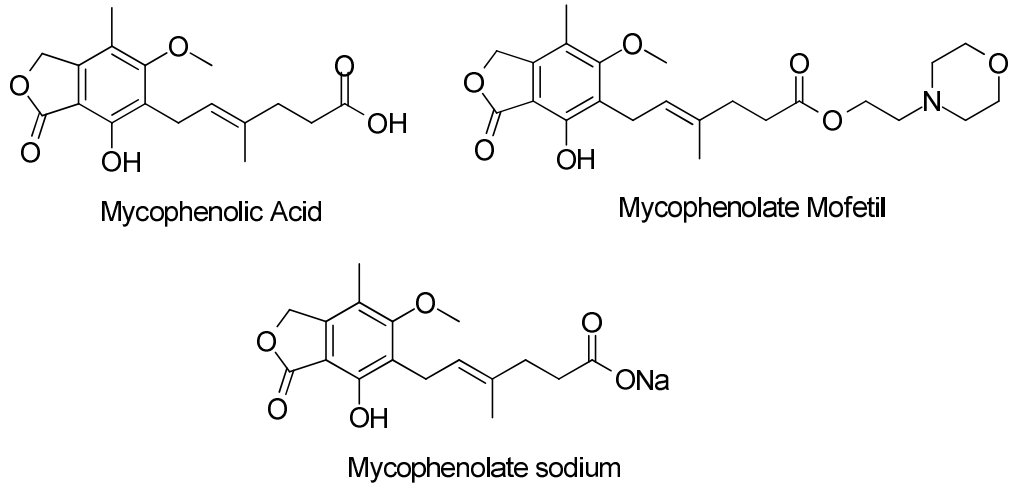

Figure 2. Chemical structures of mycophenolic acid, mycophenolate sodium and

mycophenolate mofetil.

Materials and Methods

SARS-CoV-2 (WIV04) isolated from the bronchoalveolar lavage fluid of patient was used for antiviral assay and viral titers were quantified by plaque assay. [3] Vero E6 cells were pretreated with compounds diluted in infection media for 1 h prior to infection by SARS-CoV-2 virus at MOI = 0.01. Antiviral compounds were maintained with the virus inoculum during the 2h incubation period. The inoculum was removed after incubation, and the cells were overlaid with infection media containing diluted compounds. After 24 h incubation at 37 °C, supernatants were collected. The viral RNAs in the cell culture supernatant were extracted by QIAmp 96 virus QIAcube HT kit (Qiagen, 57731), and quantified by real-time RT-PCR with Taqman probe targeting to RBD2 gene. Four-parameter logistic regression (GraphPad Prism) was used to fit the dose-response curves and determined the 50% effective concentrations (EC_{50}) of the compounds that inhibit viral replication. CC_{50} was determined with serially-diluted compounds in Vero E6 cells at 24 h post-incubation using CCK8 assay.

Results and Discussion

Mycophenolic acid, its salt mycophenolate sodium and its prodrug mycophenolate mofetil (Fig.2) are selective, non-competitive, and reversible inosine 5'-monophosphate dehydrogenase (IMPDH) inhibitors which were approved in several countries for the oral prevention of solid organ transplant rejection. Mycophenolic acid acts as a potent specific inhibitor of human lymphocyte proliferation by inhibiting human type 1 and type 2 IMPDH (hIMPDH1/2), with IC_{50} values of 19 nM and 12 nM, respectively.[4] Similarly, mycophenolate mofetil inhibited hIMPDH1/2 with IC_{50} values of 33 nM and 28 nM, respectively.[5] In addition to potent immunosuppressive activity, mycophenolic acid and mycophenolate mofetil also has broad *in vitro* activity against different viruses including coronaviruses such as HCoV-OC43 and MERS-CoV (Table 1).[6] As mycophenolic acid exhibited comparable *in vitro* antiviral activities with mycophenolate mofetil against several coronaviruses

and mycophenolate mofetil possessed higher oral bioavailability,[7] we decided to evaluate the antiviral activity of mycophenolate mofetil against SARS-CoV-2 in Vero E6 cells. The SARS-CoV-2 virus was isolated from a clinical isolate of SARS-CoV-2 infected patient. With remdesivir as positive control, the half-maximal effective concentration (EC_{50}) of mycophenolate mofetil against the SARS-CoV-2 was determined to be 0.47 μM while that of remdesivir was 0.77 μM. Cytotoxicity of mycophenolate mofetil to Vero E6 cells was measured by CCK-8 assays. In agreement with the good safety profile observed in clinic, the CC_{50} value of mycophenolate mofetil was determined to be greater than 10 μM. Accordingly, the selectivity index (SI) was estimated to be greater than 21. The human purine biosynthesis enzyme IMPDH2 regulates *de novo* nucleic acid biosynthesis and interacts with the SARS-CoV2 viral protein nsp14, which is supposed to be involved in the antiviral mechanism of mycophenolic acid or mycophenolate mofetil against SARS-CoV2.[8] The serum concentration of mycophenolic acid peaks at around 10–50 μg/ml (31.25–156.25 μM) after a 1000 mg oral dose of mycophenolate mofetil or 26.1 μg/ml (81.56 μM) after a 720 mg oral dose of mycophenolate sodium, which far exceeds its *in vitro* EC_{50} value (0.47 μM) against SARS-CoV-2.[9,10]

Table 1. Antiviral activities of mycophenolic acid and mycophenolate mofetil against five CoVs (μM).

Drug	HCoV-OC43 EC_{50}, CC_{50}	HCoV-NL63 EC_{50}, CC_{50}	MHV-A59 EC_{50}, CC_{50}	MERS-Cov EC_{50}, CC_{50}	SARS-Cov2 EC_{50}, CC_{50}
mycophenolic acid	1.95, 3.55	0.18, 3.44	0.17, 4.18	1.95, 3.21	–
mycophenolate mofetil	1.58, 3.43	0.23, 3.01	0.27, 3.33	1.54, 3.17	0.47, >10

–: not tested.

The use of mycophenolic acid or mycophenolate mofetil monotherapy has not been reported in patients with SARS-CoV or MERS-CoV infection. Interestingly, a combination therapy of IFN-β and mycophenolate mofetil has been described in a retrospective observational study in Saudi Arabia involving 51 patients and all of the 7 patients who received IFN-β and mycophenolate mofetil survived, suggesting that further study of mycophenolate mofetil in patients with MERS-CoV infection in controlled trials is reasonable.[11]

In addition, we further docked mycophenolate mofetil to potential target proteins of COVID-19,[12] dihydroorotate dehydrogenase (DHODH) ranked as top one. DHODH is an enzyme required for pyrimidine biosynthesis, mycophenolate mofetil might inhibit SARS-CoV-2 by depleting the intracellular pyrimidine pool.[13]

Conclusions

Based on its high potency against SARS-CoV-2 in Vero E6 cells, its good pharmacokinetics and clinical safety profile, mycophenolate mofetil deserves further exploration as potential treatment for COVID-19. Furthermore, as accumulating evidence suggests that a subgroup of patients with severe COVID-19 might have a cytokine storm syndrome,[14] mycophenolate mofetil as an immunosuppressant may be a good therapeutic option for the management of hyperinflammation in these patients to reduce mortality.

Acknowledgments

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