Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Combined Therapy of Ciclosporin Plus Favipiravir in the Management of Patients with Severe COVID-19, not Responding to Dexamethasone: A non-Controlled Prospective Trial

Saghar Barati a, Seyed MohammadReza Hashemian b, Payam Tabarsi c, Atefeh Abedini b, Mahshid Ashrafzadeh d, Sara Haseli b, Zahra Abtahian e, Sahar Yousefian a,b, Alireza Dastan f, Ali Sobhanian g, Farzaneh Dastan a,b,*

a Department of Clinical Pharmacy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
b Chronic Respiratory Diseases Research Center (CRDRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
c Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
d Critical Care Quality Improvement Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
e Infectious Diseases and Tropical Medicine Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
f Ernest and Julio Gallo Management Program, School of Engineering, University of California, Merced, United States
g Faculty member of Islamic Azad University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Ciclosporin
COVID-19
SARS-CoV-2
Calcineurin inhibitors

ABSTRACT

Background: Regarding the COVID-19 pandemic, potential therapeutic agents are being evaluated almost every day. Ciclosporin, a calcineurin inhibitor, is characterized by beneficial antiviral and immunomodulatory effects. The present study aimed to evaluate the efficacy of ciclosporin in managing COVID-19.

Methods: This study was a prospective non-controlled clinical trial carried out on 20 patients. Confirmed COVID-19 patients received two doses of ciclosporin (10 mg/kg and 5 mg/kg injections) 24 h apart. Mortality rate and the lengths of intensive care unit (ICU) and hospital stays were assessed for all 20 patients.

Results: The mortality rate and the need for mechanical ventilation were calculated as 50%. The percentage of ICU admission was 70%. The lengths of ICU and hospital stays were 8.13 ± 6.81 and 14.25 ± 8.55 days, respectively. The levels of ferritin and white blood cells were significantly higher after injecting the second dose of ciclosporin. Seven patients (35%) had radiologically improved lungs after ciclosporin therapy.

Conclusion: It seems that the protocol of two doses of ciclosporin in combination with favipiravir does not have favorable effects among COVID-19 patients that do not respond to dexamethasone. Controlled trials are needed to confirm the results.

1. Introduction

Since late 2019, the SARS-CoV-2 infection has spread from China to other countries, and its mortality rate and clinical manifestations are being determined almost every day [1]. However, it could take months, and perhaps years, to fully understand the origin, characteristics, and symptoms of the infection, as well as the hosts’ immune response to it [2]. Due to the proven role of the cellular immune system in the pathology of COVID-19, immunomodulatory therapies have been used in this field [3].

Several studies have shown the efficacy of different immunomodulators, including TNF-α and IL-6 inhibitors, in treating COVID-19, suggesting that the immune response might be the culprit pathology of the development of severe diseases [4,5]. Infected cells are exposed to an overload of nascent polypeptides, transcriptional machinery, and by-products of helicase activation, thus jeopardizing the maintenance of protein folding and triggering mitochondrial stress. Finally, mitochondrial proteostasis collapse would drive caspase activation and...
irreversible cell damage [6,7]. Calcineurin inhibitors may help restore the unfolded protein response (UPR) within the cytosol and rescue cells from necrosis [8]. Of note, ciclosporin has beneficial antiviral effects against RNA viruses, including the beta-coronavirus family [9,10]. Some studies have pointed out the potential useful effects of ciclosporin on the pathogenesis of COVID-19 [11]. In this respect, the present study aimed to further investigate the potential beneficial effects of ciclosporin on the pathogenesis of COVID-19.

2. Materials and methods

2.1. Setting

This study was a prospective non-controlled trial conducted on 20 patients at Dr. Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran, which is considered a referral center for COVID-19 patients.

2.2. Patients

Inclusion criteria for receiving ciclosporin were as follows: Age ≥ 18; confirmed COVID-19 infection based on the results of reverse transcription polymerase chain reaction (RT-PCR) test in the throat swab; oxygen saturation ≤ 93% despite appropriate care standards for 72 h of admission; bilateral chest involvement as evident from a computed tomography (CT) scan of the chest and not responding to dexamethasone therapy after 5 days.

Exclusion criteria included pregnancy or breastfeeding, allergy to ciclosporin, history of renal failure (estimated glomerular filtration rate lower than 30 ml/min), active infection, uncontrolled hypertension, and positive procalcitonin test.

2.3. Informed consent

This study was conducted in line with the Declaration of Helsinki and was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.NRITLD.REC.1399.058). This trial was registered in the Iranian Registry of Clinical Trials with the number IRCT20150107020592N22.

2.4. Interventions

A total of 20 eligible patients with confirmed COVID-19 were included in this study (Fig. 1). Patients received standard of care including dexamethasone 6 mg daily for the first five days, oxygen therapy, fluid support, enoxaparin 40 mg daily for deep vein thrombosis (DVT) prophylaxis, pantoprazole 40 mg daily for stress ulcer prophylaxis, and favipiravir 1600 mg twice daily for the first day followed by 600 mg BD for 14 days or until discharge. Ciclosporin was administered in patients who did not respond to dexamethasone therapy. Response to dexamethasone was defined as improvements in clinical status and laboratory parameters of the patients. The enrolled patients received

---

**Fig. 1.** The CONSORT diagram of the study.
ciclosporin (NEORAL®) at 10 mg/kg followed by 5 mg/kg on the next day. Each vial was diluted in 500 ccs of normal saline (1:50) and administered over 6–8 h. The patients’ vital signs were monitored for the first 45 min after each injection. Complete blood count, potassium, magnesium, creatinine, ferritin, C-reactive protein (CRP), white blood cells, and interleukin-6 were measured prior to injection and 24 h after injecting the second dose of ciclosporin.

2.5. Outcomes

The primary aim of this study was to evaluate the mortality rate and the lengths of intensive care unit (ICU) and hospital stays for each patient. Symptoms, including fever, cough, dyspnea, myalgia, chest pain, headache, and diarrhea, were evaluated for 14 days after admission. Laboratory results were measured before the first injection and 24 h after the second dose of ciclosporin. Any changes in chest CTs were evaluated.

Table 1
Demographic data, past medical history and baseline characteristics of the patients at admission.

|                          | N = 20 | Normal reference range |
|--------------------------|--------|------------------------|
| Age                      | 55.8 ± 12.9 | –                     |
| Gender (male)            | 9 (45%) | –                     |
| BMI (kg/m²)              | 27.4 (23.1–33.3) | 18–25               |
| Smoking history          | 2 (10%) | –                     |
| Diabetes                 | 4 (20%) | –                     |
| Malignancy (Not active)  | 1 (5%) | –                     |
| Hypertension             | 3 (15%) | –                     |
| Chronic Kidney Disease   | 1 (5%) | –                     |
| Number of comorbidities  | 0.5(0–4) | –                     |
| CRP (mg/L)               | 43.2 (5–60) | < 10                 |
| Ferritin (ng/ml)         | 759.6 (54–2000) | 20–250              |
| IL-6 (Pg/ml)             | 19.2 (2.2–70) | Should not be detected or very low levels |

Data are presented as mean (range) or percent; BMI: Body mass index; IL-6: Interleukin-6; CRP: C-reactive protein.

2.6. Statistical analysis

The results were analyzed using the SPSS v.25.0 software (IBM Corp., Armonk, NY, USA). Data were presented in the form of mean ± standard deviation or percentage. Shapiro-Wilk test was employed to evaluate the normality of data distribution. A paired sample t-test or Wilcoxon test was used to evaluate the significance of differences between the lab data measured before and after 24 h of the second dose of ciclosporin.

3. Results

The demographic data of the patients and their medical histories are shown in Table 1.

The median time before administering ciclosporin to patients was 5.5 (4.2–9) days.

Table 2 represents the trend of symptoms for all patients during the first 14 days after admission. Diarrhea and fever subsided in all patients by days 5 and 9, respectively. However, cough, myalgia, chest pain, and headaches continued until day 11. Dyspnea persisted until day 12 in one patient.

The results derived from the lab data of the patients are shown in Table 3. The results of a paired sample t-test or Wilcoxon test showed a significant difference between the data sets in terms of white blood cell and ferritin levels before treatment and 24 h after administering the second dose of ciclosporin. The mean ferritin level before the first injection was 1046.8 ± 707. This value increased to 1266.9 ± 602.1 24 h after the second dose of ciclosporin.

Table 3
Laboratory results for the patients before and after 24 h of administering the second dose of ciclosporin.

|                          | Before Ciclosporin injection | 24 h after second dose of ciclosporin | P-Value |
|--------------------------|-----------------------------|--------------------------------------|---------|
| CRP (mg/L)               | 30.9 ± 22.1 (0.8)           | 30.6 ± 21.9 (0.8)                   |         |
| Ferritin (ng/ml)         | 1046.8 ± 707 (0.03)         | 1266.9 ± 602.1 (0.03)               |         |
| IL-6 (Pg/ml)             | 34.4 ± 47.8 (0.3)           | 40.9 ± 34.6 (0.3)                   |         |
| White blood cells (<10³/µL) | 10.4 ± 4 (0.02)             | 12.3 ± 4.8 (0.02)                   |         |
| Lymphocytes (Cells/µL)   | 9.5 ± 4.5 (0.1)             | 8.7 ± 4.4 (0.1)                     |         |
| Platelet count (<10³/µL) | 259.9 ± 99.3 (0.8)          | 262.6 ± 87.7 (0.8)                  |         |
| Serum potassium (mg/dL)  | 4.2 ± 0.2 (0.1)             | 4.4 ± 0.6 (0.1)                     |         |
| Serum magnesium (mg/dL)  | 2.3 ± 0.4 (0.7)             | 2.2 ± 0.3 (0.7)                     |         |
| Creatinine (mg/dL)       | 0.9 ± 0.2 (0.4)             | 1 ± 0.4 (0.4)                       |         |

Data are presented as Mean ± SD; CRP: C-Reactive protein; IL-6: Interleukin-6.

Table 2
Symptoms of the patients during the study.

| Symptoms          | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 |
|-------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|
| Fever             | 16 (80%) | 14 (87.5%) | 12 (75%) | 12 (75%) | 10 (62.5%) | 8 (50%) | 4 (26.7%) | 2 (16.7%) | 1 (10%) | 0% (0%) | 0% (0%) | 0% (0%) | 0% (0%) |
| Cough             | 19 (95%) | 19 (100%) | 18 (94.7%) | 18 (94.7%) | 16 (84.2%) | 9 (50%) | 5 (26.7%) | 5 (26.7%) | 3 (33.3%) | 1 (10%) | 0% (0%) | 0% (0%) | 0% (0%) |
| Dyspnea           | 20 (100%) | 20 (100%) | 19 (95%) | 16 (80%) | 12 (60%) | 9 (47.4%) | 7 (43.8%) | 4 (28.6%) | 3 (21.4%) | 1 (10%) | 0% (0%) | 0% (0%) | 0% (0%) |
| Myalgia           | 16 (80%) | 16 (100%) | 15 (93.8%) | 16 (100%) | 13 (75%) | 7 (46.7%) | 3 (33.3%) | 2 (17%) | 2 (17%) | 1 (10%) | 1 (10%) | 1 (10%) | 1 (10%) |
| Chest pain        | 18 (90%) | 16 (100%) | 17 (94.4%) | 17 (94.4%) | 13 (66.7%) | 12 (75%) | 10 (50%) | 7 (45.8%) | 5 (22.7%) | 2 (17%) | 1 (10%) | 0% (0%) | 0% (0%) |
| Headache          | 12 (63.2%) | 11 (69.4%) | 9 (57.1%) | 8 (50%) | 5 (33.3%) | 3 (20%) | 1 (10%) | 1 (10%) | 1 (10%) | 1 (10%) | 1 (10%) | 1 (10%) | 1 (10%) |
| Diarrhea          | 7 (35%) | 7 (100%) | 7 (100%) | 4 (100%) | 1 (100%) | 0% (0%) | 0% (0%) | 0% (0%) | 0% (0%) | 0% (0%) | 0% (0%) | 0% (0%) | 0% (0%) |

Data are presented as percent.
after the second injection (P-Value = 0.03). The number of white blood cells also increased from 10.4 ± 4 to 12.3 ± 4.8 24 h after the infusion (P-Value = 0.02).

The mortality rate, the need for mechanical ventilation, the lengths of ICU and hospital stays, and ICU admission were evaluated in all patients. The results are shown in Table 4.

The mean time between the second dose of ciclosporin and death in non-survived patients was 4.1 ± 3.9 days. Also, the mean time from the second dose of ciclosporin until discharge from the hospital was 12.6 ± 7.8 days.

Changes in chest CT scans of the patients showed that seven patients (35%) had radiologically improved lungs; four of these patients (>50%) showed improvements (Fig. 2). All four of these patients survived. Among the other 13 patients, three (15%) did not show any improvements, and four (20%) exhibited progression. The other six patients had only one chest CT scan taken, making comparisons impossible.

None of the patients developed adverse reactions such as renal failure, acute kidney injury, electrolytes disorders, or hypertension following the use of ciclosporin. Moreover, none of the patients developed infusion reactions.

4. Discussion

Based on the results of our study, it seems that administering two ciclosporin doses in combination with favipiravir does not have a beneficial role in treating COVID-19 patients not responding to dexamethasone. Cavagna et al. performed a cohort study consisting of 385 patients receiving calcineurin inhibitor therapy due to solid organ transplantation or rheumatologic disease. The development of COVID-19 in these patients was low despite their suppressed immune status. Furthermore, most COVID-19 cases presented mild symptoms. The authors concluded that calcineurin inhibitors might have a beneficial role in treating COVID-19 disease [12]. Ciclosporin was shown to have antiviral effects against coronaviruses in-vitro, regardless of its effects.

### Table 4

|                          | N (%) |
|--------------------------|-------|
| Mortality                | 10 (50%) |
| The need for mechanical ventilation | 10 (50%) |
| ICU admission            | 14 (70%) |
| Length of ICU stay (days) | 6.1 (3–24) |
| Length of hospital stay (days) | 14.2 (6–38) |

Data are presented as percentage or mean (range); ICU: Intensive Care Unit.
on the immune system [13]. It was also identified that cyclophilin and FK506 binding protein interacted with SARS-CoV-2 proteins in the host’s immune system [9].

Galves-Romeo performed a study on moderate-to-severe COVID-19 cases and concluded that administering ciclosporin (1–2 mg/kg/day for seven days), along with clarithromycin and methylprednisolone reduces mortality and improves outcomes [14]. The dose we administered was much higher than in this previous study. It should be considered that clarithromycin is a cytoplasmic inhibitor that reduces the metabolism of ciclosporin. Hence, the plasma concentrations of ciclosporin in the previous study were probably higher than the concentration expected when administering a dose of 1–2 mg/kg/day. Methylprednisolone may also increase the serum concentrations of ciclosporin.

We believed that by considering a high dose of ciclosporin, the plasma concentrations needed for the prevention of virus replication would be achievable by the medication. Solanich, X., et al. claimed that the antiviral (and not anti-inflammatory) effects of ciclosporin will not be achieved by previously suggested doses [15]. We wanted to see if a high dose of ciclosporin, given over a short duration, would benefit severe COVID-19 cases. We decided to administer high doses for a short duration to achieve considerable plasma concentrations for antiviral effects and prevent side effects by reducing the number of administrations.

Based on what we discussed above, several studies have recommended evaluating the efficacy of calcineurin inhibitors in preventing and treating COVID-19 [16,17]. However, in our study, no therapeutic effect was observed when ciclosporin was administered to treat COVID-19. Patients’ white blood cell counts and ferritin levels significantly rose 24 h after the second dose. However, the authors believe that this phenomenon might not be related to ciclosporin, as the use of this agent is not associated with inflammation itself.

Another main point to consider is that we began administering ciclosporin almost five days after admission. The antiviral and immunomodulatory effects of ciclosporin might be achieved if it is administered earlier. Considering the drug interactions between ciclosporin and dexamethasone, as well as the potential fluctuations in serum concentrations of both medications, we decided to discontinue dexamethasone and administer ciclosporin in patients who did not respond to dexamethasone after five days. Favipiravir was continued for 14 days or until the patient was discharged. No documented interaction between ciclosporin and favipiravir was found. Favipiravir selectively prevents the replication of viral genomes by interacting with RNA-dependent polymerase (RdRp) [18]. It is currently being used as an off-label regimen for COVID-19 with the mentioned dosing regimen in the methods section based on the available published evidence [19].

The patients in our study also received favipiravir. thus, the potential therapeutic effects of ciclosporin as a single-agent therapy were not evaluated in this study.

The main limitation of our study was that we did not have a control group to evaluate the parameters by comparison. Controlled clinical trials with a larger sample size are recommended to improve the generalization of the results.

5. Conclusion

It seems that the protocol of two doses of ciclosporin in combination with favipiravir does not have favorable effects among COVID-19 patients that do not respond to dexamethasone. Further randomized controlled trials with different dosing regimens are required to confirm the results.

CRediT authorship contribution statement

Saghar Barati: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. Seyed MohammadReza Hashemian: Conceptualization, Methodology, Writing – review & editing. Payam Tabarsi: Conceptualization, Methodology, Writing – review & editing. Atefeh Abedini: Writing – review & editing. Mahshid Ashrafzadeh: Methodology, Writing – review & editing. Sara Haseli: Methodology, Writing – review & editing. Zahrab Ahbati: Methodology, Writing – review & editing. Sahar Yousefian: Data curation, Software. Alireza Dastan: Conceptualization, Methodology, Writing – review & editing. Validation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This study was approved and supported by Shahid Beheshti University of Medical Sciences.

References

[1] K. Liu, et al., Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. Chin. Med. J. 133 (9) (2020).
[2] E. Prompetchara, C. Ketloy, T. Palaga, Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic, Asian Pac. J. Allergy Immunol. 38 (1) (2020) 1–9.
[3] K.-Y. Lee, J.-W. Rhim, J.-H. Kang, Immunopathogenesis of COVID-19 and early immunomodulators, Clin. Experimental Pediatrics 63 (7) (2020) 239.
[4] F. Dastan, et al., Promising effects of tocilizumab in COVID-19: a non-controlled, prospective clinical trial, Int. Immunopharmacol. 88 (2020), 106869.
[5] P.C. Robinson, et al., Accumulating evidence suggests anti-TNF therapy needs to be given trial priority in COVID-19 treatment, Lancet Rheumatol. 2 (11) (2020) e653–e655.
[6] C.-P. Chan, et al., Modulation of the unfolded protein response by the severe acute respiratory syndrome coronavirus spike protein, J. Virol. 80 (18) (2006) 9279–9287.
[7] V. Jovaisaite, L. Mouchiroud, J. Auwerx, The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease, J. Exp. Biol. 217 (1) (2014) 137–143.
[8] I. Lebedev, et al., A novel in vitro CypD-mediated p53 aggregation assay suggests a model for mitochondrial permeability transition by chaperone systems, J. Mol. Biol. 428 (20) (2016) 4154–4167.
[9] S. Pfefferle, et al., The SARS-coronavirus-host interactome: identification of cyclophilins as target for pan-coronavirus inhibitors, PLoS Pathog. 7 (10) (2011), e1002331.
[10] A.H. de Wilde, et al., MERS-coronavirus replication induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon-α, J. General Virol. 94 (Pt 8) (2013) 1749.
[11] O. Sanchez-Pernaute, F. Romero-Bueno, Why choose cyclosporin A as first-line therapy in COVID-19 pneumonia, Reumatología. Clínica. (2020).
[12] L. Cavagna, et al., Calcineurin inhibitor-based immunosuppression and COVID-19: results from a multidisciplinary cohort of patients in northern Italy, Microorganisms 8 (7) (2020) 977.
[13] Y. Tanaka, Y. Sato, T. Sanaki, Suppression of coronavirus replication by cyclophilin inhibitors, Viruses 5 (5) (2013) 1250–1260.
[14] J.L. Gálvez-Romero, et al., Cyclosporine A plus low-dose steroid treatment in COVID-19 improves clinical outcomes in patients with moderate to severe disease: A pilot study, J. Intern. Med. 289 (6) (2021) 906–920.
[15] X. Solanich, et al., Inhibition of SARS-CoV-2 replication using calcineurin inhibitors: are concentrations required clinically achievable? J. Intern. Med. 289 (6) (2021) 926–927.
[16] M. Willicombe, D. Thomas, S. McAdoo, COVID-19 and Calcineurin Inhibitors: Should They Get Left Out in the Storm? J. Am. Soc. Nephrol. 31 (6) (2020) 1145–1146.
[17] R. Hage, C. Steinack, M.M. Schuermans, Calcineurin inhibitors revisited: A new paradigm for COVID-19? Brazilian J. Infectious Diseases 24 (4) (2020) 365–367.
[18] Y. Furuta, T. Komeno, T. Nakamura, Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase, Proc. Japan Academy Series B, Phys. Biol. Sci. 93 (7) (2017) 449–463.
[19] Q. Cai, et al., Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study, Engineering (Beijing) 6 (10) (2020) 1192–1198.