

SHORT REPORT
INFECTIOUS DISEASES

Dexamethasone vs COVID-19: An experimental study in line with the preliminary findings of a large trial

Mohammad Hossein Hosseinzadeh¹  | Amir Shamshirian²  |
Mohammad Ali Ebrahimzadeh³ 

¹Student Research Committee, School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

²Gastrointestinal Cancer Research Center, Non-Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran

³Pharmaceutical Sciences Research Center, Department of Medicinal Chemistry, School of Pharmacy, Mazandaran University of Medical Science, Sari, Iran

Correspondence

Mohammad Ali Ebrahimzadeh,
Pharmaceutical Sciences Research Center,
Department of Medicinal Chemistry, School
of Pharmacy, Mazandaran University of
Medical Science, Sari, Iran.
EEmail: zadeh20@gmail.com

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Abstract

Background: The preliminary report of the RECOVERY large randomised controlled trial indicated a promising survival effect for dexamethasone therapy of coronavirus disease 2019 (COVID-19). This study aimed to investigate the anti-hypoxic activities of dexamethasone to understand a possible mechanism of its action in hypoxia-induced lethality through experimental models of hypoxia.

Methods: In this investigation, 84 Male BALB/c mice were randomly divided into groups of seven (12 groups). Treatment groups received 10 days of dexamethasone intraperitoneal injection at both human dose (~0.1 mg/kg) and the animal dose (~1 mg/kg). Control negative and positive groups were treated with 10 ml/kg of normal saline and 30 mg/kg of propranolol, respectively. Three experimental models of hypoxia, asphyctic, circulatory, and hemic were applied in this study.

Results: The findings showed that dexamethasone significantly prolonged the latency for death in the asphyctic model concerning the control group in both humans ($P < .0001$) and animal dose ($P < .0001$). The results were also highly significant for both doses in the hemic model ($P < .001$). In the circulatory model, although a small increase was observed in death prolongation, results were not statistically significant for both doses in this model ($P > .05$).

Conclusions: This experimental in vivo investigation demonstrated an excellent protective effect for 10 days of dexamethasone treatment against hypoxia, especially in asphyctic and hemic models. In addition to promising dexamethasone outcomes, using propranolol as the positive control illustrated a very substantial anti-hypoxic effect even much better than dexamethasone in all models. It seems that propranolol would be a safe, potential, and prudent choice to invest in treating COVID-19 patients.

1 | INTRODUCTION

A novel coronavirus appeared in Wuhan, China, in December 2019 and has been named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and it induces coronavirus disease 2019 (COVID-19). The World Health Organization (WHO) declared the novel coronavirus outbreak as a pandemic on March 11, 2020.¹ At the time of writing, SARS-CoV-2 has caused >40 000 000 infections, and

>1 000 000 deaths worldwide. Despite intensive efforts, much remains unknown of many aspects of SARS-CoV-2 infection. Many treatment approaches have been proposed with many rapid pre-prints and publications with conflicting results. Hence, there is much to learn about the pathogenesis and effective treatment approaches for SARS-CoV-2 and COVID-19.

Corticosteroids (eg, methylprednisolone and dexamethasone) have anti-inflammatory, antifibrotic, and vasoconstrictive activities.

Such medications have been widely used to improve outcomes in individuals with acute respiratory distress syndrome (ARDS), pneumonia, and septic shock.^{2,3} In recent years, different well-designed clinical trials have demonstrated promising clinical benefit for corticosteroids in ARDS and septic shock.⁴⁻⁶

In this case, one of the conflicting treatment choices since the emergence of the COVID-19 was dexamethasone. The preliminary report of the RECOVERY large randomised controlled trial⁷ indicated a promising survival effect for dexamethasone therapy of coronavirus disease 2019 (COVID-19) patients at a dose of 6 mg q.d. for up to 10 days. The majority of the patients infected with SARS-CoV-2 are asymptomatic or only manifest a mild disease. However, the infection can lead to critical stages and cause acute hypoxemic respiratory failure requiring supplemental oxygen.^{7,8} Remarkably, the RECOVERY study indicated that the treatment approach was significant amongst patients with hypoxemia under the invasive/non-invasive respiratory support, but not in mild patients without hypoxemia and breathing support.

As a corticosteroid, dexamethasone widely affects innate and adaptive immunity, especially as an anti-inflammatory agent. Regardless of dexamethasone's studied activities, following the RECOVERY study results, we aimed to simulate this study's treatment approach and investigate the anti-hypoxic activities of dexamethasone to understand a possible mechanism of its action in hypoxia-induced lethality through experimental models of hypoxia.

2 | METHODS

2.1 | Ethics statement and animal treatment protocols

All the experimental procedures were performed based on the US National Institutes of Health guidelines of the Laboratory Animal Care and Use.⁹ The Animal Ethical Committee of Mazandaran University of Medical Sciences has approved the experimental protocol (IR.MAZUMS.REC.1399.546). Male BALB/c mice were purchased at 10-12 weeks of age from Royan Institute (Tehran, Iran).

2.2 | Animals

In this investigation, 84 Male BALB/c mice (32.98 ± 1.04 g) were randomly divided into groups of seven (12 groups) in polypropylene cages at ambient temperature, $25 \pm 1^\circ\text{C}$ and 45%-55% relative humidity, with a 12 hours light: 12 hours dark cycle (lights on at 7 AM). The animals had free access to standard water, pellet fuel by ad libitum access. Experiments were performed between 8:00 and 14:00 hours.

2.3 | Treatments

According to Table 1, treatment groups received 10 days of dexamethasone intraperitoneal (ip) injection at both human dose (6 mg

What's known

- The RECOVERY large randomised controlled trial indicated a promising survival effect for dexamethasone therapy of coronavirus disease 2019.
- Corticosteroids (eg, methylprednisolone and dexamethasone) have anti-inflammatory, antifibrotic, and vasoconstrictive activities.

What's new

- Dexamethasone has an excellent anti-hypoxia effect, which may reveal one of the primary mechanisms for dexamethasone's clinical benefits against COVID-19.
- Propranolol illustrated a very significant anti-hypoxic effect even much better than dexamethasone.

once daily based on body surface area = ~ 0.1 mg/kg) and the animal dose (~ 1 mg/kg for mice).¹⁰ Control negative groups were treated with 10 ml/kg of normal saline and control positive groups with 30 mg/kg of propranolol at the same time plan.

2.4 | Experimental models

Three experimental models of hypoxia; asphyctic, circulatory, and hemic were applied in this study. Asphyctic hypoxia model induced to the animals by putting them individually in a closed 300 mL glass container coated tightly with parafilm 30 minutes after the final ip injection of dexamethasone on day 11. The animals died following convulsions because of hypoxia. The latencies for death were recorded. Circulatory and Hemic hypoxia models were applied to the animals through ip injection of NaF (150 mg/kg) and NaNO_2 (360 mg/kg), respectively, on day 11, 30 minutes after the final dexamethasone treatment. Finally, the death latencies were recorded.

2.5 | Statistical analysis

The GraphPad Prism v.8 was used for Statistical Analysis. Data were presented as mean \pm SD. Tukey's multiple comparison test was used to determine the mean differences. All *P*-value less than 0.05 is considered statistically significant.

3 | RESULTS

According to Figure 1, the findings indicated that dexamethasone significantly prolonged the latency for death in the asphyctic model concerning the control group in both humans (15.05 ± 0.83 vs 21.43 ± 1.29 minutes, $P < .0001$) and animal dose (15.05 ± 0.83 vs

TABLE 1 Treatment regimens

Experiment model	Group	n	DEX _H ^a	DEX _A ^b	NS ^c	PRO ^d	Treatment period (d)
Asphyctic	GP1	7	–	–	Yes	–	10
	GP2	7	Yes	–	–	–	10
	GP3	7	–	Yes	–	–	10
	GP4	7	–	–	–	Yes	10
Circulatory	GP5	7	–	–	Yes	–	10
	GP6	7	Yes	–	–	–	10
	GP7	7	–	Yes	–	–	10
	GP8	7	–	–	–	Yes	10
Hemic	GP9	7	–	–	Yes	–	10
	GP10	7	Yes	–	–	–	10
	GP11	7	–	Yes	–	–	10
	GP12	7	–	–	–	Yes	10

Abbreviations: DEX, dexamethasone; NS, normal saline; PRO, propranolol.

^aDexamethasone human dose ~ 0.1 mg/kg.

^bDexamethasone human dose ~ 1 mg/kg.

^c10 mL/kg.

^d30 mg/kg.

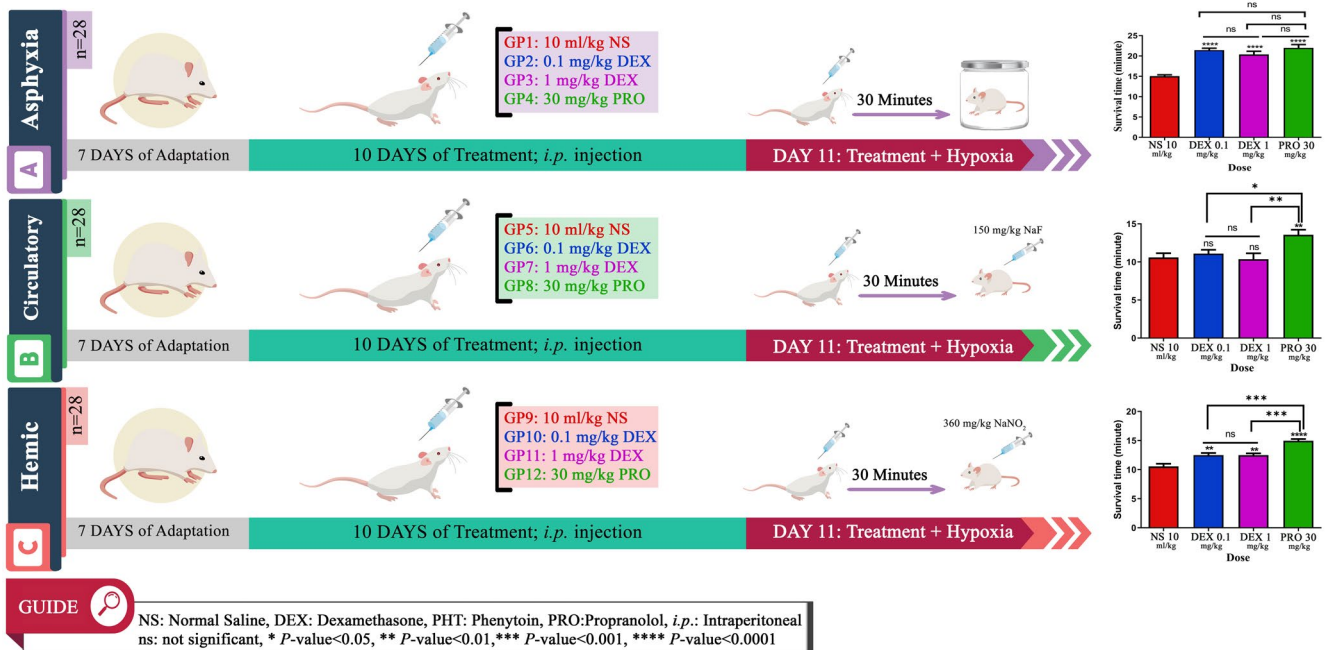


FIGURE 1 The experiment process and results

20.38 ± 1.73 minutes, *P* < .0001). The results were also highly significant for both doses in the hemic model (Human dose; 10.56 ± 1.20 vs 12.49 ± 0.96 minutes, *P* < .001, Animal dose; 10.56 ± 1.20 vs 12.50 ± 0.71 minutes, *P* < .001). In the circulatory model, although a small increase was observed in death prolongation with 0.1 mg/kg of dexamethasone (10.60 ± 1.46 vs 11.09 ± 1.24 minutes, *P* > .05), results were not statistically significant for both doses in this model (*P* > .05).

4 | DISCUSSION

After the RECOVERY press release, several multicentre RCTs have investigated the effects of corticosteroids COVID-19 patients. These studies and the WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) meta-analysis indicated a promising clinical benefit for corticosteroids against COVID-19 patients, especially in severe condition.¹¹⁻¹⁴ In this regard, some physicians may ask why

corticosteroids are beneficial in COVID-19-related ARDS patients after many heterogeneous findings of corticosteroid therapy in ARDS patients in recent decades. To find an answer to one aspect of this question, we performed an experimental study investigating dexamethasone's protective effects against hypoxia-induced lethality in mice.

Hypoxia condition leads to intense physiological stress and induces a wide range of toxic effects at the cellular level. Oxygen as a source of ROS formation is a vital component of organisms and normal redox reactions in the cell.¹⁵ It has been indicated that both hypoxia and hyperoxia can promote oxidative damage and increase the risk of morbidity and mortality.¹⁶ Studies demonstrated that hypoxia interrupts mitochondrial function and increases ROS production and oxidative stress, which could trigger apoptosis signalling.^{17,18}

The brain uses a large amount of oxygen, extremely susceptible to low oxygen levels.¹⁹ Free radicals play a critical role in signalling species in many regular physiological processes, but such radicals' excessive production leads to damage biological material. The high levels of ROS in hypoxia caused by the accumulation of reducing equivalents in the mitochondrial electron transport system.²⁰ ROS's effects can be observed mainly in specific tissues, such as the brain because it uses approximately 1/5 of the basal oxygen.

Hemoglobin is an oxygen carrier in red blood cells. Any interferences in hemoglobin's performance with oxygen transport lead to hypoxia conditions. Then, lack of oxygen in the environment, followed by mitochondrial low oxygen partial pressure, causes cell death because of inadequate energy. Available research studies illustrate that using NaF induces circulatory hypoxia, increasing the blood histamine content and decreasing the oxygen-carrying capacity.²¹ In the hemic hypoxia model induced to the mice, NaNO₂ reduces the blood's oxygen-carrying capacity by converting hemoglobin to methemoglobin and breaking the respiratory chain, preventing the cell from using oxygen to produce energy.^{22,23}

Herein, this experimental in vivo investigation, which was designed and simulated based on the RECOVERY study, demonstrated an excellent protective effect for 10 days of dexamethasone treatment against hypoxia, especially in asphyctic and hemic models. These findings may reveal one of the primary mechanisms for dexamethasone's clinical benefits against COVID-19.

More interesting, in addition to promising outcomes of dexamethasone, using 30 mg/kg of propranolol as the positive control drug in this study illustrated a very considerable anti-hypoxic effect even much better than dexamethasone in all models (Figure 1).

The propranolol is a nonselective β -blocker agent that has indicated fantastic activities such as an increase of oxygen utilisation during hypoxia,²⁴ regulation of inflammatory cytokines,²⁵ cell membrane stabilisation,²⁶ anti-thrombotic properties,²⁷ etc in different studies. Hence, this medication, which is available as 10, 20, 40, 60, and 80 mg tablets for oral administration with the maximum recommended human oral daily dose of 640 mg (FDA Reference ID: 2919389), would be a potential and prudent choice to invest for the treatment of COVID-19 patients with hypoxemia

through randomised clinical trials, especially in the continuation of the RECOVERY Collaborative Group investigation, who are highly appreciated by our team and world medical communities in these challenging times.

5 | CONCLUSION

This experimental in vivo investigation demonstrated an excellent protective effect for 10 days of dexamethasone treatment against hypoxia, especially in asphyctic and hemic models. In addition to promising dexamethasone outcomes, using 30 mg/kg of propranolol as the positive control drug in this study illustrated a very considerable anti-hypoxic effect even much better than dexamethasone in all models. It seems that propranolol would be a safe, potential, and prudent choice to invest in treating COVID-19 patients with hypoxemia through a randomised clinical trial, especially in the continuation of the RECOVERY study.

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DISCLOSURE

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All of the authors (MA.E, MH.H, and A.Sh) were contributed to study design, performing experiments, and manuscript preparation.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Animal Ethical Committee of Mazandaran University of Medical Sciences has approved the experimental protocol (IR.MAZUMS.REC.1399.546).


CONSENT FOR PUBLICATION

Not applicable.


DATA AVAILABILITY STATEMENT

Not applicable.

ORCID

Mohammad Hossein Hosseinzadeh  <https://orcid.org/0000-0002-7652-9563>

Amir Shamshirian  <https://orcid.org/0000-0002-2735-0209>

Mohammad Ali Ebrahimzadeh  <https://orcid.org/0000-0002-8769-9912>

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