AJPR
Avicenna Journal of Pharmaceutical Research
Avicenna J Pharm Res 2023;4(2)87-91. doi:10.34172/ajpr.1103
http://ajpr.umsha.ac.ir

Original Article

The Effect of Methylene Blue on COVID-19 Patients' Treatment: A Double-Blind Placebo-Controlled Clinical Trial

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Article history:
Received: October 12, 2024
Revised: December 5, 2024
Accepted: January 20, 2025
ePublished: July 31, 2025

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Abstract

Background: COVID-19 became widespread in 2019 worldwide and affected everyone. Even though it was a disease caused by a new strain of coronavirus with severe symptoms, some drugs could be effective in its treatment. It seems that methylene blue (MB), a drug for treating methemoglobinemia, can be a useful COVID-19 treatment.

Methods: Overall, 60 COVID-19 patients with an O_2 saturation of less than 85% and with no underlying diseases were chosen and included in this study. The patients were divided into the placebo control group (n=30) and the MB-treated group (n=30) and received 1 mg/kg of MB for 4 days. Some serum parameters were measured before and after 4 days of treatment. In addition, the O_2 saturation was measured every 12 hours. Then, the results were compared with each other.

Results: MB decreased the serum C-reactive protein (P=0.14), lactate dehydrogenase (P=0.08), and creatinine (P=0.12) in the COVID-19 patients non-significantly and increased the serum aspartate aminotransferase (P=0.40) and alanine aminotransferase (P=0.36) parameters in comparison with the control placebo group. However, it had no significant effect on O_2 saturation (P=0.52) in COVID-19 patients.

Conclusion: MB, a specific drug for treating methemoglobinemia, had no significant effect on COVID-19 patients.

Keywords: COVID-19, Methylene blue, O_2 saturation

Please cite this article as follows: Saffari-Chaleshtori J, Hafizi M, Baradaran A, Ghatreh-Samani K, Mohseni R, Soleimani A. The effect of methylene blue on COVID-19 patients' treatment: a double-blind placebo-controlled clinical trial. Avicenna J Pharm Res 2023;4(2)87-91. doi:10.34172/ajpr.1103

Introduction

COVID-19 has been a widespread disease since 2019 as an emerging situation worldwide, involving all the countries with a high death rate (1). This is a viral disease that involves the respiratory system and causes respiratory tract infection (2). The number of patients increased sharply in the last three years due to the rapid spread of coronavirus disease, so that it has been known as a highly contagious pandemic (3). In addition, the severe complications of this disease have led to the occurrence of severe injuries and the occurrence of other diseases, such as metabolism disorders and neurological complications (4-6). This can lead to the death of patients (7). Coronavirus is a single-stranded RNA-enveloped virus with a spike protein that helps the virus enter the host cell via endocytosis (8). Many drugs can be effective on coronavirus. Considering the urgent need for the treatment of this disease due to its sudden outbreak, many other drugs with antiviral effects have been used (9). Remdesivir is a drug that interferes with the viral RNA-dependent RNA polymerase function and is known as an antiviral agent against some human and zoonotic coronaviruses (10). Another antiviral drug, favipiravir, is a purine nucleoside analog that inhibits viral replication via the inhibition of RNA polymerase (11). Some non-antiviral drugs have been effective in coronavirus treatment, such as chloroquine (12). Further, monoclonal antibodies and protease inhibitors can be an elective treatment for coronavirus diseases (13-15). Some studies showed that methylene blue (MB), as a chemical drug, can be suitable for the prevention of coronavirus infection (16). As a drug with low cost and low side effects, MB has been historically used for methemoglobinemia

treatment. However, it has been approved for some other indications as well (17). MB could reduce methemoglobin to hemoglobin by converting the ferric ion (Fe^{3+}) to a ferrous ion (Fe^{2+}) (16). This property can be useful for coronavirus patients with respiratory indications. Furthermore, some studies demonstrated that MB reduces the reactive oxygen species by inhibiting xanthine oxidase and nitric oxide synthase (18, 19). Moreover, MB is effective in reducing cytokine production via the nuclear factor of kappa pathway inhibition (20). That is one of the most important indications in COVID patients. It seems that MB, as a versatile molecule, can be an effective compound in improving COVID patients' conditions. This clinical trial study seeks to investigate the effects of MB on coronavirus patients with respiratory indications treated with remdesivir normal treatment.

Materials and Methods

This clinical trial study was performed at Shahrekord University of Medical Sciences after receiving approval from the ethics committee (IR.SKUMS.REC.1400.111). Additionally, this clinical trial study was registered on *the Iranian Registry of Clinical Trials website* (IRCT20211012052735N1).

In this clinical trial study, 60 patients were included and treated with remdesivir as a standard treatment. These patients were divided into two groups. The control placebo group included 30 patients who received remdesivir with water as a placebo, and the MB-treated group included 30 patients who received remdesivir with 1 mg/kg MB daily and orally for four days (21).

The included criteria were individuals with no underlying diseases, no deficiencies in glucose-6-phosphate dehydrogenase, a positive real-time polymerase chain reaction test, and an O_2 saturation of less than 85%. The exclusion criteria included pregnant women, breastfeeding women, individuals allergic to MB, individuals with cardiovascular conditions and liver or kidney injuries, and individuals with a body mass index above 30. During the study, 20 individuals were excluded from each group, and the study continued with 10 patients in each group. The average age of the participants was 40.5 ± 12.9 and 45.8 ± 15.9 in the placebo and MB-treated groups, respectively. In the placebo group, there were 8 males and 2 females. The MB-treated group included 6 males and 4 females. Some biochemical factors, such as C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatinine (Cr), were measured before the study. Then, they were measured again after the individuals received 1 mg/kg of MB daily orally for 4 days. In addition, the O_2 saturation was determined every 12 hours once in both the control placebo and MB-treated groups.

Results

The three-dimensional structure of MB with a sulfur atom and three nitrogen atoms with the chemical formula [$C_{16}H_{18}ClN_3S$ and 319.85 g/mol of molecular weight (PubChem: CID:6099)] was obtained from http://www.pubchem.org/. Figure 1 shows the results of CRP. The mean of CRP in the placebo group and MB-treated group was 39.2 ± 31.1 and 55.5 ± 42.6 mg/L, respectively, at the beginning of the study. However, it was 18.4 ± 14.9 and 18.1 ± 14.7 mg/L in the placebo and MB-treated groups, respectively, 4 days after beginning the study. There was some decrease in CRP concentration 4 days after starting the study in the placebo ($P = 0.11$) and MB-treated ($P = 0.14$) groups.

The results of O_2 saturation are depicted in Figure 2. The mean of O_2 saturation in the patient and placebo groups was 81.7 ± 6.1 and 82.0 ± 4.4, respectively, at the beginning of the study. However, it was 82.8 ± 5.5 and 90.9 ± 4.3 in both groups, respectively, 4 days after beginning the study. Although there was a linear increase in O_2 saturation in both groups, the increase in O_2 saturation in the placebo group was significant ($P = 0.002$) in comparison to the treatment group ($P = 0.56$).

The results of serum ALT and AST after treating the patients with MB are displayed in Figure 3. The mean of ALT in the placebo and patient groups was 43.11 ± 11.7 and 55.89 ± 24.7 unit/L, respectively. In addition, the mean of AST was 46.33 ± 11.9 and 46.33 ± 23.8 unit/L in the placebo and patient groups, respectively, at the beginning of the study. Further, the mean of ALT in the placebo

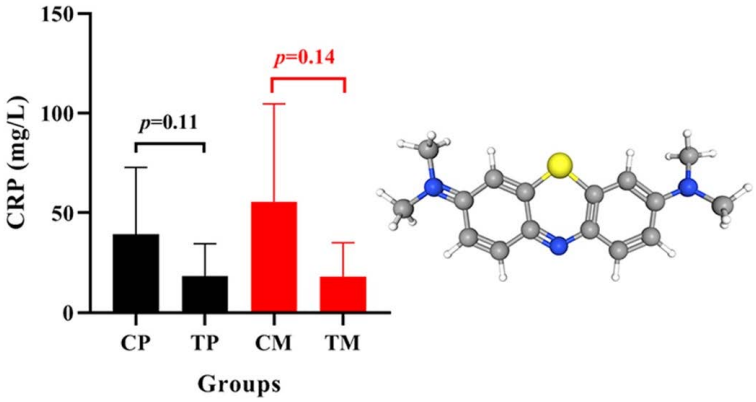

Figure 1. C-Reactive Protein (CRP). *Note*. CP: Control group before receiving the placebo; TP: Treated with the placebo group; CM: Control group before treatment with methylene blue; TM: Treated group with methylene blue (left). The three-dimensional structure of methylene blue (right)

and patient groups was 81.78 ± 52.0 and 68.71 ± 41.9 unit/L, respectively, and that of AST in both groups was 69.11 ± 46.9 and 52.43 ± 39.7 unit/L, respectively, 4 days after beginning the study. Four days at the beginning of the study, there was an increase in serum ALT in both the placebo ($P = 0.06$) and MB-treated ($P = 0.36$) groups. Moreover, an increase in serum AST was observed in both the placebo ($P = 0.20$) and MB-treated ($P = 0.40$) groups 4 days after beginning the study.

Figure 4 illustrates the results of serum LDH and serum Cr after treating the patients with MB. The mean of LDH in the placebo and patient groups was 635.55 ± 190.1 and 836.67 ± 226.2 unit/L, respectively. In addition, the mean of Cr was 0.84 ± 0.23 and 0.94 ± 0.24 mg/dL in the placebo and patient groups, respectively, at the beginning of the study. However, the means of LDH and Cr were 644.17 ± 300.9 and 597.25 ± 106.5 unit/L, as well as 0.75 ± 0.14 and 0.95 ± 0.21 mg/dL in both groups, respectively, 4 days after beginning the study. There was a decline in serum LDH 4 days after the start of the study in both the placebo ($P = 0.68$) and MB-treated ($P = 0.08$) groups. Furthermore, a decrease was found in serum Cr in

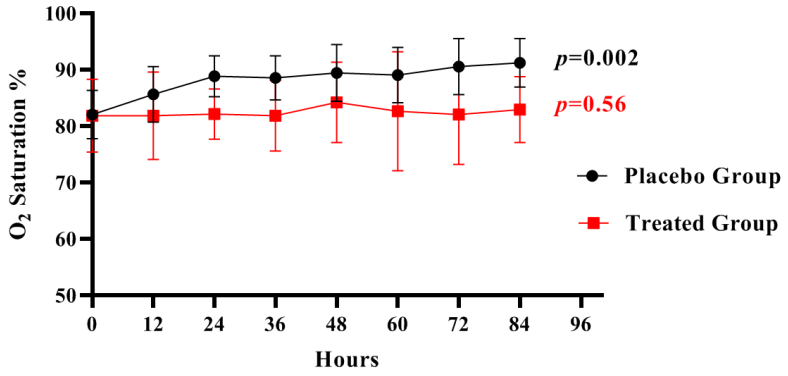

Figure 2. The O_2 Saturation in the Placebo Group and Methylene Blue-Treated Group. *Note*. Black and red lines display placebo and methylene blue-treated groups, respectively

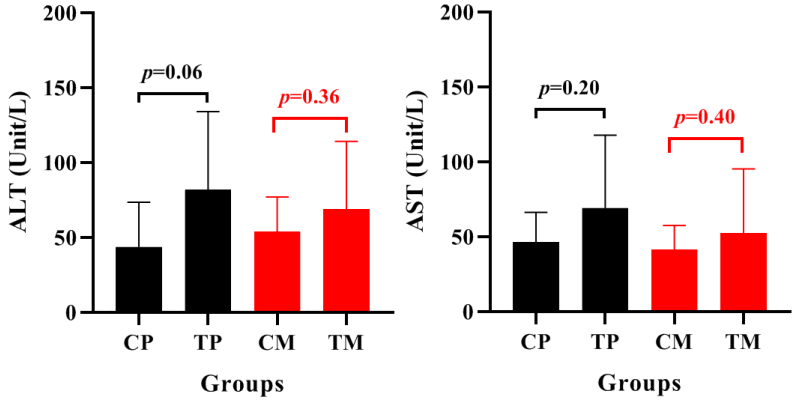

Figure 3. Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST). *Note*. CP: Control group before receiving the placebo; TP: Treated with the placebo group; CM: Control group before treatment with methylene blue; TM: Treated group with methylene blue

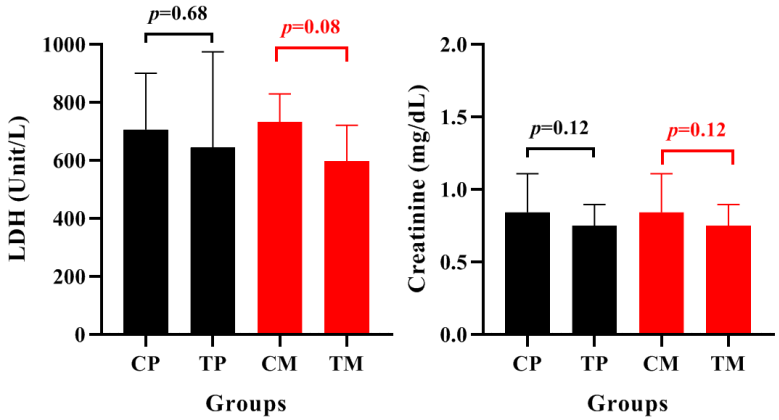

Figure 4. Lactate Dehydrogenase (LDH) and C-Reactive Protein (Cr). *Note*. CP: Control group before receiving the placebo; TP: Treated with the placebo group; CM: Control group before treatment with methylene blue; TM: Treated group with methylene blue

the placebo ($P=0.12$) and MB-treated ($P=0.12$) groups 4 days after beginning the study.

Discussion

MB, as a drug for the treatment of methemoglobinemia disease, has been suggested to be used for COVID-19 treatment in some studies (16). The most important reason for using this drug in this disease is the reduction in methemoglobin and an increase in reduced hemoglobin (17). This can be useful in increasing the O_2 saturation in patients with respiratory problems. Alamdari et al (22) found that MB increased in O_2 saturation after 3 days of treatment, which contradicts our results. There was a significant increase in O_2 saturation in patients not treated with MB (the control placebo group), while the MB-treated group demonstrated no significant increase in O_2 saturation. In this study and in accordance with O_2 saturation reports, the MB could not improve the average amount of O_2 saturation after treating patients compared to the placebo group. The level of serum CRP in COVID-19 patients was positively associated with the severity of this disease (23). Previous studies reported that MB decreases the serum CRP significantly ($P=0.005$) in COVID-19 patients (21). In the present study, MB could also decrease the serum CRP level. However, this reduction was not significant and had no significant difference from the control placebo group (Figure 1). Liver injury and an increase in serum AST and ALT levels are highly common in COVID-19; thus, choosing the best therapeutic solution to decrease these factors is one of the most important approaches to treating this disease (24). However, there was a non-significant increase in serum ALT and AST in COVID-19 patients. In this study, patients treated with MB had a lower increase in serum ALT and AST in comparison with the control placebo group (Figure 3). In a randomized, controlled, open-label clinical trial, phase 2 study, Alamdari et al demonstrated that the serum levels of ALT and AST increased in MB-treated patients more than in the control placebo group (22). Likewise, the level of serum LDH is one of the most important factors in determining liver injury, especially in COVID-19 patients (25). In the study by Alamdari et al, the amounts of serum LDH significantly decreased in COVID-19 patients treated with MB (22). Nonetheless, in the present study, the decrease in the LDH level was not significant and was less than that in the control placebo group (Figure 4). Cr is another parameter that determines kidney damage in COVID-19 patients. The serum level of Cr usually increases in these patients. It can be due to drug treatments or the other side effects of this disease (26, 27). In this study, the amount of serum Cr decreased non-significantly in the control placebo group and the MB-treated group. It seems that MB had no significant effect on the level of serum Cr in COVID-19 patients (Figure 4). However, in other studies, there was a significant decrease in the serum Cr of MB-treated patients (22). Some of the results of the present clinical trial control-placebo study differ from those of previous studies. This can be due to the low number of patients who participated in this study. Nevertheless, according to the results of this study, MB was not a significantly effective agent in COVID-19 treatment, especially in improving O_2 saturation in these patients.

Concussion

Although MB is the specific drug for treating methemoglobinemia, the findings of this study, unlike those of other previous studies, did not show the significant effect of MB on COVID-19.

Authors' Contribution

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Project administration: Akbar Soleimani.
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Supervision: Akbar Soleimani.
Validation: Ali Baradaran.
Visualization: Roohollah Mohseni.
Writing–original draft: Javad Saffari-Chaleshtori.
Writing–review & editing: All authors.

Competing Interests

The authors declare no potential conflicts of interest relevant to this article.

Ethical Approval

This study was approved by the research and ethics committee of Shahrekord University of Medical Sciences (approval No. IR.SKUMS.REC.1400.111).

Funding

This study was funded by the Deputy of Research and Technology of the Shahrekord University of Medical Sciences, Shahrekord, Iran, based on research project number:6032.

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