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Research Article

Methylene Blue as Rescue Therapy for COVID-19 Patients Who Failed to Respond to other Therapies (Final Report)

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Abstract

Background: There is debate about the efficacy and safety of antiviral drugs for treatment of COVID-19 patients. In our first cases series report, along with standard care protocol, we applied Methylene Blue (MB) as the last option of treatment to rescue 10 patients who did not respond to Remdesivir, Interferon-β, and Favipiravir therapies.

Methods: In continuing of the clinical trial, along with standard care protocol oral MB was administered to 83 severe patients who did not respond to antiviral drugs and were in critical condition.

Results: 72 patients recovered completely, and 11 patients did not survive.

Conclusions: MB is a safe and effective drug for the treatment of COVID-19 patients. MB possesses the most needed properties for therapy and can therefore be included in the clinical management of COVID-19. Considering the fungicidal activity of MB, it can be used to prevent dissemination of black fungus in the COVID-19 pandemic. Further research is needed to verify the efficacy of MB for the treatment of Mucormycosis.

Clinical trial: NCT04370288, IRCT20191228045924N120.

Keywords: COVID-19; Treatment; Methylene blue; Antiviral drug

Introduction

Coronavirus disease 2019 (COVID-19) is a systemic infectious process with severity ranging from mild forms to acute hypoxemic respiratory failure and the current treatment modalities have only a minimal impact on survival. Some major physiopathological features of COVID-19 have been identified such as: (i) direct virus-mediated cellular cytotoxicity; (ii) dysregulation of the renin–angiotensin–aldosterone system; (iii) dysregulated host response; (iv) endothelial cell injury with proinflammatory and thrombotic cascades; (v) fibrotic tissue reaction, but many other mechanisms are still unknown [1]. The available evidence from Randomized Controlled Trials (RCT) in hospitalized patients currently discourages the use antiviral drugs such as Remdesivir, Lopinavir/Ritonavir, Favipiravir and IFN-β, which had little or no effect on overall mortality, initiation of ventilation, and duration of hospital stay compared to standard care [2]. In phase 1, 2, 3 clinical trials, we showed the safety and efficacy of Methylene Blue (MB) therapy in decreasing the hospital stay and mortality of COVID1-9 patients [3-5]. In our first case series report, along with standard care protocol, we treated with oral MB as last option and rescued 10 patients who did not respond to Remdesivir, Interferon-β, and Favipiravir therapies. Our current study represents the continuation of the clinical trial of rescue therapy for patients who had previously received antiviral drugs and failed to experience clinical improvement and were in critical condition.

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Material and Methods

This study was performed at Mashhad University of Medical Sciences, Mashhad, Iran, after ethics committee approval (Clinical trial: NCT04370288, IRCT20191228045924N120) and taking written informed consent from patients. The clinical trial has been conducted according to the principles expressed in the Declaration of Helsinki. The compositions of MB syrup and powder were patented (IR-139950140003002083) (on June 1, 2020, PCT) and explained previously [3-5]. In the standard care protocol, patients receive supplemental oxygen, intravenous fluids, antiviral agents, antibiotics, anticoagulants, corticosteroids, H2 blockers, zinc, vitamin C, and vitamin D [6,7].

Results

Five cases are presents in detail.

Case 1

On November 1, 2020, a 63-year-old female was admitted to the internal medicine ward of the hospital because of high respiratory rate (30/min), reduced SpO2 (88% on room air), fever, cough, and shivering which started 5 days prior to hospitalization. Her past medical history includes diabetes, and a cerebrovascular accident (one year ago). Her RT- PCR was positive for SARS-CoV-2, and her initial workup exhibited WBC: 3.7 × 10^3 /μL with 75% neutrophils and 23% lymphocytes, platelets counts: 196 × 10^3/μL, LDH: 559 IU/L, CRP: 39 mg/dL, AST: 40 IU/L and ALT: 21 IU/L. Her lung HRCT showed diffuse bilateral GGOS (ground glass opacaties) and consolidation in the peripheral lung regions. Upper and especially lower lobes were involved. She was treated with Azithromycin (500 mg/day), Remdesivir (200 mg on the first day and 100 mg for 4 days), IFN-β (44 μg/scqod for 4 doses), Meropenem 1 gr TDS, Vancomycin 1 gr BID, and Dexamethasone (8 mg/day for 4 days). On November 8, 2020, her SpO2 bottomed down to 81%, and MB therapy (1 mg/kg every 8 hours for two days, followed by 1 mg/kg every 12 hours for the next days) was initiated. On November 14, 2020, her SpO2 reached 90%. On November 15, 2020, she achieved SpO2 of 91% (room air), respiratory rate: 16/min, WBC count: 10.3 × 10^3/μL, CRP: 0.1 mg/dL, AST: 54 IU/L, and ALT: 27 IU/L. Her VBG; before MB therapy: pH: 7.388, PO2: 43.7 mmHg, PCO2: 40.5 mm Hg, HCO3: 24.4 mm Hg, O2 Sat: 78.1%, and after MB therapy: pH: 7.349, PO2: 79.7 mmHg, PCO2: 32.85 mm Hg, HCO3: 18.0 mm Hg, O2 Sat: 95.1% revealing alleviation of her hypoxemia.

Case 2

On November 10, 2020, a 63-year-old male was admitted to the internal medicine department of the hospital because of fever, cough, and shivering, which all started 7 days prior to hospitalization, associated with tachypnea at the respiratory rate of 32/min and hypoxia at the level of SpO2 (81% on room air). His past medical history is significant for HTN. His RT- PCR was positive for SARS-CoV-2, and his preliminary blood workup manifested WBC count: 5.7 × 10^3/μL with 80% neutrophils and 15% lymphocytes, platelet count: 199 × 10^3/μL, LDH: 650 IU/L, CRP: 32 mg/dL, AST: 24 IU/L and ALT: 21 IU/L. His lung HRCT was found to have diffuse bilateral GGOS and consolidation in the peripheral lung regions. Upper and especially lower lobes were involved. He was treated with Azithromycin (500 mg/day) Remdesivir (200 mg on the first day), Meropenem 1 gr TDS, and Vancomycin 1 gr BID, and Dexamethasone (8 mg/day for 7 days). On November 12, his biochemistry revealed significant elevation of hepatic enzymes (ALT 190, AST 210), Remdesivir was discontinued (SpO2: 81% on room air), and MB therapy (1 mg/kg every 8 hours for two days, followed by 1 mg/kg every 12 hours for the next days) was initiated on the same day. On November 14, 2020, his SpO2 reached 90% (on room air). On November 15, 2020, his clinical parameters found to be SpO2: 91%, respiratory rate: 17/min, WBC: 8 × 10^3/μL, CRP: 0.1 mg/dL.

Case 3

On November 1, 2020, a 72-year-old male went to a private clinic with fever, cough, high respiratory rate (32/min), and reduced SpO2 (81% on room air), which started 7 days back. He was advised for hospitalization, but he refused and preferred to take treatment at home. The patient had DM and HTN in his past medical history. His RT- PCR was positive for SARS-CoV-2. His blood workup revealed WBC count: 6.4 ×10^3/μL with 56% neutrophils and 31% lymphocytes, platelet count: 170 × 10^3/μL, LDH: 288 IU/L, CRP: 24 mg/dL, AST: 32 IU/L, and ALT: 21 IU/L. His lung HRCT diffuse bilateral GGOS. He was treated with Favipiravir (FVP) (1600 mg BID on the first day and 600 mg BID for the next 5 days), IFN-β (44 μg/sc daily for 3 doses), and Dexamethasone (4 mg/day for 8 days). There was no response to the recommended treatment even after 7 days. His SpO2 was 81% and the patient had respiratory distress (28/min). On November 8, 2020, MB therapy (1 mg/kg every 8 hours for two days, followed by 1mg/kg every 12 hours for the next days) was initiated. After 7 days, his SpO2 reached 94% and the patient no longer had respiratory distress (respiratory rate: 16/min).

Case 4

On October 18, 2020, a 74-year-old female, with no past medical history, went to a private clinic with a fever, cough, and shivering along with a high respiratory rate (30/min) and reduced SpO2 (78% on room air), which started 7 days ago. She refused to be hospitalized and was treated symptomatically for 6 days at home. Her RT- PCR was positive for SARS-CoV-2. Her WBC count: 3.9 × 10^3/μL with 80% neutrophils and 15% lymphocytes, platelets counts: 180 × 10^3/μL, LDH: 614 IU/L, CRP: 16 mg/dL, AST: 23 IU/L, and ALT: 24 IU/L. Her lung HRCT depicted diffuse bilateral GGOS and consolidation in the peripheral lung regions. Upper and especially lower lobes were involved. On October 24, 2020, she was treated with Azithromycin (500 mg/day), FVP (1600 mg BID for the first day and 600 mg BID for the next 5 days), IFN-β (44 μg/sc daily for 4 doses), Meropenem 1 gr TDS, Vancomycin 1 gr BID and Dexamethasone (8 mg/day for 7 days) at her home. Three days later, FVP and IFN-β were discontinued because of elevated hepatic enzymes AST: 80 IU/L and ALT: 71 IU/L, exacerbation of fever and shivering along with respiratory compromised status. (SpO2 82%, respiratory rate 28/min). On November 1, 2020 MB therapy (1 mg/kg every 8 hours for two days, followed by 1mg/kg every 12 hours for the next days) was initiated. After one week, SpO2 reached 91% and respiratory rate settled down to 17/min.

Case 5

On October 10, 2020, a 44-year-old female was admitted to the internal medicine ward of the hospital because of a high respiratory rate (30/min), reduced SpO2 (75%), fever, cough, and shivering that all started 7 days prior to admission. Her past medical history was significant for splenectomy 24 years back due to the consequence of a motor vehicle accident. Her RT- PCR was positive for SARS-CoV-2. Her blood workup noted to have WBC count: 7.3 × 10^3/μL with 85% neutrophils and 13% lymphocytes, platelet count: 340 × 10^3/μL, LDH: 514 IU/L, CRP: Positive, AST: 30 IU/L, and ALT: 16 IU/L. Her lung HRCT displayed diffuse bilateral GGOS and consolidation in the peripheral lung regions. Upper and especially lower lobes were involved. She was treated with Azithromycin (500 mg/day), FVP for two days (1600 mg BID, first day 600 mg BID for the second day), Ceftriaxone 1 gr BID, Vancomycin 1 gr BID, and Dexamethasone (8 mg/day for 7 days). On October 14, 2020, her SpO2 was at 68% (on room air), FVP was discontinued, and Remdesivir was initiated. On October 16, 2020, her SpO2 further dropped down to 40%, and the patient was intubated. On October 18, 2020, SpO2 was 60%, and MB (1 mg/kg every 8 hours) was initiated. On October 18, 2020, SpO2 was 75%, and, unfortunately, the patient died. Her blood workup showed WBC count: 39.1 × 10^3/μL with 78% neutrophils and 18%

lymphocytes, AST: 220 IU/L, and ALT: 60 IU/L).

Discussion

Based on the results of the ongoing rescue therapy and RCTs, MB safely and effectively can be used for the treatment of COVID-19. In our Phase III clinical Trail, as the last option of treatment, MB saved patients who failed to respond to Remdesivir, IFN-β, FVP. MB encapsulates many of the required mechanisms for treatment: 1) Anti-viral activity against the SARS-CoV-2 virus [9,10]; 2) Anti-hypoxemia activity by converting iron from the ferric (Fe^{3+}) state to the ferrous (Fe^{2+}) state. Due to this property, MB is an approved medicine for methemoglobinemia [8]; 3) Anti-respiratory distress activity. The authors observed this effect in patients and have no explanation for its mechanism; 4) Inhibitor of nitrite production (nitrite converts ferrous iron to ferric iron in hemoglobin) by inhibiting nitric oxide synthase and guanylate cyclase in activated macrophages [11]; 5) Antimicrobial agent [12]; 6) Inhibitor of reactive oxygen species (superoxide anion and hydrogen peroxide scavenger) [13]; 7) Inhibitor of xanthine oxidase (which produces superoxide anion) [14]; 8) Anti-platelet aggregation drug [14]; 9) Anti-inflammatory agent [15,16]; 10) bronchodilator property [17]; 11) Antifungal agent [18], it is worth to mention that recently, alongside with the dramatic surge of COVID-19 in India, the number of cases of mucormycosis (Black Fungus) has risen surprisingly (about 80 times more often than in developed countries) [19,20]. Mucormycosis is a rare disease but also very serious and can be fatal, especially if diagnosis and prompt initiation of medical and surgical therapy are delayed. The intracranial involvement of mucormycosis increases the fatality rate to as high as 90% [21]. As far as for treatment, aggressive surgical debridement and antifungal agents are warranted. It is reported that in the COVID-19 pandemic, Mucormycosis is a serious threat [22] and the main drug for the treatment is amphotericin B; other antifungal like fluconazole and voriconazole have no effect. Considering the reported shortage of amphotericin B, its high cost, severe toxicity and serious side effects (acute side effects which postulated to result from proinflammatory cytokine production: nausea, vomiting, rigors, fever, hypertension or hypotension, and hypoxia; chronic side effects: nephrotoxicity include renal insufficiency, hypokalemia, hypomagnesemia, metabolic academia, and polyuria due to nephrogenic diabetes insipidus; these side effects sometimes lead to discontinuation of therapy despite a life-threatening systemic fungal infection) [23], it is necessary to take all measures to prevent it from spreading. Studies have shown that MB can destroy the mitochondria of fungus and eliminate it at concentration of 500 ppm [24]. Therefore, MB is considered as a gold drug (magic bullet) for the treatment of COVID-19. Therefore, MB is considered as "magic bullet" for the treatment of COVID-19. The effectiveness of MB for treatment can be observed in all patients who survived. It is worth mentioning that may be if MB is administered sooner in a golden time along with fluid therapy, oxygen support, antibiotics, anticoagulants, corticosteroids, and antioxidants, it can give more positive results, before multiple organ failure ensues. MB is in two forms: the oxidized form (oxidant: dark blue) and the reduced form (antioxidant, colorless). In the body, MB is reduced to LMB which is excreted primarily in urine, feces, bile [25]. MB has been widely used in various conditions, including as an antidote to paraquat poisoning, to treat ifosamide encephalopathy, to maintain blood pressure in patients with septic shock, and to support orthotopic liver transplantation [26]. There is a high levels of oxidative stress in COVID-19 patients [5]. When MB (the oxidized form) is orally used, it is converted to the reduced form [10]. Therefore, the oxidized form of MB intensifies oxidative stress in COVID-19 patients which lead to more hypoxemia. The reduced form of MB on the other hand, as an antioxidant, reduces oxidative stress and hypoxemia. Limitations of the study were the conducting of the trial in one university center with a small number of patients. In our clinical trials and recue therapy study, MB used along with other antiviral agents or as the last option of treatment. Further researches certainly be crucial to improve our understanding of the optimal place in MB therapy, which could be considered as the first choice of treatment for COVID-19.

Ethical Approval

IR.MUMS.REC.1399.122; Clinical Trials.gov Identifier: NCT04370288; April 19, 2020.

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