

SHORT COMMUNICATION

The Application of a Reduced Dye Used in Orthopedics as a Novel Treatment against Coronavirus (COVID-19): A Suggested Therapeutic Protocol

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Abstract

The severe acute respiratory syndrome caused by COVID-19 is now a global catastrophic event. Currently there is no approved drug or vaccine for the disease. Methylene blue (MB, oxidized form, blue color) has been used in many different areas of clinical medicine, ranging from malaria to orthopedics. Leucomethylene Blue (reduced form of MB, colorless) may be applied for the treatment of COVID-19 according to the scientific evidences. In severe patients, there is a cytokine storm (hyperinflammation) and high oxidative stress (OS). Inflammation and OS has a mutual correlation and exacerbate each other. In human body, MB first induces OS through absorbing electron (like a free radical) from other molecules, if the body could counteract to this OS, then reduced MB decreases OS through other mechanisms. Reduced MB could prevent inflammation, propagation of the virus RNA, and also improves hypoxia through reducing methemoglobin. Therefore, to avoid the increment of OS, we suggest using Leucomethylene Blue through the following protocol: The IV cocktail contains 50 mg MB (1mg/kg, 50-kg weight), 1000-2000 mg vitamin C, 500-1000 mg N-Acetylcysteine (or glutathione or cysteine or α -lipoic acid) and 10-20 gr urea (optional) in 100 ml dextrose 5%. Before the injection, the cocktail should be kept in a dark place for 1-2 hour to become fade or colorless.

Level of evidence: V

Keywords: Coronavirus, COVID-19, Leucomethylene blue, Methylene blue

Brief Description

In December 2019, in Wuhan, Hubei province (China), a pneumonia outbreak took place and spread nationwide quickly. A novel beta-coronavirus called COVID-19 was identified as responsible for this catastrophic pandemic disease with high lethality that spread globally (1). According to the last update (Apr 10 2020), the World Health Organization (WHO) confirmed that the infected cases and death toll are about 1,612,489 and 96,613, respectively. The WHO confirmed that 14% of the infected patients have severe disease and require hospitalization, 5% have very severe condition and require intensive care admission (mostly for ventilation),

and 4% die (2). Despite huge clinical efforts to overcome this catastrophic incidence, no drug or vaccine is currently approved for the treatment of COVID-19.

Methylene blue was first synthesized in 1876 and has been applied in many areas of clinical medicine. The first application of MB in medicine was the treatment of Malaria at 1892, then Gonorrhoea at 1897, and fever at 1908 and et cetera. Orthopedic applications of MB (blue color) are an easy and safe way to identify disruption of the joint capsule, guided debridement, a marker to find and remove tiny metallic foreign bodies embedded in the soft tissues and et cetera (3-8).

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In current medicine, the oxidized form of Methylene blue (blue color) is used, not the reduced form (colorless). The clinical applications of MB are for the treatment of pediatric and adult patients with acquired methemoglobinemia (FDA approved: 1-2mg/kg IV over 5-30 minutes), Ifosfamide-induced encephalopathy (50 mg IV every 4 hour till symptoms resolve), vasoplegic syndrome (2 mg/kg, 20-min infusion time), parathyroid imaging (3-7.5 mg/kg I.V.), Sentinel lymph node biopsy (local application of 1-5 ml of 1% MB), malaria (10 mg/kg twice a day orally), to identify disruption of the joint capsule, guided debridement, and as a marker to find and remove tiny metallic foreign bodies embedded in the soft tissues. The potential applications of methylene blue in clinical neuroscience are Alzheimer disease (10 nM-12 μ M *in-vitro*), depression, anxiety (15 mg/day orally in patients), pain (1 ml of 1% MB locally in humans) and intractable itching (10-15 ml of 0.5-1% MB locally in patients) (9).

Three main targets are considered for the treatment of COVID-19 patients:

1) Decreasing the propagation of RNA virus

MB could prevent the cytopathic effect and propagation of RNA virus (such as poliovirus) through the possible following ways: 1) There may be a mechanical effect, the easily penetrating reduced MB could competitively occupy cellular sites, necessary for virus attachment, penetration and/or multiplication, 2) Reduced MB may act through its redox-properties, completing qualitatively the defective oxidative processes of cells or by uncoupling oxidation and phosphorylation, 3) Direct or indirect virucidal effect. LMB (a lipophilic substance) penetrates into the virus through lipid membrane and attaches to RNA. (10).

2) Decreasing the hypoxia

One of the markers of hypoxia is methemoglobin resulting from the oxidation of the iron contained in hemoglobin from the ferrous to the ferric state. The oxidation is associated with decrement in the capacity of hemoglobin to carry oxygen. The treatment is intravenous infusion of MB (1-2 mg/kg body weight). The dye is reduced by NADH to leukomethylene blue (LMB) which in turn reduces the methemoglobin to hemoglobin. Patients with glucose-6-phosphate dehydrogenase deficiency (G6PDD) due to the risk of hemolytic anemia should avoid using MB (oxidized form) (11).

3) Decreasing the inflammation

Animal and human studies have shown that MB decreases inflammation, and also oxidative stress in individuals with non-impaired antioxidant mechanisms (12-15).

Inflammation and oxidative stress have a mutual correlation and exacerbate each other (15). Since MB (oxidize form) is an oxidative substance, like a free radical, it absorbs the electrons from other molecules (NADH-H⁺, NADPH-H⁺, GSH) and moderately increases the oxidative stress in the body. Healthy people can quench this oxidative stress by antioxidants. However, in severe

diseases, the oxidative stress overwhelms the body's ability to readily detoxify them, and then administering the oxidative substance increases the oxidative stress that causes more inflammation and injury.

In the human body, MB first induces the oxidative stress through absorbing electron (like a free radical) from other molecules, and then decreases the oxidative stress through other mechanisms. After injection, 65-85% of MB (oxidized form) is reduced to LMB. The oxidative effect of MB has documented that in culture media, dead cells, and senescent cells cannot reduce it to leukomethylene blue (colorless), therefore the cells remain blue (16). In all published papers, the missing point is that they use MB (oxidized form) which increases the oxidative stress, since at first MB should be reduced to LMB (a lipophilic substance), then it could penetrate into the cells. Since MB exacerbates oxidative stress, the deficiency in antioxidant defense system in diseases such as G6PDD results in intolerance to the increment of oxidative stress and there is a risk of hemolytic anemia. Also it is mentioned that methylene blue doses over 7 mg/kg paradoxically can exhibit an oxidizing effect, resulting in hemolysis and methemoglobin production (17).

On the other hand, reduced MB decreases the oxidative stress through other mechanisms such as improving mitochondrial respiration and decreasing oxidative stress in a substrate-dependent manner. The reduced MB blocks the iron containing enzymes such as xanthine oxidase that produces oxidants. MB constitutes a metabolic enhancer that accelerates the activity of the electron transport chain (18).

In severe and very severe COVID-19 patients, cytokine storm has been happening and if the oxidized form of methylene blue is given to patients, it causes more oxidative stress and consequently more inflammation, which in turn may worsen the situation.

The positive effect of MB in quenching inflammation is through the reduced form. In a recently published paper, Henry reported that both chloroquine and MB have strong antiviral and anti-inflammatory properties probably linked to the change in intracellular pH and redox. The cancer patients were treated by a combination of therapy included α -lipoic acid (800 mg twice a day), hydroxycitrate (500 mg three times a day) and Methylene blue (oxidized form: 75 mg three times a day) as well as a low carb. diet. It is assumed that at first, the antioxidant mechanism of these patients maybe quench the oxidative stress which is induced by MB and then the LMB decreases the oxidative stress and inflammation through other mechanisms (19).

MB aggregates (dimer, trimer or larger) in concentration more than 10 μ M, which limits its diffusion into the cells. MB destructs in light exposure. It is proved that the addition of urea to an aqueous solution of MB at pH 7.4 dissociates the MB molecules to monomer by forming a weak complex with MB (1:1) and also prevents the destruction of MB (20, 21). The apparent binding constant ($K=1.2 \times 0.4 \text{ M}^{-1}$) is small, ensuring that high concentrations of urea are required to saturate the complexation event. Urea has been used in pharmacology to enhance the penetration of other drugs into the

tissues and also for its anti-inflammatory effects (22). Furthermore, Urea has been used for treatment of severe chickenpox encephalopathy (intravenously), sickle cell trait hematuria (orally), and syndrome of inappropriate antidiuresis (International guidelines: orally) (23-25).

Vitamin C and N-Acetylcysteine are routine drugs that are used in the treatment of lung injury according to the guidelines.

The suggested protocol for the treatment of COVID-19 patients is using of the reduced form of MB along with antioxidants.

The protocol for sever and very sever patients (intravenously):

Add 50 mg MB (1mg/kg, 50-kg weight), 1000-2000 mg vitamin C, 500-1000 N- Acetylcysteine mg (or glutathione or cysteine or α -lipoic acid), and 10-20 gr urea (optional) into 100 ml dextrose 5%. Before injection, the cocktail should be kept in a dark place for 1-2 hour to become fade or colorless.

The protocol for infected cases (minor or moderate form, orally):

Add 50-300 mg MB (1-6mg/kg body: 50-kg weight), 1000-2000 mg vitamin C, 500-1000 mg N-Acetylcysteine (or glutathione or cysteine or α -lipoic acid), and 10-20 gr urea (optional) into 50 ml dextrose 5%. Before drinking, the cocktail should be kept in a dark place for 1-2 hour to become fade or colorless. α -lipoic acid can be taken orally.

It is suggested that these protocols also could be applied for the treatment of methemoglobinemia in all patients (even patients with G6PDD).

To perform a clinical trial in Mashhad University of Medical Sciences, we suggested a proposal (code: 990096) "application of MB for treatment of COVID-19 patients". As patent is applied, we described a hypothesis and a new therapeutic protocol by application of the

reduced (colorless) form of MB: Leucomethylene Blue. Also, the authors hypothesized that patients with severe conditions may have impaired antioxidant mechanisms which cause a mild inflammation and after viral infections, inflammation and oxidative stress exacerbate each other which lead to severe disease.

Key Points

1. Never ever use Methylene blue (the oxidized form) for treatment of COVID-19, since it increases oxidative stress and consequently inflammation.

2. The reduced form of methylene blue (Leucomethylene) should be used for treatment.

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