Rational Use of Methylene Blue in COVID-19 Treatment

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1. INTRODUCTION

In November 2020 FDA approved a clinical trial for using Methylene blue (MB) in covid treatment, and now the participants are in phase 2 trial. In 1876 methylthionine chloride (methylene blue) was synthesized by a German chemist, Heinrich Caro, which is used as an aniline-based dye for cotton dying industrial usage. Various applications within the medical field have been explored, and currently, there are >21000 entries available in PubMed for "methylene blue".

Methylene blue (MB) entered the medical field when Robert Koch and Paul Ehrlich introduced the staining of tuberculosis microorganisms using methylene blue. It is used as an anesthetic for its sensory nerve ending blocking property, and its antimicrobial activity was useful as an antimalarial therapy in the 1890s. In the modern medical field, methylene blue was majorly used for investigational purposes as contrast in many radiological diagnostic techniques. Further applications of methylene blue in various clinical indications like Alzheimer’s disease, depression, and psychosis are under investigation. Human Coronavirus invasion will cause activation of the innate and acquired immune system. This process results in the massive elimination of infected cells. Methylene blue can play a significant role in treating SARS-CoV-2 infection by inhibiting viral replication and preventing sepsis due to covid illness. Theoretically, anti-inflammatory activity, oxidative stress, and tissue damage caused by covid infection can be minimized, and thereby it can also be beneficial for the patients suffering from cytokine storm. From this review article, we conclude that methylene blue should be evaluated for its role in covid treatment at a clinical level.

Keywords: SARS-CoV-2, Antivirus activity, Cytokine storm, Nitric oxide, Antidote activity.

2. PATHOLOGY OF SARS-CoV-2

The first reported case of SARS COV 2 was found in the city of Wuhan, Hubei province republic of china, in December 2019. The genetic sequence of the novel coronavirus was studied and released in mid of January 2020. Coronavirus has trimeric spike-like structured (S) glycoprotein on its surface. This S glycoprotein initiates the binding of the virus onto the host cell surface protein angiotensin-converting enzyme 2, and this S protein is composed of subunits such as S1, which helps in the receptor binding, and S2 in the fusion of the virus envelope with the human cell membrane and release the nucleocapsid into the cytoplasm.

When the viral nucleocapsid enters into the cytoplasm, the virus's replication process starts with the translation of ORF 1a and 1b into polyproteins pp1a and pp1ab. Replicated new genomic RNA will be molded with helical nucleocapsid, which interacts with viral structural proteins. Replicated virions are transported through vesicles and released from the cell by the process of exocytosis. In this process, many proteins play a significant role, such as E protein and M protein.

Human Coronavirus invasion will cause activation of the immune system and induce apoptosis in human cells such as monocytes, macrophages, T lymphocytes, and dendritic cells that result in the activation of the innate and acquired immune system. This process results in the massive elimination of the infected cells. Studies reveal that the coronavirus damages...
dendritic cells, enhancing the spread of the virus and activating the host immune system.12

Kallikrein-Kinin System: This system consists of many proteins and peptides and possesses major physiological roles in the heart, blood vessels, skin hemostasis, ocular functions, coagulation cascade, inflammation, and pain pathway.10, SARS-CoV-2 enters into the lungs and interacts with ACE2 (Angiotensin converting enzyme) receptors and downregulates its function and this leads to activation of bradykinin receptor B1 and also increases pro-inflammatory mediators release such as TNF (Tumor necrosis factor), MIP2 (Macrophage inflammatory protein). This interaction also causes neutrophil infiltration and exaggerates inflammatory processes and can result in angioedema in infected patients.19,20

This downregulation of ACE activity causes an imbalance in the RAAS [Renin angiotensin aldosterone system] pathway, which leads to an increase in cytokine synthesis by activation of Nitric oxide (NO) and prostaglandins synthesis; this causes exudation and vascular leakage and stimulates the formation of free radicals and inhibit the production of ATP in mitochondria by altering TCA cycle and the glycolytic pathway. The appearance of pro-inflammatory cytokines such as IL-17, IL-6, IL-2, IL-18, TNF-alpha, and IL-1 beta leads to stimulation of NO synthesis.21 IL-2 is massively high in COVID-19 subjects22, and IL-2 is known to stimulate NO formation in COVID-19 subjects.23 Nitric oxide acts as a key mediator of IL-2 and causes vascular leak syndrome and hypotension.24 IL-6 upregulates inflammatory cytokines in COVID-1922, TNF-alpha and IL-6 stimulate superoxide radicals in neutrophils25,26, and hydrogen peroxide can promote the formation of IL-6 and other inflammatory mediators.27 This leads to the formation of cytotoxicity in the cell and further leads to blockage of lipid peroxidation28 and apoptosis. This is a life-threatening condition in COVID-19 patients called cytokine storm.29 Another important impact of kallikrein-kinin system dysregulation is an imbalance in coagulation cascade which tends to increase the formation of microthrombi,30 which is well-known to occur in COVID-19 patients.

3. THE PHARMACOLOGICAL ACTIVITY OF METHYLENE BLUE THAT CAN SUPPORT IT AS A THERAPEUTIC AGENT IN SARS-COV-2

3.1. Antiviral activity:

Methylene blue has a virucidal effect. It acts by interacting between the coronavirus spike protein (macromolecules) and the ACE2 receptor.31 It prevents the maturation of phagosomes and also inhibits replication and translation of virus through interaction with RNA. It prevents substrate-level phosphorylation in the mitochondria and fosters the formation of hydrogen peroxide (H2O2) ions, thereby leading to oxidative destruction of the SARS-CoV-2. It alters intracellular pH by alkalization of endosomes and lysosomes, which help to decontaminate the virus. Methylene blue and its reduced and unchanged derivative (leukoMB) can easily cross the plasma/blood transfusion barrier, which is a significant method of drug delivery. The formation of hydrogen peroxide (H2O2) ions, thereby leading to oxidative destruction of the virus. Methylene blue and its reduced and unchanged derivative (leukoMB) can easily cross the plasma/blood transfusion barrier, which is a significant method of drug delivery. The formation of hydrogen peroxide (H2O2) ions, thereby leading to oxidative destruction of the virus. Methylene blue and its reduced and unchanged derivative (leukoMB) can easily cross the plasma/blood transfusion barrier, which is a significant method of drug delivery. The formation of hydrogen peroxide (H2O2) ions, thereby leading to oxidative destruction of the virus. Methylene blue and its reduced and unchanged derivative (leukoMB) can easily cross the plasma/blood transfusion barrier, which is a significant method of drug delivery. The formation of hydrogen peroxide (H2O2) ions, thereby leading to oxidative destruction of the virus.

Experimental studies of Methylene blue in inactivation of virus in convalescent plasma therapy is explained by the following: The viral RNA of SARS CoV243,43 or MERS46 (Middle East Respiratory Syndrome), SARS-CoV-7, is detected in blood plasma. WHO predicted in early 2003 that SARS-CoV2 could contaminate blood products. So far, none of the cases have been reported due to blood transfusion, but theoretically, there could still be the possibility of a blood transfusion-related infection.38 But in this critical scenario, the safety of plasma/blood transfusion is more important.39 Convalescent plasma therapy could be an effective and rapid treatment for SARS-CoV-2.44,45 However, in this situation, it is necessary to inactivate the viral pathogen in the plasma. This can be done by photochemical treatment, which is widely used for inactivating viral and bacterial pathogens. This is done by adding methylene blue to blood products in the presence of ultraviolet light using a photochemical process. Thiamine ring of methylene blue undergoes a redox reaction to collect electrons, which are then converted to leuko-methylene blue, and then electrons are transferred to other components. It also combines with oxygen, and an energy source like metal halide lamp, halogen lamp, or fluorescent 46 lamp enters into electrochemical ground state reactions in the singlet-oxygen state. This often forms a highly reactive oxidizing form of oxygen (Boxo7, 8-dihydroguanine (BoxoGua))44,45 that damages the viral DNA or RNA of the coronavirus.46,47,48

3.2. Anti-inflammatory activity:

Cytokine release syndrome seen in the severe COVID-19 patients will exhibit a considerable increase in pro-inflammatory cytokines such as IL-17, IL-6, IL-1B, IL-8, IL-2, and TNF-alpha. This is all mediated by NOD (Nucleotide-binding oligomerization domain) -like receptor protein 3 (NLRP3), which regulates inflammation. This results in tissue damage and organ failure. Methylene blue inhibits the formation of the NLRP3 protein complex, thereby reversing the mRNA expression and alter the stimulation of cytokines like IL-12, IL-6, IL-6, IL-1 LALPalpha. Methylene blue also alters cytokine production via NF-κb (Nuclear factor kappa light chain enhancer of activated B cells) signaling pathway 31,22 and is hence useful as a therapeutic option.

3.3. Rejuvenation of Mitochondria and Superoxide inhibition:

1. Normally, cell cellular respiration takes by the process of glycolysis in cytoplasm and Krebs cycle in mitochondria. This is by the process of oxidative phosphorylation. NAD+ is the coenzyme used in cellular respiration to transport high potential energy electrons to the electron transport chain in the mitochondria. This leads to the production of energy in the form of ATP. In hypoxia conditions, the formation of NAD+ is inhibited. Hence, pyruvate, which is the product of glycolysis, reduces lactate dehydrogenase. This is an anaerobic glycolytic pathway that reduces the pH in the cell, causing acidosis. MB administered gets converted to leuko-methylene blue, which helps in the restoration of NAD+ and hence glycolysis. Methylene blue also acts as a coenzyme in the electron transport chain. It also promotes formation of acetyl CoA from pyruvate rather than lactic acid.49

2. Due to imbalance within the mitochondria of infected host cells, there is formation of free radicals such as superoxides, hydrogen peroxide, peroxyl radicals, hydroxyl radical.50 Free radicals are formed during intracellular redox reactions in mitochondria and are essential for many physiological functions such as gene expression, signal transduction, and massive amounts in COVID19 of free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed.51,52 This leads to the inhibition of endogenous proteins, nucleic acids, lipids, ROS can significantly regulate the cell proliferation adaptations and irreversible damage to the cell.53 ROS formation is inhibited by Methylene blue by blocking the xanthine oxidase enzyme.54,55. And hence useful in therapeutic options.

3.4. Nitric oxide synthase enzyme inhibition:

Nitric oxide (NO) acts as a signaling molecule between endothelial and smooth muscle through the conversion from...
L-arginine to nitric oxide, which is mediated by Nitric oxide synthase. It is mediated by endothelial-derived relaxing factor (EDRF), as a response to stimulation of certain hormones and neurotransmitters leading to stimulation of vascular endothelial cells. It causes the influx of Ca2+ ions, which leads to activation of calmodulin complex, which helps in the conversion of an inactivated form of NO to the activated form of NO. Methylen blue blocks the guanylate cyclase enzyme, which is responsible for retraction of free heme group and smooth muscle contraction. Methylen blue blocks nitric oxide synthase enzyme. Inhibition of NO synthesis can lead to decrease in IL-6 formation more than 50% and hence useful as a therapeutic option.

3.5. Antidote activity:
According to case reports of Covid-19, in some patients hypoxia is due to the formation of met-hemoglobin in the blood. Hemoglobin is formed by the combination of iron (Fe3+) and globin. Met-hemoglobin is a type of hemoglobin formed spontaneously in the human blood and is bluish chocolate brown in color. Met hemoglobin doesn't carry oxygen to the tissues, and it is reduced to hemoglobin by the NADH or NADPH-dependent methemoglobin reductase enzyme. Ingestion of certain pharmaceutical chemicals, toxins, or broad beans leads to methemoglobinemia. The higher concentration of methemoglobin in the blood decreases the oxygen supply to the cells/tissues. MB acts as an antidote for methemoglobin. When MB is administered intravenously, it converts itself into leucomethylene blue reducing heme content from methemoglobin, converting it to hemoglobin which increases oxygen delivery to the tissues.

4. PHARMACOKINETICS.
The details about the Absorption of methylene blue are poorly understood. The volume of distribution of methylene blue is found to be 10 mg/kg in the studies carried out on rats. MB is a highly protein binding drug (71-75). It is readily distributed in muscle tissue. Up to 75% of MB is excreted through urine as leucomethyleneblue. Significant amount of MB was found in bile. IV administration MB has shown 5-6.5hrs of half-life.

5. CONTRAINDICATION
Methylene blue use is contraindicated in people who have developed hypersensitivity reaction to it, Glucose-6-phosphate dehydrogenase (G6PD) deficiency as it can cause severe hemolysis. Its use is contraindicated in pregnancy (Category X) as it can cause fetal death. MB should not be combined with Antidepressants such as Selective serotonin reuptake inhibitors [SSRIs]. Selective Non-serotonin reuptake inhibitors SNRIs, Mono-amino-oxidase inhibitors [MAOIs], and Tricyclic antidepressants [TCAs] can cause serotonin release syndrome which can be life threatening.

6. DOSE OF METHYLENE BLUE
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<tr>
<th>Route Of Administration</th>
<th>Treatment Regimen</th>
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<tr>
<td>a) Intravenous(Iv)</td>
<td>Initially, give high dose Vit B complex and 2 tab of Pyridium 2 hours before MB. Followed by 5mg Vit C with 100mg MB in 200ml N saline for 4 hrs before IV infusion. Or 1mg of MB in 100ml saline over 1 hour for 3days.</td>
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<td>1mg/kg body weight in ICU admitted patients with highly oxygen requirement (SpO2 &lt;200)</td>
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<tr>
<td>b) Nebulizer</td>
<td>5ml of 0.1% MB + 1ml Dexamethasone sodium + 1 ml of levosalbutamol and ipratropium bromide respirator solution.</td>
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<tr>
<td>c) Oral</td>
<td>2-3mg/kg/day orally in 3 divided doses for 7 to 10 days (For newly diagnosed (initial) covid-19 patients)</td>
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(*) As per webinar proceedings – Dr. Golwalker’s protocol; https://youtu.be/dS1CwUdU

7. ADVERSE DRUG REACTIONS ASSOCIATED WITH THE METHYLENE BLUE:
Urine discoloration is frequently reported ADRs of MB. IV administration of MB can cause limb pain. Methylene blue can cause CNS-related side effects such as dizziness, confusion, and headache. Administration of methylene blue can cause hemolytic anemia, pulmonary edema, respiratory depression, hyperbilirubinemia, and phototoxicity, which is noticed in neonates. Hence patients should be closely monitored on this.

CONCLUSIONS:
The covid-19 disease predominantly damages lung and vascular endothelium because of the presence of ACE2 receptors, which provide the entry point. This results in the production of reactive oxygen species and excess cytokine release that causes tissue damage. The virus enters into the interstitial space, causing oxidative stress and results in apoptosis or necrosis and damage to vascular endothelial tissue and alveoli. These cytokines, ROS and RNS, enter into the systemic circulation resulting in damage of endothelium of other organs. The viral protein circulates and can lead to multi-organ failure. In covid-19, when ROS, RNS, and cytokine production are out of control and a single cytokine inhibitor can fail to show an effect.

According to current scientific research at this juncture, MB can act as a multi-modal therapy during covid-19 disease. From the above evidence, methylene blue can play a significant role in treating SARS-CoV-2 infection by inhibiting viral replication and reducing sepsis due to covid illness. By its anti-inflammatory activity, oxidative stress and tissue damage caused by covid infection can be minimized, and thereby it can also be used for covid-19 patients with mild to severe disease and also patients suffering from cytokine storms. Rational use of methylthioninium chloride (methylene blue) in SARS-CoV2 infection potentially reduces the mortality and morbidity rate. Therefore, this review article concludes that methylene blue can play a crucial role in covid treatment in all stages.

Abbreviations:
MB: Methylene blue; SARS-CoV-2: Severe acute respiratory distress syndrome-coronavirus-2; TNF: Tumor necrosis factor;
MIP2: Macrophage inflammatory protein; ACE: Angiotensin-converting enzyme; NO: Nitric oxide; RAAAS: Renin angiotensin aldosterone system; PH: Potential of hydrogen; NOD: Nudose-binding oligomerization domain; ROS: Reactive oxygen species; RNS: Reactive nitrogen species; EDRF: Endothelium-derived relaxing factor; NF-kB: Nuclear factor kappa light chain enhancer of activated B cells; G6PD: Glucose-6-phosphate dehydrogenase; SSRIs: Selective serotonin reuptake inhibitors; SNRIs: Selective Non-serotonin reuptake inhibitors; MAOIs: mono-amino-oxide inhibitors; TCA: Tricyclic antidepressants.

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