IJBPAS, July, 2024, 13(7): 3524-3534

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

A MONUMENTAL CRUSADE OF METHYLENE BLUE FROM A STAINING DYE TOWARDS A POTENTIAL DRUG CANDIDATE FOR INNUMERABLE DISEASES: A COMPREHENSIVE REVIEW

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Received 15th March 2023; Revised 8th July 2023; Accepted 23rd Oct. 2023; Available online 1st July 2024

https://doi.org/10.31032/IJBPAS/2024/13.7.8190

ABSTRACT

Methylene blue, also known as methylthionium ion, is an organic chloride salt having phenothiazine heterocyclic ring. Initially, it was synthesized as a dye. Methylene blue is an active inhibitor of enzymes like nitric oxide synthase and guanylate cyclase. For the same reason, it was used to treat cyanide poisoning and urinary tract infections in previous years. Now-a-days, methylene blue is the most promising agent to treat innumerable diseases like methemoglobinemia, psoriasis, septic shock, and ifosfamide toxicity. It is under research for the treatment of disparate diseases like malaria, bipolar disorder, AIDS related kaposi's sarcoma, west nile virus, HIV-1 virus etc. This agent has truly accomplished its journey from a staining dye to a potential drug candidate.

Keywords: Methylene blue, Methemoglobinemia, West Nile virus, Kaposi's sarcoma, Vasoplegia, Toxoplasmosis, Cytomegalovirus

INTRODUCTION

Dyes and Stains are colored compounds that can well bind to biological tissues by means of chemical reactions. Very tiny and colorless life forms like bacteria,

fungi, yeast that are invisible in naked eye but with the help of microscope and dye it can be possible to visualize them. Microscope can able to magnify tiny things in to bigger one and dyes can convert the colorless things in to color one.

Methylene blue, a synthetic, bright greenish blue organic dye, was discovered in 1876 by a German chemist Heinrich Caro and introduced in 1886. Mainly it is employed to stain bast, silk, wood, paper, leather and mordanted cotton. Also, it is useful for staining tiny biological forms, in testing milk for Mycobacterium tuberculosis infection and as a redox indicator. It is nitric oxide synthase and guanylate cyclase inhibitor. **Figures 1 (A), 1 (B)**, and 2 shows powdered, staining, and structure of methylene blue respectively **[1, 2]**.

Methylene blue can be synthesized by the reaction between N^1,N^1-Dimethylbenzene-1,4-diamine and N,N-Dimethylbenzenamine in presence of sodium thiosulphate. This was first prepared by Heinrich Caro in the year 1876 **[3]**. **Figure 3** shows synthesis of methylene blue.

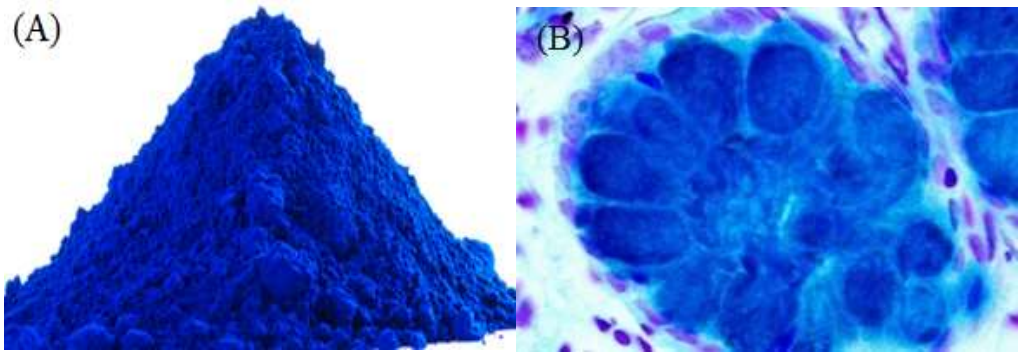

Figure 1: (A) Powdered Methylene blue (B) Staining of Methylene blue

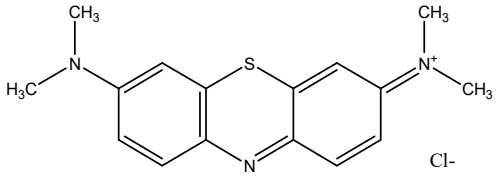

Figure 2: Structure of Methylene blue

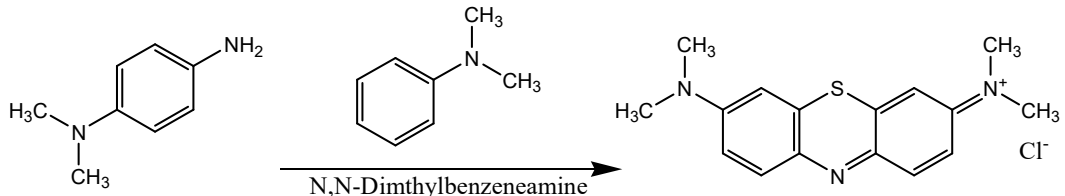

Figure 3: Synthesis of Methylene blue

Methylene blue was the first synthetic candidate molecule for the treatment of malaria before 100 years approximately. Also, at the end of 19th century, this was the first molecule to treat psychosis and as this drug contains phenothiazine nucleus, Methylene blue was a leading molecule as antipsychotic agent in the mid 20th century. In 1980s, it has been found that this drug can be useful to treat bipolar disorder and recently it has been proved that this can be effective in neurodegenerative disorder and included in the World Health Organization's List of Essential Medicines.

The purpose of the study

In this present review, we have included the potential and novel use of methylene blue as well as the research study of methylene blue in the disparate diseases. Also, in the forthcoming decades, research scientists can able to gain some dogma from this essential drug candidate for the further investigation purpose **[5, 6]**.

Methylene blue for the treatment of various diseases

Initially, Methylene blue was discovered for staining of biological tiny forms and some fibers, paper, and leather but because of its potential to treat the diseases; it can be employed for the treatment of numerous diseases.

Methylene blue in treatment of Methemoglobinemia

Oxidization of ferrous (Fe^{+2}) iron of hemoglobin in to ferric (Fe^{+3}) results in methemoglobinemia. Ferric heme (Fe^{+3}) cannot able to bind with oxygen that makes releasing of oxygen more difficult. Methylene blue is the best one for the first line treatment of this. It gets reduced in to leucomethylene blue by NADPH dependent methemoglobin reductase, which increases reduction activity of enzyme NADPH-methemoglobin reductase on methemoglobin and converts it in to hemoglobin back. So by decreasing methemoglobin level, it increases oxygen release. Methylene blue cannot be given to patient with G6PD deficiency that decreases NADPH level and cause hemolytic anemia **[7]**.

Methylene blue in the treatment of Psoriasis

Photodynamic therapy of 0.1% Methylene blue hydrogel that photo activated by light emitting diode can be effective topically in patients with resistant psoriasis plaque. Methylene blue as a photosensitizer for photodynamic therapy has found to be safe and effective treatment for the same **[8]**.

Methylene blue in the treatment of Urinary tract infections

Methylene blue is a weak antiseptic that kills bacteria in urinary tract **[9]**. As, it can able to stain other biological tissues, so cannot be given as single medication. Two combination products are available in the market that can treat urinary tract infections. One product is Prosed D/S tablets that contain Methylene blue, methanamine, phenyl salicylate, hyoscyamine and benzoioc acid whereas second product is Urelle, Urimar-T and Utira-C tablets that contains Methylene blue, methanamine, phenyl salicylate, hyoscyamine and sodium biphosphate. Other combination products are Atrosept, Dolsed, UAA, Uridon modified, Urised, Uritin **[10]**.

Methylene blue in the treatment of cyanide poisoning

Methylene blue can be utilized as an antidote for cyanide as well as carbon monoxide poisoning. The use of this drug as a cyanide antidote was first expressed by Sahlin of Lund University in 1926. The same use was established in 1930 by Eddy of Michigan University. Eddy disclosed that respiration that depressed by sodium and potassium cyanide can be well stimulated by Methylene blue and helps an animal to survive **[11, 12]**.

Methylene blue in the treatment of ifosfamide toxicity

Ifosfamide is one of the potent anticancer agent that can be used in diverse range of carcinoma that includes sarcoma, gynecological cancers, testicular cancers and lymphoma. Ifosfamide can be metabolized and converted in to chloroacetaldehyde which is toxic metabolite. This metabolite has much more side effects like CNS toxicity, myelosuppression, metabolic acidosis, nausea and vomiting. Major CNS neurotoxicity includes seizure, coma, and death whereas lethargy, confusion, delirium, drowsiness, hallucinations, change in personality can also be seen. Methylene blue is a potential candidate that introduced to treat this ifosfamide generated neurotoxicity in 1994. Several mechanisms have been suggested by which methylene blue shows its effect. This includes inhibition of monoamineoxidase (MAO) enzyme thereby decreasing chloroacetaldehyde formation and act as an electron acceptor **[13, 14]**.

Methylene blue in the Septic shock therapy

Sepsis is a life protesting disease that occurs as a response of body to the infection. It is a prevalent disease of morbidity and mortality that seen in severely ill patient. This results in critical systemic inflammatory response that occurs due to vast microorganisms. Various factors like tumor necrosis factor a, interleukin-13, and other inflammatory mediators can be stimulated by bacterial endotoxins that can activate the iNOS. iNOS

enhances production of NO that acticate GC and increases cGMP concentration in smooth muscles that leads to myocardial depression **[15]**. Injury to the tissues and organs causes life challenging lowering of blood pressure that leads to a severe fatal condition, a Septic shock. NO-cGMP pathway performs a major role in shock. Methylene blue, a potential NO-cGMP pathway inhibitor, can become a drug of choice in case of unavailability of the conventional drugs **[16, 17]**.

Methylene blue in the treatment of refractory vasoplegia

Vasoplegia is the condition that consists of low arterial blood pressure usually less than 50 mmHg. This occurs due to improper synthesis of nitric oxide. Guanylate cyclase, a soluble intracellular enzyme that is activated by nitric oxide to produce cyclic guanosine monophosphate. Methylene blue inhibits this guanylate cyclase enzyme and decreases concentration of cGMP. This results in relaxation of vascular smooth muscles **[18-20]**.

Methylene blue in the treatment of hepatopulmonary syndrome

Hepatopulmonary syndrome is caused by dilation of blood vessels in the lungs due to increase in cGMP concentration and makes the red blood cells difficult to absorb oxygen properly. This decreases PaO_2 level. As, Methylene blue is a potent inhibitor of guanylate cyclase, it decreases the cGMP concentration, and contracts blood vessels in the lungs and increases PaO_2 level **[21]**.

Methylene blue in current research

Methylene blue in the treatment of Malaria

Methylene blue was the earliest synthetic antimalarial agent and employed against all types of malaria in late 19^{th} and early 20^{th} centuries. Methylene blue, having chloroquine sensitizing property, can be given in combination with chloroquine. In the digestive organelles of *Plasmodium falciparum*, it interferes with hemoglobin and heme metabolism. It is a selective inhibitor of *Plasmodium falciparum* glutathione reductase that depletes glutathione and sensitizes the malarial parasite for the action of Chloroquine **[22-25]**. Also Methylene blue can potentiate the antimalarial action of non curative dose of chloroquine, pyrimethamine, and quinine when it is given in combination with these three agents. The combination therapy of Methylene blue with either of these three can improve the efficacy of currently employed antimalarial agents [26]. Combination therapy of Methylene blue-Amodiaquine is an advantageous backup against malaria in Africa. In combination with artemisinin derivatives, Methylene blue has an ability to quicken the parasite clearance rate **[27]**.

Methylene blue in the Bipolar disorder therapy

Patients suffering from bipolar disorder are having extensive source of disability like residual symptoms and cognitive impairment. It stabilizes function of mitochondria and generates reactive oxygen species. This role makes it to be used in the treatment of neurodegenerative disorders. The drug has treated psychotic and mood disorders for about hundred years. Being a memory enhancing drug, Methylene blue has also been employed as an additional drug to cure residual symptoms of depression and anxiety in the patients suffering from bipolar disorder **[28, 29]**.

Methylene blue in the treatment of AIDS related Kaposi's sarcoma

AIDS related Kaposi's sarcoma is a type of malignant cancer disease that involves formation of masses in skin, lymph nodes and other organs in AIDS patients. Having photophysical, photochemical and photobiological essence, Methylene blue can be contemplated as a drug for photodynamic therapy. Application of Methylene blue in combination with continuous light source (RL50®), can treat basal cell carcinoma, Kaposi's sarcoma, melanoma, viral, and fungal infections. This combination therapy has found to be successful to treat various cancerous and non-cancerous diseases with having a minimum toxicity and nil side effects **[30-32]**.

Methylene blue in the treatment of West nile virus

West nile virus is a viral disease occur as a result of biting of an infected mosquito mostly in the continental United states. This is a seasonal disease can be seen from the starting of summer till rain fall. Fever can be observed in one out of five infected patients and this may be serious as well as fatal in one out of one hundred and fifty patients. No any vaccines have been found yet that can treat WNV **[33]**. Phototreatment of methylene blue shows a better potent treatment to destroy viral RNA so inactivate WNV in plasma **[34]**. Methylene blue phototreatment is a safe and cost effective treatment to inactivate WNV **[35]**.

Methylene blue in the treatment of HIV-1 virus

HIV is the species of Lentivirus that infect the human beings. At present there are two key types of HIV. One is the most familiar, known as HIV-1 and another is somewhat rare, known as HIV-2. If HIV-1 is untreated then it influence and kills CD4 T cells, a type of immune cell that lead to Acquired Immune Deficiency Syndrome (AIDS). AIDS is a sexually transmitted disease that occurs due to

contact of blood, pre-ejaculate, semen, vaginal fluids, and breast milk that leads to continuous and progressive failure of immune system of humans **[36, 37]**. After the period of time, HIV-1 kills more and more CD4 T cells leading various types of conditions like pneumonia, tuberculosis, oral thrush, cytomegalovirus (CMV), toxoplasmosis, cryptococcal meningitis, cryptosporidiosis as well as Kaposi sarcoma and lymphoma like cancer diseases. Phototreatment of Methylene blue on HIV-1 leads to inhibition of RNA of the virus. Methylene blue affects on HIV-1 at various target sites like envelope, core proteins, inner core structures of RNA, and reverse transcriptase (RT) **[38, 39]**.

Methylene blue in photodynamic inactivation of *staphylococcus aureus*.

Addition of potassium iodide to methylene blue results in the formation of reactive iodine with short life. This causes photodynamic inactivation of *s. aureus* and effective against multi drug resistant strains **[40]**.

CONCLUSION

Methylene blue, initially synthesized as a dying agent that was used in endoscopic polypectomy, chromoendoscopy, spraying of mucosa in the gastrointestinal tract. This was the first synthetic derivative to treat malaria and psychosis. Methylene blue has some common side effects like vomiting, headache, mental confusion, shortening of breath, increase in blood pressure whereas some other side effects like allergic reactions, breakdown in red blood cells, and serotonin syndrome are also reported. Even though, these kind of side effects, this molecule has shown its effectiveness to treat various diseases like methemoglobinemia, psoriasis, urinary tract infections, cyanide and ifosfamide poisoning, septic shock, refractory vasoplegia, hepatopulmonary syndrome. Methylene blue is now under research in the treatment of bipolar disorder, AIDS related Kaposi's sarcoma, west nile virus, HIV-1 virus and also it will be the most promising agent for the treatment of these same diseases.

Abbreviations

AIDS = Acquired Immuno Deficiency Syndrome

HIV-1 = Human Immuno Deficiency Virus-1

NADPH = Nicotinamide Adenine Dinucleotide Hydrogenase

G6PD = Glucose-6-Phosphate Dehydrogenase

CNS = Central Nervous System

iNOS = Inducible Nitric Oxide Synthase

NO = Nitric Oxide

GC = Guanylate Cyclase

cGMP = Cyclic Guanosine Mono Phosphate

NO-cGMP = Nitric Oxide- Cyclic Guanosine Mono Phosphate

PaO_2 = Symbol for arterial oxygen pressure

WNV = West Nile Virus

RNA = Ribonucleic Acid

CMV = Cytomegalo Virus

RT = Reverse Transcriptase

Ethics Approval and Consent to Participate

Not applicable.

Human and animal rights

No animals/humans were employed in the studies interpreted in this review.

Conflict of Interest

Not applicable.

Conflict of Interest

The authors asserted no conflict of interest, financial or otherwise.

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