



Clinical effectiveness and prospects of methylene blue

A systematic review

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ABSTRACT

Methylene blue (MB) is a well-known pharmaceutical ingredient that is thought to have a multi-targeted therapeutic effect as an anti-malarial and neuroprotective agent and has recently been identified as a treatment for coronavirus disease 2019 (COVID-19). In this review, we present an overview of relevant clinical trials, including ongoing trials, on the therapeutic uses of MB. A search for clinical trials on clinicaltrials.gov was performed using the terms “methylene blue” and “methylthionine chloride.” This review focuses on clinical trials of MB-based therapies applied to brain diseases, cancer imaging and diagnosis, infectious diseases such as malaria or COVID-19, and cardiovascular diseases. Nanoparticle-based delivery techniques have also been briefly discussed in addition to common delivery methods.

Keywords: Diagnostic imaging; Methylene blue; Photochemotherapy; Photosensitizing agents; Reactive oxygen species

INTRODUCTION

Methylene blue (MB) or methylthionine chloride is a cationic heterocyclic compound with a photosensitizing nature and is widely used in clinical medicine, including surgery [1-3]. MB has a maximum absorption wavelength of 668 nm and exists as a redox couple in equilibrium between oxidized blue MB and reduced colorless leucomethylene blue (LMB) [4]. MB is a promising lead compound for developing therapeutics for various diseases, including viral infections, cancer and dementia [5-7]. The long history of the applications of MB can be traced back to the early studies of Paul Ehrlich on its use for tissue staining and the development of chemotherapy basics [8] and includes research on the use of MB as an anti-malarial [9] and as an antidote for the toxic side effects of methemoglobin, cyclophosphamide, and cyanide poisoning, mostly

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owing to the physiochemical potential of MB in redox chemistry [10-12]. Additionally, MB can reverse hypotension during septic shock and has been successfully applied in patients [13]. The light spectrum properties of MB allow its application in photodynamic therapy (PDT) for excisional wounds and psoriasis and as a diagnostic tool for oral and breast cancer [14-17]. The low cost and versatility of MB allow further repurposing for different clinical therapies.

MB can be administered as an oral formulation or intravenously and subcutaneously in a 1% solution form (26.74 mM) [18]. The safe dosage range for MB is <2 mg/kg [19]. For aqueous oral administration of 100 mg MB, a maximum plasma concentration of 8 μ M (25 ng/mL) was reached after 2 hours, with a plasma half-life of approximately 20 hours [20]. Upon intravenous administration, MB can reach the maximum concentration 1 to 2 hours post-injection with a half-life of approximately 5 hours and is reported to exhibit almost double the concentration in whole blood compared to that shown in oral administration [21]. It is recommended that intravenous MB dosages be limited to 1 to 2 mg/kg since a higher dosage of MB (>5 mg/kg) can cause serotonin toxicity and, rarely, anaphylactic reactions. Intravenous administration should be slow, usually over 5 to 10 minutes [22,23]. Interestingly, an elevated concentration of MB in the brain was observed upon intravenous administration, indicating that MB can penetrate the blood-brain barrier (BBB) [21]. This could be explained by the equal charge distribution on the surface of the MB molecules [24]. Following administration, MB is cleared through the kidneys and reduced to LMB and demethylated metabolites [25]. Currently, MB is approved by the U.S. Food and Drug Administration (FDA) for intravenous and oral administration in methemoglobinemia therapy and as a surgical tracing dye. As of 2022, 225 interventional studies have been registered worldwide (clinicaltrials.gov) to investigate the clinical utility of MB in areas ranging from oncology to depression, and only 102 clinical trials have been completed.

Our review explores the pharmacological aspects of MB and its applicability to various diseases. The clinical trials registry clinicaltrials.gov was queried for all results published or registered before May 2022 with the search terms 'methylene blue' and 'methylthionine chloride.' Of 171 study records identified, 81 were complete. An analysis of the clinical studies (listed in Table 1) was conducted to explain the therapeutic potential of MB.

CLINICAL STUDIES AND PERSPECTIVES OF METHYLENE BLUE-BASED THERAPY

Methylene blue as a neuroprotective agent

Some of the most prevalent, devastating, and poorly treated diseases are brain diseases such as central nervous system disorders. Because of the complex nature of the brain in contrast to other anatomical areas, the development of drugs for the treatment of brain disease has poor success rates. Researchers have found that MB has antidepressant, anxiolytic, and neuroprotective properties in animal studies as well as in human studies [26,27]. It has a stabilizing effect on mitochondrial function and a therapeutic effect on the production of reactive oxygen species (ROS). Because of these features, MB holds promise as a neuroprotective agent and treatment for neurodegenerative disorders.

The antidepressant-like activity of MB has been effective in preventing and treating cognitive disorders caused by psychosis, both in preclinical and clinical studies [28]. The nitric oxide-cyclic guanosine monophosphate (NO-cGMP) cascade modulates the extracellular levels of serotonin, dopamine, glutamate, and acetylcholine, which may contribute to the pathophysiology of depression. NO production is elevated in depression, emphasizing the importance of the NO-cGMP cascade as a biomarker for depression. MB can induce an antidepressant-like response by directly inhibiting NO synthase and guanylate cyclase [29]. However, only a few clinical trials have reported these results. In one study, a moderate cognitive-enhancing effect was seen after three months in 26 patients with posttraumatic stress disorder treated daily with 260 mg of MB compared with 16 participants awaiting treatment [27]. In another study, MB was found to have significant effects on the symptoms of depression and anxiety in patients with bipolar disorder treated with a dosage of 195 mg, with no signs of serotonergic toxicity in these patients [30].

Mitochondrial dysfunction and oxidative stress are key to the progressive nature of neurodegenerative disorders, including traumatic brain injury, Alzheimer's disease (AD), and Parkinson's disease (PD). Downstream of mitochondrial dysfunction, electron transfer impairment occurs, resulting in energy deficits and the release of ROS [31]. Therefore, improving mitochondrial respiration is important for the development of new therapies. The redox chemistry of MB, in addition to its safety, may have therapeutic benefits. MB demonstrated an alternative donor/acceptor supporting role by reducing electron leakage in mitochondria, inhibiting ROS, and improving neuronal energy production [32,33]. These results suggest

Table 1. Summary of methylene blue-based interventional clinical trials

Condition or disease	Title/NCT number	Start date	Completion date	MB dosage	Administration method	No. of participants	The stage of a clinical trial/status	Results	Ref
Alzheimer's disease	TRx0014 in Patients With Mild or Moderate Alzheimer's Disease NCT00515333	August 2004	December 2007	30 mg/day 60 mg/day 100 mg/day	Oral/tablets	323	Phase 2/Completed	On the ADAS-cog scale, significant benefits were observed in moderate cases at 24 weeks following the 138 mg/day treatment. On both mild and moderate ADAS-cog scales, benefit was observed with continued treatment for 50 weeks.	[35]
	Safety and Efficacy Study Evaluating TRx0237 in Subjects With Mild to Moderate Alzheimer's Disease NCT01689246	January 2013	November 2015	250 mg/day 150 mg/day	Oral/tablets	891	Phase 3/Completed	As a monotherapy, LMTM appears to be just as safe as methylthioninium chloride (NCT00515333). Nevertheless, LMTM does not seem to be an effective add-on treatment for patients with mild to moderate Alzheimer's.	[36]
Cognitive dysfunction	Safety and Efficacy of TRx0237 in Subjects With Alzheimer's Disease Followed by Open-Label Treatment NCT03446001	January 2018	March 2023	16 mg/day 8 mg/day	Oral/tablets	598	Phase 3/Active	NA	NA
	Methylene Blue for Cognitive Dysfunction in Bipolar Disorder NCT00214877	November 2003	October 2007	195 mg/day 15 mg/day	Oral/capsules	40	Phase 3/Completed	Montgomery-ASberg Depression Rating Scale and Hamilton Depression Rating Scale both showed meaningful improvements with active dose (195 bmg) of MB.	[30]
	Intraoperative Infusion of Methylene Blue for Prevention of Postoperative Delirium and Cognitive Dysfunction in Elderly Patients Undergoing Major Elective Noncardiac Surgery NCT04341844	January 2019	July 2020	2 mg/kg	Intravenous/ 50 mL saline	248	NA/Completed	In elderly surgical patients undergoing intraoperative intravenous 2 mg/kg MB, postoperative delirium and postoperative cognitive dysfunction were significantly reduced, while perioperative adverse events were not remarkably increased, suggesting that MB might be clinically effective and safe.	[49]
Mental disorders	Enhancing Extinction Learning in Post Traumatic Stress Disorder (PTSD) (HELP) NCT01188694	September 2009	April 2013	260 mg/day	Oral	42	Phase 2/Completed	Improvements in working memory were associated with MB effects, but not with changes in beliefs.	[27]

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Table 1. Continued

Condition or disease	Title/NCT number	Start date	Completion date	MB dosage	Administration method	No. of participants	The stage of a clinical trial/status	Results	Ref
	Effects of USP Methylene Blue on Cognitive and fMRI Brain Activity NCT01836094	August 2013	March 2016	280 mg/day	Oral	36	Early phase I/Completed	After administration of MB, the bilateral insular cortex developed an increase in activity during a psychomotor vigilance task and there was an increase in functional MR imaging activity during a short-term memory task. The use of MB also increased correct responses during memory retrieval by 7% (P=0.01).	[50]
Blood and lymph conditions	Identification and Preservation of Arm Lymphatics (DEPART) NCT04446494	June 2020	September 2025	1 mg	Intradermal injection	1,200	NA	DEPART (combination of indocyanine green and MB) can reduce the incidence of arm lymphedema for patients with breast cancer undergoing ALND without adversely affecting the incidence of regional recurrence.	[51]
	Safety, Tolerability, and Pharmacokinetic Study of Methylene Blue Following a 1 mg/kg Intravenous Dose in Healthy Adults NCT02478281	October 2012	March 2013	1 mg/kg	Intravenous injection	12	Phase I/Completed	NA	NA
	Axillary Reverse Mapping Using Methylene Blue Subcutaneous Injection Can Identify Arm Lymph Nodes and Vessels, Measuring Arm Size for Lymphedema, Histopathological Examination of Arm Lymph Nodes Included With Axillary Lymph Node Dissection NCT04137744	February 2015	August 2019	1-2 mL (dosage NA)	Subcutaneous injection	74	NA	Axillary reverse mapping and preservation of arm lymphatics reduced lymphedema rates in patients with early breast cancer without compromising oncological safety.	[52]

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Table 1. Continued

Condition or disease	Title/NCT number	Start date	Completion date	MB dosage	Administration method	No. of participants	The stage of a clinical trial/status	Results	Ref
	Methylene Blue Against Falciparum Malaria in Burkina Faso (BlueACTn) NCT02851108	October 2016	December 2016	15 mg/kg/day	Oral/tablets	100	Phase 2/Completed	MB appears to be an interesting alternative for the treatment of falciparum malaria. The effectiveness of MB needs to be improved further, although it may already be considered useful in reducing falciparum malaria transmission intensity, increasing treatment efficacy, and reducing resistance development and spread.	[53]
	Efficacy and Safety of Artesunate-amodiaquine-methylene for Malaria Treatment in Children NCT01407887	August 2011	December 2012	15 mg/kg/day	Oral/mini-tablets	180	Phase 2/Completed	<i>Plasmodium falciparum</i> gametocytes are effectively killed by a combination of MB and artemisinin.	[54]
	Phase 2 Efficacy Study of Primaquine and Methylene Blue NCT02831023	July 2016	January 2017	15 mg/kg/day	Oral/tablets	80	Phase 2/Completed	The combination of dihydroartemisinin-piperazine and MB was highly effective in preventing <i>P. falciparum</i> transmission. Primaquine and MB had a good tolerability.	[55]
	Methylene Blue in Early Septic Shock (SHOCKEM-Blue) NCT04446871	March 2017	January 2021	100 mg/500 cc of 0.9% NaCl solution	Intravenous infusion	91	Phase 2,3/Completed	NA	NA
	Fluorescent imaging in Thyroid and Parathyroid Surgery With the Fluobeam (TM) System of Fluoptics NCT01598727	May 2012	January 2013	0.4 mg/kg	Intravenous injection	10	Phase 1/Completed	MB was most effective when used at 0.4 mg/kg body weight to visualize thyroid and parathyroid glands. The median time to onset of fluorescence was 23 seconds and the median time to peak fluorescence was 41.5 seconds.	[56]
Antimicrobial/antifungal	PDT and Periodontal Treatment in DMT2 Patients (PDTDMT2) NCT01964833	October 2013	December 2015	50 µg/mL PDT 660 nm 110 mW 9 J/point 22 J/cm ²	Injection into the periodontal pocket using a syringe	44	NA/Completed	NA	[57]

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Table 1. Continued

Condition or disease	Title/NCT number	Start date	Completion date	MB dosage	Administration method	No. of participants	The stage of a clinical trial/status	Results	Ref
	A Clinical Trial Testing the Efficacy of PDT in Preventing Amputation in Diabetic Patients NCT03380403	January 2010	January 2012	1% Aqueous solution	Irrigation with syringe	34	NA/Completed	There was only one amputation among these patients. Several of these patients were cured without intravenous antimicrobial therapy. The rate of amputation was 35 times lower in the PDT group.	[58,59]
	Impact of Photodynamic Therapy as an Adjuvant to Non-surgical Periodontal Treatment on Clinical and Biochemical Parameters Among Patients Having Mild Rheumatoid Arthritis With Periodontitis NCT05122117	March 2019	January 2020	10 mg/mL PDT 660 nm 150 mW 60 mW/cm ²	Injection into the periodontal pocket using a syringe	50	NA/Completed	NA	NA
	Comparison of Efficacy and Safety Between Methylene Blue-mediated Photodynamic Therapy and 5% Amorolfine Nail Lacquer for Toenail Onychomycosis Treatment NCT03098342	February 2017	June 2017	2% MB aqueous solution PDT 630–640 nm	Irrigation with syringe	10	NA/Completed	For a limited period and for moderately severe onychomycosis, MB PDT was more efficacious than amorolfine against non-dermatophytic onychomycosis. No major adverse events were found in MB PDT groups.	[60]
Heart and blood diseases	Evaluating in Cirrhotics With Refractory Vasoplegia the Effect of Methylene Blue (CRuMBS) NCT03120637	January 2017	January 2018	2 mg/kg 0.5 mg/kg/hr	Intravenous injection	111	Phase 4/Completed	Patients with cirrhosis and refractory septic shock responded well to MB. Patients with cirrhosis suffering from septic shock independent of high-dose vasopressor therapy have an increased mortality rate; however, neither the mortality rate nor the survival rate was affected.	[61]
	Methylene Blue vs Cyanokit for Intraoperative Vasoplegic Syndrome in Liver Transplant Patients NCT04054999	November 2019	January 2024	2 mg/kg bolus administration	Intravenous bolus administration	20	Phase 4/Recruiting	In this study, MB will be tested as a pote new and perhaps superior treatment for refractory vasoplegic syndrome after liver transplant surgery.	[62]
	Methylene Blue for the Prevention of Hypotension During Hemodialysis (BLUE) NCT05092165	October 2021	December 2025	1 mg/kg 0.1 mg/kg	Intravenous bolus administration	260	Phase 2/not yet recruiting	NA	NA

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Table 1. Continued

Condition or disease	Title/NCT number	Start date	Completion date	MB dosage	Administration method	No. of participants	The stage of a clinical trial/status	Results	Ref
Antiviral	Clinical Application of Methylene Blue for Treatment of Covid-19 Patients (Covid-19) NCT04370288	April 2020	September 2020	1 mg/kg/8 hours at 14 mg/mL syrup	Oral administration/syrup	80	Phase 1/NA	On the 3rd and 5th days, the rate ratio of improvement in respiratory function was 10.1 and 3.7 times higher in the MB group than in the conventional care group. MB-treated patients stayed in the hospital for a shorter time (P=0.04), and the mortality rates were 12.5% against 22.5% in the conventional therapy group.	[48]
	Nasal Photodisinfection COVID-19 Proof of Concept Study NCT04615936	October 2020	July 2021	0.01% w/v MB PDT 670 nm 860 mW	Nasal spray	45	NA/Completed	In the early stages of COVID-19, MB PDT was found to be effective in reducing viral loads in the nasal cavity, which could help control the transmission and severity of the disease.	[63]
	COVID-19 Treatment Using Methylene Blue and Photodynamic Therapy NCT04933864	April 2020	July 2020	1 mg/kg w/water solution PDT 650 nm	Oral administration	60	Phase 1/Completed	With MB concentrations of 1 mg/kg the initial virus titers 10 ⁶ /mL and 10 ⁷ /mL were completely inhibited.	[64]
	COVID-19 Methylene Blue Antiviral Treatment (COMBAT) NCT05004805	August 2021	December 2021	18 μ/cm ² 0.02% solution	Nasal spray	24	Phase 2/Completed	NA	NA
Cancer	Identification of Sentinel Lymph Nodes With Methylene Blue and Isotope NCT00314405	April 2006	April 2008	10 mg/mL 2 mL	Subareolar intraparenchymal injections	100	NA/Completed	Combining MB with digital examination is safer than using MB alone. The use of dilute MB injections (4 mL at 1.25 mg/mL) increases MB effectiveness (90%), maintaining low complication rates.	[65]

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Table 1. Continued

Condition or disease	Title/NCT number	Start date	Completion date	MB dosage	Administration method	No. of participants	The stage of a clinical trial/status	Results	Ref
	Feasibility of One-Step Sentinel Lymph Node (SLN) Biopsy With Radiolabeled Methylene Blue (IND 70,627) NCT00784849	November 2004	April 2012	100–1,000 μ Ci dose of 125 I MB	Peritumoral or circumareolar injections	12	Phase 2/Completed	This method eliminates painful 99m Tc infusions preoperatively, reduces radiation exposure for personnel, and eliminates delays caused by non-operating room personnel in addition to eliminating painful preoperative infusions of colloid. The results of these trials warrant further research using a 1,000- μ Ci dose of 125 I MB dye in sentinel lymph node biopsies.	[66]
	Application of Surgical Navigation System in Sentinel Lymph Node of Breast Cancer Research	January 2014	September 2015	1% Solution 1 mL	Subcutaneous injection	98	NA/Completed	Using the surgical navigation system, it was possible to map the lymphatics and identify the lymph nodes in breast cancer. The system enabled surgeons to precisely locate lymph nodes during surgery.	[67,68]

NCT, National Clinical Trial; MB, methylene blue; ADAS, Alzheimer's Disease Assessment Scale; LMTM, leuco-methylthionium bis (hydromethanesulphonate); NA, not available; DEPART, identification and preservation of arm lymphatic system; ALND, axillary lymph node dissection; PDT, photodynamic therapy; COVID-19, coronavirus disease 2019.

that MB is a promising candidate for the treatment of neurodegenerative diseases.

The pathology of tau protein aggregation correlates with clinical dementia in patients with AD. Therefore, inhibitors of tau aggregation may have therapeutic potential. The phenothiazine ring in MB was found to be essential for the inhibition of heparin-induced tau filament formation. MB's inhibitory activity on tau filament formation was found to be dependent on the first and fourth repeat domains of tau protein [34]. Monotherapy with MB, which inhibits tau protein aggregation, has been tested in clinical trials [7]. Wischik et al. [35] reported that 321 patients with mild or moderate disease were treated with MB in clinical trials. Based on the cognitive subscale of the Alzheimer's Disease Scale, 138 mg daily had a moderate effect after 6 months [35]. The reduced form of MB, LMB, which retains tau-aggregation inhibitor properties, is more soluble and has an improved pKa compared to MB. However, clinical studies involving LMB as a supplemental treatment for patients with mild to moderate AD have shown negative results [36].

As a brain-active drug, MB has many desirable properties. Several factors make it worth considering as a potential therapeutic agent, such as its high solubility in aqueous media, low toxicity, ability to cross the BBB and cellular membranes, and its approval for human use. The impact of MB on mitochondrial function and neurofibrillary tangles suggests that it may be a promising neuroprotective candidate. The development of MB as a multi-tasking therapy for depression in neurodegenerative diseases, such as AD and PD, is essential. A novel therapeutic strategy can be applied when multitargeting bullet drugs are used for the treatment of depression by using currently available analogs of MB because of their physiochemical and pharmacokinetic properties. Furthermore, analogs may provide better response and reduce the risk of side effects. Despite a relatively small number of patients, recent clinical trials have shown promising results. Nevertheless, more extensive studies are required to confirm these findings.

Methylene blue in clinical practice of cancer identification

Currently, ultrasound, frozen section analysis, and visual palpation are methods used to detect tumors during surgery. All three methods are time-consuming and prone to errors [37,38]. Therefore, employing an intraoperative imaging method during surgery can be useful for identifying breast tumors and locate suspicious lesions in the resected tissues.

The FDA has approved near-infrared (NIR) fluorescence imaging by MB for intraoperative applications because it allows the accurate identification of tumor margins and sentinel lymph nodes without ionizing radiation [39]. Several studies have demonstrated the feasibility of utilizing MB-based imaging during surgery to identify tumor lesions, such as diagnosing solitary fibrous tumors in the pancreas and identifying parathyroid adenomas and paragangliomas [40]. According to a study by Tummers et al. [6], van der Vorst et al. [41], Tummers et al. [42], and an MB-based imaging approach was used to identify breast cancer. An intravenous injection of MB at a dose of 1.0 mg/kg either immediately before surgery or 3 hours before surgery produced fluorescent signals in 20 out of 24 patients with breast cancer. Even though MB does not cause serious allergic reactions, it is not without risks. Adverse reactions to MB were reported by Stradling et al. [43] in patients with breast cancer. There also have been reports of skin, fat, and parenchymal necrosis [44,45]. Due to its toxic effects on local tissues, MB should not be injected intradermally. Instead, it should be injected deep into the parenchyma.

Anti-infectious and antiviral therapy in clinical studies using methylene blue

Since the first reports of MB being used against malaria nearly a century ago, it has been replaced by chloroquine and other FDA-approved drugs owing to toxicity issues. However, MB remains an affordable and effective anti-malarial agent, particularly in patients resistant to chloroquine. MB showed the potential to inhibit glutathione reductase and reverse chloroquine resistance (CQ-sensitizing action), including the prevention of heme polymerization [46]. The daily dose of 36 to 72 mg/kg was the most effective. Apart from its well-known anti-malarial activity, it also has the potential to prevent methemoglobinemia as a complication of malarial anemia [9].

Recently, MB has been reported as a treatment option for coronavirus disease 2019 (COVID-19). COVID-19 is a natural viral lung infection that leads to increased fatality in the older generation. Sharing a common feature of the immune response to sepsis, COVID-19 activates a systemic immune response by inducing a pro-thrombotic environment enriched with proinflammatory cytokines and free radicals [47]. The application of anti-cytokine or antiviral drugs alone did not show high efficiency in COVID-19 therapy, thus leaving an unmet need for a therapeutic agent that could inhibit both cytokines and free radicals. The application of MB in COVID-19 treatment was launched in April 2020 as a parallel, randomized, interventional clinical trial [48]. Investigations into the

mechanism of action of MB focused mainly on the inhibition of toxic NO generation by blocking NO synthase as well as free radical production [5]. Several ongoing clinical studies on this topic are presented in Table 1 [27,30,35,36,48-68].

Methylene blue in the therapy of cardiovascular diseases

MB has the potential to inhibit guanylate cyclase and improve mean arterial pressure and cardiac function in septic shock, resulting in decreased cGMP and smooth vascular muscle relaxation. Additionally, MB demonstrates an improvement in arterial pressure and systemic vascular resistance [13,69,70]. A dosage of 2 mg/kg/hr of MB added to the cardiopulmonary bypass prime composition was successfully applied to prevent refractory hypotension in septic endocarditis [71]. Mortality rates in post-cardiac surgery patients with vasoplegia have been decreased by applying MB [72-74]. A single dose of MB 1.5 to 2 mg/kg was intravenously administered to patients at high risk of vasoplegic syndrome during cardiac surgery [72,75-77]. According to another case report, patients with hepatopulmonary syndrome (a pulmonary condition caused by increased endogenous NO production followed by elevated cGMP levels in the liver of patients with cirrhosis [78]) experienced improvements in alveolar-arterial oxygen pressure after MB injection over 15 minutes at a dosage of 3 mg/kg body weight [79].

METHYLENE BLUE-BASED PHOTODYNAMIC THERAPY

MB is a dye commonly used in PDT that triggers apoptosis via ROS production under NIR light [80]. Owing to its rapid absorption and clearance properties, MB is suitable for topical PDT because it reduces the risk of skin photosensitivity, a common problem after PDT. As the FDA has approved MB for clinical use, it is considered a potential photosensitizer candidate for PDT applications in cancerous and non-cancerous diseases [81,82]. PDT of tumors and nonmalignant diseased tissues is a promising alternative to chemotherapy because diseased cell death is caused by selectively locating photosensitive compounds in the target and generating cytotoxic ROS under light. An activated form of oxygen, 1O_2 , called singlet oxygen, is produced by a photosensitizer by directly absorbing energy from a light source. Known as the main cytotoxic agent associated with PDT, 1O_2 is a highly electrophilic compound capable of oxidizing the electron-rich double bonds in biological molecules and macromolecules [83,84].

While PDT works well within a spectral window of 600 to 950 nm, it shows poor penetration into deep tissue, which may adversely affect its efficiency. In terms of the application of MB for PDT, instability and bioaccumulation in endothelial cells are major impediments. It may be possible to alleviate these issues by fabricating nanoparticles that can protect and transport MB for accumulation in the target tissue, for example, by enhancing PDT at tumor sites. In addition, nanoparticles have been found to be effective in modifying pharmacokinetics and reducing adverse side effects [85].

Several studies have evaluated the PDT effects of MB in fungal diseases. In 2012, Scwingel et al. [86] assessed the effectiveness of PDT for oral candidiasis in 21 patients. A single radiation dose was applied with 450 $\mu\text{g/mL}$ MB at 660 nm, 30 mW, and 7.5 J/cm^2 for 10 seconds. While conventional treatment failed to prevent the occurrence of candidiasis in the short term, all patients in the PDT group were free from candida colonies with no recurrence of candidiasis for up to 30 days after radiation [86]. Another study demonstrated that patients with onychomycosis undergoing MB PDT showed greater improvements in the short and long-term post-PDT compared to underwent conventional therapy without PDT [87]. The optimal parameters for MB PDT in human infectious diseases need to be determined, and there is no consensus on the standardization of MB PDT protocols. To date, only a few studies have been conducted on the adjunctive use of MB PDT to treat conditions including onychomycosis, oral candidiasis, and diabetic foot infections.

MB PDT was applied to treat truncal and facial acne vulgaris, a chronic inflammatory disease of the pilosebaceous unit that has multiple pathophysiological factors [88-90]. The topical and oral treatments currently available for acne vulgaris have a limited effect, particularly in mild to moderate cases [91]. Antibiotic therapy has led to the implementation of PDT for the treatment of acne vulgaris. By targeting some of the main pathophysiological factors, topical PDT application may reduce inflammation associated with acne lesions in areas of the body which are difficult to reach with conventional therapy [92]. To overcome the problem of inadequate penetration of photosensitizers into the skin during topical treatment with PDT, nanocarriers containing MB have been developed [88-90]. MB has a high affinity for melanin, making this particularly relevant to pigmented cancer tissues in melanoma lesions [93,94]. Malignant melanomas occur when melanocytes, the pigment-producing cells found primarily in the skin, undergo malignant transformation [95]. A study reported that mitochondrial dysfunction causes apoptotic cell death when PDT

with MB derivatives is specifically directed at mitochondrial membranes. It is plausible that mitochondria are involved in MB PDT-induced tumor regression because MB is likely to bind to the mitochondrial matrix in a negative electrochemical environment [96]. Researchers have shown that MB PDT is effective against melanoma in both human and animal studies where large melanoma lesions that could not be surgically removed were effectively treated [81,97,98]. The clinical applications of MB PDT may still be limited, particularly for deeply seated hypoxic tumors. Clinical MB PDT can be more effectively utilized when targeted approaches are used, particularly in precision medicine. In recent years, many studies have focused on improving the specificity and effectiveness of MB PDT in specific cancer cells [99]. Researchers have demonstrated that using nanoparticles can increase the specificity of treatment and decrease adverse systemic effects [100-104]. MB PDT is also favorable when combined with other therapies, including immunotherapy. Of note, there are no clinical trials registered on the clinicaltrials.gov database for cancer MB PDT. However, the current preclinical interest in developing nanoparticles for MB PDT is likely to lead to the development of more effective and advanced therapies. Therefore, it can be concluded that MB PDT has tremendous potential for use in both cancerous and non-cancerous diseases.

CONCLUSION

MB is known for its accessibility, extensive safety profile, and proven versatility and has been used in medicine for decades. The fluorescence properties of this compound give it tremendous potential as a NIR fluorophore. MB imaging with NIR radiation is a promising surgical technique that requires further research. The studies included in the present review did not identify any potentially lethal side effects of PDT, suggesting that it is a safe procedure for treating mild infections if performed under supervision.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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Conception or design: AS.

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Drafting the work or revising: AS, JKY, SK.

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