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METHYLENE BLUE: A POTENTIAL NOVEL TREATMENT IN DIABETIC FOOT ULCER

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ABSTRACT

Diabetes is a chronic metabolic disease that can lead to serious consequences that impair one's quality of life if not adequately controlled. One of the undesirable complications is a diabetic foot ulcer. It is estimated globally that every 30 seconds, a leg is amputated due to diabetic foot, and thus can lower the quality of life. Recent studies have used a low-cost dye known as methylene blue as an anti-microorganism agent, and this sparks the idea of exploring more of its possible benefits. This literature review aimed to outline the beneficial roles of methylene blue in diabetic foot ulcer treatment. According to the findings, it is said that methylene blue may play a role as an anti-microorganism agent through its contribution to wound healing and invasive surgical prevention such as limb amputation. All the pooled articles showed a promising outcome of MB from the reduction of wound size in a shorter healing period with no adverse effects reported. Hence, methylene blue may have a promising role to be an effective agent in treating diabetic foot ulcers.

Keywords: Diabetic Foot Ulcer; Methylene Blue; Photodynamic Therapy; Wound Healing

INTRODUCTION

Diabetes is a chronic metabolic illness defined by high levels of blood glucose (or blood sugar), which causes significant damage to the heart, blood vessels, eyes, kidneys, and nerves over time.[1] According to the International Diabetes Federation (IDF) Diabetes Atlas, around 463 million individuals are diagnosed with diabetes, and is estimated to elevate to 700 million by 2045.[2] Diabetes mortality rate has jumped to 24,6% per 100.000 population in 2020.[3] Indonesia itself ranks seventh in the world in terms of the number of people affected by diabetes.[2] This number excludes those who have undiagnosed diabetes or are at high risk of developing diabetes. Unmonitored blood glucose may progress the disease to its complex stage and surge the risk for three common diabetes microvascular complications, namely diabetic nephropathy, diabetic retinopathy, and diabetic neuropathy.[4]

In the United States, every 17 seconds, someone is diagnosed with diabetes, and every day 230 Americans with diabetes suffer from an amputation as a result of diabetic foot ulcer (DFU) from neuropathic damage. Throughout the world, it is estimated that a leg is amputated every 30 seconds, and 85% of these amputations are due to diabetic foot ulcers.[5] This condition is developed by a diabetic neuropathy that coexists with peripheral artery disease (PAD), which accounts for more than half of major limb amputations in the United States, reaching 50.000 cases each year.[6] Several therapies have been used to prevent the worsening of diabetic foot ulcers. Regimens of antibiotics such as silver sulfadiazine, murpicin, trimethoprim, vancomycin, and many others[7] were used as a main treatment for DFU. Besides having the antibiotic treatment, other DFU management including glycaemic control, pharmacological therapy, vascularisation improvement, debridement, the use of offloading devices, proper wound

dressing, negative pressure wound therapy, maggot therapy, growth factors and skin substitutes, as well as multidisciplinary team input should be considered. Delayed treatment can lead to major limb amputation due to the rapidly progressing soft-tissue infections.[8] Therefore, the mentioned therapy above may be helpful to prevent the delay of wound healing management. However, to date, these therapy modalities were not yet sufficient to lessen the morbidity of DFU. The slow healing chronic wounds in DFU frequently require proper antibiotic regimens which unfortunately may induce antibiotic resistance. The authors aimed to explore low-cost novel treatment which provide faster healing, minimize the risk of antibiotic resistance, and decrease the amputation rate. This leads to the idea of application of methylene blue (MB), as known to be a type of dye used as a photosensitizer (phenothizianium group). Other dyes that have similar role as a photosensitizer are the group of hematoporphyrin, cyanine, phytotherapeuic agents, and phtyalocyanine, in which each has specific wavelength activation. Several group have been specifically explored for its benefit, so did the MB in its unique antimicrobial role.[9] Thus, MB, as one of the most widely used dye in the antimicrobial study, will be investigated for its role in DFU in this study.

Diabetes Mellitus Pathophysiology

Diabetes mellitus (DM) can be classified into several types regarding each of its causes. According to the American Diabetes Association (ADA)[10], diabetes can be classified into 1) Type 1 Diabetes, 2) Type 2 Diabetes, 3) Specific types of diabetes due to other causes, and 4) Gestational diabetes mellitus. Among these four classifications, diabetes type 2 accounts for approximately 90-95% of all diagnosed diabetes cases.[10] Type 2 Diabetes Mellitus (T2DM) covers individuals with relative (rather than absolute) insulin insufficiency and peripheral insulin resistance.[10] T2DM is characterized by insufficient insulin secretion of pancreatic islet β-cells, tissue insulin resistance (IR), and an insufficient compensatory insulin secretory response.[11] Several mechanisms known to cause the development of T2DM primarily are defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond to circulating insulin.[12] Insulin resistance itself may develop as a result of excess body weight and primarily occur in overweight or obese T2DM patients.[11] Both genetics and the environmental condition can be addressed as the non-modifiable and modifiable risk of T2DM. Non-modifiable risk includes certain ethnicities which are more prone to diabetes, such as Native Americans[13] and genetic predisposition, whereas modifiable risks include obesity, sedentary lifestyle, and unhealthy diet.[11]

Diabetic foot ulcer (DFU) is an injury spotted in all layers of skin, which, accompanied by necrosis or gangrene, is usually found in the feet' soles. This condition is commonly caused by the occurring diabetic neuropathy and/ or PAD and has the potential to cause morbidity and mortality.[14,15] These outcomes are due to the complex metabolic disturbances in T2DM patients that take a role in the damage of many areas in the nervous system.[16] The cause of the ulcer itself is multifactorial, such as neuropathic damage and poor blood circulation. The neuropathic damage in DM patients is a result of excess oxidative stress on nerve cells due to hyperglycemia. Poor blood circulation, as seen in PAD, may disturb the distribution of nutrition and oxygen to distal extremities.[11] Other causes that contribute are poor glycemic control, calluses, foot malformation, improper foot care, ill-fitting footwear, dry skin condition, etc.[17]

It is known that demyelinated motor neurons and motor endplates may damage muscles atrophy of the foot and may cause the foot musculature imbalance in its flexors and extensors. This condition causes anatomical deformities and eventually skin ulcerations[15,18,] as seen in classic Charcot foot in longstanding patients with DM. The damage to the autonomic nerve also diminishes the function of the sweat gland, resulting in less moisturized skin, leading to epidermal cracks and skin breakdown.[18]

Furthermore, the sensory loss caused the ulcer to be painless, resulting in further trauma due to the patients'unawareness, and this loss of protectivesensation is the most common cause ofulceration in DM patients.[15]

The diagnosis of DFU can be challenging since PAD, neuropathy, or impairedleukocyte functions in diabetic patients maydeplete the local inflammatory response and local infection manifestation.[15] Differential diagnoses that should be considered are venous leg ulcers, ulcers of mixed venous, arterial leg ulcers, and arterial origin and vasculitic ulcers.[19] Grading and classification of DFU can help determine the best treatment. Wagner classification (Table 1**.)** is currently used to classify DFU.[20]

Table 1. Wagner Classification of DFU [20]

Grade	Lesion
0	Intact Skin
1	Superficial ulcer of the skin or subcutaneoustissue
2	Ulcers extend into tendon, bone, or capsule
3	Deep ulcer with osteomyelitis or abscess
4	Gangrene of toes or forefoot
5	Midfoot or hindfoot gangrene

People with certain risks are more likely to develop ulcers or have amputations, such as poor glycaemic control, peripheral neuropathy with loss of protective sensation (LOPS), cigarette smoking, foot deformities, pre-ulcerative callus or corn, PAD, history of foot ulcer, amputation, visual impairment, and chronic kidney disease (CKD) (especially patients on dialysis).[21] Low-risk complications patients with no foot anatomical abnormalities should be taught regarding proper foot care, appropriate decreasing-pressure footwear, and a careful glycaemic evaluation. In addition, a level of hemoglobin A1C of 7% or below must be maintained to prevent patients from developing microvascular complications.[15] On the other hand, patients with high-risk classification may require advanced care, and surgical intervention is an option.

Amputation, as a consequence of DFU, is the process of removing a limb or its part by sectioning one or more bones, while disarticulation is surgery through the joint surface. People undergoing amputationneed to adapt to new physical loss andchanges in the pace of life in interpersonal, social, and professional interactions.[22]

METHODS

The literature review examined articles that were sought in electronic databases including PubMed, Embase, Scopus, and Web of Science from 2011 to August 2021. This study includes clinical trial, experimental, and observational studies in diabetic foot ulcer patients. Both authors thoroughly read, investigate, and retrieve the suitable research which discussed the desired topic. Studies that applied methylene blue (MB) as a treatment indiabetic foot ulcers and provided healingoutcomes in comparison to non-MB treatment were eligible for review. Authors retrieved studies with language restriction toEnglish. We manually exclude article duplicates, non-human studies, and inaccessible articles. Boolean operators "AND" and "OR" were used as a search strategy to keywords, comprising: ((methylene blue) OR (chromoson) OR (urolene blue) OR (blue 9 basic) OR (methylene blue n) OR (methylthioninium chloride) OR (blue swiss)) AND ((diabetic ulcer) OR (foot ulcer diabetic) OR (diabetic feet) OR (foot diabetic)). This review covered a total of seven relevant studies with its retrievable data. This review covered a total of seven relevant studies with its retrievable data.

RESULTS

Table 2. Characteristics of the Included Studies

Author	Study Protocol	Outcomes
Coutts et al., 2014[23]	Role: Wound dressing (anti-microorganism) Patients: 14 (DFU, LU) Age: 18-65 years old Intervention: Blue foam dressing containing gentian violet and methylene blue (GV/MB) Evaluation: Change in wound size was determined by comparing wound size observed at weeks 2 and 4.	As much as 57% of patients had a decrease in wound size. Not changing: 1 patient (7%). Increase in woundsize: 5 (36%)
Woo and Heil, 2017[24]	Role: Wound dressing (anti-organism) Patients: 29 patients with chronic wounds > 2 weeks, >18 years old, DFU, PrU, VLU, ALU, MLU, SW. Intervention: Standard wound care and gentian violet or methylene blue (GV/MB), then covered with secondary dressing. Evaluation: Dressings were changed at least 3 times/week during the 4-week period Other intervention:	A significant reduction was observed in: 1. Mean surface area, mean pressure ulcer scale for healing (PUSH) score, 2. Mean wound surface area covered with devitalized tissue, and 3. Mean of infection score over the 4-week study period. The combination of antibacterial properties, autolyticdebridement effects and absorption capabilities of the GV/MB dressing contributed the effectiveness in promoting wound healing.

	Antibiotics	
Lullove2017[25]	Role: Wound dressing (anti-microorganism) Patients: 53 (22 DFU, 28 VLU, 3 PrU) Intervention: All patients received treatment twice weekly in the first 4 weeks. DFU patients received weekly treatment (cleansing/surgical debridement application of collagen extracellular matrix (CECM) and gentian violet/methylene blue (GV/MB) antibacterial polyurethane (PU) foam dressings) until the wound closed.	95,5% of DFU patients had recovered completely by week 20. The other recovered completely by week 24.
Tardivo et al., 2014[26]	Role: Preventing amputation Patients: 16 people in control group, 18 treatment (35-83 years old). All patients presented deep-tissue wound, abscess formation, and osteomyelitis(Wagner Grade 3 classification). Intervention: Photodynamic therapy, using methylene blue (MB) and O-toluidine blue (Labsynth Products, São Paulo Brazil) as photosensitizers. Light absorption at 664 nm and 630 nm, respectively. Light was positioned 10 cm above the infected tissue and used for 10 minutes. Treatments were done twice a week.	All patients in the control group ended up getting amputation. In photodynamic therapy (PDT) group, accelerated healing of the fistulas and tissue reconstruction were found. As much as 17 out of 18 patients were considered cured. The only amputation case was due to recurrent ulcer that did not heal like the previous treatment.

Tardivo et al., 2017[27]	Role: Nullification of surgical debridement (preventing amputation) Patients: 57 subjects, 40 of which received photodynamic therapy (PDT) in non-debrided (NDP), and the 17 others were initially debrided before receiving PDT. Intervention: Photodynamic therapy, using MB and O- toluidine blue (Labsynth Products, São Paulo Brazil) as photosensitizers. Light absorption at 664 nm and 630 nm, respectively. Light was positioned 10 cm above the infected tissue and used for 10 minutes.	All patients with PDT had healing ulcers and complete bonereconstruction. Intervention with PDT performed faster healing time (29 days shorter) in patients of the NDP group, and average of 106 ± 77 days and 135 ± 67 days, respectively. One case of debrided patients(DP) underwent amputation. No patient had adverse reactions from PDT.
Carrinho et al., 2018[28]	Role: Anti-microorganism Patients: 12 (6 PDT group and another 6 in control group) All were treated with collagenase/chloramphenicol ointment. Intervention: Photosensitizer (PS) used is MB dye 0.01% applied along the border and the center of the wound. Red laser therapy (660 nm, 30mW, 8 sec, 6 J/cm^2, beam area of 0.04mm^2)were given 5 minutes after the drop of PS. The therapy was done three times per week, totaling 10 sessions. Evaluation: The Ulcer Healing Index and the wound area reduction were calculated for both groups.	PDT group showed greater reduction of the diabetic ulcer area and lesion compared to the control group.
Kashef et al., 2011[29]	Role: Anti-microorganism (bactericidal)	Bactericidal effect depends on the light dose, not on the concentration of methylene blue.

	Population: 3 bacteria strains (*Staphylococcus aureus*,*Staphylococcus epidermidis, Escherichia coli*) isolated from DFU infection were subculture on nutrient agar (Merck) aerobically at 37°C for 18-24 hours. Intervention: Photosensitizer & light source: Methylene blue(MB) from Sigma, UK. MB solution in sterile PBS (pH=7.4), filter-sterilized and kept in the dark. Irradiation used a 35 mW diode Laser (Lasotronic – UK) that emitted 660 nm. Adding MB (from 25 µg/ml to 100 µg/ml), the wells were left in the dark for 30 minutes (pre-irradiation time), and then exposed to a measured dose of laser light at a fluence rate of 0.091 W/cm^2 for 10 minutes corresponding to a light dose of 54.6 J/cm2. Each experimental condition was tested 5 times and in 4 occasions (controls whichcontained neither MB nor received irradiation (L-S-), incubation with MB in the dark (L-S+),irradiation in the absence of MB (L+S-), and irradiation in the presence of MB (L+S+).	Methylene blue(MB) photosensitization using red laser light(109.2 J/cm2) was able to achieve reductions of 99.03% in *S. aureus*, 98.95% in *S. epidermidis.*, and 92.23% in *E. coli.* In the absence of MB, irradiation did not result in significant kills in 3 organisms ($p > 0.05$).

ALU, arterial leg ulcer; CECM, collagen extracellular matrix; DFU, diabetic foot ulcer; DP, debrided patients; GV, gentian violet; LU, leg ulcer; MB, methylene blue; MLU, mixed leg ulcer; NDP, non-debrided patients; PDT, photodynamic therapy; PS, photosensitizer; PrU, pressure ulcers; PU, polyurethane; SW, surgical wounds; VLU, venous legulcers.

DISCUSSION

From the pooled studies, the authors spotted two major benefits of methylene blue (MB) utilization in treating foot ulcers among 199DFU patients. These benefits are the anti-microorganism role through photodynamic therapy (PDT) and wound dressing application, also the preventive role from invasive procedures such as limb amputation.

Role of Methylene Blue-Photodynamic Therapy (MB-PDT)

Methylene blue has long been known as a therapy in methemoglobinemia, malaria, septic shock, urinary tract infections[30,31] , vasoplegic syndrome post cardiothoracic surgery[32], and chronic urolithiasis.[31] It is also recently utilized as a photosensitizer (PS) in photodynamic therapy (PDT) which contribute to eliminating the virus infection.[33]

Photodynamic therapy (PDT) is a two-step procedure, starting with the administration of a light-sensitive PS that will later receive irradiation of light with specific wavelengths.[34] Photodynamic therapy has been implemented in tackling various medical challenges, such as treating multidrug resistant microbial infections, since it has fast efficacy outcomes.[35] Currentutilization of this non-invasive treatment wascommonly applied in cancer therapy and the wound healing process. The wound's accessibility to skin-light therapy makes PDT suitable for the treatment of superficialwound infections due to its topical photosensitizer delivery.[29] The effects of PDT were observed from the activation of PS by light energy. Combined with molecular oxygen, it can activate a photochemical reaction that creates one of the forms of reactive oxygen species(ROS), the oxygen singlet ($^1O_2$).[34] ROS were considered toxic to microorganisms and several cells, such as cancer cells since ROS have the ability to eradicate them.[36] ROS were known to oxidize various biomolecules, inducing oxidative stress effectively and further causing biological damage, as observed in bacteria under PDT.[37] These findings open an opportunity for DFU treatment.

Former research conducted by Shen et al. (2020)[38] showed the result of a quick healing wound process (within 1-3 days) from bacterial infection under the treatment of methylene blue as a PS in PDT (MB-PDT). On a note that the frequency and wavelength used varied according to the wound severity. Moreover, all subject samples from this study did not show any recurrences or side effects after 3-12 months of follow-up. A recent study indicatedthat it might reduce the emergence of antibiotic resistance, yet the exact mechanism remained uncertain. [29,37,39]

In this review, the PDT-positive effect can be well-noticed in vivo study by Carrinho et al.[28] and an experimental study by Kashef et al.[29] (see Table 2.) According to the research data,[28] MB-PDT treatment in DFU was able to speed up diabetic ulcer recovery. In Kashef et al.[29] studies, the antibacterial role of MB-PDT to bacteria isolated from DFU patients indicated that MB-PDT was able to limit bacterial growth, as shown by the measurement of bacterial colony forming unit.

Besides MB's role as an anti-microorganism, it was also known that the converted light energy in PDT was useful to cells viability since it might surge ATP mitochondrial production, boost the releaseof serotonin and endorphins, as well as increase local blood circulation, cellular proliferation, and protein synthesis.[40] Oyama (2020)[41] also stated that PDT played a crucial role in the entire process ofwound healing, particularly in the early stage, by controlling the infection by eradicating bacteria and promoting the proliferation of fibroblasts and collagen synthesis. These roles may help DFU to heal faster, as observed in Tardivo et al.[26,27] studies. Hence, undesirable invasive procedures may be hindered.

Role of Methylene Blue PolyvinylAlcohol (PVA) Foam Dressing

The application of MB as a wound dressing was also found to significantly improve wound healing in diabetic foot ulcers.[23,24,25] According to Woo et al.[24] retrospective 4-week studies, themean wound surface area was decreased by 42,5%

compared to standard therapy. The mean Pressure Ulcer Scale for Healing (PUSH) score on chronic wounds fell by 19,5% following four weeks of therapy with the GV/MB antibacterial PVA foam dressing. A similar result was found in a studyconducted by Coutts et al.[23] , showing that at week 4, 63% of patients with diabetic foot ulcers experienced a decreasing superficialand deep bacterial burden. On top of that,one patient from the study developed complete wound closure by week 4. A study by Lullove[25] , also discovered that 38.1% of the wound area was closed by the end of week 4. In the next eight weeks, 90,6% of the wound area was closed, and by week 20, 21 out of 22 (95,5%) DFU patients had their wound area completely healed.[23] The shorter healing time might be attributed to a smaller wound area, younger age, and lower BMI in some hospitalized patients. Moreover, all subjects from these three studies did not report any adverse effects or ulcer recurrences.

Lullove[25] reported a case of diabetic foot ulcer, which fully healed at week 15 by the treatment of GV/MB as a wound dressing. The patient presented an ankle wound with an exposed tendon, in which, after five weeks of regular debridement along with extracellular matrix (ECM) and GV/MB antibacterial PVA dressing, the tendon was eventuallycovered, and the wound was 20% closed. As of week 6, the patient solely received GV/MB dressing twice weekly, resulting in a fully closed wound by the end of week 15.

The GV/MB foam has the ability to trap, absorb, and inhibit exudate associated with bacterial growth by unfavorably altering the oxidation-reduction potential within the bacterial cell.[24] This bacteriostatic role may assist the host resistance to minimize further bacterial damage and facilitate the proliferative cell in the wound healing process.[42] Gentian violet and methyleneblue (GV/MB) absorbent, antibacterial dressings are available in several forms: foam wafer, packing material, and ostomy ring dressings.[24]

According to a study by Coutts, 2014[23] MBdressing reduced both the superficial and deep bacterial burden in wounds among63% of DFU patients. Interestingly, the finding is similar to a case series by Sharma in 2017,[43,] which involved a more costly nanocrystalline silver dressing. However, the deep bacterial burden with nanocrystalline dressing did not differ in quantity. This finding indicates that GV/MBdressing is cheaper and more efficient in decreasing bacterial burden in DFU patients.

Methylene Blue as Prevention to Amputation

A study by Tardivo et al.[26] showed the outcome differences between patients who were treated with a combination of antibiotic-PDT compared to those with antibiotic--debridement therapy. All patients that were only treated by antibiotics and debridement ended up suffering amputation. On the other hand, patients with PDT therapy resulted in faster healing of the fistulas and tissue reconstruction. Interestingly, the amputation rate in the PDT group was markedly lower, only 0.029 times compared to the control group ($p = 0.002$).

Precaution and Limitation in Methylene blue Blue Application

There are several issues to consider before administering MB therapy since it may be contraindicated in some individuals. People with glucose-6-phosphate dehydrogenase (G6PD) deficiency are known to be contraindicated due to its adverse effect on hemolysis.[44] Forthose who do not have any contraindications, the recommended safe dosage is 1-2 mg/kg.[45] An unusual incidence of skin necrosis in a female breast cancer patient who had a peritumoral injection of MB dye for sentinel lymph node biopsy localization was recorded, suggesting that more study regarding MB and the skin is necessary.[46]

Limitation of this review

According to the authors' best knowledge, no study has yet examined the potential of MB in DFU patients. As a result, this paper may provide a valuable overview of findings for

future research. Some limitations include the small number of subjects and the lack of a comparative study between methylene blue and other dyes. However, replication studies with a larger sample size may be performed to verify the MB mechanisms in DFU patients.

CONCLUSION

Methylene blue may be a promising agent that contributes to DFU wound healing through two mechanisms: 1) as an anti-microorganism through MB-PDT and wound dressing and 2) as a prevention of limb amputation by increasing local blood circulation, cellular proliferation, and protein synthesis. A greater population should be involved in further studies to ensure MB's role and provide robust efficacy in treating DFU patients.

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