International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Pharmacological Review of Diabetic Foot Ulcer-Inducing Methods and its Healing

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Abstract: ***A metabolic illness called diabetes mellitus is characterized by numerous long-term consequences impacting practically all bodily systems. One of the most common side effects of diabetes is foot ulcers. The exact pathophysiology of a diabetic foot ulcer is clearly unknown and it may be induced due to various factors. Impaired diabetic wound repair is mainly caused by diabetic neuropathy, peripheral vascular disease, and aberrant cellular, and cytokine / chemokine activity. Treatment for DFU is based on the severity of the ulcer which is assessed by the Wagner ulcer classification system. Furthermore, the most preferred treatment for foot ulcersis antibiotic therapy, revascularization, debridement, off-loading, and amputation. This review also discusses screening methods for diabetic foot ulcers in rats such as in vitro and invivo models.***

Keywords: Diabetic foot ulcer, Wound healing, Diabetes Mellitus, Foot care

1. Introduction

Diabetes mellitus (DM) is one of the most common chronic metabolic syndromes characterized by an increase in blood glucose level (hyperglycemia), and impairment in the secretion of insulin. The incidence of diabetes mellitus is rapidly increasing worldwide. According to epidemiological studies, it is estimated that by 2030, diabetic cases will be more than 360 million worldwide. There are currently 61.2 million diabetics in India; by 2030, it is estimated to be 101.2 million [1]. There are various complications associated with diabetic patients such as diabetic foot ulcers, neuropathy, peripheral vascular disease, retinopathy, etc [2].

Diabetic foot ulcer (DFU) is the most severe chronic complication among all other complications. DFU defines a non-traumatic lesion of the skin (partial or full thickness) on the foot in a person with diabetes that does not heal right away. The skin break is caused by a variety of events, and once an ulcer has formed, several things prevent it from healing quickly [3]. The reasons for the skin break and the reasons for the delay in healing will change over time from person to person. Various factors may predominate at various stages of the healing process.

The majority of Diabetes mellitus-related amputations start with a foot ulcer [4]. DFU is currently regarded as a significant cause of morbidity and a key reason for hospitalization in diabetic patients [5, 6]. According to estimates, DFU accounts for 20% of hospital admissions among DM patients [7]. If the proper care is not given, DFU can result in infection, gangrene, amputation, and even death [7]. On the other hand, once DFU has appeared, there is a higher chance that the ulcer may progress and require amputation [8]. Overall, patients with DM had a 15 times greater rate of lower limb amputation than patients without diabetes. DFU is thought to be the cause of 50% to 70% of all lower limb amputations [8].

Etiology

There are various risk factors associated with Diabetic foot ulcers. These factors are as follows [9-12]:

- Peripheral neuropathy (sensory, motor, autonomic)
- Foot deformity (hammer toe, bunion, Charcot, etc)
- Trauma
- Improperly fitted shoes
- Peripheral arterial disease
- Callus
- History of prior ulcers/amputations
- High plantar foot pressures
- Limited joint mobility (neuroarthropathy)
- Uncontrolled hyperglycemia
- Chronic renal insufficiency
- Diabetes duration
- Older age
- Poor knowledge of diabetes

The major factor in the development of DFU is neuropathy and peripheral artery disease. Ischemia, callus, and edema are additional variables that are connected to the ulceration causative pathway. Since foot ulcers are the key antecedent events leading to amputation, several risk factors such as gender (male), duration of DM longer than 10 years, advanced age, high body mass index, prior ulceration, and other comorbidities, such as retinopathy, glycated haemoglobin level, limited joint mobility, foot deformity (Charcot foot, prior partial foot amputation, etc), high plantar pressures, and inappropriate foot self-care habits are also predisposing factors for amputation [10, 12].

Pathogenesis:

Typically, a diabetic ulcer develops in three stages. The callus-forming phase is the initial stage. The callus is a result of neuropathy. Sensory neuropathy causes sensory loss, which results in continuing trauma; while motor neuropathy physically deforms the foot [13]. Another contributing aspect is autonomic neuropathy, which causes the skin to dry out.

Finally, repeated stress to the callus causes subcutaneous bleeding, and over time, it erodes and develops into an ulcer [14].

Patients with diabetes mellitus also experience vascular compromise due to extensive atherosclerosis of the tiny blood arteries in the legs and feet, which is another factor contributing to diabetic foot infections [15]. Healing is slowed down because blood cannot get to the wound, which finally causes necrosis and gangrene [16]. Diabetes mellitus also leads to foot deformity which causes increased pressure on plantar tissue and results in ulceration [17].

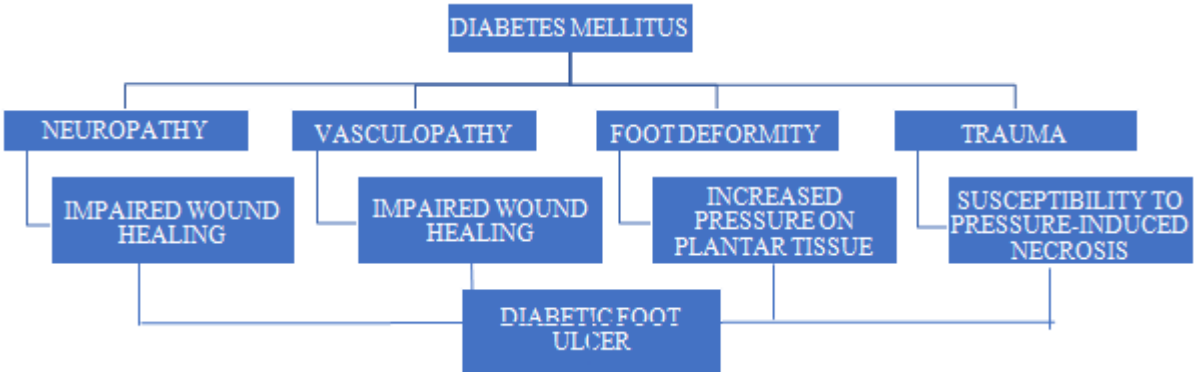

Figure 1: Pathogenesis of diabetic foot ulcer

Assessment of Foot Ulcer:

The seriousness of diabetic foot ulcer is identified on a scale of 0-5 using the Wagner Ulcer classification system [18] [19]:

- 0: no open lesions; may have healed lesion
- 1: superficial ulcer without penetration to deeper layers
- 2: deeper ulcer, reaching tendon, bone, or joint capsule
- 3: deeper tissues involved, with abscess, osteomyelitis, or tendonitis
- 4: gangrene in a portion of the forefoot or heel
- 5: extensive gangrenous involvement of the entire foot

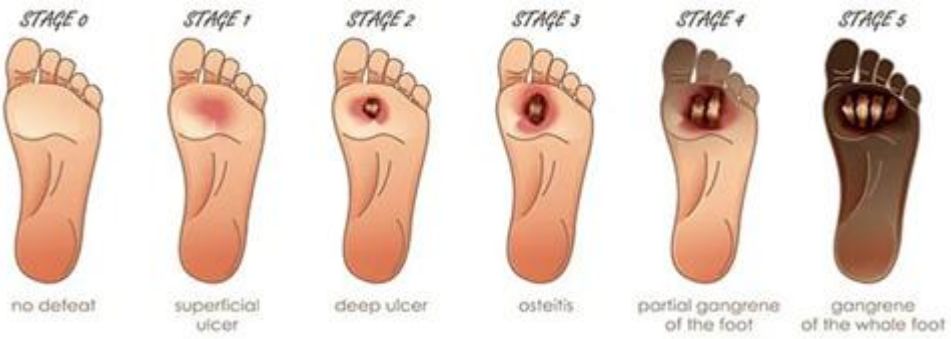

Figure 2: Stages of diabetic foot ulcer

Treatment and management:

The primary goal in the treatment of DFUs is to obtain wound closure and avoid lower extremity amputation [20]. The main strategies involved in the treatment are identification of the at-risk foot, treatment of the acutely diseased foot, and prevention of further complications [21]. The first step in the treatment of DFU is to treat infections. Antibiotics are the first-line drugs prescribed depending on patients and ulcer characteristics [22]. The next step is to treat the underlying peripheral vascular disease which leads to inadequate delivery of antibiotics due to inadequate blood supply limiting the oxygen supply. Hence revascularization improves blood supply which leads to a higher chance of ulcer healing [23]. The next step is to remove calluses or do local debridement [24].

Rest, elevation, and removal of pressure (off-loading) are essential components in the management of DFU [25]. Total contact casting (TCC) half-shoes, short-leg walkers, and felted foam dressings are the most widely used off-loading treatments [26]. The total contact cast (TCC) is used in the management of neuropathic ulcers due to its proven ability to redistribute pressure, which promotes expeditious wound closure [27] [28]. TCC is the most efficient of these when evaluated by the rate of wound healing [29] [30]. However, TCC is not widely utilized primarily due to its inherent drawbacks (possible secondary skin lesions and inability to daily assess the wound) [31]. Even if the pressure reduction is noticeably less than TCC and the patient's compliance cannot be guaranteed, other off-loading devices (such as the half shoe and short leg walker) are simpler to use and are more well-liked by the patient [32]. Another category of off-loading devices is felted foam dressing, which provides tailored pressure relief and, when used with a surgical shoe or half-shoe, is more efficient than a short-leg walker or crutches [33]. For clean, non-healing wounds, vacuum-assisted closure can be used. Hyperbaric oxygen therapy can also be considered if the wound doesn't heal in 30 days [34]. One of the mainstays of ulcer therapy is the debridement of necrotic, callus, fibrous, and senescent tissues [35]. Adequate debridement must always precede the application of topical wound healing agents, dressings, or wound closure procedures [36].

Name of the Drug	Mechanism of Action	Adverse Effects
ANTI-BIOTICS: • Vancomycin	Provides resistance against aerobic Gram-positive bacteria.	Nephrotoxicity
• Ceftazidime • Cefepime	Third-generation cephalosporin which covers both aerobic and anaerobic bacteria	Serious adverse nervous system effects
• Piperacillin-tazobactam	Aminopencillin and a beta-lactamase inhibitor. Especially protects against MRSA, Enterobacteriaceae, Pseudomonas, and obligate anaerobes	Diarrhoea
• Clindamycin	Clindamycin covers Gram-positive (exceptEnterococcus) and anaerobic infections	Diarrhea and gastrointestinal upset
• Amoxicillin-Clavulanate	It is a relatively broad-spectrum oral antibiotic with Gram-positive, Gram-negative, and anaerobic coverage.	Mild diarrhea, gas, vomiting, stomach pain, headaches, itching, and vaginal yeast infections.
• Ampicillin-Sulbactam	It is effective against many Staph and Strep species, E. coli, Proteus species, Morganella, Clostridium, and Bacteroides species. It does not provide coverage for MRSA or Pseudomonas.	Mild gastrointestinal upset and skin hypersensitivity.
Anti-Diabetic Agents: • Alogliptin • Pioglitazone • Ertugliflozin • Semaglutide • Teneligliptin	Sodium glucose co-transporter 2 (SGLT2) inhibitor, glucagon-like peptide-1 receptor agonist	Hypoglycemia, hypoglycemic coma, hypersensitivity, hepatotoxicity, drug-induced erythema multiforme, photodermatitis.
Anti-Fungal: • Metronidazole	It is effective against obligatory and facultative anaerobes such as Clostridium, Eubacterium, Peptococcus, Peptostreptococcus, Bacteroides fragilis, and Fusobacterium species	Flushing, tachycardia, palpitations, nausea, and vomiting.
Wound Healing Agent: • Becaplermin	Binds to the beta platelet-derived growth factor (PDGF) receptor, a tyrosine kinase receptor. It includes promoting the chemotactic recruitment and proliferation of cells involved in wound repair and enhancing the formation of granulation tissue.	Rare but serious side effects may include allergic reactions such as rash, itching, swelling (especially of the face/tongue/throat), dizziness, and trouble breathing.
Anti-Platelets: • Clopidogrel	It selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet P2Y12 receptor and the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, thereby inhibiting platelet aggregation.	Diarrhea, stomach pain, Indigestion, or heartburn.

2. Screening Methods

Invitro Methods:

1) MTT ASSAY [37]:

For quantitative evaluation of cell viability and proliferation, MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) assay is used, in which only viable cells could reduce MTT to insoluble purple formazan. Thus, the intensity of the purple color represents the number of viable cells. For MTT assay, the cells are cultured in a 24-well microtiter plate at a density of 5×103 cells per 100 µl of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS and 1% antibiotic and antimycotic solutions (termed as normal growth media) for 48 h. Then the medium is supplemented with different concentrations of test drug that included 7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 parts per million (ppm). HDF cells are then incubated in a humidified atmosphere of 5% CO2 at 37ºC and the medium is changed every day (wherever applicable). MTT assay is performed after 48 h post-treatment. To evaluate the number of viable cells, 300 µl of MTT solution is added into each well and incubated for 4 h at 37ºC in dark. The formazan crystals that are formed by living cells are solubilized with DMSO and the absorbance is measured at 570 nm with background subtraction at 690 nm using a multimode plate reader (TECAN, Infinite M 200).

2) Invitro Wound Healing Assay [38]:

The in vitro wound healing assay is carried out as described by Liang et al., 2007. Human Dermal Fibroblast (HDF) cells are seeded on 24-well tissue culture dishes (106 cells/well). The cells are incubated for 48 h at 37°C with 5% CO2. When confluence is reached, cell monolayers are incubated in a serum-free medium for 12 h. The monolayers are then gently scratched with a sterile pipette tip to create the wound and extensively rinsed with medium to remove all cellular debris. Then an indicated concentration of plant extracts is added and incubated for 24, 48, and 72 h. The rate of cell migration and healing is assessed by placing the cells under an inverted microscope and an image is obtained.

Invivo Method:

Induction of Diabetic foot Ulcer in Animals:
Firstly, Diabetes is induced in animals using any one of diabetes-inducing agents such as Streptozotocin, alloxan, etc. Blood glucose level is estimated using glucometers. Then animals showing blood glucose levels of more the 250 mg/dl are subjected to wound creation [39]. An ulcer is caused by creating either an excision or incision wound on the dorsal side of the animal [39]. Due to hyperglycemia and impaired wound healing, a diabetic ulcer will be induced.

Diabetes-Induced Using Streptozotocin:
Streptozotocin is dissolved in 100 mM citrate buffer (pH 4.5) and the calculated amount of the dose (60 mg/kg) of the fresh solution is injected intraperitoneally into overnight fasted rats and (40 mg/kg) for mice. Blood glucose is checked using a glucometer 48 h later and animals showing blood glucose values more than 250 mg/dl are included in the experiments and termed as diabetic [40].

Diabetes-Induced Using Streptozotocin with Nicotinamide:
Diabetes is induced by a single intraperitoneal injection of streptozotocin (STZ) (50 mg/kg body. wt) dissolved in 0.1 M of cold citrate buffer (pH 4.5) 15 min after the intraperitoneal administration of nicotinamide (110 mg/kg body wt) in overnight fasted rats (Masiello et al., 1998). Induction of diabetes is confirmed by tail vein blood glucose estimation using a glucometer (One Touch Horizon, Johnson & Jonson, Mumbai, India) after 72 h. After two weeks, rats with blood glucose levels>250 mg/dl are deemed as diabetic and used for the experiment [41].

Diabetes-Induced Using Alloxan:
Diabetes mellitus is induced in male albino Wistar rats by a singleintraperitoneal injection of a freshly prepared solution of alloxan monohydrate (10 mg/kg body weight) in physiological saline after overnight fasting for 12 h. The development of hyperglycemia in rats is confirmed by plasma glucose estimation 72 h post alloxan injection. The rats with fasting plasma glucose levels of 160-220mg/dL are used for this experiment [42].

Surgical Wound Invivo Models

- **Excision wound invivo model:**

Rats are anesthetized by a single intraperitoneal injection of sodium thiopentane (50 mg/kg body wt) dissolved in PBS (Mackraj et al., 2008). The dorsal fur of the animals is shaved with a sterilized blade and a 2cm2 (2 X 2) full-thickness excision wound is created on the back of the rat (Figure-3) [43] [45].

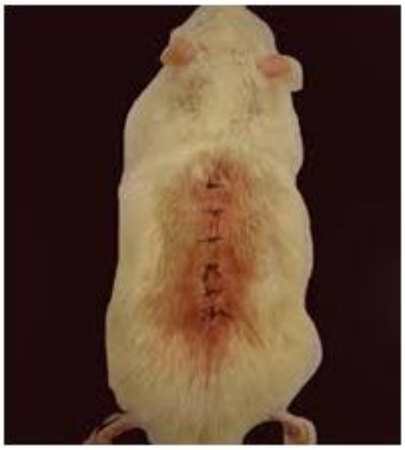

Figure 3: Excision Wound Model

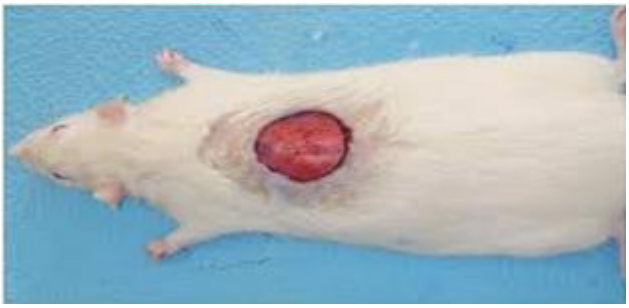

Figure 4: Incision Wound Model

- **Incision wound in vivo model**

A 6 cm long para vertebral incision wound is created on either side of the male rats. After mopping the wound dry, intermittent sutures are placed 1 cm apart using black cotton threads (Figure-4). The sutures are removed on day 7 and the tensile strength of the wound is determined on day 8 [44] [45].

Ex-Vivo Evaluation:

- Estimation of nucleic acid [46]
- Estimation of DNA [47]
- Protein estimation [48]
- Estimation of total collagen [49]
- Measurement of Hydroxyproline [49]
- Estimation of uronicacid [50]
- Estimation of lipid peroxide [51]

3. Conclusion

The elements of etiology, pathophysiology, examination, and treatment that can assist and assure the successful healing of foot ulcers in diabetes patients have been described above. When possible, these methods should be implemented to lower the significant morbidity and risk of severe consequences brought on by foot ulcers. Although improvements in the management of chronic diabetic wounds are encouraging, it is important to not disregard the underlying pathophysiologic problems that cause ulcers in the first place. No known therapy will be helpful without concurrent care of ischemia, infection, and sufficient off-loading. The screening methods are helpful in inventing new treatments for these severe complications.

While not all diabetic foot problems can be avoided, they can be significantly decreased in frequency with the right management and prevention strategies. The most effective way to obtain favorable rates of limb salvage in high-risk diabetic patients is to use a multidisciplinary team strategy that incorporates the skills of many different types of healthcare practitioners.

Acknowledgement

We wish to acknowledge the help and support rendered by the management of C. L. Baid Metha College of Pharmacy, Chennai, Tamilnadu, India

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