Natural Biologics Accelerate Healing of Diabetic Foot Ulcers by Regulating Oxidative Stress

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Abstract

Difficult or even non-healing diabetic foot ulcers (DFU) are a global medical challenge. Although current treatments such as debridement, offloading, and infection control have resulted in partial improvement in DFU, the incidence, amputation, and mortality rates of DFU remain high. Therefore, there is an urgent need to find new or more effective drugs. Numerous studies have shown that oxidative stress plays an important role in the pathophysiology of DFU. The nuclear factor erythroid 2-related factor (Nrf2) signaling pathway and the advanced glycedated end products (AGEs)-receptor for advanced glycation endproducts (RAGE), protein kinase C (PKC), polyol and hexosamine biochemical pathways play critical roles in the regulation of oxidative stress in the body. Targeting these pathways to restore redox balance can control and alleviate the occurrence and development of DFU. Natural biologics are a major source of potential drugs for these relevant targets, and their antioxidant potential has been extensively demonstrated. Here, we discussed the pathophysiological mechanism of oxidative stress in DFU, and identified natural biologics targeting these pathways to accelerate DFU healing, in order to provide a new or potential direction for clinical treatment, nursing and related basic research of DFU.

Keywords: diabetic foot ulcers; diabetic wound; oxidative stress; natural biologics; targeted therapy

1. Introduction

The incidence and prevalence of diabetes mellitus (DM) is increasing worldwide. In 2021, 536.6 million people in 20–79-year-olds had diabetes worldwide, which is estimated to rise to 783.2 million in 2045 [1]. DFU are a common complication of DM. It is estimated that 19–34% of diabetic patients will develop DFU [2]. With the increase in patients with diabetes, the number of patients with DFU is increasing. Importantly, DFU not only have a high incidence [3], but also a high rate of disability and mortality [4,5], and once DFU appear, they can be difficult to heal or even become nonhealing. They lead to great suffering both physically and mentally. Current treatments for DFU mainly include debridement, offloading, and infection control [6], but the effect is not ideal. Therefore, more effective treatment strategies need to be explored. Although the exact mechanism of the occurrence and development of DFU is still unclear, there is no doubt that oxidative stress plays a key role in DFU [7].

Oxidative stress is a physiological process, and a reasonable level of oxidative stress can promote wound healing [8,9]. However, in DFU, there is an imbalance between the body’s reactive oxygen species (ROS) levels and antioxidant enzymes [10]. The pathological oxidative stress not only damages the stages of inflammation, proliferation, and remodeling, making each stage unable to proceed smoothly and orderly, but also leads to vascular, neuropathy, or local infection, and ultimately worsens DFU [7]. Nrf2 is the central regulator of oxidative stress [11]. Damage to the AGEs-RAGE, PKC, polyol and hexosamine biochemical pathways is the key to the imbalance of redox levels [12]. Therefore, seeking drugs targeting the Nrf2 signaling pathway and AGEs-RAGE, PKC, polyol and hexosamine pathways to promote the body’s redox balance may become an important strategy to accelerate the healing of DFU.

Natural biologics have always been a research hotspot in the world due to their advantages of less toxicity and side effects, wide sources, and cost efficiency. Many natural biologics can scavenge oxygen free radicals or increase antioxidant levels by targeting the Nrf2 signaling pathway, AGEs-RAGE, PKC, polyol and hexosamine pathways and ultimately play a beneficial role in the repair of DFU.
Therefore, the aim of this review was to investigate the role of oxidative stress in the pathophysiology of DFU through a literature review. We then introduced the Nrf2 signaling pathway and AGEs-RAGE, PKC, polyol and hexosamine pathways as pharmacological targets, providing up-to-date information on natural biologics that target these pathways to accelerate DFU healing.

2. Oxidative Stress in DFU

Oxidative stress was defined as: “an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage” [13]. When the skin barrier structure is destroyed, oxygen immediately flows in, and the injury-induced O2 influx fuels local ROS production [14]. At low concentrations, ROS act as cellular messengers to accelerate wound healing [8,9]. However, in DFU, abnormalities in the Nrf2 signaling pathway and AGEs-RAGE, PKC, Polyol and Hexosamine biochemical pathways result in excessive oxidative stress on the wound surface, which is one of the main reasons for the difficulty in healing of DFU. The results of clinical specimen experiments showed that there was uncontrolled oxidative stress and decreased antioxidant capacity in DFU, which resulted in redox imbalance [10]. Excessive oxidative stress can damage all stages of DFU repair, such as inflammation, proliferation, and remodeling.

2.1 Excessive Oxidative Stress Causes DFU to Stagnate During the Inflammatory Phase

In patients with DFU, continuous hyperglycemia will lead to excessive oxidative stress. Oxidative stress induced by hyperglycemia can enhance neutrophil gene expression and secretion of S100A8 and S100A9 [15,16]. S100A8/A9 promotes leukocytosis via increased proliferation of Bone Marrow progenitor cells [17]. For example, S100A8/A9 induces macrophages to secrete more proinflammatory mediators, such as IL6 [15]. Moreover, sustained hyperglycemia exposure increases neutrophil metabolism, and ROS production as a byproduct through the mitochondrial electron transport chain also increases [16,18]. ROS in the neutrophils stimulate NETosis, captivating and killing pathogens via neutrophils extracellular traps (NETs) [19], through both the NOX-dependent and NOX-independent pathways [16]. Reasonable formation of NETs is beneficial to the normalization of the immune microenvironment, but excessive formation of NETs worsens the abnormal inflammatory response of wounds, which is also the key pathological manifestation of diabetic wounds (DW) that is difficult to heal [20–22]. In addition, NETs also reduce the responsiveness of neutrophils to lipopolysaccharide, leading to an imbalance of M1/M2 macrophages [23]. Neutrophils and macrophages are the core cells in the inflammatory phase, and their abnormal function or phenotype often leads to a continuous low-efficiency inflammatory reaction on the wound. Natural biologics play an import role in decreasing inflammatory response and oxidative stress markers [24]. Solving the problem of pathological oxidative stress in the wound and precise regulation of the start and end of inflammation are the key to promote the repair of DFU.

2.2 The Balance of Redox Homeostasis is the Basis for the Smooth Progress of the DFU Proliferation Phase

Excessive ROS production in DFU leads to ROS accumulation in endothelial cells, which mediates endothelial dysfunction [25,26]. Inhibition of oxidative stress can enhance the proliferation of human umbilical vein endothelial cells and human skin fibroblasts, restore angiogenesis, promote granulation tissue formation and healing of epithelialization, overall demonstrating markedly improved healing of DW [27]. In addition, fibroblasts promote keratinocyte proliferation through Jun-dependent expression of PTN and SDF-1 [28], which is beneficial to epithelial reformation [29]. However, in DFU, excessive oxidative stress impaired the proliferation and differentiation of fibroblasts [27]. In DFU, intense oxidative DNA damage, low vascular endothelial growth factor (VEGF) and transforming growth factor β1 (TGF-β1) expression, and increased apoptosis all contribute to delayed healing of DFU [30]. Excessive oxidative stress results in a harsh microenvironment for DFU; and endothelial cells, fibroblasts, etc., cannot proliferate ideally to promote the smooth progress of wound proliferation.

2.3 Imbalance in Redox Homeostasis Causes DFU Remodeling Disorder

In DFU, excessive oxidative stress leads to impaired proliferation of fibroblasts. Fibroblasts play an important role not only in reepithelialization but also in remodeling. Fibroblast derived keratinocyte growth factor-1 (KGF-1), also known as fibroblast growth factor 7, accelerates wound contraction by promoting TGF-β1-induced fibroblast contraction via the TGF-β1/Smad signaling pathway [31]. KGF-1 deficiency can significantly reduce the wound contraction rate during healing [32]. Myofibroblasts are responsible for contracting dermal tissue and they can produce several components of the extracellular matrix (ECM) such as collagens of types I, III, IV, and V to promote tissue remodeling [33]. TGF-β1 is also required for fibroblast differentiation into myofibroblasts [31]. However, the expression levels of TGF-β1 were significantly lower in DFU [34]. Ilya A Demyanenko et al. [35] confirmed that the mitochondria-targeted antioxidant 10-(6′-plastoquinonyl) decyltriphenyolphosphonium can not only reduce the number of neutrophils, promote the M1/M2 macrophages to restore balance, also promote fibroblast migration and increase the number of myofibroblasts, eventually improving DW healing. Therefore, remodeling is also impaired by excessive oxidative stress.
3. Signaling Pathways in the Regulation of Oxidative Stress in DFU

3.1 Nrf2 Signaling Pathways

Nrf2, a member of the cap ‘n’ collar subfamily of basic region leucine zipper transcription factors [36], maintains the balance of redox in the body by regulating many genes, including but not limited to GSH, catalase (CAT), heme oxygenase 1 (HO1) and quinone oxidoreductase 1 (NQO1) [37,38]. Research targeting Nrf2 shows great potential and application value in cancer [39], Alzheimer’s disease [40], cardiovascular disease [41] and DW [42] or chronic wound healing [43].

In a normal physiological environment, Nrf2 specifically binds to the amino-terminal Neh2 domain to mediate the ubiquitination and degradation of Nrf2, thus maintaining a low intracellular concentration [44]. When the body is in a state of oxidative stress, the accumulation of ROS or electrophiles will weaken the interaction between Nrf2 and Keap1, thus releasing Nrf2 and inhibiting Nrf2 ubiquitination, leading to the enhancement of Nrf2 nuclear translocation and the formation of heterodimerizes with one of the small Maf proteins, and binding with antioxidant response elements to activate the transcription of antioxidant protein genes. Ultimately, it acts as an antioxidant and protects cells from stress factors [45,46]. Therefore, low levels of oxidative stress can be regulated by Nrf2 to promote the recovery of redox homeostasis. However, continuous excessive oxidative stress will lead to the imbalance of ROS, free radicals, etc., and antioxidants in the body, which is one of the pathological mechanisms that mediate the development of DFU. Under hyperglycemia, the activation of Nrf2 is inhibited, and the expression of antioxidant genes including NQO1 and HO1 are significantly down-regulated, which exacerbates the occurrence and development of oxidative stress [37]. The study of Rajan Teena et al. [47] using human clinical specimens showed that Nrf2 expression was significantly reduced in type 2 DM subjects and DFU subjects compared with normal glucose tolerant subjects. Perilesional skin tissues from patients with diabetes are often in a more severe state of oxidative stress. Although the compensatory Nrf2 pathway is activated due to high oxidative stress, such compensatory Nrf2 activation cannot restore the redox balance of the body [42]. Activation impairment of Nrf2 not only aggravates oxidative stress, but also causes abnormal inflammation of the wound surface [37]. Moreover, due to severe oxidative damage to the skin tissue of diabetic patients, cell apoptosis often occurs [42]. Silencing Nrf2 by siRNA not only increases cell apoptosis, but also slows down the migration rate of HaCaT cells [42], and the Nrf2 transcription factor is also a novel target of KGF [48]. An Abnormal Nrf2 pathway is not conducive to the smooth progression of wound proliferation and remodeling. There is no doubt that targeted activation of Nrf2 is an effective way to accelerate healing of DFU. The team of Min Li et al. [37] and Ying Li et al. [49] confirmed this idea in streptozotocin-induced diabetic rat wound models using dimethyl fumarate, an Nrf2 activator. In addition, Nrf2 also promotes angiogenesis through multiple pathways, such as MALAT1/HIF-1α [50], which is also beneficial for the healing of DFU. It has been reported that hyperbaric oxygen therapy, which is commonly used in clinical practice, can accelerate wound repair by activating Nrf2 and stimulating angiogenesis [51]. In conclusion, the persistent and high level of oxidative stress on the DW is closely related to the refractory DW, and the disorder of redox homeostasis is mainly related to abnormal Nrf2 in the tissue of DFU.

3.2 AGEs-RAGE Pathways

AGEs are a heterogeneous group of modified molecular species [52] that play a central role in oxidative stress. The accumulation of AGEs in vivo can be not only produced endogenously but also acquired by exogenously. For endogenous AGEs, the most classic is the Maillard reaction. The Maillard reaction is a complex network of amino acids and reducing sugars [53]. This non-enzymatic reaction can generate intermediate carbonyl precursors of AGEs, and glucose can also generate dicarbonyls through autoxidation, the polyol pathway and lipid peroxidation, which greatly contribute to the formation of AGEs [54]. Worldwide, the age-standardized prevalence of daily smoking was 25.0% for males and 5.4% for females [55]. Cigarette smoke promotes the production and activation of advanced glycation end products [56]. Food is also an important source of AGEs in the body. Approximately 10–30% of AGEs in food can be absorbed into the systemic circulation [54,57]. AGEs/RAGE interactions can activate or strengthen numerous signaling pathways to enhance oxidative stress such as the Ras-mediated extracellular signal-regulated kinase (ERK1/2), stress-activated protein kinase/cJun N-terminal kinase (SAPK/JNK), mitogen-activated protein kinase (MAPK), and Janus kinase signal transducer and activator of transcription (JAK/STAT) pathways [54].

Under normal circumstances, there are only moderate amounts of AGEs in the body. However, for DFU patients, the continuous high glucose environment leads to the continuous accumulation of AGEs in the body [58,59]. Moreover, many diabetic patients are obese, and the obese patients often acquire more exogenous AGEs orally [60]. The binding of AGEs to RAGE promotes the production of ROS and the inflammatory cascade by reinforcing oxidative stress. For example, excessive accumulation of AGEs decreased the migratory and adhesive functions of neutrophils [61], inhibited the influx of early macrophages, and disrupted the phagocytic function of M1 macrophages [62], which is not conducive to the formation of an early proinflammatory microenvironment on the wound surface. At the late stage of wound healing, accumulated AGEs promote overactivation of the NLRP3 inflammasome by gener-
PKC and RAGE binding can affect Ras homolog family members A/Rho kinase signaling to inhibit the anti-inflammatory function of exocytosis [64], putting the wound in a continuous stage of an ineffective inflammatory response. The adverse immune microenvironment prevented the wound from progressing to the stage of proliferation and remodeling from the stage of inflammation. In the proliferation phase, AGEs significantly inhibited HaCaT cells proliferation and migration by down-regulating the expression of miR-146a and upregulating the expression of an anchoring protein 12 (AKAP12) [65]. Moreover, excessive activation of the AGEs-RAGE pathway disrupts collagen I maturation and prevents its deposition in the ECM, which also further impairs DFU remodeling [66,67]. In addition to these adverse consequences, unfortunately for patients with DFU, the accumulation of AGEs in tissues was independently correlated with vascular lesions that may lead to ischemic lesions in diabetic feet, leading to the adverse consequences of amputation [68,69]. The accumulation of AGEs is also a risk factor for the development of diabetic neuropathy [70], which may cause the patient’s feet to lose protective pain sensation. Undoubtedly, vascular and neuropathy are an added hazard for an already difficult-to-heal DFU. More troubling is that AGEs induced the release of extracellular DNA (eDNA) by positively affecting sigB transcription and downregulating IgA expression, thereby promoting the formation of S. aureus biofilms [71], which may aggravate DFU local infection. AGEs not only adversely affect the inflammatory, proliferative, and remodeling phases of DFU repair, but also codamage DFU by inducing or aggravating vasculopathy, neuropathy, and local infection. Targeting AGEs-RAGE pathways could be a good way to treat DFU.

3.3 PKC Pathways

Protein kinase C isozymes (PKCs), serine-threonine protein kinases, are widely distributed in mammalian tissues and play a critical role in many physiological functions such as regulating cell growth and proliferation, senescence, and apoptosis [72,73]. They are grouped into three categories according to their domain composition: conventional PKCs (PKCα, PKCβI, PKCβII, PKCγ), novel PKCs (PKCδ, PKCe, PKCη, PKCθ), and atypical PKCs (PKCζ, PKCλ/ι) [74,75]. Conventional PKCs are activated by diglycerol and Ca2+, novel PKCs are activated by diglycerol, and atypical PKCs are regulated by protein:protein interactions [76]. All PKCs remain in an autoinhibited conformation until they bind to the correct second messengers or protein scaffolds for atypical PKCs [77], thus releasing the pseudosubstrate and activating PKC [76,78]. Activation of PKC regulates substrate phosphorylation and downstream signal transduction.

In the high-glucose environment of DFU, PKC is continuously activated [79]. Excessive activation of PKC aggravates the neutrophil respiratory burst and releases a large number of NETs [80,81]. Sushant Kumar Das et al. [82] also confirmed that the expression of PKCβII was significantly upregulated in DFU, which primes neutrophils to undergo NETosis and promotes PKCβII mediated downregulation of the VEGF-dependent Akt/eNOS pathway. The respiratory burst of neutrophils, the occurrence of NETosis and the reduction of VEGF, which worsen inflammation, endothelial cell damage, and ECM degradation, are important reasons for the difficult healing or even nonhealing of DFU. In addition, excessive activation of PKC signaling also damages the vascular endothelium. With the further research, the abnormal expression of PKC in DFU does not remain at the level of animal and cell experiments. Zhichuan Liu et al. [10] also verified the significant upregulation of p-PKCβ in DFU at the population level. High glucose levels may impair the vascular endothelium by activating the PKCβ3-p66shc signaling pathway [10]. Inhibition of PKC signaling promotes the stabilization of endothelial barrier function by reducing moesin phosphorylation [83]. Furthermore, PKCδ protein and mRNA levels were increased by 7-fold and 3-fold, respectively, in discarded tissues obtained from active DFU compared with levels detected in control (without diabetes) tissues [84]. Increasing PKCδ expression aggravates the difficulty in DFU healing by impairing fibroblasts. Equally worrisome is that the PKC signaling pathway is closely related to peripheral neuropathy, and abnormal PKC can exacerbate peripheral neuropathy in diabetic patients [85]. Numbness and sensory disturbances are common symptoms of peripheral neuropathy that cause protection from painful stimuli. This is an important reason why the injured feet cannot be detected and treated in time, resulting in wound infection, deterioration, and missed optimal treatment time. An abnormal PKC signaling pathway not only intensifies oxidative stress but also impairs the healing of DFU. However, currently, in view of the special complications of diabetic foot, the most studied category is conventional PKCs (PKCβ), and other categories need to be further explored.

3.4 Polyol Pathways

The polyol pathway includes a family of monomeric nicotinamide adenine dinucleotide phosphate (NADPH) dependent aldo-keto reductase enzymes that catalyze the conversion of carbonyl compounds to sugar alcohols [72]. Part of glucose can be converted into sorbitol via aldolase reductase (AR). Then sorbitol is slowly converted to fructose by sorbitol dehydrogenase hindering antioxidant activity [86]. This step uses NAD+ as a cofactor. Substrate-driven reactions mediated by NADPH and NAD+ play a significant role in maintaining the redox balance [87]. However, compared to nondiabetic wounds, higher levels of oxidative stress were present in the tissue of DFU [47]. The increased sorbitol pathway activity is one of the important mechanisms for the development of DFU [88].
AR is the rate-limiting enzyme for this reaction. Under normal conditions, AR has a very low affinity for glucose, and no more than 3% of glucose is processed through this pathway [89]. However, under the condition of continuous hyperglycemia, hexokinase is saturated, and then AR is continuously activated [90], which consumes NADPH and reduces the function of GSH reductase impairing antioxidant activity [86]. The accumulation of sorbitol may cause osmotic damage and lead to cell death. At present, there is no direct evidence that excessive accumulation of sorbitol in DFU tissue can aggravate the death of cells that are beneficial to wound healing such as endothelial cells. Further research is needed. However, the team of Bradley P Mudge [91] confirmed that the level of GSH in DFU was significantly lower than that in nondiabetic wounds. Treatment with local GSH accelerated the recovery of redox levels, which reduced wound tissue biofilm production, increased antibiotic sensitivity and ultimately promoted the healing of DFU [92]. What also bothers us is that the abnormal increase of AR in DFU may make DFU fall into a worse situation [93]. In addition, fructose was abnormally increased in DFU tissue. Excessive fructose is metabolized by fructokinase to produce overly acetyl-CoA, which increases protein acetylation and results in protein dysfunction [94,95]. Fructose can generate AGEs [96], which are detrimental for the repair of DFU. Continuous abnormal oxidative stress in DFU increases the collagen I-to-collagen III ratio in collagen accumulation and lipid atrophy and may eventually lead to amputation in DFU patients [95]. Therefore, an abnormal polyol pathway may hinder the repair of DFU.

3.5 Hexosamine Pathways

The hexosamine pathway (HP) is identical to the first two steps of glycolysis. The glycolytic intermediate fructose-6-phosphate is converted to glucosamine-6-phosphate under the action of glutamine-fructose-6-phosphate aminotransferase, the rate-limiting enzyme of HP [97]. Subsequent enzymatic steps then lead to acetylation and activation using UTP to produce the amino sugar UDP-N-acetylglucosamine (UDP-GlcNAc) [98]. UDP-GlcNAC is an important metabolic compound for the formation of glycosyl chains of proteins and lipids [99]. Moreover, under the action of O-glycosyltransferase (OGT), UDP-GlcNAC can modify the protein O-GlcNAC [100]. The HP is composed of a series of anabolic reactions [98] and plays an important role in the pathophysiology of diabetes complications [101–103].

Under normal blood glucose levels, only 2–5% of fructose-6-phosphate enters the HP [104]. However, in diabetic patients, excess fructose-6-phosphate is shunted into the HP. The increase in HP flux leads to an increase in UDP-GlcNAC levels, which in turn increases the flux through OGT, leading to an increase in O-GlcNAC levels [99,105]. O-GlcNAC modification is a posttranslational modification that can regulate inflammatory response by target-
of redox homeostasis in the body and ultimately promote DFU repair. We identified some natural biologics targeting Nrf2 to promote DFU healing. Here, we briefly list a few natural biologics and describe how the targeted activation of Nrf2 can promote the repair of DFU. Nefertine (Nef), a bisbenzylisoquinoline alkaloid obtained from the seed embryos of Nelumbo nucifera, has various pharmacological effects such as anti-oxidative, anti-inflammatory, anti-arrhythmic and anti-thrombotic. In addition, it can increase the sensitivity of chemotherapy drugs, such as paclitaxel, cisplatin and doxorubicin [121–123]. Juan Li et al. [124] showed that Nef can improve insulin sensitivity, which significantly reduced blood glucose levels. Local application of Nef in diabetic rat wounds can activate the Nrf2 signaling pathway, reduce Keap1 expression, increase the expression of downstream factors antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR), and increase the expression of collagen-1, TGF-β and α-SMA in ECM. In addition, Nef can also reduce the production of TNF-α, IL-1β and other inflammatory factors. These results suggested that Nef improved the redox homeostasis of the wound through the targeted activation of Nrf2 and promoted the repair of DW from multiple dimensions of inflammation, remodeling and blood glucose levels. Moreover, targeted activation of Nrf2 to reverse oxidative stress in wounds can not only play a beneficial role in the inflammation and remodeling stages of DW but also improve the proliferation, angiogenesis and neuropathy of DW (important pathological mechanisms or manifestations of the occurrence and development of DW). Resveratrol [125] is a good example of alleviating the effects of oxidative stress on the proliferation and migration of HUVECs through targeted activation of Nrf2, thereby promoting wound proliferation. In addition, procyanidin B2 [126] and rutin [127] promoted DW repair by targeting the Nrf2 pathway to promote angiogenesis and improve neuropathy, respectively. For more examples, please see Table 1 (Ref. [30,62,124–149]). These findings indicate that Nrf2, as a classical and core signaling pathway to maintain redox homeostasis in the body, plays a main role in the development of DFU. Targeted activation of the Nrf2 pathway can increase the expression of downstream factor antioxidant enzymes to exert antioxidant activity, restore the body’s redox homeostasis, thereby mediating the smooth progress of various stages of inflammation, proliferation and remodeling in DW repair, promote angiogenesis and improve neuropathy to prevent the deterioration of DFU and ultimately promote the healing of DFU. At present, many natural biologics targeting Nrf2 have been reported, but screening more efficient drugs to promote DFU healing, so as to promote their clinical transformation and ultimately promote the recovery of clinical DFU patients is our ultimate goal. Therefore, further exploration is needed.

4.2 AGEs-RAGE Pathways

AGEs-RAGE pathways is a key factor in maintaining the body’s redox balance. Abnormal activation of AGEs-RAGE signaling leads to damage to DFU in all stages. Vascular, neuropathy and local infections also cause patients with DFU to face the threat of amputation or death. Targeting the AGEs-RAGE signaling axis can inhibit abnormal inflammatory responses by regulating the NF-kB/NLRP3 [150], RAGE/RhoA/ROCK signaling pathway [151] or immune cells [64] to normalize the immune microenvironment. Previous studies have reported that topical application of pyridoxamine, a natural vitamin B6 analog, reduced the accumulation of AGEs in the wound tissue of diabetic mice, promoted the influx of macrophages in the early stage of tissue repair, improved the dysfunctional inflammatory response, and accelerated wound healing [62]. Moreover, targeting AGEs-RAGE signaling pathways can also be beneficial in the proliferation and remodeling stages of wound healing such as Centella cordifolia and Shixiang Plaster. Centella cordifolia reversed collagen migratory defects and restored the spreading and attachment of fibroblasts, endothelial cells and keratinocytes over the glycated ECM [133]. Ji Fei et al. [134] also used Shixiang Plaster, a traditional Chinese medicine, to promote the reepithelialization of DW by reducing the expression of AGEs and RAGE. In addition, as mentioned above, the accumulation of AGEs in vivo is inseparable from exogenous acquisition. Diets with a low AGEs content have effectively improved insulin sensitivity and reduced insulin resistance and cholesterol levels, which are beneficial for DFU repair [152]. There is no doubt that targeting AGEs-RAGE has great potential for the healing of DFU. Natural biologics targeting AGEs/RAGE can also prevent the further deterioration of DFU by regulating angiogenesis or neuropathy. For instance, the extracts of Momordica charantia (MC) induce ERK1/2 phosphorylation and tube formation through the AGEs-RAGE pathway, promoting angiogenesis [135], which may improve diabetic foot microcirculation and prevent diabetic foot ischemic lesions. In addition, salvianolic acid A (SalA), the main efficacious, water-soluble component of miltiorrhiza bunge, also improved peripheral blood perfusion, vasodilation responsiveness and peripheral nerve function by targeting AGEs, which to some extent hindered the development or deterioration of DFU [136]. These research results showed that natural biologics, in addition to their own advantages such as low toxicity and wide sources, were also efficacious for DFU. However, the combination of these natural biologics, which play different roles in different stages of wound healing, may be better for seeking the optimal prescription. Moreover, the current research on the mechanism of natural medicines and diseases mostly focuses on the indirect mechanisms, and the research on the direct mechanisms is not deep enough. This may give us the illusion that natural medicines are less effective. Therefore, an in-depth understanding the exact target of DFU, using
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<td>Luteolin</td>
<td>Inactivation of NF-κB and upregulation of Nrf2</td>
<td>Streptozotocin induced diabetic male rats</td>
<td>Excision wound model (wound diameter is 15 mm)</td>
<td>Intraperitoneal injection</td>
<td>Once a day for 14 days</td>
<td>DW healing is accelerated</td>
<td>[131]</td>
</tr>
<tr>
<td></td>
<td>Bee venom</td>
<td>Activated pathways</td>
<td>Streptozotocin induced diabetic male mice</td>
<td>Excision wound model (wound diameter is 8 mm)</td>
<td>Subcutaneously injected</td>
<td>Once a day for 15 days</td>
<td>DW healing is accelerated</td>
<td>[132]</td>
</tr>
<tr>
<td>Targeted Pathways</td>
<td>Natural Biologies</td>
<td>Mechanism</td>
<td>Human/Animal/Cell</td>
<td>Wound</td>
<td>Intervention Dose</td>
<td>Administration route</td>
<td>Administration frequency</td>
<td>Findings/Outcomes</td>
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<tr>
<td>AGEs-RAGE Pathways</td>
<td>pyridoxamine (PM)</td>
<td>Reduced the accumulation of AGEs, promoted the influx of macrophAGEs in the early stage of tissue repair</td>
<td>Human monocytic cell (THP-1), Male db/db T2DM mice</td>
<td>Excision wound model (wound diameter is 6 mm)</td>
<td>100 µM (THP-1), 100 µM/20 µL</td>
<td>Co-incubation, Topically applied, then wound was covered with a transparent dressing (3M, 1624W)</td>
<td>Once a day until day 10 post-injury</td>
<td>DW healing is accelerated</td>
</tr>
<tr>
<td>Centella Cordifolia</td>
<td>Inhibited the expression of AGEs and improved antioxidant capacity</td>
<td>The human vascular endothelial cell line (EAhy926), The mouse embryonic fibroblasts (NIH3t3) cell, The human keratinocytes cell line (HaCat)</td>
<td>EAhy926: 2 mM; NIH3t3: 500 µg/mL; HaCat: 500 µg/mL</td>
<td>Co-incubation</td>
<td>/</td>
<td></td>
<td>Restored the spreading and attachment of endothelial, fibroblast and keratinocyte cells over the glyated ECM</td>
<td>[133]</td>
</tr>
<tr>
<td>Shixiang Plaster</td>
<td>Inhibited the expression of RAGE and AGEs, Promoted angiogenesis and granulation tissue formation</td>
<td>Streptozotocin induced diabetic Sprague-Dawley rats</td>
<td>Excision wound model (wound diameter is 5 mm)</td>
<td>Topical application at a thickness of 2 mm over the wound</td>
<td></td>
<td></td>
<td>DW healing is accelerated</td>
<td>[134]</td>
</tr>
<tr>
<td>Momordica Charantia</td>
<td>Blocked RAGE, induced ERK1/2 phosphorylation and tube formation to promote angiogenesis</td>
<td>bovine aortic endothelial cells (BAEC)</td>
<td>10, 50 or 75 µg/mL</td>
<td>Co-incubation for 72 hours</td>
<td></td>
<td></td>
<td>promoted angiogenesis</td>
<td>[135]</td>
</tr>
<tr>
<td>Salvinolic acid A (SalA)</td>
<td>Decreased AGEs levels, vascular eNOS expression, and blood glucose, lipid, vWF and malondialdehyde levels</td>
<td>streptozotocin-induced type 2 diabetic rats</td>
<td>1 and 3 mg/kg</td>
<td>Per os (p.o) for 10 weeks</td>
<td></td>
<td>Once a day</td>
<td>Improved diabetic plantar microcirculation and peripheral nerve function</td>
<td>[136]</td>
</tr>
<tr>
<td>PKC Pathways</td>
<td>Curcumin</td>
<td>Inhibited PKC-α and PKC-β1 activity</td>
<td>streptozotocin-induced type I diabetic rats</td>
<td>100 mg/kg</td>
<td>p.o. for 8 weeks</td>
<td>Once a day</td>
<td>Diabetic nephropathy is attenuated</td>
<td>[137]</td>
</tr>
<tr>
<td>Pueraria tuberosa extract</td>
<td>Downregulated the PKC-α and NF-κB pathway to normalize the inflammatory microenvironment</td>
<td>streptozotocin-induced diabetic nephropathy rats</td>
<td>100 mg/100 g and 50 mg/100 g</td>
<td>p.o for 20 days</td>
<td></td>
<td></td>
<td>Inhibited the abnormal inflammatory response in diabetic nephropathy</td>
<td>[138]</td>
</tr>
<tr>
<td>sasa borealis water-extract</td>
<td>Inhibiting the activation of PKC β3 and NADPH oxidase</td>
<td>HUVEC</td>
<td>1 and 10 µg/ mL</td>
<td>Co-incubation</td>
<td></td>
<td></td>
<td>Blocked chronic high glucose-induced endothelial apoptosis</td>
<td>[139]</td>
</tr>
<tr>
<td>Verbascoside</td>
<td>Inhibiting PKC/HMGB1/RAGE/NFκB signaling</td>
<td>Smulow-Glickman (S-G) gingival epithelial cell line</td>
<td>25, 50 and 100 µM</td>
<td>Co-incubation for 24 hours</td>
<td></td>
<td></td>
<td>Mitigated the Suppressed Cell Proliferation and Wound Healing Capacity of Gingival Epithelial Cells under High Glucose Condition</td>
<td>[140]</td>
</tr>
<tr>
<td>Targeted Pathways</td>
<td>Natural Biologies</td>
<td>Mechanism</td>
<td>Human/Animal/Cell</td>
<td>Wound</td>
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<tr>
<td>Polyol Pathways</td>
<td>chlorogenic acid</td>
<td>Enhanced hydroyproline content, decreased malondialdehyde/nitric oxide levels, elevated reduced-glutathione</td>
<td>streptozotocin-induced diabetic rats</td>
<td>Excision wound model (wound diameter is 15 mm)</td>
<td>50 mg/kg</td>
<td>Intraperitoneal injection</td>
<td>Once a day</td>
<td>DW healing is accelerated</td>
</tr>
<tr>
<td>AgNPs</td>
<td>Increased GSH peroxidase activity and GSH content</td>
<td>streptozotocin-induced diabetic rats</td>
<td>Incision wound model (straight incisions of 8 cm Length on both sides of the vertebraland closed with interrupted sutures 1 cm apart)</td>
<td>10, 30 µg/kg</td>
<td>Topically applied</td>
<td>Once a day for 21 days</td>
<td>DW healing is accelerated</td>
<td>[142]</td>
</tr>
<tr>
<td>propolis</td>
<td>Increased the GSH and GSH: GSSG ratio, depleted TNF-α, and increased IL-10 levels</td>
<td>Diabetic patients with foot wounds</td>
<td>Propolis (Beepolis®) used was 3% in propylene glycol preparation manufactured</td>
<td>Topically applied (Propolis spray was applied to cover the wound surface in each dressing from week 0 until cicatrization or 8 weeks as a maximum, whichever occurred first.)</td>
<td>There was a decrease in the wound area by an average of 4 cm² in the propolis group compared with the control group, which reduced 3 cm²</td>
<td>[143]</td>
<td></td>
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<tr>
<td>polyphenolic fraction</td>
<td>Inhibited aldose reductase activity as well as their expression in diabetic animals</td>
<td>Diabetic peripheral neuropathy was induced by streptozotocin and maintained</td>
<td>100, 200 mg/kg</td>
<td>p.o for 30 days</td>
<td>/</td>
<td>Ameliorated diabetic peripheral neuropathy</td>
<td>[144]</td>
<td></td>
</tr>
<tr>
<td>Hexosamine Pathways</td>
<td>APE Increased hexosamine levels</td>
<td>Strepototozin-induced type 2 diabetic Sprague-Dawley rats</td>
<td>Excision wound model (wound diameter is 8 mm)</td>
<td>100, 200 and 400 mg/kg (p.o), 5, 10 and 20% APE cream (topically applied)</td>
<td>p.o for 7 days and topically applied for 28 days</td>
<td>p.o (once a day) topically applied (twice a day, approximately 1 mm thick)</td>
<td>DW healing is accelerated and neuropathy is ameliorated</td>
<td>[145]</td>
</tr>
<tr>
<td>ethanolic extract of Melia dubia</td>
<td>Improved total collagen and hexosamine</td>
<td>Female Wistar rats</td>
<td>Excision wound model (wound diameter is 2 cm) and Incision wound model (para-vertebral straight incisions of 6 cm Length and closed with interrupted sutures 1 cm apart)</td>
<td>40 mg/kg b. wt, 200 μL</td>
<td>Topically applied</td>
<td>Once a day until the wounds heal completely</td>
<td>DW healing is accelerated</td>
<td>[146]</td>
</tr>
<tr>
<td>Solanum xanthocarpum</td>
<td>Increased hexosamine levels</td>
<td>streptozotocin-induced diabetic rats</td>
<td>Excision wound model (wound diameter is 10 mm)</td>
<td>10% gel (topically applied) and 200 mg/kg (p.o)</td>
<td>Topically applied and orally once a day for 14 days</td>
<td>DW healing is accelerated</td>
<td>[147]</td>
<td></td>
</tr>
<tr>
<td>embelin</td>
<td>Increased the level of hexosamine</td>
<td>Streptozotocin-induced diabetic rats</td>
<td>Excision wound model (wound diameter is 2 cm) and Incision Incision wound model (para-vertebral straight incisions of 5 cm Length and closed with interrupted sutures 1 cm apart)</td>
<td>5% embelin cream (topically applied), 25 and 50 mg/kg (p.o)</td>
<td>Topically applied and p.o Once a day</td>
<td>Wound contraction was significantly increased and epithelialization was promoted; Diabetic wounds healing is accelerated</td>
<td>[148]</td>
<td></td>
</tr>
<tr>
<td>Withania coagulans</td>
<td>Decreased level of hexosamine</td>
<td>Streptozotocin-induced diabetic rats</td>
<td>Excision wound model (wound diameter is 10 mm)</td>
<td>10% w/w cream (topically applied), 500 mg/kg (p.o)</td>
<td>Topically applied and p.o Once a day for 16 days</td>
<td>DW healing is accelerated</td>
<td>[149]</td>
<td></td>
</tr>
</tbody>
</table>
high-throughput screening technology to efficiently and accurately screen natural biologics targeting AGEs-RAGE pathways, and using combination therapy may be an important direction to promote DFU repair.

4.3 PKC Pathways

Abnormal activation of PKC is an important pathological mechanism for the occurrence and development of diabetes and its complications. Ruboxistaurin successfully reversed endothelial progenitor cell dysfunction and prevented the excessive formation of NETs by targeting PKC β/II activation [82]. However, the safety of Ruboxistaurin is questionable. A clinical study showed that 87.5% of volunteers experienced one or more treatment-emergent adverse events during the study period such as shoulder pain, Dupuytren contracture and upper respiratory infection [153]. Therefore, the search for safer and more efficient PKC-targeted inhibitors may become a new direction for promoting the repair of DFU. As previously mentioned, natural biologics have become the priority choice for many researchers to study disease treatment strategies due to their rich sources, affordability, low toxicity, and side effects. Although there are few direct studies on natural biologics targeting PKC to promote the healing of DFU, we found through a literature search that there are many natural biologics that can improve diabetes and its complications by regulating PKC. For example, wogonin can prevent hyperglycemia by blocking PKC activation via diacylglycerol [154]. Curcumin [137], Breviscapine [155] and Pursaria tuberosa extract [138] play a beneficial role in diabetic nephropathy by regulating PKC. Sasa borealis water-extract blocks chronic high glucose-induced endothelial apoptosis by the blunting activation of βII and NADPH oxidase promoted [139]. Furthermore, verbascoside downregulated expression of the inflammatory cytokines IL-6 and IL-1β by inhibiting PKC/HMGB1/RAGE/NFκB signaling and ultimately may be beneficial to oral wound healing in diabetic patients [140]. From these findings, we speculate that these PKC-targeted natural biologics for diabetes and its other complications may be potential strategies for DFU therapy because of their same or similar pathogenesis.

4.4 Polyol Pathways

There is no doubt that an abnormal polyol signaling pathway plays a harmful role in DFU repair. Normalizing the polyol signaling pathway may have unexpected benefits for the repair of DFU. As mentioned above, an abnormal polyol signaling pathway will reduce the function of GSH reductase and damage the antioxidant capacity [86]. AgNPs, produced by a unicellular spherical cyanobacterium with photo- and hetero-trophic capabilities, also accelerate DW repair by intensifying GSH peroxidase activities and GSH content [142]. In addition to its high antioxidant capacity, GSH can help to normalize the immune microenvironment [156]. Of course, due to the low toxicity and side effects of natural biologics, they are easy to clinically convert. For example, in a clinical trial, topical application of propolis to DFU also accelerated wound repair by increasing the the GSH and GSH/glutathione disulfide (GSSG) ratio [143]. Moreover, Deniz Bagdas [141] confirmed that systemic antioxidant therapy with chlorogenic acid, a dietary antioxidant, can ultimately promote DW healing by increasing reduced GSH. Dietary care, an important auxiliary strategy to maintain the body’s blood glucose and redox balance [157–159], combined with other treatment strategies may result in better outcomes. Because oxidative stress may impair wound healing through neuropathy and lead to loss of protective sensation for patients with chronic DFU, drugs that target polyol pathways in natural biologics to reverse neuropathy are also being sought. Suman Samadar and Raju Koneri [144] confirmed that the polyphenolic fraction, isolated from Symphyocladia latisulcata, can inhibit aldose reductase activity and expression, thereby improving peripheral neuropathy. Therefore, it can be inferred that targeting the polyol pathway may be an effective auxiliary strategy for promoting DFU healing. However, although there are many natural biologics targeting the polyol signaling pathway [160,161], there are few studies on the application of DFU, and more relevant studies are focused on diabetic retinopathy. For example, total lignans from Fructose Arctii improve diabetic retinopathy by inhibiting aldose reductase [162]. Moreover, resveratrol (3,4′,5-trihydroxy-stilbene) [163] and zinc supplementation [164] can also target the polyol pathway to improve diabetic lens lesions. This may be due to the complex mechanisms such as an abnormal inflammatory response, microvascular damage, neuropathy, and local infection, which cause diabetic feet to be difficult to heal or even nonhealing. There are too many confounding factors to exclude when researchers conduct experiments. It is also possible that DFU, unlike retinopathy, will greatly threaten the patient’s life in a short period of time. However, as an increasing number of people suffer from the high incidence and disability rate of DFU, there will surely be an increasing number of studies on improving the natural biologics of diabetic foot via the polyol pathway effects of natural biologics in the future.

4.5 Hexosamine Pathways

As mentioned above, for diabetic patients, the increase in hexosamine flux in the body leads to an increase in the flux through OGT, which in turn increases the level of O-GlcNAc [99,105]. Lowering protein O-GlcNAc expression reverses delayed wound closure caused by hyperglycemia [117]. However, studies on natural biologics targeting O-GlcNAc to promote DFU healing are lacking. Interestingly, it was reported that the hexosamine of DW tissue decreased [99,105], there are few stud-
The potential therapy of natural biologics for DFU. Oxidative damage plays an important role in the pathology of DFU, including leading to arrest DFU healing in the inflammatory stage, which then impairs wound proliferation and remodeling. Nrf2 signaling Pathway and AGEs-RAGE, PKC, Polyol and Hexosamine biochemical pathways are key regulatory targets in DFU. The treatment of natural biologics with antioxidant properties, targeting these signaling pathways and biochemical pathways, can effectively improve the inefficient inflammatory response of DFU, promote the smooth progress of wound proliferation and remodeling, and finally promote the healing of DFU.

The application of natural biologics, such as ethanolic extracts of Melia dubia and Solanum xanthocarpum (more examples are shown in the Table), during the wound repair in diabetic rats promotes the deposition of hexosamine, which eventually accelerates the wound repair. Theoretically, increased activation of the HP leads to an increase in its downstream product, O-GlcNAc, which impairs wound repair. This is contrary to the results of increased HP flux in diabetic patients. One important reason may be caused by the different model construction and detection methods. Clinical diabetes is often divided into type I and type II. Type I diabetes is mainly caused by immune-mediated destruction of pancreatic beta cells, and patients with type II diabetes face both insulin resistance and relative insulin deficiency. The pathogenesis of type I and type II diabetes is different, and the method of constructing animal models is also different. It is critical to construct and select a full-thickness injury model of diabetic rat skin specifically. Moreover, direct evidence for changes in HP flux in DFU is lacking. There may be other regulatory mechanisms of HP in DFU. Therefore, in order to better study clinical DFU, it is necessary to collect clinical specimens, use emerging technologies to seek direct evidence, and conduct an indepth study of the specific mechanisms of the onset and difficult healing of DW.

5. Conclusion and Prospects

These studies have shown that continuous exposure to hyperglycemia leads to abnormalities in the Nrf2 signaling pathway and AGEs-RAGE, PKC, polyol and hexosamine biochemical pathways, which results in excessive oxidative stress and an imbalance of redox levels in the body. Excessive oxidative stress on the wound will trigger the release of proinflammatory mediators and trigger an inflammatory cascade, which will cause DFU to be in a stage of persistent low-efficiency inflammatory response state and impair the proliferation and remodeling stages of wound repair. These factors are the bases for the onset, progression, and deterioration of DFU and therapies targeting these pathways have been shown to be effective in promoting the healing of DFU (Fig. 1). This review not only describes the pathology and key mechanisms of oxidative stress in DFU, natural bio-
logics targeting Nrf2 signaling pathway and AGEs-RAGE, PKC, polyol and hexosamine biochemical pathways promoting the healing of DFU are also summarized. Some of these natural biologics have been tested in DW models with promising results. They show the therapeutic potential of antioxidation and anti-inflammatory, promoting proliferation and remodeling, and some natural biologics can also promote wound angiogenesis and improve peripheral neuropathy.

However, targeted natural biologics promoting DFU are more focused on the Nrf2, AGEs-RAGE and HP, while direct studies on the other pathways are currently a shortcoming, but there are many studies on other complications of diabetes (similar to the pathogenesis of DFU). It should also be noted that the specific mechanism of the HP in promoting DW repair is still unclear. Filling in the research gap is bound to happen. Furthermore, due to heterogeneity in animal selection, model construction, blood glucose levels, dosing start, end time, route of administration, frequency of administration, we were unable to identify which natural biologics show better efficacy in the treatment of DFU. The bioavailability, stability and solubility of these natural biologics in vivo need further confirmation. In addition, hyperglycemia is closely related to oxidative stress. Hyperglycemia can upregulate markers of chronic inflammation and contribute to increased ROS generation, which ultimately cause pathological oxidative stress. Conversely, pathological oxidative stress can lead to insulin resistance and impaired insulin secretion [165]. Proper treatment of hyperglycemia and inhibition of pathological oxidative stress are crucial for the healing of DFU. On the basis of proper control of hyperglycemia (including dietary care [166]), the application of natural biologics may achieve better curative effect. And in the study of DFU, it is important not only to focus on the effect of natural biologics on DFU healing, but also consider whether they have an effect on blood glucose level. Of course, many synthetic compounds have been produced as antidiabetic agents [167], and it is also an important direction to search for drugs that can promote DFU healing from these antidiabetic agents. Metformin [168] and insulin [169] are good examples. Moreover, seeking high-efficiency drug delivery systems (such as bioactive nanoparticle delivery systems) and combination therapy of multiple pathways (rather than targeting one pathway alone) are better options for promoting the repair of DFU. However, greater thought, investigation, and verification are still required for both drug selection (based on targets such as signaling networks or metabolic pathways or on different stages of wound healing) and the order of administration. However, it is certain that natural biologics have a wide range of sources and excellent cost effectiveness, which can reduce the economic burden of DFU patients to a certain extent. Therefore, it is crucial to devise strategies to study the effects of these natural biologics on patients with DFU, ethically, without compromising patient interests and obtaining informed consent.

Abbreviations

DFU, diabetic foot ulcers; Nrf2, nuclear factor erythroid 2-related factor; AGEs, advanced glycated end products; RAGEs, receptor For Advanced Glycation Endproducts; PKC, protein kinase C; DM, diabetes mellitus; ROS, reactive oxygen species; NETs, neutrophil extracellular traps; DW, diabetic wounds; VEGF, endothelial growth factor; TGF-β1, transforming growth factor β1; KGF-1, keratinocyte growth factor 1; CAT, catalase; HO1, heme oxygenase 1; NQO1, quinone oxidoreductase 1; ERK1/2, extracellular signal-regulated kinase; SAPK/JNK, stress-activated protein kinase/cJun N-terminal kinase; MAPK, mitogen-activated protein kinase; JAK/STAT, Janus kinase signal transducer and activator of transcription; AKAP12, a anchoring protein 12; eDNA, extracellular DNA; PKCs, protein kinase C isozymes; NADPH, nicotinamide adenine dinucleotide phosphate; AR, aldolase reductase; GSH, glutathione; HP, hexosamine pathway; UDPGlcNAc, UDP-N-acetylglucosamine; OGT, O-Glycosyltransferase; Nef, neferine; SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; ECM, extracellular matrix; MC, momordica charantia; SalA, salvianolic acid A; GSSG, glutathione disulfide; APE, apple peel extract; EPC, endothelial progenitor cell; p.o, per os; eNOS, endothelial nitric oxide synthase; S-G, Smulow-Glickman.

Author Contributions

QC, JS and AL conceptualized this study and wrote the manuscript. BL and WH created the figures and tables. ZJ, XB, LH and SZ contributed to the literature search and provided help and advice on grammar. SG, JW and QC reviewed and modified the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Hinz B. Formation and Function of the Myofibroblast during


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