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Lower Limb Ulcers in Diabetic Patients: Molecular and Cellular Mechanisms

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Abstract

The disease diabetes depictures a heterogeneous group of metabolic disorders that arise as a result of hyperglycemia due to the deficit of secretion and/or insulin action. How it promotes systemic alterations, short and long-term complications have high impact for de health system of countries. Among its long-term complications, foot ulcer is the one that generates more hospital admissions. These wounds often become chronic due to a series of molecular and cellular aberrations of the healing process, being the main mechanisms the following: high concentrations of matrix metalloproteinases (MMPs), neuropathy, high probability of infection and non-physiological inflammatory response, oxidative stress, excessive formation of AGEs (advanced glycoxidation end-products), deficient neoangiogenesis, imbalance between metabolism and nutrient delivery, inadequate concentrations of growth factors and gene expression regulators, and cellular abnormalities. With better scientific understanding of these events and physiological healing, new approaches to disease can provide more satisfactory results to the treatment.

Keywords: Diabetic foot; Wound healing; Wounds and injuries; Diabetes mellitus; Advanced glycosylation end-products

Introduction

Diabetes mellitus represents a group of heterogeneous metabolic disorders that arise as a result of hyperglycemia due to deficiency in secretion and/or insulin action. Included in definition are two types of diabetes: type 1, resulting from the destruction of insulin-producing pancreatic β-cells; and type 2, which results from the peripheral resistance of that enzyme [1]. The prevalence of diabetes has been increasing in epidemic proportions [2]. Currently, the disease affects 170 million people worldwide [3], with an expected 430 million people by 2030. Likewise, the number of patients with complications, particularly long-term complications such as cardiovascular diseases, nephropathy, retinopathy, neuropathy and ulcers in the lower limbs will be increased [1]. Among these conditions, the latter is responsible for a greater number of hospital admissions [2], with annual expenditures on its care reaching five billion dollars in the United States [4]. In addition, it is estimated that one in four patients with diabetes will develop chronic foot injuries at some point in their lives [5]. Ulcers in diabetic patients can be defined as lesions that involve loss of epithelium and may extend to the dermis and deeper layers, sometimes involving bones and muscles [2]. However, in diabetic patients, foot ulcers often take long time to heal due to a series of molecular and cellular aberrations of the healing process [6], being the main ones: high concentration of metalloproteinases (MMPs), neuropathy, high probability of infection and non-physiological inflammatory response, oxidative stress, excessive formation of AGEs (advanced glycoxidation products), deficient neoangiogenesis, imbalance between metabolism and nutrient delivery, inadequate concentrations of growth factors and regulators of gene expression, and cellular abnormalities [6].

High Concentration of MMPs

Physiologically, proteases that degrad extracellular matrix (MMPs) are under the fine control of plasmatic inhibitors (e.g.: alpha2-macroglobulin) or tissue inhibitors of metalloproteinases (TIMPs) [7]. However, in the chronic wounds of diabetics there is an increase concentration of MMP-2, MMP-8 and MMP-9, along with a decrease of TIMPs. This imbalance leads to the destruction of

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proteins and growth factors required to initiate a proliferative phase, helping to park the wound in the inflammatory phase of healing. As a possible explanation for this phenomenon there is evidence suggesting that macrophages of diabetics exaggerate metalloproteinases and that cytokines IL-1 (interleukin-1), and TNF-α (tumor necrosis factor-α) are at high levels in the wounds in question. These last two signals have as their functions to increase the secretion of proteases and to diminish the inhibitors of them [1].

Neuropathy

Neuropathy is the most common source of ulcers in diabetic individuals [2], with involvement of motor, sensory and autonomic components of the peripheral nervous system [7]. This process as a whole leads to a decrease in the secretion of neuropeptides (substance P and peptide related to the calcitonin gene), which induces damage in the production of growth factors, chemotaxis and cell proliferation [6]. The autonomic component of diabetic neuropathy induces a drop in pre-capillary resistance, which favors capillary flow, increasing luminal hydrostatic pressure and shear forces, generating an inflammatory response that, in turn, causes thickening of the basal membrane of the vessel. This end result compromises healing by: restricting nutrient transport; Induce a decrease in the elastic capacity of the vessel, with consequent limited vasodilatation and functional ischemia; Decrease maximal hyperemia and impair self-regulation of the capillary [6,8]. Sensory diabetic neuropathy causes insensitivity to protective symptoms against pressure and heat, which increases the likelihood of developing ulcers and the growth in extension of existing ones [7,9]. The motor deficit of the peripheral nervous system ends up creating undue physical stress on the insensible foot, leading to anatomical deformities (e.g.: arched feet, claw toes), which contribute to the formation of ulcers [7].

Non-Physiological Inflammatory Response and High Probability of Infection

Chronic foot ulcers usually park in the inflammatory phase, with consequent impaired formation of granulation tissue. It has been suggested that the longer a wound remains in the inflammatory phase, the greater the number of microcellular defects accumulate, further reducing the probability of cure [6].

Hyperglycemia impairs the efficiency of this inflammatory response through changes in the functions of neutrophils, macrophages and lymphocytes. More particularly, macrophages and neutrophils have their deficient cytotoxic and phagocytic abilities [1,8]. Evidence suggests that these alterations in the activities of these cells are due to low local oxygen pressure, since impaired processes consume high concentrations of this gas [8].

With regard to wound infection, it has been raised that its incidence in diabetic ulcers is greater than in other chronic wounds. This reflects both the decreased function of leukocytes and the fact that the normally present compression and physical stress end up leading to an increase in local bacterial load [7]. There is also evidence suggesting that there is a more substantial increase in the number of microorganisms when there is necrotic tissue. In addition, a relationship was established between the number of local bacteria and poor healing: when more than 10^5 bacteria are found per gram of tissue, there is a high probability that the tissue repair is deficient, with this number dropping to 10^3 when β streptococci dealing with hemolytics [8]. Thus, in order to combat an increased bacterial load, it would be expected, physiologically, that there was an increase in the ratio of CD4 to CD8 (CD4 / CD8) lymphocytes. However, in an analysis of the margins of wounds and ulcers of diabetics, there was a decrease in this relation, with an increase in the number of CD8 cells and a decrease in CD4. These results suggest that CD8 cells may play an active role in the diabetic's poor healing, since evidences raise them as acting in the harmful alteration of the physiological tissue repair [6].

Oxidative Stress

Increasing evidence suggests causal linkage between hyperglycemia and oxidative stress, which leads to cellular damage and various complications associated with diabetes. Several mechanisms generate this oxidative stress, being involved: ascorbic acid (vitamin C), protein kinase C (PKC), polio pathway, hexosamine pathway and oxygen free radicals [6]. Hyperglycemia interferes with cellular transport of ascorbic acid (powerful antioxidant) in emplastia, including fibroblasts. Glucose has a structure similar to ascorbic acid, and can competitively inhibit its transport to the cell membranes [8]. The diabetic also presents an increased activity of PKC, which increases oxidative stress by activation of the enzyme NADPH (nicotinamide adenine dinucleotide phosphate reduced) oxidase and consequent depletion of NADPH. This latter effect reduced the synthesis of NO (nitric oxide) and reduced glutathione, increasing a production of oxygen free radicals [6]. The high concentrations of glucose end up saturating a hexokinase enzyme of the glycolytic pathway, causing a portion of this monosaccharide to end up following an alternative route of degradation: via the poliois, made possible by the aldosose reductase. As a final result, it is formed by sorbitol, and the reactions that preceded its formation used NADPH. In this way, the concentration of this last substance ends up decreasing still more [6,10]. Diabetes was also characterized by increased activity of the hexosamine biosynthetic pathway, a qualitative conversion of NADH (reduced nicotinamide adenine dinucleotide) to NADPH by inhibition of glyceraldehyde-6-phosphate dehydrogenase [6]. In the disease in question, an increase in the activities of the arachidonic acid cascade, xanthine oxidase, NADPH oxidase and semi-ubiquinone component of the mitochondrial electron transport chain leads to the formation of oxygen free radicals. Among them is superoxide, which can react with NO and give rise to a peroxynitrite, or be converted by a superoxide dismutase to a nitrogen peroxide. The latter, in turn, can generate hydroxyl radicals, in the presence of cuprous and/or ferrous ions [6,11]. Superoxide anion limits a vasodilator biodiversity by reacting with NO. Already titanium peroxide contributes to the perpetuation of inflammation and premature apoptosis of matrix-producing cells. Finally, hydroxyl radicals interact with proteins, lipids and DNA, inducing conditions such as: reperfusion injury, dementia and atherosclerosis [12].

Excessive Formation of AGEs

Excess formation of AGEs (glycotoxins) has been recognized as an important pathophysiological mechanism of diabetes complications. These substances are products of advanced glycoxidation, a set of reactions that consists of: reaction between carbonyl groups of aldehydes and N-terminal or amino-free portions of proteins; Subsequent formation of Schiff bases (unstable); Conversion to Amadori products (stable); Oxidative reactions in a small part of the latter and, thus, irreversible generation of AGEs (Figure 1) [5,13]. In addition, AGEs can be generated by reactions between sugars and amino groups of phospholipids, and guanyl nucleotides of DNA; with the possibility of being formed by sources not directly

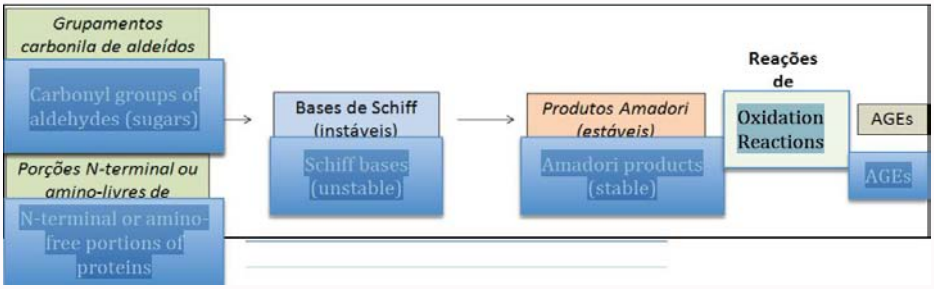

Figure 1: Schematization of the formation of AGEs.

related to glucose, such as by oxidation of amino acids and lipids [14,15]. The concentration of Amadori products is in equilibrium with that of glucose and, consequently, is high in hyperglycemic. Therefore, there is also an increase in the formation of AGEs in these individuals. However, in the intracellular medium, glucose has the lowest glycation potential among sugars, with some intermediates of the glycolytic pathway and dicarbonyl compounds being more relevant. The most potent glycating agent is methylglyoxal, which is detoxified in a glyoxalase I and II catalyzed conversion, and reduced glutathione [5]. Although the scientific literature addresses more endogenous formation of AGEs, a large portion of these substances come from exogenous sources. Among them are tobacco and foods exposed to high temperatures and processed industrially [5,14,16-18]. The main mechanisms of how AGEs are related to the complications of diabetes are: accumulation of these substances in the extracellular environment, interaction with RAGEs (receptor for advanced glycoxidation products) and intracellular formation of glycotoxins [5,14,19]. It is noted that the plasma level of RAGEs are higher in diabetic patients than in non-diabetic patients, stimulating this interaction [20]. The presence of glycation products in the myelin of the peripheral nerves leaves it susceptible to phagocytosis by macrophages, contributing to segmental demyelination. In addition, evidence suggests that AGEs impair nerve regeneration [5,14]. The accumulation of glycotoxins in the extracellular environment creates aberrant cross-links between collagen molecules, impairing the interaction of cells with the matrix and, consequently, cell migration. Glycation of matrix proteins also induces apoptosis, contributing to the hypocellularity observed in diabetic wounds. In a peculiar way, fibroblasts exposed to AGEs had no changes in the rate of secretion of collagen I, but decreased that of hyaluronic acid [5,14]. Extracellular glycotoxins also contribute to alter the bioactivity of growth factors, impairing their structures and their receptors [14]. The AGE/RAGE interaction triggers cascades of intracellular signaling, culminating, among other results, with gene modulation. Through this mechanism, these substances lead to the following harmful effects: failure of neovascularization induced by functional ischemia; Thickening of the basement membrane of vessels; glycation of LDL (low density lipoprotein), impairing its uptake by LDLR (low density lipoprotein receptor) and facilitating phagocytosis by macrophages, contributing to the formation of foam cells; oxidative stress; supra-regulation of NF-κB (nuclear factor-κB), transcription factor that increases the expression of inflammatory cytokines; The expression of MMPs, TNF-α, IL-1, VCAM-1 (vascular cell adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1) and eNOS (endothelial NO synthase) [14,19]. Evidence suggests that intracellularly formed glycotoxins also cause cell deficiencies. A phenomenon in favor of this assertion is the modification of bFGF (basic fibroblast growth factor) in endothelial cells, with a decrease in the proliferation of fibroblasts and keratinocytes [5].

Poor Neoangiogenesis

In physiological tissue repair, fibroblasts, macrophages and keratinocytes secrete VEGF (vascular endothelial growth factor) into the bloodstream, which activates eNOS from the bone marrow and thus increases NO production. This substance, on the other hand, induces migration of EPCs (endothelial progenitor cells) into the circulation and, through secretion of SDF-1α (factor derived from stromal cells-1α) by the myofibroblasts and keratinocytes of the wound, end up being attracted to the bed where they promote angiogenesis [1,12]. However, diabetic wounds present a lower migration of EPCs to circulation, which consists of low intensity neoangiogenesis. Using rats as a model, it was found that diabetics had lower eNOS activity and SDF-1α production, explaining the phenomenon observed [1].

Unbalance between Metabolism and Nutrient Delivery

Part of the abnormal metabolism of diabetic ulcer may be due to a low concentration of ATP (adenosine triphosphate) and glucose, but high lactate. When increased activity of macrophages, fibroblasts and other leukocytes in the margins of chronic wounds of diabetics was observed, it was suggested that incompatibility between increased cellular metabolism and insufficient delivery of nutrients may contribute substantially to the deficiency in healing [6]. Inadequate concentrations of growth factors and gene expression regulators with respect to the presence of growth factors in diabetic ulcers, there was a decrease in the expression of PDGF (platelet-derived growth factor), FGF (fibroblast growth factor), IGF-1 (insulin-1), IL-8 (interleukin-8), IL-10 (interleukin-10), steopontine and TGF-β1 (transforming growth factor-β1). On the other hand, supra-regulation of IL-1, TNF-α and angiopoi etin-21 was observed [1,21]. Decreased concentrations of osteopontine and increased angiopoietin-2 are consistent with the delay in angiogenesis, since the former induces the process and the latter inhibits it [1]. Recently, an important role of micro RNA in the pathology of chronic diabetic wounds has also been discovered. Physiologically, a precursor of micro RNA is converted by the enzyme dicer to micro RNA, whose function is to inhibit translation of specific messenger RNAs [22].

When analyzing diabetic and non-diabetic rats, there was a 40% decrease in dicer enzyme in the first group, suggesting a substantial change in cellular gene expression. It has also been found that biological mechanisms related to the mechanism described may play a direct role in the decline of keratinocyte proliferation in diabetic ulcers. HIF-1α (hypoxia-1α inducing factor) is a supra-regulated

transcription factor in diabetes and stimulates expression of micro RNA 210. This, in turn, inhibits expression of the gene encoding E2F3 transcription factor, which stimulates mitotic activity of keratinocytes. Simplified, increase in HIF-1α concentration inhibits keratinocyte proliferation [1].

Cellular Abnormalities

The keratinocytes and fibroblasts of diabetic ulcers present different phenotypes from the wounds of healthy people. The former have altered migration, proliferation, differentiation and secretion factors of growth factors, while the latter have altered morphology, senescence, deficient myofibroblast differentiation, low migratory and proliferative capacities, and aberrant secretory function [1,3,14]. Given the great advances in the scientific understanding of physiological healing and mechanisms of chronic wounds of diabetics, new approaches to pathology may provide more satisfactory results to their treatment [7].

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