The role of advanced glycation end products in the hyperinflammatory response of diabetic wounds

Abstract

Chronic wounds are a frequent complication in diabetic patients and contribute significantly to morbidity and mortality. In the hyperglycaemic state, advanced glycation end products (AGEs) form, affecting collagen cross-linking, cytokine, nitric oxide and growth factor production and molecular signalling pathways. AGE receptors are upregulated and as it activates NF-kappaB, a host of inflammatory cytokines are expressed, exacerbating the self-sustaining inflammatory response seen in diabetic wounds. Therapeutic strategies include inhibiting the formation of AGEs, either directly or by inhibiting the formation of intermediates, and blocking the AGE receptor. A more complete understanding of the biology of the diabetic wound healing process is imperative in the development of successful treatment of diabetic wounds.

Keywords: advanced glycation end products, hyperinflammatory response, diabetic wounds

Introduction

Diabetes is a common metabolic syndrome prevalent in 1-2% of the global population, and it affects about 3.5 million South Africans. Insulin deficiency or resistance are the main causes of the hyperglycaemic state in diabetes. Diabetes is also a major risk factor for critical limb ischaemia and, as a result, a significant and frequent complication in diabetic patients is the development of non-healing diabetic ulcers and wounds which may be associated with significant morbidity, including the loss of limbs.

Normal wound healing is an orderly process of clot formation, inflammation and angiogenesis, followed by granulation tissue formation with wound contraction, re-epithelialisation, scar formation and remodelling. This process may be disrupted in diabetic patients, resulting in a chronic wound with healing delayed for up to two months, as cellular growth is limited or absent and the epidermis does not close over the wound. The current consensus is that diabetic wounds develop from the hyperglycaemic state and are exacerbated by underlying immunological, vascular, and neurological disorders, and deranged cytokine expression and activity, and are possibly compounded by an additional bacterial burden. The inflammatory phase of wound healing has been implicated as a key early event which determines the subsequent outcomes in the wound healing cascade, and imbalances in this acute phase have been closely linked to the pathology of diabetic wounds. Furthermore, the oxidative milieu created in hypoxic states, with high levels of reactive oxygen species, elevated arginase and reduced nitric oxide synthase activity, disrupts the normal redox state, leading to cellular DNA damage and disruption of the normal healing process.

The non-enzymatic glycosylation of collagen, with subsequent advanced glycation end product (AGE) formation, is one of the main biochemical abnormalities common to diabetes and is known to predispose ligaments to stiffness. Mechanical stresses during ambulation are further increased by diabetic peripheral neuropathies, causing loss of sensation and coordination of foot and leg muscles. Although this non-enzymatic Maillard reaction, involving a reaction between the carbonyl group of a reducing sugar and the nucleophilic group on an amino acid with the subsequent formation of an AGE, has been studied extensively since the beginning of the 20th century, the significance of AGEs in the pathology of diabetic complications and aging processes has only emerged in the last 30 years.

In general, AGEs contribute to diseases in the human body by accumulating on nucleic acids, proteins and lipids. Clinically, these widespread actions infiltrate various systems of the human body. The mechanisms by which AGEs have been implicated in diabetic complications include the following:

- Inappropriate cross-linking of collagen, causing decreased elasticity and tensile strength of the extracellular matrix and clinically causing limited joint mobility.
- Glycation of cytoskeletal proteins following changes to nerve fibres, playing a role in diabetic neuropathy. The latter pathology is particularly common in the diabetic foot and ultimately leads to the development of diabetic foot ulcers. While vascular insufficiency may be a large contributing factor to the development of diabetic foot ulcers, it is the AGEs that infiltrate nerve cells, both peripheral and autonomic, that cause aberrations long before clinical manifestations are evident. AGEs have not only been implicated in the development of diabetic foot ulcers, but have also been reported to be related to the severity of neuropathic foot ulceration.
- Oxidative stress of adhesion molecules, cytokines and growth factors, which are all involved in diabetic nephropathy.
- Inappropriate activation of cellular signalling cascades affecting gene expression when AGEs bind to receptors on endothelial cells or are deposited on other cells. An example of the latter includes the deposition of AGEs on the epithelial
basement membrane of the cornea, playing a causal role in the pathogenesis of diabetic keratopathy.18

- Increased AGE deposition in atherosclerotic lesions, which are correlated with the degree of atheroma. AGEs have been implicated in the vasculopathies that develop in diabetic patients. Atherosclerosis, common to all, is considerably accelerated in diabetes. This may be attributed to increased AGE formation in diabetic vasculature that result in functional alterations of endothelial cells, macrophages and smooth muscle cells.15

Surprisingly, clinicians make use of the process of AGE formation when they use the haemoglobin A1c (HbA1c) concentrations as a measure of diabetic status (Figure 1). During the synthesis of AGEs, Amadori products are formed, reflecting on the metabolic stress accompanying diabetes.14,15

Although the prevention of AGE formation has been the main aim of many therapeutic targets, the complexity of the normal and chronic wound environments is not fully understood. As a result, therapeutic advancements have been limited.19

This review considers the current evidence on the role of AGEs and AGE receptors (RAGEs) in diabetic complications and their roles in the wound healing process.

The formation of advanced glycation end products

The initial step in the formation of AGEs is the reaction between the carbonyl group of glucose and a protein amino group, either the N-terminus or a side chain, to form an imine, or Schiff base. This unstable intermediate base forms a more stable Amadori product, of which the formation of HbA1c from haemoglobin is one example. As the concentrations of the Amadori products are in equilibrium with the blood glucose concentrations, they provide a long-term measure of glucose control in the diabetic subject. Some of the formed Amadori products irreversibly oxidise further to form the stable AGEs (Figure 1). Diet and other factors, such as smoking, are major contributors to the formation of AGEs.12

Glucose is a six-carbon chain molecule with a terminal carbonyl group, which reacts with the alcohol at position C5 to form the familiar textbook cyclic structure, with an equilibrium existing between the open chain and cyclic structure. The carbonyl group is protected in the latter, and such cyclic structures are relatively unreactive compared to other open-chain intermediates in the pathways of glucose and fructose metabolism. Such reactive intermediates, including glyceraldehyde-3-phosphate, dicarbonyl glyoxal, methylglyoxal and 3-deoxyglucosone, are recognised precursors of AGEs.3,12 Of all these AGE precursors, methylglyoxal, formed from glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, has been identified as one of the most reactive glycating agents.12

In diabetic mice, where gastric ulcer healing was significantly delayed, Naito et al recently (2009) showed that methylglyoxal reacts with the protein peroxiredoxin 6 in the mucosa around the gastric ulcer. After oral administration of an inhibitor of the methylglyoxal
reaction, the delay in ulcer healing was overcome without altering the blood glucose levels. This implies that the inhibition of methylglyoxal also prevented the formation of AGEs.

Methylglyoxal is inactivated by the enzymes glyoxalase I and II, which convert methylglyoxal in the presence of reduced glutathione to S-D-lactoylglutathione and D-lactate. AGE formation was effectively prevented in endothelial cells overexpressing glyoxalase, suggesting the importance of this pathway in preventing AGE formation.

Interaction between advanced glycation end products and their receptor, leading to cellular derangement

The receptor of AGEs, RAGE, is part of the immunoglobulin superfamily of receptors and is expressed at minimal levels in tissue not subjected to hyperglycaemic environments. RAGEs do not recognise any specific peptide sequence but rather structures such as β-sheet and fibril protein structures. As AGEs form and accumulate, RAGEs are upregulated at the accumulation sites. As a result of the interaction between ligand and receptor, many cellular functions are affected. One specific effect is the activation of nuclear factor (NF)-κB in smooth muscle cells, endothelial cells, neural cells and mononuclear phagocytes (monocytes and macrophages). As this transcription factor regulates many genes involved in immune and inflammatory responses, its activation is linked to the expression of various inflammatory cytokines (the interleukins IL-6 and IL-1α, and tumour necrosis factor-α, TNF-α), growth factors and additional RAGE molecules, causing an amplification of a self-sustaining inflammatory response seen in diabetic-related wounds. Goova et al restored effective wound healing in diabetic mice by blocking the RAGE molecule with a truncated version of the receptor known as soluble (s) RAGE, which is similar to extracellular RAGE on a molecular level, but lacks the cytosolic domain necessary for activating its signalling pathways. This study showed no difference in the expression or activities of TNF-α in the sRAGE-treated animals. Although more inflammatory cells infiltrated the wound by 10 days, and accelerated blood vessel formation was observed, IL-6 and matrix-metalloproteinase (MMP)-3 and -9 were similar. However, sRAGE limited the inflammatory phase and healing was rapid, whereas in the diabetic the inflammatory phase was sustained, and after 21 and 35 days, both the expression and activity of these MMPs and cytokines were significantly increased. The MMPs caused collagen breakdown whereas with RAGE this effect was blocked, enhancing wound healing. The results implicate RAGEs in delayed wound healing by sustaining the inflammatory phase.

The effect of AGEs on cytokines and growth factors

Several studies have shown that AGE formation has an effect on macrophage function, promoting the release of various growth factors and/or cytokines. The histological findings of Loots et al demonstrated significantly elevated macrophage and B-cell populations in chronic venous and diabetic ulcers. Significant increases in vascular endothelial growth factor (VEGF), IL-8 and TNF-α release occurred when human-derived macrophages were stimulated with human serum albumin (HSA) or bovine serum albumin (BSA), modified by glycation products. Similarly, Berbaum et al studied the release of proinflammatory cytokines by macrophages when stimulated with glycated-BSA (AGE-BSA) and lipopolysacharides (LPS). Their results showed that, upon stimulation, monocyte chemoattractant protein (MCP-1) and TNF-α were released in a time-dependent manner. Unstimulated cells also released MCP-1, but at levels that were much lower than those in the stimulated groups.

Together these studies suggest that:

• A hyperglycaemic environment could induce an increase in proinflammatory mediators, possibly amplifying the inflammatory feedback loop on the macrophages.
• An increased expression of RAGEs in the hyperglycaemic state may mediate this inflammatory process in the macrophage populations.
An important aspect of wound healing and regeneration is the formation of granulation tissue. The latter is a matrix of endothelial cells, fibroblasts, inflammatory cells and new blood vessels that are enmeshed in a ground substance with the eventual aim of connective tissue replacement. It also provides a network for keratinocytes to move across, so as to recover the voided area and re-establish the protective barrier provided by the skin. Hehenberger et al showed that fibroblasts in the diabetic setting exhibit a decreased rate of proliferation.27 However, it was determined that the changes were due to changes in the cells themselves prior to wounding, and not an effect of the wound environment. Furthermore they showed that the fibroblasts had a decreased potential for responding to growth factor mitogenic signals. This decreased fibroblast mitogenic potential was restored with the addition of heparin. More recently, similar findings were noted by Rao et al in macrophages grown in culture, where a modified heparin treatment was found to block the binding of the RAGE molecule to its specific ligand.28 Finally, Porter-Otin et al showed that growth factor signalling pathways are deranged in an AGE-precursor environment and the effects of the epidermal growth factor receptor (EGFR) were diminished as AGEs inhibited the phosphorylation of the tyrosine residues, thereby blocking the signal transduction pathway regulated by EGFR.29

To date, although the effects of growth factors such as epidermal growth factor (EGF), transforming growth factors (TGF-α and –β), transforming growth factor alpha in healing cutaneous wounds. Am J Pathol. 1991;138:1307-1313. 

Conclusion

A complication in diabetic patients is the development of difficult-to-treat, non-healing chronic wounds requiring specialised care. These wounds develop in hyperglycaemic states and are exacerbated by deregulated immunological, vascular and neurological systems. AGEs are not enzymatically synthesised, and therefore an approach of targeting key enzymes cannot be employed. Although AGE formation can be limited by strict glycaemic control, therapeutic approaches have included inhibiting the formation of AGEs, either directly, or by inhibiting the formation of intermediates, such as methylglyoxal, and blocking the RAGE receptor. A more complete understanding of the biology of the diabetic wound healing process is imperative in the development of future successful therapeutic strategies for diabetic wounds.

References

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