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DIABETIC DELAYED WOUND HEALING AND THE ROLE OF SILVER NANOPARTICLES

Manish Mishra, Hemant Kumar, Kamlakar Tripathi*
Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, INDIA-221005.

Diabetes mellitus is most common disease of the altered glucose homeostasis. Diabetics have impaired wound healing and impaired formation of coronary collaterals. The abnormal apoptosis or angiogenesis may cause many of the clinical manifestations of diabetes. Silver has been known to have effective bactericidal properties for centuries. Nowadays, silver-based topical dressings have been widely used as a treatment for infections in burns, open wounds, and chronic ulcers. Silver nanoparticles are novel nanosized and highly crystalline antibacterial agent which carries Ag^+ ions by ion-exchanging. This review is an attempt to illustrate the molecular signaling of the apoptosis involved in delayed wound healing and the role of silver nanoparticles in earlier healing.

Keywords: Diabetes mellitus, Apoptosis, Wound Healing, Nanoparticles

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1. Introduction

Diabetes is a disease of altered glucose homeostasis and persistent hyperglycemia lead to advanced glycation end product (AGE) which are primarily responsible for the damage of cells which have a slow turn over (like neuronal tissue). This abnormality is the cause of some of the late complications of diabetes manifestation in the form of tropical ulcer and also vascular abnormality due to autonomic nervous dysfunction resulting in vascular stasis and poor wound healing. The acute metabolic hyperglycemic complication affects those cell line which proliferate rapidly and in the absence of proper glycemic control, the cellular maturity does not occur and this can result into poor quality of wound healing which may manifest either in poor capillary bed formation by endothelial cell, weak reticular network laid by fibroblast and abnormal epithelial cell migration. The other cellular component of the blood like macrophages and lymphocytes may also become weak and could result into wound infection and may compromise with other cellular and humoral immune response. Wound healing is a complex process and has been the subject of intense research for a long time. The recent emergence of nanotechnology has provided a new therapeutic modality in silver nanoparticles for use in wounds. Nonetheless, the beneficial effects of silver nanoparticles on wound healing remain unknown. Nowadays, silver-based topical dressings have been widely used as a treatment for infections in open wounds and chronic ulcers. Ag+-loaded zirconium phosphate nanoparticle is a novel nanosized and highly crystalline antibacterial agent which carries Ag^+ ions by ion-exchanging. It can also protect the host material from oxidation and discoloration and have been often used as additive for wound dressing.

*Corresponding author: Kamlakar_tripathi@yahoo.co.in

2. Diabetes

One of the common degenerative diseases affecting people in the world today is diabetes mellitus. People with diabetes mellitus have five times the risk of having heart disease as people without diabetes. More than 60% of people with end-stage renal disease are people with diabetes. Diabetes is the leading cause of blindness in the United States. Diabetes mellitus is among the top 10 causes of death either directly or indirectly, yet our understanding of its pathophysiology and management is incomplete. WHO estimates that by 2025 as many as 200–300 million people worldwide will have developed type 2 diabetes.[1] South East Asian countries have the highest burden of diabetes [1,2], and the projections of the International Diabetes Federation on the prevalence of diabetes mellitus and impaired glucose tolerance (IGT) for the year 2005 is, respectively, 7.5 and 13.5% [1,2].

3. Apoptosis

Apoptosis is a complex network of biochemical and molecular pathways with fine regulatory mechanisms that control the death event (apoptosis) in a cell. Unlike necrosis, apoptosis is the part of normal development, termed as physiologic apoptosis, though it also occurs in a variety of diseases and known as aberrant apoptosis. One area of the particular importance is the wound-healing process in which apoptosis is responsible for the removal of inflammatory cells and the evolution of granulation tissue into scar tissue [3]. Wound repair process in healthy individuals depends on several interrelated processes, including the migration of inflammatory cells into the wound area to colonize the provisional matrix, proliferation of fibroblasts and vascular cells, apoptosis, and synthesis of extracellular matrix proteins to reconstitute dermal architecture. Aberrant apoptosis can lead to abnormal wound healing by removing granulation tissue [4]. Apoptosis is the end point of an energy-dependent cascade of molecular events initiated by certain stimuli. It is a complex process that can be divided into the following 4 separable but overlapping processes: induction, detection, effector, and removal [5]. The cell receives an apoptotic signal in the induction stage. Various external stimuli can trigger apoptosis, including nutrient deprivation, cytokine depletion, ionizing radiation, and mechanical or oxidative stress [6-8]. The cell then integrates numerous signals derived from signal transduction pathways to decide whether to commit to apoptosis. When the cell commits to apoptosis, the signal to activate death machinery is detected and transduced to downstream effectors. Various regulators then carry out the apoptotic response, [9-11] and finally the cell is removed by phagocytosis. Each step of the apoptosis process requires the concerted effort of many molecules, and among the most influential ones are the caspases, the Bcl-2 family of proteins, Fas-FasL and p53.

Cysteine-dependent aspartate directed proteases (caspases) play an important part in apoptosis. At least 14 caspases have been identified. All caspases have 3 domains in common: a large subunit (20 kd), a small subunit (10 kd), and an NH2-terminus. Caspases initially become active by selectively cleaving after aspartic acid and assembling into heterotetramers. In general, they can be categorized into 3 groups. The first group (caspase-1, caspase-4, and caspase-5) plays a role in inflammatory response [12]. The second group is composed of initial transducers (caspase-2, caspase-8, caspase-9, caspase-10), and the members of the third group are effectors (caspase-3, caspase-6, and caspase-7) [13-15]. Caspase activity is regulated by a family of cellular proteins, the inhibitor of apoptosis that probably acts by inhibiting caspase-3, caspase-7 and caspase-9 [14-15]. The inhibitors of apoptosis are the first class of endogenous cellular inhibitors of caspases to be found in mammalian species, [18] and at least 8 members are known. Caspase activity is triggered by apoptotic protease activating factor-1 (Apaf-1). Fas/CD95/Apo-1 is a cell surface receptor that transduces apoptotic death signals following activation and has been implicated in triggering apoptosis in infected or damaged cells in disease states [19]. Eighteen members of the Bcl-2 family of proteins have been discovered. As effectors of the apoptosis pathway, the Bcl-2 family of proteins either promotes or prohibits apoptosis. Proapoptotic proteins include Bax, Bad, and Bak, Bid whereas Bcl-2 and Bcl-xL, Bcl_w are antiapoptotic proteins [20-21]. The p53 often regarded as the guardian or molecular policeman, p53 controls the fate of

damaged cells by detecting and arresting the cell cycle [22-23]. The p53 protein is localized to the nucleus, and when called into action, it functions primarily by controlling the transcription of several other genes[24].

4. Normal wound healing

Neutrophils are the first cells to arrive at the wound, as they perform an essential function defending against invading organisms. Their activity is also implicated in local and distant tissue damage through the release of oxygen-free radicals and proteases. Most neutrophils entering a wound perform their task of eliminating microorganisms, undergo apoptosis, and afterward are rapidly and efficiently consumed by macrophages in a process that does not lead to further inflammation. The cycle of inflammation and apoptosis is interrupted in this case.

The mechanisms of neutrophil apoptosis are beginning to be elucidated. In vitro studies suggest that the tumor necrosis factor α is involved in the signaling of neutrophil apoptosis [24]. The interaction of these granulocytes with the β2 integrins (CD11b/CD18) potentiates the extent of apoptosis [25]. Macrophages also undergo apoptosis, although little is known about the mechanisms of the process. Tidball and St Pierre reported that macrophages predominate in the inflammatory response and rapidly disappear through apoptosis [26]. Two populations (ED1 and ED2) underwent apoptosis at 2 different rates. The ED1 macrophages were eliminated rapidly, but the ED2 cells persisted for longer periods. The precise mechanism is not clear, but it has been proposed that transforming growth factor-α 1 is a growth factor that has a high probability of being involved in the down regulation of inflammation. Another likely mediator involved in apoptosis of inflammatory cells during the healing process is the antiproliferative protein p53. Antoniades et al examined p53 expression during the healing of cutaneous wounds in swine [27]. It was found that p53 expression was decreased during the periods of rapid proliferation but then increased during the later stages of healing. The expression was in opposition to the expression of platelet-derived growth factor. These results suggest that p53 levels may decrease when rapid proliferation is needed but then increase to turn off the proliferative process with wound maturation, different cell populations need to be eliminated. Fibroblasts need to be downregulated along with a concomitant decrease in vascularity. Early studies suggest that endothelial cells undergo apoptosis followed by the removal of myofibroblasts [28]. In vitro studies suggest that c-myc is involved in fibroblast apoptosis in a process that involves the interaction of fas and fasL on the cell membrane [29, 30]. Growth factors, especially insulin-like growth factor-1, block the apoptosis pathway to allow for an increase in fibroblast proliferation. In contrast, another class of growth factor may be involved in the downregulation of fibroblast activity in wounds.

Apoptosis signals are also involved in the regulation of collagen degradation by inducing collagenase activity. Bian and Sun found that p53 binds to the promoter of the human type IV collagenase (matrix metalloproteinase 2) gene [31]. These findings suggest that apoptosis signals may also participate in the down-regulation of collagen deposition by both decreasing fibroblast numbers (thus decreasing collagen synthesis) and also by activating collagenase activity. The additive effect of reduced growth factor expression, increased extracellular matrix turnover, and nitric oxide generation may result in the fibroblast and vascular cell apoptosis [32].

5. Impaired wound healing

Impaired wound healing is a common complication of diabetes mellitus [33]. Healing in patients with diabetes mellitus is characterized by reduced tensile strength of wounds when compared with controls, suggesting either defective matrix production or deposition. In the human mammal, diminished perfusion resulting from the presence of peripheral arterial disease as well as decreased sensory nerve function caused by peripheral neuropathy may contribute to impaired healing [34, 35]. It is presumed that diabetic complications result from periods of poor glycemic control. However, aberrant growth factor expression or factors secondary to diabetes, such as advanced glycation and cross-linking of matrix protein, may also be involved [4]. Growth factor

involvement has been implicated not only in diabetic wounds but also in other diabetic complications, such as diabetic retinopathy and nephropathy [36]. It was previously discussed in this article that in the normal process of wound healing, apoptosis is involved in the loss of granulation tissue, including fibroblasts and small vessels, occurring late as scars [3, 37]. By comparison, in a diabetic wound, apoptosis is increased throughout the healing process. This suggests aberrant control of cell survival. In contrast, in a person without diabetes, it occurs mainly in the late phase of healing during scar formation [38] It has been shown that high ambient glucose concentration, linked to vascular complications in diabetes, in vivo modulates messenger RNA expression of fibronectin, collagen, tissue-type plasminogen activator and plasminogen activator inhibitor and induces delayed replication followed by excess cell death in cultured vascular endothelial cells [39].

Increased production of reactive oxygen species (ROS) in the case of hyperglycemia leads to ROS-mediated mitochondrial release of cytochrome C followed by activation of caspase-3, leading to hyperglycemia induced myocardial cell apoptosis [40-41]. Partial inhibition of increased glucose levels by insulin almost completely prevents myocardial cell death; otherwise, it could be argued that there is a significant increase in apoptosis with an increase in the levels of blood sugar. Dysregulation of apoptosis in response to hyperglycemia is generalized all over the body, leading to impaired wound healing along with the involvement of other target organs. Contrary to popular opinion that diabetic foot is caused by neuropathy and peripheral vascular disease, it now appears that dysregulated apoptosis is emerging as a major cause of the diabetic foot wound[54]. It is associated with other microvascular complications of diabetes mellitus, giving the evidence of generalized increased apoptosis all over the body. The effect of hyperglycemia is independent of the hyperosmolar effects of glucose, as experiments with manitol did not increase apoptosis significantly [41].

6. Role of Silver nanoparticles in wound healing:

Silver has been used in the clinical setting as an antimicrobial for over a century, and silver nitrate is still a common antimicrobial used in the treatment of chronic wounds [42]. Silver nitrate causes a significant amount of staining of virtually any surface with which it comes into contact [43] and can also cause irritation to tissues. Silver sulfadiazine was introduced in the 1960s to overcome some of the shortcomings of silver nitrate, but both are limited in the clinic due to the need for a high frequency of application, inactivation of much of the silver by wound fluid and the formation of a pseudo-eschar. New silver-impregnated dressings such as Acticoat were designed to overcome these limitations, in particular the rapid inactivation of silver. In these dressings, as silver is consumed by interaction with target cells or inactivated by protein and anion complexes in wound fluid, additional silver is released, thus producing a sustained, steady supply of active silver. Silver exerts its antimicrobial effects by interfering with the respiratory chain at the cytochromes [44]. Silver ions also interfere with components of the microbial electron transport system, bind DNA and inhibit DNA replication [45-47]. Silver is effective against a broad range of aerobic, anaerobic, Gram-negative and Gram-positive bacteria, yeast, filamentous fungi and viruses [42, 48-52]. In addition to the antimicrobial properties, silver also appears to have anti-inflammatory properties, as suggested by the loss of rubor in chronic wounds treated with colloidal silver [49]. An ideal Ag^{+} donor site dressing material would promote healing, cause minimal pain to the patient, prevent infection, result in minimal scarring, and be inexpensive and easy to use. A dressing which possesses all of these qualities has yet to be developed, but currently many dressing materials meet some of these criteria to varying degrees Rakel et al. [53] concluded that dressings which provide a moist healing environment such as calcium alginate, transparent films, or hydrocolloids were associated with the fastest healing times. Furthermore, hydrocolloid and transparent film dressings were more likely to result in a smooth stable epithelial surface than air exposure, xenografts, gauze, or calcium alginate dressings. Donor site pain was lowest with transparent film dressings, and, in increasing order, higher with hydrocolloids, Biobrane, Tulle gauze, and Scarlet Red. Thus, based on the literature published over the last 30 years, it would

appear that transparent films and hydrocolloids come closest to meeting the criteria of fast, stable healing and reduced donor site pain.

7. Conclusions

This article was an attempt to review the process of wound healing and the role of silver nanoparticles in wound healing. The Silver nanoparticles and Ag^+ carriers can be beneficial in delayed diabetic wound healing as diabetic wounds are affected by many secondary infections. These nanoparticle can help the diabetic patients in early wound healing with minimal scars. Further researches are recommended in this field.

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