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Diabetes foot ulcers: A novel treatment strategy

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Abstract

Foot ulcers are common in 12-25 percent of diabetic patients. Preventing, controlling and treating of these kind chronic wounds are of the major clinical challenges.
Evidence based documents revealed that DFU (Diabetic Foot ulcer) is a chronic wound type originating from disturbed cellular and molecular mechanisms that have to be in its functional form to overcome its problem. In diabetes and some other chronic based diseases, harmonized acting machine causes chronic phases that result in conditions as foot ulceration and related complications seen commonly in diabetes.
DFU needs to be transformed into acute phase in order to be healed in a physiological manner. Disturbed mechanisms have to be corrected reversely and to achieve such a goal it is essential to better understanding of disturbing factors responsible for biological abnormalities. Factors associated with DFU are as cellular and molecular recruitment and function impairments and there is need to repair these mechanisms. For this, we believe that the activated Th-1 cells (T helper-1 Cells) might have a critical role in regulation of the several effector functions of the cellular and molecular mechanisms essential to the body to act the best. Evidences and our successful results urge us to suggest this regulatory role for effector cells and molecules generated through activation of Th-1 cells as a treatment strategy.

Keywords
Diabetic Foot Ulcer, Treatment, Activation, Th-1 cells

Introduction

Foot ulcers occur in almost 25 percent of diabetic patients (1) and due to its pathogenesis they may hardly healed and some of unhealed ones may face with major problems as end organ lost (2). The major targets of the diabetes complications are the patients, their family and the whole society. Therapy yearly cost of diabetic foot ulcer (DFU) is over $1 billion and the yearly cost of chronic wounds is over than$25 billion in the United States (3,4). Despite this the most serious and life threatening factor is the stress originating from organ lost that in turn acts as the most serious immunosuppressive factor; while these patients are targets of immunosupport instead.

Treatment of DFU is one of the major clinical challenges globally. Current therapy protocols are based upon control of infection; debridementation of necrotic tissues and amputation and despite globally challenges, protocols through which we can overcome the problem is remained to be challenged. Due to current therapy methods the attitude of fighting against external causative

factors are challenged more effectively rather than internal pathogenic parameters.

As an injury disrupts an organized functioning network; so it is essential to rebuild and restructure the damaged organization. As part treatment of injured tissues as skin wounds need to a harmonic orchestrate function of complexes of cellular and molecular events as recognition of the injury, emigration, proliferation, Extra Cellular Matrix (ECM) structure and remodeling (5,6).

Wound healing is a concert of simultaneously occurring processes rather than a series of discrete steps; so cellular recruitment by inflammatory mediators, growth factors, cytokines and mechanical forces should be appropriate to initiate and complete the healing process. It will be transformed into a chronic phase if any of the disturbing factors persists. Physiologically clot formation and an inflammatory response are seen as a morphologic characteristic of wound processing. Through such a process damaged endothelium triggers the healing via leading the platelets to aggregate along with clot formation. Platelets then produce cytokines, which include PDGF (Platelets Derived Growth Factor), TGF-alfa (Transforming Growth Factor) and PDEGF (Platelets Derived Endothelial Growth factor) (7). These cytokines influence wound healing directly or indirectly. PDGF is the first FDA approved recombinant product that is used for wound healing purposes (8). Clot formation despite its haemostatic capacity, prevents external infecting agents, reduces exudates (9) and prepares an appropriate environment to inflammatory cells to function their best.

Of the infiltrated cells, Neutrophils are the first phagocytes recruit into the injured site. They remove bacteria, necrotic tissues and other debris components that prevent normal healing. Neutrophils then removed by the act of macrophages. It has been shown that these cells and some related cytokines as GM-CSF (Granulocyte Monocyte-Colony Stimulating Factor), G-CSF (Granulocyte- Colony Stimulating Factor) and IL-8 (Interleukine-8) are decreased in edges of chronic wounds (10). These mechanisms that proven to be critical in physiologic wound healing, are impaired in diabetes. Keratinocytes and endothelial cells found in the injured sites are known to have cellular and molecular regulatory potentials (11). Collagen as the major ECM protein especially in the skin, deposits the injured site to produce suitable environment to the cells and molecules to harmonize the orchestrate (12). Procollagen synthesis, recruitment of repair cells as macrophages, fibroblasts and others take place when oxygen tension is normal (13). Tissue oxygen tension, failure of angiogenesis, decreased endothelial progenitor cell (EPC) production, mobilization and homing and other critical mechanisms responsible for normal healing are impaired in diabetes (13,14).

Factors associated with DFU and its non-healing nature

DFUs are common as in almost 25% of diabetic patients (1). This is shown to be caused by several mechanisms. One of such mechanisms is decreased response to stimulatory factors leading to decreased local blood flow and angiogenesis. Stimulator and responder factors involved in normal healing are impaired in diabetes; so complications as DFUs originate from chronic nature of the disease. In fact cellular and molecular network of homeostatic organization is not only disturbed in diabetes; but also is not strong enough to establish a physiological healing process. Macrophages as the most effective regulatory cells of the innate immunity are decreased or are functionally failed in diabetes (5). Hyperglycemia is reported as a predisposition factor of failure in leukocyte function (15). Respiratory burst and production of cannexin is disturbed in Neutrophils of diabetic patients (16). C- reactive protein and CD8 (Cellular Determinant) positive lymphocytes in diabetics are higher than control subjects (17). Production of growth factors, cytokines, collagen synthesis and many other effective factors involved in normal healing, are impaired in diabetes (18).

In addition to these vital defected events, there are also cell migration and phagocytes and phagocytosis disturbances, failures in primary responses to cytokines and production defects /or insufficient response of cytokines especially IL-1 beta (Interleukine-1) (10, 19). IL-1 beta is one of the most critical factors in initiation of a proper inflammation and known as the proinflammatory cytokine (10, 20). As this critical cytokine is one of the central factors involved in establishment of appropriate inflammatory reactions; its defect may enhance cellular infiltration of leukocytes as macrophages and Neutrophils; therefore may be one of the important factors of transforming into the chronic phase seen in diabetes and delay/or non healing wounds and predisposition of infection. Defect of vasculogenesis is well documented in diabetes and in DFUs especially around injures sites (21). Decreased oxygen tension around the injured sites results in decreased cellular recruitments including macrophages, fibroblasts and also in production defect of collagen synthesis (19). Angiogenesis is one of the most important factors triggered in normal healing following the injury (21, 22). This is achieved through vasodilatation by providing the cellular and molecular regulatory mechanisms. Mechanisms needed in physiologic healing as events involved in proinflammatory, inflammatory and cellular and molecular modulators are impaired in diabetes.

Nitric oxide (NO) has been shown to be one of the main involved stimulators of vasodilatation and produced from several resources during phagocytosis and tissue hypoxia (22). Primary source of this vital gas is macrophages, Keratinocytes, fibroblasts and endothelial cells. An important reported production pathway of this gas is achieved via activation of eNOS (endothelial nitric oxide synthase) that is one of this kind enzyme's isomers present in humans (4, 22). This enzyme is decreased or inhibited in diabetes (22). It acts as vasodilatation, angiogenesis and repair promoting agent (4,22). For achieving this, The NO stimulates production of EPCs (endothelial precursor cells) and their mobilization in the circulation (4,22). In this stage these programmed vital cells have to find injured tissue. This goal is achieved by a kind of crucial chemokine known as SDF1-alfa(Stromal Cell Derived Factor) produced by stromal cells that lead to these cells (EPCs) to home in needed sites (4,22). The above vital mechanism is impaired in diabetes. Increased enzymes as MMPase (Matrix Metalloproteinase) and decreased rates of TIMPase (Tissue Inhibitor of Metalloproteinase) activity are documented in diabetics (23). These kind enzymes and several cellular and molecular mechanisms have to be in equilibrium with their inhibitors to the body to sustain its homeostasis.

The mentioned mechanisms are examples of impaired mechanisms that shift the physiologic mechanisms to the pathologic ones as seen in diabetes. As previously shown, normal healing process is achieved by the harmonized and organized act of a complexity of cellular and molecular events. The events that have to recognize, organize, proliferate, differentiate and maturate the cells and mobilize and remove and replace the needed elements through a programmed manner. Inflammatory step and needed factors to initiate and continue it normally is impaired in diabetes and DFUs.

From this, one may postulate that factors essential for establishment of homeostasis in cellular and molecular basis are impaired in diabetes. These are as defect in regulatory roles of cells as T-cells, macrophages, fibroblasts, endothelial cells and Keratinocytes.

Physiological healing is achieved by the act of a harmonized complex of cells together with initiation, stimulation, activation, inhibition and remodeling processes. Best concern of etiologic factors leading to an ulcer, its chronic transformation and non-healing nature of the wound and beneficial impact of some therapy and care methods by determining mechanisms involved in normal healing and correction of impaired steps may help us to search, improve and establish alternative sophistical ways for therapy purposes.

It should be remembered in mind that the major reason for chronic transformation of DFUs is hyperglycemia that is achieved in combination with other predisposition factors in diabetes. It is the physiologic healing process caused by impaired inflammation that has failed to function normally in these patients. Normal inflammation leads to an organization that enables the body to resolve its problems. One of the most altered mechanisms is insufficient clot formation that acts as a morphologic criterion in wound healing evaluation. This process prepares an appropriate medium to improve the healing process followed by next steps. Once clot is formed it meant that: it is possible to control infection, 2- it keeps the circulation tight as its nature together with keeps inside the body from invasive agents, 3- There is possibility of the best cellular and molecular interactions, and 4- The improving capacity to jump into the next phase is available. The above process is disturbed in diabetes; because diabetic patients especially those with DFUs lack such a phenomenon. Presence of exudates, surface and depth invasive nature of DFUs probably due to mechanisms as: Presence of bacterial contamination caused by staphylococci, streptococci followed by anaerobic bacterium (24) and impaired leukocytes' infiltration and function. Infection is easily being controlled by use of proper antibiotics; but it is not sufficient enough to trigger the normal healing by its alone; because defects in leukocyte infiltration as refiners of the wound surface and other cells are disturbed and are targets of peculiar concern. The next step will be normally initiated only when the previous step continued to reach this phase successfully. Proliferation of cells as myofibroblasts leading to wound edges to be contracted and decreasing its size and granulation tissue formation comes through the successful inflammation that achieved in physiologic wound healing process. Decreased or lack of IL-1 and TNF-Alfa (Tumor Necrosing Factor) as the major proinflammation cytokines determine the impairment present in inflammation phase that results in at least non harmonized cellular and molecular functional networks.

One of the leading steps in wound healing process is angiogenesis that in turn triggered by the act of endothelial cells via production of VEGF (Vascular endothelial Growth Factor) (22). This phenomenon is achieved by vasodilatation that is triggered by tissue hypoxia (13). An important approved report is the critical role of nitric oxide (22). No is produced by activation of Endothelial Nitric Oxide Synthase (eNOS) in several tissues a phenomenon that is failed in diabetes (22,25). Epithelialization is one of the most critical steps of wound healing. It should be synthesized mainly in BM(Bone Marrow) and entered in circulation to take a trip to reach its home (injured site). Thus to

achieve this despite its production, there is need to its mobilization and homing. These achieved by the act of eNOS activation and SDF1- Alfa produced by stromal cells of the ECM. In diabetes this chemokine (SDF1) and others as IL-8 are decreased. (22,25).

It is evidently clear that proper use of antibiotics will lead to control of infection; but as healing process is achieved in different and distinct way, it will be remained unhealed in presence of other underling mechanisms. Therapeutic implementation of PDGF and factor XIII (Plasma Transglutaminase) may reduce exudates; but do not complete the healing process. Alternatively implementation of G-CSF and GM-CSF increase the cellular infiltration; but the wound healing happens when every thing goes in its way. Keratinocytes derived GM-CSF has been shown to have a central role following tissue injuries (11). This molecule that is also secreted by other cells has been shown to have numerous roles in tissue repair mechanisms. They are as proliferation and emigration effects on endothelial cells, Keratinocytes, macrophages, fibroblasts, monocytes and denderitic cells. In addition it stimulates release and modulation of factors as IL-1, IL-6(Interleukin-6), TNF/Alfa, IFN/gamma (Interferron) and M-CSF(Monocyte-Colony stimulating factor), the molecules that have been shown to have critical roles in wound healing (9, 11). Regarding such reports there are more emphasize in some parts; for example expression of mRNA(Messenger RNA) for IFN/gamma, IL-6 and TGF/beta in addition to the high rate production of GM-CSF had been higher in patients of DFUs (26). It is well documented that these factors in combination with some other mechanisms had been to have critical roles in neoangiogenesis as an essential event for epithelialization and improving the healing process (26). Kinds of T- cells known as γδ- T(Gamma Delta T cells) cells are found early in embryonic life and epidermis of humans reported to have central role in healing of wounds (27). These are denderitic in shape and believed to be derived from langerhance cells that are the main macrophages of the skin surfaces (27).

Hypothesis

In depth analysis of factors involved in normal wound healing processes and impaired mechanisms seen in non-healing ones, confirms that healing is a concert of overlapping interactions of several cellular and molecular machine that have to work in an harmonized fashion to overcome the problem; so each break caused by pathogenic factors may enhance the organization fail relatively in its normal function. The role of macrophages is well defined in the immune system (28). They are as initial defense, antigen presentation and effector functions. Low counts of macrophages and some other critical cells in diabetes and suppressed levels of several molecules especially GM-CSF, TGF/β and most importantly IFN/γ, elevated amounts of IL-1R (Interleukine 1 receptor) in activated T cells and T cells of CD4+CD25+ (IL-2R) (Interleukine 2 Receptor) confirms the central roles of macrophages in respect with its activation to gain effector potentials and leader characteristics; a phenomenon that strongly suggestive of Th-1 cell activation.

Evaluation of the hypothesis

IFN-gamma has other biological effects as chemotactic potency (11, 26). IFN/gamma expression in higher rates suggests that effector cells as macrophages may have central roles in normal wound healing. Depleted in macrophages and failures of leukocyte functions have been reported in animal models.

Another confirmatory role of this hypothesis is depletion of CD3 expression in diabetes. This molecule triggers T cells enter to G1(Of Cell Cycle) phase following proper activation (27). This is the macrophages that activate T cells when they have to present antigens. IL-1 produced by macrophages triggers activation of T-cells. T cells are shown to express more IL-1Rs and produce IL-2 (receptor for this is increased in activated T-cells). Type of response needed is determined by activated T-cells. Production of IFN/γ triggers macrophage activation to initiate a cellular immune response organization (26). Macrophages organize this by production of IL-12 (29). In such a manner Th-2 cells and humoral immunity are not involved directly. Activation of Th-1 cells could have therapeutic potentials. This would be achievable through regulation and modulation leading to correction of impaired cellular and molecular network, directly and indirectly. Evidences based on worldwide literature and our successful experiments have revealed that it would be the case and importantly it is cheap, safe and easily applicable.

Conclusion

Th-1 cells activation may lead to complete healing of chronic wounds as DFUs. We have found such significant effects through our pilot study on several cases of DFU using safe formulations available inside the country. We suggest this mode of action for controlling and treatment purposes. Activators of Th-1 cells are available as optimized safe adjuvants. This mode of action is safe,cheap and its technology is avilble here in the country. It will be the future not only in regard with DFU; but also in many other aspects of chronic diseases. It would be considered globally in close future.

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