Original Research Paper

Volume - 10 | Issue - 11 | November - 2020 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Biological Science

REINING VARIOUS FACTORS TO RECTIFY THE CHRONIC WOUND HEALING IN THE DIABETIC CONDITION

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ABSTRACT Chronic wound-healing, one of the serious complications with the prevalence of diabetes remains the prime focus of wound care research. The present review explores various research approaches from gene therapies to administration of chemical compounds with the promising delivery systems to rectify the concatenation of impaired wound healing phases with an objective of the development of the various techniques combination to have a synergistic effect.

KEYWORDS : wound, inflammation, nanoparticles, GM3 synthase, VEGF, PDGF

INTRODUCTION

Diabetes affects about 500 million people in the world and the chronic ulcer is one of the grave ailments associated with it, accompanied by the augmentation of inflammation and ROS level, a diminution of Angiogenesis and vitiated keratinocyte and fibroblast migration (Dunnill et al., 2017). Wound healing comprises four overlapping phases viz. homeostasis, inflammation, proliferation and remodelling. Blood platelets on being exposed to the collagen spur the release of various clotting factors, platelet-derived growth factor (PDGF) and transforming growth factor (TGF-β) and migration of inflammatory cells e.g. proteases and ROS releasing neutrophils and macrophages to fend off pathogens in the ensuing inflammation phase. Fibroblasts migrate to the cleaned wound bed for deposition and remodelling of extracellular matrix and collagen and eventually its cross-linking remodelling and granulation tissue replacement and devascularization. But in the malfunctioned or chronic wounds, the concatenation of the aforementioned events is impaired with undue neutrophils infiltration and its adjunct degradative enzymes collagenase and ROS protracting the inflammatory phase (Chleboun et al., 1995).

Inhibiting Neutrophils NETs Forming Potential:

Subsequently, after infiltrating the wound, neutrophils secrete NETs (Neutrophil Extracellular Traps) to trap the pathogen in a meshwork of decondensed chromatin. The Histone citrullination, post-translational modification of a/a arginine in the Histone protein into the a/a citrulline through peptidylarginine deiminase (PAD4) catalysis, decondenses the chromatin. Wong et al (2015) manifested the links between neutrophils PAD4 expression, NETosis, and thereby impairing the wound healing in diabetes. They also demonstrated the insulin resistance by the role of neutrophils elastase and upsurge in the NET-related biomarkers in the circulation (Wong et al., 2015). Wang et al (2019) demonstrated the NADPH oxidase and high glucose-induced ROS overproduction as the crucial cause of the NETs formation. With the two inhibitors of NADPH oxidase, which catalyses the superoxide free radical production, viz. Apocynin and Diphenyleneiodonium (DPI), they observed a reduction in the neutrophils NETs forming potential even in comparison with the high glucose-induced normal rat and human neutrophils and also with both diabetic rats and T2DM patients neutrophils respectively (Wang et al., 2018).

Rectifying Antioxidant Mechanisms By Curbing The Keap1 Upregulation:

In another prior research, wound healing got ameliorated through a cationic lipid and supercharged coiled-coil α-helical pentamer protein complex nanoparticle-delivered 21-23 nucleotide long fragment of siRNA targeted against Keap1 (Kelch-like ECH-associated protein), a key repressor of Nrf2 (Nuclear factor erythroid 2 like) which is a central regulator of redox mechanisms in order to rectify ROS imbalance (Rabbani et al., 2017). In the cytoplasm, Nrf2 is bound to Keap1, which is targeted for ubiquitination and proteasomal degradation. Under the oxidative stress, covalent modification in cysteine-rich and electrophile sensing regions of Keap1 prevents ubiquitination of Nrf2 and Nrf2 dissociates from its repressor, Keap1 and enters into the nucleus to bind to an antioxidant response elements in the promoter region of a wide array of genes, which are involved in protein stability and defence against oxidative stress. In diabetes, Keap1 is upregulated and Nrf2 subsequently degraded, causing a dearth of antioxidant mechanisms(Baird & Dinkova-Kostova, 2011).

Angiotensin Receptor Blocker Treatment:

Renin Angiotensin Aldosterone System (RAAS or RAS), a hormone system, which regulates blood pressure and fluid balance, is found to be dysregulated in both ageing and diabetes with high cutaneous AT_1R and AT_2R (Angiotensin Type 1 and 2 Receptor) ratio. AT_2R properties of cell differentiation, anti-apoptotic, and anti-inflammatory effects counter the AT_1R effects. Influence of AT_1R and AT_2R on the wound healing may be phase-dependent (Hao et al., 2011). Obstruction of the AT_1R, which augments inflammatory signalling during the initial wound healing stage, led to a slower closure rate, which is putatively as a result of disrupted inflammatory phase and vitiated transition to the proliferation and remodelling phases. Also, $AT_1R^{-/-}$ mice showed retarded healing, supported by a substantial reduction in proliferating cell nuclear antigen (PCNA) and phosphohistone H3 in their healing skin vis-à-vis controls (Faghih et al., 2015). But when AT_1R was blocked with 1% Valsartan, an Angiotensin receptor blocker (ARB) also known as Angiotensin II receptor antagonist, in diabetic mice and ageing diabetic porcine models at the transitioning of inflammatory to proliferative phase, led to an escalated healing rate with revamped dermal parameters viz. enhanced blood flow to the wound, re-epithelization and collagen accumulation, which provides a scaffold for healing cells and increased tensile strength thereof (Abadir et al., 2018). Immediately after wounding in diabetic mice, an oral Angiotensin receptor blocker (ARB) -Losartan treatment also manifested an expedited wound healing (Kamber et al., 2015). SMADs are the prime signal transducer of TGF-β receptor. A failed phosphorylation of the SMAD pathway was found in the cells from the chronic wound (Kim et al., 2003). An accelerated wound healing has been observed with the administration of exogenous TGF-β and SMAD3 in the wounds and also with the selective activation of SMAD2, SMAD3 and co-SMAD4 and inhibition of SMAD1,2,5 and 9 with 1% Valsartan treatment (Abadir et al., 2018). The other enhanced parameters of the healing skin were a higher expression of CD31 (PECAM-1), VEGF receptor 2, α-Smooth Muscle Actin (α-SMA), and Mitogen-Activated Protein Kinase (MAPK) and an increase in the mitochondrial content in the tissues obtained from the wound bed (Benigni et al., 2009).

Lactate And VEGF Administration:

In humans, wound healing depends prominently on re-epithelization while wound contraction in mice. To simulate a human wound, silicon splints are fastened to the skin, skirting the wound of a mouse, to preclude wound closure and to enhance re-epithelization. Oedema and localized Ischemia restrain nutrient delivery and oxygen. A proper angiogenesis is indispensable for the wound healing and VEGFa is a powerful proangiogenic therapeutic aid (Moulin et al., 2000). It was also alluded that dermal cells migration and proliferation at the wound site can be as a result of hypoxic condition and lactate(Johnson & Wilgus, 2014). Chereddy et al manifested synergistic effect of lactate and VEGF to expedite the wound healing through VEGF administration using 10 mg of ~ 200 nm PLGA {Poly(lactic-co-glycolic acid)} nanoparticles (PLGA-VEGF NP) with ~ 75% encapsulation efficiency in a splinted mouse. To study wound healing through re-epithelization prominently, silicone splints were used to avoid wound contraction. There was a substantial healing effect from day 5 and day 10 for non-diabetic and diabetic respectively. On HaCaT human Keratinocyte, PLGA-VEGF NP demonstrated substantial mitotic induction effect. VEGF may modify stemness and

proliferation of Keratinocytes (Chereddy et al., 2015). Among the principal tyrosine kinase receptors VEGFR1, VEGFR2, and VEGFR3, VEGFR2 is expressed predominantly in blood vascular endothelium and main mediator of angiogenesis and vascular permeability. VEGF binding to VEGFR2 induces tyrosine residue phosphorylation, leading to protein kinase B (PKB or Akt), mitogen-activated protein kinase (MAPK) pathway activation, which induced proliferation and withstrained apoptosis. By VEGF and its endothelial-specific receptor VEGFR2 upregulation, HUVECs responded to high lactate level and thereafter by means of poly ADP ribosylation (PAR)-dependent mechanism, enhancing angiogenic potency (Kumar et al., 2007).

Knock Down Of Gene Expressing The MT Severing Enzymes:

Directional dermal FBs and keratinocytes migration, cell adhesion and wound closure are driven by the spatiotemporal interaction of Rho GTPase (Rho A, Rac1 and Cdc 42), actin microfilaments, microtubules (MTs) and their associated proteins. Indispensable factors for wound healing- gene expression, cell polarity, Actin remodelling and vesicular trafficking are regulated by Rho GTPases. Rac1 induces lamellipodial, a cytoskeletal actin protrusion and Cdc 42 induces filopodial or microspikes, cytoplasmic projection in the leading strand of migrating cells, while RhoA impels fibre contraction and focal adhesion between cells and extracellular matrix through transmembrane protein integrin(Hall, 1992). Contractile force is transmitted from the basal actomyosin ring to the underlying substrate of the crawling cells and originates inward-pointing traction layer while the outward-pointing traction layer is originated from the lamellipodial force (Brugués et al., 2014). Three ATP dependent MT severing enzymes viz. Katanin, plastin and fidgetin have been discovered hitherto. These enzymes encompass roles of new MT growth, release of the MTs from centrosomes and their intracellular transportation towards the leading edge and slicing MTs into small fragments. Charaffedine et al. asseverate that MT severing enzyme fidgetin like protein (FL2) impedes MT polymerization and Rac1 local activation for lamellipodial protrusion. In their study, rapid wound closure was achieved by enhanced cellular migration with the help of Tetramethyl orthosilicate nanoparticle-mediated siRNA delivery to knock down FL2 in a murine full-thickness excisional and burn wounds(McNally & Vale, 1993).

Knock Down Of Gene Expressing The GM3 Synthase:

Similarly, with a nanoparticle, siRNA was delivered to knock down ganglioside-monosialic acid 3 synthase (GM3S) in a diabetic mouse model to improve cellular migration. Gangliosides GM1 and GM3 restrain the cell growth by extending the G-phase of the cell cycle and make cells unresponsive to stimulation by epidermal growth factor, fibroblast growth factor, and platelet-derived growth factor. Ganglioside-Monosialic Acid (GM3) in the skin is found to be a critical cause of insulin resistance (Randeria et al., 2015). GM3 synthase (aka GM3S or ST3 Gal-V or SAT-I), required for GM3 synthesis, was knocked down through an siRNA pathway, which consequently escalated keratinocyte migration and proliferation in a splinted 6 mm diameter full thickness wounds (in vivo) of diet-induced obese diabetic mice and scratch assay (in vitro) by a topical application of an Aquaphor® dispersed 15 nM spherical nucleic acid (SNA) gold nanoparticle of 13 nm diameter (Giljohann et al., 2009). The sense strand of the duplexed siRNA linked by propylthiol to the gold nanoparticle was found to be capable of traversing the stratum corneum of an intact skin (Zheng et al., 2012). Epidermal Growth Factor Receptor and Insulin-like Growth Factor-1 Receptor were found to be activated in GM3S SNA treated wounded diabetic skin, confirmed by Immunohistochemical staining. In comparison with the untreated wound, granulation tissue area was raised four-fold by the time of closure and Platelet Endothelial Cell Adhesion Molecule (PECAM-1 aka CD31), a parameter for wound vascularity was increased by almost two-fold (Randeria et al., 2015).

Topical Application Of Synthetic ds miR-132 mimics And Knock Down Of miR-378a:

The human genome encodes more than a hundred thousand of micro RNAs and some are employed in the tissue repair mechanism. The expression of miR-132, which regulates several inflammation-related signalling pathways e.g. Toll-like receptor, NOD (Nucleotide binding oligomerisation domain)-like receptor, NF- κB, and TNF signalling pathways, restrains pro-inflammatory cytokines/ chemokines released by macrophages, monocytes, and Keratinocytes and promotes the proliferation of epidermal keratinocytes and neovascularization, was found to be low in diabetes (D. Li et al., 2015). To expedite the inflammatory-proliferative phase transition, miR-132 was locally replenished by topical application of the Pluronic F127 gel containing the synthetic double-stranded miR-132 mimics loaded liposomes in the wounded skin of leptin receptor-deficient (db/db) type-2 diabetic mice model (X. Li et al., 2017). miRNA targets the similar mRNAs because of their highly conserved seed region. miRNA is silenced by anti-miRNA oligonucleotides (AMOs), which are chemically modified to elude the nucleotidase degradation. Besides the seed region, anti-miRNA construct binds to the central loop of miRNA precursor to prevent it to be functional. To study the tissue regeneration, miR-378a was knocked down by AMO and increases the expression of vimentin and integrin β-3, which expedited fibroblast migration and differentiation in vitro and improved healing of a 5 mm diameter, wound in vivo. Vimentin generates traction forces and integrin β -3, which is an integral cell surface protein for cell adhesion and signal transmission, accelerates fibroblast migration (Eckes et al., 2000). Upregulation of integrin β-3 fostered tube-like structure in vitro and angiogenesis in vivo, ostensibly activating the VEGF signalling. Also in the ischemic wounds, the miR-210 level was found to be surged, which promotes stabilization of Hypoxia-inducible factor 1-α (HIF1-α) (pseudohypoxia), causing feeble keratinocyte proliferation and impaired wound re-epithelization (H. Li et al., 2014).

Other Successful Gene Therapies For Diabetic Wound Healing:

The cardinal cause of the chronic wound healing is the scarcity of growth factors due to the proteasomal degradation by neutrophils released elastase. Inefficient delivery to the target cells, short shelf life, large and repeated doses of PDGF-β are the constraints in treating chronic wounds topically (Pierce et al., 1995). In prior research, substantially higher degree of wound healing i.e. epithelization, keratinisation, fibrocollagenation and blood vessel formation in streptozotocin-induced diabetes rats in 10 days were manifested with a single subcutaneous cationic liposome injection deploying integrin receptor targeting RGDK (Arginine-Glycine-Aspartic Acid-Lysine) lipopeptide1 and rh PDGF-β plasmid DNA to a proangiogenic fibroblast cells, affirmed through Western Blot and Histopathological staining (Bhattacharyya et al., 2009). Various successful gene therapies for diabetic wound healing in mice comprise siRNA-mediated prolyl-hydroxylase domain 2 knockdown for fibroblast proliferation and angiogenic factors augmentation (Wetterau et al., 2011), siRNA knockdown of ganglioside GM3 synthase (Randeria et al., 2015), p53 si RNA knockdown to amplify vasculogenic mediators (Nguyen et al., 2010), siRNA knockdown of xanthine dehydrogenase to slump down the ROS upsurge (Weinstein et al., 2015), plasmid VEGF delivery through hyaluronic acid matrix metalloproteinase hydrogels (Tokatlian et al., 2015).

CONCLUSIONS

To rectify the concatenation of Wound healing phases, different combinations of gene therapies and administration of other factors should be done. The synergistic effect of these various approaches can elucidate a better insight into the healing process and can treat the chronic wound healing more effectively.

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