Role of Growth Factors in the Treatment of Diabetic Foot Ulceration

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PRINCIPLES OF WOUND HEALING AND GROWTH FACTOR THERAPY

Wound healing is the process of tissue repair and the tissue response to injury. It is a complex biological process involving chemotaxis, cellular reproduction, matrix protein production and deposition, neovascularization, and scar remodeling (1). Growth factors are polypeptides that control the growth, differentiation, and metabolism of cells, and regulate the process of tissue repair (2,3). The role of growth factors in wound healing and specifically in diabetic ulcer healing is the subject of this chapter.

The three phases of wound healing—inflammation, fibroplasia, and maturation—are each controlled by growth factors that are present in only small amounts, yet powerful in their influence on wound repair. There is great interest in manipulating the cellular environment of the wound with proteins, growth factors, and gene therapy.

The first phase of wound healing is the inflammatory response, initiated immediately after the injury (4). Vasoconstriction limits hemorrhage to the site of wounding. As blood vessels are damaged and blood leaks from within the lumen, platelets come into contact with collagen in the wall of the vessel beneath the endothelium. Platelets are activated by the collagen and initiate coagulation. Serotonin and thromboxane are released and enhance vasoconstriction locally, keeping the healing factors within the wound. Simultaneously, vasodilatation occurs, allowing new factors to be brought into the wound. Vasodilatation is mediated by histamine, released by the platelets, mast cells, and basophils. There is also an increase in vascular permeability allowing blood borne factors to enter this area. Arachidonic acid is produced and serves as an intermediate for production of prostaglandins and leukotrienes. These proteins are intense vasodilators which increase vascular permeability, along with histamine, bradykinin, and complement. Thromboxane also increases platelet aggregation and local vasoconstriction.

Platelets control hemorrhage by initiating clotting through the coagulation system. The intrinsic system is activated by Hageman factor, known as factor XII, as it comes into contact with collagen. In the presence of kininogen, a precursor of bradykinin and prekallikrein, factor XII activates factor XI, then factor IX, and then factor VIII. Thromboplastin triggers a response in the extrinsic system. Thromboplastin is formed as phospholipids and glycoproteins are released by blood coming into contact with the injured tissues. Factor VII is activated in the presence of calcium. Both the intrinsic and
extrinsic systems activate the final common pathway, producing fibrin and leading to fibrin polymerization. To balance the coagulation cascade, the fibrinolytic system is activated. This system monitors clotting to prevent coagulation from extending beyond the wound. It is activated by the same factors that initiate coagulation and thus regulates the process.

The complement cascade is activated by platelets and neutral proteases. This system produces potent proteins known as anaphylotoxins, which cause mast cells to degranulate and release histamine. Substances released by the inflammatory process are chemoattractants for neutrophils. These cause margination of white blood cells and then migration of these white blood cells into the wound. The neutrophils are phagocytes for bacteria. Wounds can heal without white blood cells, but the risk of infection is increased. Neutrophils produce free oxygen radicals and lysosomal enzymes for host defense. The neutrophils are later removed from the wound by tissue macrophages.

Monocytes enter the wound space and become tissue macrophages. They take over control of the wound environment by the third day. Wounds cannot heal without the macrophage. These cells regulate the production of growth factors including platelet-derived growth factor (PDGF), tumor necrosis factor (TNF), and transforming growth factor (TGF)-β; thus they control protein production, matrix formation, and remodeling. Extracellular matrix (ECM) is a group of proteins in a polysaccharide gel made up of glycosaminoglycans and proteoglycans produced by the fibroblast. These proteins are structural such as collagen and elastin, or are involved in controlling cell adhesion such as fibronectin and laminin (5). Thrombospondin and von Willebrand factor are other adhesion molecules. Fibronectin is also a chemoattractant for circulating monocytes and stimulates its differentiation into tissue macrophages.

The second phase of wound healing is fibroplasia and begins with macrophages and fibroblasts increasing in number in the wound, whereas white blood cells decrease as fewer enter the wound. The inflammatory response ends as the mediators of inflammation are no longer produced, and those already present are inactivated or removed by diffusion or by macrophages. Fibroplasia begins around the fifth day following injury and may continue for 2 weeks. This begins the process of matrix formation, especially collagen synthesis. Angiogenesis is the process of rebuilding the blood supply to the wound (6). Fibroblasts are attracted to the wound and replicate in response to fibronectin, PDGF, fibroblast growth factor (FGF), TGF-β, and C5a, a product of the complement system. Fibroblasts produce proteoglycans and structural proteins. The cellular matrix is made up of hyaluronate and fibronectin which allow for cellular migration through chemotactic factors formed in the wound. Fibronectin binds proteins and fibroblasts in the matrix and provides a pathway along which fibroblasts can move. Fibronectin also plays a role in epithelialization and angiogenesis.

Collagen is the most common protein in the mammalian world and is produced by the fibroblast. It is a family of at least 12 proteins, rich in glycine and proline, and bound in a tight triple helix. Cross-linking between the three strands of collagen provides for a very stable molecule, resistant to breakdown. Macrophages control the release of collagen from fibroblasts through growth factors such as PDGF, epidermal growth factor (EGF), FGF, and TGF-β. Collagen is remodeled for several years in a healing wound. Elastin is the other major structural protein and contains proline and lysine. It is present as random