

**CELLULAR AND MOLECULAR MECHANISMS OF ULCER HEALING
2005: LESSONS FROM GASTROINTESTINAL ULCERS**

Andrzej S. Tarnawski, M.D., D.Sc.

Department of Medicine, Veterans Affairs Medical Center, Long Beach, 5901 East Seventh Street, Long Beach, California 90822, and the University of California, Irvine, Irvine, California 92697.

Running head: mechanisms of ulcer healing

Andrzej S. Tarnawski, M.D., D.Sc.
VA Long Beach Healthcare System
Gastroenterology Section 111G
5901 E. 7th Street
Long Beach, CA 90822-5201
USA
Phone: (562) 826-5437
Fax: (562) 826-8016
e-mail: atarnawski@yahoo.com

Abbreviations:

EGF – epidermal growth factor
PDGF – platelet derived growth factor
KGF – Keratinocyte growth factor
HGF – hepatocyte growth factor
TGF β - transforming growth factor beta
VEGF – Vascular endothelial growth factor
Ang - angiopoietin
SRF – serum response factor
HIF – hypoxia inducible factor
TNF α - tumor necrosis alpha
IL-1 α - interleukin
MAPK – mitogen activated protein kinase
Erk – extracellular regulated kinase

Abstract:

In this lecture, I review cellular and molecular mechanisms of gastrointestinal ulcer healing as a paradigm for ulcer healing process. Ulcer healing, a genetically programmed repair process, includes inflammation, cell proliferation, re-epithelialization, formation of granulation tissue, angiogenesis, interactions between various cells, matrix and tissue remodeling, all resulting in scar formation. All these events are controlled by cytokines and growth factors (EGF, PDGF, KGF, HGF, TGF β , VEGF, angiopoietins) and transcription factors activated by tissue injury in spatially and temporally coordinated manner. These growth factors trigger mitogenic, motogenic and survival pathways utilizing Ras, MAPK, PI-3K/Akt, PLC- γ and Rho/Rac/actin signaling. Hypoxia activates pro-angiogenic genes (e.g. VEGF, angiopoietins) via HIF, while serum response factor (SRF) is critical for VEGF-induced angiogenesis, re-epithelialization and muscle restoration.

EGF, its receptor, HGF and Cox2 are important for epithelial cell proliferation, migration reepithelialization and reconstruction of gastric glands. VEGF, angiopoietins, nitric oxide, endothelin and metalloproteinases are important for angiogenesis, vascular remodeling and mucosal regeneration within ulcer scar. Circulating progenitor cells are also important for ulcer healing. Local gene therapy with VEGF + Ang1 and/or SRF cDNAs dramatically accelerates esophageal and gastric ulcer healing and improves quality of mucosal restoration within ulcer scar. Future directions to accelerate and improve healing include the use of stem cells and tissue engineering.

Key words: growth factor, re-epithelialization, granulation tissue, signaling, gene therapy

Extended abstract

This lecture summarizes the cellular and molecular mechanisms of ulcer healing. While focus is on gastric ulcer, the general principles of healing and the cellular and molecular events described in this paper apply to the healing of other gastrointestinal tract ulcers (esophageal, duodenal and intestinal), other external and internal ulcers and tissue injury. An ulcer is a deep defect in the esophageal, gastric, duodenal or intestinal wall penetrating the entire mucosal thickness and the muscularis mucosae (**Figure 1**) (1-3).

Figure 1
Gastric Ulcer - Histology



Histology

Histologically, an ulcer consists of two major structures; a distinct ulcer margin formed by the adjacent non-necrotic mucosa - the epithelial component, and granulation tissue at the ulcer base, which consists of fibroblasts, macrophages and proliferating endothelial cells forming microvessels (**Figure 1**) (1-3).

Ulcer healing is a complex process, which involves cell migration, proliferation, reepithelialization, angiogenesis, and matrix deposition, all ultimately leading to scar formation (1-9) (**Figure 2**). All these processes are controlled by growth factors, transcription factors and cytokines (2, 3, 9-11).

Figure 2 Healing of Gastric Ulcer



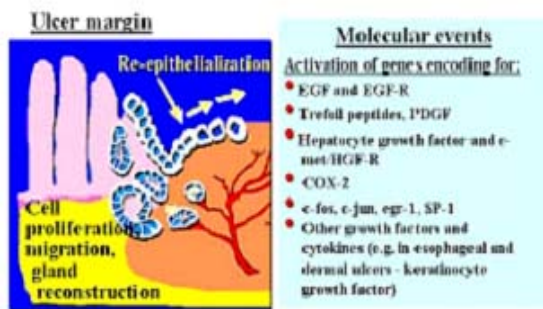
A. Tarnowski 2000

Cellular and molecular events in the ulcer margin.

Mucosa of the ulcer margin forms a characteristic “healing zone” (1-3). The epithelial cells lining glands of the ulcer margin undergo de-differentiation, express epidermal growth factor receptor (EGF-R) and actively proliferate (2, 3, 12). Proliferation is essential for ulcer healing, because it supplies epithelial cells crucial for re-epithelialization of the mucosal surface and reconstruction of gastric glands (2, 3, 7). These cells migrate from the ulcer margin onto the granulation tissue to re-epithelialize the ulcer base. In addition, the epithelial cells from the base

of the ulcer margin form tubes composed of ulcer-associated cell lineage, which invade granulation tissue migrate toward the surface, branch and undergo transformation into gastric glands within the ulcer scar (3, 7, 13) (**Figure 2**). Growth factors are the major stimuli for cell proliferation, division, migration and re-epithelialization are (1, 3, 9-11). In addition to the initial pool of growth factors derived from the platelets, macrophages and injured tissue, ulceration triggers in cells lining mucosa of the ulcer margin, genes encoding for the growth factors (e.g. EGF, bFGF, HGF, VEGF and PDGF) and Cox2, in a well synchronized spatial and temporal manner (3, 11, 14).

Figure 3 Gastric Ulcer Healing



These growth factors produced locally, activate epithelial cell migration and proliferation via autocrine and/or paracrine actions (**Figure 3**).

Recent study indicates that in addition to utilizing cells from the mucosa of the ulcer margin, epithelium of damaged or ulcerated mucosa can be regenerated by bone marrow-derived adult stem cells (15). Okamoto and co-workers demonstrated

that bone marrow cells can re-populate epithelium of human gastrointestinal tract and this process is increased by ~ 50-fold in gastric ulcer (15).

Re-epithelialization, the migration of epithelial cells from the ulcer margin to restore epithelial continuity, is essential for cutaneous and gastrointestinal wound/ulcer healing **Figures 2 and 3** (1, 3, 7, 9, 10), because a continuous epithelial "barrier" protects granulation tissue against mechanical and chemical injury or infection.

The cell migration is dependent on the transcription factors and cytoskeletal rearrangements (16, 17). The cytoskeleton (actin filaments, microtubules, intermediate filaments, focal adhesions and their associated proteins) plays an important role in cell structure, shape and mobility (16-18). Cell migration requires polymerization of G-actin into F-actin and formation of stress fibers. Recent study demonstrated that, role of serum response factor (SRF), it is crucial for ulcer re-epithelialization and muscle restoration (16).

Signaling events in the mucosa of the ulcer margin during ulcer healing: (**Figure 3**).

In vivo studies on experimental gastric ulcers in rats, demonstrated that ulceration triggers overexpression of EGF and its receptor, EGF-R, in epithelial cells of the ulcer margin (21) and that healing of the epithelial component of ulcers involves activation of the EGF-R – MAPK (Erk) signal transduction pathway (21-23) (**Figure 3**).

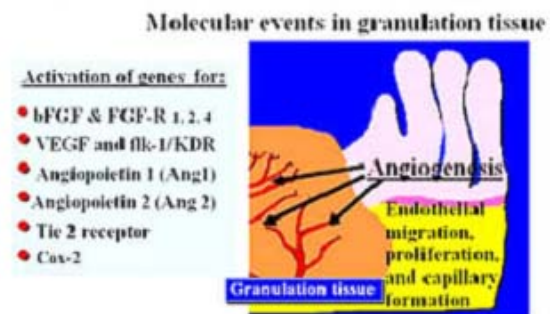
Cellular and molecular events in granulation tissue: angiogenesis (Figure 4). Granulation tissue develops at the ulcer base within 48-72 hours after ulceration (1-3, 6, 7, 24). Granulation tissue consists of proliferating connective tissue cells, i.e. macrophages, fibroblasts and proliferating endothelial cells, which form microvessels through the process of angiogenesis (1-3, 6, 7, 24). Migration of fibroblasts into the granulation tissue and their proliferation are triggered by growth factors: TGF β , PDGF, EGF, FGF and cytokines: TNF α and IL-1 derived from inflammatory cells, activated endothelial cell and macrophages (1). Granulation tissue supplies connective tissue cells (synthesizing extracellular matrix) for restoring the lamina propria and microvessels for the restoration of the microvasculature within ulcer scar (1-3, 24). **Angiogenesis** - formation of new microvessels from preexisting vessels – is essential for healing of chronic gastroduodenal ulcers (1, 24-26).

The growth of granulation tissue and generation of new microvessels through angiogenesis is stimulated by bFGF, VEGF, PDGF, angiopoietins and possibly by other growth factors and cytokines, including IL-1 and tumor necrosis factor-alpha (TNF- α) (3, 25, 26) **Figure 4.**

VEGF is a fundamental regulator of angiogenesis. Its binds to at least two specific receptors; VEGF-R1 or flt-1 and VEGF-R2 or flk-1/KDR expressed mainly on endothelial cells (27), initiates phosphorylation of numerous cytosolic proteins involved in signal transduction that triggers endothelial cell proliferation, migration and microvascular tube formation (27).

Hypoxia is one of the best characterized stimuli for the induction of VEGF production. Hypoxia triggers accumulation of a hypoxia-inducible factor (HIF-1) that binds to and activates the VEGF gene promoter (27). Angiogenesis is critically dependent on SRF (28).

Figure 4 Gastric Ulcer Healing



Several new angiogenic factors have been identified (in addition to bFGF and VEGF) namely, angiopoietins and Tie2, neuropilin, ephrin/Eph, leptin and CXCR-4. Angiopoietin-1 and -2 (Ang1 and Ang2) appear to be involved in angiogenic processes occurring subsequent to the actions of VEGF and its receptors (34-36). Overexpression of Tie2, Ang1 and Ang2 occurs in early phase of gastric ulcer healing (39, 40) and likely stimulates angiogenesis in granulation tissue by the above mechanisms (36). MAPK and Phosphatidylinositol 3-kinase (PI-3K), is another important kinase which has been shown to be activated by most mitogens and has been implicated as a critical factor in the regulation of endothelial cell proliferation, survival and motility (41).

The extracellular matrix and mesenchymal to epithelial interactions during ulcer healing.

Extracellular matrix (ECM) is secreted locally by fibroblasts epithelial, smooth muscle and endothelial cells and assembles into a network in the spaces surrounding cells (1, 42, 43). It sequesters water and minerals and binds growth factors. ECM consists of: fibrous structural proteins such as the collagens and elastins, adhesive glycoproteins including fibronectin and laminin and an amorphous gel composed of proteoglycans and hyaluronan. The above components assemble and form an interstitial matrix and the basement membrane (1, 42, 43). Collagens are the most abundant structural proteins of extracellular matrix. Collagen types I, II and III interstitial or fibrillar collagens and are the most abundant (1, 42). Collagens IV, V and VI are nonfibrillar and are present predominantly in the basement membrane (11, 42, 43). Integrins are transmembrane glycoproteins, cell surface receptors that mediate cell attachment to ECM and regulate cell to matrix signaling and interactions (1, 43). Interaction of integrin receptors with the cell and ECM takes place in focal adhesions where integrins link to intracellular cytoskeletal complexes (1, 43).

The cell growth, differentiation and migration are thus regulated in part by extracellular matrix interaction with epithelial and endothelial cells via integration of multiple signaling: (a) initiated by growth factors, cytokines and growth inhibitors and (b) derived from the ECM components and transmitted via integrins (1, 42, 43). A cross-talk between those pathways and signaling systems represents integration of cell to matrix interactions.

Tissue remodeling. The replacement of granulation tissue with a connective tissue scar involves changes in the composition of the ECM. The growth factors that stimulate synthesis of collagen

and other connective tissue components also modulate the synthesis and activate of metalloproteinases, enzymes that degrade these ECM components (1). The net result of ECM synthesis versus its degradation is remodeling of the connective tissue— an important feature of ulcer healing (1). Degradation of collagen and other ECM proteins accomplished by matrix metalloproteinases, enzymes dependent on zinc ions for their activity (1, 44, 45).

Activated MMPs are rapidly inhibited by specific tissue inhibitors of metalloproteinase (TIMP), which are produced by most mesenchymal cells, thus preventing uncontrolled action of these proteinases (1, 45). Collagenases and their inhibitors are spatially and temporally regulated during gastric ulcer healing (45). They are essential in the remodeling of connective tissue necessary for tissue defect repair and scar formation (44, 45).

Temporal and spatial gene expression during ulcer healing. Epithelial/mesenchymal interactions and back-up. Sequential analysis of gene expression during gastric ulcer healing allowed to distinguish genes involved in early response: EGF-R, c-fos, c-jun, egr-1, Sp-1, TFF-2/SP, which are all activated shortly after ulcer formation (within 30 min – 2 hrs), intermediate response genes: EGF, bFGF, PDGF and VEGF (activated within 6 hrs – 2 days) and late response genes: HGF, ITF, c-met/HGF-R (activated within 14 days). It should be also pointed out that some of the growth factors are produced by epithelial cells, e.g. TP, EGF, TGF α , while others, e.g. PDGF, VEGF, HGF, bFGF and KGF by mesenchymal cells.

Esophageal ulcer healing follows the same general pattern of healing as gastric ulcers, but in addition, keratinocyte growth factor (KGF) plays an important role (5). The same is true for duodenal and intestinal ulcers. They contain specialized cells that provide some tissue variation and specificity to the ulcer healing process.

Non-steroidal anti-inflammatory drugs (NSAIDs) and *H. pylori* toxins interfere with the ulcer healing by inhibiting, epithelial cell proliferation, migration and angiogenesis and by blocking growth factor-triggered signaling pathways (33, 46, 47).

Quality of ulcer healing. Previous studies demonstrated that re-epithelialized mucosa of grossly “healed” experimental gastric ulcers has prominent histological and ultrastructural abnormalities (7, 8): reduced height, dilation of gastric glands, increased connective tissue and a disorganized microvascular network. These prominent abnormalities may interfere with the mucosal defense and cause ulcer recurrence when ulcerogenic factors are present. Therefore, the

quality of mucosal structural restoration may be the most important factor in determining future ulcer recurrence. Several studies indicate that topically acting ulcer healing drugs such as antacids and rebamipide may improve quality of ulcer healing in experimental models and in human ulcers (48-50).

Gene therapy for ulcer healing. Gene therapy specifically applied to accelerate the healing of chronic gastric and/or esophageal ulcers, has not been explored to date except for our experimental studies (51, 52). These studies demonstrated that a single local injection of plasmids encoding VEGF₁₆₅ and Ang1 significantly increased angiogenesis and accelerated gastric and esophageal ulcer healing (51). Co-injection of both plasmids encoding VEGF₁₆₅ and Ang1 resulted in a more complete restoration of gastric glandular structures within the ulcer scars and also led to the formation of more mature vessels (51). Szabo and co-workers successfully applied gene therapy with adenoviral plasmids or naked DNA of VEGF and PDGF to duodenal ulcers and demonstrated that this treatment accelerates duodenal ulcer healing (53, 54).

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