Esophageal and Gastrointestinal Microcirculation: Essential for Mucosal Protection, a Target for Injury, and a Critical Component of Injury and Ulcer Healing

Andrzej S. Tarnawski, Jianyuan Chai, and Michael K. Jones

Summary. Blood flow through the network of mucosal microvessels in the gastrointestinal tract is essential for delivery of oxygen and nutrients to all mucosal constituents. The endothelial cells lining the microvessels, although protected by prostaglandins, are major targets for injury by various noxious factors, such as ethanol, nonsteroidal anti-inflammatory drugs (NSAIDs), ischemia–reperfusion, and free radicals. Endothelial injury results in formation of thrombi, microvascular stasis, and hypoxia, leading to tissue necrosis in the form of erosions or ulcers. Quantitative histologic, Transmission (TEM) and scanning electron microscopy (SEM) studies show that acute mucosal injury such as erosion triggers angiogenesis in the mucosal microvessels bordering necrosis: endothelial sprouting, formation of endothelial tubes ultimately leading to restoration of microvessels in regenerating tissue. The major molecular trigger for the initiation of angiogenesis in injured esophageal and gastric mucosa is accumulation of hypoxia inducible factor-1α (HIF-1α), which activates the genes encoding vascular endothelial growth factor (VEGF), its receptor, and angiopoietins that regulate angiogenesis. During healing of esophageal or gastric ulcers, granulation tissue, consisting of fibroblasts and proliferating endothelial cells forming microvessels, develops at the ulcer base. The newly formed microvessels, supported by fibroblasts and smooth muscle cells, sprout into the ulcerated area and restore the microvasculature in the ulcer scar. The molecular mechanisms stimulating angiogenesis in granulation tissue include activation of HIF-1α, egr-1, and genes encoding basic fibroblast growth factor, VEGF, angiopoietin-1, angiopoietin-2, their receptors, and cyclooxygenase-2. The latter co-localizes with upregulated VEGF in esophageal and gastric ulcers. The scars of healed ulcers demonstrate prominent microvascular abnormalities detected by cast/SEM

Department of Gastroenterology, VA Medical Center, Long Beach and the University of California, Irvine, 5901 East 7th Street, Long Beach, CA 90822-5201, USA

and vascular permeability studies. NSAIDs inhibit angiogenesis by interfering with MAP/Erk2 kinase, actin cytoskeleton, and D_1 cyclin. Such actions are likely responsible for NSAID interference with ulcer healing. Our studies demonstrated that gene therapy with a single local injection of VEGF and angiopoietin-1 cDNAs stimulates angiogenesis, promotes restoration of microvascular network, and accelerates healing of experimental gastric and esophageal ulcers.

Key words. Endothelial cell, Hypoxia, Angiogenesis, Vascular endothelial growth factor, Gene therapy

Gastric Microcirculation

Blood flow through microvessels (capillaries, arterioles, and collecting venules) is crucial for maintaining the structure and functions of all tissues including esophageal and gastrointestinal mucosa [1–5]. The microcirculation is critical because it delivers oxygen and nutrients to all tissues and cells and removes toxic metabolites [3]. The vascular cast/scanning electron microscopy studies of normal gastric mucosal microvasculature demonstrated a very dense capillary network in the gastric mucosa in lamina propria adjacent to glandular epithelial cells (Fig. 1) [6].

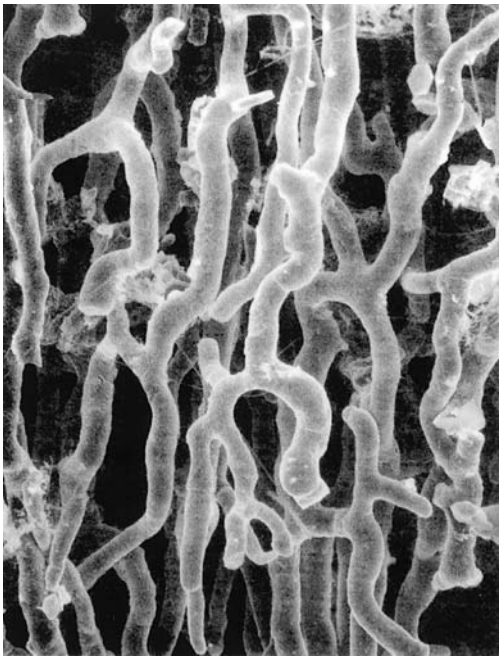

Fig. 1. Vascular capillary casts of normal gastric mucosa in rat. Vasculature was filled with Mercox resin (Dainippon, Tokyo, Japan). Thirty minutes later, the stomach was removed, cut into small pieces and immersed in 20% NaOH for 6 h to dissolve tissue external to the cast. Desiccated specimens were coated with gold/palladium (60:40) and examined under a scanning electron microscope (5500, Hitachi, Tokyo, Japan) at 10 kV. Reprinted, with permission, from our previous publication [6]

Microvascular Blood Flow, its Regulation, and its Response to Injurious Factors

The microvascular endothelium generates potent vasodilators such as nitric oxide (NO) and prostacyclin, which protect the gastric mucosa against injury and oppose the mucosal damaging action of vasoconstrictors such as leukotriene C_4, thromboxane A_2, and endothelin [3–5]. When the gastric mucosa is exposed to an irritant, or when acid back-diffusion occurs, there is, in response, a marked and rapid increase in mucosal blood flow [3–5], which allows removal and/or dilution of the back-diffusing HCl and/or noxious agents. This response is essential for mucosal defense, because its abolishment through mechanical restriction of blood flow leads to the development of hemorrhagic necrosis. Holzer [7] characterized the mechanisms responsible for this hyperemic response and demonstrated that it is mediated by sensory afferent nerves. Stimulation of gastric sensory nerves leads to the release of neurotransmitters such as calcitonin gene-related peptide (CGRP) and substance P in the nerve terminals, located within or close to the large submucosal vessels [7–9]. Calcitonin gene-related peptide exerts a mucosal protective action most likely through vasodilatation of submucosal vessels mediated by NO generation [9,10]. Ablation of the sensory afferent nerves (by chronic, large dose treatment with capsaicin) abolishes the hyperemic response and greatly increases the susceptibility of gastric mucosa to damage [4,7,9].

Nitric oxide, a potent vasodilator, is generated from L-arginine by an enzyme, NO synthase (NOS), which is expressed in endothelial cells as two isoforms: a constitutive (eNOS) and an inducible (iNOS) [3–5,9,11]. In the gastric mucosa, NO plays a major role in mucosal defense by regulating the amount of blood entering mucosal circulation. Endogenous and exogenous NO protects the gastric mucosa against injury by ethanol and endothelin-1, while inhibition of NOS (resulting in reduced NO generation) increases gastric mucosal injury [3,5].

Endothelial cells of microvessels are the major targets for injury by various noxious factors such as ethanol, nonsteroidal anti-inflammatory drugs (NSAIDs), ischemia–reperfusion, and free radicals. Gastric mucosa exposed to ulcerogenic and necrotizing agents such as aspirin, indomethacin, and other nonselective drugs (NSAIDs), bile acids, alcohol, ischemia, or corrosive agents develops characteristic morphologic, ultrastructural, and functional changes reflecting injury [3,4,11,12]. Damage to the microvascular endothelium leads to microvascular stasis, cessation of oxygen delivery, and transport of nutrients [3,4,8]. Microvascular damage occurs early during mucosal injury, precedes necrosis of glandular cells, and adds an ischemic component to the direct toxic injury of the cells [11].

Our endoscopic, histologic, and ultrastructural studies demonstrated that, after intragastric administration of ~50% ethanol (a concentration comparable to that in hard liquors), endothelial capillary injury occurs as early as 5 min (Fig. 2B) and leads to thrombi formation and microvascular stasis (Fig. 2C), resulting in ischemia and hypoxia, which in turn lead to local necrosis in the form of erosions [11]. Our study also demonstrated that in human gastric mucosa endothelial cells are also the major targets of prostaglandin-induced protection against alcohol-induced injury [12].

Arteriovenous (AV) shunting blood flow is another potential mechanism of mucosal injury. Kitajima's group demonstrated using intravital microscopy that AV blood shunting takes place in the gastric mucosa after thermal injury [13]. Such blood shunting can cause mucosal hypoperfusion resulting in hypoxia, which may contribute to erosion formation [13].

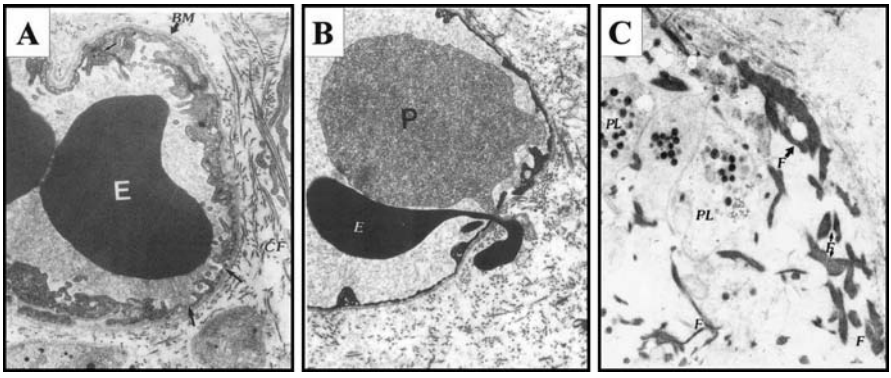

Fig. 2A–C. Transmission electron micrographs of human gastric mucosal microvessels. **A** The structure of normal capillary vessel in the mid mucosa. The capillary wall and endothelial cell cytoplasm is normal with a characteristic fenestration (*arrows*). *BM*, basement membrane; *E*, erythrocytes in the capillary lumen; *J*, junction between two neighboring endothelial cells; *CF*, collagen fibers (×17 400). **B** Electron micrograph of human gastric mucosa 5 min after intragastric alcohol administration. The continuity of the capillary wall is broken and erythrocyte (*E*) together with coagulated plasma (*P*) are leaking into lamina propria (×17 400). **C** Transmission electron micrograph of the gastric mucosa 15 min after alcohol administration. Part of a necrotic capillary wall is shown with aggregated platelets (*PL*) and deposited fibrin (*F*) (×21 450). Reprinted, with permission, from our previous papers [11,12]

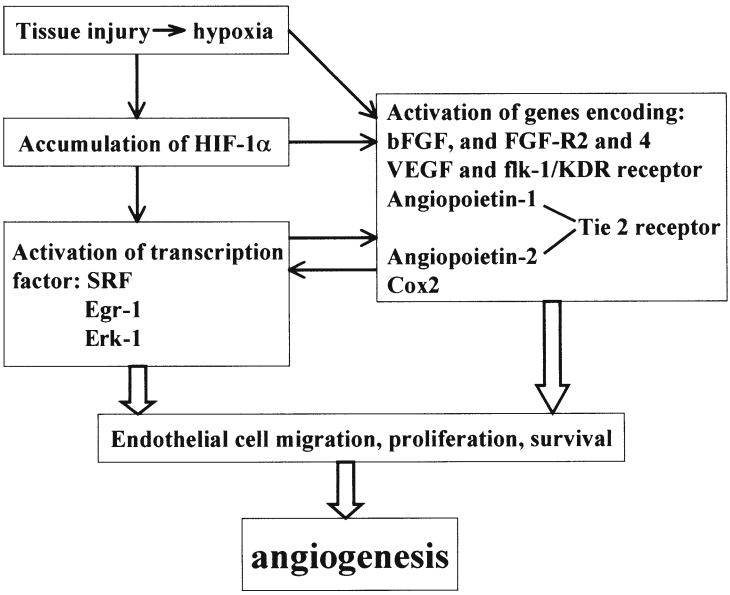

FIG. 3. Diagrammatic representation of molecular events leading to activation of angiogenesis. *HIF-1α*, hypoxia inducible factor-1α; *SRF*, serum response factor; *Erk*, extracellular regulated protein kinase; *Egr*, early growth response; *VEGF*, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; *KDR*, kinase domain receptor; *Cox*, cyclooxygenase

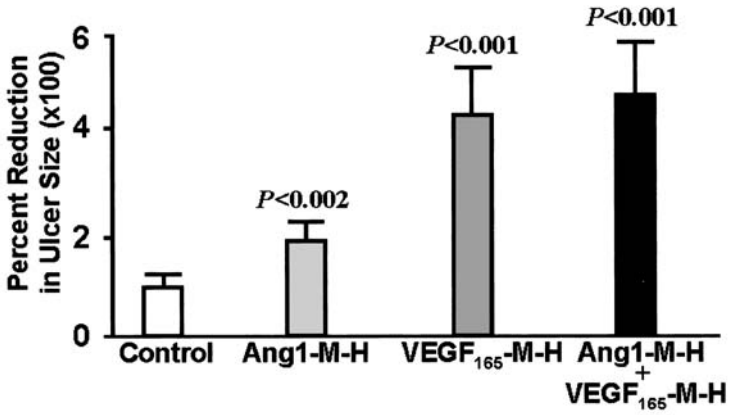

FIG. 4. Gene therapy with angiogenic growth factor cDNAs encoding vascular endothelial growth factor (*VEGF*) and angiopoietin-1 (*Ang1*) accelerates gastric ulcer healing: quantitative data for ulcer size reduction. Percent reduction in ulcer size was measured at 14 days after ulcer induction and injection of either control plasmid (set to 100%), Ang1–M-H, $VEGF_{165}$–M-H, or the combination of Ang1–M-H+$VEGF_{165}$–M-H ($n = 18$ for each group). Reprinted, with permission, from our previous paper [35]

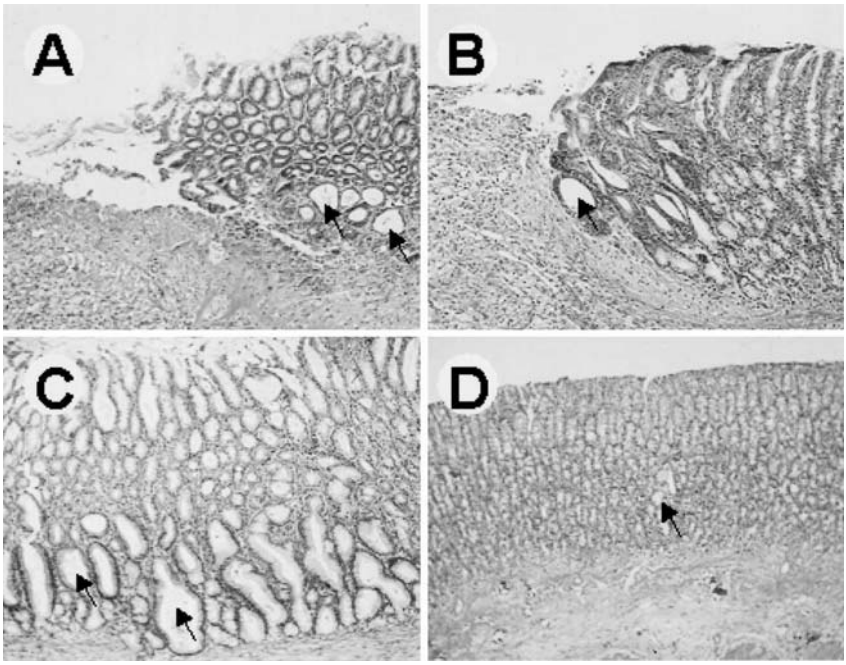

Fig. 5A–D. Gene therapy with VEGF and Ang1 improves mucosal healing assessed histologically. Histologic illustration of ulcer healing in representative groups at 14 days. Single injection of plasmids expressing $VEGF_{165}$ and Ang1 accelerates ulcer healing and scar formation. **A** In the control group (empty vector), a large ulcer is present with a moderate amount of granulation tissue. In the basal portion of the ulcer margin mucosa, several dilated glands are present. **B** Representative ulcer from the Ang1-treated group. Granulation tissue is more developed, compared to the control group, reaching about the height of the ulcer margin. Several tubes are budding into the granulation tissue (*arrow*). **C** In the VEGF-treated group 33% of the ulcers were completely healed. Mucosal scar contains several dilated gastric glands (*arrows*), but restoration of the mucosal architecture in the scar, even if imperfect, was accomplished. **D** In the VEGF+Ang1-treated group, 44% of the ulcers were completely healed. Restoration of the architecture is almost complete except for a few slightly dilated glands (*arrow*). × 100. Reprinted, with permission, from our previous paper [35]

Angiogenesis in Acutely Injured Mucosa and its Regulation

Angiogenesis, formation of new capillary blood vessels, is a fundamental process essential for embryonic development, tissue growth and reparative processes such as wound healing [14–17]. Under these conditions, the resting phenotype of endothelial cells is changed to an angiogenic phenotype [15].

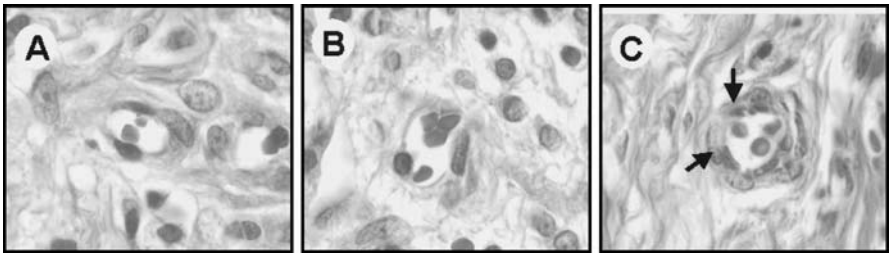

FIG. 6A–C. Gene therapy with VEGF plus Ang1 improves restoration of well-found and mature microvessels. Photomicrographs of granulation tissue stained by H&E. **A** Granulation tissue of ulcers injected with control plasmid; **B** plasmid-encoding $VEGF_{165}$; or **C** the combination of plasmids encoding both $VEGF_{165}$ and Ang1. The microvessels of the granulation tissue from the ulcers injected with either the control plasmid or the plasmid encoding $VEGF_{165}$ contained primarily only endothelial cells with very few pericytes. In contrast, the microvessels of the granulation tissue from the ulcers injected with the combined plasmids expressing both $VEGF_{165}$ and Ang1 appeared to be more well-formed and contained more pericytes (*arrows*). Pericytes were identified by their close adherence to the endothelium and characteristic shape of being flat toward the abluminal surface of the endothelium and convex toward the surrounding collagen. × 400. Reprinted, with permission, from our previous paper [35]

As a result, the microvascular endothelial cells from preserved microvessels at the wound edge migrate, proliferate and initiate re-establishment of a microvascular network through the process of angiogenesis [14,17]. Our studies demonstrated that 24 h after alcohol injury, at the edge of non-necrotic gastric mucosa, about 6%–8% of mucosal microvessels undergo angiogenesis: basement membrane dissolution, endothelial cell budding into the extravascular space, tube formation and, ultimately, reconstruction of a capillary network [14]. Angiogenesis in injured gastric mucosa is associated with a significant increase in the expression of Ki-ras mRNA and Ras protein and a significant 4–6-fold increase in vascular endothelial growth factor (VEGF) mRNA and protein levels that trigger gastric mucosal angiogenesis in response to ethanol injury [14]. The gastric mucosal angiogenesis is strongly stimulated by VEGF, basic fibroblast growth factor (bFGF), prostaglandin E_2, and prostacyclin [18].

Ulcer Formation: Importance of Vascular Lesions

Experimental studies have clearly demonstrated that vascular and microvascular changes are the earliest events in, as well as the basis for, the development of experimental gastric ulcers [19]. These vascular changes (thrombi, constriction) cause mucosal ischemia, free radical formation, and cessation

of nutrient delivery, all resulting in ischemic necrosis of the mucosa and muscularis mucosae [19].

Ulcer Healing: Reconstruction of the Microvessels Through Angiogenesis in Granulation Tissue

Granulation tissue develops at the ulcer base within 48–72 h after ulceration [19]. It consists of proliferating connective tissue cells, namely, macrophages, fibroblasts, and proliferating endothelial cells, which form microvessels through the process of angiogenesis [19]. Granulation tissue is important for the ulcer-healing process because it supplies microvessels and connective tissue cells for restoration of the microvasculature within the ulcer scar [19]. The growth of granulation tissue and generation of new microvessels through angiogenesis is stimulated by bFGF, VEGF, platelet-derived growth factor (PDGF), angiopoietins (Ang1 and Ang2), ephrins, leptin, cyr61, prostaglandins, and cytokines, including interleukin-1 and tumor necrosis factor-α [18,20].

Vascular endothelial growth factor is a potent angiogenic growth factor and is an endothelial cell-specific mitogen because its receptors, flt-1 and flk-1, are restricted predominantly to endothelial cells, although other cells such as endothelial cell precursors and some cancer cells also express these receptors (reviewed in [18,20]). Vascular endothelial growth factor (originally identified as a vascular permeability factor) is produced by vascular smooth muscle cells, tumor cells, and endothelial cells [20], and has been implicated in stimulation of the normal physiological angiogenesis involved in wound and ulcer healing [14,16,20]. Activation of the flk-1 receptor tyrosine kinase in response to VEGF is necessary for VEGF-induced angiogenic sprouting, which is facilitated by activation of matrix metalloproteinases that degrade the extracellular matrix [20]. Vascular endothelial growth factor, bFGF, and Ang1 stimulate endothelial cell proliferation and migration that result in microvascular network assembly [15,16,20]. Vascular endothelial growth factor production is stimulated by PDGF, transforming growth factor β1, and bFGF, as well as by cytokines, NO, E-series prostaglandins (PGs), and hypoxia [18,20]. Hypoxia stimulates VEGF production via the hypoxia-inducible transcription factor (HIF)-1, which accumulates in response to hypoxia and activates transcription from a hypoxia response element (HRE) binding site in the VEGF promoter [18–20]. Vascular endothelial growth factor is also regulated post-transcriptionally under hypoxia [20–27]. For example, it has been shown that VEGF mRNA is stabilized under hypoxia by binding of the Elav-like protein, HuR, to specific sequences within the 3′-untranslated region, thereby increasing the VEGF mRNA half-life > 2-fold and compounding the increases in VEGF mRNA levels produced by HIF-induced transcription under hypoxia [27].

The angiogenic peptides Ang1 and Ang2, and the endothelial specific receptors Tie1 and Tie2, are involved in angiogenic processes occurring subsequent to the actions of VEGF and its receptors [14,15,22,29–31]. It is known that Ang1 acts to stabilize vessels and promotes vessel maturation and, therefore, Ang1 *does* function subsequent to the actions of VEGF. However, Ang2 functions to inhibit the Ang1–Tie2 interaction, thereby destabilizing vessels and allowing VEGF access to receptors on endothelial cells. Ang2 probably does not function subsequent to VEGF but rather promotes VEGF function and attracts recruitment of vascular smooth muscle cells [30,31].

We have demonstrated that esophageal ulceration induces HIF-1α protein expression and VEGF gene activation reflected by increased VEGF mRNA (240%) and VEGF protein (310%) levels. HIF-1α protein was expressed in microvessels bordering necrosis where it co-localized with VEGF [28].

NSAIDs Interfere with Angiogenesis

Endothelial cells of the microvessels are also major targets of NSAID-induced injury to gastrointestinal mucosa [32]. Moreover, NSAIDs inhibit angiogenesis (reviewed in [18,33,34]). Based on the experimental data and the literature, the mechanisms by which NSAIDs inhibit angiogenesis appear to be multifactorial and likely include local changes in angiogenic growth factor expression, alteration in key regulators and mediators of VEG, increased endothelial cell apoptosis, inhibition of endothelial cell migration, recruitment of inflammatory cells and platelets, and/or thromboxane A_2-mediated effects (reviewed in [18]). Some of these mechanisms include: inhibition of mitogen-activated, or extracellular regulated protein kinase (Erk) activity; suppression of cell cycle proteins; inhibition of early growth response (Egr-1) gene activation; interference with HIF-1 and VEGF gene activation; increased production of the angiogenesis inhibitor, endostatin; inhibition of endothelial cell proliferation, migration, and spreading; and the induction of endothelial apoptosis (reviewed in [18,34]).

Gene Therapy with Angiogenic Growth Factors

We have previously reported that endogenous VEGF is required for unimpeded healing of mucosal injury [13]. As a follow-up of this work, we performed studies to determine whether a single local injection of VEGF and Ang1 cDNA (gene therapy), with limited duration of target gene expression, affects healing of experimental gastric ulcers [35]. This study demonstrated that a single injection of plasmid DNA-encoding VEGF increases angiogenesis in granulation tissue by 7 days after injection; and that this increase

strongly correlates with a reduction in ulcer size by more than 400% at 14 days compared with controls injected with nonexpressing plasmid [35]. Simultaneous injection of both the VEGF and Ang1 expression plasmids did not significantly decrease ulcer size beyond that resulting from injection of the VEGF expression plasmid alone (possibly indicating that the angiogenic response to VEGF has reached a maximum). However, with the combined treatment of VEGF+Ang1, the microvessels in the granulation tissue contained more pericytes, were better developed, and the structural restoration of gastric mucosa within the scar was more complete [35]. A neutralizing anti-VEGF antibody abolished VEGF gene therapy-induced accelerated ulcer healing, indicating an essential role for VEGF and enhanced angiogenesis in ulcer healing [35]. Gene therapy with cDNA encoding VEGF also significantly enhances angiogenesis and accelerates healing of experimental esophageal ulcers, indicating essential roles of VEGF and angiogenesis in esophageal ulcer healing [28].

Pharmacological Stimulation of Angiogenesis for Gastric Mucosal Injury and Ulcer Healing: Present and the Future

Experimental studies demonstrated that therapy with bFGF, PDGF, and/or VEGF accelerates angiogenesis and accelerates healing of duodenal ulcers in rats [36,37]. Our studies (described above) have demonstrated efficacy of gene therapy with angiogenic growth factors for the healing of experimental gastric and esophageal ulcers [28,35]. However, application of the above therapies for human gastrointestinal ulcers will require at least 5–7 years. While application of angiogenic growth factors and gene therapy for angiogenesis-based healing of human gastrointestinal ulcers still requires clinical trials, a currently available drug, rebamipide, has been shown to activate genes encoding angiogenic growth factors (e.g. VEGF > 7-fold, HB-EGF > 5-fold), and cyclooxygenase > 9.3-fold in gastric mucosal cells and, in addition, to directly stimulate angiogenesis [38,39].

Acknowledgments. This study was supported by the Medical Research Service of the Department of Veterans Affairs, Merit Reviews (A.S.T. and M.K.J) and Research Enhancement Award Program (A.S.T.).

References

1. Guth PH, Leung FW (1987) Physiology of the gastric circulation. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Raven, New York, pp 1031–1054

2. Crissinger KD, Granger N (1995) Gastrointestinal blood flow. In: Yamada T (ed) Textbook of gastroenterology, 2nd edn. Lippincott, Philadelphia, pp 518–545
3. Tarnawski A (1998) Cellular and molecular mechanisms of mucosal defense and repair. In: Bioregulation and its disorders in gastrointestinal tract. Blackwell Science, Tokyo, pp 3–17
4. Tarnawski A, Jones MK, Baatar D, et al. (2002) Role of angiogenesis and angiogenic growth factor in mucosal repair and ulcer healing. In: Cho CH, Wang JY, Wang JH (eds) Karger gastrointestinal mucosal repair and experimental therapeutics, vol. 25. pp 101–116
5. Kawano S, Tsuji S (2000) Role of mucosal blood flow: a conceptual review in gastric mucosal injury and protection. J Gastroenterol Hepatol 15:D1–D6
6. Ichikawa Y, Tarnawski A, Sarfeh IJ, et al (1994) Distorted microangio-architecture and impaired angiogenesis in gastric mucosa of portal hypertensive rat. Gastroenterology, 106:706–708
7. Holzer P (1991) Sensory nerves and neuropeptides in gastroenterology. From basic science to clinical perspectives. Adv Exp Med Biol 298:3–17
8. Tarnawski A, Sarfeh IJ, Lu S-Y, et al (1993) Quality of ulcer healing: evidence for impaired restoration of sensory CGRP nerves in the scar of experimental gastric ulcer. Eur J Gastroenterol Hepatol 5(3):S81–S85
9. Peskar BM, Plate S, Stroff T (1997) Gastroprotective effects of gut peptides: Role of afferent neurons, calcitonin gene-related peptide and nitric oxide. In: Cheli R, Iaquinto G, Szabo S (eds) Gastroduodenal mucosal damage: problems of protection and healing. Medserve, Milan, pp 70–83
10. Arai K, Ohno T, Saeki T, et al (2003) Endogenous prostaglandin 12 regulates the neural emergency system through release of calcitonin gene related peptide. Gut 52(9): 1242–1249
11. Tarnawski A, Stachura J, Gergely H, et al (1988) Microvascular endothelium: A major target for alcohol injury of the human gastric mucosa: histochemical and ultrastructural study. J Clin Gastroenterol 10(suppl 1):S53
12. Tarnawski A, Stachura J, Hollander D, et al (1988) Cellular aspects of alcohol-induced injury and prostaglandin protection of the human gastric mucosa. J Clin Gastroenterol 10(suppl 1):S35–S45
13. Yoshida M, Wakabayashi G, Ishikawa H, et al (2002) Arteriovenous shunting blood flow is intravitally observed in the stomach after thermal injury in rats. Keio J Med 51(4):193–200
14. Jones MK, Itani RM, Wang H, et al (1999) Activation of VEGF and Ras genes in gastric mucosa during angiogenic response to ethanol injury. Am J Physiol 276:G1345–G1355
15. Folkman J, D'Amore PA (1996) Blood vessel formation: what is its molecular basis? Cell 87:1153–1155
16. Carmeliet P (2003) Angiogenesis in health and disease. Nat Med 9:653–693
17. Tarnawski A, Hollander D, Stachura J, et al (1991) Role of angiogenesis in healing of experimental gastric ulcer. In: Halter F, Garnet A, Tytat GNJ (eds) Mechanisms of peptic ulcer healing. Kluwer, Dordrecht, pp 165–171
18. Tarnawski AS, Jones MK (2003) Inhibition of angiogenesis by NSAIDs: molecular mechanisms and clinical implications. J Mol Med 81:627–636
19. Tarnawski A, Hollander D, Stachura J, et al (1999) Vascular and microvascular changes—key factors in the development of acetic acid-induced gastric ulcers in rats. J Clin Gastroenterol 22(suppl 1):S148–S157

20. Ferrara N, Gerber H-P, LeCourter J (2003) The biology of VEGF and its receptor. Nat Med 9:669–676
21. Pugh CW, Ratcliffe PJ (2003) Regulation of angiogenesis by hypoxia: role of the HIF system. Nat Med 9:677–684
22. Risau W (1997) Mechanisms of angiogenesis. Nature 386:671–673
23. Levy AP, Levy NS, Goldberg MA (1996) Post-transcriptional regulation of vascular endothelial cell growth factor by hypoxia. J Biol Chem 271:2746–2753
24. Gerber HJP, Condorelli F, Park J, Ferrara N (1997) Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes: Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. J Biol Chem 272:23659–23667
25. Kelly BD, Hackett SF, Hirota K, et al (2003) Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor-1. Circ Res 93(11):1074–1081
26. Yamakawa M, Liu LX, Date T, et al (2003) Hypoxia-inducible factor-1 mediates activation of cultured vascular endothelial cells by inducing multiple angiogenic factors. Circ Res 93(7):664–673
27. Levy NS, Chung S, Furneaux H, et al (1998) Hypoxic stabilization of vascular endothelial growth factor mRNA by the RNA-binding protein HuR. J Biol Chem 13; 273(11):6417–6423
28. Baatar D, Jones MK, Tasugawa K et al (2002) Esophageal ulceration triggers expression of hypoxia-inducible factor-1α and activates vascular endothelial growth factor gene. Am J Pathol 161:1449–1457
29. Davis S, Aldrich TH, Jones PF, et al (1996) Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 87:1161–1169
30. Maisonpierre PC, Suri C, Jones PF, et al (1997) Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277:55–60
31. Iivanainen E, Nelimarkka L, Elenius V, et al (2003) Angiopoietin-regulated recruitment of vascular smooth muscle cells by endothelial-derived heparin binding EGF-like growth factor. FASEB J 17(12):1609–1621
32. Tarnawski A, Stachura J, Gregeley H, et al (1990) Gastric microvascular endothelium: a major target for aspirin-induced injury and arachidonic acid protection. An ultrastructural analysis in the rat. Eur J Clin Invest 20(4):432–440
33. Tarnawski A, Stachura J, Douglass TG, et al (1991) Indomethacin impairs quality of experimental gastric ulcer healing: A quality histologic and ultrastructural analysis. In: Garner A, O'Brien PE (eds) Mechanisms of injury, protection and repair of the upper gastrointestinal tract. Wiley, Chichester, pp 521–531
34. Jones MK, Wang H, Levin E, et al (1999) Inhibition of angiogenesis by NSAIDs. Insight into the mechanisms and implications for cancer growth and ulcer healing. Nat Med 5:1418–1423
35. Jones MK, Kawanaka H, Baatar D, et al (2001) Gene therapy for gastric ulcers. Single local injection of VEGF and angiopoietin-1 DNAs dramatically accelerates gastric ulcers healing and improves quality of scar. Gastroenterology 121:1040–1047
36. Folkman J, Szabo S, Stovroff M, et al (1991) Duodenal ulcer. Discovery of a new mechanisms and development of angiogenic therapy that accelerates healing. Ann Surg 214:414–427
37. Szabo S, Folkman J, Vincze A, et al (1997) Modulation of vascular factors by VEGF/VPF is sufficient for chronic ulcer healing and acute gastroprotection (abstract). Gastroenterology 122:A303

38. Tarnawski A, Chai J, Chiou S-K, et al (2003) Rebamipide—a novel ulcer healing drug—strongly stimulates angiogenesis by activating genes inducing angiogenic growth factors, and Cox2. A key to its ulcer healing action? In: Pasteur Institute Euroconference Paris, Proceedings A-38
39. Tarnawski AS, Chai J, Pai R, et al (2004) Rebamipide activates genes encoding angiogenic growth factors and Cox2 and stimulates angiogenesis: a key to its ulcer healing action? Dig Dis Sci 49(2):202–209
40. Yoshida M, Wakabayashi G, Ishikawa H, et al (2002) Arteriovenous shunting blood flow is intravitally observed in the stomach after thermal injury in rats. Keio J Med 51(4):193–200