An imbalance between VEGF and endostatin underlies impaired angiogenesis in gastric mucosa of aging rats

Amrita Ahluwalia,1 Michael K. Jones,1,3 Xiaoming Deng,2,4 Zsusanna Sandor,1,3 Sandor Szabo,2,4 and Andrzej S. Tarnawski1,3

1Medical Service, Veterans Affairs Long Beach Healthcare System, University of California, Irvine, California, and 2Diagnostic and Molecular Medicine, Veterans Affairs Long Beach Healthcare System, University of California, Irvine, California; 3Department of Medicine, University of California, Irvine, California; and 4Department of Pathology, University of California, Irvine, California

Submitted 24 April 2013; accepted in final form 18 June 2013

Ahluwalia A, Jones MK, Deng X, Sandor Z, Szabo S, Tarnawski AS. An imbalance between VEGF and endostatin underlies impaired angiogenesis in gastric mucosa of aging rats. Am J Physiol Gastrointest Liver Physiol 305: G325–G332, 2013. First published June 20, 2013; doi:10.1152/ajpgi.00127.2013.—Gastric mucosa of aging individuals exhibits increased susceptibility to injury and delayed healing. Our previous studies in young rats showed that healing of mucosal injury depends on and is critically dependent on VEGF and angiogenesis. Since angiogenesis in aging gastric mucosa has not been examined before, in this study we examined the extent to which angiogenesis is impaired in gastric mucosa of aging vs. young rats and determined the underlying mechanisms with a focus on mucosal expression of VEGF (proangiogenic factor) and endostatin (antiangiogenic factor). Aging rats had significantly impaired gastric angiogenesis by ~12-fold, 5-fold, 4-fold, and 3-fold, respectively (vs. young rats; all P < 0.001) at 24, 48, 72, and 120 h following ethanol-induced gastric injury and reduced delayed healing of mucosal erosions. In gastric mucosa of aging (vs. young) rats at baseline, VEGF expression was significantly reduced, whereas endostatin levels were significantly increased (P < 0.05 and P < 0.01, respectively). In contrast to young rats, gastric mucosal VEGF levels did not increase following ethanol-induced injury in aging rats. MMP-9 enzyme activity was significantly higher in gastric mucosa of aging vs. young rats both at baseline (2.7-fold) and 24 h (3.8-fold) after ethanol injury (both P < 0.001). Since endostatin is generated from collagen XVIII by MMP-9, this finding can explain the mechanism of increased endostatin expression in aging gastric mucosa. The above findings demonstrate that reduced VEGF and increased endostatin result in the impaired angiogenesis and delayed injury healing in gastric mucosa of aging rats.

aging; gastric mucosa; angiogenesis; mucosal healing; VEGF; endostatin

GASTRIC MUCOSA OF AGING humans and animals (referred to in the present study as “aging gastric mucosa”) exhibits increased susceptibility to injury (17–19, 29, 32, 36, 37, 50) and delayed healing (19, 20, 33, 41, 51, 52). Experimental studies showed that gastric mucosa of aging rats has increased susceptibility to injury by a variety of damaging agents such as ethanol, aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), hypertonic saline, bile acids, and restraint-induced stress (17, 19, 31–34, 36, 46, 50). Human studies fully confirmed experimental data and demonstrated that patients over 65 yr of age have significantly reduced gastric generation of prostaglandins (7, 16) and increased gastric mucosal injury by aspirin and other NSAIDs (28, 29, 32, 38). Clinical studies from the Veterans Affairs Long Beach Healthcare System (VALBHS) clearly demonstrated that NSAIDs, especially in combination with alcohol, are the most frequent cause of erosive gastritis and upper gastrointestinal bleeding (30). Older patients taking low-dose aspirin or NSAIDs also have a much greater absolute risk of gastrointestinal complications than younger patients. The risk of ulcer complications in subjects younger than 50 yr was less than 0.5% whereas it was ~4% in patients 70–79 yr of age (18, 38, 41). Therefore, aging gastropathy is an important issue, especially since it is estimated that by the year 2020 more than 16% of US population will be over 65 yr of age and that nearly 20 million will be 85 yr or older (18). Our previous experimental study identified some of the mechanisms responsible for the increased susceptibility of aging gastric mucosa to injury, including hypoxia, increased expression of PTEN and proapoptotic caspase-3 and -9, and reduced expression of survivin (50). That study also showed that some of these mechanisms have direct human relevance (50). Although the mechanisms underlying increased susceptibility of aging gastric mucosa to injury were identified, the targets and the mechanisms responsible for the impaired healing of aging gastric mucosa remain incompletely defined.

Healing of gastric injury requires reestablishment of the mucosal microvasculature through angiogenesis (new blood vessel formation from preexisting vessels) that is sine qua non for the delivery of oxygen and nutrients to the healing site (5, 11–13, 21–23). Previous studies suggest that healing of injured gastric mucosa is delayed in aging vs. young rat and mouse models (19, 20, 33, 36, 41, 51, 52). One previous study postulated that angiogenesis is impaired in the gastric mucosa of aging rats (25). However, this conjecture was based solely on the assessment of VEGF messenger RNA (mRNA) whereas angiogenesis itself was not examined (25). Thus delineation of the regulatory mechanisms underlying impaired angiogenesis and delayed injury healing during aging is lacking, and angiogenesis in aging gastric mucosa has not been examined before.

In general, angiogenesis is regulated by a balance between proangiogenic growth factors including VEGF (VEGF-A; the most potent and endothelial-specific angiogenic growth factor) (5, 11–13, 21–23) and antiangiogenic factors such as endostatin and angiostatin (4, 43, 48). VEGF is produced by endothelial cells, smooth muscle cells, and myo/fibroblasts (11, 12). VEGF is upregulated in wounded tissues and it initiates and regulates angiogenesis (5, 22, 23). Our previous study demon-
strated that, in injured gastric mucosa of young rats, VEGF gene expression is activated, and that VEGF plays a critical functional role in promoting angiogenesis and acute gastric injury healing (22). Treatment with VEGF or gene therapy with VEGF cDNAs accelerates the healing of gastric and duodenal ulcers in young rats (8, 23). Decreased levels of proangiogenic growth factors can lead to reduced angiogenic capacity in the cardiac vasculature, which has been shown in aging individuals (42, 44, 45).

Endostatin and angiostatin are endogenous inhibitors of angiogenesis (4, 43, 48). They inhibit endothelial cell proliferation and migration in vitro and induce apoptosis in some endothelial cells (10). Endostatin and angiostatin are generated by cleavage of collagen XVIII and plasminogen, respectively, by matrix metalloproteinases (MMPs) (4, 39). However, the expression of neither endostatin nor angiostatin in aging and/or young gastric mucosa has been examined before.

Our overall hypothesis was that aging gastric mucosa has impaired angiogenesis and healing because of an imbalance between proangiogenic and antiangiogenic factors. Specifically, we hypothesized that aging gastric mucosa exhibits decreased levels of VEGF and a concomitant increase in the level(s) of endostatin and/or angiostatin (potent antiangiogenic factors). We examined this hypothesis in vivo in young and aging rat gastric mucosae.

MATERIALS AND METHODS

All experimental studies in young and aging rats were approved by the Subcommittee for Animal Studies (Institutional Animal Care and Usage Committee) of the VALBHS. We used Helicobacter pylori and virus-free male Fisher F-344 rats: 3 mo of age (young; n = 30) and 24 mo of age (aging; n = 30), purchased from the National Institute on Aging. Studies were performed in rats fasted for 18 h. The selection of male rats for this study was made to assure comparability with our previous studies and studies by other groups that were performed mainly in male rats (22, 25, 49, 50).

Ethanol injury in rats. Young and aging rats were given either saline (control) or 50% ethanol (8 ml/kg body wt) by gavage as described previously (22) and were euthanized at 0, 24, 48, 72, and 120 h after ethanol treatment. The stomachs were excised, opened longitudinally along the greater curvature, rinsed, and photographed. The areas of macroscopic hemorrhagic necrosis predominantly localized to corpus mucosa were measured by use of a computerized video analysis system (MetaMorph 7.0, Molecular Devices, Downingtown, PA). Macroscopic mucosal damage was expressed as a percentage of total mucosal area, as described in our previous study (49). Standardized cross sections of gastric mucosa from the corpus region were used for quantitative histology, immunohistochemistry, assessment of angiogenesis, and healing of injury. Gastric mucosal scrapings obtained from the entire mucosa between erosions were used for molecular biology studies.

Angiogenesis. We used our previously established model of in vivo angiogenesis in gastric mucosa in young rats (22). This model of angiogenesis utilizes ethanol-induced gastric mucosal injury in rats. Ethanol itself, unlike NSAIDs, does not directly inhibit angiogenesis and is therefore the preferred model for the assessment of angiogenesis in gastric mucosa in response to acute injury. Assessment and quantification of in vivo angiogenesis in gastric mucosa of young and aging rats was performed similar to our previous study (22). Specimens of normal gastric mucosa or injured gastric mucosa (gastric erosions) were fixed in 4% paraformaldehyde for 4 h, subsequently transferred to 0.5 M sucrose in phosphate-buffered saline for 24 h, and stored at −80°C until cutting. Frozen mucosal sections were cut and immunostained for factor VIII-related antigen (factor VIII RA) by using a specific antibody (1:100, Dako, Carpinteria, CA) followed by FITC-conjugated secondary antibody to visualize endothelial cells lining microvessels (22, 23). Immunofluorescence was evaluated via a Nikon Optiphot microscope. Angiogenesis was quantified by counting (under ×400 magnification) the total number of microvessels and the number of microvessels exhibiting sprouting endothelial tubes reflecting in vivo angiogenesis in 20 randomly selected fields of mucosal erosions as described in our previous study (22). Angiogenesis was expressed as the percent of mucosal microvessels (mean ± SD) demonstrating sprouting endothelial tubes per total number of microvessels in section areas of the mucosa bordering necrosis similar to our previous study (22).

Gastric mucosal healing. Gastric erosion healing at 48, 72, and 120 h after ethanol was assessed and quantified histologically on hematoxylin and eosin-stained sections by measuring the reduction in size of deep erosions (penetrating more than 0.2 mm of the mucosal thickness) vs. erosion size at 24 h, similar to our previous study (49).

Reepithelialization. Restitution of the continuity of the gastric surface epithelial layer outside deep erosions was quantified by measuring the length of the mucosa devoid of the surface epithelium and is expressed as a percentage of total mucosal length for each mucosal section studied similar to our previous study (49).

Transmission electron microscopy. Standardized gastric wall specimens containing macroscopic erosions and normal mucosal tissues were fixed in glutaraldehyde and processed for transmission electron microscopy (TEM) as described in our previous study (49) and examined under a Phillips 300 transmission electron microscope operated at 60 kV.

Reverse-transcription real-time quantitative PCR. Total cellular RNA was isolated from snap-frozen gastric mucosal scrapings obtained from the entire mucosa between erosions by use of TRIzol reagent (Invitrogen, Carlsbad, CA). Total RNA (1 μg) was treated with deoxyribonuclease I and reverse transcribed by using the GeneAmp RNA-PCR kit (Applied Biosystems, Foster City, CA) as described in our previous studies (1, 50). The mRNA levels of VEGF and glyceraldehyde-3-phosphate dehydrogenase were quantified by real-time PCR using prevalidated QuantiTect assays (Qiagen, Valencia, CA) and the iCycler real-time PCR detection system (Bio-Rad, Hercules, CA). Relative mRNA levels were calculated by the 2-ΔΔCt method and glyceraldehyde-3-phosphate dehydrogenase was used as a reference.

Western blot analysis. Rat gastric mucosal scrapings obtained from the entire mucosa between erosions were homogenized with a Polytron homogenizer in lysis buffer and processed routinely for Western blotting as described in our previous studies (24, 50). Expression of VEGF, endostatin, and angiostatin was determined by Western blotting using respective specific antibodies and normalized by using β-actin as an internal control reference. The primary antibodies used were against VEGF (1:250, Santa Cruz Biotechnology, Santa Cruz, CA), endostatin (1:200, Lab Vision, Fremont, CA), angiostatin (1:200, Novus Biologicals, Littleton, CO), and β-actin (1:1,000, Sigma, St. Louis, MO).

Substrate gel electrophoresis (zymography). MMP-9 activity was measured by zymography as described in our previous study (9). Briefly, proteins isolated from mucosal scrapings were resolved on a 10% SDS-PAGE gel containing 1 mg/ml gelatin under nonreducing conditions. MMP-9/NGAL complex (Calbiochem, San Diego, CA) was used as a control. The gels were washed in 2.5% Triton X-100 for 30 min and then incubated overnight at 37°C in substrate buffer (50 mM Tris pH 7.5, 0.2 M NaCl, 5 mM CaCl2, and 0.02% Brij 35). Gels were stained with 0.25% Cooomassie blue and destained for 1 h. Gelatinolytic activity was detected as clear bands on a blue background.

Statistical analysis. Results are expressed as means ± SD from at least five animals. Student’s t-test was used to determine statistical significance, and a P value less than 0.05 was considered statistically significant. Comparisons of data between multiple groups were made by analysis of variance.
RESULTS

Angiogenesis is impaired in aging gastric mucosa. We examined in vivo angiogenesis in gastric mucosa of young and aging rats in response to ethanol-induced injury using immunofluorescence staining for the endothelial marker factor VIII RA, similar to our previous study (22). With this technique, endothelial cells stain green and are easily visualized under an epifluorescence microscope. In normal (uninjured) gastric mucosa, factor VIII RA showed a regular pattern of distribution in endothelial cells lining the microvessels (Fig. 1A) and sprouting endothelial tubes were absent. In ethanol-injured gastric mucosa of young rats at 24 h, the areas bordering necrosis showed numerous endothelial tubes (reflecting in vivo angiogenesis) that had sprouted from existing preserved microvessels toward the erosion (Fig. 1B). In contrast, sprouting endothelial tubes in injured gastric mucosa of aging rats at 24 h were virtually absent (P < 0.001 vs. young) demonstrating that angiogenesis is dramatically impaired in gastric mucosa of aging vs. young rats (Fig. 1C). Quantitative evaluation showed that angiogenesis in gastric mucosa of young rats was significantly higher vs. aging rats 24 h following ethanol-induced injury by 12.1-fold (P < 0.001) (Fig. 1D). Furthermore, angiogenesis in gastric mucosa of young rats following ethanol-induced injury was significantly and time dependently increased (all P < 0.001) (Fig. 1D). In contrast, angiogenesis in gastric mucosa of aging rats was significantly reduced vs. young rats at each study time after ethanol-induced injury (Fig. 1D).

We next examined the ultrastructural features of angiogenesis with focus on capillary basement membrane continuity, endothelial budding into the extravascular space, endothelial tube formation, and capillary vessel reconstruction using TEM. As shown in Fig. 2, the initial phase of angiogenesis within 24 h following ethanol-induced injury to young gastric mucosa was characterized by a focal dissolution of basement membrane and endothelial cell budding and migration into the extravascular space. Following this initial phase of angiogenesis, 48–72 h after injury, emigrated endothelial cells formed endothelial tubes that sprouted toward the erosion, migrated, developed lumina, and ultimately restored the capillary vessels (Fig. 2). In contrast, TEM studies of ethanol-injured aging gastric mucosa revealed no or minimal features of angiogenesis: endothelial budding and tube formation (data not shown).

Healing of mucosal erosions is delayed in aging gastric mucosa. We examined the healing rate of ethanol-induced deep mucosal erosions in young and aging gastric mucosa at 48–120 h following injury induction. Gastric erosion size at each study point was compared with that at 24 h following injury induction. As shown in Fig. 3, aging rats had significantly decreased gastric erosion healing vs. young rats at each study time, 48–120 h following injury induction by 4.5-fold, 3.8-fold, and 2.9-fold, respectively (all P < 0.001). These results further showed that healing of gastric mucosal injury closely correlated with angiogenesis (correlation coefficient r = 0.992; P < 0.001).

Fig. 1. Angiogenesis in gastric mucosa of young and aging rats in response to ethanol-induced injury. A–C: photomicrographs of gastric mucosa-immunofluorescence staining for factor VIII-related antigen. A: in normal mucosa (uninjured control), immunostaining shows a regular pattern of distribution in endothelial cells lining gastric mucosal microvessels. Magnification ×200. B: in the gastric mucosa bordering necrosis of young rats 24 h after ethanol administration, numerous endothelial tubes, reflecting angiogenesis, are present (arrows) and sprout toward the erosion. Magnification ×500. C: in contrast, in injured gastric mucosa of aging rats sprouting endothelial tubes are virtually absent, reflecting impaired angiogenesis. Magnification ×500. D: quantitative analysis of angiogenesis in injured gastric mucosa of aging rats at 24, 48, 72, and 120 h following ethanol administration demonstrated significantly reduced angiogenesis vs. that of young rats (*P < 0.001). Values are means ± SD from 5 animals.
Restitution of the surface epithelium is decreased in aging gastric mucosa. We examined the restitution of surface epithelium at the erosion margin and between erosions in young and aging gastric mucosa 24 h following ethanol administration. As shown in Fig. 4, aging gastric mucosa had significantly decreased restitution of gastric surface epithelium by 7.7-fold \( (P < 0.001) \) vs. young gastric mucosa. In young rats, 96% of the surface epithelium continuity was restored vs. only 69% in aging gastric mucosa. Even in the areas of aging gastric mucosa with restored surface epithelium, the restitution was incomplete and was flat.

Reduced VEGF gene expression and decreased levels of VEGF protein in aging gastric mucosa. Since VEGF is a critical, rate-limiting factor for initiating and promoting angiogenesis and mucosal healing in gastric mucosa of young rats as shown in our previous studies (22, 23), we examined VEGF mRNA (Fig. 5A) and protein (Fig. 5B) expression in gastric mucosa of young and aging rats. As shown in Fig. 5, VEGF mRNA and protein expression levels in aging gastric mucosa were 2-fold and 1.3-fold lower, respectively \( (P < 0.05) \), vs. young gastric mucosa at baseline. Furthermore, following injury VEGF mRNA and protein expression levels did not increase in aging gastric mucosa whereas they were increased by 1.9-fold \( (P < 0.01) \) and 1.8-fold \( (P < 0.05) \), respectively, in gastric mucosa of young rats following injury (Fig. 5A and B).

Increased levels of endostatin protein in aging gastric mucosa. Since angiogenesis reflects a balance between proangiogenic and antiangiogenic factors, we examined the expression of the antiangiogenic proteins endostatin and angiostatin in aging and young gastric mucosa. Endostatin levels were 1.7-fold higher both at baseline and 24 h after ethanol-induced injury \( (P < 0.01) \) in aging (vs. young) gastric mucosa (Fig. 6A). Angiostatin
levels were not significantly different between gastric mucosa of aging and young rats (data not shown).

**Increased MMP-9 enzyme activity in aging gastric mucosa.** Since endostatin is generated from collagen XVIII by the enzymatic action of MMP-9, we also measured the activity of MMP-9 by zymography. MMP-9 enzyme activity was significantly higher in aging (vs. young) gastric mucosa by 2.7-fold ($P < 0.01$) at baseline and by 3.8-fold ($P < 0.001$) 24 h after ethanol-induced injury (Fig. 6B). This can explain the mechanism of increased endostatin expression.

**DISCUSSION**

This is the first study to directly demonstrate that angiogenesis in gastric mucosa of aging rats is significantly impaired and that the impairment in angiogenesis is associated with reduced mucosal expression of VEGF and increased mucosal expression of the antiangiogenic factor endostatin. Although a recent study did examine VEGF mRNA expression in gastric mucosa of aging rats at baseline, that study did not assess VEGF protein levels nor did it examine in vivo angiogenesis (25). Moreover, angiogenesis in gastric mucosa at baseline is negligible and is only activated and detectable in response to injury. Our study quantitatively examined in vivo angiogenesis in response to injury and mucosal healing in gastric mucosa of young and aging rats and demonstrated that the impaired angiogenesis in aging gastric mucosa following ethanol-induced injury was strongly associated with delayed healing of both deep erosions and surface epithelium.

Angiogenesis is essential for postnatal growth and reparative processes such as wound healing after tissue injury (5). Under these conditions angiogenesis is strictly regulated and limited to a relatively brief time period (days) that accommodates healing, after which angiogenesis is inhibited (5, 13, 21). This is in contrast to the unregulated and persistent angiogenesis

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**Fig. 4.** Restitution of the surface epithelium between erosions is decreased in aging gastric mucosa. A: in young gastric mucosa between erosions there is almost complete restitution of surface epithelium 24 h following ethanol administration. B: aging gastric mucosa at the erosion margin and between erosions shows incomplete and flat restitution of surface epithelium. C: quantitative analysis of the restitution of surface epithelium in young and aging gastric mucosa at 24 h following injury induction. The length of the gastric mucosa without restored gastric surface epithelium was measured and expressed as a % of the total length of the mucosal section. Aging gastric mucosa had significantly decreased restitution of gastric surface epithelium at the erosion margin and between erosions ($P < 0.001$). Values are means ± SD from 5 animals.

**Fig. 5.** VEGF mRNA and protein expression in gastric mucosa of aging and young rats. A: real-time RT-PCR determination of VEGF gene expression at baseline and 24 h after ethanol injury. The mRNA levels were determined by the $2^{-ΔΔCt}$ method and VEGF mRNA levels were normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA levels. B: Western blotting determination of VEGF protein levels at baseline and 24 h after ethanol injury. Values are means ± SD of 3 independent experiments. VEGF mRNA and protein levels in young gastric mucosa were significantly lower than in young gastric mucosa at baseline (†$P < 0.05$) and did not increase following ethanol injury. In contrast, both VEGF mRNA and protein levels in young gastric mucosa significantly increased following injury (***$P < 0.01$; *$P < 0.05$, respectively).
Fig. 6. Effect of ethanol injury on endostatin expression and the activity of matrix metalloproteinase (MMP-9, the enzyme that generates endostatin) in gastric mucosa of aging and young rats. A: Western blotting determination of endostatin levels. Values are means ± SD of 3 independent experiments. Endostatin levels are significantly higher in aging (vs. young) gastric mucosa at baseline (†P < 0.01) and further increased 24 h after ethanol-induced injury (**P < 0.01). B: zymography analysis of MMP-9 enzyme activity. Values are means ± SD of 3 independent experiments. In aging gastric mucosa MMP-9 enzyme activity was significantly increased at baseline (vs. young) (†P < 0.01) and further increased 24 h after ethanol-induced injury (**P < 0.001).

occurring in some pathological processes such as tumor growth and metastases (5, 13, 21). Blood microvessels (capillaries, arterioles, and collecting venules) consist of endothelial cells adherent to basement membranes. Endothelial cells carry genetic information to proliferate and form tubes, branches, anastomoses, and a capillary network (5, 13, 21). Under normal physiological conditions this genetic information is suppressed in most tissues and the endothelial cells within microvessels remain quiescent (resting phenotype) and their turnover rate is very low (5, 13, 21). In certain situations (e.g., wound healing), the resting phenotype is changed to an angiogenic phenotype (5, 13, 21). In some tissues the microvascular endothelial cells of preserved microvessels at the wound edge bordering necrosis migrate, proliferate, and attempt to reestablish a microvascular network through the process of angiogenesis. Angiogenesis in gastric mucosa has not been examined except in our previous study, which demonstrated that ethanol-induced gastric injury in young rats triggers significant increases in VEGF mRNA and protein expression in the areas bordering necrosis and that neutralizing anti-VEGF antibody reduces angiogenesis and delays healing of injured gastric mucosa in young rats (22). That study established the causal relationship between angiogenesis and VEGF gene expression in injured gastric mucosa of young rats (22). Moreover, we previously demonstrated that VEGF gene therapy stimulates angiogenesis and healing of gastric ulcers in young rats (23). Therefore, VEGF plays a crucial role in gastric angiogenesis and healing of both acute and chronic gastric injury. However, our previous studies did not examine either VEGF expression or angiogenesis in aging gastric mucosa.

Our present study demonstrates that in aging gastric mucosa VEGF expression is significantly lower vs. young rats at baseline and that, unlike young gastric mucosa, VEGF expression does not increase following ethanol-induced injury. The present study also demonstrated significantly higher levels of the antiangiogenic protein endostatin in aging (vs. young) gastric mucosa, both at baseline and 24 h after ethanol-induced injury. Therefore, an angiogenic imbalance resulting in reduced expression of proangiogenic VEGF and increased expression of antiangiogenic endostatin in gastric mucosa of aging rats is likely the key mechanism underlying impaired angiogenesis and delayed healing of aging gastric mucosa.

Gastric mucosal injury by ethanol includes both focal deep mucosal necrosis (erosions) caused by microvessel injury and resulting hypoxia and superficial injury limited to extensive exfoliation of the surface epithelium (47, 49). Following superficial mucosal injury, the continuity of the surface epithelium is promptly (minutes to hours) reestablished by the process referred to as epithelial restitution (47). This process involves migration of the epithelial cells from the gastric pits and upper regions of the glands bordering injury to cover the denuded mucosal surface. Our present study was focused on angiogenesis in aging (vs. young) gastric mucosa in response to injury and on the relationship between angiogenesis and the healing of erosions and the roles played by VEGF and endostatin. However, for comparison we also examined restitution of the surface epithelium in mucosal areas bordering the erosion and outside deep erosions. The latter study demonstrated that in aging gastric mucosa restitution of the surface epithelium outside deep erosions is significantly impaired in aging vs. young gastric mucosa. This likely results from impaired migration of the surface epithelial cells due to hypoxia with inadequate oxidative phosphorylation essential for kinase function, reduced expression of survivin, inadequate activation of the growth factors regulating epithelial cell migration (e.g., EGF, TGF-α), and/or basement membrane injury in aging gastric mucosa (2, 27, 47).

Our previous study demonstrated hypoxia in aging gastric mucosa (50). Since hypoxia is a potent stimulus for VEGF gene activation (14, 22), one would expect that aging gastric mucosa should have increased VEGF protein expression. However, our present study demonstrates that this is not the case;
aging gastric mucosa does not have higher VEGF levels despite increased tissue hypoxia, indicating that aging gastric mucosa loses its sensitivity to hypoxia at least with respect to VEGF gene activation.

Our present study also showed a significant increase of endostatin, an important antiangiogenic factor in aging gastric mucosa of rats. The expression of endostatin in gastric mucosa has not been examined previously. Moreover, our present study identified the potential mechanism responsible for the increased endostatin levels in aging gastric mucosa. We found that aging gastric mucosa has significantly increased activity of MMP-9, the enzyme that generates endostatin from collagen XVIII. Taken together, our findings of reduced VEGF and increased endostatin levels in aging gastric mucosa clearly demonstrate that there is an imbalance between proangiogenic and antiangiogenic factors, which inhibits angiogenesis and delays healing of injured gastric mucosa. The clinical relevance of this finding is supported by a recent study demonstrating that increasing age is associated with increased serum endostatin concentrations (3). Furthermore, endostatin inhibits angiogenesis in several experimental models (4, 35, 43, 48).

Regarding ulcers, the incidence of gastric and duodenal ulcers, as well as associated complications (e.g., bleeding), is increasing in old-aged populations worldwide (16, 38, 41). The increased risk of complications may be due to impaired healing. Nevertheless, extensive literature searches on angiogenesis, endostatin and MMP-9 expression in the mucosa of aging humans yielded no information indicating that these topics have not yet been explored. Angiogenesis has been shown to be impaired in some other (nongastric) aging tissues such as heart, skeletal muscle, and brain and is associated with reduced blood flow, decreased capillary vessel density, and impaired wound healing (15, 42, 44, 45). However, none of these studies were related to angiogenesis in aging gastric mucosa nor did they examine the balance between proangiogenic and antiangiogenic factors.

The importance of proper regulation of angiogenesis is reflected in other pathological diseases such as cancer and rheumatoid arthritis. Increased angiogenesis is present in cancer and has been attributed to elevated levels of proangiogenic molecules, mainly VEGF, and antiangiogenic compounds are used for cancer therapy (6). Our study, demonstrating an angiogenic imbalance in aging gastric mucosa, may also have potential implications for slower tumor growth in aging individuals (26, 40). Although aging is associated with increased cancer prevalence, the growth of histologically similar tumors has been demonstrated to be slower in models of older vs. young animals (26, 40).

The identification of mechanism of impaired angiogenesis in response to injury in aging gastric mucosa provides a rationale for earlier expanded diagnosis and new therapeutic approaches such as enhancing angiogenesis, e.g., via short-term VEGF protein or gene therapy (23) or endostatin inhibitors.

In conclusion, this study demonstrated that angiogenesis is impaired in aging gastric mucosa and that VEGF expression levels are significantly reduced, whereas endostatin protein levels are significantly increased vs. the gastric mucosa of young rats. This represents a significant shift in the angiogenic balance toward inhibition of angiogenesis and is a key underlying mechanism for the impaired angiogenesis and delayed healing of aging gastric mucosa.

ACKNOWLEDGMENTS

The authors acknowledge the contributions of the late Dr. J. Stachura MD, PhD (Professor and Chairman Department of Pathology, University Medical School, Krakow, Poland and former Visiting Professor at the University of California, Irvine) in interpreting some of histological and TEM studies.

GRANTS

This research was supported by the VA Merit Review (to A. S. Tarnawski).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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