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FRONTIER

Increased susceptibility of aging gastric mucosa to injury: The mechanisms and clinical implications

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Abstract

This review updates the current views on aging gastric mucosa and the mechanisms of its increased susceptibility to injury. Experimental and clinical studies indicate that gastric mucosa of aging individuals-"aging gastropathy"-has prominent structural and functional abnormalities *vs* young gastric mucosa. Some of these abnormalities include a partial atrophy of gastric glands, impaired mucosal defense (reduced bicarbonate and prostaglandin generation, decreased sensory innervation), increased susceptibility to injury by a variety of damaging agents such as ethanol, aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), impaired healing of injury and reduced therapeutic efficacy of ulcer-healing drugs. Detailed analysis of the above changes indicates that the following events occur in aging gastric mucosa: reduced mucosal blood flow and impaired oxygen delivery cause hypoxia, which leads to activation of the early growth response-1 (egr-1) transcription factor. Activation of egr-1, in turn, upregulates the dual specificity phosphatase, phosphatase and tensin homologue deleted on chromosome ten (PTEN) resulting in activation of pro-apoptotic caspase-3 and caspase-9 and reduced expression of the anti-apoptosis protein, survivin. The imbalance between pro- and anti-apoptosis mediators results in increased apoptosis and increased susceptibility to injury. This paradigm has human relevance since increased expression of PTEN and reduced expression of survivin were demonstrated in gastric mucosa of aging individuals. Other potential mechanisms operating in aging gastric mucosa include reduced telomerase activity, increase in replicative cellular senescence, and reduced expression of vascular endothelial growth factor and importin-α-a nuclear transport protein essential for transport of transcription factors to nucleus. Aging gastropathy is an important and clinically relevant issue because of: (1) an aging world population due to prolonged life span; (2) older patients have much greater risk of gastroduodenal ulcers and gastrointestinal complications (*e.g.*, NSAIDs-induced gastric injury) than younger patients; and (3) increased susceptibility of aging gastric mucosa to injury can be potentially reduced or reversed pharmacologically.

Key words: Aging gastric mucosa; Injury; Phosphatase and tensin homologue deleted on chromosome ten-PTEN; Survivin; Apoptosis; Hypoxia

Core tip: This review focuses on aging gastric mucosa and its increased susceptibility to injury. The following events occur in aging gastric mucosa: reduced mucosal blood flow and hypoxia, upregulates PTEN that activates pro-apoptotic caspases and reduces anti-apoptosis protein, survivin. The imbalance between pro- and

anti-apoptosis mediators results in increased apoptosis and increased susceptibility to injury. Aging gastropathy is an important and clinically relevant issue because of: (1) an aging world population; (2) older patients have much greater risk of gastroduodenal ulcers and gastrointestinal complications (*e.g.*, non-steroidal anti-inflammatory drugs-induced gastric injury) than younger patients; and (3) increased injury of aging gastric mucosa can be reversed pharmacologically.

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BIOGRAPHY

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, University of California Irvine, Editor-in-Chief, *World Journal of Gastroenterology* (Figure 1), Graduated (MD) from the University Medical School, Krakow, Poland, where he also received PhD (pathology) and DSc (gastroenterology) and served as Assistant and Associate Professor and V-Chair, Department of Gastroenterology. Following gastrointestinal fellowship at the University of Missouri, Columbia, MO, United States, he was appointed as Associate Professor (1982-1986) and full Professor (1986-present) at the University of California, Irvine, United States. He served as: Associate Chair, American Gastroenterological Association/EGD 1997-1999 and 2008-2010; Scientific Director, Shimoda Symposia on Mucosal Defense in Japan (8x), Chairman of Research Fora at DDW/AGA annual meetings (12 times; 1996-2011), Chair, Pasteur Institute Euroconference 2005 and as Chair and or Co-chair of 68 International Symposia.

Publications, presentations and grants: 347 full, peer reviewed publications [*Lancet*, *Nature Med*, *JCI*, *Gastroenterology* (over 30 papers), *Hepatology*, *Gut*, *FASEB J*, *Am JPathol, Am J Physiol*, *AmJ Gastroenterol* and others]; 20 book chapters; 507 presentations at international and United States meetings; 20 peer reviewed funded grants (NIH, VA Merit Review 1984-present), 4 United States patents. Clinical and Research interest: endoscopic, histologic, functional assessment of injury and protection of gastrointestinal mucosa; cellular and molecular mechanisms of gastroduodenal and esophageal ulcer healing-role of growth factors, signaling pathways, angiogenesis, non-steroidal anti-inflammatory drugs (NSAIDs), prostaglandins and *Helicobacter pylori* (*H. pylori*) toxins; injury and protection of portal hypertensive gastric mucosa and aging gastric mucosa; confocal endomicroscopy and molecular imaging; gene therapy. Received numerous prestigious academic honors including Glaxo International Research Award, Athalie-Clarke, Merentibus Medal, Peregrinator of Science awards and others. Memberships: American Gastroenterological Association (Fellow), American College of Gastroenterology (Fellow), British Society of Gastroenterology, Japanese Society of Gastroenterology (Honorary), Hungarian Society of Gastroenterology (Honorary), American Society for Investigative Pathology, Association of American Physicians (by election) and others. Editorial Boards-9 scientific journals. Sixteen of his former GI Clinical Fellows and/or Residents or Research postdoctoral fellows hold academic positions in United States Medical Schools (4 being Chairs of Departments). Twenty of his former international trainees and/or associates hold academic positions abroad (France, Germany, Hungary, Japan, Poland, Sweden, Switzerland).

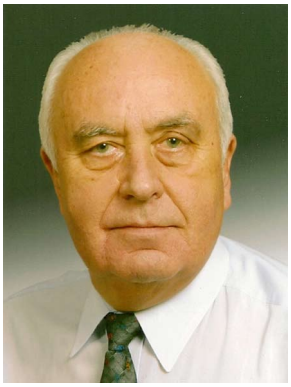

Figure 1 Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, University of California, Irvine; and VA Long Beach Health Care System, 5901 E. Seventh Str., Long Beach, CA 90822, United States; Honorary Professor, Chinese University of Hong Kong; Editor-in-Chief, *World Journal of Gastroenterology*.

INTRODUCTION

Experimental and clinical studies indicate that the gastric mucosa of aging individuals (which we refer to herein as aging gastric mucosa and/or "aging gastropathy"-the term that we proposed earlier[1]) has prominent structural and functional abnormalities *vs* young gastric mucosa[1-3] that impair gastric mucosal defense.

Gastric mucosal defense and its impairment in aging

Mucosal defense in normal stomach, its particular components, and the mechanism of gastric mucosal injury have been reviewed in detail in previous papers[4-6]. Under normal conditions, gastric mucosal integrity is maintained by defense mechanisms (Figure 2), which include pre-epithelial, epithelial and post-epithelial components[4,5]. The pre-epithelial component: mucus-bicarbonate-phospholipid "barrier"-constitutes the first line of gastric mucosal defense[4]. The epithelial component consists of a continuous layer of surface epithelial cells interconnected by tight junctions and forming the epithelial "barrier". These epithelial cells generate and secrete bicarbonate, mucus, phospholipids, trefoil peptides, prostaglandins (PGs) and heat shock proteins[4]. The integrity of the epithelial cell layer is maintained by continuous cell renewal that is accomplished by proliferation of progenitor cells regulated by growth factors, prostaglandin E_2 and survivin-an anti-apoptosis and mitosis-promoting protein[4]. The post-ep-

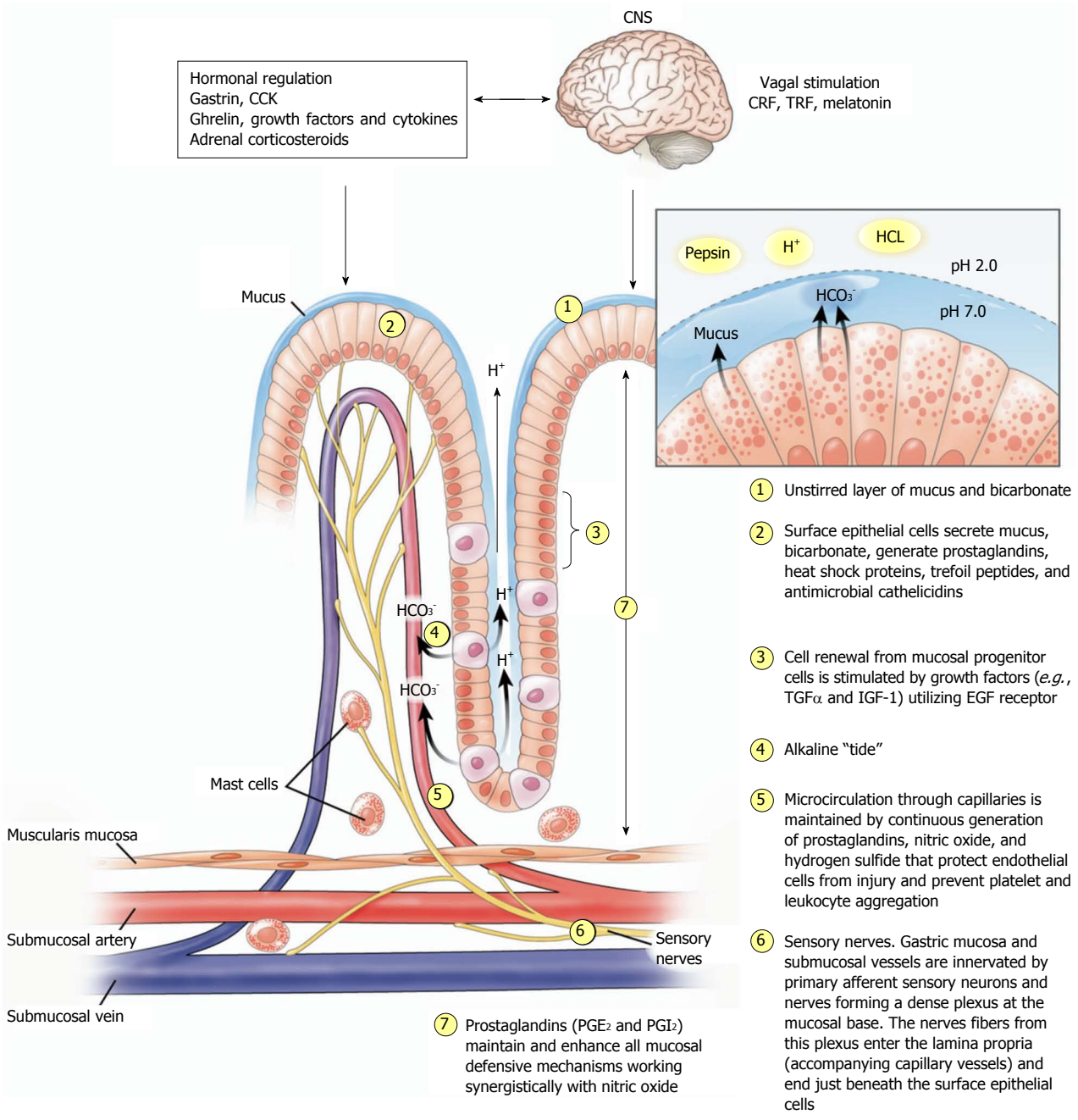

Figure 2 Gastric mucosal defense. Schematic representation of gastric mucosal defense mechanisms. Reproduced with permission from Laine, Takeuchi and Tarnawski[4]. (1) "Unstirred" layer of mucus/bicarbonate/phospholipids above surface epithelial cells constitutes the first line of defense. It maintains a pH of approximation 7.0 (close to the physiological cell pH) at the surface epithelial cells, while pH in the lumen is about 1.0-3.0; (2) the surface epithelial cells secrete mucus, bicarbonate and synthesize prostaglandins and heat shock proteins; (3) mucosal cell renewal from mucosal progenitor cells is driven by growth factors (transforming growth factor α and insulin like growth factor-1α) utilizing the epidermal growth factor receptors). Expression of survivin in epithelial progenitor cells prevents apoptosis and is the key for "immortality" of these cells under normal conditions; (4) "Alkaline tide"-parietal cells secreting HCl into the gastric gland lumen concurrently secrete bicarbonate into the lumen of adjacent capillary blood vessels. Bicarbonate is transported to the surface and contributes to the first line of defense; (5) mucosal microcirculation through the capillary microvessels is essential for delivery of oxygen and nutrients. Endothelial cells of microvessels generate prostaglandins, mainly PGI_2 (prostacyclin) and nitric oxide, which exert vascular and mucosal protective actions; (6) sensory nerve stimulation by H^+-ion or other irritants causes release of neurotransmitters such as calcitonin gene related peptide (CGRP) and substance P in nerve terminals, which induce vasodilatation and enhance mucosal blood flow; and (7) continuous generation of prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) by the gastric mucosal cells is crucial for the maintenance of mucosal integrity. Almost all of the above (1-6) mucosal defense mechanisms are stimulated or facilitated by endogenous or exogenous prostaglandins. CRF: Corticotrophin-releasing factor; TRF: Thyrotropin-releasing factor; CCK: Cholecystokinin.

ithelial component of mucosal defense includes continuous blood flow through mucosal microvessels lined with endothelial cells forming an endothelial "barrier", sensory nerves releasing calcitonin gene-related peptide (CGRP) and hence regulating mucosal blood flow; and, the generation of PGs and nitric oxide[4,5]. The structural elements of normal gastric mucosal defense were reviewed and discussed in detail in our previous paper[4] and are presented in Figure 3. Importantly, gastric mucosal defense is also regulated by the central nervous system through

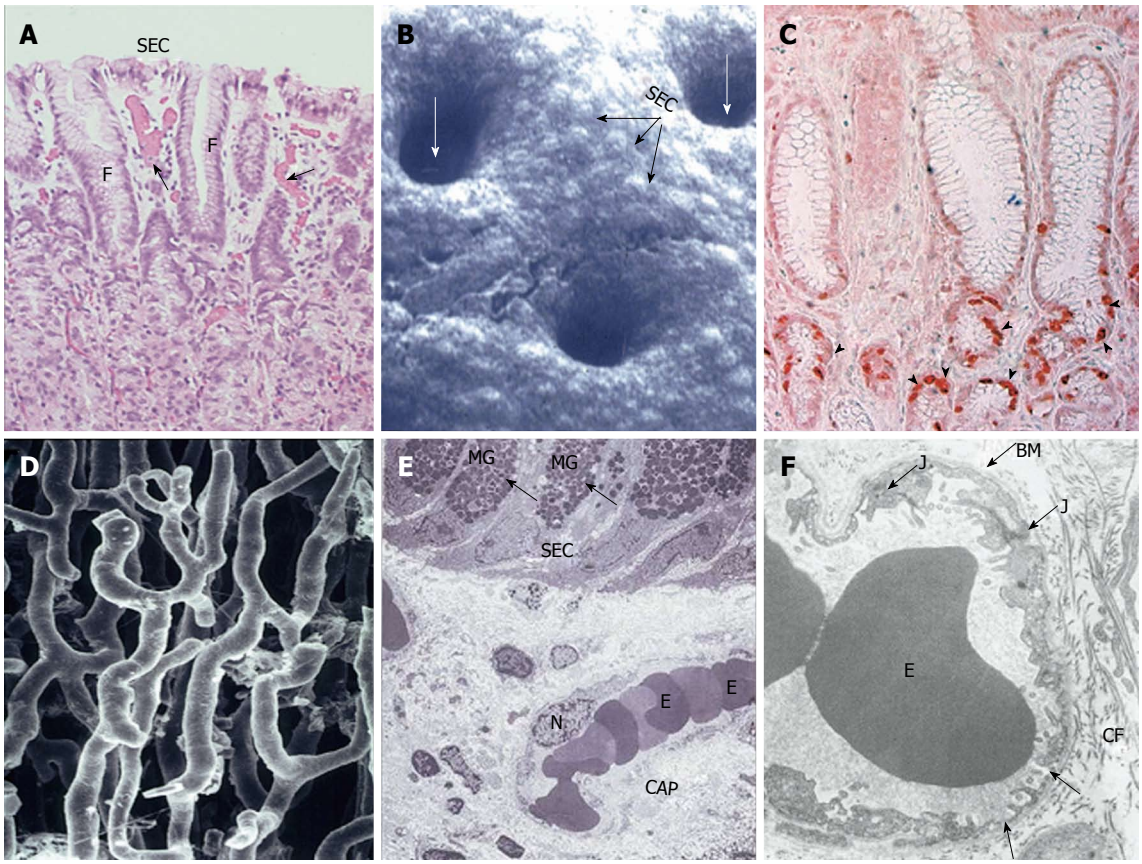

Figure 3 Structural components of gastric mucosal defense: surface epithelial cells, progenitor cells and blood microvessels. Reproduced with permission from Laine, Takeuchi and Tarnawski[4]. A: Histology of upper part of human gastric mucosa visualizing surface epithelial cells (SEC), foveoli (F), and upper gland area. (Hand E staining; original magnification, × 50). Blood microvessels with erythrocytes in the lumen are present in the lamina propria (arrows); B: Scanning electromicrograph of human gastric mucosal luminal surface. The unstirred mucus gel layer is not seen because of dissolution during fixation. Individual *SEC* are clearly visible as are lumina of the gastric pits (white arrows). Reproduced with permission from Tarnawski *et al*[7]; C: Immunostaining of human gastric mucosa with survivin (anti-apoptosis protein) antibody. Survivin is strongly expressed (brown-red staining) in the epithelial progenitor cells located in the foveolar/neck area (arrowheads). Reproduced with permission from Tarnawski *et al*[1]; D: Vascular cast study of capillary blood vessels in the gastric mucosa using Mercox resin. The remaining components of the mucosa were dissolved with concentrated NaOH. Reproduced with permission from Ichikawa, Tarnawski *et al*[8]; E: Transmission electron micrograph of normal human gastric mucosa. SEC contain dark mucus granules (MG, arrows). Below the surface epithelial cells, a capillary blood vessel (CAP) with erythrocytes (E) in the lumen is present in the lamina propria. N, nucleus of endothelial cell lining capillary vessel (original magnification, × 2000). Reproduced with permission from Tarnawski *et al*[9]; F: Transmission electron micrograph of a portion of human gastric capillary blood vessel. The structure of the capillary wall and endothelial cell cytoplasm is normal with a characteristic fenestration (arrows) allowing transport. BM: Basement membrane; E: Erythrocytes in the capillary lumen; J: Junction between two neighboring endothelial cells; CF: Collagen fibers. Original magnification, × 17400. Reproduced with permission from Tarnawski *et al*[9].

vagal innervation, the release of corticotrophin-releasing factor, thyrotropin-releasing factor, melatonin and others; by hormones including gastrin, cholecystokinin, adrenal corticosteroids; and by growth factors and cytokines[4].

Gastric mucosal injury occurs when injurious factors "overwhelm" a normal, intact mucosal defense or when the mucosal defense is impaired[4,5]. The mechanisms of mucosal injury and its repair were described in detail in our previous publications[5,6].

Impaired gastric mucosal defense in aging individuals

Previous studies showed that aging gastric mucosa has impaired mucosal defense including reduced mucus and bicarbonate secretion, decreased prostaglandin generation, reduced nitric oxide synthase (NOS) activity; and, impaired sensory nerve responses to luminal acid[10-16]. Lee and Feldman demonstrated in Fisher 344 rats *in vivo* that gastric mucosal prostaglandin synthesis is significantly reduced in aging *vs* young rats; and, that aging rats are more susceptible to aspirin-induced acute gastric mucosal injury[10]. Gronbech and Lacy examined in young and aged Fisher 344 rats damage of gastric mucosa by exposure to either 80% ethanol for 30-45 s or 1 mol/L NaCl for 10 min followed by saline in a chambered stomach model[11]. They found that the mucosal lesions were significantly more extensive, and epithelial restitution was significantly reduced and delayed in aging *vs* young rats after both types of injury[11]. In separate experiments, they monitored changes in gastric mucosal blood flow using a laser-Doppler flow-meter and demonstrated that young rats had a marked increase in gastric mucosal blood flow in response to 1 mol/L NaCl, luminal acid challenge, and capsaicin treatment; and, that these responses were abolished in aging rats[11]. Moreover, aging rats had a lower density of CGRP (+) positive nerve fibers around gastric submucosal blood

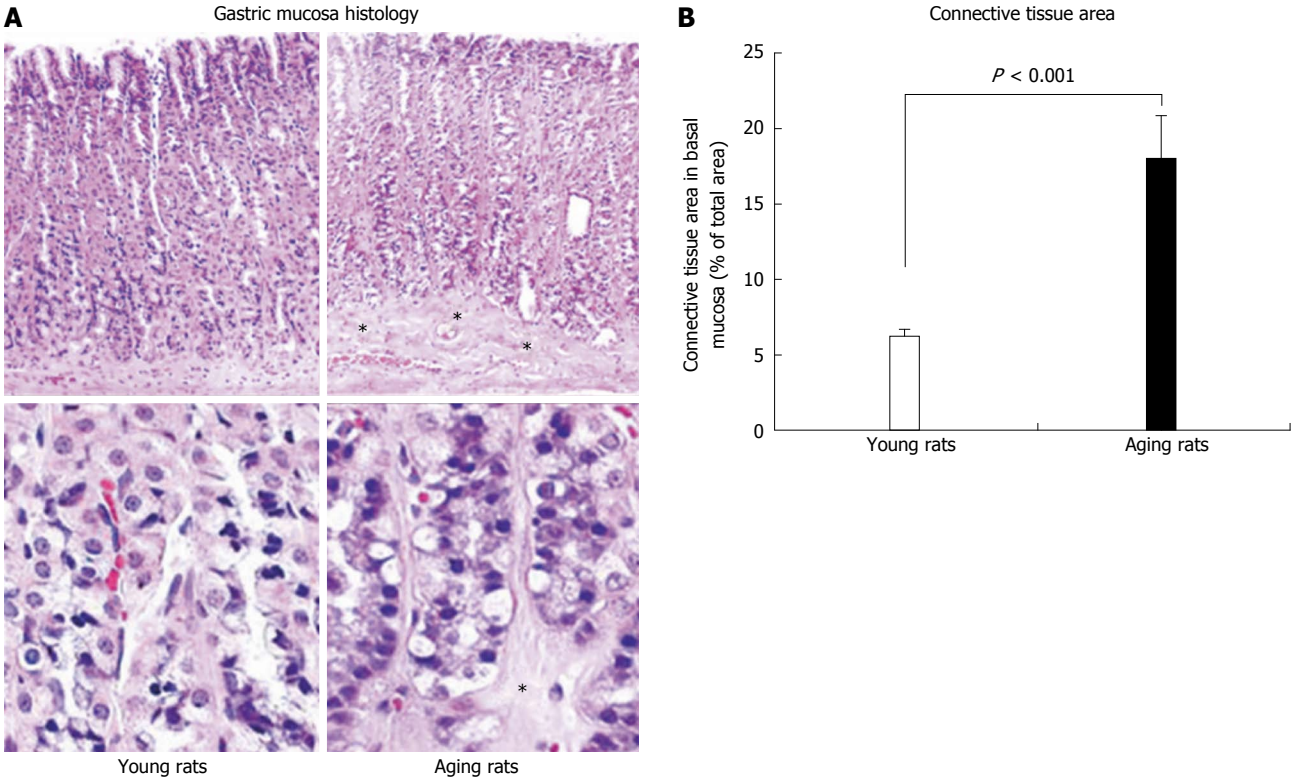

Figure 4 Photomicrographs of gastric mucosa in young and aging rats. In gastric mucosa of aging rats there is partial atrophy of gastric glands in the basal mucosa and their replacement with connective tissue (*). A: Hematoxylin and eosin staining at low magnification (x 100) is shown in the upper panels and higher magnification (x 500) is shown in the lower panels; B: Quantification of connective tissue in the lower one third of the gastric mucosa shows a significant increase in connective tissue replacing glandular cells in aging rats. Quantification of the number of inflammatory cells in gastric mucosa shows no inflammation (only a minimal number of inflammatory cells) and no difference between young and aging rats indicating that atrophic changes are not accompanied by an inflammation. Reproduced with permission from Tarnawski *et al*[1].

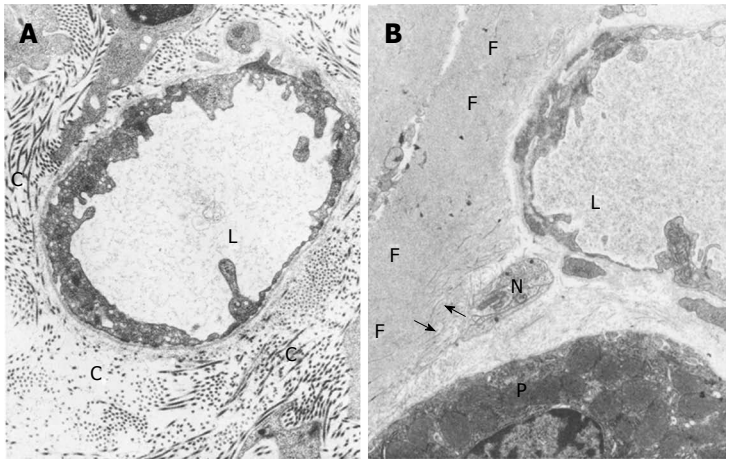

Figure 5 Transmission electron microscopy. A: Transmission electron micrograph of perivascular connective tissue from a 3-month-old control rat from the basal portion of the oxyntic mucosa. The connective tissue shows numerous collagen fibers (C); L, Microvessel lumen. Magnification x 19000; B: Transmission electron micrograph of perivascular connective tissue from an old rat. In the basal portion of the oxyntic mucosa, collagen fibers are mostly absent and replaced by rudimentary collagen fibers (arrows) and deposits of amorphous fibrillar material (F). P: Parietal cells; L: Blood microvessel lumen; N: Nerve bundle. Magnification x 19000. Reproduced with permission from Hollander, Tarnawski *et al*[2].

vessels and decreased mucosal release of prostaglandin E2 compared to young rats[11]. These data demonstrated impaired gastric mucosal defense and reduced gastric epithelial restitution in aging rats, which were related to the lack of hyperemic response to mucosal injury likely due to reduced CGRP (+) nerve fibers and decreased prostaglandin generation in aging gastric mucosa[11]. Other studies demonstrated aging-related changes in gastric mucosal glycoprotein synthesis, reduced gastric mucosal bicarbonate secretion and reduced gastric mucosal blood

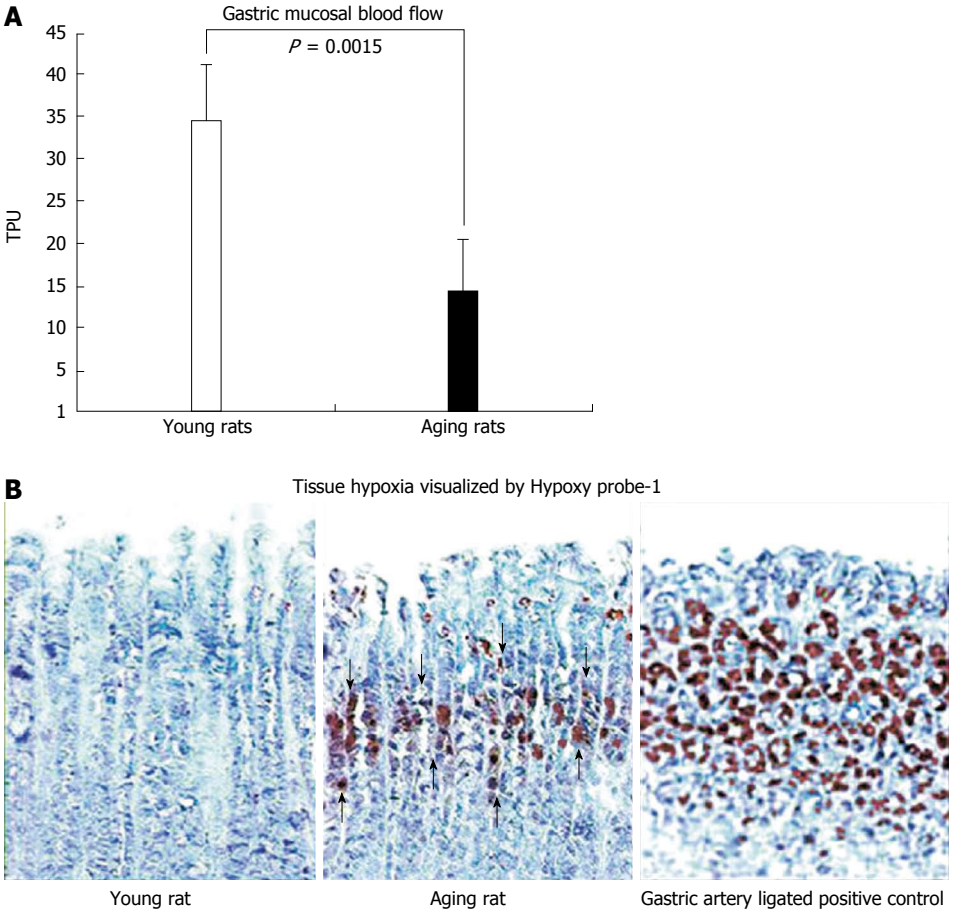

Figure 6 Gastric mucosal blood flow measured with BLF21 Laser-Doppler flow meter and mucosal hypoxia visualized by Hypoxy probe-1. A: In gastric mucosa of aging rats at baseline, mucosal blood flow, expressed in perfusion units, is significantly reduced by approximation 60% (*vs* young rats; $P = 0.0015$). Such a dramatic reduction in blood flow likely leads to chronic hypoxia; B: Photomicrographs of rat gastric mucosa. Gastric mucosal hypoxia is visualized by immunohistochemical staining utilizing the small molecular marker, pimonidazole HCl (Hypoxy probe-1), which binds selectively to oxygen starved cells[30]. In young rats, Hypoxy probe-1 staining is negative in both connective tissue and epithelial cells of the gastric mucosa demonstrating the absence of hypoxia. In aging rats, positive staining is strongly expressed (brown staining) in the upper and mid-mucosa, mainly in the progenitor and parietal cell zone (arrows), reflecting severe hypoxia in these cells. As a positive control we used gastric mucosa of young rats that had all major gastric arteries ligated for 1 h. A strong accumulation Hypoxy probe-1 is present in the majority of epithelial cells (brown staining) reflecting profound cell hypoxia. Reproduced with permission from Tarnawski *et al*[1].

flow in aging *vs* young rats[12-14]. Importantly human studies confirmed clinical relevance of these experimental findings. Cryer *et al*[17] and Goto *et al*[18] demonstrated in humans an age-associated decrease in gastric mucosal prostaglandin concentration *vs* young individuals. In another human study, Feldman and Cryer[19] showed that aging is associated with a significant reduction in gastric bicarbonate, sodium ion and non-parietal fluid secretion. Since mucosal defense is significantly reduced in aging gastric mucosa, not surprisingly one can anticipate increased susceptibility of aging gastric mucosa to injury.

Increased susceptibility of aging gastric mucosa to injury

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 NSAIDs, hypertonic saline, bile acids, cold restraint-induced stress and other factors[10,11,20-25]. Human studies fully confirmed these experimental findings and demonstrated that patients over 65 years of age have significantly increased gastric mucosal injury by aspirin and other NSAIDs[23,26-29]. Older patients taking low-dose aspirin or NSAIDs also have a much greater absolute risk of gastrointestinal (GI) complications than younger patients. Patrono *et al*[28] reported that the risk of ulcer complications in subjects under 50 years of age was below 0.5% while the risk was nearly 4% in subjects aged 70-79 years and approximately 6% in subjects over 80 years of age. Even though a 2-fold increase in risk with low-dose aspirin is consistent across the different age groups, the incidence of complications and the absolute increase in complications with aspirin *vs* controls is dramatically higher in the older population due to their higher baseline risk[28]. Furthermore, the concurrent use of other medications (*e.g.*, NSAIDs) that increase the risk of bleeding in low-dose aspirin users also increases with age[26-29].

Structural abnormalities of aging gastric mucosa

In a previous study we analyzed structural changes in gastric mucosa of aging (*vs* young) rats by quantitative histology[1]. That study demonstrated a partial atrophy of

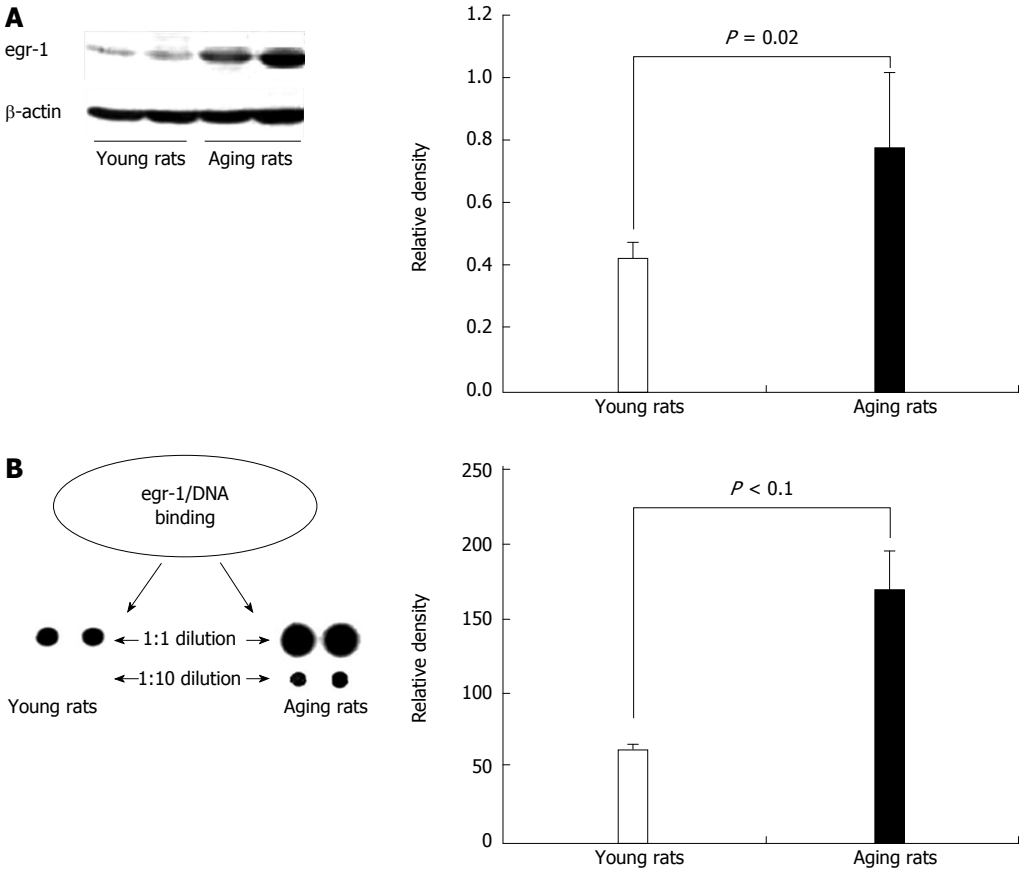

Figure 7 Increased expression of early growth response-1 and increased early growth response-1 transcriptional activity in gastric mucosa of aging *vs* young rats. A: Representative Western blotting demonstrate increased early growth response-1 (egr-1) protein expression in gastric mucosa of aging (*vs* young) rats; B: Assessment of egr-1 transcriptional activity in gastric mucosa of young and aging rats was performed using the TranSignal™ TF-TF Interaction Array (Panomics, Redwood City, CA). The egr-1 *cis*-element is spotted in duplicate: in the first row DNA was spotted without dilution; in the second row DNA was diluted ten times (1:10). In gastric mucosa of aging rats there is a significant, 2.7-fold increase (*vs* that of young rats; $P < 0.02$) in binding of egr-1 protein to its GC-rich *cis* elements that are highly expressed in the PTEN gene promoter. Reproduced with permission from Tarnawski *et al*[1].

gastric glands and their replacement with increased connective tissue in the basal one third of the mucosa (Figure 4A). Quantification of connective tissue in the lower one third of the gastric mucosa shows a significant approximately 3 fold increase in connective tissue replacing glandular cells in aging rats (Figure 4B). These findings were independently confirmed later by another group[3].

In a separate study using transmission electron microscopy (TEM) (Figure 5) we demonstrated prominent histologic and ultrastructural alterations in gastric mucosa of aging rats including disorganized collagen fibrils in connective tissue immediately adjacent to capillary blood vessels (Figure 5B)[2]. We postulated that these changes could interfere with nutrient and oxygen transport and hence lead to hypoxia as well as the accumulation of toxic metabolites[2].

Mechanisms of aging gastropathy-novel insight

While previous studies showed reduced gastric mucosal blood flow in aging rats, those studies did not examine mucosal hypoxia directly. To fill this gap we examined gastric mucosal blood flow in young (3 mo of age) and aging (24 mo of age) rats using a laser Doppler flowmeter as well as determined mucosal oxygenation[1] using the specific Hypoxy-1 probe, which visualizes tissue and individual cell hypoxia[30]. In addition, we also examined expression of early growth response-1 (egr-1), a transcription factor (which is activated by hypoxia) and expression of dual phosphatase and tensin homologue deleted on chromosome ten (PTEN). PTEN is a dual specificity phosphatase that inhibits the PI3K/Akt signaling pathway crucial for cell survival and therefore promotes apoptosis[31-34]. Furthermore, the same study[1] examined apoptosis in the gastric mucosa of aging *vs* young rats using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) nick-end labeling (TUNEL) method and also quantified expression and activation of the apoptosis-inducing executioner proteases: caspase-3 and caspase-9 described in our previous paper[35], as well as the anti-apoptosis protein, survivin described in our previous studies[36,37]. That experimental study[1] showed that gastric mucosa of aging rats exhibits: (1) Significantly reduced mucosal blood flow (by approximation 60%) compared with gastric mucosa of young rats (Figure 6A) resulting in marked hypoxia (reflected by the accumulation of Hypoxia-1 probe) of the upper and middle gastric mucosa, mainly in parietal and progenitor cells (Figure 6B). It should be noted that a recent human

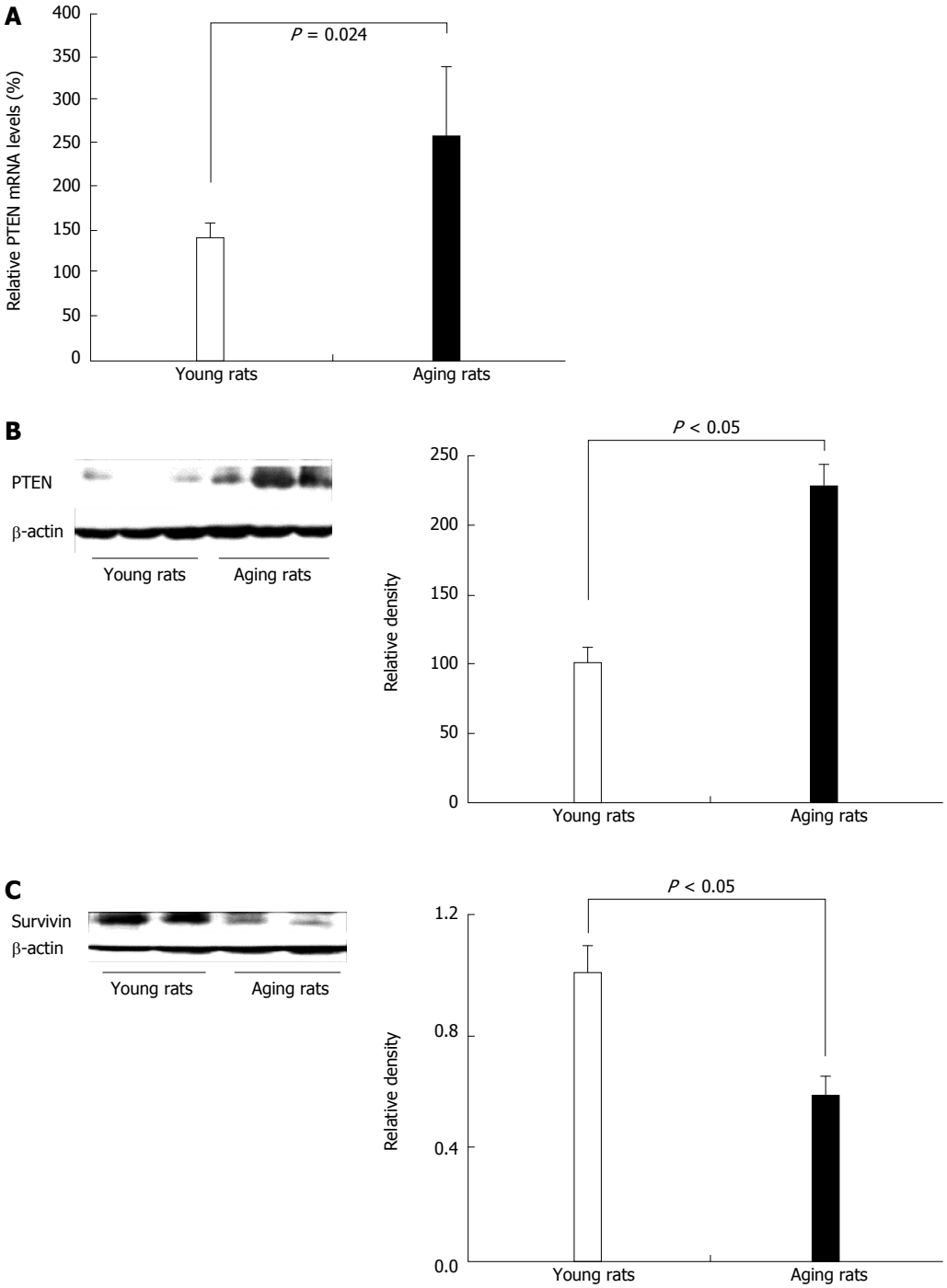

Figure 8 Increased expression of dual phosphatase PTEN and reduced expression of survivin in gastric mucosa of aging *vs* young rats. A: Real time PCR; B: Representative Western blotting showing a significant increase in phosphatase and tensin homologue deleted on chromosome ten (PTEN) mRNA and protein expression, respectively in gastric mucosa of aging *vs* young rats; C: Representative Western blotting showing a significant decrease in survivin (anti-apoptosis protein). Reproduced with permission from Tarnawski *et al*[1].

study fully confirmed these experimental findings and demonstrated abnormalities in gastric submucosal vessels and gastric submucosal arteriolar dysfunction in elderly patients, which may lead to reduced blood supply[38]; (2) Increased expression of egr-1 protein, which is activated by hypoxia, and increased egr-1 transcriptional activity (Figure 7); (3) Increased expression of PTEN mRNA and protein, and reduced expression of survivin (Figure 8). This is mechanistically important since increased PTEN arrests cell growth and inhibits cell survival by reducing survivin and inducing apoptosis[33,34]; (4) Significantly increased apoptosis demonstrated by TUNEL assay (Figure 9A and B); (5) Significantly increased expression of cleaved caspase-3 and caspase-9, which induce apoptosis (Figure 10); and (6) Significantly increased susceptibility to ethanol-induced injury compared with gastric mucosa of young rats (Figure 11A). The crucial mechanistic role of PTEN in the increased susceptibility of aging gastric mucosa to injury is evidenced by the finding that down-regulation of PTEN protein expression by local admin-

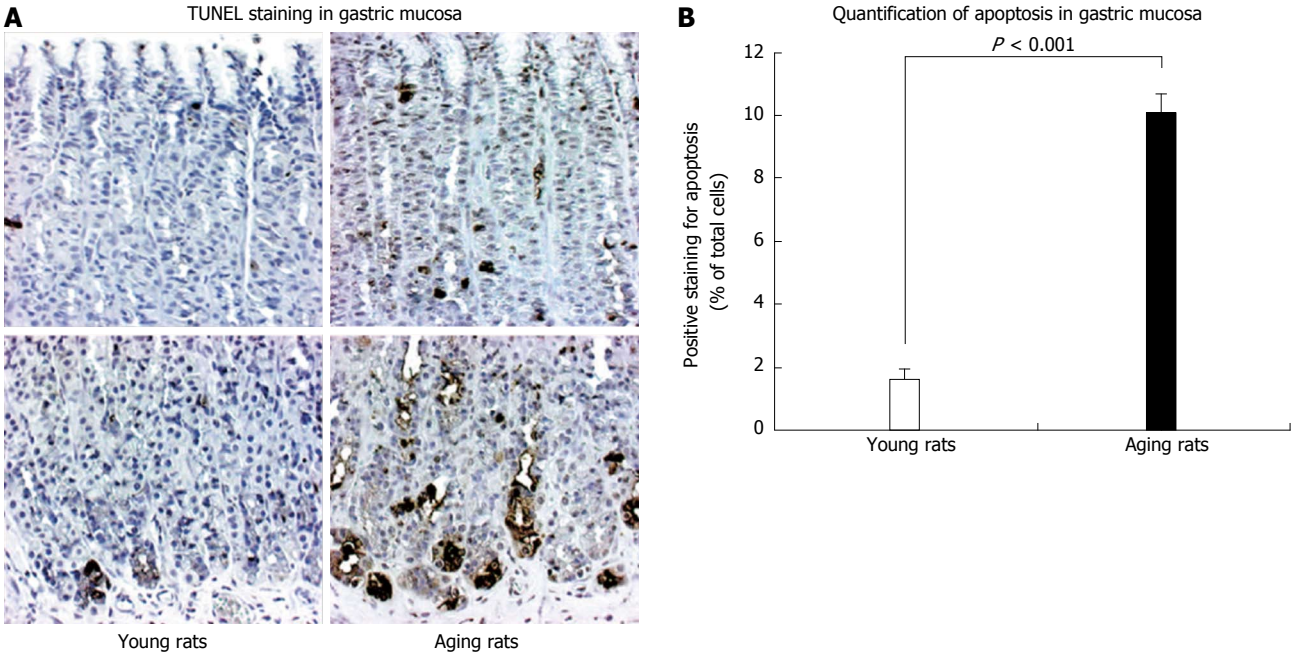

Figure 9 TUNEL staining for apoptosis in gastric mucosa of young and aging rats. A: The photomicrographs of gastric mucosa of young and aging rats at baseline (magnification x 100). *In situ* cell death (apoptosis) detection by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was used to visualize apoptotic-positive cells (brown staining); B: Quantification of the number of positively labeled cells demonstrated that gastric mucosa of aging rats exhibits a significantly increased number of apoptotic cells *vs* mucosa of young rats. The increased apoptosis prominently involved epithelial cells at the basal mucosa explaining atrophy of the basal gastric glands shown in Figure 4. Reproduced with permission from Tarnawski *et al*[1].

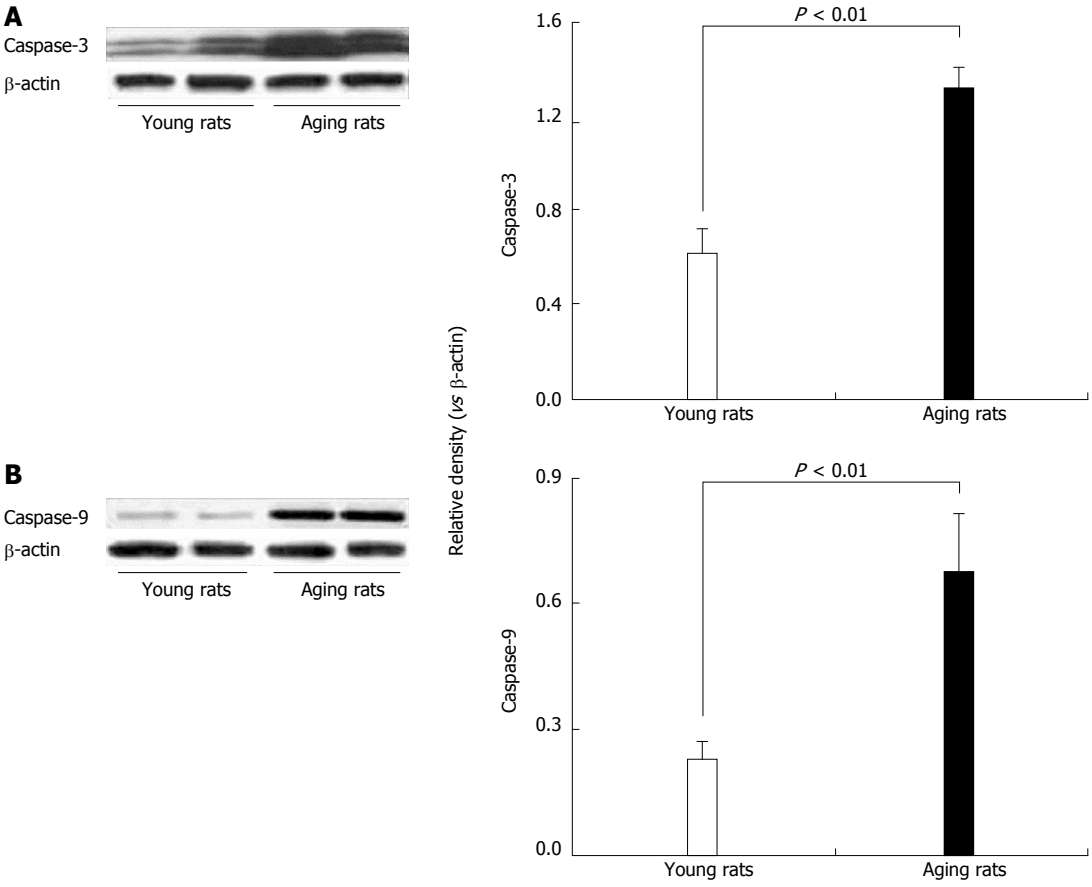

Figure 10 Expression of cleaved caspase-3 and caspase-9 protein levels by Western blotting in gastric mucosa of young and aging rats. In gastric mucosa of aging rats there is a significant increase in apoptotic cis-inducing (A) cleaved caspase-3 and (B) caspase-9 compared to gastric mucosa of young rats. Reproduced with permission from Tarnawski *et al*[1].

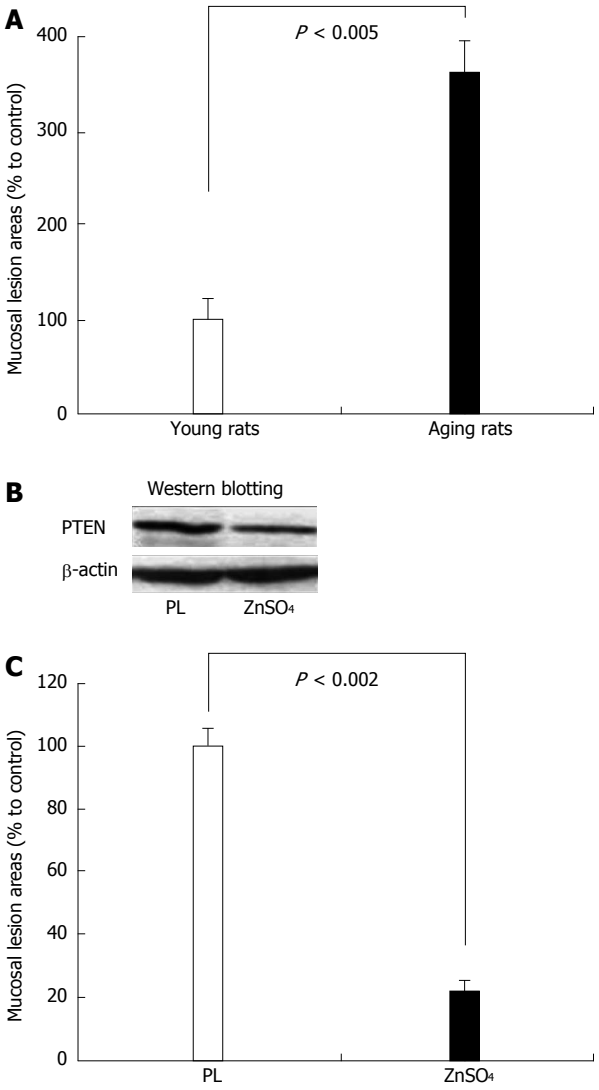

Figure 11 Extent of ethanol-induced gastric mucosal injury in young and aging rats. A: Three hours after intragastric administration of 8 mL/kg of 50% ethanol, gastric mucosal injury is significantly increased in aging rats *vs* young rats; B: Intragastric administration of $ZnSO_4$ for 4 h (2 mL 0.5% solution) downregulates phosphatase and tensin homologue deleted on chromosome ten (PTEN) protein expression in gastric mucosa of aging rats *vs* placebo control (PL); C: Intragastric administration of $ZnSO_4$ to aging rats for 4 h completely reverses the increased susceptibility of gastric mucosa to ethanol-induced injury indicating a causal relationship between PTEN and mucosal injury. Reproduced with permission from Tarnawski *et al*[1].

istration of $ZnSO_4$ completely reversed the increased susceptibility of gastric mucosa of aging rats to ethanol-induced injury[1] (Figure 11B and C).

We also tested human relevance of these experimental findings. These studies[1] demonstrated that gastric mucosa of aging humans has increased expression of PTEN; and, reduced expression of survivin, anti-apoptotic and mitosis-promoting protein (Figure 12)[1], which is a major target for NSAIDs-induced gastric mucosal injury[36,37].

Other mechanisms of impaired gastric mucosal defense in aging gastric mucosa

Other abnormalities including reduced telomerase activity[39], cellular senescence[40] and increased lipid peroxidation also significantly contribute to impaired gastric mucosal defense and increased susceptibility to injury of aging gastric mucosa. Shortening of telomeres or loss of telomere function seen during aging, results in activation of DNA damage checkpoint responses[39]. In aging gastric mucosa these events result in a biologically irreversible state of cell-growth arrest or cellular senescence[40], which increases susceptibility to injury from damaging agents.

In addition, our recent study in rats identified in aging gastric mucosa reduced expression of vascular endothelial growth factor (VEGF)-which is a pro-angiogenic factor and protects gastric endothelial cells[41]. Our subsequent study showed that reduced VEGF expression in aging gastric mucosa is mediated by the downregulation in gastric endothelial cells of importin-α, nuclear transport protein essential for transport of transcription factors to the nucleus[42].

One of the potential factors and targets operating in aging may be Klotho-a membrane protein related to β glucuronidase. Mutation of this protein has been associated with human aging and circulating levels of Klotho protein decline with age[43]. Klotho deficient mice have many features of human premature aging syndrome-progeria[43,44]. Klotho overexpression in mice extended lifespan of mice by 19%-31% *vs* normal mice[44]. A study examining Klotho expression in mice demonstrated that in normal mice Klotho is expressed in the stomach, mainly in the myenteric plexus and the loss of Klotho (in homozygous Klotho$^{-/-}$ mice) causes depletion of interstitial cells of Cajal and their progenitors resulting in gastric motor dysfunction[45]. Our preliminary data indicate that in contrast to Klotho deficient mice with premature aging syndrome, in normally aging rats Klotho expression in gastric mucosa in epithelial and vascular compartments is similar to that in young rats.

A summary of structural, functional and biochemical abnormalities of aging gastric mucosa is presented in Table 1.

DISCUSSION

Detailed analysis of the above changes indicates the following sequence of events taking place in aging gastric mucosa: (1) reduced mucosal blood flow and impaired oxygen delivery causes hypoxia, which leads to activation of the egr-1 transcription factor; (2) Activated egr-1 in turn upregulates PTEN, which induces cleavage-mediated activation of the pro-apoptotic proteases, caspase-3 and caspase-9[1]. In addition, upregulated PTEN exerts a pro-apoptotic action by reducing expression of the anti-apoptosis protein, survivin; and (3) This imbalance between pro- and anti-apoptosis factors results in increased apoptosis and increased susceptibility to injury[1].

We also tested human relevance of this concept and demonstrated increased expression of PTEN and reduced expression of survivin in human gastric mucosa of aging individuals[1]. This clearly indicates the human relevance of our experimental findings and also can ex-

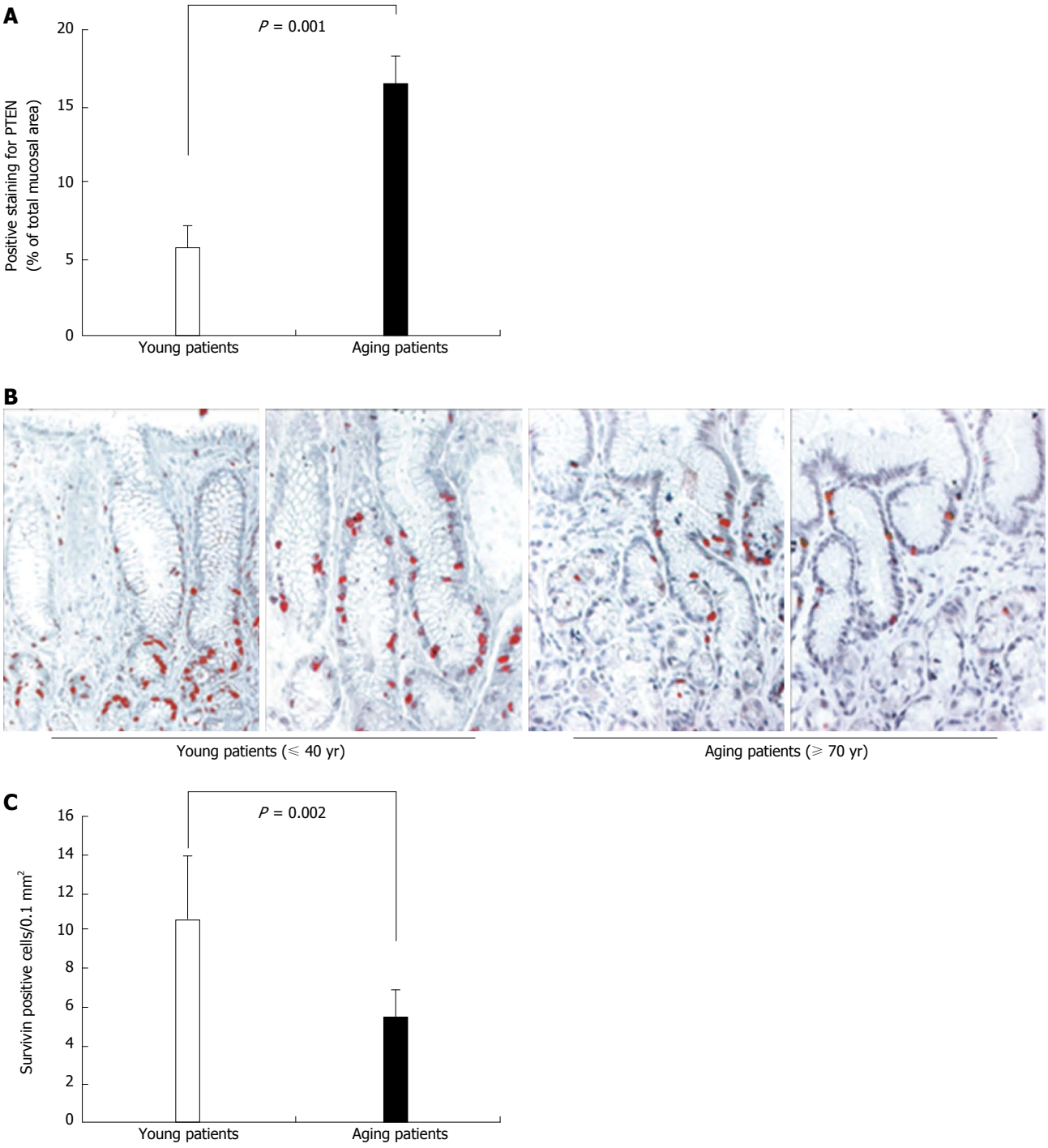

Figure 12 Human relevance: expression of phosphatase and tensin homologue deleted on chromosome ten and survivin in gastric mucosa of young and aging individuals. A: Quantification of phosphatase and tensin homologue deleted on chromosome ten (PTEN)-positive cells in gastric mucosal sections demonstrated a significantly increased number and significantly increased mucosal area of positively stained cells, mainly epithelial cells in aging (≥ 70 years of age) *vs* young patients. The threshold of positive staining was set at level 75 on scale 0-255. Reproduced with permission from Tarnawski *et al*[1]; B: Photomicrographs of representative sections of human gastric mucosa of young and aging patients immunostained for survivin[1]. In gastric mucosa of young patients (40 years of age and younger), survivin expression is strong (brown-red staining) in the nuclei of the progenitor cells; C: Quantification of the number of positively stained cells for survivin demonstrated significantly reduced survivin expression in the gastric mucosa of aging patients (70 years of age or older) *vs* young patients as reflected by significantly fewer positively stained cells. Reproduced with permission from Tarnawski *et al*[1].

plain the increased susceptibility of aging human gastric mucosa to injury as a consequence of the mechanisms leading to the imbalance between pro-apoptotic PTEN and anti-apoptotic survivin, which were established in our studies utilizing a rat model[1].

A subsequent study by another group[3] fully confirmed our findings including the basal atrophy of gastric glands in gastric mucosa of aging rats and the increased expression of egr-1, PTEN, and caspase-9 and also showed reduced mRNAs for CGRP and neuronal NOS.

Table 1 Structural, functional and biochemical abnormalities of aging gastric mucosa

Partial atrophy of gastric glands in the basal one-third of the mucosa and their replacement with connective tissue
Degenerative changes in parietal and chief cells and accumulation of disorganized collagen fibrils in connective tissue immediately adjacent to capillary blood vessels. The latter most likely interferes with transport of oxygen and nutrients
Reduced sensory innervation and abolished hyperemic response to mild and moderate irritants
Reduced bicarbonate and prostaglandin generation and secretion
Reduced (by 60%) mucosal blood flow and profound hypoxia of epithelial cells
Increased expression of egr-1 and its transcriptional activity-most likely responsible for activation of the *PTEN* gene
Increased expression of PTEN mRNA and protein (pro-apoptosis protein) and reduced expression of survivin (anti-apoptosis protein); this imbalance results in increased apoptosis
Increased apoptosis
Other abnormalities: reduced telomerase activity, cellular senescence, increased lipid peroxidation, impaired hypoxia sensor in endothelial (and epithelial?) cells, increased reactive oxygen species, downregulated or mutated Klotho protein in some submucosal neural elements, and dysregulated mitochondrial-nuclear communication
Decreased importin-α expression in endothelial cells of gastric mucosa leading to reduced activation and expression of vascular endothelial growth factor, which is a pro-angiogenic factor and protects gastric endothelial cells

All the above changes underlie increased susceptibility of aging gastric mucosa to injury by a variety of factors including aspirin and other non-steroidal anti-inflammatory drugs, ethanol, ischemia/reperfusion and others, and these changes most likely impair injury healing. PTEN: Phosphatase and tensin homologue deleted on chromosome ten.

It is important to point out that the use of rats for the studies on aging has several advantages[1]. Fisher F-344 rats obtained from the National Institute on Aging (NIA, United States) have a very similar, almost identical, genetic background; and, we can analyze the entire mucosa and gastric wall in a standardized fashion, which is impossible in human endoscopic biopsy specimens[1]. Importantly, we can ensure the absence of *H. pylori* and viral infections because the F-344 strain is tested for these and the animals sent to investigators are *H. pylori* and viral free. Moreover, absence of damaging environmental factors, such as smoking or drugs (*e.g.*, NSAIDs) in rats eliminates possible confounding influences that are difficult, if not impossible, to completely control for with human biopsy specimens[1]. Therefore, the rat model utilized in our studies can differentiate aging-related mucosal changes from mucosal changes resulting from various environmental factors[1].

Investigating available mucosal protective agents we found that hydrotalcite (Al and Mg containing antacid) exerted a protective action on aging gastric mucosa in rats, similar to that afforded by $ZnSO_4$[1], and significantly reduced injury of aging gastric mucosa induced by ethanol and NSAIDs by preserving endothelial cells of mucosal blood vessels and epithelial progenitor cells[46]. Since hydrotalcite is clinically available in some countries (*e.g.*, China and most European countries), this finding may have an important clinical application.

As described above, there is increased apoptosis and increased executioner caspase activity in gastric mucosa of aging rats[1,3]. A recent study demonstrated that melatonin given for 3 wk significantly reduced caspase-3 levels in gastric mucosa of aging rats[47]. In another study, cell proliferation, telomerase activity and lipid peroxidation were examined in gastric mucosa of young and aging rats[48]. Telomerase activity was significantly reduced in aging *vs* young rats while lipid peroxidation was increased. Treatment with melatonin for 3 wk significantly increased telomerase activity and reduced lipid peroxidation in gastric mucosa of aging rats[48]. The authors concluded that melatonin may reverse changes in aging gastric mucosa by inhibiting the replicative cellular senescence through both a stimulatory effect on telomerase activity and a suppressive effect on lipid peroxidation[48].

Significance and clinical implications of increased susceptibility of aging gastric mucosa to injury

In many countries (*e.g.*, China, Japan, Western Europe and United States) the population is aging. For example, in the United States it is estimated that approximates 16% of the population will be ⩾ 65 years of age by 2020[49]. This aging population is increasingly using aspirin for cardiovascular and cerebrovascular events and/or prophylaxis; and, is using aspirin and other NSAIDs (the most widely used drugs worldwide) for arthritis and musculoskeletal ailments[29,50]. Clinical studies demonstrated that patients' age is a significant predictor of gastric injury and its complications[26,29]. In a long-term prospective study of 34701 arthritic patients, Laine *et al*[26] examined the risk factors for NSAIDs-associated upper gastrointestinal events. They found that an age of ⩾ 65 years was a significant predictor of NSAIDs-induced risk of bleeding, perforation, obstruction or ulcer and their complications. They concluded that age ⩾ 65 years, prior upper GI clinical events and low dose aspirin are main risk factors for these complications[26].

Another recent study[29] listed age > 70 years among the major factors associated with increased risk of upper GI complications in patients on a low dose aspirin prophylaxis/treatment. Since, aspirin and NSAIDs are the most widely used drugs worldwide and cause a higher rate of gastric complications in elderly patients, the issue of aging gastropathy has important clinical implications. Moreover, since gastric mucosal defense is impaired in aging, it is likely that injury caused by other noxious factors such as ethanol, bile reflux, chemotherapeutic agents, *etc.* is also increased in aging individuals. Importantly increased injury susceptibility of aging gastric mucosa can

be potentially reduced or reversed pharmacologically *e.g.*, by using prostaglandin analogs (misoprostol), Al-Mg containing antacids (hydrotalcite), and/or GI sparing novel NSAIDs[51], melatonin and others.

H. pylori and gastric mucosal defense in relation to aging

There is relatively little information pertaining to this topic and the available information is mainly related to peptic ulcer disease, GI bleeding, *H. pylori* and NSAIDs in relation to aging. The interactions and a molecular crosstalk between *H. pylori* and human gastric mucosa (without focus on aging) were recently reviewed by Ricci *et al*[52]. In general, the prevalence of *H. pylori* infection increases with age and is present in 40%-60% of asymptomatic elderly individuals and in more than 70% of elderly patients with gastroduodenal diseases[53-55]. Since gastric mucosal defense in aging individuals is impaired, not surprisingly peptic ulcer disease in elderly patients is an increasingly frequent occurrence[53-55].

The outcome of infection and its pathological consequences depend on the type of *H. pylori* (*e.g.*, Cag A+), duration of infection, changes in the gastric mucosa (*e.g.*, superficial gastritis, atrophic gastritis, metaplasia, dysplasia), gastric acid secretory status, and many other variables. *H. pylori* infection may temporarily activate cyclooxygenase 2 and, consequently, the generation of protective prostaglandins, which in experimental conditions may reduce acute mucosal damage by ethanol or acid[56]. However, most studies indicate that *H. pylori* infection (especially Cag A+) has a negative effect on gastric mucosal defense by reducing surface hydrophobicity, impairing mucin production rate, impeding the tightening of tight junctions between the surface epithelial cells in response to acid, disrupting the gastric mucosal "barrier" and inducing loss of survivin and a decrease in gastric cell viability[57-62].

A recent study[63] evaluated gastric mucosal "barrier" defects using confocal laser endomicroscopy and TEM in *H. pylori* (-) *vs H. pylori* (+) patients. In gastric mucosa (outside intestinal metaplasia) the paracellular permeability was significantly (18-fold) increased in *H. pylori* (+) patients *vs H. pylori* (-) patients[63]. After eradication of *H. pylori* the paracellular "barrier" dysfunction significantly improved indicating a causal relationship between *H. pylori* infection and gastric mucosal "barrier" dysfunction[63]. In intestinal metaplasia areas of the gastric mucosa, mucosal permeability was increased in both *H. pylori* (+) and *H. pylori* (-) patients[63]. In elderly patients using NSAIDs *H. pylori* infection is associated with an increased ulcer incidence[53-55,64-67] and *H. pylori* eradication reduces peptic ulcer incidence in NSAIDs users, especially those new to NSAIDs and within the Asian population[65]. The same paradigm applies to low dose aspirin users[66,67]. Therefore from the practical point of view *H. pylori* eradication should be recommended for elderly patients before starting chronic NSAIDs therapy and especially before instituting low dose aspirin therapy in *H. pylori* (+) patients with preserved gastric acid secretion[66,67].

While the precise effects of *H. pylori* infection on mucosal defense in aging gastric mucosa has not been examined, based on the existing experimental and clinical studies indicating that *H. pylori* impairs gastric mucosal defense, one can speculate that *H. pylori* infection will further decrease mucosal defense in aging individuals. The correlation between *H. pylori* infection and mucosal defense in aging stomach deserves in our opinion a separate editorial article.

Molecular abnormalities in aging-future directions

Previous and more recent studies identified hypoxia, increased reactive oxygen species, and abnormal expression of various factors such as PTEN, survivin, caspases 3 and 9, in aging gastric mucosa. However, the key switch that triggers these changes will need to be elucidated through future studies. It is conceivable, and perhaps very likely, that some of the impairments resulting from aging pertain not only to the gastric mucosa but also other tissues; and, that some key targets and mediators may be similar, *e.g.*, in endothelial cells from various tissues. While some cellular and molecular targets and mechanisms operating in aging tissues have been identified, *e.g.*, increased reactive oxygen species, mitochondrial dysfunction, reduced sirtuin 1, impaired nuclear-mitochondrial communication[68], deficiency of the anti-aging transmembrane protein, Klotho, dysfunction of the hypoxia sensor (HIF-1α)[42,69] and impairment of the metabolic sensor (AMPK)[42,69], the fundamental master switch triggering these events still remains elusive and requires further research.

CONCLUSION

Aging gastric mucosa-"aging gastropathy" has impaired mucosal defense and increased susceptibility to injury by aspirin, NSAIDs, ethanol and other injurious factors. In the last decade research uncovered novel mechanisms underlying impairment of mucosal defense in aging gastric mucosa including partial atrophy of gastric glands, reduced gastric mucosal blood flow with resulting profound hypoxia, increased expression of egr-1 and PTEN, reduced expression of survivin and significantly increased apoptosis due to increased expression of activated caspase-3 and caspase-9. Other abnormalities identified in aging gastric mucosa include reduced expression of growth factors (*e.g.*, VEGF), impaired hypoxia sensor, decreased telomerase activity, cellular senescence, and increased lipid peroxidation. These findings provide a better understanding of aging gastropathy, which because of an increasing aging population and increased use of aspirin and other NSAIDs (most widely used drugs) worldwide has major clinical implications and impact. While some cellular and molecular targets and mechanisms operating in aging tissues have been identified, the fundamental master switch triggering these events still remains elusive and requires further research.

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