Invited Review

Gastric mucosal injury and repair: effect of aging

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Summary. Although the gastric mucosa of healthy adult animals possesses the inherent capacity to promptly repair (often within 24 h) after a minor to moderate injury, aging appears to diminish its reparative capacity. At least two different repair mechanisms are thought to participate in full repair of the damaged gastric mucosa: the initial rapid process of mucosal restitution begins by migration of viable epithelial cells from gastric pits and glands; the subsequent slower process is replacement of lost cells by cell division. Intracellular events that regulate these processes are poorly understood, nor do we know how they may be affected by aging. However, evidence is accumulating which suggests that a number of gastrointestinal hormones/growth factors, most notably EGF and TGF-α may play a critical role in regulating gastric mucosal reparative processes. Since EGF and TGF-α exert their physiological actions by activating the intrinsic tyrosine kinase (Tyr-k) activity of their common receptor, the EGF-R, studies have been performed to assess the role of EGF-R Tyr-k in regulating mucosal reparative processes during aging. Recent data suggest that the age-related decline in mucosal repair after acute injury could in part be due to decreased activation of EGF-R Tyr-k. In addition, polyamines and prostaglandins are also thought to be involved in gastric mucosal reparative processes. Although the involvement of polyamines in gastric mucosal reparative processes during aging has not yet been studied, decreased mucosal prostaglandin levels in the aged are thought to be a causative factor for the increased susceptibility of the mucosa to injury. These and other relevant matters are discussed in the current review.

Key words: Aging, Mucosal cell proliferation, Gastric mucosal injury, Cytoprotection, Tyrosine kinases, EGF, TGF-α, Prostaglandins

Introduction

Unlike other organs (e.g. liver, pancreas and kidneys) where growth ceases with adolescence, gastrointestinal (GI) mucosal growth, including that of the stomach is maintained by constant cell renewal. This renewal in the adult mucosa is assured by sustained proliferation of the precursor cells. Resulting cell production compensates for cellular exfoliation from the surface epithelium which sustains the delicate balance necessary to maintain a constant number of cells in a healthy adult tissue.

Gastric mucosal surface epithelium plays an important role in protecting the mucosa not only from the deleterious effects of luminal acid and pepsin but also from various ingested drugs, particularly non-steroidal anti-inflammatory drugs (NSAID) and ethanol as well as from physical stress. Each of them either alone or in combination with others may induce gastric mucosal injury. However, over the past decade, a number of in vivo and in vitro studies have demonstrated that the gastric mucosa of healthy adult animals possesses a remarkable capacity for rapidly restoring epithelial continuity, often within 24 h, after minor to moderate injury (Yeomans, 1976; Svanes et al., 1982; Rutten and Ito, 1983; Ito et al., 1984; Critchlow et al., 1985; Ito and Lacy, 1985; Lacy et al., 1992; Paimel et al., 1993; Fligiel et al., 1994). At least two different mechanisms are thought to participate in full repair of the gastric mucosa after an acute injury. The rapid process of mucosal healing (termed «restitution»), which is essential to protect the underlying submucosa from digestion by luminal acid and proteases, begins within minutes after injury and is accomplished by migration of viable cells from areas adjacent to or just beneath the denuded areas (Svanes et al., 1982; Rutten and Ito, 1983; Ito et al., 1984; Paimel et al., 1993). A slower process is replacement of lost cells by cell division (Silen, 1987). The early mucosal reepithelialization or restitution occurs too rapidly to be accounted for by cell division (Svanes et al., 1982; Lacy and Ito, 1984), which in rats peaks 16-18 h after an acute mucosal injury (Yeomans,
In this communication, the age-related changes in gastric mucosal injury and regulation of subsequent reparative processes are described. In addition, recent observations on adaptive cytoprotection are also summarized.

**Aging and gastric mucosal injury**

The incidence of many GI dysfunctions, including gastric and duodenal ulcers, increases with aging (Geokas et al., 1985; Steinheber, 1985), which could partly be attributed to increased susceptibility of the gastric mucosa to various damaging agents together with impediment of the repair process. Since intake of aspirin and other NSAIDs are generally higher among the elderly, studies have been performed to determine the frequency of NSAID-induced injury in elderly subjects. Epidemiological data suggest that aging is associated with increased NSAID-induced gastric mucosal injury (Fries et al., 1989; Soll et al., 1991). Our investigation with hypertonic saline, which virtually eliminates the surface epithelium, revealed a 67% higher lesion index (percent damaged area) in the gastric mucosa of 24-month-old Fischer-344 rats than in their 4-month-old counterparts (Majumdar et al., 1989). Electron microscopic examination also revealed that although administration of hypertonic saline virtually eliminated the surface epithelium in both young and aged rats, the extent of injury in older animals extended beyond the surface epithelium (Edgerton et al., 1991). In aged rats, epithelial cells in the deeper parts of the gastric glands demonstrated severe swelling with vacuolization and disintegration of the cell organelles, with dying and dead cells (Edgerton et al., 1991). Aspirin and ethanol has also been shown to produce a greater number of lesions in aged than in young rats (Gronbech and Lacy, 1994; Lee and Feldman, 1994). Taken together, these observations suggest that aging is associated with reduced mucosal protective mechanisms that may predispose aged animals to mucosal injury. Although the underlying biochemical mechanisms for this increased susceptibility are poorly understood, several investigators have reported that gastric mucosal prostaglandin (PG) content decreases with aging in humans (Cryer et al., 1992a,b; Goto et al., 1992). Also, in rats gastric mucosal eicosanoid formation and synthesis of PGI2 have been shown to decrease with aging (Bunck et al., 1988; Greenberg et al., 1989). These results suggest that the decrease in PG content may be a causative factor for the increased susceptibility of the elderly to gastric mucosal injury. Recently, Gronbech and Lacy (1995) have monitored the gastric blood flow between young and aged rats in response to 1M NaCl, acid challenge and capsaicin for 60 min. Their data suggest that impaired mucosal defense and reduced restitution in aged rats is related to lack of hyperemic response caused by mucosal injury and H+ back diffusion, which is thought to be due to decreased density of calcitonin gene-related peptide (CGRP) in nerve fibres and prostaglandin biosynthetic capacity of the mucosa. Lee (1996) has recently demonstrated a significant decline in mucosal blood flow in aged Fischer rats, and has suggested this to be one of the causative factors in age-related decline of mucosal repair. In addition to the above mentioned factors, the incidence of Helicobacter pylori infection, which is recognized to be an important factor in duodenal ulcer recurrence, has also been found to be higher in older diabetes mellitus patients (Oldenburg et al., 1996). Aging by itself is also associated with a higher incidence of H. pylori infection (Oldenburg et al., 1996). Clearly, further studies are needed to evaluate the roles of various endogenous and exogenous factors in the age-related rise in the frequency of gastric mucosal injury.

**Regulation of mucosal reparative processes**

Although the gastric mucosa of healthy adult animals possesses a remarkable capacity to repair promptly after an acute injury, regulatory mechanisms which direct this repair as well as the potential modulating effects of aging are poorly understood.

**Mucosal restitution**

The process of early mucosal restitution, which is accomplished by cell migration, begins within minutes after injury and is completed within a few hours. In vitro studies using frog mucosa in the Ussing chamber have
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Control 4 Month 24 hr After Inj

1 hr After Inj 6 hr After Inj

1a
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Control

1 hr After Inj

24 Month

6 hr After Inj
demonstrated that the surface epithelium is restored within a few hours of exposure to 1M NaCl (Svanes et al., 1982; Paimel et al., 1993). A similar phenomenon was also observed in rats (Ito et al., 1984). Although similar studies have not been performed during aging, we have demonstrated that whereas in young rats gastric epithelium shows signs of re-epithelialization as early as 6 h after 2M NaCl-induced injury and is essentially complete by 24 h, in aged rats only intermittent surface cells can be seen 24 h after injury (Fig. 1,b). The partial re-epithelialization of the gastric mucosa of young rats that is found to occur within 6 h postinjury is thought to be the result of increased cell migration during this period. Aging appears to attenuate this process (Fig. 1a,b).

A number of growth factors, including bFGF (Mustoe et al., 1991; Paimel et al., 1993), intestinal trefoil factor (Ashwood et al., 1993; Hauser et al., 1993; Dignass et al., 1994; Playford et al., 1995) as well as EGF and TGF-α (Blay and Brown, 1985; Barrandon and Green, 1987; Polk et al., 1992; Tarnawski et al., 1992a,b), all of which stimulate cell migration, are thought to play a role in this process in healthy young animals. However, their role in mucosal cell migration during aging remains to be evaluated. Similarly, polyamines, which are known to be essential for cell proliferation (Pegg, 1988) and for normal repair of gastric and duodenal ulcers, have also been shown to stimulate IEC cell migration in vitro (McCormack et al., 1993) and thus may also play a role in the mucosal restitutional process.

In assessing the role of growth factors in mucosal restitutional process, Polk et al (1992) have demonstrated a marked rise in TGF-α, but not EGF, in the gastric mucosa of young rats within 30 min after an acute injury. They suggested that local production of TGF-α may have an important role in the early reparative phase of the gastric mucosa. In aged rats, we have also observed a marked rise in gastric mucosal TGF-α levels at 30 min after hypertonic saline induced injury (Relan et al., 1995a,b). Yet, mucosal restitution, as determined by re-epithelialization of the gastric mucosa, appears to be attenuated (Fligiel et al., 1994). Since TGF-α exerts its physiological action by activating the intrinsic tyrosine kinase of its receptor, EGF-receptor (EGF-R) (Ullrich and Schlessinger, 1990), we postulated that decreased activation of EGF-R tyrosine kinase may in part be responsible for the diminished mucosal repair in aging animals. To evaluate the role of EGF-R tyrosine kinase in the early reparative phase of the gastric mucosa, we have examined the time course changes in total and EGF-R tyrosine kinase activity in young adult rats over a period of 60 min after hypertonic saline induced injury (Relan et al., 1995b). Injury caused an immediate rise in both total and EGF-R tyrosine kinase activity in the gastric mucosa with the maximal stimulation occurring at 30 min after injury (Fig. 2). When gastric mucosal EGF-R tyrosine kinase activity was examined in young and aged at 30 min after injury, we have observed that the enzyme activity and the expression of the receptor protein in the gastric mucosa of both age groups were significantly increased at 30 min postinjury; however, the magnitude of stimulation was lower in aged than in young rats, when compared with the corresponding basal levels (Relan et al., 1995b). A similar phenomenon was also noted for phospholipase C activity (PLC) and tyrosine phosphorylation of PLC-γ (Relan et al., 1995b). Taken together, the results suggest that TGF-α is less effective in enhancing activation of EGF-R tyrosine kinase in aged rats. This could partly be attributed to increased...
sensitivity of the aged gastric mucosa to EGF-family of peptides such that low doses are stimulatory, whereas high doses of these peptides may inhibit the physiological actions of these peptides in aged rats. Basis for this hypothesis comes from our previous observation that administration of a relatively high dose of EGF, which causes a significant stimulation in mucosal proliferative activity and tyrosine kinase activity in the gastric mucosa of young rats, produces marked inhibition in aged animals (Majumdar and Arlow, 1989). Our recent studies further substantiate this observation. We have observed that in isolated gastric mucosal membranes from aged rats, the concentration of TGF-α required to achieve the maximal stimulation of EGF-R tyrosine kinase is about 1/1000th of that required to attain the same in young animals (Majumdar et al., 1996). Because injury causes a significant increase in TGF-α production in the gastric mucosa of both age groups (Relan et al., 1995a,b), it is likely that elevated levels of endogenous TGF-α may prevent the injury-induced stimulation of EGF-R tyrosine kinase in aged rats through an autocrine/paracrine mechanism. Another possibility could be that the maximal stimulation in mucosal EGF-R tyrosine kinase in aged rats after injury occurs at a different time than in young animals. Undoubtedly, further studies are needed to evaluate the participation of EGF-R and other intracellular events in early reparative phase of the gastric mucosa during advancing age.

Mucosal cell proliferation

Stimulation of mucosal cell proliferation that occurs subsequent to the restitutional process is also thought to be one of the crucial events for full repair of the damaged gastric mucosa. A number of observations support this postulation. Earlier, Willems et al. (1971) suggested that the early burst in gastric mucosal proliferative activity that occurs after feeding is the consequence of shedding of surface cells. Similarly, gastric mucosal injury/erosion produced by intragastric administration of hypertonic saline, 1-methyl-3-nitrosoguanidine (MNNG) or aspirin result in a prompt rise in mucosal proliferative activity, as evidenced by increased DNA synthesis (Furihata et al., 1984), thymidine kinase (Majumdar et al., 1989) and ornithine decarboxylase (ODC) activity (Furihata et al., 1984; Thirumalai et al., 1987; Edgerton et al., 1991). Also, we have reported that when gastric mucosal explants from MNNG-injured or control (vehicle treated) rats are maintained in organ culture for 36 h, ODC activity in both rises slowly during the first 9 h, whereas the ODC activity in explants from injured rats increases sharply, revealing a 900% increase above the control at 12 h, then decreases slightly over the next 24 h (Lans et al., 1990). Furthermore, it has been reported that EGF and TGF-α, both of which stimulate gastric mucosal cell proliferation (Johnson and Guthrie, 1980; Johnson, 1987; Rutten et al., 1993; Podolsky, 1995), accelerate gastric mucosal repair (Konturek, 1990; Konturek et al., 1992; Brzozowski et al., 1993). A similar phenomenon has also been reported for gastrin (Takeuchi and Johnson, 1979), an antral hormone that also stimulates oxyntic gland mucosal cell proliferation (Johnson, 1987). More recently, Babyatsky et al., (1996) have demonstrated that intragastric administration of trefoil peptides protects against ethanol- and indomethacin-induced gastric injury in rats. It has been suggested that trefoil peptides may contribute to surface mucosal defense. In addition, polyamines, which are required for cell proliferation (Pegg, 1988), have also been implicated in mucosal repair (Wang and Johnson, 1992; Brzozowski, 1993). Although the intracellular events that regulate gastric mucosal cell proliferation have not been fully elucidated, mucosal injury induced by either stress or indomethacin has been shown to stimulate the expression of a number of proto-oncogenes, including c-myc, c-fos and c-Ha-ras (Ito et al., 1990; Wang and Johnson, 1994), suggesting that they may be critically involved in the mucosal reparative process. Our recent data suggest that tyrosine kinases, and specifically the enzyme associated with EGF-receptor, may play a critical role in gastric mucosal regeneration at least partly through cell proliferation (Majumdar et al., 1989, 1996a,b; Majumdar, 1990). Tyrosine kinases, which are associated with receptors of a number of growth factors and products of many proto-oncogenes (Adamson, 1987; Ullrich and Schlessinger, 1990), are known to play an important role in proliferation, differentiation and transformation of cells (Hunter and Cooper, 1985; Yarden and Ullrich, 1988). That tyrosine kinases may play a role in gastric mucosal cell proliferation after injury came from our earlier observation, that in young rats, stimulation of gastric mucosal proliferative activity at 6 h after hypertonic saline induced injury was accompanied by a marked rise in mucosal tyrosine kinase activity and tyrosine phosphorylation of a number of mucosal membrane proteins, including a protein with M₅ of 170 kDa, that corresponds to the molecular mass of EGF-receptor (Majumdar et al., 1989). Moreover, this injury-induced stimulation of mucosal proliferative activity could be greatly attenuated by genistein (Majumdar, 1990), an inhibitor of tyrosine kinases. More recently, we have observed that the stimulation of gastric mucosal proliferative activity [as evidenced by proliferating cell nuclear antigen (PCNA)] in young adult rats at 24 h postinjury is accompanied by a rise in EGF-receptor tyrosine kinase activity and these

Fig. 3. Photomicrograph of the gastric mucosa from 4-month-old rats showing PCNA immunoreactivity (proliferative index) and the surface epithelium at 24 h after administration of water (control), 2M NaCl (injury), 2M NaCl and tyrphostin, and water and tyrphostin. Tyrphostin in 30% DMSO was administered (IP) 2 hours before the administration of water or 2M NaCl, and at 2, 4 and 12 hours after the administration of water or NaCl x 500. (From Majumdar et al., J. Lab. Clin. Invest. 129, 173-178, 1996; with permission).
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PCNA Immunoreactivity

Control

24 h-Injury

24 h-Inj + Tyrp

Tyrphostin 51
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Increases are also associated with extensive regeneration of the surface epithelium (Fig. 3). In addition, we have also observed that suppression of EGF-receptor tyrosine kinase activity by administration of tyrphostin-51 [an inhibitor with a greater specificity for EGF-receptor tyrosine kinase (Levitzki, 1992)] during the 24 h reparative period markedly attenuates the degree of mucosal regeneration (Fig. 3). These findings are analogous to our recent observations of increased colonic mucosal cell proliferation after administration of azoxymethane (Relan et al., 1995a), and are also similar to those reported for human and guinea pig keratinocytes in which tyrphostin had been shown to inhibit EGF-dependent proliferation (Dvir et al., 1991). Earlier studies have also demonstrated that gastric mucosal injury caused by either alcohol or hypertonic saline enhances the expression of EGF-R protein in the gastric mucosa (Majumdar, 1990; Tarnowski et al., 1992b). In our investigation, we have also observed a marked rise in EGF-receptor mRNA levels at 24 h after injury (Majumdar et al., 1996). However, this increase was not affected by tyrphostin (Majumdar et al., 1996), suggesting that tyrosine kinase activity of EGF-receptor rather than the level of the receptor is essential in promoting the regenerative processes of the gastric mucosa.

Little is known of how the regenerative processes of the gastric mucosa are affected by aging. Previously, we compared the early changes in gastric mucosal proliferative activity in young (4-month) and aged (24-month) Fischer-344 rats 6 h after intragastric administration of hypertonic saline. Mucosal injury caused a significant stimulation in proliferative activity in both age groups. However, the magnitude of mucosal proliferative activity could not be related to the degree of mucosal damage. Thus, although the extent of damage (percent damaged area) was higher in aged rats, the magnitude of stimulation in proliferative activity was considerably lower in these animals, compared to water-fed controls (Majumdar et al., 1989). This could not be attributed to severe mucosal damage by 2M NaCl since no ulceration or bleeding was observed in any of the animals (Majumdar et al., 1989; Edgerton et al., 1991; Figliel et al., 1994). Since the induction of proliferative activity is one of the crucial events in rapid repair of the mucosa after injury, we have suggested that the relatively small increase in mucosal proliferative activity in aged rats 6 h after hypertonic saline induced injury may result in diminished mucosal repair in these animals. In a further effort to evaluate the reparative process during aging, we have recently examined the degree of re-epithelialization of the mucosal surface in 4- and 24-month old Fischer-344 rats at 1, 6 and 24 h after hypertonic saline-induced injury (Fligiel et al., 1994). Our results reveal that whereas the injured gastric mucosa of young rats show signs of re-epithelialization as early as 6 h postinjury which is completed by 24 h, in aged rats only intermittent surface cells are seen at 24 h after injury (Fig 1a,b). At this time, the possibility that the healing process takes longer time in aged than in young animals cannot be totally disregarded. Detailed time course analysis of mucosal reparative processes are, therefore, essential to fully evaluate the age-related changes in mucosal repair.

In the gastric mucosa, proliferating mucous neck cells migrate upward or downward differentiating into various types of cells. During recovery from injury, surface cells are replaced by relatively undifferentiating cells. As dead cells and injured cells are sloughed off the surface, new surface cells differentiate into mature cells which exhibit the normal morphological and physiological characteristics. Therefore, it is conceivable that the age-related changes in epithelial cell differentiation may also affect the full repair of the gastric mucosa. Although no information is available on this aspect, indirect evidence suggests that aging diminishes the rate of cell differentiation in both gastric and small intestinal mucosa (Holt et al., 1985; Moshier et al., 1993). To evaluate whether aging may affect the rate of maturation/differentiation of gastric mucosal cells after injury, we have recently examined the relative amount and cellular distribution of mucins (the major structural component of mucous layer) by periodic acid Schiff (PAS) staining in 4 and 24 months old rats at 1, 6 and 24 h after hypertonic saline induced injury. We observed that whereas the young rats showed numerous positively stained cells in the mucous neck region and along the epithelial surface of the gastric mucosa already at 6 h after injury, aged rats showed only scattered cells with cytoplasmic staining along the neck and surface area; intensity of staining was also lower in aged than in young rats at 24 h after injury (unpublished observation). Clearly, further experiments are required to fully assess the age-related alterations in gastric mucosal cell differentiation during mucosal repair.

Adaptive cytoprotection

Although there is no information of whether aging affects gastric adaptive cytoprotection, we have briefly summarized the current information on this topic. Robert et al., (1983) described «adaptive cytoprotection» as the ability of the gastric mucosa to develop enhanced resistance to injury following exposure to a mild irritant. Gastric mucosal injury from the irritant would gradually improve and ultimately resolve with continued exposure to the irritant (Chaudhury and Robert, 1980; Robert et al., 1983). The dose and frequency of administration of the mucosal toxin profoundly determined the spectrum of mucosal injury and the efficiency of adaptation. For example, with higher doses of aspirin the relative severity of initial mucosal injury is greater as is the time between injury and subsequent resolution or adaptation (Graham et al., 1988). Early studies strongly suggested that mucosal adaptation was mediated by endogenous release of prostaglandins. Konturek et al., (1982) demonstrated that exposure of the gastric mucosa to mild irritants resulted in increased generation of prosta-
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