Bulgarian Academy of Sciences

Acta morphologica et anthropologica, 4
Sofia • 1997

Stress ulcer : a morphological approach

S. Arbak, F. Şen*, F. Ercan*, I. Alican***, N. Aslan**, B. Yeğen***, S. Oktay**, K. Berkman****

Departments of Histology and Embryology, Pharmacology**, Physiology***, Marmara University, Faculty of Medicine, Istanbul, Turkey*

The effects of starvation and cold-restraint stress on the rat stomach mucosa were investigated. Mucosal damage was evaluated using light, transmission (TEM) and scanning (SEM) electronmicroscopy. Light microscopy revealed hemorrhagic areas as well as cellular damage in the mucosa. At TEM level, severe degenerative changes were especially observed at parietal cells and chief cells of the fundic mucosa. SEM demonstrated a totally necrotic appearance of the mucosa at the ulcer region with widened gastric pits devoid of lining epithelium. We conclude that experimental stress conditions produce severe gastric mucosal damage.

Key words: stress ulcer, gastric mucosa, morphology, microscopy.

Introduction

In literature, it is mentioned that stress conditions cause severe damage in the gastric mucosa [1, 2, 3] which was thought to occur as a result of autonomic nervous system activation [2, 3, 4]. In our study, we want to investigate rat fundic mucosa in an experimental stress ulcer model at light, transmission and scanning electronmicroscopy and we want to relate these morphological observations to the ulcer pathophysiology.

Materials and methods

Wistar albino rats of both sexes (200-250 g) were fed a standard diet and water ad libitum. The control group (n=8) was deprived of food for 3 h. Animals in the stress group (n=13) were immobilized in plastic restraining devices at 2-4 °C for 3 hours following a 48 h of starvation period. After stress application, animals were decapitated. Fundus material was taken from the macroscopically defined ulcer regions. For light microscopy, fundus specimens were fixed in 10 % formalin solution. The sections from paraffin blocks (4-5 μm) were stained with Haematoxylin-Eosin and investigated at Olympus BH-2 light microscope. The stomach was also examined macroscopically to determine the ulceration index as described by S e n a y et al. [5].

For transmission electronmicroscopic (TEM) examination, tissue specimens were fixed in 5 % phosphate-buffered glutaraldehyde and postfixed in 1 % OsO_4 solution for 1 hour. Following Epon 812 embedding, semithin sections of 1 μm were stained with Azur B and examined at Olympus BH-2 light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and examined at a JEOL 1200 SX transmission electronmicroscope.

For scanning electronmicroscopy (SEM), tissue samples, fixed and dehydrated as mentioned above, were transferred into amyl acetate solution and then dried in a critical point dryer (Biorad). Specimens were coated with gold using a sputter-coater (Biorad 502 SC) and examined in a scanning electronmicroscope (JEOL 5200).

Results

Control group

Light microscopical examination of control group revealed a normal rat fundic mucosa. Intact surface mucous cells were columnar. Gastric pits were of the expected depth. Neck portion of the gastric glands consisted of mucous neck cells while their bases contained parietal cells, chief cells and enteroendocrine cells. Mucous neck cells were irregular in shape with a basally located nuclei. Parietal cells were pyramidal with one or two centrally located nuclei. Their cytoplasms were intensely eosinophilic. Chief cells were noticed with a rounded and intense basophilic cytoplasm (Fig. 1).

Fig. 1. Control group: normal state of rat fundic mucosa is observed. H. E. stain, ×132

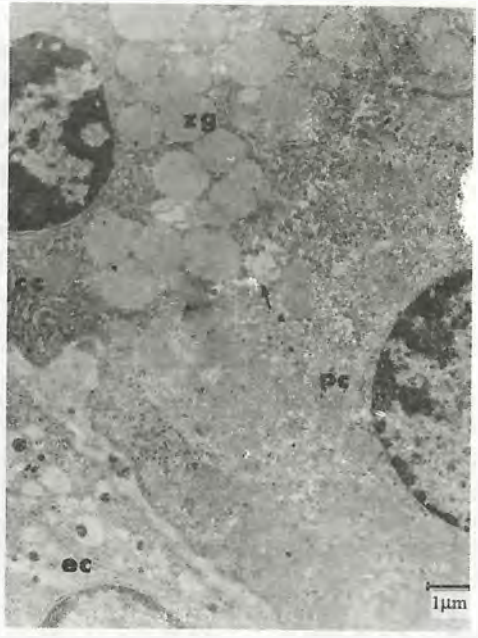

Fig. 2. Control group at TEM level: parietal cells (pc), chief cells (cc), endocrine cells (ec) reflect a normal ultrastructure.

Arrow – intracellular secretory canaliculi; zg – zymogenic granules

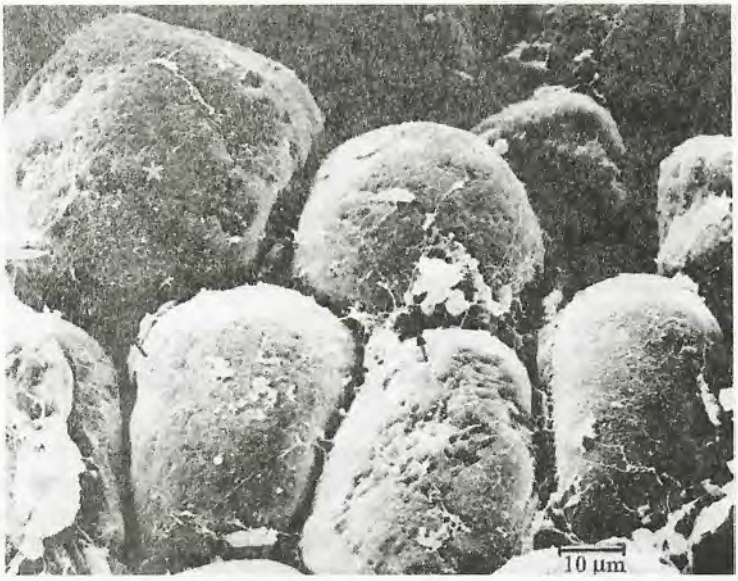

Fig. 3. SEM investigation of the control group: tightly bound polygonal surface mucous cells (*) and narrow openings to the gastric pits (arrow)

At TEM level, parietal cells were distinguished with their charactheristic intracellular secretory canaliculi lined by numerous microvilli. Adjacent to canaliculi, an extensive tubulovesicular system was observed. Numerous mitochondria were present around the intracellular secretory canaliculi. Chief cells represented typical protein-secreting cell ultrastructure with extensive granular endoplasmic reticulum and well-developed Golgi complex. Endocrine cells were within the fundic glands with their characteristic secretory granules. Granular endoplasmic reticulum and Golgi complex were sparse (Fig. 2).

Scanning electronmicroscopical investigation of the control group revealed closely packed, polygonal surface mucous cells and narrow opening to the gastric pits (Fig. 3)

Stress group

At light microscopy, stress group fundic mucosa showed a prominent cellular damage. Disrupted surface mucous cells were vacuolated and had pyknotic nuclei. In addition to luminal damage, cells lining the fundic glands were also disrupted and exfoliated (Fig. 4). In that group, distinct extravasated free erythrocytes both in lamina propria and luminal surface reflected an obvious hemorrhage which was also observed at muscularis mucosa, submucosa and muscularis layers indicating a true ulcer formation (Figs. 4, 5) which was confirmed by an ulcer index of 13,5—3,3 mm.

Ultrastructurally, at TEM level, we especially investigated parietal and chief cells. Pyknotic nuclei of parietal cells presented prominently enlarged perinuclear spaces (Fig. 6). In contrast to extremely widened intracellular secretory canaliculi reflecting an extreme acid secretion, tubulovesicular system was restricted (Figs 6, 7, 8). Numerous mitochondria around the canaliculi possessed degenerated cristae (Figs 6, 8). Chief cells' nuclei represented enlarged perinuclear spaces (Fig. 7).

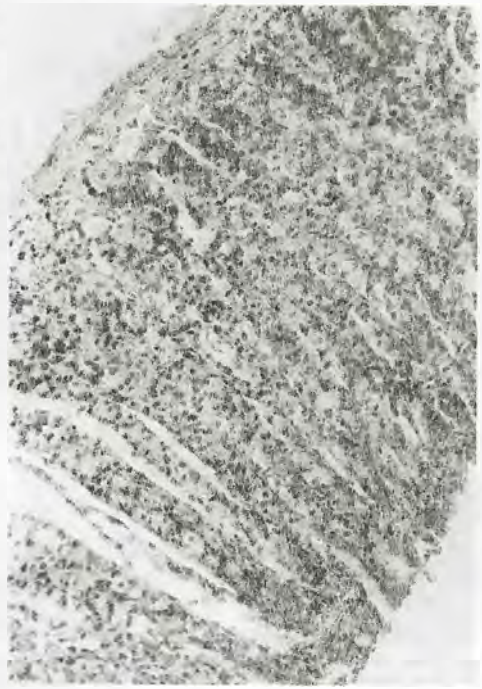

Fig. 4. Light microscopy of the stress group. H. E. stain, ×33

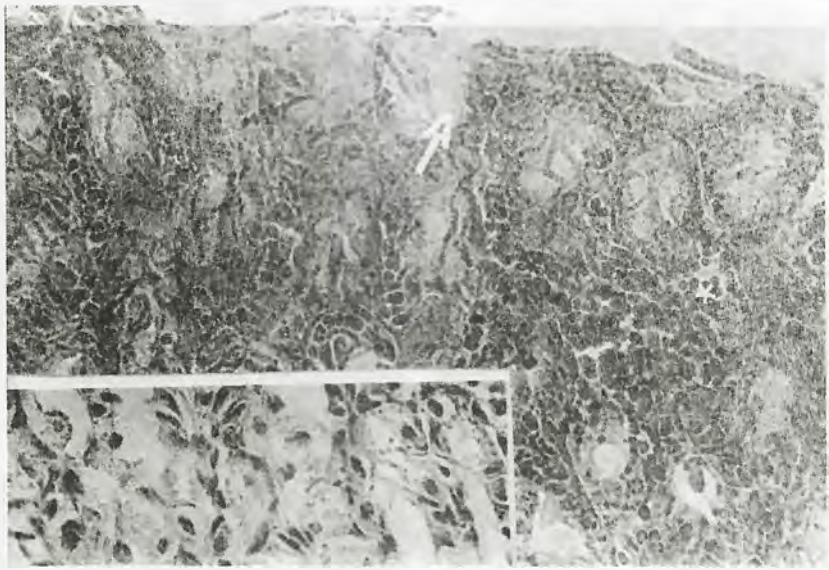

Fig. 5. Stress group light micrograph indicates disrupted surface mucous cells (arrow). Distinct free erythrocytes at the mucosa are seen. Inset: vacuolated glandular cells. H. E. stain, ×132

Fig. 6. Stress group at TEM level shows pyknotic nucleus (nu), mitochondrial crista damage (*), enlarged intracellular secretory canaliculi (arrow) at a parietal cell
ec — endocrine cell

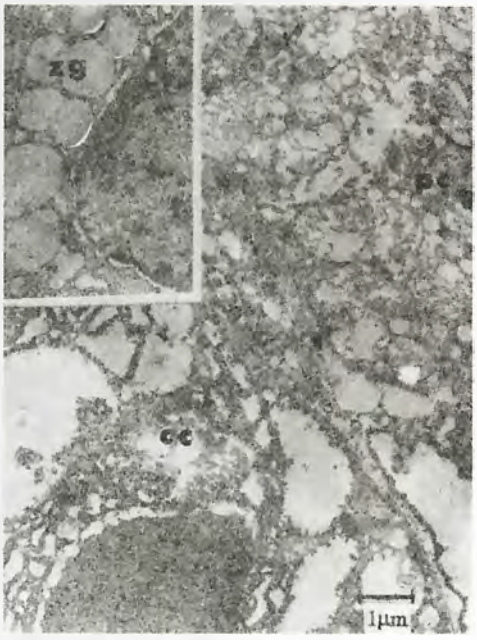

Fig. 7. TEM observation of the stress group: cellular damage at the parietal cells (pc) and chief cells (cc) is observed. Inset: zymogenic granules (zg) at a chief cell

Fig. 8. Stress group electronmicrograph: widened intracellular secretory canaliculi (*) and mitochondrial degeneration at a parietal cell

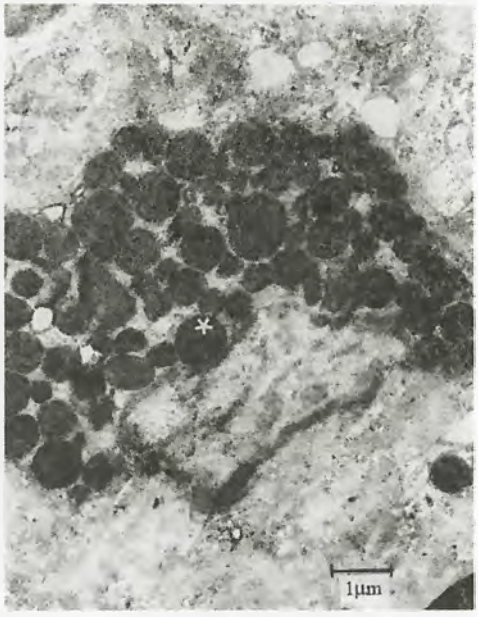

Fig. 9. Mast cell with numerous secretory granules (*) is observed at the stress group electronmicrograph

Fig. 10. Periphery of the ulcer zone; surface mucous cells were flattened with irregular cell borders. Wide openings to the gastric pits (*), few erythrocytes, necrotic area (arrow)

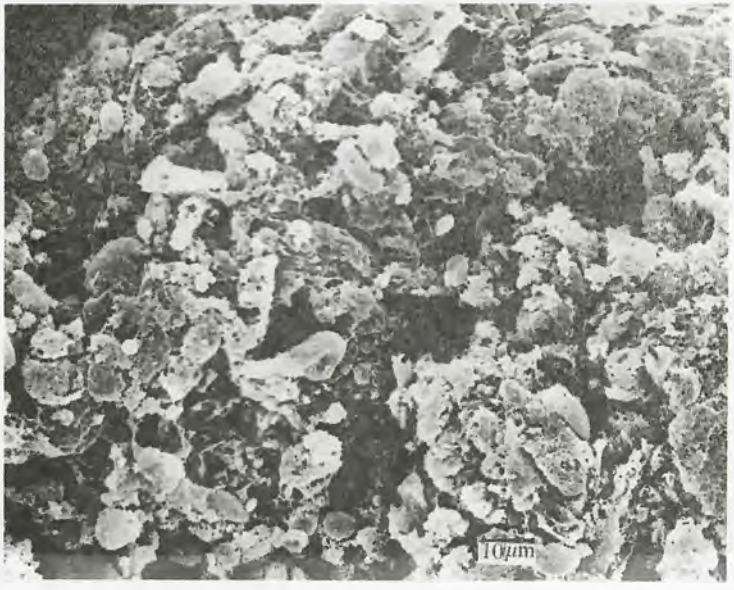

Fig. 11. Ulcer area; remnants of degenerated epithelial cells. Gastric pits (*) are widened and devoid of epithelium

Fig. 12. Hemorrhage area; Erythrocytes with distorted morpohology. Characteristic echinocytes (arrow) are present

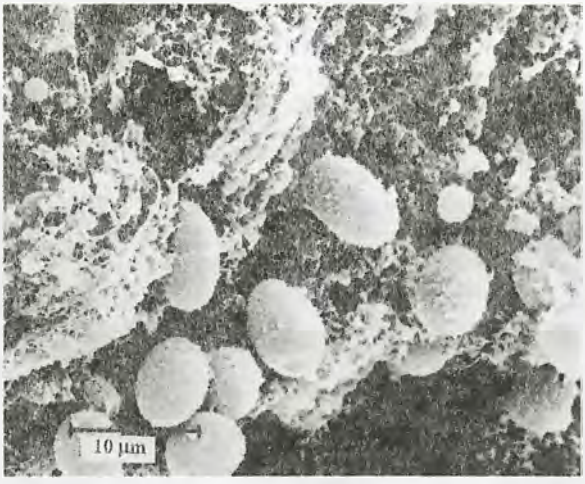

Fig. 13. Individual epithelial cells without cellular junctions. A characteristic fibrin coat (arrow) is present

Extremely dilated endoplasmic reticulum membranes reflected a high degree of cell damage. Abundant secretory granules filled the apical cell cytoplasm (Fig. 7). Endocrine cells were observed with their secretory granules. Their organelles did not reflect a cellular damage (Fig. 6). Mast cells, as a connective tissue component, were noticed with numerous granules which seemed to be increased (Fig. 9).

Scanning electronmicroscopical investigations revealed that:

a) In region with a normal appearance close to the ulcer area, flat epithelial cells and loose cellular interaction were observed. Few scattered erythrocytes were dispersed (Fig. 10).

b) In ulcer area, a totally necrotic appearance was dominant. Epithelial cells were no longer observed individually. Only degenerated remnants of cells were present. Gastric pits were observed widened and devoid of lining epithelium (Fig. 11). In the centre of ulcer, a hemorrhagic area was noticed with numerous erythrocytes of abnormal morphology (echinocytes) as a sign of extravasation (Fig. 12). A characteristic network of fibrin coat and individual epithelial cells without cellular junctions were also determined at the SEM observations of the ulcer group (Fig. 13).

Discussion

According to some investigators, stress ulcer formation is primarily mediated by a marked increase in gastric muscular contractility and ischemia [4, 6]. Parallel to the ulcer formation, both an increase in gastric lipid peroxidation level and a decrease in glutathione level were described [7]. Investigators mentioned the high degree of gastric mucosa cellular degeneration and related it to increased levels of lipid peroxidation as a result of stress application [7, 8, 9]. Calcium was also mentioned as an important point in the ulcer formation in which increased gastric muscle Ca^{++} levels were correlated with the magnitude of gastric lesions observed [10, 11].

We define the parietal and chief cells as the most affected cells of the stress group. Extremely widened intracellular secretory canaliculi of the parietal cells implied an extreme HCl secretion (Figs 6, 7, 8). While those canaliculi were swollen, tubulovesicular structures around them were restricted (Figs 7, 8). That morphological appearance was interpreted as a membrane transport form tubulovesicular structures toward the widened intracellular secretory canaliculi. In literature, that reverse correlatoin between them was mentioned [12]. Chief cells of the stress group were noticed with extremely dilated granular endoplasmic reticulum membranes. Apical secretory granules reflected their storage phase (Fig. 7). Those findings were correlated with literature mentioning an obvious increase in pepsinogen synthesis at the acidic medium. It could be explained that extreme HCl secretion by parietal cells might induce pepsinogen synthesis of the chief cells [13].

Some authors suggested that histamine release from mast cells could induce HCl secretion [9, 13, 14]. The mast cells with numerous granules at the stress group could support that suggestion (Fig. 9).

Hemorrhagic regions observed at light and scanning electron microscopical level were distinct especially at the upper gastic mucosa (Figs 4, 5, 10, 12). Those observations were parallel to the literature [15, 16]. We want to correlate that findings to the cellular damage observed at TEM level (Figs 6, 7, 8). A delay in vascular supply due to the hemorrhage could cause an ischemia and that may provoke the mentioned cellular degeneration.

As a conclusion, individual findings at the level of light, transmission and scanning electron microscopy were parallel and showed correlation with the physiologic and biochemical approaches of the experimental stress ulcer.

References

1. Tamatsu, S., N. Yamaguchi et al. Brain corticotropin-releasing factor acts as inhibitors of stress-induced gastric erosion in rats. — Life Sci., **47**, 1990, 925-932.
2. Hernandez, E. D., P. Morin, A. B. Salaiz et al. Brain ACTH prevents stress gastric lesions in rats. — Brain Res. Bulletin., **25**, 1990, 605-607.
3. Henke, P. G., R. M. Sullivan, A. Ray. Interactions of thyrotropin-releasing hormone (TRH) with neurotensin and dopamine in the central nucleus of the amygdala during stress ulcer formation in rats. — Neurosci. Lett., **91**, 1989, No 1, 95-100.
4. Kleiman, R. L., C. G. Adair, K. S. Ephagrave. Stress ulcers: Current understanding of pathogenesis and prophylaxis. — Drug Intell. Clin. Pharm., **22**, 1988, No 6, 452-460.
5. Senay, E. C., R. J. Levine. Synergism between cold and restraint for rapid production of stress ulcers in rats. — Proc. Soc. Exp. Biol. Med., **124**, 1967, 1221-1223.
6. Robert, A., F. W. Leung, D. G. Kaiser, P. H. Guth. Potention of aspirin-induced gastric lesions by exposure to cold in rats. Role of acid secretion, mucosal blood flow and gastric mucosal prostanoid content. — Gastroenterol., **97**,1989, 147-158.
7. Yeğen, B., A. Dedeoğlu, I. Aykaç, Oktay, Yalçin. Effect of cold-restraint stress on glutathione and lipid peroxide levels in the liver and glandular stomach of rats. — Pharmacol. Res., **22**, 1990, No 1, 45-48.
8. Mizuni, T., M. Doteuchi. Lipid peroxidation : A possible role in gastric damage induced by ethanol in rats. — Life Sci., **38**, 1986, 2163-2167.
9. Szelengi, I., K. Brune. Possible role of oxygen free radicals in ethanol-induced gastric mucosal damage in rats. — Digestive Dis. Sci., **33**, 1988, 865-871.
10. Koo, M. W., Ch. Cho, Cw. Ogle. The antiulcer effect of verapamil in relation to gastric calcium levels in stressed rats. — Pharmacol. Biochem. Behav., 1989, 34-73.
11. Szeleng, I. Calcium, histamin and pentagastrin: Speculations about the regulations of gastric acid secretion at cellular level. — Agents Actions, **10**, 1980, 187-190.
12. Junqueira, L. C., J. Carneiro, R. O. Kelley. Basic Histology, 6th Ed. Appleton and Lange, 1989.
13. Johnson, R. L. Physiology of Gastrointestinal Tract. 2nd Ed. New York, Raven Press, 1987.
14. Otani, K. S., Yamaura, Y. Yuda et al. Histochemical studies on the anti-ulcer effect of bamboo grass in rats. — Int. J. Tissue React., **12**, 1990, No 6, 319-332.
15. Brant, E. V., L. S. Karmen, S. Gregory et al. Prostaglandin-induced gastric mucosal protection against stress injury. — Ann. of Surg., **209**, 1989, No 3, 289-296.
16. Van Ritter, C., Frcs. Hinder, L. G. Oosthuizen et al. Gastric mucosal lesions induced by hemorrhagic shock in baboons. — Digestive Dis. and Sci., **33**, 1988, No 7, 857-864.