

## Factors influencing intercellular spaces in the rat esophageal epithelium

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### Abstract

**AIM:** To evaluate the effect of acute stress, hydrochloric acid, ethanol, aspirin, and prednisolone on the intercellular spaces of the esophageal epithelium.

**METHODS:** Part I, male Sprague-Dawley rats were randomly divided into eight groups and treated with the damaging or control factors. The esophagus of each rat was macroscopically inspected. Histological changes in mucosal biopsies were examined by light microscopy, and the widths of intercellular spaces were determined by transmission electron microscopy (TEM). Part II, in part I, we found that acute stress and aspirin induced dilated intercellular spaces (DIS) of the esophageal epithelium. Therefore, the effect of acid suppression pretreatment with esomeprazole on esophageal epithelial DIS induced by water immersion and restraint stress (WRS) and aspirin was further investigated to determine the association of DIS with acid reflux. After administration of 0.9% sodium chloride solution or esomeprazole solution orally for five days, rats underwent WRS or intragastric administration of aspirin solution. Esophageal epithelial intercellular spaces were investigated by TEM.

**RESULTS:** (1) The five damaging factors produced no lesions or inflammation in esophageal mucosa of rats under either gross or routine histological inspections. Esophageal epithelial intercellular space diameters in stress and aspirin groups were significantly greater, nearly three or two-fold respectively, than those in their corresponding control groups (stress model:  $0.38 \pm 0.05 \mu\text{m}$  vs  $0.13 \pm 0.02 \mu\text{m}$ ,  $P < 0.01$ ; aspirin model:  $0.32 \pm 0.12 \mu\text{m}$  vs  $0.19 \pm 0.05 \mu\text{m}$ ,  $P < 0.01$ ). Neither intragastric administration of hydrochloric acid or ethanol, nor hypodermic injection of prednisolone produced DIS compared with their corresponding control groups (hydrochloric acid model:  $0.24 \pm 0.03 \mu\text{m}$  vs  $0.19 \pm 0.05 \mu\text{m}$ ,  $P > 0.05$ ; ethanol model:  $0.25 \pm 0.10 \mu\text{m}$  vs  $0.19 \pm 0.05 \mu\text{m}$ ,  $P > 0.05$ ; prednisolone model:  $0.20 \pm 0.03 \mu\text{m}$  vs  $0.14 \pm 0.03 \mu\text{m}$ ,  $P > 0.05$ ); and (2) No significant difference in the intercellular space diameters was observed between the group pretreated with esomeprazole and the control group, in both the stress and aspirin models (stress model:  $0.35 \pm 0.05 \mu\text{m}$  vs  $0.37 \pm 0.05 \mu\text{m}$ ,  $P > 0.05$ ; aspirin model:  $0.24 \pm 0.02 \mu\text{m}$  vs  $0.27 \pm 0.03 \mu\text{m}$ ,  $P > 0.05$ ).

**CONCLUSION:** Acute stress and aspirin can induce DIS of the esophageal epithelium in rats, and it is not correlated with acid reflux.

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**Key words:** Esophagus; Dilated intercellular spaces; Stress, acute; Aspirin; Reflux, acid

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## INTRODUCTION

Gastroesophageal reflux disease (GERD) covers a broad range of symptoms and signs arising from the reflux of gastric contents into the esophagus. Gastric acid is the most important injury factor in the refluxate. In patients with GERD, only one third of those with heartburn have endoscopic evidence of erosive esophagitis (EE), and the remainder have non-erosive reflux disease (NERD). Mucosal damages are not visible under endoscopy in patients with NERD<sup>[1]</sup>. Several diagnostic tests have been established to diagnose GERD, but there is not a gold standard for diagnosing NERD. Many existing methods, such as ambulatory 24-h esophageal pH or bile monitoring, are not satisfactory due to their poor sensitivity, specificity, and reproducibility<sup>[2]</sup>. For example, about 50% of patients with NERD were found to have physiological values of acid exposure time with ambulatory 24-h esophageal pH monitoring<sup>[3-5]</sup>. Therefore, more effective methods need to be developed for the diagnosis of NERD.

Dilated intercellular spaces (DIS) have been observed in both the early stage of acid-perfused rabbit esophageal epithelium<sup>[6]</sup> and the esophageal biopsies of patients with GERD, including NERD and EE<sup>[7,8]</sup>. Studies reported that three or six months of acid suppression therapy with omeprazole led to a recovery of DIS in more than 90% patients with GERD, accompanied by regression of heartburn<sup>[9]</sup>. Thus, DIS has been considered as a feature of esophageal epithelial damage induced by gastric acid reflux, and serves as a marker for new methods to diagnose GERD, especially NERD<sup>[10-14]</sup>.

However, the specificity of DIS is questionable. In patients with other esophageal disorders, either with or without acid reflux, DIS was also observed, suggesting that refluxed acid is not the only factor that results in DIS<sup>[15,16]</sup>. A number of common damaging factors, such as ethanol, hydrochloric acid, nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and stress, have been known to induce stomach lesions. The aim of this study was to evaluate the effect of acute stress, hydrochloric acid, ethanol, aspirin, and prednisolone on the intercellular spaces of the esophageal epithelium in rats.

## MATERIALS AND METHODS

### Animals

Adult male Sprague-Dawley (S-D) rats, weighing 190-210 g, were used in the experiments. The animals were kept in individual cages, and provided with food and water *ad libitum*. They were housed in an air-conditioned room with a temperature of  $23 \pm 1^\circ\text{C}$ , humidity of 55%, and a 12 h light/dark cycle. All rats were acclimated under the same conditions for three days before study.

### Models

In part I we evaluated the effect of different damaging factors on the intercellular spaces of the esophageal epithelium in rats. Subsequently, the effect of esomeprazole pretreatment on esophageal epithelial DIS induced by

water immersion and restraint stress (WRS) and aspirin was further investigated (part II).

**Part I :** After a three-day acclimation, 45 rats were randomly divided into eight groups (five or six rats per group) and treated as follow: (1) Normal control group (NC): rats remained untreated in their home cage for seven hours, and subsequently were anesthetized using diethyl ether; (2) Normal saline (NS) intragastric administration group (NSIG): rats were anesthetized one hour after intragastric administration of 1 mL of 0.9% sodium chloride solution (NS); (3) NS hypodermic injection group (NSHD): rats were given hypodermic injection of 2 mL of 0.9% sodium chloride solution at cervical part once a day for four days, and anesthetized eight hours after the last injection; (4) WRS group: the four limbs of each rat were bounded on a board, and the rats were immersed in water in a head-up vertical position up to the level of the xiphoid process at a temperature of  $20 \pm 1^\circ\text{C}$  for seven hours before they were anesthetized<sup>[17,18]</sup>; (5) Hydrochloric acid intragastric administration group (HCLIG): rats were anesthetized one hour after intragastric administration of 1 mL of 0.7 mol/L hydrochloric acid; (6) Ethanol intragastric administration group (EIG): rats were anesthetized one hour after intragastric administration of 1 mL of 100% ethanol; (7) Aspirin intragastric administration group (AIG): rats were anesthetized one hour after intragastric administration of 1 mL of aspirin solution (300 mg/kg, dissolved in 2% sodium bicarbonate solution); and (8) Prednisolone hypodermic injection group (PHD): rats were given hypodermic injection of 2 mL of prednisolone acetate injection (250 mg/kg per day) at cervical part once a day for four days, and anesthetized eight hours after the last injection. All rats were fasted for 24 h but allowed free access to water before anesthesia. The esophagi and stomachs of rats were isolated, opened, and macroscopically inspected. Esophageal mucosal biopsies 0.5-1 cm above Z-line were then obtained to prepare paraffin sections for light microscopy and ultra-thin sections for transmission electron microscopy (TEM).

**Part II :** (1) WRS model: 12 rats were randomly divided into two groups averagely: control group (C) and esomeprazole group (E), which were given 0.9% sodium chloride solution (2 mL, once a day) or esomeprazole solution (5 mg/kg per day, dissolved in 2 mL of distilled water, once a day) orally for four days, respectively. Rats were then fasted for 24 h but allowed free access to water. On the 5th day, two hours after 0.9% sodium chloride solution or esomeprazole solution were administered, all rats underwent WRS for seven hours as described in part I, and were then sacrificed under ethyl ether inhalation. Esophageal mucosal biopsies 0.5-1 cm above Z-line were taken to measure the width of intercellular spaces with TEM; and (2) Aspirin model: 12 rats were divided into two groups, and treated as described in WRS model for the first four days. On the 5th day, rats received intragastric administration of 1 mL of aspirin solution (300 mg/kg, dissolved in 2% sodium bicarbonate solution) two hours after 0.9% sodium chloride solution or esome-

prazole solution were administered. One hour later, the rats were anesthetized using ethyl ether. Esophageal mucosal biopsies 0.5-1 cm above Z-line were taken to measure the width of intercellular spaces with TEM.

### Histology

Biopsies of esophageal mucosal tissues were fixed in formalin immediately after being removed from the rats, and were then embedded in paraffin wax. Serial 5  $\mu\text{m}$  sections were cut and stained with hematoxylin and eosin (HE) for routine histological evaluation by light microscopy.

### Transmission electron microscopy

Specimens of esophageal mucosae were fixed in glutaraldehyde immediately after being removed from the rats, rinsed, and processed for TEM. The specimens were post-fixed in 1% buffered osmium tetroxide. They were then dehydrated through a graded acetone series and embedded in Araldite. Blocks were trimmed, and ultra-thin sections on copper grids were post-stained with uranyl acetate and lead citrate. Each specimen was analyzed with a transmission electron microscope (JEOL JEM-1230) and then photographed at an accelerating voltage of 80 kV. Photographs of at least 10 fields were magnified at  $\times 5000$ .

Ten transmission electron photomicrographs of biopsy specimens from each rat were obtained. In particular, the suprabasal layer of the esophageal mucosa was examined in each image. Photographs with an internal scale marker were digitized and then each field was evaluated using Adobe Photoshop 7.0 software. According to Tobey *et al.*<sup>[7]</sup>, at least 10 randomly selected perpendiculars transects to adjacent membranes were drawn and measured in each image for a total of 100 measurements in each case. Each transect was drawn at a distance not closer than 1  $\mu\text{m}$ .

### Statistical analysis

The measurements obtained were used to calculate the mean intercellular space scores and the mean scores of the minimal and maximal intercellular spaces for each subject and for all cases as a whole. Scores are reported as mean  $\pm$  SD. Statistical significances were determined using one-way analysis of variance (ANOVA) and LSD-*t* test for comparisons among multiple groups, and using Student's *t*-test for unpaired samples between two groups. A *P*-value of less than 0.05 was considered statistically significant.

## RESULTS

### Effect of different damaging factors on intercellular spaces of the esophageal epithelium

Multiple congestion, erosions, ulcers, and hemorrhage in the gastric mucosa were observed grossly in the WRS, HCLIG, EIG, AIG, and PHD groups, while in the other three control groups the gastric mucosa remained intact with no apparent lesions. The esophageal mucosae of all the groups were also examined by gross inspection, and no evident inflammation or lesions were found in any rat. In addition, under light microscopy with magnifica-

**Table 1** Effect of damaging factors on intercellular space diameters of esophageal epithelium ( $\mu\text{m}$ , mean  $\pm$  SD)

Groups	Cases	Mean IS	Mean of minimal IS	Mean of maximal IS
NC	5	0.13 $\pm$ 0.02	0.09 $\pm$ 0.02	0.18 $\pm$ 0.03
NSIG	5	0.19 $\pm$ 0.05	0.10 $\pm$ 0.01	0.29 $\pm$ 0.12
NSHD	5	0.14 $\pm$ 0.03	0.09 $\pm$ 0.02	0.20 $\pm$ 0.02
WRS	6	0.38 $\pm$ 0.05 <sup>b</sup>	0.20 $\pm$ 0.06 <sup>b</sup>	0.64 $\pm$ 0.08 <sup>b</sup>
HCLIG	6	0.24 $\pm$ 0.03	0.14 $\pm$ 0.02	0.40 $\pm$ 0.10
EIG	6	0.25 $\pm$ 0.10	0.16 $\pm$ 0.07	0.37 $\pm$ 0.13
AIG	6	0.32 $\pm$ 0.12 <sup>d</sup>	0.19 $\pm$ 0.07 <sup>d</sup>	0.47 $\pm$ 0.17 <sup>d</sup>
PHD	6	0.20 $\pm$ 0.03	0.13 $\pm$ 0.02	0.30 $\pm$ 0.07

<sup>b</sup>*P* < 0.01 vs NC; <sup>d</sup>*P* < 0.01 vs NSIG. IS: Intercellular spaces; NC: Normal control group; NSIG: Normal saline (NS) intragastric administration group; NSHD: NS hypodermic injection group; WRS: Water immersion and restraint stress group; HCLIG: Hydrochloric acid intragastric administration group; EIG: Ethanol intragastric administration group; AIG: Aspirin intragastric administration group; PHD: Prednisolone hypodermic injection group.

tion of  $\times 100$  and  $\times 400$ , no histological evidences of inflammation (intraepithelial inflammatory infiltration, epithelial cellular swelling, submucous dropsy, telangiectasia, papillae elongation, or hyperplasia of the basal layer) were observed in the esophageal mucosa from all eight groups.

The mean and mean scores of the minimal and maximal intercellular space diameters of esophageal epithelium were determined using TEM (Table 1, Figure 1A-H). The mean intercellular space diameter in the WRS group was nearly three times greater than that in the NC group (0.38  $\pm$  0.05  $\mu\text{m}$  vs 0.13  $\pm$  0.02  $\mu\text{m}$ , *P* < 0.01). The mean intercellular space diameter in the AIG group was greater, by nearly two-fold, than that in the NSIG group (0.32  $\pm$  0.12  $\mu\text{m}$  vs 0.19  $\pm$  0.05  $\mu\text{m}$ , *P* < 0.01). The comparison of mean scores of the minimal and maximal intercellular space diameters showed similar results to the comparison of mean between WRS and NC groups, and AIG and NSIG groups. No difference in mean and mean scores of the minimal and maximal intercellular space diameters was observed between HCLIG and NSIG groups, EIG and NSIG groups, and PHD and NSHD groups (*P* > 0.05).

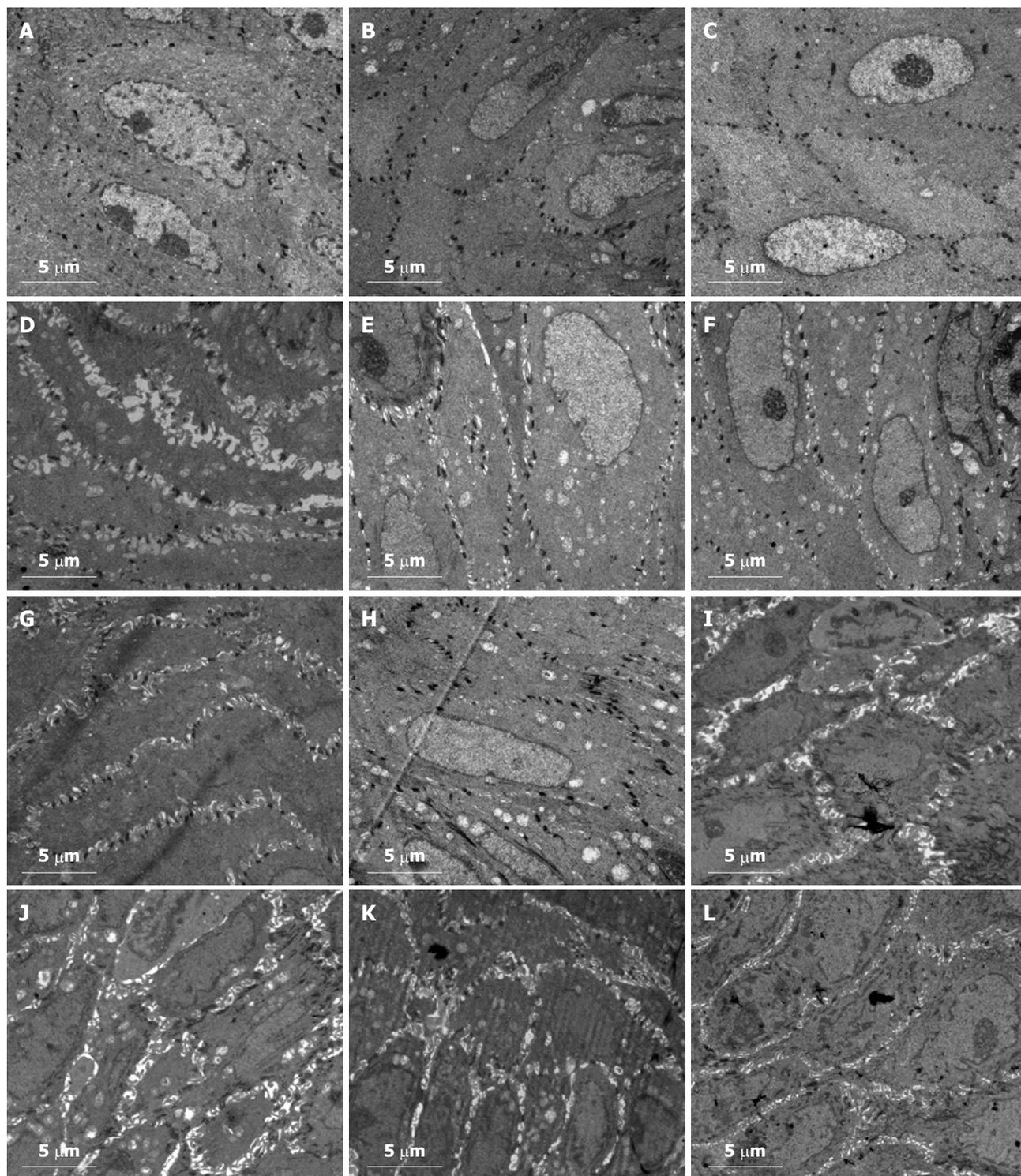
### Effect of esomeprazole pretreatment on esophageal epithelial DIS induced by WRS and aspirin

Esomeprazole inhibits gastric acid secretion with suppression of esophageal acid exposure, therefore the effect of esomeprazole pretreatment on esophageal epithelial DIS induced by WRS and aspirin was further investigated to determine the association of DIS with acid reflux.

In both the WRS model and the aspirin model, no significant difference in mean and mean scores of the minimal and maximal intercellular space diameters was observed between the group pretreated with esomeprazole and the control group (*P* > 0.05) (Table 2, Figure 1I-L).

## DISCUSSION

DIS of esophageal epithelium in patients with GERD was first reported 30 years ago<sup>[19]</sup>. It was later detected at



**Figure 1** Transmission electron photomicrographs of the esophageal epithelium, showing intercellular spaces in rats of different groups in Part I (A-H) and Part II (I-L) (magnified at  $\times 5000$  by transmission electron microscopy). A: Normal control group; B: Normal saline (NS) intragastric administration group; C: NS hypodermic injection group; D: Water immersion and restraint stress (WRS) group; E: Hydrochloric acid intragastric administration group; F: Ethanol intragastric administration group; G: Aspirin intragastric administration group; H: Prednisolone hypodermic injection group; I: NS + WRS group; J: Esomeprazole + WRS group; K: NS + aspirin group; L: Esomeprazole + aspirin group.

the early stage of acid-induced epithelial injury of rabbit esophagus<sup>[6,20]</sup>. Using TEM, Tobey *et al*<sup>[7]</sup> demonstrated that DIS was a feature of reflux damage to the human esophageal epithelium, irrespective of whether the patient had erosive or non-erosive disease. However, TEM is expensive and thereby difficult to apply to routine biopsies.

Scientists have been trying to establish an equivalent of TEM with light microscopy to detect DIS<sup>[21-23]</sup>. Although DIS can be seen under light microscopy, TEM has higher resolution and better quality of visualization. In 2003, DIS was shown to be an extremely sensitive marker of damage in GERD, duodenal gastro-esophageal reflux (DGER)

Table 2 Effect of esomeprazole pretreatment on esophageal epithelial DIS induced by WRS and aspirin ( $\mu\text{m}$ , mean  $\pm$  SD)

Damaging factors	Mean IS		Mean of minimal IS		Mean of maximal IS	
	Control group	Esomeprazole group	Control group	Esomeprazole group	Control group	Esomeprazole group
WRS	0.37 $\pm$ 0.05	0.35 $\pm$ 0.05	0.27 $\pm$ 0.05	0.25 $\pm$ 0.04	0.49 $\pm$ 0.05	0.45 $\pm$ 0.07
AIG	0.27 $\pm$ 0.03	0.24 $\pm$ 0.02	0.19 $\pm$ 0.02	0.18 $\pm$ 0.02	0.35 $\pm$ 0.05	0.32 $\pm$ 0.03

and NERD, and hence was thought to be the most appropriate marker to evaluate damage in NERD<sup>[24]</sup>.

In patients with GERD, contact with, and damage to, the esophageal epithelium by gastric acidic refluxate causes microscopic defects, such as DIS. DIS allows luminal acid to diffuse between the intercellular spaces, access the esophageal nociceptors in the intraepithelial sensory nerve endings, and trigger a signal by virtue of changing the pH in the intercellular environment, thereby eliciting symptoms of heartburn and pain<sup>[25]</sup>. Among the antisecretory drugs, proton pump inhibitors (PPIs) offer rapid symptomatic relief in the highest percentage of patients with GERD, due to their inhibition of gastric acid secretion with suppression of esophageal acid exposure. Studies demonstrated that three and six months of acid suppression therapy with omeprazole, respectively, led to a complete recovery of DIS in 92.1% and 97.4% of the patients with GERD, accompanied by regression of heartburn<sup>[9]</sup>. Similar results were also shown in other two studies<sup>[26,27]</sup>. In addition, recently studies have also shown that dilation of intercellular space diameter occurred along the distal and proximal esophageal epithelium in NERD patients and could be responsible for the enhanced perception of proximal acid reflux<sup>[2]</sup>. In an experimental model of non-erosive acid-damage esophageal epithelium in rabbits, DIS developed in association with, and as a marker of, reduced trans-epithelial resistance and increased shunt permeability<sup>[28]</sup>. All these findings indicate that DIS could be a sensitive histopathological marker of NERD and be related to acid reflux.

However, the specificity of DIS to diagnose NERD is questionable, as it is also present in patients without GERD but with other esophageal disorders. Takubo *et al.*<sup>[6]</sup> evaluated nine histological changes that have been previously described in the esophageal mucosa of patients with GERD, including DIS, and found that there was no significant difference in the incidences of DIS between the GERD and esophageal cancer patients. DIS was also found in children with esophagitis due to causes other than acid reflux (Candida infection, food allergy, and eosinophilic esophagitis)<sup>[15]</sup>. We designed this study to evaluate whether the common damaging factors, including acute stress, hydrochloric acid, ethanol, aspirin, and prednisolone, can induce DIS of the esophageal epithelium and investigated the association of DIS provoked by any of these factors with acid reflux.

Stress, ethanol, NSAIDs, and corticosteroids are common exogenous injury factors, while acid is an important endogenous injury factor. They have been known to induce damage to the gastric mucosa at certain densities and doses. In this study, lesions were grossly observed

in the gastric mucosa of rats under these conditions, but the esophageal mucosa remained intact, with no evident lesions or inflammation, under both gross and histological inspections. Possibly the contact time of gastric and esophageal mucosa with stimulants is different, or the sensitivities of different epithelial types to stimulants are different.

Stress has been known to induce all kinds of mental and physical disorders. Up to 60% of patients with GERD report an increase in symptoms related to stressful life events<sup>[4,29,30]</sup>. WRS has been widely used to induce gastric lesions in animals, providing a model system for studying stress-induced ulcers in humans<sup>[31,32]</sup>. This model involves elements of physical stress in addition to psychological stress. Factors known to play a role in the induction of gastric lesions by WRS include gastric acid, oxygen-derived free radicals, cytokines, and bioactive amines<sup>[33]</sup>. We found that the intercellular space diameter of the esophageal epithelium in the WRS group was nearly three times greater than that in the control group ( $P < 0.01$ ), suggesting that acute stress is able to induce DIS of the esophageal epithelium. This result also correlates with recent studies by Farré *et al.*<sup>[34]</sup>. Twenty percent to thirty percent of the patients treated with NSAIDs have symptoms in the digestive tract. NSAIDs are able to block cyclo-oxygenase-1 (COX-1) and inhibit the synthesis of physiological prostaglandins (PGs); or damage the mucosa of digestive tract directly as a poor organic acid. In our study, the intercellular space diameter of esophageal epithelium in the aspirin intragastric administration group was significantly greater than that in the NS intragastric administration group ( $P < 0.01$ ), indicating that aspirin could provoke esophageal epithelial DIS. Combined with the results showing there were no gross lesions or histological evidence of cell necrosis and inflammation in the esophageal mucosa, both in the stress group and the aspirin group, we speculate that DIS is an early and sensitive marker of esophageal damage, and appears before the changes observable in gross or detectable with routine histological inspections. In a recent study, Shebani *et al.*<sup>[35]</sup> showed increased intercellular space diameters in the epithelium in bronchial biopsies of asthmatics versus healthy controls, also suggesting that DIS might simply represent the early epithelial response to diverse insults, regardless of its nature and the type of epithelium.

Acid is the most important endogenous injury factor, and the major component is hydrochloric acid. Gastric mucosal injury induced by ethanol is considered "non-acid related" injury, because acid does not make the main contribution in the injury<sup>[36-38]</sup>. We could not detect significant differences in the esophageal epithelial inter-

cellular space diameters between the hydrochloric acid intragastric administration group and the NS intragastric administration group ( $P > 0.05$ ), or between the ethanol intragastric administration group and the NS intragastric administration group ( $P > 0.05$ ) in our study. This discrepancy is likely due to the dose and action time we used in our experiment. Possibly hydrochloric acid and ethanol were not refluxed or the refluxed time was too short under our conditions. Though gastric mucosal lesions were produced in rats, there were no evident lesions or inflammation either in gross or in routine histological inspections. In addition, the intercellular space diameters of the esophageal epithelium did not change. Further studies regarding continuous perfusion with hydrochloric acid or ethanol into the animal's esophagus are required to investigate the effect of hydrochloric acid and ethanol on intercellular spaces of the esophageal epithelium. Our study also showed that there was no difference in the intercellular space diameter between the prednisolone hypodermic injection group and the NS hypodermic injection group ( $P > 0.05$ ). However, other studies showed that incidence rates of peptic ulcers usually increase after treatment with corticosteroids<sup>[39]</sup>. We currently do not know the exact reason for this difference. It is possible that prednisolone has no effect on intercellular spaces of the esophageal epithelium. Further studies need to be conducted to determine the reason.

PPIs provide more effective acid suppression and are more effective at reaching target pH values  $> 4$  for  $\geq 18$  h per day<sup>[40]</sup>. Our study showed that there were no significant differences in intercellular space diameters of the esophageal epithelium of rats between the group pretreated with esomeprazole and the control group both in the WRS model ( $P > 0.05$ ) and the aspirin model ( $P > 0.05$ ). This suggests that pretreatment with esomeprazole had no effect on DIS of esophageal epithelium induced by acute stress or aspirin. DIS was still present after acid suppression, indicating that DIS induced by acute stress and aspirin is not correlated with acid reflux. Similar results were also observed by Bradley *et al.*<sup>[29]</sup>, who showed that acute stress did not increase esophageal acid exposure. Besides acid reflux, other noxious contents of the refluxate, such as bile, were reported to be able to impair mucosal integrity and provoke dilated intercellular spaces after short exposure of the esophageal mucosa to bile acid in both acidic and weakly acidic conditions<sup>[41]</sup>. Moreover, more than 50% of patients with NERD have normal esophageal acid exposure<sup>[4]</sup>. No difference in intercellular space diameters was observed between NERD pH-positive and pH-negative patients<sup>[8]</sup>. Therefore DIS is not related solely to acid reflux.

In conclusion, we find that acute stress and aspirin can induce DIS of the esophageal epithelium in rats, which appears before macroscopically observable changes and before changes detectable with routine histological inspection. This ultrastructural change provoked by acute stress and aspirin is not related to acid reflux, suggesting that other damaging factors can also induce DIS of esophageal epithelium. Therefore DIS is an early, sensitive, but non-specific, ultrastructural feature of NERD. These results

are beneficial for the diagnosis and differential diagnosis of NERD, and provide initial insight to the mechanism of this disease.

## COMMENTS

### Background

The incidence of gastroesophageal reflux disease (GERD) is rising rapidly. Symptoms of GERD arise from the exposure of increased acid gastric contents into the lower part of the esophageal mucosa. In patients with GERD, two-thirds of them have non-erosive reflux disease (NERD) with invisible mucosal damages under endoscopy. However, there is not a gold standard to diagnose NERD due to the poor sensitivity, specificity, and reproducibility of many existing methods. A prime candidate for this role is dilated intercellular spaces (DIS) in esophageal epithelium, which has been a recent research hotspot.

### Research frontiers

DIS has been considered as a feature of esophageal epithelial damage induced by gastric acid reflux, and serves as a marker for new methods to diagnose NERD. However, the specificity of DIS is questionable. In this study, the esophageal mucosal damage in response to various factors in rats, including acute stress, hydrochloric acid, ethanol, aspirin, and prednisolone, were evaluated.

### Innovations and breakthroughs

Recent studies have highlighted the importance of refluxed gastric acid in esophageal epithelial DIS. This study demonstrated that acute stress and aspirin can induce DIS of the esophageal epithelium in rats, and it appeared before changes that can be seen in gross and routine histological inspections. Furthermore, DIS induced by acute stress and aspirin was not correlated with acid reflux. Thus, our study suggests that DIS is an early, sensitive, but nonspecific, ultrastructural feature of NERD.

### Applications

By showing that DIS is not related solely to acid reflux, this study is beneficial for the diagnosis and differential diagnosis of NERD, and provides initial insight to the mechanism of this disease.

### Peer review

A well done investigation in rats that examines esophageal mucosal damage in response to various factors.

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