Mast Cell Stabilizator and Antioxidant Effects of Epidermal Growth Factor (EGF) on Gastric Mucosal Injury Induced by Ethanol in Rats

Serdar Erkasap¹, Nilufer Erkasap², Erinc Aral³, Tulay Koken⁴, Ahmet Kahraman⁴, Yasemin Aydin², Sezgin Yilmaz⁵, and Ersin Ates¹

¹Osmangazi University, Medical Faculty
Department of General Surgery
Eskisehir, Turkey

²Osmangazi University, Medical Faculty
Department of Physiology
Eskisehir, Turkey

³Osmangazi University, Medical Faculty
Department of Histology & Embryology
Eskisehir, Turkey

⁴Kocatepe University, Medical Faculty
Department of Biochemistry
Afyon, Turkey

⁵Kocatepe University, Medical Faculty
Department of General Surgery
Afyon, Turkey

Abstract

The role of epidermal growth factor (EGF), a polypeptide containing 53 amino acids, on protection and repair of ethanol-induced gastric mucosal injury was investigated in rats. In addition, the effects of EGF on the gastric damage were evaluated histopathologically. We used 48 Spraque-Dawley rats which were divided into three groups as control rats, ethanol treated rats and ethanol+EGF treated rats. The ethanol group was given a gastric gavage containing 1 ml of 80% ethanol (v/v) prepared in distilled water. EGF (100 µg/kg) was given by intragastric gavage 30 min before the administration of ethanol.

We studied histopathological evaluation and the histochemical heterogeneity of mast cells and its degree of degranulation. Besides, gastric tissue malondialdehyde (MDA), protein sulfhydryl groups (SH), and protein carbonyl levels were measured. EGF treatment stabilized mast cell degranulation and had lower polymorphonuclear leukocytes (PMNL) infiltration, ulcer index, histamine, and MDA; protein carbonyl levels were also lower, compared to the non-treated animals. EGF exerts a protective effect on gastric mucosa to ethanol-induced gastric injury probably through antioxidant and mast cell stabilizing mechanism.

Key Words: epidermal growth factor (EGF), ethanol induced gastric mucosal injury, mast cell, oxidative stress

Introduction

Successful healing of gastric ulcer depends on the enhancement of the defensive mechanisms or the decrease in the challenging forces leading to ulcer formation. Exposure of the gastric mucosa to a damaging agent such as ethanol may cause intensive injury with loss of surface epithelium, and denudation.
of lamina propria (23). A significant amount of histamine is released in the gastric mucosa after damage with concentrated ethanol (5). In addition, neutrophil accumulation in the gastric mucosa has been reported to play a significant role in gastric mucosal damage following ethanol administration. Increased neutrophil infiltration after ethanol treatment was reported to be a histamine mediated process via adhesion molecules expression and potentiation of chemoattractant on neutrophils (8). Histamine is also known to increase arteriolar vasodilation and capillary permeability, decrease total peripheral resistance and eventually to increase acute inflammation (12). Mast cells have been considered a major source of histamine in human gastric mucosa (3). Various stressful stimuli cause mast cell degranulation and release of the granular products, especially histamine, and which is known to be a major functional product of mast cells (27, 29). The ulcerogenic effect of histamine on the stomach of experimental animals has also been reported (21). Takeuchi et al. report that mast cell stabilizers protect the gastric mucosa against ethanol-induced gastric lesions (25). Besides, a high correlation was observed between mucosal histamine release and lipid peroxide production in LPS and ethanol induced gastric injury (10, 13).

Reactive oxygen species (ROS) are continuously produced during normal physiologic events and are removed by antioxidant defense mechanisms. In pathological conditions, ROS are overly produced and result in lipid peroxidation and oxidative damage (4). Some studies have revealed that ROS and lipid peroxidation are implicated in the pathogenesis of ethanol- and stress-induced gastric mucosal injuries in rats. (2, 7-9, 11, 20, 28).

The process of ulcer healing involves growth factors that appear to play a crucial role in the stimulation of reconstruction of damaged mucosal structures. Among these factors, the most relevant are epidermal growth factor (EGF), transforming growth factor alpha (TGFα), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) (15). Epidermal growth factor (EGF) is a polypeptide containing 53 amino acids (14, 17, 26). It is mainly produced by submaxillary gland (14, 17, 26) but can also be found in duodenal Brunner’s glands of rodents and human beings (26). EGF stimulates the restitution and proliferation of mucosal cells at ulcer margin, leading to reepithelization of the mucosal scar and reconstruction of glandular structure and acceleration of the healing process. However, the beneficial effect of EGF and its relation with mast cells still remain unknown.

So this study aims to examine mast cell stabilizing and antioxidant effects of EGF on ethanol-induced gastric lesions in rats. For this purpose, we studied the effects of EGF on histopathological evaluation, the histochemochemical heterogeneity of mast cells, its degree of degranulation, the subsequent histamine levels on ethanol induced gastric ulcer. In addition, the effects of EGF on protein carbonyl content, total protein sulfhydryl (SH) content and changes in lipid peroxidation have also been evaluated.

Materials and Methods

Sprague-Dawley rats (200-250 g) of either sex were used. They allowed drinking water ad libitum, but they were deprived of food for 12 h before the experiments. All procedures were performed in sterilized conditions. The rats were divided into 3 groups; group 1 (control) (n: 16): physiologic saline was given by gavage, group 2 (n: 16): 1 ml 80% ethanol (v/v) in distilled water was given by gavage and group 3 (n: 16): EGF (100 µg/kg) was given by gavage, 30 min before the administration of 1 ml 80% ethanol. All experimental procedures were based on the guidelines of the by Osmangazi University Ethic Committee, number: 193.

Biochemical Assays

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Animals were killed by cervical dislocation 1 h after the administration of ethanol from each group (n: 8), and their stomachs were removed and opened along the lesser curvature, rinsed with ice-cold saline then frozen at - 20°C until assay. The stomach tissues were homogenized in 0.1 M phosphate-buffer (pH 7.4) with an Ultra Turrax homogenizer (IKA T18 basic, Wilmington, NC, USA). The homogenates were centrifuged at 5000 rpm, at 4°C for 10 min. The lipid peroxidation level was monitored by determining the end products of lipid peroxidation, MDA, using thiobarbituric acid method (22). Protein sulfhydryl groups, which reflect antioxidant activity, were measured by using Ellman’s reagent 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). The thiol-disulfide interchange reaction between DTNB and thiol is the basis of this spectrophotometric assay (18). Protein carbonyls, resulting from the oxidation of proteins, were measured with dinitrophenylhydrazine, as described by Levin et al. (19). Tissue histamine levels were measured with use of a rat histamine enzyme-like immunoasorbent assay (ELISA) kit (IBL Immuno-biological Laboratories, Hamburg, Germany).

Morphological Examination

Animals were killed by cervical dislocation 1 h after the administration of ethanol from each group (n: 8), with stomachs removed and opened along the
The rats stomachs were pinned out flat in a standard position for macroscopic examination, and scoring of ulcer was performed with the help of a magnifying glass. Lesion size (mm) was determined by measuring each lesion; its greatest diameter was recorded in the case of petechial lesions. Five such lesions were considered to be the equivalent of a 1 mm ulcer. The sum of the total ulcer scores in each group of rats divided by the number of animals was expressed as the mean ulcer index (UI). The stomach tissue was fixed in 10% neutral formaline and embedded in paraffin. Sections from tissue blocks taken from ulcerated areas were stained with hematoxyline-eosin for routine histological examination.

**Light Microscopy and Histochemistry**

From paraffin blocks, 6 µm-thick sections were sliced in every 30 µm with a microtome. Mast cells were assigned to the one of following categories: 1) alcian blue-stained cells, showing only blue granules; 2) alcian blue and safranin-stained cells, showing a mixture of blue and red granules or granules of intermediate color; and 3) safranin-stained cells, showing only red granules. Mast cells are counted by using an eyepiece micrometer (OC-M) (X40, 2000 µm²) in selected sets of six serial sections of each animal. Polymorphonuclear leukocytes (PMNL) infiltration was evaluated, on sets of six serial sections stained with hematoxylin-eosin for each rat, by semiquantative scale graded from 0 to 2+ in which 0 denoted no infiltration, 1+ mild, 2+ severe infiltrations.

**Results**

Intragastric administration of ethanol-induced gastric damage in the rat illustrated in Fig. 1. Treatment of rats with EGF (100 µg/kg) prevented gastric mucosal ulcers induced by the administration of ethanol in Fig. 2. Ulcer indices (UI) and PMNL infiltration are shown in Fig. 3 (a) and (b), respectively. In the ethanol group, UI and PMNL infiltration were

![Fig. 1. Gastric mucosal damage found in the ethanol group superficial loss of normal mucosa (H & E, Orig. mag×33).](image1)

![Fig. 2. The mucosa containing normal gastric glands in the EGF treated group (H & E, Orig. mag×33).](image2)

![Fig. 3. (a) The effect of EGF on mean ulcer indices. *P < 0.05, significantly different from ethanol induced group (n:8); (b) The effect of EGF on PMNL infiltration. UI=2, *P=0.008, significantly different from ethanol induced group (n: 8).](image3)
significantly different from that of the control and EGF treated groups. Histamine level was significantly increased in the ethanol group according to control and EGF treatment groups. EGF treatment decreased histamine level to the control level (Fig. 4). Ethanol administration causes degranulation of mast cells but EGF treatment inhibited the degranulation of mast cells (Fig. 5 and Fig. 6). Histochemical heterogeneity, degranulation pattern, and the numbers of mast cells are reported in Table 1, and total numbers of blue and red stained mast cells were given in Table 2.

MDA is a metabolic product of lipid peroxide and carbonyl levels were significantly increased in ethanol group, compared with that of the control and EGF treatment. EGF treatment decreased MDA and carbonyl levels to the control levels (Fig. 7 and Fig. 8). Sulphydryl level increased significantly in the EGF treatment group, compared with that of the control and ethanol groups (Fig. 9).

**Discussion**

EGF has been shown to protect the gastric mucosa against injury induced by stress or various necrotizing agents such as absolute ethanol, hypertonic saline, and aspirin. EGF also enhances healing of experimental peptic ulcer (14, 16). Although the mechanism of its gastroprotective action is unclear, the trophic effect on gastro-duodenal mucosa, enhancement of mucus production, increase in gastric mucosal blood flow and anti-secretory activity are the probable mechanisms suggested (14). It can also decrease mucosal lipid peroxidation, as evidenced by lowered MDA levels (1).

Histamine is known to be released significantly in the gastric mucosa mainly from mast cells after damage by concentrated ethanol (5). It increases blood flow and vascular permeability during acute inflammation (8). In addition, it is implicated in
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microvascular dysfunction and gastric mucosal injury (6). It was also reported that acid back diffusion after barrier disruption is associated with an increased release of histamine (24). In this study we found that ethanol administration increased significantly tissue histamine levels. Besides, the numbers of blue-staining mast cells were found to be increased after ethanol administration. It indicated that ethanol increased the only histamine containing (blue-staining) mast cell degranulation. The increased level of mast cell degranulation was also confirmed by microscopic evaluation (Fig. 5). These results also correlated with the increased tissue histamine levels (Fig. 4). These observations support the principal role of mast cells in ethanol-induced gastric ulcer.

Although the effect of histamine on gastric injury has been widely investigated, the precise effect of EGF on mechanisms related to histamine has not been evaluated yet.

In the present study, we found that EGF treatment decreased the histamine level resulting from the inhibition of mast cell degranulation as evidenced by decreased number of blue-staining degranulated mast cells. It indicates that EGF is a potential mast cell stabilizer and the inhibition of mast cell degranulation contributes to the protection of gastric tissue against ethanol induced gastric injury.

Histamine stimulates the neutrophile infiltration evoked by several chemotactic factors through H2 receptors, which were located on the parietal cell membrane, and subsequently leading to increased lipid peroxidation (8). In this study, we found that ethanol increased gastric tissue MDA and carbonyl levels and decreased the sulfhydryl level. Ethanol is known to increase the xanthine oxidase (XO) activity which subsequently initiate the production of XO-derived oxidants and proinflammatory mediators from endothelium (4, 8). These mediators then activate neutrophile infiltration during pathological states. Reactive oxygen species play an important role in the pathogenesis of acute experimental gastric lesions (4, 8, 13, 28). ROS damages the cellular membrane through lipid peroxidation which leads to the release of lysosomal enzymes and further tissue damage (4, 13)

Tepperman et al. has previously shown that EGF was associated with a reduction in mucosal neutrophil infiltration (26). Our results showed that EGF treatment decreased tissue MDA, carbonyl levels and increased sulfhydryl levels. This result indicated that EGF has an antioxidant effect on gastric injury induced by ethanol administration. This effect might be mediated either by histamine or its direct antiinflammatory effect.

It has been suggested in this present study that, with the involvement of the protective mechanisms mentioned above, EGF treatment prevented gastric

Table 1. Histochemical heterogeneity of submucosal mast cell

<table>
<thead>
<tr>
<th>Groups</th>
<th>Red-Stained Mast Cells</th>
<th>Blue-Stained Mast Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Degranulated</td>
<td>Granulated</td>
</tr>
<tr>
<td>Control (n: 8)</td>
<td>1.0±0.62</td>
<td>6.5±1.2*</td>
</tr>
<tr>
<td>Ethanol (n: 8)</td>
<td>0.75±0.31</td>
<td>0.5±0.37</td>
</tr>
<tr>
<td>Ethanol+EGF (n: 8)</td>
<td>0.75±0.31</td>
<td>1.1±0.47</td>
</tr>
</tbody>
</table>

*; P < 0.05 significantly different from Ethanol and EGF treated groups  
**; P < 0.05 significantly different from Control and EGF treated groups  
***; P < 0.05 significantly different from Control and Ethanol groups

Table 2. Total submucosal mast cell numbers

<table>
<thead>
<tr>
<th>Groups</th>
<th>Degranulated</th>
<th>Granulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n: 8)</td>
<td>1.0±0.6</td>
<td>1.5±0.4</td>
</tr>
<tr>
<td>Ethanol (n: 8)</td>
<td>10.37±1.6*</td>
<td>2.37±0.49</td>
</tr>
<tr>
<td>Ethanol+EGF(n:8)</td>
<td>1.87±0.39</td>
<td>10.6±2.7**</td>
</tr>
</tbody>
</table>

*; P < 0.05 significantly different from Control and EGF treated groups  
**; P < 0.05 significantly different from Ethanol group

![Fig. 9. The effect of EGF on serum sulfhydryl level *P < 0.05, significantly different from ethanol induced group (n: 8).](image-url)
mucosal lesion both macroscopically and microscopically proven by significantly lowered ulcer indices and PMNL infiltration seen in ethanol rats.

In conclusion, the results of this study suggested that the gastroprotective effects of EGF on gastric lesions induced by ethanol could be related to its mast cell stabilizer and antioxidant effects.

References