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Effect of Oral TGF-A Formulations on ASA Induced Duodenal Ulcer and The Role of Lipid Peroxidation in The Healing Process

[Oral TGF-A Formülasyonunun ASA ile Oluşturulmuş Duodenal Ülser Üzerine Etkisi ve İyileşme Sürecinde Lipid Peroksidasyonun Rolü]

Çiğdem Özer[1],
Bilge Gönül[1],
Nevin Çelebi[2],
Gülay Yetkin[2],
Çiğdem Elmas[3],
Deniz Erdoğan[3]

[1] Faculty of Medicine, Department of Physiology, University of Gazi, Ankara, 06500, Turkey
[2] Faculty of Pharmacy, Department of Pharmaceutical Technology, University of Gazi, Ankara, 06510, Turkey
[3] Faculty of Medicine, Department of Histology and Embriology, University of Gazi, Ankara, 06500, Turkey

Yazışma Adresi
[Correspondence Address]

Çiğdem Özer, MD, PhD

Gazi University, Faculty of Medicine, Department of Physiology
06500, Beşevler/Ankara, TURKEY
Tel: +90 312 2024612
Fax: +90 312 2124647
E-mail: ozercigdem@yahoo.co.uk

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ABSTRACT

Objective: This study was designed to investigate the effects of aqueous or Microemulsion solution containing Transforming Growth factors-alpha (TGF-a) and/or administration of aprotinin on healing rate of duodenal ulcer induced by acetylsalicylic acid, and to examine the relationships between oxidative processes and TGF-a formulation during duodenal ulcer healing by measuring malondialdehyde, glutathione and total nitric oxide levels.

Methods: Male Wistar albino rats were divided into nine groups 1. Control , 2. Acute Ulcer, 3. Chronic Ulcer (untreated), 4. Physiological saline (PS) 5. PS containing TGF-α (10μg/kg) , 6. Microemulsion (ME), 7. Microemulsion containing TGF-α (T-ME), 8. Microemulsion containing aprotinin (AME) 9. Microemulsion containing TGF-α and aprotinin (T-AME). Acute duodenal lesions were induced by intragastric administration of acidified acetylsalicylic acid (150 mg/kg dissolved in 0.2 N HCl) to rats Ulcer areas were detected planimetrically. Tissue malondialdehyde, glutathione and total nitric oxide levels were measured by spectrophotometric methods The ultrastructural changes in duodenum observed by Transmission Electron Microscopy (TEM).

Results: T-ME and/or aprotinin application decreased the duodenal ulcer areas and malondialdehyde levels and increased glutathione and total nitric oxide levels. Histological evaluation indicated that the best regeneration was observed in T-ME and T-AME groups.

Conclusions: TGF-α in microemulsion dosage form accelerates the healing rate of duodenal ulcer and reduces oxidative stress.

Key Words : Duodenal ulcer, TGF-α, microemulsion, aprotinin, oxidant stress, TEM

Conflict of Interest: There is no conflict of interest among the authors who contributed to the present study.

ÖZET

Amaç: Çalışmanın amacı; Asetil salisilik asit ile oluşturulmuş duodenal ülserin iyileşme hızı üzerine Mikroemülsiyon içeren Transforming Growth factors alpha (TGF-a) ve/veya aprotinin verilmesinin etkilerini araştırmak, ve bununla birlikte duodenal ülser iyileşmesi süresince TGF-a formülasyonu ve oksidatif olaylar arasındaki ilişkiyi malondialdehit, glutatyon ve total nitrit oksit düzeyleri ile ilişkilendirerek incelemektir.

Gereç ve Yöntemler: Çalışmada Wistar albino cinsi erişkin erkek sıçanlar dokuz gruba ayrıldı.

1. Kontrol, 2. Akut ülser, 3. Kronik ülser (tedavi edilmeyen), 4.Serum fizyolojik (PS), 5.Serum fizyolojik içeren TGF-α(10µg/kg), 6.Mikroemülsiyon (ME), 7.Mikroemülsiyon içeren TGF-α (T-ME), 8. Mikroemülsiyon içeren aprotinin (AME), 9.Mikroemülsiyon içeren TGF-α ve aprotinin (T-AME). Akut duodenal lezyonlar intragastrik olarak asidifiye asetilsalisilik asit (150 mg/kg, 0.2 N HCl içinde) verilerek yapıldı. Ülser alanları planimetrik olarak ölçüldü. Doku malondialdehit, glutatyon ve total nitrik oksit düzeyleri spektrofotometrik olarak ölçüldü. Doudenumdaki yapısal değişiklikler Geçirgen Elektron Mikroskobu (TEM) ile değerlendirildi.

Bulgular: T-ME ve/veya aprotinin uygulaması ülser alanını küçülttüğü, malondialdehit düzeyini düşürdüğü ve glutatyon ve total nitrik oksit düzeyini yükselttiği bulundu. Histolojik olarak ise en iyi doku yenilenmesi, T-ME ve T-AME gruplarında gözlendi.

Sonuçlar: TGF-α' nın Mikroemülsiyon formları duodenal ülserin iyileşme hızını arttırdığı ve oksidatif stresi azalttığı bulunmuştur.

Anahtar kelimeler: Duodenal ülser, TGF-α, mikroemülsiyon, aprotinin, oksidatif stres, TEM

Çıkar Çatışması: Katkıda bulunan yazarların hiçbir çıkar çatışması bulunmamaktadır

Introduction

Use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as acetylsalicylic acid (ASA) is known to cause the impairment in mucosal defenses that are well recognized and clinically emphasized with respect to the gastrointestinal tract. NSAIDs are most often ascribed to the impairment of prostaglandin synthesis and the disturbances in mucosal blood flow and superoxide generation [1]. Ulcer healing is a complex process. The repair of gastric ulcer requires the reconstitution of epithelial structures and the underlying connective tissue, including vessels and muscle layers. Several growth factors were implicated in this process [2].

Growth factors and their receptors play important roles in cell proliferation, migration, tissue injury repair and ulcer healing. Among these factors TGF-α and epidermal growth factor (EGF) play important roles in the natural mechanism of wound healing. These growth factors are homologous peptides produced in the gastrointestinal tract and show a similar spectrum of biologic activities [3,4]. Also TGF-α and EGF are structurally related peptides that exert their biological activities through interaction with a common cell-surface receptor [TGFα/EGF receptor (TGF-α/EGFR)]. Experimental evidences have demonstrated that reactive oxygen species and lipid peroxidation are involved in the pathogenesis of gastric mucosal injuries by NSAIDs [5] and some stress forms [6-8]. Polyunsaturated fatty acids of cellular membranes are degraded by lipid peroxidation with subsequent disruption of membrane integrity, suggesting that lipid peroxidation induced by oxygen radicals is an important cause of the damage and destruction of cell mebranes [9]. Lipid peroxidation can also be evaluated by the measurement of malondialdehyde (MDA) [10] . Glutathione (GSH) is an endogenous antioxidant found in all animal cells. It reacts with free radicals and can provide protection from singlet oxygen, hydroxyl radical and O_2^- [11]. Several reports indicate that tissue injury, induced by various stimuli, is coupled with GSH depletion [12,13].

Nitric oxide (NO) plays an important regulatory role in maintaining gastric mucosal integrity. Cooperation between NO and prostaglandins in gastroprotection was reported previously [13]. In particular, NO interacts with neuropeptides and prostaglandins to maintain mucosal integrity in basal conditions [14]. The release of NO in the ulcerated mucosa contributes to tissue repair as demonstrated by the fact that treatment with NOS inhibitors delays such a process [15].

In basal conditions, nitric oxide (NO) plays an important regulatory role in maintaining gastric mucosal integrity via interacting with neuropeptides and prostaglandins [14]. The release of NO in the ulcerated mucosa contributes to tissue repair as demonstrated by the fact that treatment with NOS inhibitors delays such a process [15].

TGF-α can lose its biological activity in the ratio of 2-5 fold in gastric juice [16]. There have been several approaches to administration of peptides orally. One of these approaches is using microemulsion (ME) formulations to prevent the degradation of peptides in the gastrointestinal system. Due to their improved drug solubilization, and long shelf life, these microemulsions are used as drug delivery systems of peptides, which can be administered orally [17,18]. In this study, we thus used a ME dosage form of TGF-α, as previously described [5,19], to minimize the enzymatic degradation of TGF-α in gastrointestinal tract (GIT). Immunohistochemical studies have shown that, TGF-α immunoreactivity is found in duodenum, especially duodenal epithelium, Brunner's gland [20], villus enterocytes and may have an important role in mucosal healing [21].

Previous studies documented that intragastric (i.g.) administered ME containing TGF-α or TGF-α plus aprotinin, a non-specific serine protease inhibitor, increased TGF-α levels in rat duodenum tissue [22]. In addition we demonstrated that gastric acid secretion was significantly reduced by ME containing TGF-α or TGF-α plus aprotinin and also gastric mucus levels increased and gastric ulcer areas decreased remarkably in groups that were treated with TGF-α in ME [19].

This study was designed to investigate the effects of aqueous or ME solution containing TGF-α and/or i.g. administration of aprotinin on healing rate of duodenal ulcer induced by ASA, and to examine the relationships between oxidative processes and TGF-α formulation during duodenal ulcer healing evaluated by MDA, GSH and NOx (total nitrates and nitrites) levels.

Methods

Materials

ASA was donated by Bayer Türk Drug Manufacturer. mTGF-α was purchased from Sigma (USA). Labrafil M 1944 CS (unsaturated polyglycolysed glycerides) was donated by Gattefosse® (France). Arlacel 186 (glycerolmonooleate-propylene glycol) and Brij 35 (polyoxyethylene lauryl ether) were produced by ICI Pharmaceuticals® (England). Absolute alcohol was supplied by Riedel-de Haen (Germany) All other chemicals for biochemical evaluation were of the best quality available.

Methods

Preparation of the Microemulsion Formulation

The w/o ME was prepared by modifying the ME formulation which was described in previous studies [4,23]. Surfactant: co-surfactant ratio was chosen as 2.5:1.0. Oil phase were prepared using Labrafil M 1944 CS, Arlacel 186:Brij 35 [1,5], absolute alcohol and distilled water; surfactant, co-surfactant and aqueous phase, respectively. TGF-α (10μg/kg) was added into water phase 20.

Animals and Study Design

The following experiments were approved by the Ethics Commitee of Gazi University Medical Faculty for the care and use of laboratory animals (DHE 00-07). Male Wistar albino rats (n=83) weighing 200±20 g were used in the study. They were divided into nine groups consisting of 8-10 animals each and allowed free access to standart diet and water ad libitum. Acute duodenal lesions were induced by i.g. administration of acidified ASA (150 mg/kg dissolved in 0.2 N HCl) to rats which were fasted for 24 hr before the experiments but had free access to water. The design of the experimental animals groups are shown in Table 1.

Determination of Ulcer Score

The rats were killed by thiopental (50mg/kg) anesthesia at the end of experiments. The duodenum was removed. Lesion area was measured by planimetrically under a dissection microscope. Lesion area was determined by measuring each lesion along its greatest diameter under a microscope. Five such lesions were taken as the equivalent of a 1 mm^2 ulcer lesion area (ulcer score) [17]. The ulcer score was expressed as square millimeters.

Determination of the Lipid Peroxidation (MDA) and GSH Levels

Duodenum samples were obtained after measuring lesion areas and freezed immediately by a liquid nitrogen, than kept in –70° C deepfreeze until analyses were performed. MDA levels [24], and GSH levels were measured by spectrophotometric methods [25]. Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS). Briefly, tissue samples were homojenized in ice-cold trichloroacetic acid (1 g tissue in 10 ml 10% trichloroacetic acid) in a tissue homogenizer (Heideloph Diax 900, Germany). Following centrifugation of the homogenate at 3000 rpm for 10 min (Hermle Z 323 K, Germany), 750 µl of supernatant was added to an equal volume of 0.67% (m/v) thiobarbituric acid and heated at 100°C for 15 minutes. The absorbances of the samples were measured at 535 nm. Lipid peroxide levels are expressed in terms of MDA equivalents using an extinction coefficient of 1.56 x 10^5 mol^{-1} cm^{-1}.

The GSH levels were determined by a modified Ellman method [25]. Briefly, after centrifugation of homogenate at 3,000 rpm for 10 min. 0.5 ml of supernatant was added to the 2 ml of 0.3 M Na_2HPO_4 2 H_2O solutions. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. The GSH levels were calculated using an extinction coefficient of 13,600 mol^{-1} cm^{-1}.

Measurements of MDA and GSH were carried out at room temperature using a spectrophotometer (UV 1208, Shimadsu, Japan).

Determination of the total NOx levels

NOx levels were obtained from Elisa reader by vanadium chlorure (VCl_3)/Griess assay. Prior to NOx determination tissues were homogenized in five volumes of phosphate buffer saline (pH 7) and centrifuged at 2000Xg for 5 min. 0.25 ml 0.3 M NaOH was added to 0.5 ml supernatant. After incubation for 5 min. at room temparature, 0.25 ml of 5% (w/v) $ZnSO_4$ was added for deproteinization. This mixture was then centrifuged at

Table 1. Design of experimental animal groups

Code	Application
CONT	Healthy rats (n=8)
Ulcer groups	
AU	150mg/kg ASA suspended in 1.5 ml of 0.2 N HCl was administered i.g, (n=8)
CU	Untreated rats with ASA, (n=10)
PS	Physiologic saline solution without TGF-α was administered i,g. for 2 days (n=9)
T-PS	TGF-α, dissolved in PS (10µg/kg day) was administered i,g. for 2 days (n=9)
ME	Microemulsion (0.75ml/100g daily) without TGF-α was administered i.g. for 2 days (n=10)
T-ME	ME containing TGF-α (10µg/kg day) was administered i.g. for 2 days (n=10)
AME	ME containing aprotinin, 3000 KIU/ml was administered i.g.for 2 days (n=9)
T-AME	ME containing TGF-α and aprotinin was administered i.g.for 2 days (n=10)

ASA : Acetylsalicylic acid
CONT : Control
AU : Acute ulcer
CU : Chronic ulcer
TGF-α : Transforming Growth factors alpha
ME : Microemulsion

3000Xg for 20 min and supernatants were used for the assays. Nitrate standart solution was serially diluted. After loading the plate with samples (100 µl), addition of vanadium III chloride (VCl_3) (100 µl) to each well was rapidly followed by addition of Griess reagents, sulphanilamide (SULF) (50 µl) and N-(1-naphtyl) ethylenediamide dihyrochloride (NEDD) (50 µl). After incubation (usualy 30-45 min), absorbance of the samples were measured at 540 nm using an ELISA reader [26].

Electron microscopic studies

Evaluation was made by electron-microscopic examination of the duodenum specimens. Eight rats from each group were examined by TEM and one specimen was taken from each rat yielding eight specimens for each group. One cm of dissected duodenal parts was used for TEM and immunohistochemical experiments. Tissue samples were prepared for TEM. The pieces were placed in 2.5% glutaraldehyde and then in phosphate buffer saline post fixative 1% osmium tetraoxide. The specimens were firstly embedded in Dodecyenyl Succinic Anhydride (DDSA) + Araldyt CY 212 (1:1, v/v) for overnight at room temparature, then 24 hr at 40° C and 48 hr at 60° C. Thin sections were stained with lead citrate and uranyl acetate and were photographed using Carl Zeiss EM 900 electron microscope.

If degeneration criteria were observed more or less during the evaluation of duodenum slices, then degeneration was considered positive; however, if no degeneration criteria were observed it was accepted as negative. For example, if Rough Endoplasmic Reticulum (RER) sisternaes are seperated from each other, degeneration was accepted to be present without considering the amount of dilatation. Also wiping out of the crista, completely or partially and disruptions in their configurations partially or completely were accepted as signs of degeneration.

The duodenum sections obtained from the other groups were compared with the results of the control group in regard to degeneration parameters. The numbers for duodenum specimen having degeneration are shown quantitatively in Table 2. If there were no degeneration criteria observed, the result is shown as 0 (zero) and maximum degeneration is represented by the number 5 (five).

Statistical Analysis

Data are presented as means ± S.E.M. Groups of data were compared with an analysis of variance (ANOVA) and by nonparametric Mann-Whitney U test using SPSS for Windows 7.0 pack. Values of $p<0.05$ were regarded as significant.

Results

Appereance of the lesions were homogenous. The effect of ME containing TGF-α and/or aprotinin on duodenal ulcer score are shown in Figure 1. The results indicated that the mean ulcer areas reduced significantly in all treated groups with TGF-α (T-ME, T-AME and T-PS) compared to their control groups (ME, AME, PS) ($p<0.001$). Ulcer areas of T-ME and T-AME treated groups were significantly lower than the T-PS groups ($p<0.001$).

The results for duodenal tissue MDA levels are shown in Figure 2. Tissue MDA levels were significantly lower in the T-ME and T-AME treated group than those in AU, CU, PS, T-PS, ME and AME treated groups ($p<0.01$), but they were similar with CONTROL group.

Duodenal tissue GSH levels are shown in Figure 3. The GSH levels were higher in both T-ME and T-AME groups when they compared with the other groups. The differences were not statistically significant and similar between Control, T-ME, and T-AME groups.

Duodenal tissue NOx levels are shown in Figure 4. The NOx levels were increased in both T-ME and T-AME groups. The differences were statistically significant between all groups.

Table 2. Total results from the study groups, *n* number of rats.

Electron-microscopic criteria	Cont (*n*=8)	AU (*n*=8)	CU (*n*=10)	PS (*n*=9)	T-PS (*n*=9)	ME (*n*=10)	T-ME (*n=10)*	AME (*n*=9)	T-AME (*n*=10)
Kristolysis	0	1	2	4	3	0	2	3	1
Giant vacuoles	0	5	0	0	0	3	0	0	0
Dilated RER sisternaes	0	5	3	0	0	2	0	0	0
Junctional complex dilatation	0	4	2	0	3	3	0	0	0
Lipid granules	0	0	1	0	1	0	0	0	0
Myeline like figures	0	0	1	0	4	0	0	0	0
Microvillus degeneration	0	5	0	2	2	0	0	0	0

*0 (zero): no degeneration criteria and 5 (five): maximum degeneration criteria as shown.

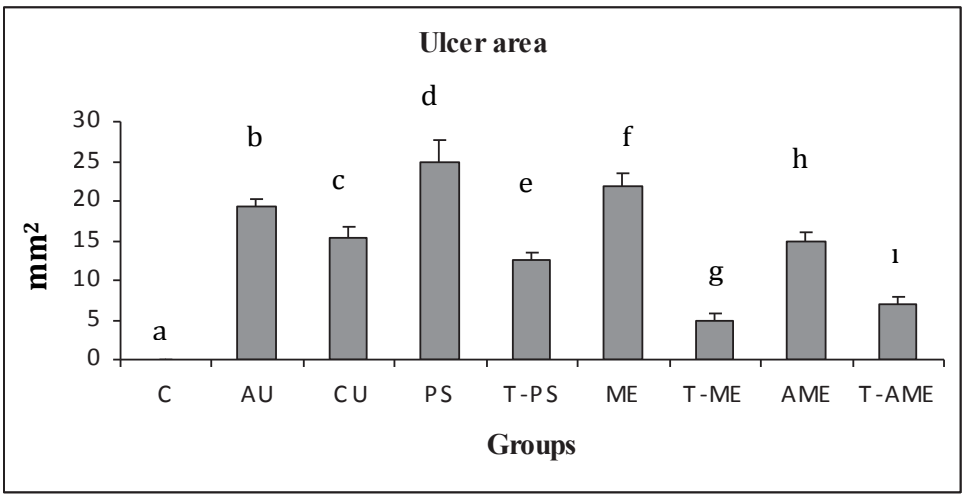

Figure 1. Ulcer scores in different experimental groups. The results are expressed as mean ± S.E.M. Difference statistically significant: p<0.05: b-c, b-h, c-f, g-I; p<0.0: c-d, d-h, e-f, f-h; p<0.001: b-e, b-g, b-ı, c-ı, d-e, d-g, d-ı, e-g, e-ı, f-e, f-ı, g-c, g-f, g-h, h-ı by Mann-Whitney U test.

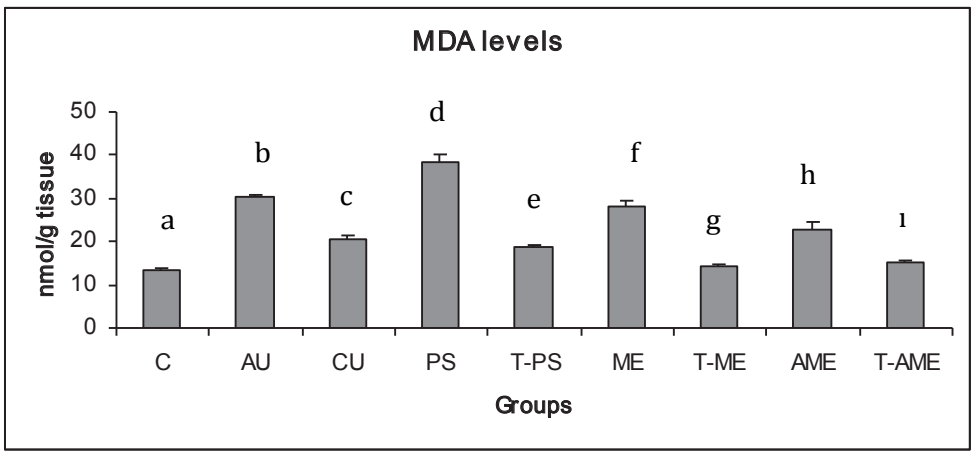

Figure 2. MDA levels in different experimental groups. The results are expressed as mean ± S.E.M. Difference statistically significant: p<0.05: b-h; p<0.01: a-b, a-c, a-e, a-h, b-c, b-d, b-e, c-f, c-ı, e-ı, g-h, h-I; p<0.001: a-d, a-f, b-g, b-ı, c-d, c-g, d-g, d-e, d-h, d-ı, e-f, e-g, f-g, f-ı by Mann-Whitney U test.

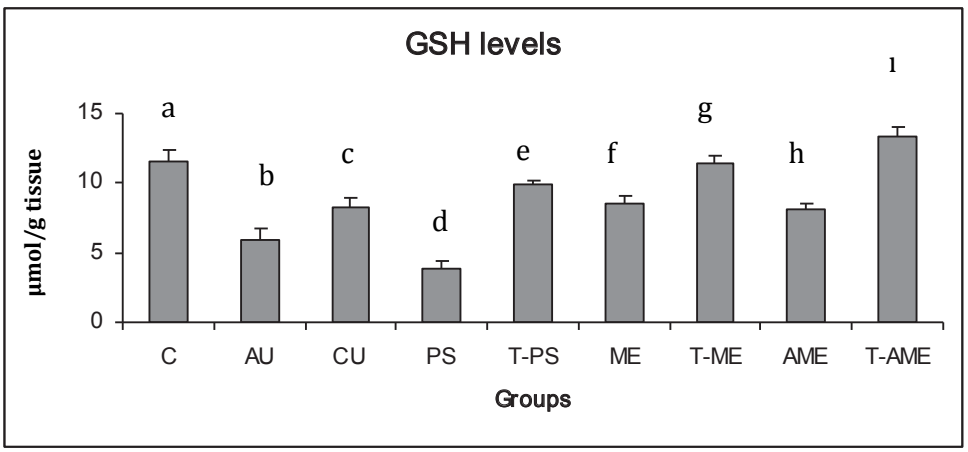

Figure 3. GSH levels in different experimental groups**.** The results are expressed as mean ± S.E.M. Difference statistically significant: p<0.05: a-e, b-c, b-d, b-f, b-h, c-g, c-e, e-f, e-g, e-h, f-g, g-h; p<0.01: a-b, a-c, a-f, a-h, b-e, b-g, c-d, d-h; p<0.001: a-d, b-ı, c-ı, d-e, d-f, d-g, d-ı, e-ı, f-ı, h-I by Mann-Whitney U test.

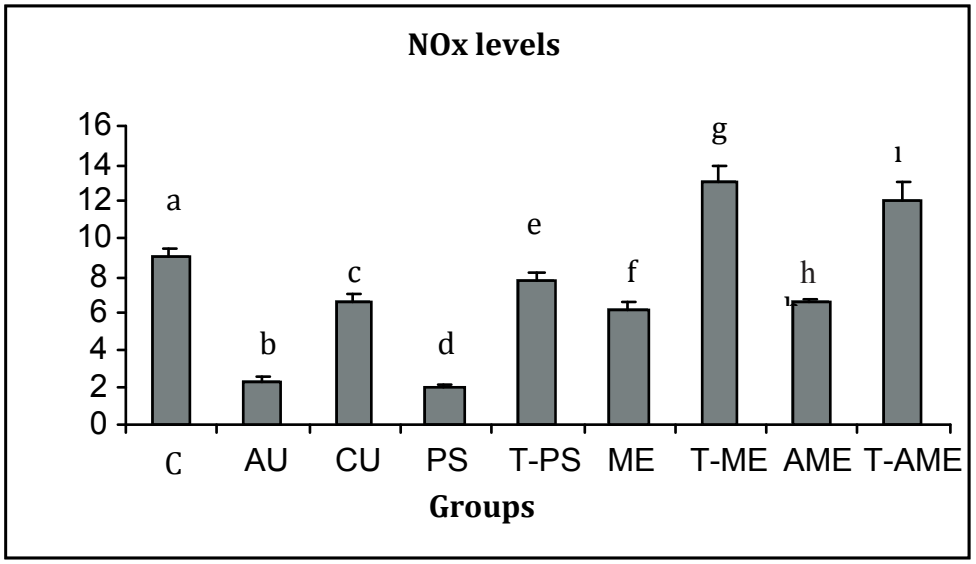

Figure 4. NOx levels in different experimental groups. The results are expressed as mean ± S.E.M. Difference statistically significant: $p<0.05$: a-ı, c-e, e-f, e-h; $p<0.01$: a-c, a-h; $p<0.001$: a-b, a-f, a-g, b-c, b-e, b-g, b-h, b-ı, c-ı, e-ı, f-ı, h-I; $p<0.0001$: a-d, b-g, c-d, c-g, d-e, d-f, d-g, d-h, d-ı, e-g, f-g, g-h by Mann-Whitney U test.

Electron microscopy results

The duodenal tissues of control rats were found to be completely normal (Figure 5a). There were significant differences in the appearence of giant vacuoles, dilated RER sisternaes makes large vacuole like structures in same areas, dilatation in junctional complexes and loss of microvilluses were seen when duodenal samples of AU group was compared to the control group (Figure 5b), Although CU group had better histologic findings some duodenal cells had degenerative areas in this group also. Dilatation between the junctional complexes was still prominent. Dilatation in RER sisternaes, crystolysis in mitochondrions were observed, but normal appearence of microvilluses were seen in this group (Figure. 5c). PS group shown degenerative findings, but not as severe as in AU and CU groups. There were still crystalysis in the mitochondria and there were some degenerative areas in the microvilluses. However, junctional complexes and RER sisternaes were seemed to be normal in this group (Figure 5d). There were also significant differences in the accumulation of lipid droplets, fraction in junctional complexes, occurence of myeline like vacuoler figures and cristalysis in mitochondria when duodenal samples of T-PS group was compared to the control group (Figure 5e). On the other hand, there were no significant changes in ME groups (Figure 5f) , exept the vacuoler deposition and dilatation in junctional complexes in some areas and AME group except crystolysis in some mitochondrions (Figure 5h). Finally there were no significant changes in T-ME group (Figure 5g) and T-AME group indicating there was almost no degeneration in duodenal cells (Figure 5ı).

Discussion

Acute gastric mucosal lesions represent an important clinical problem. Animal models allow recognizing details of pathomechanisms of gastric damage. Among the various experimental ulcer models, the ASA application model is frequently used [27,28,29]. In our previous studies, the effect of i.g. administration of ME formulation of TGF-α on healing of acute gastric ulcer induced by ASA in rats were investigated [5,19]. In the present study, ME and aqueous solution of TGF-α and/or aprotinin were used on the healing of duodenal ulcer induced by acidified ASA. Previous studies have demonstrated that reactive oxygen specimens are involved in ASA induced gastric mucosal damage due to an enhancing effect of lipid peroxidation and attenuation of mucosal antioxidant mechanisms [30,31].

In the present study, ulcer index and MDA levels were significantly elevated in the duodenal tissue after intragastrically acidified ASA aplication (Figure 1, Figure 2). On the other hand in this group GSH and NOx levels were significantly lower than the control (Figure 3, Figure 4). These results show that the antioxidant system is affected by ASA application.

Increase in levels of MDA indicates an enhanced lipid peroxidation leading to tissue injury and a failure in scavenging of excess free radicals.

TGF-α has been proposed to play a major role in the maintenance of gastric mucosal integrity and to participate in the mechanism of ulcer healing via the stimulation of cell proliferation and migration and enhancement in the gastric blood flow (GBF) at the ulcer edge [32]. There is also evidence that the increased TGF-α contribu-

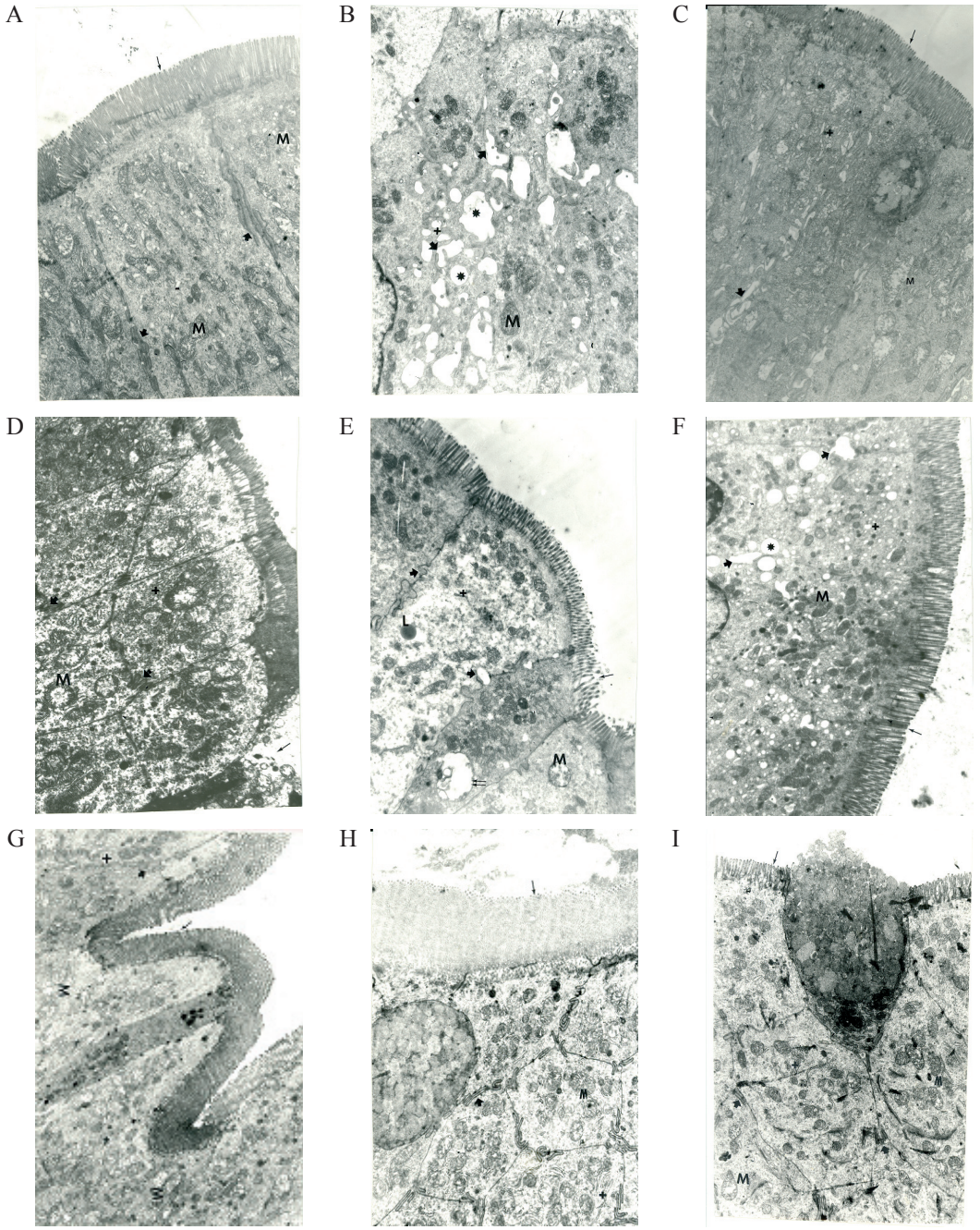

Figure 5. TEM appearance of duodenum tissue in experimental groups.
Control group (a) ; Duodenal cell of control group; microvillus (thin arrow), mitochondrion (M) and cell to cell junctional complexes (➔) (Uranyl acetate- Lead citrate X3000).
AU group (b) ; Cells of AU group; giant vacuoles (★), dilatation in junctional complexes (➔), dilated RER sisternaes (✚), degenerated and loss of microvillus (®) (Uranyl acetate-Lead citrate X3000).
CU group (c); CU group; dilatation in junctional complexes (➔), dilated RER sisternaes (✚), crystolysis in mitochondrion (M) and normal appearence of microvillus (thin arrow) (Uranyl acetate-Lead citrate X3000).
PS group (d) ; Electron microscopy of PS group; crystolysis in mitochondria (M), junctional complexes (➔), RER sisternaes (✚) and degenerated microvillus (®) (Uranyl acetate-Lead citrate X3000).
T-PS group (e) ; T-PS group; mitochondria with crystolysis (M), fructured junctional complexes (➔), myeline like degenerated figures (double arrow), lipid droplets (L) and normal RER sisternaes (✚) (Uranyl acetate-Lead citrate X3000).
ME group (f) ; Microvillus (®) , mitochondria (M), RER sisternaes (✚), big vacuoles (★), and dilated junctional complexes (➔) are seen in the ME group (Uranyl acetate-Lead citrate X3000).
T-ME group (g) ; Microvillus (®) , mitochondria (M), RER sisternaes (✚), and junctional complexes (➔) are seen in the T-ME group (Uranyl acetate-Lead citrate X3000).
AME group (h) ; Microvillus (®) , mitochondria (M), RER sisternaes (✚), and junctional complexes (➔) are seen in the AME group (Uranyl acetate-Lead citrate X3000).
T-AME group (ı) ; Mitochondrion (M), (✚) RER sisternaes, (➔) junctional complexes , (®) microvillus are seen in the T-AME group (Uranyl acetate-Lead citrate X3000).

te to increased cyclooxygenase-2 expression in H. Pylori infected mucosa resulting in the increased prostaglandin synthesis [33]. The results of present study show that, treatment with ME containing TGF-α and/or aprotinin (T-ME and T-AME) for 2 days significantly reduced the acidified ASA induced duodenal mucosal injury (Figure 1) and significantly inhibited the increase in MDA levels of duodenal mucosa compared to T-PS and the other groups (Figure 2).

Various routes of administering TGF-α have been studied. Although orogastric administration has been reported to be one of these application, degradation of the peptide in the lumen is an important problem [18]. Bastaki et al. reported that, subcutaneous administration of TGF-α to be more effective than oral route on gastric lesions [34]. Therefore we used ME formulation to minimize the enzymatic degradation of the factor in GIT. Although these results show, the efficacy of both ME carrier (T-ME, T-AME) and aqueous solution containing TGF-α (T-PS) on duodenal lesions, the ME formulation of this peptide (T-ME, T-AME) at the same dose (10 μg/kg) seem to be more effective for healing of ASA-induced duodenal ulceration (Figure 1). The most prominent suppression of lipid peroxidation was observed in T-ME and T-AME treated groups. Also maximal increase of GSH activity occurred in these two groups. Our results are in similar with the results of previous study about decreased MDA levels in ASA induced gastric ulcer [5]. These findings supports Ogle and co-workers suggesting that ME formulation prevents the degradation of peptides in gastrointestinal system [17].

It was reported that intracellular stores of GSH were sensitive to skin ischemia in rats and therefore, may regulate the early temporal course of wound healing [35]. Our results show decreased levels of GSH in duodenum tissue of untreated groups (AU, CU, PS groups) and increased duodenal levels of GSH in TGF-α treated groups (Figure 3). Increased GSH and decreased MDA levels and smaller ulcer areas in the groups which were treated with ME containing TGF-α supported the previous reports suggesting the failure of GSH dependent defence system results in accumulation of free radicals which can cause membrane damage by lipid peroxidation [5,36].

Gastric microcirculation has an important role in damage and protection of gastric barrier. The main factors which regulate gastric blood flow are prostaglandins, sensory peptides released from endings of afferent nerves, and NO [13]. The wound healing mechanism that are triggered by NO appear to be diverse, involving inflammation, angiogenesis, and cell proliferation and also collagen accumulation and acquisition of mechanical strength of incision wounds [37,38]. All of these processes are controlled by defined cytokine cascades in many cases, NO appears to modulate these cytokines [39]. The protective role of NO is also effective against exogenous injurious agents. The ability of endogenous NO to protect the mucosa is effective against a wide variety of damaging agents. Moreover, NO acts as the endogenous mediator for the gastroprotective actions of different antiulcer agents, several hormones and modulators of neural activity [14]. In the present study NOx levels increased significantly in the ME containing TGF-α treated groups (Figure 4) while the ulcer areas decreasing. A relationship between the upregulation of TGF-α and an increased production of nitric oxide synthase in the ulcer area was reported [40]. TEM results about ulcer healing and biochemical changes in lipid peroxidation were also positively affected by increased NOx levels in TGF-α treated group. The fact that ME containing TGF-α application increased both GSH and NOx levels and decreased MDA level in the duodenum suggests that the benefical effects of TGF-α on duodenal ulcer healing might be mediated by NO originating from duodenum.

Conclusion

Our findings suggested that an increase of oxygen-derived free radicals and decrease of GSH level in duodenal mucosa may be involved in the pathogenesis of ASA-induced duodenal ulcers in rats. ASA induced duodenal lesions occur due to an impairment of duodenal microcirculation and excessive proinflamatory cytokine release and suppression of antiulcer TGF-α expression. Therefore the healing affects of ME containing TGF-α and/or aprotinin solutions on duodenal ulcers pathogenesis might be associated with their effects on microcirculation and excessive proinflamatory cytokine release.

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