The Role of Transforming Growth Factor α Formulation on Aspirin-Induced Ulcer Healing and Oxidant Stress in the Gastric Mucosa

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Abstract
Purpose. Transforming growth factor (TGF) α accelerates wound healing, especially in gastric ulcers. Transforming growth factor α can be affected by acid and pepsin in the gastric juice. Oxidative stress also plays a role in the formation of gastric lesions. This study was designed (1) to investigate the effects of microemulsion dosage form on the healing of gastric ulcers, and (2) to determine the relationship between oxidative mechanisms and TGF-α during ulcer healing.

Methods. Gastric ulcers were induced in Wistar rats (male, 200 ± 25 g), by 150 mg/kg acidified aspirin application. The animals were divided into five groups consisting of 7–11 animals. The rats were killed after ulcer induction with aspirin (acute ulcer), or 2 days after ulcer induction (chronic ulcer), or after the daily application of microemulsion and TGF-α for 2 days. The ulcer area was measured planimetrically. Thiobarbituric acid reactive substance, glutathione, and gastric mucus levels of tissues were measured by spectrophotometric methods. The total nitric oxide level was measured by a VCl₃ / Griess assay. Statistical comparisons were made by an analysis of variance and the Mann-Whitney U-test.

Results. The ulcer area and malondialdehyde level of gastric tissue both decreased and the glutathione level increased to intact gastric tissue levels, while the mucus and total nitric oxide levels increased significantly after the application of intragastric TGF-α.

Conclusion. These findings suggest that TGF-α accelerates the healing process after aspirin-induced gastric injury, and a relationship was observed between this application and the oxidative reactions.

Key words Microemulsion form of transforming growth factor α · Rat gastric ulcer · Glutathione · Thiobarbituric acid reactive substance · Malondialdehyde · Nitric oxide

Introduction
Animal models of any human disease aim to mimic the human condition as much as possible, so experimental manipulations can be carried out that contribute to an understanding of that disease. After nonsteroidal anti-inflammatory drugs (NSAIDs) were introduced, a whole new class of human ulcers appeared. Animal studies then confirmed the mechanisms of induction of the NSAID ulcer. Conventional NSAIDs such as aspirin and indomethacin inhibit the cyclo-oxygenase enzyme activity, the rate-limiting step in the production of prostaglandin from precursor membrane phospholipids.¹

Epidermal growth factor (EGF), transforming growth factor α (TGF-α), amphiregulin, heparin-bind-EGF, poxvirus growth factors, crypto, and heregulin are included in the EGF family. These molecules all bind to the same receptor, epidermal growth factor receptor (EGFR). Transforming growth factor α appears to be the primary physiological ligand for the EGFR. Accordingly, in the gastrointestinal tract TGF-α may be the most important ligand for EGF. Since both EGF and TGF-α promote cell proliferation, stimulate cell migration, and inhibit gastric acid secretion, it is likely that these two growth factors play a pivotal role in ulcer healing.² There have been many reports about the roles of growth factors on gastric ulcer healing.³,⁴

The superoxide anion radical, hydrogen peroxide, and extremely reactive hydroxyl radical are common products of life in an aerobic environment. These radicals cause oxidative damage in the living organisms.
Living organisms generate a variety of antioxidant molecules and enzymes in preventing damage, such as melatonin, glutathione (GSH), glutathione peroxidase, superoxide dismutase, and catalase. In recent years oxidative stress has been implicated in a wide variety of degenerative processes, diseases, and syndromes, including acute inflammatory problems such as wound healing.

The role of free radicals has been indicated as a background for the development of gastric mucosal damage produced by intragastric administration of ethanol, acid, base, and concentrated salt solutions. The extent of lipid peroxidation (LP) can be estimated by the measurement of malondialdehyde (MDA), while the changes in the GSH level indicate a part of the defensive mechanism against free radicals. Oxidative stress also plays a role in the formation of gastric lesions. Nitric oxide (NO) interacts with neuropeptides and prostaglandins to maintain mucosal integrity in basal conditions. The protective role of NO is also effective against exogenous injurious agents. Nitric oxide acts as an endogenous mediator for the gastroprotective actions of different antiulcer agents, several hormones, and modulators of neural activity. Gastroprotection induced by NO has been demonstrated against NSAIDs, acid, ischemia/reperfusion, and stress effects on ulcer induction. Recently, investigators have implicated NO to play a role in the regulatory forces on various cellular activities of the inflammatory and proliferative phases of wound healing. In many cases NO appears to modulate some cytokines. Childress and Stechmiller reported that an acceleration in ulcer healing depends, at least in part, upon an upregulation of TGF-α and increased NO production at the ulcer margin.

Transforming growth factor α can be affected by acid and pepsin of gastric juice causing a 2–5-fold loss of biological activity. We thus used a microemulsion formulation of TGF-α (TGF-α ME) to minimize the degradation of the factor in the present study. Some pharmaceutical properties and effects of this form have been published in our previous report. These were: (1) gastric acid secretion was significantly reduced after the intragastric administration of TGF-α ME; (2) based on confocal laser scanning microscopy, the imaging the TGF-α levels in the control group were observed at almost normal physiological concentrations — however, the levels in the TGF-α ME group were higher than the control group’s levels; (3) a histological evaluation of gastric mucosa samples revealed the best recovery to be obtained in the TGF-α ME-treated group. The surface mucous cells and parietal cells of corpus were evaluated by transmission electron microscopy (TEM). The results of TEM indicated that untreated groups showed degenerative changes in surface mucous epithelia which included the deformations of junctions, the separation in surface cell membranes, and a decrease in the number of mucous granules. However, in the TGF-α ME group, the cells showed the same normal structure as that seen in the control group cells.

This study was designed (1) to investigate the effects of TGF-α ME dosage form prepared in the Pharmaceutical Technology Department of Gazi University Pharmaceutical Faculty on the healing of gastric ulcers, based on the mean ulcer area and mucus secretion in 2 days; and (2) to determine the relationship between the oxidative mechanisms and TGF-α during ulcer healing based on the MDA, GSH, and NO levels.

Materials and Methods

Preparation of the ME Formulation

The microemulsion was prepared by a modification of ME as previously described. The microemulsion formulation was prepared using Labrafil M 1944CS, Arlacel 186:Brij 35 (5:1), absolute alcohol, and bidistilled water as the oil phase surfactant, cosurfactant, and aqueous phase, respectively. Next, 133 μg TGF-α was added to 100 ml ME.

Animals and Study Groups

Gastric ulcers were induced in Wistar rats (male, weight 200 ± 25 g), by intragastric 150 mg/kg aspirin (acetysalicylic acid; ASA) in 1.5 ml 0.2 N HCl (acidified aspirin) application. This ASA dose and healing period were chosen after studying the dose, time, and gastric ulcer area relationships in Wistar male rats as shown in Fig. 1. The animals were divided into five groups consisting of 7–11 animals and were allowed free access to normal chow and water for 3 days. The rats were killed under thiopental anesthesia immediately after ulcer induction. The last group was made up of control rats.

Determination of the Ulcer Score

The stomach was removed on the third day (2 days of treatment) under thiopental anesthesia, and the area of ulceration was measured planimetrically, using an operating microscope. The pH of gastric content was measured by a pH indicator strip. All measurements were performed by the same person who was unaware of the