Gut, 1986, **27**, 1443–1449

Effect of sialoadenectomy and synthetic human urogastrone on healing of chronic gastric ulcers in rats

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SUMMARY The effect of extirpation of the submandibular glands, an exocrine organ for epidermal growth factor/urogastrone (EGF/URO), and the effect of oral administration of synthetic human (EGF/URO) on healing of chronic gastric ulcers in rats has been investigated. Removal of the submandibular glands delayed healing of chronic gastric ulcers when examined after 50, 100, and 200 days. Oral administration of synthetic human EGF/URO stimulated gastric ulcer healing when examined after 25 and 50 days of treatment. The effect of synthetic human EGF/URO was comparable with that of cimetidine. The combined administration of synthetic human EGF/URO and cimetidine further increased healing of gastric ulcers compared with administration of each substance. Neither synthetic human EGF/URO, nor removal of the submandibular glands had any influence on gastric acid secretion. This study showed that the submandibular glands influence healing of chronic gastric ulcers and suggest that EGF/URO participate in healing of chronic gastric ulcers in rats.

Gastric ulcers are thought to result from an imbalance between protective and aggressive factors in the stomach.[1] The protective factors comprise a series of interrelated mechanisms including secretion of mucus and bicarbonate, changes in the mucosal blood flow and content of prostaglandins.[2] Saliva may also be a protective factor in the stomach, the main components being mucus and bicarbonate which have a high buffer capacity.[3] An increasing number of biological active peptides have been found to be secreted in an exocrine manner from the salivary glands – for example, renin, amylase, nerve growth factor, and epidermal growth factor (EGF).[4 5]

Epidermal growth factor is a polypeptide with a chemical structure and biological activity similar to urogastrone (URO), originally isolated from human urine.[6] Epidermal growth factor/urogastrone (EGF/URO) has been localised to the submandibular glands, Brunner's glands and kidneys of both rodents and man.[7–9] The peptide has a number of different effects such as stimulation of cellular growth and cellular differentiation and inhibition of gastric acid secretion.[6 10] In the rat, secretion of EGF/URO from the submandibular glands is mainly exocrine and oral administration of the peptide has been reported to prevent the development of experimental gastric lesions,[11 12] but the effect of EGF/URO on healing of gastric ulcers remains to be clarified.

The purpose of the present study was to elucidate the influence of the submandibular glands and the effect of oral administration of synthetic human EGF/URO on healing of chronic gastric ulcers in rats. Furthermore, the effect of synthetic human EGF/URO was compared with cimetidine, a histamine H_2-receptor antagonist, regarding ulcer healing as well as gastric acid secretion.

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Received for publication 22 April 1986

Methods

ANIMALS

Female Sprague Dawley rats weighing approximately 200 g were used throughout the study. Before the experiments, the rats were fasted overnight with free access to water in raised meshbottom cages to prevent coprophagy.

EXPERIMENTAL PROCEDURE

Gastric ulcer studies

Chronic gastric ulcers were induced by a slight

modification of the acetic acid method.[13] Under ether anaesthesia a laparotomy was made and a round glass mould (diameter 6 mm) was placed on the serosal surface at the fundo-antral junction of the stomach. Acetic acid 100 µl (17·5 mol/l) was poured into the mould and removed after 120 seconds. In preliminary studies this method was found to destroy the outer muscular layer of the stomach and induce chronic gastric ulcers in all rats. A recovery period of seven days was allowed before further experiments. The influence of the submandibular glands on healing of chronic gastric ulcers was investigated in 60 rats, who had the submandibular glands removed 30 days before ulcer induction. Rats in groups of 15 were killed after 50, 100, 150, and 200 days.

Ulcer healing was studied in 120 rats who had a gastric ulcer induced and received one of the following agents in the drinking water: synthetic human EGF/URO 5 nmol/kg × day, cimetidine 2 mmol/kg × day or synthetic human EGF/URO 5 nmol/kg × day plus cimetidine 2 mmol/kg × day. In each group 20 rats were killed after 25 and 50 days. In a control study, 85 rats had a gastric ulcer induced as described and were killed in groups of 20 after 25 and 50 days and in groups of 15 after 100, 150 and 200 days.

To evaluate the results, the stomach and duodenum were fixed *in situ* by intraluminal injection of 10% formalin. The organs were removed, cut open and suspended on a polyethylene plate in 10% formalin for 24 hours, washed with water and stained with periodic acid-Schiff reagent. The organs were studied under a stereomicroscope and the ulcerated area was photographed and specimens taken out for histological examination and stained with periodic acid-Schiff and haematoxylin-Aurentia. On the photographs the outlines of the original ulcer and the part that had not healed could be identified. The size of the original and remaining ulcer was measured planimetrically using a Hewlett Packard 9874 A digitiser (Hewlett Packard Company, Palo Alto, CA). The results were corrected for the magnification of the photographs. The size of the orginal and remaining ulcer was expressed in mm^2 and the size of the regenerated mucosa was given in percent of the size of the original ulcer.

Effect of extirpation of the submandibular glands on gastric acid secretion

Twenty rats were prepared with a chronic gastric cannula according to the method of Lane.[14] Ten of the rats had the submandibular glands removed at the same occasion. After a postoperative period of two weeks all rats had a 0·8 mm polyethylene catheter placed in a jugular vein for infusion. The gastric cannula was opened and the stomach was rinsed out with saline through the fistula whereafter the rats were placed in Bollman cages for collection of gastric acid secretion. After one hour, basal gastric acid secretion was collected for 60 min. Thereafter an infusion of pentagastrin (Peptavlon, ICI, UK) 25 µg/kg × h was started and continued for 120 min during collection of acid secretion.

Acid secretion after intragastric infusion of EGF/URO

Twenty rats were equipped with a gastric cannula. Ten of the rats also had a gastric ulcer induced. Two weeks later gastric acid secretion was collected in all rats. After one hour of spontaneous secretion a 0·8 mm polyethylene catheter was passed through the cannula into the stomach for infusion of synthetic human EGF/URO 0·2 nmol/kg × h in a volume of 1 ml/h. The infusion continued for three hours whereafter the catheter was removed and basal gastric acid secretion collected for 60 min during iv infusion of saline 0·154 mol/l (2 ml/h). The intragastric infusion of synthetic human EGF/URO was then resumed for two hours whereafter pentagastrin-stimulated (25 µg/kg × h) gastric acid secretion was collected for 60 min.

Absorption of synthetic human EGF/URO

Ten rats had a chronic gastric ulcer induced as described above. After 10 days the rats had a laparatomy made together with another 10 rats who served as controls. Through an incision in the forestomach, a polyethylene catheter was placed with the tip in the fundic part of the stomach. The catheter was fixed with a purse string suture in the forestomach. After one hour of recovery an infusion of synthetic human EGF/URO 0·2 nmol/kg × h in a volume of 2 ml/h started and continued for three hours. Thereafter blood was drawn from the inferior vena cava for determination of the concentration of synthetic human EGF/URO.

LABORATORY ANALYSES

The hydrogen ion concentration in gastric acid secretion was determined by titration with NaOH using an autotitrator ABU-12 (Radiometer, Copenhagen, Denmark). From the hydrogen ion concentration and volume the acid output was calculated. Synthetic human EGF/URO was determined in undiluted rat serum as described.[15] The antibody used was 1589. Iodinated peptide and calibration standards were synthetic human EGF/URO. The calibration curve was performed in charcoal stripped serum from untreated rats. Detection limit of the assay was 0·07 nmol/l serum.

STATISTICAL ANALYSIS

Statistical evaluation of the data was done by Mann-

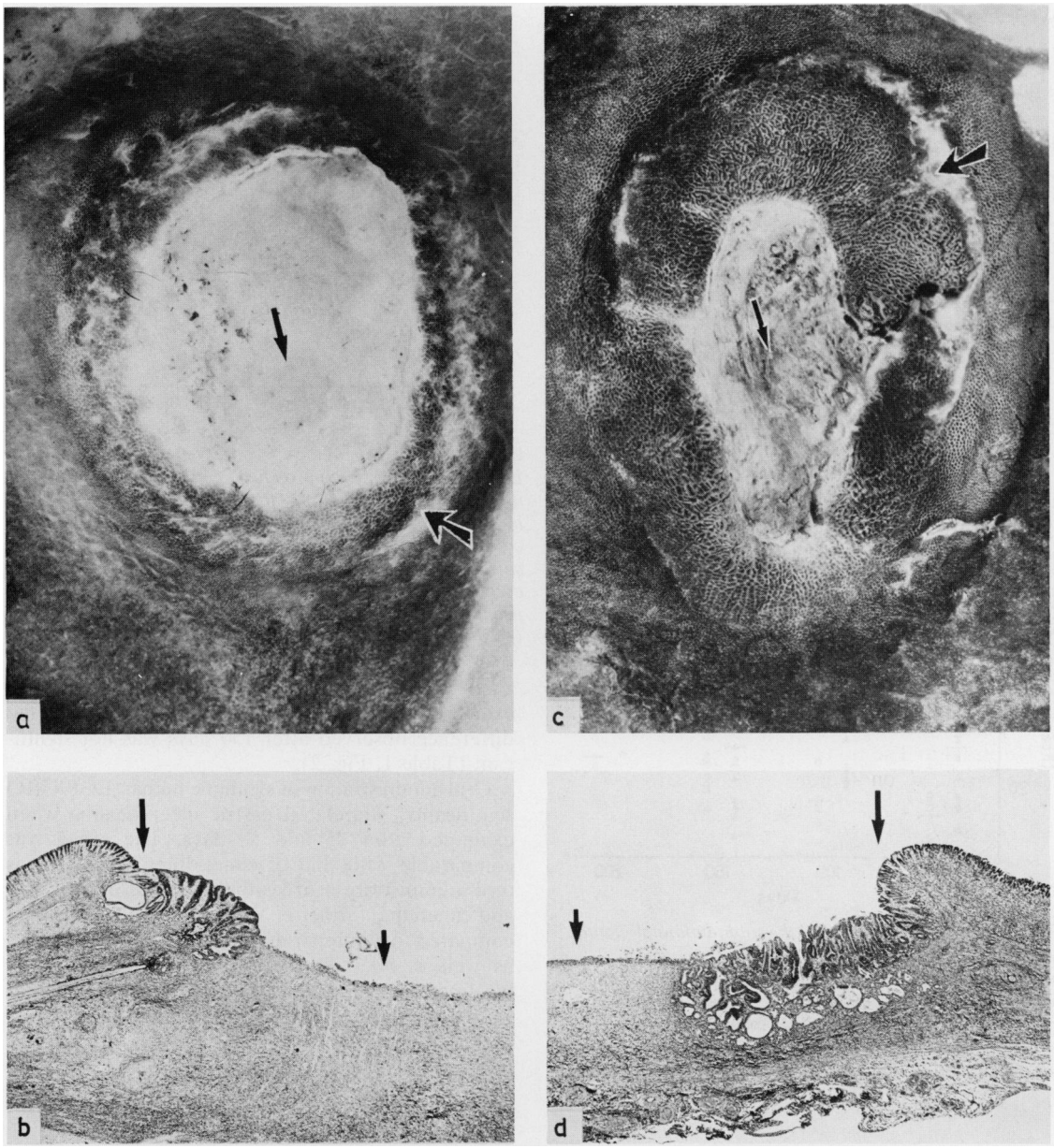

Fig. 1 *Stereomicroscopic and histologic appearance of ulcers from treated and untreated rats. The large arrow heads indicate the border between the part of the mucosa that has not been ulcerated and the part that has regenerated. The small arrow heads indicate the remaining unhealed part of the ulcer. (a) Stereomicroscopic appearance of ulcer from an untreated control rat (50 days). The regenerated part of the mucosa is rather narrow and characterised by large irregular gastric pits (PAS). (b) Corresponding histological section of the edge of the ulcer. The regenerated mucosa has mucous, pyloric-like glands although the ulcer is situated in the fundic part of the stomach. At the edge of the ulcer large cystic glands are present. (PAS-haematoxylin-Aurentia). (c) Stereomicroscopic appearance of ulcer from a rat given synthetic human urogastrone 5 nmol/kg×day for 50 days. Only a minor part of the ulcer remains to heal (PAS). (d) Histological section of a part of the same ulcer. The ridge in the right part of the photomicrograph is the primary margin of the ulcer (PAS-haematoxylin-Aurentia).*

Whitney's test for unpaired observations. Probability values ≤0·05 were considered significant. Analysis of variance was performed by Krushall-Wallis test. All results are given as medians and total ranges.

Results

Chronic gastric ulcers were induced in 265 rats. Six died within 10 days after ulcer induction because of perforation and peritonitis. In the remaining rats, the contour of the original ulcer and remaining ulcer could readily be identified and measured (Fig. 1). In control rats ulcer healing ranged from 13% after 25 days to 45% after 200 days (Figs. 2 and 3).

Extirpation of the submandibular glands significantly delayed healing of chronic gastric ulcers

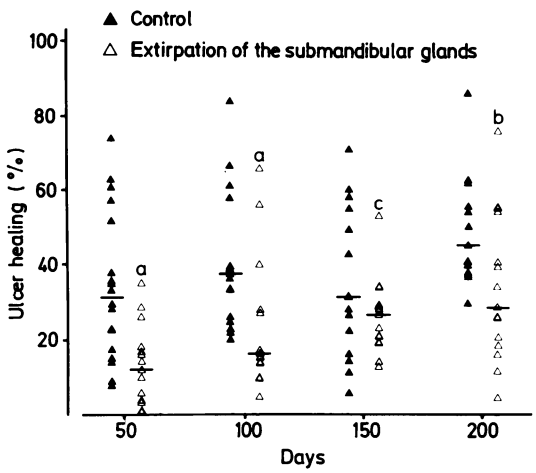

Fig. 2 *Effect of extirpation of the submandibular glands on healing of chronic gastric ulcers in rats. Controls and rats without the submandibular glands were killed after 50, 100, 150 and 200 days. (a) p<0·01 compared with the corresponding controls. (b) p<0·05 compared with the corresponding controls. (c) No significance compared with the corresponding controls.*

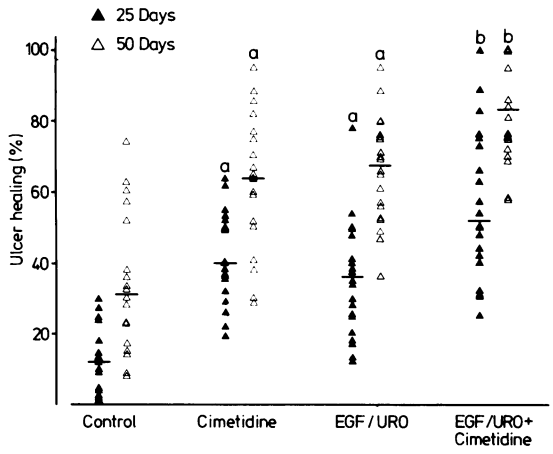

Fig. 3 *Comparison of the effect of synthetic human EGF/URO and cimetidine on healing of chronic gastric ulcers after 25 and 50 days of treatment. Horizontal bar indicate the median. a: p<0·01 compared with corresponding controls. b: p<0·05 compared with cimetidine and p<0·05 compared with EGF/URO. In each group ulcer healing was significantly more pronounced after 50 days of treatment than after 25 days (p<0·05).*

examined after 50, 100 and 200 days while the difference observed after 150 days was not significant (Table 1, Fig. 2).

Oral administration of synthetic human EGF/URO significantly stimulated gastric ulcer healing when examined after 25 and 50 days. The effect was comparable with that of cimetidine. Simultaneous oral administration of synthetic human EGF/URO and cimetidine further enhanced gastric ulcer healing compared to administration of each substance for 25 as well as 50 days (Fig. 3). The effect of each treatment procedure on ulcer healing was significantly more pronounced after 50 days than after 25 days(Fig. 3).

Basal and pentagastrin stimulated gastric acid

Table 1 *Effect of sialoadenectomy (SLA) on healing of chronic gastric ulcers in rats.*

	50 days		*100 days*		*150 days*		*200 days*	
	Control	*SLA*	*Control*	*SLA*	*Control*	*SLA*	*Control*	*SLA*
Ulcer healing %	31 (8–74)	12* (0–35)	37 (20–84)	16* (5–66)	31 (6–71)	26† (13–53)	45 (29–86)	29‡ (5–76)
n	18	15	15	15	15	15	14	15

Values are given as medians and total ranges. *: p<0·01 compared with controls. †: no significance compared with controls. ‡: p<0·05 compared with control.

secretion was unchanged after extirpation of the submandibular glands (Table 2). Intragastric infusion of synthetic human EGF/URO 0·2 nmol/kg × h had no influence on gastric acid secretion in rats with chronic gastric ulcers (Table 3). After intragastric infusion of synthetic human EGF/URO 0·2 nmol/kg × h small amounts of the peptide could be measured in serum from control rats, median 0·08 nmol/l, total range <0·07–0·14 nmol/l, and from rats with chronic gastric ulcers, median 0·12 nmol/l, total range <0·07–0·16 nmol/l. The difference between the two groups was not significant.

Discussion

Chronic gastric ulcers can readily be induced in rats by topical application of acetic acid on the serosal surface of the stomach.[13] In controls none of the ulcers had healed completely after 200 days as reported previously.[16 17] A decisive factor in development of a chronic gastric ulcer seems to be destruction of the outer muscular layer of the stomach.[18] We found in a preliminary study that rats with chronic gastric ulcers showed confined perforation of the tunica muscularis externa and recently we have shown that chronic duodenal ulcers can only be induced if the ulcer penetrates the tunica muscularis externa.[19]

In the present study removal of the submandibular glands delayed healing of chronic gastric ulcers. Removal of the submandibular glands has previously been demonstrated to decrease the volume of saliva by approximately 60% and the total output of EGF/URO in saliva.[11] This suggests that a decrease in the level of EGF/URO in gastric juice may be a factor leading to delayed healing of chronic gastric ulcers in rats.

Oral administration of synthetic human EGF/URO increased healing of chronic gastric ulcers examined after 25 and 50 days of treatment. The effect of synthetic human EGF/URO was comparable with that of cimetidine, a histamine H_2-receptor antagonist, which inhibits gastric secretion of acid. Combined administration of synthetic human EGF/URO and cimetidine further enhanced gastric ulcer healing which suggests that healing of chronic gastric ulcers in rats is more rapidly obtained by a combination of a growth promoting agent with inhibition of gastric acid secretion.

The role of the submandibular glands and saliva in healing of gastric ulcers is largely unknown. Saliva has a high buffer capacity that could decrease the acidity of gastric juice and thus enhance ulcer healing.[3] Salivary mucus might also act as a protective surface gel and prevent damage from the gastric juice. In addition, peptides that stimulate healing of wounds such as nerve growth factor and EGF/URO, have been isolated from the submandibular glands and saliva.[20–22] The submandibular glands exhibit exocrine as well as endocrine secretion of peptides such as EGF/URO,[23] but the concentration of EGF/URO in saliva is considerably higher than in plasma – for example, the concentration of salivary EGF/URO during stimulation in rats increased by a factor 50–100 while no changes in the serum concentration was observed.[11] It is therefore tempting to suggest that the influence of submandibular EGF/URO on the gastric mucosa is due to the exocrine secretion from the glands. Desalivation in the rat is followed by a decreased resistance of the gastric mucosa to damaging agents such as bile salt solutions[24] and recently sialoadenectomy was reported to decrease the DNA synthesis and contents of DNA and RNA in the

Table 2 *Influence of sialoadenectomy (SLA) on basal and pentagastrin-stimulated gastric acid secretion in rats.*

Treatment	*n*	*Basal gastric acid secretion μmol H⁺/60 min*	*Pentagastrin-stimulated acid secretion μmol H⁺/60 min*
Controls	10	47 (26–146)	388 (248–408)
SLA	10	42 (24–77)	318 (222–400)

Values are given as medians and total ranges. No statistical difference was found between controls and SLA.

Table 3 *Effect of intragastric (ig) infusion of synthetic human EGF/URO on basal and pentagastrin-stimulated acid secretion in rats with chronic gastric ulcers.*

Treatment	*n*	*Basal gastric acid secretion μmol H⁺/60 min*	*Pentagastrin-stimulated acid secretion μmol H⁺/60 min*
Controls (saline ig)	10	45 (21–82)	306 (178–357)
Rats with gastric ulcers (EGF/URO ig)	10	61 (32–76)	289 (224–471)

Values are given as medians and total ranges. No statistical difference was observed between the individual groups. Synthetic human EGF/URO was administered in a dose of 0·2 nmol/kg×h in a volume of 1 ml/h.

gastric mucosa, an effect that could partly be prevented by administration of a salivary gland extract.[25] These observations suggest a physiological function of the salivary glands in protection of the gastric mucosa.

Systemic administration of EGF/URO strongly inhibits gastric acid secretion.[26] Removal of the submandibular glands had no influence on acid secretion nor did intragastric infusion of EGF/URO influence acid secretion though small amounts of the peptide could be detected in the circulation. This suggests that the beneficial effect of EGF/URO on ulcer healing is caused by factors other than inhibition of acid secretion.

The mode of action of EGF/URO on the gastric mucosa is still unknown. EGF/URO acts on its target cells after binding to specific membrane receptors[27] which have been identified in the mucosa of the small intestine[28] and recently high affinity binding sites for EGF/URO was localised on the gastric epithelium.[29] After binding of EGF/URO to the receptor, a number of immediate effects have been observed such as alterations in cytoskeleton organisation, cell surface proteins and increased hyaluronic acid synthesis, all of which occur minutes after exposure to EGF/URO.[30–32] The ability of the peptide to prevent the development of experimental gastric mucosal lesions induced by aspirin or cysteamine is probably caused by the immediate effects of EGF/URO.[12 33] The results reported in this study may rather be due to the delayed effects of EGF/URO which include increased synthesis of DNA and RNA and thus a stimulation of epithelial growth.[34]

Fibroblasts and collagen are important factors in the natural healing process of gastroduodenal ulcers and both are increased in the presence of EGF/URO.[35 36] This suggests that EGF/URO may take part in healing of chronic gastric ulcers not only by formation of new surface epithelium but also by formation of the underlying connective tissue.

The amounts of synthetic human EGF/URO administered in the present study are comparable with the amounts of EGF/URO measured in saliva during adrenergic stimulation of the salivary glands in rats.[11] The observed effect of EGF/URO might therefore mimic a physiological function of the peptide.

In conclusion this study suggests that the submandibular glands play an important role not only in protection of the gastric mucosa, but also in healing of experimental gastric ulcers. We found synthetic human EGF/URO to promote healing of chronic gastric ulcers to the same extent as cimetidine. As EGF/URO has also been found in the human submandibular glands and saliva,[7] the role of EGF/URO in pathogenesis and healing of gastric ulcers in man should be investigated.

This work was supported by the Danish Medical Research Council (12–4680), the Danish Hospital Foundation for Medical Research, Region of Copenhagen, The Faroe Islands and Greenland (49/83) and the Novo Foundation. The authors thank Jette Schousboe and Henry Pløger for skilful technical assistance. Synthetic human urogastrone was supplied by G D Searle and Co Ltd. and Imperial Chemical Industries PLC.

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