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SOLCOSERYL IN PREVENTION OF STRESS-INDUCED GASTRIC LESIONS AND HEALING OF CHRONIC ULCERS

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Solcoseryl, a deproteinized extract of calf blood, protects the gastric mucosa against various topical irritants and enhances the healing of chronic gastric ulcerations but the mechanisms of these effects have been little studied. This study was designed to elucidate the active principle in Solcoseryl and to determine the role of prostaglandisn (PG) and polyamines in the antiulcer properties of this agent. Using both, the radioimmunoassay and radioreceptor assay, EGF-like material was detected in Solcoseryl preparation. Solcoseryl given s. c. prevented the formation of stress-indused gastric lesions and this was accompanied by an increase in the generation of PGE_2 in the gastric mucosa. Similar effects were obtained with EGF. Pretreatment with indomethacin, to suppress mucosal generation of prostaglandins (PG), greatly augmented stress-induced gastric ulcerations and antagonized the protection exerted by both Solcoseryl and EGF. Solcoseryl, like EGF, enhanced the healing of chronic gastroduodenal ulcerations. This effect was abolished by the pretreatment with difluoromethylornithine, an inhibitor of ornithine decarboxylase, the key enzyme in the biosynthesis of polyamines. The healing effects of Solcoseryl and EGF was also reduced by prednisolone which decreased the angiogenesis in the granulation tissue in the ulcer area. These results indicate that Solcoseryl 1. contains EGF-like material, 2. displays the protective and ulcer healing effects similar to those of EGF and involving both PG and polyamines and 3. acts *via* similar mechanism as does EGF.

Key words: *peptic ulcer, healing, prostaglandins, polyamines*

INTRODUCTION

Previous studies demonstrated that Solcoseryl (Solco Ltd. Basel, Switzerland) a protein free dialyzate of calf blood and containing various inorganic and organic substances (amino acids, low molecular weight polypeptides, phospholipids etc.), possesses gastroprotective (1, 2) and ulcer healing properties in animals and in men (3, 4). The major mechanism of this gastroprotection by Solcoseryl was thought to be the maintenance of the mucosal blood supply in the mucosa exposed to topical irritants. This effect has been attributed to the stimulation of the biosynthesis of vasodilating prostaglandins

(PG) and/or to the suppression of mucosal formation of vasocontrictive leukotrienes (5). As mucosal blood flow is known to be reduced after exposure to stress (2, 6, 7, 8) possibly due to the reduction of PG biosynthesis (8, 9), the question remains whether Solcoseryl could reverse the changes in mucosal circulation and PG biosynthesis evoked by stress conditions.

Solcoseryl was shown in numerous, well controlled studies to represent a valuable aid in the treatment of wounds such as caused by termal injury, irradiation, trauma and circulatory disorders (10—13). These effects have been attributed to the ability of Solcoseryl to enhance the formation of granulation tissue (14) and to increase the processes of epithelialization (15), angiogenesis (16) and of oxygen utilization in the perinecrotic tissue of the wound (10).

Solcoseryl also increased the rate of healing of chronic gastroduodenal ulcerations (3, 4). Since this agent does not affect gastric acid secretion or plasma gastrin level (3), the ulcer healing effects of Solcoseryl have been attributed to the stimulation of proliferation of epithelial and non-epithelial cells (3, 14, 15, 17). Growth factors have been implicated in the wound healing (18) and appear to act through the activation of specific membrane receptors, the induction of ornithine decarboxylase (ODC) and polyamine formation (19, 20). The question arises whether the ulcer healing effects of Solcoseryl involve similar the mechanisms to those activated by growth factors.

MATERIAL AND METHODS

Identification of EGF — material in Solcoseryl

Standard Solcoseryl (40 mg/ml) and concentrated Solcoseryl (200 mg/ml) preparations (gift from Solco Ltd, Basel, Switzerland) were used. EGF present in the latter preparation was extracted by adsorption onto octadecylsilylsilica (SEP—PAK C_{11}) cartridges (Water Associates, Miliford, MA (and finally eluted with 1 ml 100% ethanol and 1% trifluoroacetic acid (4:1, v/v) into incubation via and dried under N_2. The dried material was then dissolved in 1 ml of phosphate buffer and applied for the identification to G-50 Sephadex (Sigma) supperfine columns (1 × 100 cm) for gel filtration. Fractionation was carried out at 15°C and the columns were eluted with 0.5 M phosphate buffer at pH 7.4 containing 0.15 M NaCl abd 1% sodium azide. The columns were with blue dextran, $K_2Cr_2O_7$ and cytochrome C. Fractions of 1 ml were collected and EGF was equilibrated determined in each sample by radioimmunoassay (RIA) and radioreceptor assay (RRA). Pure urogastrone (10 ug) (gift of Dr. Y. Yamazaki, Hitachi Chemical, Ibaraki, Japan) was also eluted on the same G-50 Sephadex columns and EGF was examined in each ml collected fraction using specific RIA as described previously (20). For the comparison, labelled ^{125}I hEGF (Amersham, UK) was also eluted on G-50 Sephadex column and the radioactivuty was measured in each of the collected 1 ml samples.

RIA of EGF present in the standard and concentrated Solcoseryl in the original preparations and in the samples eluted from the G-50 Sephadex gel filtration column was performed according to the method described previously (21). Briefly, hEGF antiserum (kindly provided by Dr. H-Gregory, ICI, UK) was used in the final dilution of 1:210,000. This antiserum cross-reacted in abou. 90% with rat (rEGF) and mouse (mEGF) but exhibited no cross reactivity with structurally unre-

lated gut peptides such as gastrin, CCK, secetin, GIP or PP. Iodinated (3-^{125}I-Iodothyrosyl) peptide and the hEGF were calibration standards (Amersham, UK). The detection limit of the assay was 0.1 ng/ml. The interassay and intraassay precisions were about 14 and 10%, respectively.

RRA of EGF was adopted after Imai et al. (22) and Yip et al. (23). Male mouse livers were washed thoroughly by perfusion and homogenized in 10 vol of 50 mM Tris-HCl, 10 mM $MGCl_2$, pH 7.4 with Polytron PF10. The homogenate was spun at 16 000 × g for 20 min, the pellets were suspended in equal volume of buffer and stored at −20°C. For RRA, this crude membrane fraction was diluted to bind approximately 30% of ^{125}I-EGF added about 10 000 cpm. Final incubation mixture was 300 µl and all dilutions were made with 0.1% BSA in Tris-HCl, $MgCl_2$, pH 7.4. After standing at room temperature for 1 h, 2 ml of cold buffer was added and the test tubes were spun at 2 000 × g at 0°C for 15 min. Finally, the pellets were recounted in gamma counter (LKB, Vienna). Non-specific binding was determined in the presence of excessive hEGF (1 µg). Concentrated Solocoseryl preparation (200 mg/ml) was used for comparison of the binding curves with those of standard hEGF.

Measurement of mucosal capacity for PGE2 biosynthesis.

The PG biosynthetic capacity in the oxyntic mucosa of intact and indimethacin treated rats without or with addition of EGF (100 µg/kg s.c.) or Solcoseryl (1 ml/h) was determined as described previously (24). Briefly, the oxyntic mucosa (about 50 mg) was scraped by glass from the musculature and rinsed in a buffer solution containing 50 mM Tris-HCl, pH 8.4. The tissue sample was placed in a test preweighed Eppendorf vial, weighed and then 1 ml of Tris buffer was added to each vial. The sample was finally minced (for 15 s) with scissors, washed and centrifuged (3000 × g) for 10 s, the pellet being suspended again in 1 ml of Tris. Each sample was incubated on the vortex shaker for 1 min, then centrifuged again for 15 s. The supernatąant was transferred to a second vial containing indomethacin (10 µl of 20 mM) and kept at −20°C until radioimmunoassay. PGE_2 immunoreactivity was determined using a RIA kit (New England Nuclear, Munich FRG). The capability of the mucosa to generate PGE_2 was expressed in nanograms (ng) per gram of wet tissue weight.

Determination of angiogenesis in chronic ulcerations.

Newly formed blood vessels in the granulation tissue of the gastric ulcer base was measured using a modification (25) of the carmine dye technique of Kimura et al (26). Following 7 days after ulcer production in rats treated with EGF or Solcoseryl alone and in combination with prednisolone (50 mg/kg/day), the stomach was resected and opened along the greater curvature and the ulcer area was measured by planimetry. The ulcer was then excised from the surrounding normal tissue along its margin. The granulation tissue of the ulcer base was weighed and placed in 4 ml of 3 M NaOH for 30 min at 30°C. After the addition of 1 ml of 10 M HCl, the solution was filtered and exactly 2 h after dissolving the tissue, the absorbance of the ultrafiltrate was measured at 530 nm with spectrophotometer (Beckman, Berlin, FRG). The total carmine content of each specimen was determined from the calibration curvetfor the carmine. In evaluation of angiogenesis within the ulcer base, the term carmine density was designed and this represents the total carmine content divided by the ulcer area (ulcer index). Control specimens were obtained from the same parts of the stomach of ulcer-free rats.

Production of acute and chronic gastroduodenal ulcerations

Acute gastric lesions were induced in male Wistar rats, weighing 160—200 g and fasted about 20 h using the technique described by Takeuchi et al (27). Briefly, animals were placed in special stress cages causing immobilization and immersed into 23°C water to the rat's xyphoid process

for 6 h period. Several groups of animals were used in stress induced ulcerations. One group was sacrificed without stress and was used to determine the value of mucosal generation of PGE_2 n the intact mucosa. In other series including three groups, animals were exposed to stress for 6 h and infused s.c. throughout the experimental period with saline (1 ml/h), EGF (50 μg/kg-h) or Solcoseryl (1 ml/h). In next series, the animals were given indomethacin (5 mg/kg i.p.) and subdivided into 4 groups exposed to 6 h stress and infused with vehicle (saline) (1 ml/h), EGF (50 μg/kg-h), Solcoseryl (1 ml/h) or 16,16-dimethyl-PGE_2 (10 μg/kg s.c.).

After 6 h of stress the animals were killed by cervical dislocation. The stomach was removed, opened along the greater curvature and then rinsed in ice-cold saline. The stomach was spread flat, rinsed with cold saline and inspected for growth lesions. The number and the area of lesions in oxyntic gland mucosa was counted using planimeter (Morphomat 10, Opton, FRG). and presented as means ± SEM of ulcer number for each experimental group.

Chronic ulcerations were induced in 180—200 g male Wistar rats using our modification (3) of the acetic acid method described by Okabe et al (28). Briefly, under ether anesthesia, the abdomen was opened and the stomach and duodenum were exposed. A plastic tube 4.2 mm^2 in diameter was applied tightly to the serosal surface of the anterior wall of the distal portion of the body of the stomach and then to the serosal surface of the duodenum about 5 mm beyond the pylorus. Approximately 70 μl of 100% acetic acid was poured into the tube on the surface of the stomach for 20 s. Similarly, 70 μl of 75% acetic acid was poured into the tube on the doudenum for 10 s. This method caused an immidiate necrosis of the entire thickness of the mucosa exactly within the area of acetic acid application and resulted in the formation within next few days of chronic gastric and duodenal ulcerations that did not penetrate into the surrounding tissues and healed within 2—3 weeks.

Several groups of rats were used. Group A was treated with vehicle (saline), group B received hEGF, 3 times daily at a dose of 10 μg/kg, group C was given Solcoseryl 10 ml/kg per day given i.g. in three equal doses, group D was treated with difluoromethylornithine (DFMO) (Merrel Dow Pharmaceutical, Cincinnati, OH) (200 mg/kg i.p., group E obtained DFMO+EGF and group F — DFMO+Solcoseryl. All animals received only water on the day of operation and then were maintained on the Purina chow diet and tap-water ad libitum for the duration of the experiment.

To evaluate the results the animals were killed after 7 days of treatment. Under ether anesthesia the stomach and duodenum were removed and rinsed with cold saline. The area of gastroduodenal ulcers were mesured planimetrically and the area was expressed in mm^2. The mucosa of the half of the stomach containing intact mucosa was rececte and the mucosa was scraped from the oxyntic gland area.

Statistics

Results are presented as means ± SEM. Means were compared by analysis of variance and were considered to be significantly different if $P < 0.05$.

RESULTS

The concentration of EGF-like material in the standard preparation of Solcoseryl (40 mg of dry blood extract per ml) averaged about 0.4 ng/ml (Table 1). The concentrated Solcoseryl preparation (200 mg of dry blood extract per ml) contained about 2 ng/ml of EGF. With the progressive dilution of the concentrated Solcoseryl there was a gradual decline in the EGF immunoreactivity in the solution (*Table 1*).

Table 1

	Immunoreactive EGF ng per ml
Standard Solcoseryl (40 mg/ml)	0.39 ± 0.11
Concenrtated Solcoseryl (200 mg/ml)	2.18 ± 0.18
Solcoseryl (200 mg/ml)	
Dilution 1 : 2	0.95 ± 0.14
Dilution 1 : 4	0.42 ± 0.09
Dilution 1 : 8	0.26 ± 0.05
Dilution 1 : 16	0.12 ± 0.04

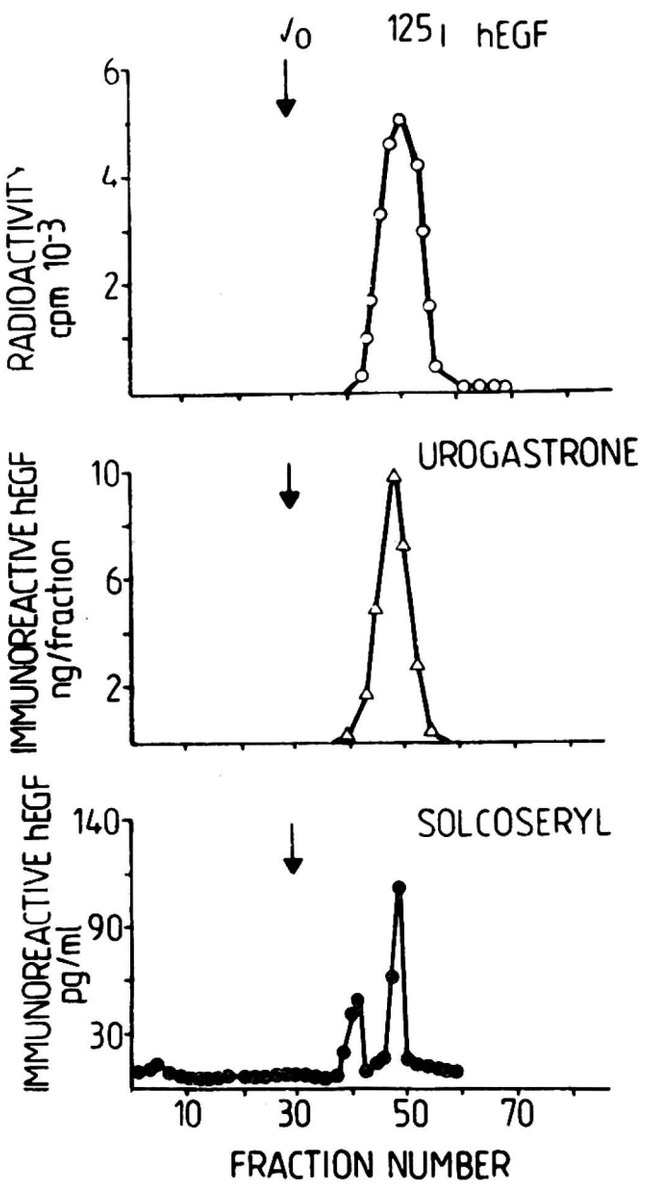

Fig. 1. Sephadex G-50 gel exclusion chromatography of iodinated hEGF (upper panel), urogastrone (middle panel) and concentrated Solcoseryl preparation (lower panel).

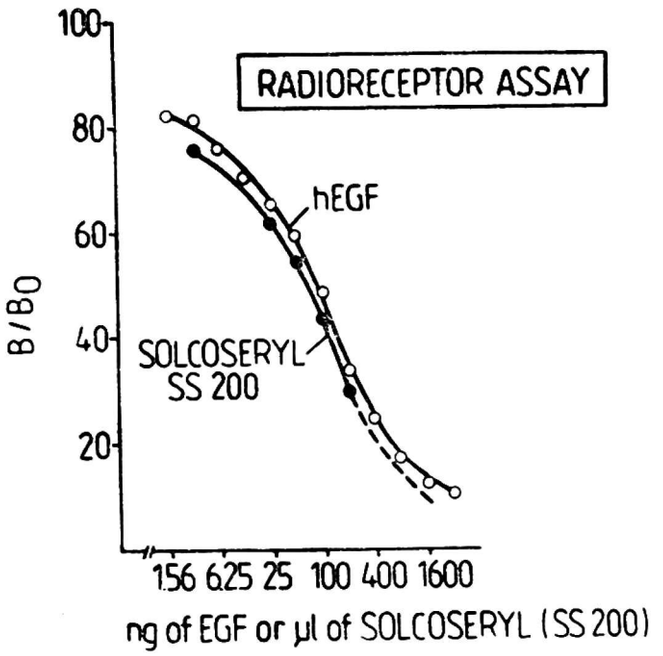

Fig. 2. Radioreceptor assay of EGF using hEGF and concentrated Solcoseryl preparation.

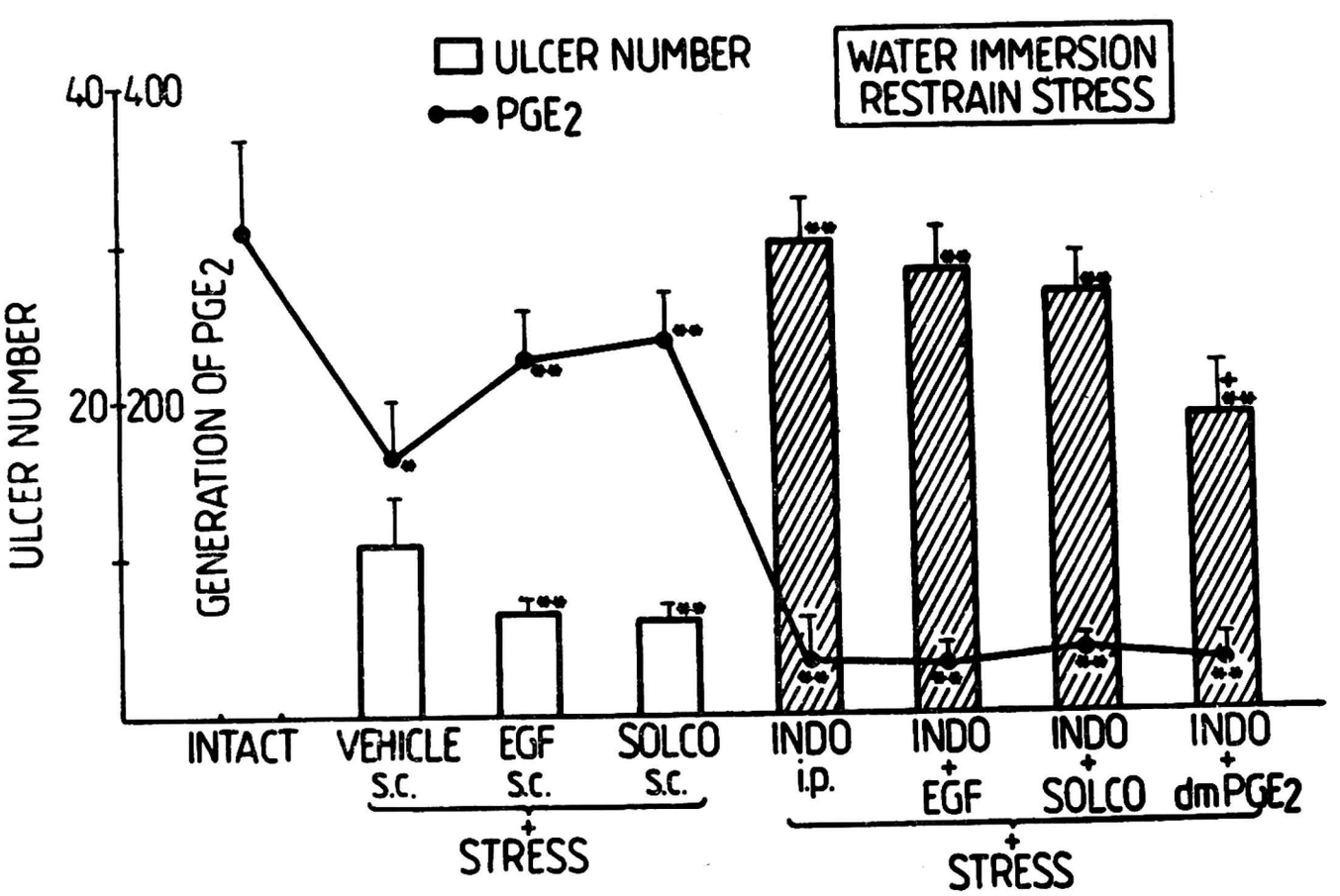

Fig. 3. The number of stress induced gastric ulcerations and mucosal generation of PGE_2 in rats without and with pretreatment with indomethacin (5 mg/kg i.p.) alone or in combination with EGF (50 µg/kg—h s.c.). Solcoseryl (1 ml/h s.c.) or 16,16 dimethyl PGE_2 (10 µg/kg s.c.). For comparison, the value of PGE_2 in the intact oxyntic mucosa is included. Mean ±SEM of 8—10 experiments on 8—10 rats.

Fig. 1 shows that human EGF and urogastrone merged as a single radioactivity peak from the Sephadex G-50 column. EGF present in Solcoseryl was eluted from the column in two peaks, one smaller and the other major peak ocurring at the same position as EGF or urogastrone. Radioreceptor assay shows that EGF material present in Solcoseryl preparation generated parallel binding curves on that of standard hEGF over the whole range of the assay concentrations (*Fig. 2*).

Water immersion and restraint stress for 6 h caused visible lesions in the mucosa of the oxyntic gland area (*Fig. 3*). No macroscopic evidence of damage occurred at that time in either forestomach or the antrum. Damage to the stomach appeared as elongated bands of erosions following the long axis of the lumen. The number of gastric ulcer lesions averaged about 31 ± 7 per rat in vehicle treated rats. The appearance of gastric ulcerations was accompanied by about 50% reduction in the mucosal generation of PGE_2. Subcutaneous infusion of EGF (50 μg/kg-h) or Solcoseryl (1 ml/h) resulted in a significant reduction in the mean lesion number and caused significant increase

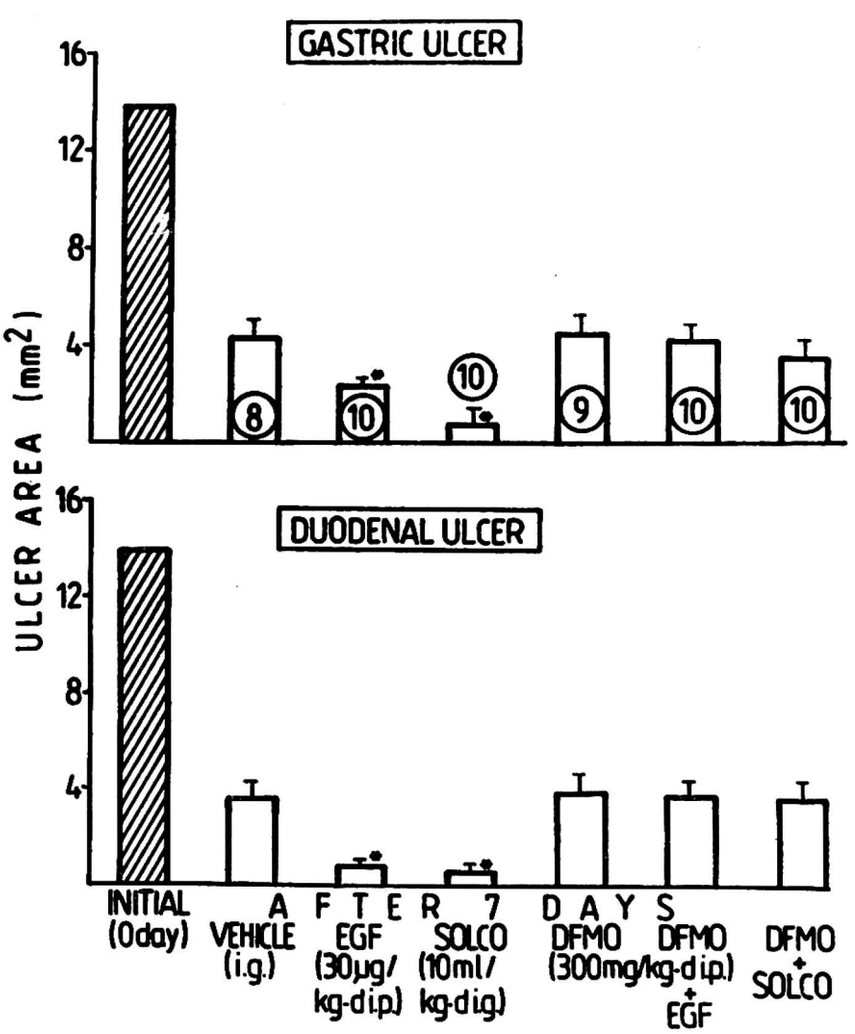

Fig. 4. Mean area of acetic acid induced gastric and duodenal ulcerations treated with vehicle (saline), EGF (30 μg/kg per day i.g.) or Solcoseryl (10 ml/kg per day i.g.) in rats without and with administration of DFMO. Means ±SEM of 8—10 rats per each experimental growth. Asterisk indicates significant decrease below the control value obtained in rats treated with saline (vehicle).

in the mucosal generation of PGE_2. Pretreatment with indomethacin (5 mg/kg i.p.) increased the number of gastric ulcerations about three folds as compared to stress animals without indomethacin. The mucosal generation of PGE_2 was reduced in indomethacin treated rats by over 90% when compared to the intact gastric mucosa. Administration of EGF or Solcoseryl in the same doses which produced significant reduction in stress ulcerations, failed to affect the number od gastric lesions in indomethacin treated animals. In contrast, injection of 16, 16-dimethyl-PGE_2 (10 μg/kg s.c.) resulted in a significant decrease (by about 25%) of the number of gastric lesions in indomethacin treated rats.

The effect of 7 day treatment with EGF (30 μg/kg per day) and Solcoseryl (10 ml per kg per day) in rats with chronic gastric and duodenal ulcers are shown on *Fig. 4*. Both EGF and Solcoseryl were effective in a significant acceleration of the healing of both gastric and duodenal ulcerations. Pretreatment with DFMO (200 mg/kg i p.) delayed the healing of gastroduodenal ulcers and abolished the enhancement of this healing induced by EGF or Solcoseryl.

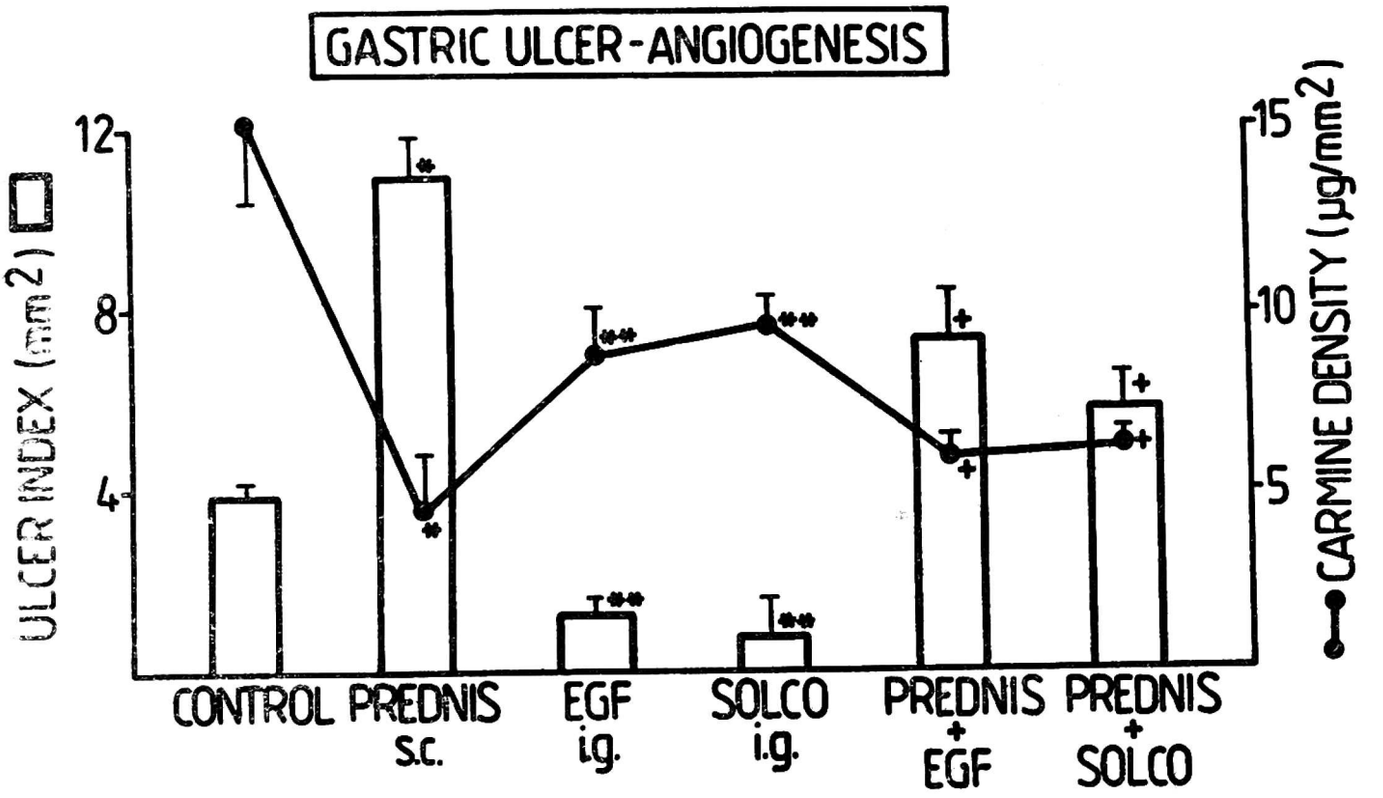

Fig. 5. Mean afea of gastric ulcerations induced by acetic acid and the density of carmen dye in the granulation tissue at the ulcer base in rats without any treatment (control) and in rats receiving daily treatment with prednisolone (50 mg/kg—day s.c.), EGF (30 μg/kg—day i.g.), Solcoseryl (10 ml/kg—day i.g.) and the combination of EGF or Solcoseryl and predinsolone. Means ±SEM of 8—10 rats per each experimental group. Single asterisk indicates significant change as compared with control values. Double asterisks indicate significant decrease as compared with the control values. Cross indicates significant decrease as compared with the value obtained with prednisolone.

Treatment with prednisolone (50 mg/kg/day s.c.) for 7 days significantly increased the ulcer area as compared to the control rats injected with saline. The carmine density in prednisolone treated rats fell to about 30% of the control value. In rats receiving treatment with EGF or Solcoseryl, the healing rate of gastroduodenal ulcers was significantly enhanced as compared to control animals treated with vehicle. The combination of prednisolone and EGF or Solcoseryl resulted in a significant acceleration of ulcer healing as compared to that obtained with prednisolone alone. Carmine density in the ulcer base in these animals also showed significant increase above the value obtained with prednisolone alone.

DISCUSSION

This study demonstrates for the first time that Solcoseryl preparation contains EGF-like immunoreactivity as determined by both radioimmunoassay and radioreceptorassay. The content of EGF-like material in Solcoseryl preparations increased with the concentration of the dry blood extract in the preparations. EGF present in Solcoseryl showed similar elution profile on gel chromatography to that of urogastrone or radio-labelled hEGF. The presence of specific EGF-like material is also confirmed by the generation with Solcoseryl of parallel binding curve on that of a standard hEGF in radio-receptor assay.

Although EGF-like material was found in Solcoseryl preparations it is not clear whether this material is responsible for the gastroprotective and ulcer healing properties of the drug. The major evidence supporting the concept of EGF mediation in the antiulcer effect of Solcoseryl was provided by the comparison of the activity of Solcoseryl and EGF in the prevention of stress ulcerations and in the healing of chronic gastroduodenal ulcers. As shown in this study both Solcoseryl and EGF were effective in the prevention of stress-induced gastric lesions and of the reduction in mucosal generation of PGE_2. The involvement of mucosal PG in the protection by both Solcoseryl and EGF is supported by our finding that the pretreatment with indomethacin, that reduced by over 90% mucosal generation of PGE_2, almost completely abolished the gastroprotection by Solcoseryl and EGF. However, the content of EGF in the hEGF preparations used in this study was several orders of magnitude higher than that in the Solcoseryl preparation. It is reasonable to assume, therefore, that EGF is not likely to play any major role in the biological action of Solcoseryl and other, as yet unspecified gastroprotective compounds, are involved. In this respect it should be emphasized that Solcoseryl contains phospholipids which were shown previously to protect the mucosa against various ulcerogens probably due to the enhancement

of the hydrophobicity of the gastric mucosal surface and strengthening of mucosal barrier (29, 30).

This study confirms that Solcoseryl, like EGF (3, 25), is highly effective in acceleration of the healing process of chronic gastroduodenal ulcerations induced by acetic acid. The mechanism of this enhancement is not clear but the trophic effects on the gastric mucosa may be of crucial importance for both these agents because the prevention of the growth promoting activity by blocking ODC activity with DFMO (31) virtually abolished the ulcer healing effect of these drugs. Since ODC is the key enzyme in the biosynthetic pathway of mucosal polyamines (32) it may be accepted that both EGF and Solcoseryl act, at least in part, via the stimulation of the synthesis of polyamines, which are essential for mucosal growth and repair (32, 33). Solcoseryl, as other growth factors, may also activate S6-kinase resulting in the multiple phosphorilation of the S6-protein located in the 40s ribosomal subunits. Such phosphorilation is believed to be a prerequisite for the initiation of both DNA- and protein synthesis (34, 35).

It is well known that wound healing involves several integrated processes such as the replication of epithelial cells and fibroblasts (fibroplasia), the synthesis and deposition of granulation tissue and angiogenesis (18, 36). Hypoxia in the wound area is detrimental to some of these processes and the extra supply of oxygen is known to stimulate the healing process (37). Both EGF and Solcoseryl are highly effective in stimulation of the proliferation of fibroblasts and angiogenesis in the granulation tissue (10—17). This is confirmed in part by the observation that prednisolone which reduces angiogenesis (25) delays ulcer healing and this is accompanied by the decrease of angiogenesis as determined by carmine dye technique. The restoration of angiogenesis by EGF or Solcoseryl in prednisolone-treated rats could contribute to the acceleration of ulcer healing by these agents observed in this and previous studies (14). The activation of angiogenesis by Solcoseryl or EGF probably involves the increase of EGF receptors in the granulation tissue and the increase in binding of EGF at the ulcer edge as reported by Hansson et al (38). The activation of EGF receptors could result in the proliferation of endothelial cells, neovascularization and increased oxygen supply in the ulcer area. All these effects could explain the ulcer healing activity of EGF and Solcoseryl observed in this report.

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