● **CELLULAR MECHANISMS OF DUODENAL ULCER HEALING. IS THE QUALITY OF HEALING INFLUENCED BY TREATMENT?**
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Limited clinical data indicate that mucosa of healed duodenal ulcers (DU) displays prominent histologic abnormalities (*Gastro 1985;88:1390*). Using a rat model of DU, we studied: a) the dynamic and cellular events taking place during ulcer healing and b) whether treatment with sucralfate (SCR) and/or omeprazole (OME) may influence the quality of healing as reflected by restoration of duodenal mucosal architecture. **METHODS:** DU were produced in male rats (n=94) by focal (4.0mm i.d.) duodenal serosal application of acetic acid. Rats were gavaged 2x daily for 7 or 14 days with 2ml of: a) Placebo (PLA), b) SCR, 500mg/kg or c) OME, 50mg/kg. **STUDIES:** 1) Ulcer size; 2) quantitative histology including: a) residual necrosis; b) height and composition of restored villi and crypts; c) connective tissue to epithelial cells ratio (C/EpR); d) number and size of dilated gland-like structures and e) cell proliferation. **RESULTS:** Ulcer size at 14 days (mean±SE) was 2.5±0.5mm in the PLA group, 1.1±0.2 in OME group and 1.2±0.2 in SCR group (both $p<0.01$ vs PLA). In the PLA group, ulcers were well developed at 7 days, with dilated gland like-epithelial tubes (at the base of ulcer margins) lined with poorly differentiated, actively (2.6-fold increase) proliferating cells invading granulation tissue. Mucosal scars were flat, with rudimentary villi (height ↓ to 240±30μm; $p<0.01$), few crypts, and increased C/EpR to 1.5±0.2 (vs 1.0±0.15 in normal controls; $p<0.01$). In the SCR group, the ulcer margins were better developed (height ↑ 24% vs PLA), epithelial cells were more differentiated, and at day 7, re-epithelialization and reconstruction of villi was more advanced. The mucosal scars had better restored villi (320±30μm; $p<0.01$ vs PLA), 3.5-fold fewer dilated gland-like tubes and more balanced connective to epithelial ratio (C/EpR 1.2±0.15; $p<0.05$). In the OME group, the ulcer margins were thinner vs PLA and SCR. Within the scar, villi were very flat or absent and often a single layer of epithelium devoid of goblet cells covered granulation tissue. C/EpR was 2.4±0.4; $p<0.01$ vs PLA and SCR. **CONCLUSIONS:** 1) Scars of healed experimental DU display prominent structural abnormalities, which may be the basis for ulcer recurrence. 2) Restoration of duodenal mucosal architecture within the scar is much better in the SCR than in the OME or PLA treated groups, reflecting superior quality of healing provided by the SCR.

● **SUCRALFATE TREATMENT ACTIVATES EGF GENE AND ENHANCES EXPRESSION OF bFGF AND FGFR-2 GENES IN GASTRIC MUCOSA. THE MOLECULAR MECHANISM OF ITS ULCER HEALING ACTION?**
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Sucralfate (SCR) is a non-systemic ulcer healing drug, which reduces ulcer relapse and improves the quality of ulcer healing. The precise cellular and/or molecular mechanism(s) of SCR's ulcer healing action remain unknown. Since epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and its receptors (FGFRs) play important roles in ulcer healing, we studied whether sucralfate treatment affects expression of genes encoding for EGF, bFGF, FGFR-2 and their respective proteins in the gastric mucosa. **METHODS:** 68 male rats received 500mg/kg SCR or placebo, twice daily for either 3, 7, or 14 days and gastric mucosal specimens were obtained. **STUDIES:** (A) The expression of EGF, bFGF, FGFR-2 and β-actin mRNAs was determined with reverse transcription polymerase chain reaction (RT/PCR) using specific primers. The PCR products were quantified with a videoimage analysis system. (B) Mucosal sections were immunostained with specific antibodies against EGF, bFGF and FGFR-2 and fluorescence quantified using videoimage system. **RESULTS:** SCR treatment produced a significant increase in EGF mRNA: 1,105%, 645% and 1,443%, respectively at 3, 7 and 14 days; (all $p<0.001$ vs control mucosa). Also, bFGF's mRNA increased: 406%, 251%, and 393%, respectively, at 3, 7 and 14 days ($p< 0.001$ vs normal mucosa). The FGFR-2 mRNA increased: 226% and 179% at 7 and 14 days ($p<0.01$ vs normal mucosa). Immunohistochemical staining of mucosal sections demonstrated a corresponding, significant increase in respective proteins. For EGF, the increased (>2,200% at 14 days) signal was localized to the surface epithelial and mucous neck cells; for bFGF, the increased signal (420% at 14 days) was mainly localized to the endothelial cells of microvessels, their basement membranes, lamina propria and macrophages. **CONCLUSIONS:** 1) SCR treatment triggers activation of the EGF gene. It also enhances expression of bFGF and EGFR-2 genes in the gastric mucosa with a resulting increase in mucosal concentration of all these peptides. 2) Since EGF and bFGF are crucial for cell proliferation, migration, re-epithelialization and angiogenesis, the above actions of SCR provide the molecular mechanism for the ulcer healing action of this drug.

● **RANITIDINE BISMUTH CITRATE PROTECTS GASTRIC MICROVASCULAR ENDOTHELIUM AND EXTRACELLULAR MATRIX FROM ETHANOL INJURY.**
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In previous studies, we found that the H_2 receptor antagonists cimetidine and ranitidine (*Am J Med 1985;79:19-23*), as well as, famotidine and nizatidine (*Gastro 1989;96:A505*) are not able to protect the gastric mucosa against ethanol (ETOH) injury, an acid independent type of injury. In the present study, we assessed the efficacy of ranitidine bismuth citrate (RBC; GR 12311X) in the protection of the gastric mucosa against ETOH-injury. Special attention was focused on microvascular endothelium, which is one of the major targets of ETOH injury and on extracellular matrix components [(ECM): fibronectin (FN), its receptor (FNR) and laminin (LM)] which provide structural support for gastric mucosal endothelial and epithelial cells. **METHODS:** 48 male rats received i.g. either: A) placebo; B) ranitidine (RAN 30 or 100mg/kg) or C) RBC (30 or 100mg/kg), and 2hrs later 8ml/kg 100% ETOH. **STUDIES** of the gastric mucosa at 5 min at 3 hrs after ETOH: 1) macroscopic necrosis; 2) quantitative histology including assessment of superficial and deep (>200μm) necrosis 3) distribution and expression of: a) vimentin (VM, endothelial marker), b) FN, c) FNR and d) LM were analyzed and quantified with a videoimage system after immunostaining with specific antibodies. **RESULTS:** ETOH produced extensive microvascular injury (endothelial necrosis, rupture of capillaries, platelets adherence, thrombi formation) within 5 min. By 3hrs necrosis involved 45±4% of mucosa and deep histologic necrosis involved 52±4% of mucosal strip length. In areas of deep necrosis, VM fluorescence in microvascular endothelium was reduced from 240±30 units (in normal mucosa) to 46±6; $p<0.001$, reflecting its severe damage. Expression of FN, FRN and LM was decreased by 4-, 3- and 5-folds, respectively, reflecting injury and depletion of ECM. Pretreatment with RBC (100 mg/kg) but not RAN, reduced microvascular injury at 5 min after ETOH (5.5-fold; $p<0.01$), reduced gross necrosis 5-fold and deep histologic necrosis 5.8-fold (all $p<0.01$). Also, fluorescent signals for VM, FN, FNR, and LM, were only slightly (5-30%) reduced vs normal, 3hrs after ETOH. **CONCLUSIONS:** 1) RBC protects gastric mucosa against ETOH injury. 2) Gastric microvascular endothelium and extracellular matrix are targets of ETOH-injury and RBC afforded protection. 3) RBC is a unique H_2 receptor antagonist with true cytoprotective properties.

● **IGF-I STIMULATES DNA SYNTHESIS IN ESOPHAGEAL MUCOSA.**
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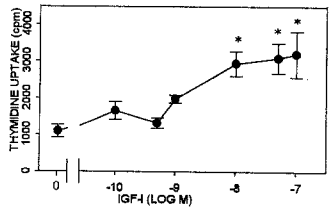
Esophageal mucosal repair remains an uncertain domain. Recent studies suggest a critical role for exogenous growth factors in mucosal proliferation. **Aim:** This study examines the effect of Insulin Like Growth Factor-I (IGF-I) on a rabbit esophageal explant model *in vitro*. **Methods:** The esophagus of New Zealand white rabbits was resected and cut into standardized blocks with mucosa trimmed to allow a canopy of muscle to serve as a base for newly proliferating cells. The explants were incubated in defined media at 37° C, 5% CO_2. Media was supplemented with IGF-I, TGF-α, or EGF. The rate of DNA synthesis was measured by ^{3}H-Thymidine incorporation. Explants were pulsed with 3.6 μCi/ml ^{3}H-Thymidine 18 hours before harvesting the tissue on the 4th day. The explants were then weighed, dessicated, solubilized and counted in a ß-scintillation counter. Data represent ^{3}H-Thymidine uptake (cpm)/mg dry weight (mean ± SEM; *$p<0.05$ vs no growth factor, ANOVA-Bonferoni t-test, n ≥ 6/group). Epithelial proliferation from the cut edges of the mucosa was confirmed with light and electron microscopy.



Results: The explant model allowed us to culture the tissue for up to two weeks. Cell proliferation was stimulated by IGF-I (171% vs control), TGF-α (159%), and EGF (132%). IGF-I mitogenesis was dose dependent with maximal effect at 10nM. Hematoxylin and eosin staining and electron microscopy confirmed growth of multilayer neo-mucosa tissue of epithelial origin with deposition of basement membrane. **Conclusions:** (1) The esophageal mucosal explant model allows growth of new mucosa in vitro. (2) IGF-I stimulates esophageal mucosal DNA synthesis in a dose dependent manner. IGF-I may enhance mucosal repair by stimulating epithelial proliferation and has potential therapeutic application in esophageal injury.