Ulcer Healing Effect Of Anti-Ulcer Agents: A Comparative Study

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Citation

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Abstract

Gastric ulcer being the most prevalent gastrointestinal disorder is considered as a major therapeutic target. Large numbers of drugs ranging from proton pump inhibitors, H2 receptor antagonists to cytoprotective agents are being used for treatment of this disease.

Purpose of the study: In the present study, we have made an attempt to compare the ulcer healing activity of four well known drugs, omeprazole, misoprostol, ranitidine and sucralfate which are known to act against ulcers through different routes. Celecoxib was used as negative control.

Material and method: Ulcers were produced in Sprague-dawley rats by application of 40% acetic acid. Different drugs were given to ulcerated rats to check the effect of drugs on healing of ulcers. Further confirmation of extent of healing was done by histopathology of ulcerated stomach.

Results: We found omeprazole being most effective drug followed by misoprostol, ranitidine, sucralfate while celecoxib delayed the normal ulcer healing. Omeprazole has reduced the ulcerated area to 2.0mm2 in comparison to controls of 19.5mm2, showing 89.74% healing in comparison to controls on 15th day.

Conclusion: We conclude from the study that drugs which not only decrease the acid secretion but also increase cytoprotective activities are more effective in ulcer healing. The study provides further support to the fact that cyclooxygenase-2 iso-enzyme which induces prostaglandin synthesis plays a vital role in ulcer healing as depicted by delayed healing by celecoxib.

INTRODUCTION

Among different disorders of gastrointestinal system, peptic ulcer is the one which is more prevalent and have greatest clinical impact. Ulcer is characterized by disruption of mucosal integrity leading to local defect or excavation due to active inflammation[1]. Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, H. pylori and NSAID's) and local mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins). Integrity of gastro duodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors[2].

Clinically, regulation of gastric acid secretion is considered as major therapeutic target in the management of disease[3]. Among clinically established drugs, H_2 blockers (ranitidine etc) and proton pump inhibitors (omeprazole etc) are most widely used as anti-ulcer drugs in addition to the cytoprotective agents like sucralfate and misoprostol.

Many experimental investigations have been undertaken to elucidate the etiology of development of ulcers induced by various means, however ulcer healing process has been given a lesser consideration even though of same importance. Ulcer healing consists of reconstruction of mucosal architecture and is a dynamic, active process of filling the mucosal defects with epithelial and connective

tissue cells. It encompasses cell proliferation, division and migration[4,5,6]. Prostaglandins (PGs) and growth factors play an important role in healing of ulcers. Synthesis of PGs is governed by the expression of inducible cyclooxygenase-2 (COX-2) isozyme in gastric mucosa during healing process, further, COX-2 expression is enhanced in gastric epithelial cells after treatment with growth factors in vitro and in vivo during acetic acid induced gastric damage[7,8,9].

In the present study, we have compared four anti-ulcer drugs, omeprazole, misoprostol, ranitidine and sucralfate for their ulcer healing activity with a negative control celecoxib.

MATERIALS AND METHODS

ANIMALS

Sprague-Dawley rats (National Animal Laboratory Center, Central Drug Research Institute, Lucknow) of either sex, weighing 160-180 gm were kept in an environmentally controlled rooms (25±2C, 12 h light and dark cycle), inside raised mesh bottom cages to prevent coprophagy. The animals were deprived of food, and had free access to water for 18 h before ulcer induction. Each group consists of 10 animals.

INDUCTION OF ACETIC ACID INDUCED ULCERS

Gastric ulcers were induced by local application of acetic acid to Serosal surface of the stomach as previously reported[10]. Briefly, the abdomen of animals was opened under ether anesthesia and stomach was exposed. Serosal surface of glandular portion was exposed to 60µl of 40%acetic acid by using a round ring of 6mm in diameter. 90sec later, the acid solution was removed and wiped with filter paper and abdomen was closed. Thereafter, the animals were fed normally. The test drugs or vehicle were administered orally for 2 weeks starting from 3 days after the application of acid. After treatment, on 5th and 15th day, the animals were sacrificed and their stomachs were removed. Each stomach was then opened along the greater curvature. The ulcerated area (mm^2) was determined under zoom stereomicroscope with a square grid (X10). After determination of ulcer size, stomachs were fixed with 10% formalin for histological study.

DRUG TREATMENT

Omeprazole (Sigma chemicals, USA), suspended in 0.5% carboxymethyl cellulose, misoprostol (Zydus-Cadila, India), ranitidine (Zydus-Cadila,India), sucralfate (Menarini Raunaq, India) and Celecoxib (Dr. Reddy's Lab., India), suspended in water were administered orally once daily. The doses of omeprazole, ranitidine, sucralfate and celecoxib were given as 10, 5, 500 and 10mg/kg body weight respectively whereas misoprostol was administered at 100µg/kg body weight.

HISTOLOGICAL ANALYSIS

Histological studies were performed according to the methods previously described[11]. At autopsy, small pieces of tissue, including ulcers, were embedded in paraffin and sectioned at 5µm in an automated microtome. Haematoxylin and eosin staining was done. Tissue contraction, regeneration of the ulcerated mucosa, formation of granulation tissue, glands arrangement and inflammatory exudates were observed under stereomicroscope.

STATISTICAL ANALYSIS

All data are expressed as mean ±SEM. Statistical analysis was performed using one way ANOVA followed by Dunnett's multiple comparison test. Data was computed for statistical analysis by using Graph Pad PRISM software.

RESULTS

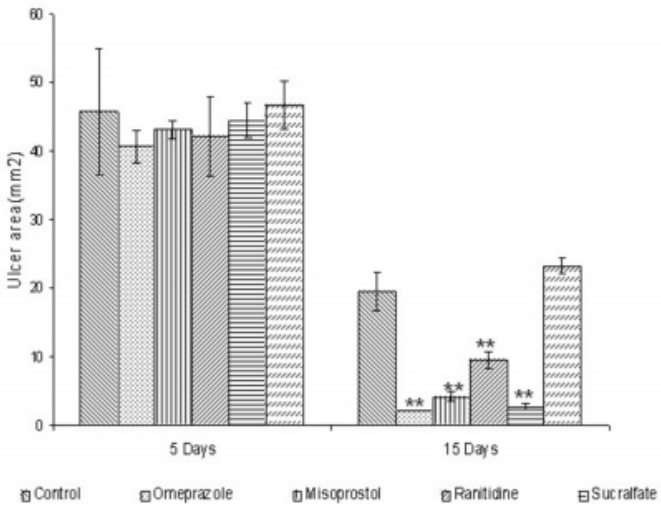
EFFECT OF OMEPRAZOLE, RANITIDINE, MISOPROSTOL, SUCRALFATE AND CELECOXIB ON HEALING OF ACETIC ACID INDUCED GASTRIC ULCER

Three days after serosal application, gastric ulcers were developed with an incidence of 100%. Repeated administration of standard anti-ulcer drugs significantly healed ulcers as evaluated by the ulcerated area.

The ulcerated area in omeprazole, ranitidine, sucralfate, misoprostol and celecoxib treated groups on 5th day was 40.6±2.4, 43.25±1.32, 42.2±5.8, 44.5±2.59 and 46.75±3.64mm^2 respectively, in comparison to 45.75±5.92mm^2 in the control group however, on 15th day the ulcerated area was 2±00, 4.25±0.63, 9.5±1.26, 2.75±0.49 and 23.25±1.11 mm^2 respectively in comparison to 19.5± 2.72 mm^2 in the control group (Fig 1).

Figure 1

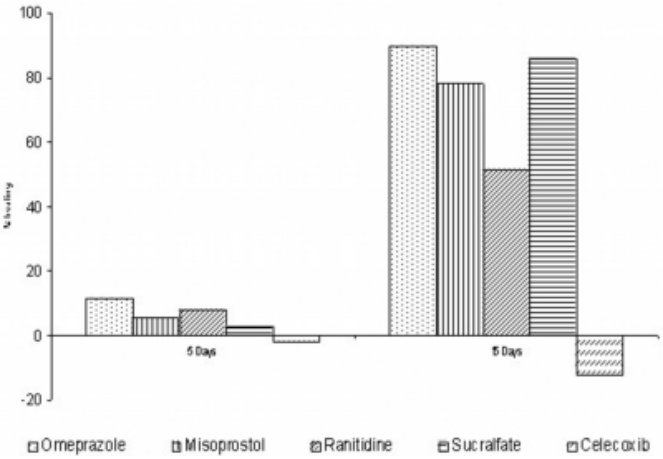
Figure 1: Effect of omeprazole, misoprostol, ranitidine, sucralfate and celecoxib on area of ulceration after 5th and 15th day of treatment. ** P



The effect of healing was calculated with respect to ulcerated area of control. The % healing of omeprazole, ranitidine, sucralfate, misoprostol and celecoxib on 5th day was 11.25, 5.46, 7.75, 2.7 and-2.1% respectively whereas on 15th day the healing was 89.74, 78.20, 51.28, 85.89 and -12.2% respectively as shown in Fig 2.

Figure 2

Figure 2: Effect of omeprazole, misoprostol, ranitidine, sucralfate and celecoxib on percentage of healing of gastric ulcer.



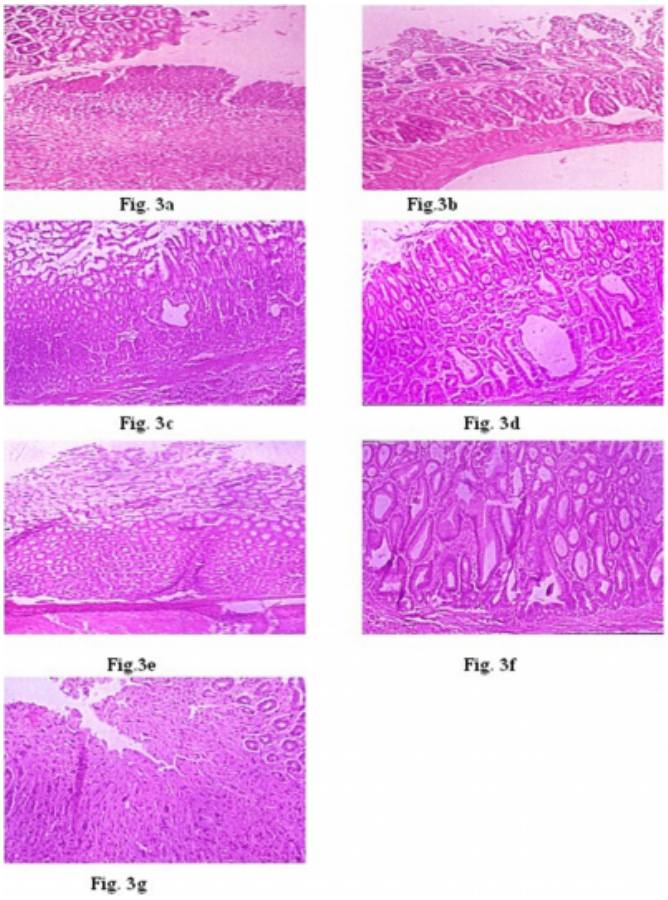
HISTOPATHOLOGY OF STOMACHS TREATED WITH OMEPRAZOLE, RANITIDINE, MISOPROSTOL, SUCRALFATE AND CELECOXIB IN ACETIC ACID INDUCED ULCER

After 5 days of treatment, control, omeprazole, ranitidine, misoprostol, sucralfate and celecoxib treated rats showed sharply defined mucosal ulcer in stomach. Damaged mucosal epithelium, glands, inflammatory exudates, proliferated fibroblast and cellular debris was found in the ulcerated wall of stomach (Fig. 3a). No sign of healing was seen in any of the treated groups, similar to control rats.

On 15th day of the study, control rats showed reduced inflammatory exudates along with some extent of mucosal regeneration, glandular organization and reduced size of ulcer (Fig. 3b). Omeprazole treated rats at this point showed clear evidence of restoration of mucosal epithelium (reepithelization), almost complete clearance of inflammatory exudates and good secretory activity of normally arranged glands. Some superficial remodeling was also seen (Fig.3c). In misoprostol treated rats, picture was almost similar as in omeprazole treated rats. Minimal inflammatory exudates and proper organization of glands was seen (Fig.3d). In ranitidine treated rats, few inflammatory exudates with cellular debris were present. Mucosal epithelium was not formed completely and less organized glands were seen (Fig. 3e). In sucralfate treated rats, glands were at the proliferative stage with few fibroblasts, cellular debris and mucosal inflammatory exudates (Fig.3f). The celecoxib treated group has not shown any healing in comparison to control group. Celecoxib treated rats had more inflammatory exudates, disorganized glands and a bigger size of ulcer that of controls (Fig.3g).

Figure 3

Figure 3: Histological examination of stomachs treated with different drugs in acetic acid ulcer model under 100 X magnification. (a and b) controls (5 days &15 days respectively), (c-g) 15th day examination of omeprazole, misoprostol, ranitidine, sucralfate and celecoxib treated stomachs respectively.



DISCUSSION

In this study, we compared the effect of various clinical agents on healing of ulcers induced by acetic acid. We observed that among different anti-secretory and cytoprotective agents, omeprazole was found to be most effective drug. Omeprazole produced highest protection of 89.74% on 15th day, followed by misoprostol, ranitidine and sucralfate.

Validity of acetic acid induced ulcer model for ulcer healing studies was substantiated by Tsukumi et al, 1994[12]. Acetic acid solution consistently induces penetrating ulcers and lacks adhesion of ulcer base to underlying liver. Ulcers are caused by hyper-secretion of acid in this model. Efficacy of omeprazole and ranitidine confirms that inhibition of acid may enhance healing of ulcers. Our results comply with previous reports, where omeprazole was found to enhance healing of ulcers due to its potent anti-secretory effect. In addition to anti-secretory activity higher protective effect of omeprazole may be due to its gastric activity also. It has already been shown that repeated treatment with proton pump inhibitors increase the level of PGs in the gastric mucosa, which is considered to be chief mediators in gastric cytoprotection[13,14,15]. This could be due to the fact that omeprazole might have increased the expression of COX-2 isoform in ulcerated mucosa during healing process because COX-2 mediated PGs generation is known to dominate the COX-1 mediated protection[16]. Further, efficacy of omeprazole can be substantiated by its trophic effects on damaged gastric mucosa mediated by serum gastrin. Proton pump inhibitors are reported to increase the levels of serum gastrin which is supposed to accelerate the regeneration of ulcerated mucosa[3], [17]. Similarly efficacy of another anti-secretory agent, ranitidine may be due to its inhibitory effect on acid secretion. However, less efficacy indicates lesser acid inhibiting effect than omeprazole. Further, reports indicate that anti-secretory effect of ranitidine is of short duration[18]. Previous reports also indicate the quicker healing effect of omeprazole than ranitidine is because proton pump inhibitors directly inhibit acid secretion[19].

Role of PGs alone may not be sufficient in accelerating the healing process as is evident from lesser efficacy of misoprostol. Even though misoprostol accelerated healing process more than other agents but its effect is less than that of omeprazole. Protective effect of misoprostol is due to its prevalent direct cytoprotective effect of PGs coupled with anti-secretory effect[20,21]. Moreover relative delay in ulcer healing is could be due to reduction in level of both directly acting PGs and indirectly acting PGs which in turn reduces the ulcer healing by affecting other component involved in ulcer healing.

Efficacy of sucralfate is due to its protective effect by forming a coat over ulcer base which prevents the direct effect of acid on ulcer base. It neither effect acid secretion nor generate prostaglandins[2], which could be the possible reason for sucralfate being least effective in ulcer healing.

Celecoxib, a selective COX-2 inhibitor showed delayed healing in our study. COX-2 is the key enzyme for the formation of PGs in ulcerated mucosa. COX-2 protein enhances angiogenesis and improves the gastric mucosal blood flow and induces various growth factors responsible for ulcer healing. COX-2 expression is upregulated in the margins of healing of gastric ulcers [22], [23] which supports the concept that COX-2 represents a further line of defense

necessary for maintenance of mucosal integrity and ulcer healing. Similarly in our studies inhibition of PGs generation by selective COX-2 inhibitors attenuated the healing of ulcers which is in agreement with earlier studies on mice[24]. This further supports importance of COX-2 in ulcer healing.

Histologically, omeprazole increased the rate of regeneration of the ulcerated mucosa. In general gastrin is known to have a trophic action on the gastric mucosa[25]. As omeprazole increases serum gastrin level, the accelerated regeneration of the ulcerated mucosa is likely due to an increase in serum gastrin level. Omeprazole shortened muscularis mucosa. Although the acceleration of tissue contraction may be based on their inhibition of acid secretion, but precise mechanism is unknown. Similarly, ranitidine also act via gastrin, regenerating mucosa. Sucralfate had no effect on tissue contraction or regeneration of mucosa but accelerates the healing through the protection of basic fibroblast growth factor from gastric acid. Therefore, sucralfate probably increases the thickness of the ulcer base through the same mechanism. Misoprostol along with accelerating regeneration of mucosa, stimulates formation of granulation tissue, resulting in thickness of the ulcer base. Celecoxib delays healing procedure by decreasing epithelial cell proliferation, angiogenesis and maturation of granulation tissue in ulcer margin that is why it delays healing of ulcer.

CONCLUSIONS

In conclusion, drugs which not only decrease acid secretion but also increase cytoprotective activity possibly by enhancing PGs level have greater efficacy in ulcer healing. Omeprazole was found to be most effective as it carries both these properties while other drugs which have either of these properties are less effective. Moreover, it was observed that inhibition of COX-2 enzyme attenuated the healing of ulcers as exhibited by delayed healing activity of celecoxib in comparison to high activity of misoprostol and omeprazole. Use of selective COX-2 inhibitors as anti-inflammatory agents, should be well justified prior to usage in case of gastric ulcer patients. In such conditions usage with anti-secretory agents coupled with NSAID's should be more preferred choice than COX-2 inhibitors.

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