Short communication

Combination therapy of gamma-aminobutyric acid derivative promotes proton pump inhibitor based healing of reflux esophagitis in animal model

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ABSTRACT

Background: The present study was undertaken to elucidate the effect of omeprazole and baclofen on experimental esophagitis in albino rats.
Methods: Groups of rats, fasted overnight received normal saline (3 ml/kg, ip; sham control) or toxic control (3 ml/kg, ip) or omeprazole (30 mg/kg, ip) or baclofen (20 mg/kg, ip) or their combinations, were subjected to the pylorus and forestomach ligation.
Animals were sacrificed after 6 h and evaluated for the gastric pH, volume of gastric juices, total acidity, esophagitis index and free acidity. Esophageal tissues were further subjected to estimations of TBARS, GSH, catalase and SOD.
Results: Treatment with omeprazole and baclofen significantly inhibited the gastric secretion, total acidity and esophagitis index. The treatment also helped to restore the altered level's oxidative stress parameters to normal.
Conclusion: The beneficial effect of omeprazole and baclofen against GERD could be conglomerately attributed to the antisectretory action of omeprazole and reduction in the tracheal lower esophageal sphincter release rate by baclofen.
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Introduction

Gastroesophageal reflux disease (GERD)/gastric reflux disease or acid reflux disease is apparently the most frequently occurring functional foregut disorder and accounts for approximately 75% of esophageal pathology in the industrial countries. GERD is the most common outpatient gastroenterological diagnosis in the USA, with a prevalence rate of 10–20% and an annual incidence of 0.38–0.45% in the western world [1]. GERD significantly reduces health-related quality of life and intrude a marked economic dead weight on the healthcare system [19]. Recent investigations have reported that the mucosal damage in GERD is due to several causative agents in the refluxate [12] that stimulate mucosal and sub-mucosal cells to release mediators, eliciting an inflammatory reaction and leading to visceral hypersensitivity and other symptoms of GERD. Inflammatory process seems to play a key role in the underlying mechanisms of the symptoms and pathogenesis of other gastrointestinal conditions as well, such as functional dyspepsia and irritable bowel syndrome [2].

Omeprazole is a proton pump inhibitor (PPI) used in the treatment of dyspepsia, peptic ulcer disease, gastroesophageal reflux disease, laryngopharyngeal reflux and Zollinger–Ellison syndrome. It is most acknowledged antisecretory agent in terms of symptomatic relief and mucosal healing in GERD [6]. Baclofen is a derivative of gamma-aminobutyric acid (GABA) and a recent study has divulged that baclofen reduces the tracheal lower esophageal sphincter release rate (TLESR) by 40–60% and reflux episode up to 48% (by increasing esophageal sphincter basal pressure and accelerating gastric emptying) [7,9]. The recidivating nature of GERD requires long term maintenance therapy and none of the currently available medications provides long term cure [15]. Tiberiu and Fass [19] in their report has estimated that 10–40% of patients with GERD fail to reciprocate symptomatically, either partially or completely, to a standard dose of PPI. About 90% of PPI treated GERD patient are partially or completely unresponsive to standard dose/duration of PPI therapy, for such patients either increased dose/duration of the same therapy or using amalgamation therapies can provide more symptomatic relief for long duration [5,11]. In view of above, the present study was ventured to inquest the effect of monotherapy and combination therapy of baclofen and omeprazole on experimentally induced esophageal lesions in animal model.

Materials and methods

Drugs and chemicals

Baclofen and omeprazole were procured from Sun Pharma Ltd., Mumbai, Maharashtra, India and Dr. Reddys Laboratories Ltd., Hyderabad, Andhra Pradesh, India respectively. All other chemicals were purchased from S.D. Fine Chemicals, Mumbai, India.

Animals

Wistar strain albino rats (175–200 g) were obtained from Indian Veterinary Research Institute, Izatnagar, Bareilly (UP). Animals were housed under standard conditions of temperature (25 ± 1 °C) with 12 h light/dark cycle and had a free access to commercial pellet diet and water ad libitum. The animals were given a week's time to get acclimatized with the laboratory condition, before experimentation. The study was approved by the Institutional Animal Ethics Committee (Ref: 43/1279/ac/SU/IAEC/2012).

Induction of esophagitis

Animals were randomized and divided into five groups of six animals each. Groups of rats, fasted overnight received normal saline (3 ml/kg, *ip*; sham control) or toxic control (3 ml/kg, *ip*) (forestomach and pylorus ligated) or omeprazole (30 mg/kg, *ip*) or baclofen (20 mg/kg, *ip*) or their combinations. After 1 h, coeliotomy was performed and esophagitis was induced by ligating the forestomach and pylorus with 2-0 silk suture under pentobarbitone (50 mg/kg, *ip*) anesthesia. After 6 h, animals were sacrificed by cervical dislocation and the chest was opened with a median incision, the tissue esophagus and stomach were removed. The stomach was opened along the greater curvature, and the esophagus was dissected out by extending the dissection line along the major axis. The tissue was washed with normal saline and examined for lesions. The severity of the erosions was scored and the index was calculated by dividing the total score by ten, which was designated as esophagitis index [14].

Erosion (mm)	Score
1 or less	1
1–2	2
>2	3

The volume of gastric juice, total acidity and pH were measured as described subsequently under "gastric secretion in pylorus ligated rats" [8].

Estimation of free radical generation

Esophageal tissue was minced well, homogenized in ice-cold 0.01 M Tris–HCl buffer, pH 7.4 and subjected to the estimations of thiobarbituric acid reactive substances (TBARS) [10], tissue glutathione (GSH) [16], catalase [3] and superoxide dismutase (SOD) [1].

Statistical analysis

All data were presented as mean $\pm$ SD and analyzed by one way ANOVA followed by Student–Newmans–Keuls test for the possible significance identification between the various groups. Statistical significance was compared to control [(a) $p < 0.05$, (b) $p < 0.01$, (c) $p < 0.001$] and toxic control [(x) $p < 0.05$, (y) $p < 0.01$, (z) $p < 0.001$]. Statistical analysis was carried out using Graph Pad Prism 3.0 (Graph Pad Software, San Diego, CA).

Results

Ligating the forestomach and pylorus augmented reflux esophagitis in all the animals marked by macroscopically visible necrosis and cogent ulceration in the esophagus. Treatment with monotherapy of omeprazole and baclofen significantly constrained the lesion formation in esophagus. Omeprazole significantly inhibited the esophagitis index (1.24 ± 0.14) in comparison with toxic control (4.32 ± 0.24) (Fig. 1). The unification therapy of omeprazole and baclofen produced 74.31% inhibition of esophagitis index respectively. The combination of omeprazole and baclofen, inhibited the esophagitis index, decreased the volume of gastric juices, total acidity and increased the gastric pH to a sententious level, suggesting the possible synergistic effect (Fig. 2). There was a momentous increase in the TBARS generation (7.44 ± 0.48 nmol of MDA/mg of protein) in toxic control in collation to normal control (0.92 ± 0.18 nmol of MDA/mg of protein) indicating reactive oxygen species (ROS) generation and oxidative stress. Our results suggest that the reflux esophagitis produces free radical species that attack lipid components, leading to lipid peroxidation. Concomitant administration of the omeprazole and baclofen as a monotherapy significantly repressed the lipid peroxidation manifested by decreased TBARS levels, i.e. 3.21 ± 0.30, 3.25 ± 0.27 and 4.92 ± 0.50 respectively.
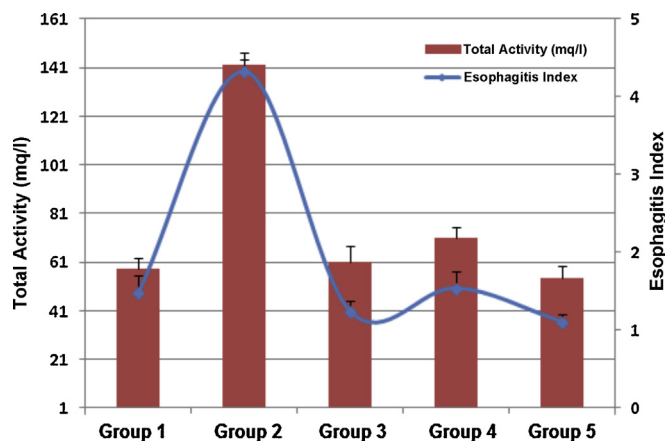

Fig. 1. Effect of omeprazole and baclofen on total acidity and esophagitis index in experimental animals subjected to pylorus and forestomach ligation (please refer Table 1 for treatment schedule of a particular group) (values are mean $\pm$ SD), each group contains 6 animals.

Fig. 2. Effect of omeprazole and baclofen on volume of gastric juices and pH in experimental animals subjected to pylorus and forestomach ligation (please refer Table 1 for treatment schedule of a particular group) (values are mean $\pm$ SD), each group contains 6 animals.
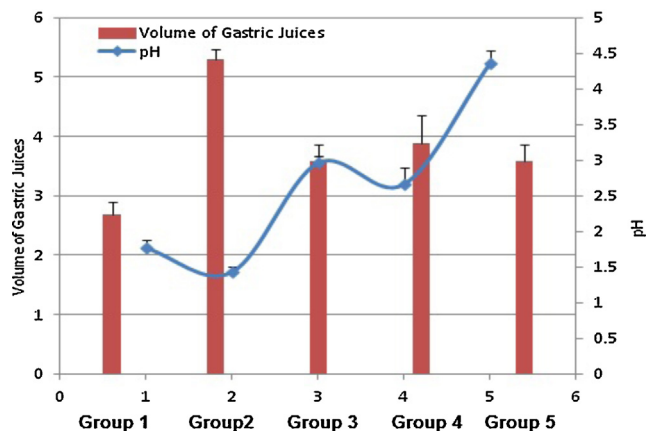
Table 1
Effect of omeprazole and baclofen on GSH, TBARS, SOD and catalase in esophageal tissue.

S. no.	Treatment	GSH (mg%)	TBARS (nmol of MDA/mg of protein)	SOD (SOD/mg of protein)	Catalase (nmol of H_2O_2/min/mg of protein)
Group-I	Sham control	238.37 ± 34.22[***]	0.92 ± 0.18[***]	6.22 ± 0.81[***]	31.87 ± 3.01[***]
Group-II	Toxic control	155.07 ± 24.15	7.44 ± 0.48	1.77 ± 0.13	7.98 ± 3.01
Group-III	Omeprazole	195.12 ± 23.21[*]	3.21 ± 0.30[*]	4.22 ± 0.52[**]	18.38 ± 2.011[**]
Group-IV	Baclofen	165.17 ± 17.11	3.53 ± 0.27[*]	3.55 ± 0.48[*]	11.73 ± 132
Group-V	Omeprazole + baclofen	217.49 ± 23.94[***]	2.48 ± 0.21[***]	4.67 ± 0.52[**]	23.68 ± 2.27[***]

Each group contains six animals; all data are presented as mean $\pm$ SD. Statistical significance compared to toxic control using; one way ANOVA followed by Student–Newman–Keuls test.
[*] $p < 0.05$ was considered statistically significant.
[**] $p < 0.01$ was considered statistically significant.
[***] $p < 0.001$ was considered statistically significant.

Amalgamation therapy of omeprazole and baclofen decreased the TBARS levels to 2.48 ± 0.21, pointing the synergestic efficacy of the both. Glutathione (GSH) acts as an antioxidant co-enzyme of GSH peroxidase, which is utilized, to yield oxidized glutathione. In our study, we found that GSH levels in the normal control and toxic control animals was 238.37 ± 34.22 mg% and 155.07 ± 24.15 mg%, respectively. Experimental esophagitis decreased the level of SOD in toxic control (1.77 ± 0.13) and treatment with omeprazole and baclofen significantly helped to restore the same (i.e. 4.22 ± 1.88, 3.55 ± 0.48, units of SOD/mg of protein) in analogs to normal control (6.22 ± 0.87 units of SOD/mg of protein) (Table 1). The catalase activity in the toxic control animals was decreased to 7.98 ± 0.69 nmol of H_2O_2/min/mg of protein in comparison to normal control (i.e. 31.87 ± 3.01 nmol of H_2O_2/min/mg of protein).

Discussion

Omeprazole is a proton pump inhibitor and has a dual mechanism of action: H^+K^+ATPase inhibition and gastric mucosa carbonic anhydrase (CA) inhibition. The antisecretory mechanism of omeprazole involves inhibition of gastric mucosa CA II and CA IV, which are located in abundance in the parietal cell and in its secretory canaliculi walls. This inhibition potentiates the inhibitory effect of omeprazole on the proton pump [13]. The dual inhibitory potential of omeprazole helps in providing relief and mucosal healing in GERD and the same can implicate in the present experiment as well. The beneficial effects of baclofen observed in the present study could be attributed to its ability to reduce TLESR by increasing esophageal sphincter basal pressure and accelerated gastric emptying [2]. Thus, the effect against GERD could be conglomerately attributed to the antisectretory action of omeprazole and reduced TLESR rate by baclofen, which seems to accounts for decrease in gross volume of gastric juice secretion, total acidity and esophagitis index in the present experiment.

Previous studies have elaborated the role of free radicals in pathogenesis of the reflux esophagitis in experimental animals. GERD has been reported to increase malondialdehyde, a stable product of lipid peroxidation and a sensitive marker of membrane damage [4,20]. Treatment with omeprazole and baclofen increased the level of blood GSH significantly with omeprazole exhibiting the maximum effect. Free radical damage lead to dissipation of GSH in the first few hours of oxidative stress, directing decreased GSH level (a marker of short term oxidative stress) [18]. Decrease in the levels of GSH represents its increased utilization by the cells due to oxidative stress and treatment with omeprazole and baclofen alone or in combination has significantly helped to revert the same. Restoration of GSH could be attributed either due to decreased oxidative stress or increased biogenesis of GSH. It would be lucrative to mention that combination therapy of omeprazole with baclofen illustrated maximum antioxidant effect. Similar patterns of results were contemplated for the antioxidant defense of SOD and catalase in control and treatment groups. The antioxidant enzymes, SOD and catalase constitute the major supportive team of defense against free radicals. SOD by scavenging the superoxide radical generates H_2O_2 and molecular oxygen. Catalase existing in the cells catalyses the dismutation of H_2O_2 (produced due to the scavenging effect of SOD) to water and molecular oxygen. Previous studies have advocated the decrease in SOD levels in the course of oxidative stress [17,19]. We observed a momentary decrease in SOD level in animals subjected to reflux esophagitis (control), which could be the repercussion of physiological reimbursing mechanisms to affray oxidative stress in early phase. Thus, the synchronic decrease in the catalase activity is cardinal with decrease in SOD activity to negate the detrimental effects of oxidative stress, which was observed in our study. Concomitant administration of omeprazole and baclofen alone or in combination altered the catalase levels significantly toward regularization. Restoration of the altered antioxidant enzymes further confirms the efficacy of the current therapy.

It is noteworthy that baclofen has been divulged to retain its ameliorative effects even after long term use, which is not the case for present therapeutic alternatives for GERD and no untoward effect was observed in animals treated with omeprazole and baclofen either alone or in combination. Our results suggest the possible therapeutic potential of combination therapy of omeprazole and baclofen against reflux esophagitis without any untoward effect. However, additional studies are required to be undertaken to confirm the efficacy of current proposed therapy against GERD.

Conflict of interest

We declare that we do not have conflicts.

Funding

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