Evaluation of antioxidant and anti-inflammatory activity of Omeprazole against experimentally induced colitis

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Abstract

Aim: The objective of the study is to assess the possible antioxidant and anti-inflammatory activities of Omeprazole against acetic acid-induced colitis in albino rats. Materials and Methodology: In vitro antioxidant activity such as Lipid peroxidation and anti-inflammatory activity such as protein denaturation assay of Omeprazole were carried out. Omeprazole 2.5 mg/kg i.p was given to the test group. The animals were sacrificed after 24 hours and colon/body weight ratio and ulcer score was evaluated after the removal of the colon. The biochemical parameters like nitrate levels and protein concentration were determined from tissue homogenates. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Tukey’s t test. Results: In vitro results showed that the drug posses both antioxidant and anti-inflammatory activity. There was a significant improvement in colon/body weight ratio, ulcer score and biochemical parameters in Omeprazole treated group compared to the colitis group which proved the antioxidant and anti-inflammatory activity.

Keywords: Ulcerative colitis, antioxidant, anti-inflammatory, Omeprazole.

Introduction

Inflammatory bowel disease (IBD) is a medical term that describes a group of conditions in which the intestines become inflamed. Two major types of IBD are Crohn’s disease (CD) and Ulcerative colitis (UC). UC affects the large intestine, whereas CD can occur in any part of the intestines. UC is a worldwide, idiopathic, chronic inflammatory disease characterized by an acute phase of inflammation of the colonic mucosa with inflammatory changes followed by an inactive and quiescent periods.¹

Omeprazole is a proton pump inhibitor which is extensively used for the therapeutic control of acid related disorders. The novel antioxidant and anti apoptotic role of Omeprazole at a dose of 2.5 mg/kg to block gastric ulcer through scavenging of hydroxyl radical have been reported.²

Proton pump inhibitors (PPI) have been shown to exert antioxidant and anti-inflammatory action beyond acid suppression.³ This provides a new opening for PPI in the treatment of many anti-inflammatory diseases. Therefore the aim of the study was to evaluate the effect of Omeprazole on acetic acid induced colitis in rats.
Materials and Methods

Lipid peroxidation

Human peripheral lymphocyte cell extract was used for induction of lipid peroxidation. The presence of oxidative compound will result in peroxidation of intracellular lipids. 30µl of supernatant was taken and made up to 500 µl with cold phosphate buffer saline. 0.1 ml of the different concentration of the samples was added. 2ml of thiobarbituric acid reagent (15% trichloroacetic acid, 0.8% thiobarbituric acid, 0.25 N HCl) was added to all the test tubes followed by boiling in a water bath for 15 min at 95°C. After cooling to room temperature, the thiobarbituric acid residue substrate (TBARS) was measured at 532 nm spectrophotometrically and referred to the observance of the standard glutathione, which was treated in the same way and the percentage inhibition was calculated.

Inhibition of protein denaturation

The reaction mixture consisted of the sample and 1% aqueous solution of bovine albumin fraction, the pH of the reaction mixture was adjusted using a small amount of 1N HCl. Aspirin was taken as the standard drug. The sample was incubated at 37 ° C for 20 min and then heated to 51° C for 20 min, after cooling the samples the turbidity was measured at 660nm and the percentage inhibition was calculated.

Induction of colitis

After overnight fasting, intrarectal administration of 1 ml 4% AA solution using 8-cm- soft 6F pediatric catheter. After induction of colitis, the animals were divided into different groups. In this experiment, a total of 18 animals was used. The animals were divided into three groups of six animals each according to the following manner given below.

Group I: Control (Normal saline p.o)
Group II: Colitis group (Acetic acid i.r)
Group III: Omeprazole group (Omeprazole 2.5 mg/kg i.p + Acetic acid i.r)

The drug solutions were prepared using normal saline. The control animals received only normal saline p.o, colitis group received intra rectal administration of acetic acid and the test group received Omeprazole 2.5 mg/kg i.p given 30 min prior to the acetic acid administration.

Tissue sample

Animals were sacrificed by cervical dislocation 24 h after the last treatment. The abdomen was opened and the distal colon was rapidly excised and opened longitudinally along the mesenteric ridge. The fecal contents were removed and the colon was washed with 0.9% (w/v) saline and placed with mucosal surfaces upward over glass plate or slide chilled with ice. The ratio of 8cm segment distal colon to body weight was calculated as an index of colonic tissue edema.

Evaluation of colonic damage

The macroscopic mucosal damage was evaluated and validated by the grading scale introduced by Morris. Scores were:

0 = no ulcer,
1 = mucosal erythema only,
2 = mild mucosal edema, slight bleeding or slight erosion,
3 = moderate edema, bleeding ulcers or erosions,
4 = severe ulceration, erosions, edema and tissue necrosis and perforation.

Estimation of protein

It was determined by the method of Lowry et al. 0.2 ml of the protein solution was taken in test tubes and 2 ml of alkaline copper sulphate solution was added and incubated at room temperature for 10 min. 0.2 ml of the Folin-Ciocalteau reagent was then added and then incubated for 30 min and the absorbance was measured at 660nm. The concentration was determined from the standard curve plotted using bovine serum albumin.

Estimation of nitrate level

100 µl of the sample were incubated with 100 µl of Griess reagent (1% Sulphanilamide, 0.1% Naphthyl ethylene diamine and 3% Phosphoric acid) at room temperature for 10 min and the nitrate concentration will be determined by the absorbance at 540 nm. The standard curve was obtained using the known concentrations of sodium nitrite.

Results

Lipid peroxidation
Omeprazole significantly inhibits lipid peroxidation in a dose dependent manner compared to the standard glutathione. The IC$_{50}$ value obtained from Standard was found to be 26.51, and that of Omeprazole was 30.78 µg/ml (Figure 1).

**Figure 1:** Lipid peroxidation of Omeprazole using Glutathione as the standard

Inhibition of protein denaturation

Omeprazole prevents the denaturation of protein and significant activity was found at 250µg/ml compared to the standard Aspirin.

The percentage inhibition of Aspirin at 100 and 200 µg/ml were 74.4 and 80.1, while that of Omeprazole was 52.3 and 63 respectively (Figure 2).

**Figure 2:** Inhibition of protein denaturation Omeprazole using Aspirin as standard

Acetic acid administration causes significant macroscopic ulcerations and inflammation of rat colon with significant mucosal injury compared to the normal control animals. Omeprazole pretreatment results in a near normalization of colon architecture compared to the colitis group (Figure 3 and 4).

**Figure 3:** Acetic acid induced colitis

**Figure 4:** Omeprazole treated colon

Discussion

The in vitro antioxidant lipid peroxidation study showed that Omeprazole significantly inhibited lipid peroxidation in peripheral blood lymphocytes. This inhibitory effect of Omeprazole was greater with increasing concentrations and the IC$_{50}$ was found to be 30.78µg/ml. Omeprazole inhibited lipid peroxidation and exhibited DNA protective effect in normal human peripheral blood mononuclear cells exposed to H$_2$O$_2$-induced oxidative stress through intracellular ROS scavenging and indirect action through induction of anti-oxidative enzymes.$^{11}$

Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs have shown a dose dependent ability to thermally induced protein Denaturation.$^{12}$ Omeprazole may possibly inhibit the release of the lyzosomal content of neutrophil at the site of
inflammation. These lysosomal constituents include bactericidal enzymes and proteases, which upon extracellular release cause further tissue inflammation and damage.\textsuperscript{13} The results revealed that Omeprazole prevents the denaturation of proteins and significant activity was found at 250\textmu g/ml.

The animals were sacrificed after 24 hours of the induction of colitis and the colon/body weight ratio was determined to quantify the inflammation.\textsuperscript{14} Acetic acid induced colitis resulted in severe ulcerative inflammation of the rat colon evidenced by the increase in colon/body weight ratio of colitis rats. Pretreatment of Omeprazole resulted in a significant reduction of colon/body weight ratio

The clinical activity score introduced by Morris was used to evaluate the severity of the colonic inflammation.\textsuperscript{8} The colitis group proved to be an excellent model of inflammation as evidenced by the highly increased clinical activity. Significant damages of the intestinal tissue including ulceration, erosions, perforations, colonic edema etc had been observed in the colitic group. A decrease in the progression of the disease pathogenesis following the treatment of Omeprazole was observed, which was characterized by a decrease in the lesion area. The results obtained demonstrate that Omeprazole reduced the inflammation of the bowel and have protective effects on acetic acid induced colitis in rats.

Nitric oxide (NO) is an unconventional intracellular messenger playing a vital role in various pathological and physiological processes.\textsuperscript{15} NO is an oxidant as it reacts with reactive oxygen species (ROS). It results in cellular damage as the oxidation process is not specific. It also forms peroxynitrite anion (ONOO\textsuperscript{−}) by reacting with ROS, which is enough to avoid the action of antioxidant system.\textsuperscript{16} In the study, the colonic nitrate level was significantly increased by inoculation of rats with acetic acid. The elevation of nitrate level is indicated for the inflammation. Intrarectal administration of 4\% acetic acid resulted in increase of NO production, which was converted to peroxynitrite which mediates oxidative damage to biomolecules. The pretreatment with Omeprazole reduced the levels of the nitrate compared with UC rats. NO in cells produced during inflammation rapidly converted to nitrite, after conversion to nitrate can be determined as an indicator for NO production.

Chronic inflammation is associated with considerable disturbances of protein metabolism.\textsuperscript{17} In the present study, tissue protein production was explored for the inflammation induced by acetic acid that mimics human IBD. The study revealed that during inflammation, there is an increase in the protein synthesis in the colon, which seems to be a relevant tool to study the nature of protein disturbances linked to UC.\textsuperscript{18} Additional studies would be necessary to establish which parts of the colon (mucosa, submucosa and muscularis layer) are involved in the global increase in protein synthesis. The study showed that there was an increased level of protein in colitis rats compared to the normal group. Omeprazole treated group showed a reduction in the protein level after the induction of colitis.

**Conclusion**

Hence it can be concluded from the study that Omeprazole possess potent activity against UC due to its antioxidant and anti inflammatory activity. It also puts forward a new indication of Omeprazole as a drug for treatment of IBD.

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**References**


