Journal of Scientific and Innovative Research 2014; 3(3): 352-356
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2014; 3(3): 352-356

Received: 16-01-2014
Accepted: 27-05-2014

Sibi P. Ittiyavirah
Deaprtment of Pharmacology, University College of Pharmacy, Cheruvandoor campus, Ettumanoor P.O, Kottayam, Kerala- 686631, India

Shenika MS
Deaprtment of Pharmacology, University College of Pharmacy, Cheruvandoor campus, Ettumanoor P.O, Kottayam, Kerala- 686631, India

Correspondence:
Dr. Sibi P. Ittiyavirah
Asistant Professor & Head, Deaprtment of Pharmacology, University College of Pharmacy, Cheruvandoor campus, Ettumanoor P.O, Kottayam, Kerala- 686631, India
E-mail: ittiyavirah@gmail.com

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

Sibi P. Ittiyavirah, Shenika MS*

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.[4]

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 º C for 20 min and then heated to 51º C for 20 min, after cooling the samples the turbidity was measured at 660nm and the percentage inhibition was calculated.[5]

Induction of colitis

After overnight fasting, intrarectal administration of 1 ml 4% AA solution using 8-cm- soft 6F pediatric catheter.[6] 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.[7]

Evaluation of colonic damage

The macroscopic mucosal damage was evaluated and validated by the grading scale introduced by Morris.[8] 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, oedema and tissue necrosis and perforation.

Estimation of protein

It was determined by the method of Lowry *et al*[9]. 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.[10]

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).

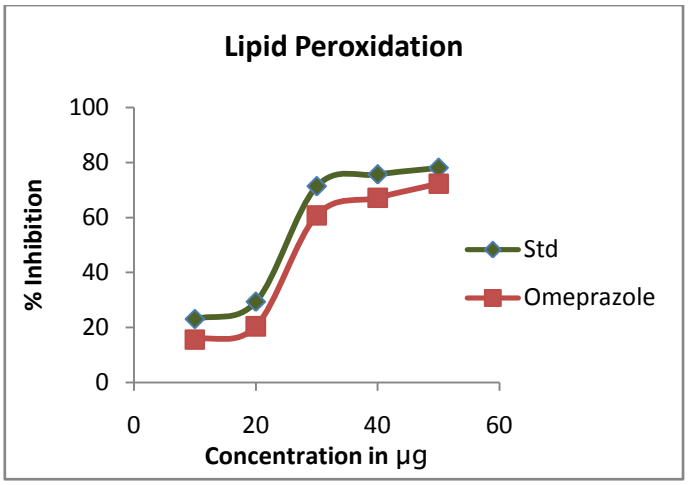

Figure 1: 1Lipid peroxidation of Omeprazole using Glutathione as the standard

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).

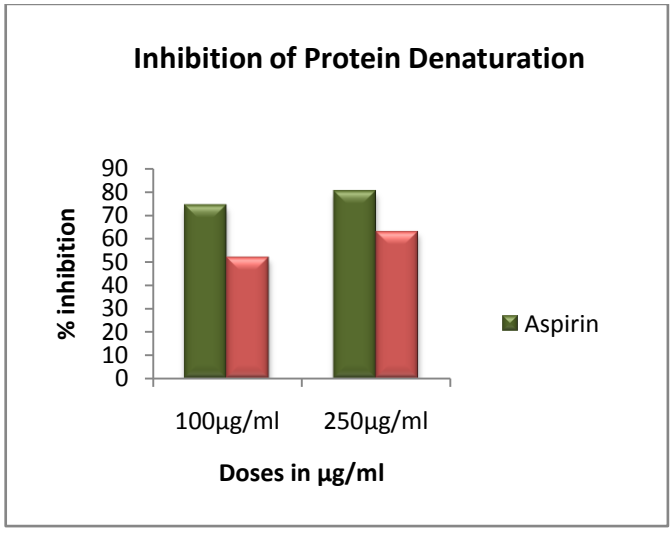

Figure 2: Inhibition of protein denaturation Omeprazole using Aspirin as standard

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).

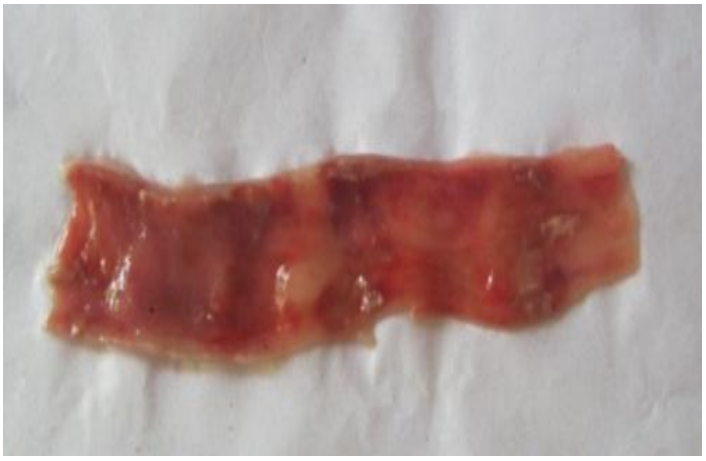

Figure 3: Acetic acid induced colitis

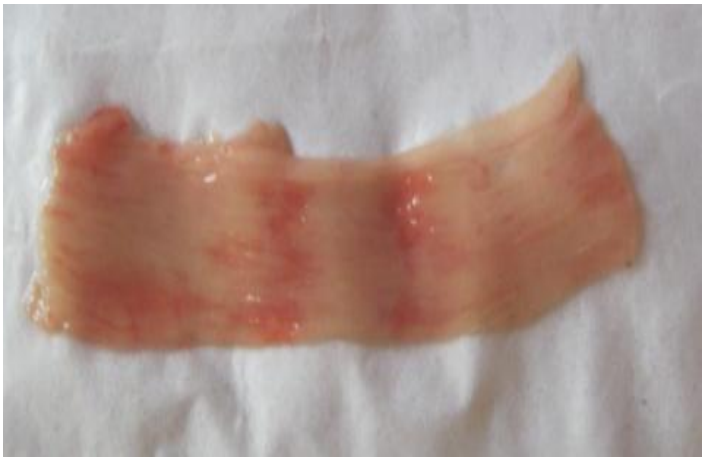

Figure 4: Omeprazole treated colon

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_2O_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.[13] The results revealed that Omeprazole prevents the denaturation of proteins and significant activity was found at 250µg/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.[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.[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.[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 ($ONOO^-$) by reacting with ROS, which is enough to avoid the action of antioxidant system.[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.[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.[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.

Acknowledgement

The authors like to acknowledge AICTE for providing scholarships. We express our gratitude and thankfulness for the Institutional Animal Committee for the permission for doing animal studies.

References

1. Geboes K. Chapter 18: Histopathology of Crohn's disease and ulcerative colitis. In: Satsangi J, Sutherland LR. Inflammatory Bowel Diseases (4th edition). New York: Churchill-Livingstone; 2003: 255-276.

2. Biswas K, Bandyopadhyay U, Chattopadhyay I, Varadaraj A, Ali E, Banerjee RK. A novel antioxidant and antiapoptotic role of omeprazole to block gastric ulcer through scavenging of hydroxyl radical. J Biol Chem. 2003; 28;278(13):10993-1001.

3. Kanho R, Hirofumi M,,Tsuyoshi K, Yumiko N, Osamu S, Jumpei U, Aki H, Ichinosuke H, Hiroko PI,Hideyuki JM. Lansoprazole inhibits mitochondrial superoxide production and cellular lipid peroxidation induced by indomethacin in RGM1 cells. J Clin Biochem Nutr. 2011 July; 49(1): 25–30.

4. Bochra G, Riadh BM, Malek MJ, Rihab NB, Noureddine A, Saloua L, Khaled H. Hydroxytyrosol supplementation inhibits oxidative DNA damage, suppresses protein-lipid oxidation and modulates antioxidant enzymes in human peripheral blood lymphocytes. J Med Plants Res 2011; 5: 4863-4869.

5. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol 1968; 20:169- 173.

6. Elife E , Cansel T, Burak UZ , Arif K, Cemile K, Reyhan B, Özlem A. The Effects of Caffeic Acid Phenethyl Ester (CAPE) on Acetic Acid Induced Colitis in Rats. The New J Med. 2010; 27: 106-112.

7. Yue G, Sun FF, Dunn C, Yin K, Wong PY. The 21-aminosteroid Tirilazad mesylate can ameliorate inflammatory bowel disease in rats. J Pharmacol Exp Ther 1996; 276: 265–270.

8. Morris G, Beck P, Herridge M, Depew W, Szewczuck M, Wallace J. Hapten induced model of inflammation and ulceration in rat colon. Gastroenterol. 1989; 96:795-803.

9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951; 193 (1): 265–75.

10. Lowry OH, Rosebrough NJ, Farr AL Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-675.

11. Stefania A, Diego I, Valentina M. Hydroxytyrosol, a natural antioxidant from olive oil, prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells. Free Biol Med 2005; 38: 908-909

12. Chou CT. The anti-inflammatory effect of Triptergium wilfordii Hook F on adjuvant- induced paw edema in rats and inflammatory mediators release. Phyto Res 1997;11: 152-4.

13. Das SN, Chatterjee S. Long term toxicity study of ART-400. Ind Indigenous Med 1995; 16: 117-123.

14. Rasha HM. The impact of Vitamin B6 supplementation on experimental colitis and colonic mucosal DNA content in female rats fed high sucrose diet. Aus J Bas Appl Sci 2011; 5: 1051-1060

15. Gow AJ, Farkouh CR, Munson DA, Posencheg MA Ischiropoulos H. Biological significance of nitric oxide-mediated protein modifications. Amer J Physiol Lung Cell Mol Physiol 2004; 8:262-287

16. Perner A, Andresen L, Normark M, Fischer HB, Sorensen S, Eugen OJ. Expression of nitric oxide synthases and effects of L-arginine and L-NMMA on nitric oxide production and fluid transport in collagenous colitis. Gut 2001, 49: 387-394.

17. Mercier S, Breuill´e D, Mosoni L, Obled C, Patureau MP. Chronic inflammation alters protein metabolism in several organs of adult rats. J Nutr 2002; 132: 1921–1928.

18. Mimoun EY, Denis BE, Isabelle P, Stephanie B, Laurent M, Philippe D, Caroline B. Increased tissue protein synthesis during spontaneous inflammatory bowel disease in HLA-B27 rats. Clin Sci 2003; 105: 437–446.