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Effect of Ascorbic Acid on Aceclofenac Induced Gastric Mucosal Damage: Role of Antioxidant Enzymes and Lipid Profile

S. DARBAR, A. BOSE, N. CHATTERJEE, B. ROY, U. MANDAL, K. VEERAN GOWDA, A. GHOSH and T.K. PAL*
Department of Pharmaceutical Technology, Jadavpur University
Kolkata-700 032, India
Tel: (91)(33)24146967; Fax: (91)(33)24146186
E-mail: tkpal12@gmail.com

The present work has been undertaken to study the effect of ascorbic acid, a potent antioxidant on experimental gastric ulceration induced by aceclofenac and their possible anti-oxidative mechanism to cure ulcer. Gastric mucosal damage was produced in rats by administering aceclofenac (90 mg/kg/d) orally. Preadministration of ascorbic acid (200 mg/kg/d) as a food supplement, decreased the ulcer index, lipid peroxidation, conjugated diene and protein carbonyl content and increased the antioxidant enzyme levels. The lipid levels were maintained at near normalcy when treated with ascorbic acid in aceclofenac-administered rats. The major mechanism involved appears due to free radical scavenging action and changes in lipid profile.

Key Words: Aceclofenac, Ascorbic acid, Lipids, Antioxidant.

INTRODUCTION

Reactive oxygen species (ROS) have been thus been regarded as highly toxic agents responsible for a wide variety of tissue damage. Recently, interest has been focused on the role of ROS in gastroduodenal pathogenesis related to gastric hyper secretion and gastroduodenal mucosal damage. ROS has been implicated in gastric mucosal damage by non-steroidal anti-inflammatory drugs[1]. The lipids content of mucus gel, along with its renewable quality, appear to play a major role in the inherent resistance of the mucosa to injury[2]. While in gastric disease condition, imbalance in lipid metabolism occurs, leading to disturbance of the mucosal integrity.

Gastrointestinal nuisance symptoms and serious complications can result from the use of Non-Steroidal Anti-inflammatory Drugs (NSAIDs)[3]. In a two-pronged attack, NSAIDs injure the mucosa by causing topical injury to gastrointestinal mucosa and by producing systemic effects induced by prostaglandin depletion. Because prostaglandins are essential

to the maintenance of intact gastrointestinal defenses as well as normal platelet function, NSAIDs both promote ulcer formation and enhance bleeding[4]. World over, 35 million people consume these drugs on a daily basis and about 30 % of these users may develop GI toxicity of sufficient degree requiring a physician's intervention. It has also been estimated that one-third of the cost of treating arthritis patients relates to treatment of the side effects of NSAIDs. Observative calculations estimate that *ca.* 1,07,000 patients are hospitalized annually for non-steroidal antiinflammatory drug (NSAID)-related gastrointestinal (GI) complications and at least 16,500 NSAID-related deaths occur each year[5].

Aceclofenac sodium a prodrug in the aryl-acetic acid class is a commonly used NSAID in several countries. Aceclofenac is an oral non-steroidal anti-inflammatory drugs (NSAIDs) that is effective in the treatment of painful inflammatory diseases and has been used to treat more than 75 million people worldwide[6]. Chronic used of aceclofenac, damages gastrointestinal mucosa by irritant action, causing alteration in mucosal permeability and/or suppression of prostaglandin synthesis. Aceclofenac is highly protein and has antipyretic, analgesic and anti-inflammatory effects, is an inhibitor of arachidonic acid level. The use of oral non-steroidal antiinflammatory drugs is associated with upper gastrointestinal complications, particularly perforated and bleeding peptic ulcer[7]. The exact mechanism is not known but it is probably related to the decrease in the fatty acid entering the cell or release from the cell.

A group of metabolic products called free radicals can damage liver and stomach cells and promote inflammation, impairing vital functions such as energy production. The body's natural defenses against free radicals (*e.g.*, antioxidants) are inhibited by aceclofenac consumption, leading to increased gastrointestinal damage.

Ascorbic acid may protect lipids and lipoproteins in cellular membranes against oxidative damage caused by toxic free radicals at early stage. The antioxidant function of ascorbic acid is related to its reversible oxidation and reduction characteristics[8,9].

The aim of this study was to investigate the possible gastro protective effects of ascorbic acid on the antioxidant enzymes and the lipid profiles and free radical damage of gastric mucosal cells caused by aceclofenac in rats.

EXPERIMENTAL

Aceclofenac and ascorbic acid were obtained as a gift from Dey's Medical Stores (Mfg.) Ltd, Kolkata, India. All other chemicals and biochemicals used were of high analytical grade.

All the experiments were carried out with male albino Wister rats; weighing 120-130 g was obtained from Indian Institute of Chemical Biology, Kolkata, India. The animals were housed in polypropylene cages (47 cm × 34 cm × 20 cm) maintained in controlled temperature and under a 12 h light-dark cycle with normal pellet diet (Hindustan liver, Kolkata) food and water *ad libitum*. The experiments were performed after the approval of Institutional Animal Ethics Committee (IAEC).

Experimental study design: The rats were divided into 3 groups of 6 animals each (n = 6) as follows: Group I animals served as control. Group II animals received a single dose of aceclofenac (90mg/kg/d) orally for a period of 28 d. Group III animals received aceclofenac (90mg/kg/d) orally and pre treatment with ascorbic acid (200 mg/kg/d) as a food supplement for 28 d.

Preparation of gastric mucosal homogenate: The stomach was removed and kept in ice-cold phosphate buffer (pH 7.2). It was cut along greater curvature and the scrapped mucosa was weighed and homogenized in ice-cold phosphate buffer. The homogenate was used for the assay of lipid peroxidation[10] protein carbonyl content[11], conjugated dienes[12], glutathione (GSH) content[13], superoxide dismutase (SOD) activity[14], catalase (CAT) activity[15], glutathione peroxidase (GPx) activity[16], total cholesterol[17], phospholipids[18], triglycerides[19] and free fatty acids (FFA)[20] were determined by using standard biochemical kit obtained from Merck, Germany. Protein was estimated by the method of Lowry *et al.*[21].

Statistical analysis: The results were presented as the mean ± SD. Student's 't' test was used to analyze statistical significance. p-values less then 0.05 were considered significant.

RESULTS AND DISCUSSION

Ascorbic acid reduced lipid peroxidation, protein carbonyl content and conjugated dienes as compared to aceclofenac control. GSH content was found to decrease in gastric mucosa of aceclofenac administered rats when compared with the group I controls. The decreased content of GSH was restored to near normal in ascorbic acid pretreated animals (Table-1). Aceclofenac caused significant alteration in SOD, CAT and GPx activity, the important antioxidant enzymes of the mucosa. Ascorbic acid an increased in the concentration of SOD, CAT and GPx activity when compared to aceclofenac administered groups (Table-2). In gastric mucosa, significant alteration in lipid levels was observed in aceclofenac induced rats (Table-3). Pretreatment with ascorbic acid significantly abated the levels of phospholipids and triglycerides when compared to aceclofenac control group.

TABLE-1
EFFECTS OF ASCORBIC ACID ON LIPID PEROXIDATION, PROTEIN CARBONYL CONTENT, GLUTATHIONE LEVEL OF THE GASTRIC MUCOSA

Parameters	Group I	Group II	Group III
Lipid peroxidation MDA content (n mol/mg protein)	0.40±0.02	0.71±0.02†	0.50±0.02‡
Protein carbonyl content (n mol/mg protein)	1.39±0.01	2.91±0.01†	1.71±0.03‡
Glutathione content (n mol/mg)	55.33±1.23	36.46±1.69†	49.38±1.78‡
Conjugated dienes (μ mol/100 g tissue)	0.38±0.01	0.66±0.02†	0.42±0.02‡

Values are expressed as mean ± SD (n = 6).
†$p < 0.001$ as compared to group I; ‡$p < 0.001$ as compared to group II.

TABLE-2
LEVELS OF ANTIOXIDANT STATUS IN GASTRIC MUCOSA OF CONTROL AND EXPERIMENTAL GROUP

Parameters	Group I	Group II	Group III
SOD (IU/mg protein)	4.74±0.16	2.37±0.37†	4.47±0.25‡
CAT (μmol/H_2O_2/mg protein)	4.10±0.09	2.31±0.14†	4.21±0.12‡
GPx (n mol/GSH/mg protein)	210.67±1.97	141.17±2.14†	201.17±3.19‡

Values are expressed as mean ± SD (n = 6)
†$p < 0.001$ as compared to group I; ‡$p < 0.001$ as compared to group II.

TABLE-3
EFFECT OF ASCORBIC ACID ON LIPID PROFILE IN ACECLOFENAC INDUCED RATS

Parameters	Group I	Group II	Group III
Cholesterol (mg/g tissue)	43.92±1.64	30.50±1.37†	39.87±1.54‡
Triglycerides (mg/g tissue)	59.69±1.95	40.15±1.94†	52.52±0.86‡
Phospholipids (mg/g tissue)	73.88±1.21	50.79±1.54†	69.07±0.51‡

Values are expressed as mean ± SD (n = 6)
†$p < 0.001$ as compared to group I; ‡$p < 0.001$ as compared to group II.

The role of ROS in ulcer generation by various factors has recently attracted the attention of many investigators. Lipid peroxidation leads to less of membrane fluidity, ion transport and membrane integrity of the surface epithelial cell and helps to generate gastric lesions[22]. Aceclofenac cased significant increase in lipid peroxidation and protein carbonyl content with significant decrease in the mucosal glutathione level, indicating that lesions were due to oxidative damage caused by ROS. Pretreatment with ascorbic acid prevented these alterations in stress condition; ulcer is developed mainly due to oxidative damage by OH^- generated from derangements of the antioxidant enzymes. Severe depletion of GSH affects the synthesis of

2 major cellular polymers, *i.e.* proteins and DNA. Elevation of GSH status in gastric mucosal cell can be achieved by ascorbic acid after aceclofenac administration.

Free radical scavenging enzyme such as SOD, catalase and GPx are the first line of cellular defense against oxidative injury. The equilibrium between these enzymes is an important factor for the effective removal of ROS in intracellular organelles[23]. Aceclofenac treated rats showed decreased activity of SOD and catalase in stomach. A decrease in the activity of these antioxidant enzymes can lead to the formation of oxygen and hydrogen peroxide, which intern can form the toxic hydroxyl radical (OH). The decrease in the activity of SOD and catalase may be due to gastric mucosal cell damage. The increased activity of catalase and SOD is associated with decreased levels of lipid peroxidation in the ascorbic acid (200 mg/kg/d) treated aceclofenac group. These can result in decreased formation of toxic intermediates.

The enzyme glutathione peroxidase (GPx) is a well known first line of defense against oxidative stress, which intern requires glutathione as co factor. GPx catalyses the oxidation of GSH to GSSG at the expance of the H_2O_2. The observe decreased activity of GPx in these study might be due to increased conc. of hydroperoxide or due to decreased conc. of GSH in aceclofenac induced rats. Pretreatment with ascorbic acid as a food supplement increases the activity on GPx in Aeclofenac treated rats.

Stimulation of lipid influence lipid metabolism in a biological system. The lipids content apparently determines the degree of resistance of mucin to peptic degradation and contribute significantly to mucus viscosity, hydrophobicity and impedance to hydrogen ion diffusion[24]. Aceclofenac reduces phospholipids concentrations, which leads to altered surface hydrophobicity and weakened gastric mucosal barrier. These lipids are also known to exert the greatest impact on the physiochemical characteristics of mucus. The results presented in this report demonstrated that intragastic administration of ascorbic acid not only possess antioxidant property but also increase the lipid composition in the gastric mucosal surface when compared to the respective controls and there by providing anti-ulcerogenic efficiency.

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