ALIMENTARY TRACT AND PANCREAS

Colonic mucosal antioxidant enzymes and lipid peroxide levels in normal subjects and patients with ulcerative colitis

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Abstract. Oxygen-derived free radicals have been implicated in the pathogenesis of ulcerative colitis. Mammalian tissues contain antioxidant systems that offer protection from the damaging effect of these active species. In the present study, the activity of the antioxidant enzymes catalase, glutathione peroxidase, glutathione transferase and glutathione reductase were measured in rectal biopsies from patients with ulcerative colitis and compared with that obtained from normal subjects. A significant decrease in the activity of glutathione transferase was observed in ulcerative colitis (48.32 ± 6.73 units/mg protein, mean ± S.E.) compared to normal (68.20 ± 6.83, P = 0.015). There was no difference in the activity of other antioxidant enzymes between controls and ulcerative colitis. Myeloperoxidase, a marker for neutrophil infiltration, was considerably increased in ulcerative colitis while malondialdehyde, the end product of lipid peroxidation, was not increased. The reduced activity of glutathione transferase in ulcerative colitis may be an additive factor in the pathogenesis of mucosal damage in this disease.

Key words: antioxidant enzymes, lipid peroxide, ulcerative colitis.

INTRODUCTION

Oxygen-derived free radicals mediate mucosal damage in several gastrointestinal diseases including ischaemia of the bowel. Recent studies indicate that these free radicals may also be involved in the pathogenesis of mucosal damage in inflammatory bowel disease.\(^1,2\) Infiltration of polymorphonuclear leucocytes (which are capable of producing free radicals) into the lamina propria is a hallmark of active ulcerative colitis. In inflammatory bowel disease, these cells as well as peripheral blood monocytes and intestinal macrophages have been shown to produce increased amounts of free radicals.\(^3,4\) Production of these active species has been demonstrated in experimental colitis in animals as well as in biopsies taken from patients with ulcerative colitis.\(^5,6\) The mucosa has several antioxidant systems which prevent damage to cellular components by these active species. There are no data on antioxidant enzymes in the intestinal mucosa of patients with inflammatory bowel disease. Superoxide dismutase and metallothionein, two endogenous copper/zinc (Cu/Zn) containing proteins involved in radical scavenging, are reported to be decreased in intestinal mucosa from patients with Crohn's disease and ulcerative colitis.\(^7\) The present study was undertaken to examine the activity of antioxidant enzymes and lipid peroxides in biopsy samples of rectal mucosa obtained from patients with ulcerative colitis.

METHODS

Chemicals

Reduced and oxidized glutathione, 2-thiobarbituric acid, 1-chloro-2, 4-dinitrobenzene, cumene hydroperoxide, NADPH and O-dianisidine were all obtained from Sigma Chemical Co., St Louis, MO, USA. All other chemicals used were of analytical grade.

Subjects

The control population consisted of 36 patients (21 male) with a diagnosis of haemorrhoids or irritable bowel syndrome, who were undergoing flexible sigmoidoscopy. Their ages ranged from 23 to 53 years (median 39 years). Thirty-one patients (23 male) with active ulcerative colitis, either at first presentation or during relapse, were also studied. Their ages ranged from 26 to 69 years (median 39 years).

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Four of these patients had only a proctitis, eight had left-sided colitis and the rest had pancolitis. None of these patients had a bacterial or parasitic superinfection responsible for exacerbation of colitis. Eleven patients had not previously been treated. The rest were on treatment with mesalazine (3), sulphasalazine (5), mesalazine with steroid enemas (3), or sulphasalazine with steroid enemas (9). Clinically, 10 of the patients had disease of mild severity, 10 had moderately severe disease and two had severe disease at the time of study.

Methods

In all patients, two biopsies of the rectal mucosa were taken 10 cm from the anal verge using an FB 24E (Olympus Co., Tokyo, Japan) biopsy forceps. Informed consent was obtained from all subjects. Biopsy specimens were obtained from non-ulcerated but inflamed mucosa of patients with confirmed ulcerative colitis and from macroscopically normal mucosa of the control subjects. Tissue samples were stored immediately at −20°C and processed within 4 days. Tissue samples were homogenized in 1 mL of 0.1% EDTA, 0.5 M Tris-HCl buffer, pH 7.1 and the homogenate was used for measurement of various enzymes and lipid peroxide content as described below. Glutathione transferase activity was measured spectrophotometrically using 1-chloro-2, 4-dinitrobenzene as substrate. One unit of enzyme is the amount required to conjugate 1 μmol of substrate with glutathione in 1 min. Specific activities were expressed as units per mg protein. Glutathione peroxidase activity was measured by following the change in absorbance of NADPH with reduced glutathione (GSH) and cumene hydroperoxide as substrate. One unit is defined as the amount of enzyme required to bring about oxidation of 1 nmol of NADPH per minute. Catalase activity was estimated by measuring change in absorbance at 240 nm using hydrogen peroxide as substrate. One unit is the activity that disproporionateates H₂O₂ at the rate of 10⁻³ absorbance per s. GSH reductase was determined by measuring the change in absorbance of NADPH at 340 nm using oxidized glutathione (GSSG) as substrate. One unit is the amount of enzyme needed to convert 1 nmol of NADPH per min. Myeloperoxidase was measured by following the absorbance of O-dianisidine at 550 nm as described. One unit is defined as that degrading 1 μmol of peroxide per min. The lipid peroxide content of the tissue was measured by the thiobarbituric acid reaction and expressed as nmol of malonaldehyde formed per mg protein. Protein was estimated using bovine serum albumin as standard.

Statistics

All values are reported as mean ± standard error. Differences between means were assessed using the two-tailed Wilcoxon rank test.
RESULTS

The activities of the various enzymes studied in the rectal mucosa are shown in Fig. 1. The activity of glutathione transerase was significantly decreased in patients with ulcerative colitis (48.32 ± 6.73 units/mg protein) compared to normal controls (68.20 ± 6.83 units/mg protein). This difference was statistically significant (P = 0.015). When analysed according to the severity of colitis, glutathione transerase activity was 55.62 ± 10.3 units/mg protein in 10 patients with mild disease, compared to 45.25 ± 8.47 units/mg protein in 19 with moderate disease and 38.90 ± 21.88 units/mg protein in two patients with severe disease. No significant difference was seen in glutathione peroxidase, catalase and glutathione reductase activities between normal and ulcerative colitis.

Myeloperoxidase activity and lipid peroxide levels are shown in Fig. 2. Myeloperoxidase activity was several-fold higher in ulcerative colitis patients (n = 7; 0.782 ± 0.035 units/mg protein, mean ± s.e.) as compared to the normal controls (n = 12; 0.069 ± 0.019 units/mg protein; P = 0.028). Though statistically not significant, there was a higher level of lipid peroxide in ulcerative colitis biopsies (n = 24; 2.125 ± 0.054 nmol/mg protein) compared to normal mucosa (n = 26; 1.199 ± 0.124 nmol/mg protein).

DISCUSSION

The present study reports for the first time the activities of several antioxidant enzymes in the colonic mucosa in ulcerative colitis. Antioxidant enzymes have been earlier shown to be present in the normal human colonic mucosa. The enzymes measured in biopsies in this study serve the following functions: Catalase and glutathione peroxidase to remove hydrogen peroxide (H₂O₂) which, in addition to directly causing damage, generates very reactive hydroxyl radicals in the presence of transition metals. Glutathione is used as a cofactor by the antioxidant enzymes, resulting in the production of oxidized glutathione. Oxidized glutathione formed in the reaction is reduced to GSH by the action of glutathione reductase. Glutathione transerase may also help to detoxify free radicals, although its major function is in xenobiotic metabolism.

Ulcerative colitis is characterized by an accumulation in the colonic mucosa of inflammatory cells that release oxygen free radicals as part of their bactericidal activity. In the present study, myeloperoxidase (a marker of neutrophil polymorphs) was found to be increased several-fold in the rectal mucosa of patients with ulcerative colitis. A similar increase in myeloperoxidase activity in ulcerative colitis has been shown earlier. These cells, as well as monocytes and intestinal macrophages, produce large amounts of free radicals in inflammatory bowel disease. The therapeutic benefit of 5-aminosalicylic acid in ulcerative colitis may result from its inhibition of cyclo-oxygenase and lipooxygenase as well as reduction of the local generation of highly reactive oxygen species.

Membrane lipids are a target for free radical-induced damage, and measuring lipid peroxides in tissue provides an index of free radical activity. In this study, although patients with ulcerative colitis tended to have higher concentrations of lipid peroxides in the mucosa than controls, the actual levels were not very high. The normal gastrointestinal mucosa is resistant to lipid peroxidation in vivo and the low level of peroxides seen may be due to this resistance. Increased lipid peroxidation has been reported in colorectal cancer, but the absolute levels of lipid peroxides are not very high and are similar to those obtained in this study.

In comparison to tissues such as liver, levels of antioxidant enzymes in the normal colonic mucosa are low, suggesting that these enzymes may not be the primary defence against oxidant injury in the colonic mucosa. In the present study, no significant differences were noted in mucosal activity of antioxidant enzymes between ulcerative colitis and control with the exception of glutathione transerase. However, it is possible that the apparently normal values in ulcerative colitis may represent an inadequately sensitive assay.
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gute rise in enzyme levels after oxidative stress. Studies in patients with inactive colitis may be useful in confirming this possibility.

The mucosal activity of glutathione transferase was decreased in patients with ulcerative colitis in this study. An over-expression of this enzyme has been shown in malignant tissues. The decreased levels of this enzyme in ulcerative colitis may have possible significance in pathogenesis, because of its dual function as an antioxidant enzyme and in xenobiotic metabolism. The possible role of glutathione transferase in inflammation and inflammatory bowel diseases is not shown. Glutathione-S-transferases are a multifunctional family of enzymes present in the cytosol of most cells, and are especially geared to detoxification. This enzyme system catalyses a number of interactions between glutathione and electrophilic centres on hydrophobic substrates. Various classes of substrates have been identified which react with the reduced form of glutathione by substitution, addition, or reduction reactions such as reduction of nitrosoquinones, prostaglandin endoperoxides and organic peroxides, including lipid peroxides. In humans, the individual glutathione transferases are very closely related in amino acid composition, substrate specificity and immunological characteristics. As detoxifying enzymes, transferases catalyse lipid peroxide metabolism and remove the metabolites from possible interactions with DNA and proteins.

Impaired colonic mucosal metabolism of other xenobiotics has been described in ulcerative colitis. It is possible that defective metabolism of xenobiotics may lead to the absorption of various molecules from the colon, which may then contribute both to carcinogenesis in the colon and to hepatobiliary damage in inflammatory bowel disease.

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