Endogenous antioxidant status in neoplastic and adjacent tissues in 1,2-dimethylhydrazine-induced colon cancer in rats: effects of olsalazine

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There is much evidence suggesting a possible role of reactive oxygen-derived substances in the pathogenesis of both ulcerative colitis and colon cancer. The antioxidant effects of 5-aminosalicylic acid (the active moiety of olsalazine) on induction of colon cancer in an experimental model using 1,2-dimethylhydrazine were studied in male Wistar rats. The levels of reduced glutathione were significantly (P < 0.01) decreased (by ~50%) in neoplastic tissues of rats receiving 1,2-dimethylhydrazine alone and olsalazine treatment significantly (P < 0.01) reduced the extent of this alteration. Adjacent tissues from rats receiving either carcinogen alone or carcinogen and olsalazine showed comparable levels of glutathione and these were significantly (P < 0.01) lower than corresponding control values and higher than corresponding values from neoplastic tissues. Activity of the glutathione regenerating enzyme glutathione reductase was significantly (P < 0.01) decreased (by ~40%) in neoplastic colonic tissue and this alteration was unaffected by olsalazine treatment. Neither carcinogen nor olsalazine treatment caused alterations in activity of glutathione reductase in adjacent tissue as compared with corresponding control values. Activity of the glutathione utilizing enzyme glutathione peroxidase was significantly (P < 0.01) increased (almost doubled) in neoplastic tissue of rats treated with carcinogen alone. Olsalazine treatment significantly (P < 0.01) reduced the elevation in glutathione peroxidase activity in neoplastic tissues of rats treated with the carcinogen. Glutathione peroxidase showed comparable activity in adjacent tissue from rats treated with either carcinogen alone or a combination of carcinogen and olsalazine and these values were significantly (P < 0.01) lower than corresponding control values. Colonic neoplastic tissues from all experimental groups of animals showed a small, but statistically significant (P < 0.05), decrease in superoxide dismutase activity compared with that in corresponding tissues from control animals.

Materials and methods

Animals and carcinogenesis induction

Male Wistar rats weighing 150–160 g were housed in suspended cages with wire mesh floors to prevent coprophagia and were isolated in a carcinogen containment laboratory as previously described (13). Relative humidity (40%) and temperature were constant and a 12 h light/12 h dark cycle was imposed using automated switching devices. Each rat was administered s.c. injections of DMH dihydrochloride (Aldrich Chemical Co. Inc., Milwaukee, WI) once weekly for 12 weeks at a dosage of 25 mg/kg prepared as a 0.5% solution in 1 mM EDTA (Mallinckrodt Chemical Works, St Louis, MO) adjusted to pH 6.5 with sodium bicarbonate. Previous studies in our laboratory have established this to be an effective regimen for colon tumor induction (17–20). Experimental animals were divided into 2 groups: one received DMH only, the other group was treated concomitantly with DMH and liquid OLS (Kabi Pharmacia, Uppsala, Sweden) diluted daily in drinking water at a dose of 12 mg/kg. Rats were weighed on a weekly basis throughout the experimental period and killed 4 weeks after the final injection of DMH, along with a separate group of age-matched controls not receiving carcinogen. Animals were weighed weekly and there were no statistical differences in mean animal

Abbreviations: RODS, reactive oxygen-derived substances; SOD, superoxide dismutase; GPX, glutathione peroxidase; GRD, glutathione reductase; GSH, glutathione; DMH, 1,2-dimethylhydrazine; OLS, olsalazine; 5-ASA, 5-aminosalicylic acid.
weights in any of the experimental groups during the period of injections or at the time of death.

Tissue preparation and histopathology

Animals were killed by an overdose of urethane (2 g/kg given by i.p. injection) followed by cardiac excision and the large intestine was immediately removed, flushed with cold isotonic saline and opened longitudinally. With tissues placed on an ice-cold metal plate, normal mucosa and normal mucosa immediately adjacent to neoplastic lesions were removed by gentle scraping using a blunt scalpel. Neoplastic lesions on the surface of the colon were isolated and the outer mucosal layer was removed using a sharp scalpel. A small portion each of tumor or adjacent tissue was separately processed in buffered formalin for histopathological confirmation of neoplastic or non-neoplastic status. Tissue samples (0.2-0.4 g mucosa from neoplastic lesions and 0.15-0.2 g adjacent mucosa or control mucosal tissue) were homogenized on ice in 50 mM Tris, 0.1 M ethylenediaminetetraacetic acid, pH 7.6 (10% w/v) using two 15 s bursts of a Polytron (Brinkman, Westbury, NY) at 25% maximal speed. Aliquots of homogenate (0.2 ml) for basal glutathione estimation were taken and the remainder was centrifuged for 15 min at 12 000 g in an Eppendorf microcentrifuge at 4°C. Supernatant fractions were assayed for endogenous antioxidants as described below.

Basal glutathione assay

Basal GSH levels were measured by adding 0.2 ml 0.9% saline containing 2.0 mM sodium azide followed by 0.1 ml 25% trichloroacetic acid to the 0.2 ml homogenate on ice. The samples were mixed and centrifuged for 5 min at 12 000 g. The resulting supernatants were assayed for acid-soluble sulphydryl groups by adding 0.04 ml 3 mM 5,5'-dithiobis-(2-nitrobenzoic acid) and measuring the absorbance at 412 nm after 10 min. Since, in most tissues, >90% of acid-soluble sulphydryl groups are contributed by GSH, the term 'basal GSH' as used in this manuscript indicates acid-soluble sulphydryl groups.

Antioxidant enzyme activities

GRD activity was assayed by the method of Long and Carson (21) and expressed as μmol NADPH oxidized to NADP/min/g wet tissue. GPX activity was assayed by the method of Paglia and Valentine (22) and the activity was expressed as μmol NADPH oxidized to NADP/min/g wet tissue, using an extinction coefficient for NADPH at 340 nm of 6.22×10⁵. Cu,Zn-SOD activity was measured by the method of Winterbourn et al. (23) and expressed as U SOD/g wet tissue, 1.0 U being defined as that amount of enzyme causing half-maximal inhibition of nitroblue tetrazolium reduction. A Cecil spectrophotometer (CE 292 digital ultraviolet spectrophotometer) was used for the measurement of SOD activity, while all other assays were performed at 25°C using a Perkin-Elmer model Lambda 6B dual beam spectrophotometer.

Statistical analysis

Results were analyzed using single factor ANOVA followed by application of the Tukey test to assess the significance of specific inter-group differences.

Results

Figure 1 summarizes results obtained for measurements of basal GSH levels in neoplastic and adjacent colonic mucosal tissues of DMH- and DMH+OLS-treated and control rats. The level of GSH in DMH-induced neoplastic tissues was ~50% that of corresponding tissue samples from control rats. OLS treatment significantly (P < 0.01) reduced the decrease in levels of GSH, although these were still significantly (P < 0.01) lower than those of corresponding tissues from control animals. The level of GSH in tissues adjacent to tumor sites in levels of GSH, although these were still significantly (P < 0.01) reduced the decrease OLS treatment significantly (P < 0.01) caused a statistically significant decrease in the activity of GPX in neoplastic tissues, without affecting that in adjacent tissues. Tissue samples adjacent to neoplasms in DMH- and DMH+OLS-treated rats

antioxidant enzyme in DMH-induced neoplastic tissue was almost double that in corresponding tissue from control animals. OLS treatment caused a statistically (P < 0.01) significant decrease in the activity of GPX in neoplastic tissues, without affecting that in adjacent tissues. Tissue samples adjacent to neoplasms in DMH- and DMH+OLS-treated rats
exhibited significant ($P < 0.01$) decreases in the activity of this enzyme compared with that in corresponding specimens from control animals.

Figure 4 shows the activity of SOD in DMH- and DMH+OLS-treated and control rats. The activity of SOD in neoplastic or adjacent tissues showed a small but significant ($P < 0.05$) decrease as compared with that of corresponding colonic mucosa from control animals.

**Discussion**

Involvement of RODS in the initiation and progression of neoplasms is well documented (26–30). Much research has been undertaken to determine the possible relationship between the incidence of colon cancer and antioxidant micronutrient consumption. Bostick et al. (31) reported a strong inverse association between vitamin E intake and colon cancer in a prospective Iowa Women’s Health Study. Vitamin E is a lipid-soluble antioxidant that can scavenge free radicals and protect biological membranes against lipid peroxidation. Roncucci et al. (32) also reported marked effects of antioxidant vitamins (A, C and E) in reducing the occurrence rate of adenomas in a total of 23 human large intestine tumors compared with non-neoplastic tissue, the active moiety of sulfasalazine, the drug most commonly used in the treatment of ulcerative colitis. Allgayer et al. (16) used spin trap electron spin resonance spectroscopy to show that this active moiety has superoxide and hydroxyl radical scavenging properties. These investigators also reported that OLS scavenges superoxide radicals.

There is evidence that antioxidant enzymes are inducible in both bacterial and eukaryotic cells (41,42). It has also been reported that $H_2O_2$ and other oxidants can induce the antioxidant heme oxygenase in human skin fibroblasts (43). The observed increase in GPX activity in neoplastic tissue (Figure 3) may, therefore, be the result of enzyme induction, possibly in response to an oxidative insult. Indeed, the reduction in GPX induction following OLS treatment may reflect a decreased oxidative stress arising from the oxygen radical scavenging activity of 5-ASA (14–16). It should be noted that OLS contains two molecules of 5-ASA linked by an azo bond which can be broken down by bacteria in the colon, thereby releasing the active oxygen radical scavenging moeity. The proposed induction by DMH of the GSH utilizing enzyme GPx was paralleled by an ~40% reduction in the activity of the GSH regenerating enzyme GRD. Since these two enzymes are crucial in determining the turnover of GSH in tissues, the combined effect of these two alterations could contribute to the nearly 50% reduction in the level of this important non-enzymatic antioxidant component. Colonic mucosal tissue immediately adjacent to neoplastic lesions showed activities of GRD which were comparable with those in non-neoplastic tissue, but there was a significant reduction in the activity of GPX. Non-enzymatic scavenging in the face of an increase in oxidative stress may also play a role in the depletion of GSH. Paralleling our finding of a progressive decrease in the level of the non-enzymatic antioxidant GSH in neoplastic lesions, immediately adjacent tissue and control tissue, Quershi et al. (26), studying azoxymethane-induced colonic tumors in rats, showed a progressive increase in chemiluminescence, an index of oxidative activity, in the order neoplastic tissue > adjacent tissue > control tissue. Our finding in adjacent tissue of a decrease in GPX activity, which was the opposite of the situation in neoplastic tissue, was associated with an increased level of GSH. Taken together our findings suggest a direct relationship between alterations in tissue GSH levels and in the activity of GPX. More generally, the presence of detectable alterations in antioxidant components in tissues immediately adjacent to neoplastic tissue is suggestive of a ‘field effect’ in the process of tumorigenesis.

Hoffman et al. (44) reported an increase in GPX activity in association with a reduction in SOD and catalase activities in a total of 23 human large intestine tumors compared with two control mucosal samples. Our results parallel theirs, although we found a larger increase in GPX activity along with a smaller decrease in SOD activity than they observed in the neoplastic tissues compared with normal mucosa. This difference in the magnitude of the changes in the activity of antioxidant enzymes might be a reflection of species-related variations in antioxidant profiles in the gastrointestinal tract, as has been observed in several species studied in our laboratory (24).

Given the free radical scavenging effects of the active moiety (5-ASA) of OLS (10–12), our results would be consistent with the involvement of RODS in this model of carcinogenesis. If such were the case one could predict that OLS might exert a protective effect against DMH-induced colon cancer. Further
studies are needed to explore this issue and the possible preventative role of OLS in this animal model of chemically induced colon cancer.

In summary, the present study describes alterations in endogenous antioxidant components in colonic mucosa of rats treated with the carcinogen DMH with or without OLS. DMH treatment was associated with the development of colonic neoplasms that showed a statistically significant reduction in the activity of GRD and levels of GSH along with a marked increase in the activity of GPX. OLS treatment produced a reduction in the DMH-induced alterations in antioxidant profiles of neoplastic tissues. Adjacent tissues also showed alterations in antioxidant components, although these were less severe and, in some cases, qualitatively different from those in neoplastic tissue. Our data are consistent with an oxidative etiology in DMH-induced tumor formation in rat colon, as previously suggested by Hoffman et al. (44) in human tumors of the large intestine. Further studies in this area would certainly seem warranted and, in particular, investigation of the possible preventive effects of exogenously administered antioxidants on the development of human colon cancer, and possibly other gastrointestinal malignancies.

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References

Effects of obalazine on endogenous antioxidant status


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