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Chemopreventive potential of zinc in experimentally induced colon carcinogenesis

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Abstract

The present study was performed to evaluate the efficacy of zinc treatment on colonic antioxidant defense system and histoarchitecture in 1,2-dimethylhydrazine- (DMH) induced colon carcinogenesis in male Sprague–Dawley rats. The rats were segregated into four groups viz., normal control, DMH treated, zinc treated, DMH + zinc treated. Colon carcinogenesis was induced through weekly subcutaneous injections of DMH (30 mg/kg body weight) for 16 weeks. Zinc (in the form of zinc sulphate) was supplemented to rats at a dose level of 227 mg/L in drinking water, *ad libitum* for the entire duration of the study. Increased tumor incidence, tumor size and number of aberrant crypt foci (ACF) were accompanied by a decrease in lipid peroxidation, glutathione-*S*-transferase, superoxide dismutase (SOD) and catalase. On the contrary, significantly increased levels of reduced glutathione (GSH) and glutathione reductase (GR) were observed in DMH treated rats. Administration of zinc to DMH treated rats significantly decreased the tumor incidence, tumor size and aberrant crypt foci number with simultaneous enhancement of lipid peroxidation, SOD, catalase and glutathione-*S*-transferase. Further, the levels of GSH and GR were also decreased following zinc supplementation to DMH treated rats. Well-differentiated signs of dysplasia were evident in colonic tissue sections by DMH administration alone. However, zinc treatment to DMH treated rats greatly restored normalcy in the colonic histoarchitecture, with no apparent signs of neoplasia. EDXRF studies revealed a significant decrease in tissue concentrations of zinc in the colon following DMH treatment, which upon zinc supplementation were recovered to near normal levels. In conclusion, the results of this study suggest that zinc has a positive beneficial effect against chemically induced colonic preneoplastic progression in rats induced by DMH.

Keywords: Dimethylhydrazine; Antioxidant status; Zinc; Colon cancer

1. Introduction

Colon cancer is one of the leading causes of cancer related deaths in both men and women in western countries, including the Unites States (Landis et al., 1999). It is frequently a pathological consequence of persistent oxidative stress, leading to DNA damage, mutations in cancer related genes, as well as epigenetic silencing of tumor suppressor genes (Goel et al., 2001, 2006; Bartsch and Nair, 2002; Boland et al., 2005). The end consequence of such a genomic instability is cellular overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Oxidative DNA

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damage may participate in ROS-induced carcinogenesis (Breimer, 1990). Formation of hydroxylated bases of DNA is considered an even important event in chemical carcinogenesis (Breimer, 1990; Bartsch and Nair, 2002). This adduct formation interferes with normal cell growth by causing genetic mutations and altered normal gene transcription.

The public health impacts from colon cancer has spawned a growing interest in prevention trials and among these dietary micronutrients are viewed as promising agents in colon cancer prevention. Both epidemiological and experimental studies suggest that colon cancer is strongly influenced by nutritional factors, including the quantity and composition of dietary fat (Willett et al., 1990). Several investigators have over many years, conducted research on agents with potential chemopreventive properties and have elucidated their modes of action. Although a full explanation of the intricacies of the causes, development and control of colon cancer is awaiting further research, however, there is enough scope to explore the use of new agents as interventions at various stages of cancer for the better management of the patients suffering from this pathological condition.

Nowadays, some recent evidence has indicated role of zinc in carcinogenesis. Zinc has been ascribed vital roles in the metabolism and interaction of malignant cells (Schrauzer, 1977). Zinc replenishment has been shown to induce apoptosis in esophageal epithelial cells, thereby providing growth inhibition for the development of esophageal cancer (Fong et al., 2001). The zinc content of leukemia cells has also been found to be reduced and reports indicate that zinc deficiency does enhance the carcinogenic effects of nitroso-methylbenzylamine (Fong et al., 1978). It has also been shown that a frequent biochemical characteristic of prostate cancer is the marked decrease in zinc levels in the malignant cells, thus providing compelling evidence that the lost ability of the malignant cells to accumulate zinc is an important factor in the development and progression of prostate malignancy (Costello et al., 2004). Keeping in mind these limitations of the existing literature, the present study was designed to explore the possibility of zinc being used as a measure of long term prophylactic therapy in delaying the compounding events, leading to the development of colon tumors. These aims were achieved by evaluating its role in the regulation of key enzymes involved with the oxidative stress mechanisms, as well as investigations on the histological alterations in the colon of rats subjected to 1,2-dimethylhydrazine (DMH) (an organo-specific carcinogen) treatment.

2. Materials and methods

2.1. Chemicals

1,2-Dimethylhydrazine (DMH), NADPH, GSH, NBT, DTNB, were procured from Sigma–Aldrich company (Delhi, India). Zinc sulphate was purchased from E. Merck.

2.2. Animals

Male Sparque–Dawley rats in the weight range of 120–150 g were procured from the Central Animal House, Panjab University, Chandigarh. The animals were housed in polypropylene cages under hygienic conditions in the departmental animal house. Before initiating the experiments, the animals were adapted to the laboratory conditions for a week. Necessary approvals were obtained from the Ministry of Social Justice and Empowerment for the use of experimental animals in this study.

2.3. Experimental design

Animals were segregated into four treatment groups. Animals in Group I served as normal controls and were given water and diet *ad libitum*. Rats in this group in addition were also administered with 1 mM EDTA–saline subcutaneously per week, which was used as the vehicle for the treatment of DMH treated animals. Animals in Group II were given a weekly subcutaneous injection of DMH at a dose level of 30 mg/kg body weight dissolved in 1 mM EDTA–normal saline (pH 6.5), for a total duration of 16 weeks (Soler et al., 1999). Group III animals were given zinc in the form of $ZnSO_4 \cdot 7H_2O$ in drinking water *ad libitum* at a dose level of 227 mg/L of drinking water (Goel et al., 2005). Animals in Group IV were given a combined treatment of DMH as well as zinc in a similar manner as was given to Group II and Group IV animals, respectively.

2.4. Colon tumor analysis

After the terminal sacrifice following 16 weeks of DMH treatment, colons were excised from the rats, blotted dry, cut open longitudinally and the inner surface was examined for the visible macroscopic lesions. Tumors were easily discernable in the inflamed sections of the colon. The number of tumors was noted for tumor incidence and multiplicity studies. Tumor size was also noted and the three main axes of each macroscopic tumor from rats were measured using a vernier caliper with 0.1 mm graduation.

2.5. Preparation for aberrant crypt foci (ACF) counting

The entire colon was removed and washed thoroughly with 0.9% NaCl, cut longitudinally and fixed with 10% buffered formaldehyde solution overnight. The colon was then stained with 0.2% methylene blue for 3–5 min in saline in order to iden-