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Tissue zinc levels in precancerous tissue in the gastrointestinal tract of azoxymethane (AOM)-treated rats

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Abstract

Alterations in tissue zinc levels have been documented in patients with gastrointestinal tract malignancies and more frequently, in those with colonic cancer. However, the precise role of tissue zinc in carcinogenesis is not well elucidated.

This study, using a well-established colon cancer model in rats, was designed to investigate the relationship of tissue zinc to the carcinogenic process. The aim was to examine tissue zinc levels in the preneoplastic tissues and to study the changes that occur during transition of mucosa from normal to preneoplastic state.

Six-week old rats were given a single dose subcutaneous injection of azoxymethane (AOM) (30 mg/kg body weight) and sacrificed after 1, 2, 5, and 9 months of the treatment. Plasma zinc levels showed a significant decrease ($p<0.05$) at 9 months compared with controls. Tissue zinc levels showed a significant decrease in the large intestine at 1 and 2 months ($p<0.05$) and at 5 and 9 months ($p<0.01$), in the small intestine at 2, 5, and 9 months ($p<0.05$), and in the stomach at 5 and 9 months ($p<0.05$). The maximum percent decrease (45%) in tissue zinc was observed in the large intestine at 9 months. Tissue copper zinc super oxide dismutase (CuZnSOD) activity was assessed in the body of the stomach, small intestine, and large intestine and compared with the control group. There was a significant fall in CuZnSOD levels in the small intestine at 9 months ($p<0.05$) and in the large intestine at 5 and 9 months ($p<0.01$). Two of these six rats showed histological evidence of precancerous lesions in the mucosa of the colon. This study suggests that the decrease in plasma zinc, tissue zinc and activity of CuZnSOD is associated with development of preneoplastic lesions in the colonic mucosa.

Keywords: Colon carcinoma; Azoxymethane; Zinc; CuZnSOD; Precancerous

Introduction

In the developed countries, the colon is a common site for malignancies of the gastrointestinal tract, which are also a leading cause of death. Many contributing factors such as lifestyle, nutrition and obesity have been

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postulated to play a role in the development of these malignancies (Mohandas and Desai, 1999).

Zinc is necessary for the functioning of all living systems. It is an essential trace element, important for the stabilization and function of numerous metalloenzymes involved in protein synthesis, protein catabolism, energy metabolism, and RNA and DNA synthesis (Sky Peck, 1986). Since zinc was first reported as a constituent of carbonic anhydrase, in 1940, it has now been identified as a component of more than 200 mammalian metalloenzymes. These metalloenzymes are activated by zinc incorporation. Zinc plays an important role in protein metabolism in humans and is necessary for maintenance of normal levels of proteins. The role of zinc in RNA, DNA polymerases, its inhibitory effects on phosphodiestrases and its activating effect on membrane-bound adenyl cyclase; all suggest a role for zinc in carcinogenesis (Vallee and Goldes, 1984). It is well established that zinc plays an essential role in a number of biological processes, through its action as an activator or inhibitor of enzymatic reactions, by competing with other elements and proteins for binding sites, and influencing permeability of the cell membrane. It is therefore reasonable to assume that zinc could exert an action directly or indirectly on the carcinogenic process (Sunderman, 1984)

The role of zinc in carcinoma development has been the subject of debate, and reports of zinc values in the biological fluids from cancer patients are often conflicting and contradictory (Ehud et al., 1983).

Significant alterations (either an increase or decrease) from the normal distribution of tissue zinc concentrations have been reported to occur in patients with various forms of cancer (Valcovic, 1980). Low plasma zinc levels have been observed in patients with cancer of the colon, bronchus, and digestive system (Karcioglu et al., 1980). Ehud et al. showed a decrease in tissue zinc levels in large bowel cancer and stomach cancer compared with normal tissues (Ehud et al., 1983). The mechanism by which serum zinc and tissue zinc decrease in various cancerous tissues is still obscure; neither has it yet been established, whether these altered zinc levels contribute to the malignant state.

Oxidative stress caused by reactive oxygen metabolites, result in damage to cellular structure, and this has frequently been implicated in the initiation and promotion phases of carcinogenesis. Super oxide dismutases (SOD) are metalloenzymes that play a vital role in the protection of aerobic cells against oxygen toxicity and cytosolic CuZnSOD has been shown to contain both copper and zinc atoms (Cindy and Davis, 1999).

Superoxide anion (O_2^-) is a free radical, produced by partial reduction of molecular oxygen in several metabolic processes. In the presence of two molecules of hydrogen gas, two molecules of oxygen are converted to H_2O_2 and O_2. The rate of this reaction is greatly increased by SOD. This enzyme, which is essentially absent from anaerobic cells, is important as a scavenger of oxygen-free radicals that otherwise would lead to damage of the cell membrane and biological structure (Jansen and Bosman, 2000).

Tissue CuZnSOD activity is reduced in a number of malignancies. However, in other malignancies variable findings have been observed. Some tumors have less CuZnSOD in comparison with the more metabolically active tissues (Grigolo et al., 1998). The SOD plays an important role in the body's defense mechanisms against the deleterious effects of O_2 free radicals in biological systems. Study by Jansen and Bosman (2000) showed that SOD was decreased in gastric malignant tissues compared with normal tissues. These findings led to the hypothesis that early mucosal alterations, which are preneoplastic lesions, might lead to a decrease in tissue zinc concentration and zinc-related enzyme CuZnSOD activity in the large intestine and to possible changes in other parts of the gastrointestinal tract.

The DNA alkylating agent azoxymethane (AOM), which is primarily activated in the liver, induces a high incidence of initiation and promotion steps of precancerous lesions in the colon of rats (Guda et al., 2003). Numerous studies have been carried out to investigate the protective effect of various chemicals, drugs and food items on the induction and development of azoxymethane-induced colon tumors in rats (Davies et al., 1999; Tao and Pereira, 1997; Seraj et al., 1997). However, so far, no studies have been reported on the tissue zinc levels and activities of zinc-containing enzymes in AOM induced colon carcinomas.

Decrease in SOD activity and in tissue zinc levels have been linked to malignancy in patients. In order to investigate their relevance in the development of the precancerous process in the colon, we have used a well-established AOM colon carcinoma model in the rat.

Materials and methods

Animals

Six-week-old adult Wistar rats (100–120 g) obtained from the institutional animal house were housed in polypropylene plastic cages, in an animal-holding room under controlled conditions with $25 \pm 2\,^{\circ}C$, $50 \pm 10\%$ humidity, and 12 h light–dark cycles. The rats were allowed water and food ad libitum, observed daily and weighed weekly. This study was approved by the animal experimentation ethics committee of our institution.

Chemicals

AOM, stock Zn standard (1002 μg/ml), bovine serum albumin, triton X100, bathocuproindisulfonate sodium