Calcium, Vitamin D, and Colon Cancer

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Abstract

Calcium contributes to the progression of epithelial cells through all phases of the proliferative cycle and into stages of cell differentiation; intracellular concentrations of calcium that are required for cell renewal, however, are lower than those required for epithelial-cell differentiation. These effects of calcium are modulated by interactions with 1,25-dihydroxyvitamin D₃, phosphate, and fatty acids, all of which are partly dependent on dietary intake.

In rodent models, increased dietary calcium inhibited hyperproliferation of colon epithelial cells induced by increased levels of fatty acids or bile acids present in the colon. When carcinogens induced hyperproliferation of colon epithelial cells the hyperproliferation was decreased by added dietary calcium, and in several animal models the occurrence of carcinogen-induced carcinomas of the colon decreased with increased dietary calcium.

A nutritional stress diet, designed to represent human Western dietary intake of calcium, phosphate, vitamin D, and fat, produced hyperproliferation and hyperplasia in the colons of rodents; these effects were reduced by increasing dietary levels of calcium. Decreased levels of ornithine decarboxylase also were reported in human and rodent colon mucosa exposed to increasing levels of calcium.

In human subjects at increased risk for familial colon cancer, hyperproliferation of colon epithelial cells was reduced after oral dietary supplementation with calcium. In epidemiological studies, several investigators reported inverse correlations between levels of dietary calcium intake and the incidence of colon cancer. Extrapolation of the data have suggested a protective effect of total calcium intakes above 1500 to 1800 mg/day.

Introduction

Colorectal cancer is one of the cancers of highest incidence in the United States and in other countries where Western-style diets are generally consumed. An increased dietary intake of fat has been associated with an increased rate of colon cancer in high-risk geographic regions (1). In animal studies, dietary fat appears to act as a promoter rather than as an initiator of colon carcinogenesis (2).

The presence of excessive amounts of free bile acids and fatty acids unbound by calcium in the colon lumen was put forward in 1984 as a possible causative factor. It was suggested that increased dietary fat promotes colon cancer by increasing the levels of free ionized fatty acids and unconjugated bile acids in the colon contents (3). In the ionized state, free fatty acids can be very irritating and toxic to the colon epithelium. In animal studies, it was demonstrated that orally administered calcium supplements decreased such effects of bile acids and fatty acids on the colon epithelium, presumably because these ionized fatty acids and bile acids were converted to insoluble calcium soaps (4, 5). This action of medium- and long-chain fatty acids and bile acids in the colon lumen on the availability of calcium to epithelial cells has focused attention on the broader importance of calcium in normal cell structure and function and on the interactions of dietary calcium, vitamin D, fat, and phosphate in affecting one or more aspects of the carcinogenic process.

Calcium in Cellular Structure and Function

Although only 1% of body calcium is in soft tissues, calcium is key for the maintenance of proper structure of many components of the cell, including the membranes and many organelles. Calcium is also a pivotal regulator of a variety of cellular functions, as a major second messenger that carries signals from the plasma membrane and other intracellular stores to activate a large number of cellular functions (6, 7). Many of these effects of calcium are mediated by a class of calcium-binding proteins, of which calmodulin, found in all eukaryote cells, appears to be the most versatile.

The dynamics of calcium entry into cells is closely regulated; the average concentration within cells (10 μM) is 10,000 times lower than that outside the cell (1 mM), thereby providing concentration-driven forces for calcium entry into cells. Small changes in this flux are signals for many cell functions, directly or indirectly, and, therefore, the dynamics of the flux are well regulated to achieve calcium balance and ensure normal cell function. Small perturbations, even within the normal range of external calcium concentration, can thus have major effects on the dynamics of calcium flux into cell compartments, altering or stressing cell functions and affecting tissue biology.

Other inorganic ions, such as phosphate, sodium, and potassium, and the hormone 1,25-dihydroxycholecalciferol, which is biologically derived from vitamin D₃, are involved in aspects of calcium flux in the cell. A balance in the exterior concentrations of all these components is needed to enable the cells to function in a normal manner.

Membrane viscosity or rigidity and inversely related permeability are partly dependent on the local calcium concentration, probably by a combination of ionic and coordinate bonding to the phosphatidester groups of the phosphatides comprising the bilayers of cell and organelle membranes (8, 9).

Calcium and Cell Proliferation

In cell and organ culture studies, lowering the external concentration of calcium reduces cell responsiveness to normal external stimuli and leads to hyperproliferation and poor differentiation of cells (10-12). Normal cell function and differentiation can usually be restored by returning the external calcium concentration to normal. These effects were demonstrated in rat esophageal epithelial cells (11), murine epidermal cells (13), and, more recently, human colon epithelial cells (14). Proliferation, particularly of epithelial cells, is normally a tightly regulated process that supplies fresh, undamaged, and nonmutated cells to organ surfaces exposed to chemical and biological challenges, e.g., the colon epithelium. Hyperproliferation of colon epithelial cells is a consistent early biomarker of...
increased risk of colon cancer (15, 16). In a recent monograph, Whitfield (17) indicated that calcium contributes to the progression of epithelial cells through all phases of the proliferation cycle and into stages of cell differentiation. However, intracellular concentrations of calcium that are required for cell renewal (i.e., proliferation) are lower than those required for epithelial cell differentiation (17). These effects of calcium are modulated by interactions with 1,25-dihydroxy-vitamin D<sub>3</sub> (calcitriol), phosphate, and fatty acids, all of which are partly dependent on dietary intake (18).

After direct exposure of normal human colon epithelial cells to physiological levels of calcium in vitro, the cells responded with decreased proliferation (14); however, when colon epithelial cells became transformed to adenomas and carcinomas, the cells became unresponsive to calcium in short-term tissue culture (19). Direct exposure to calcium also induced more quiescent proliferation of other normal epithelial cells, including keratinocytes and mammary, esophageal, bronchial, and urothelial epithelial cells, and led to terminal differentiation and growth inhibition of some cell types (17).

### Dietary Calcium in Animal-Colon Models

Intrarectal administration of a bile acid, deoxycholic acid, to mice produced marked irritation and toxicity to the colon epithelium, followed by hyperproliferation and repair. Oral supplementation of calcium to a level twice the normal intake prevented toxicity and subsequent hyperproliferation (4). Oral calcium had similar ameliorative effects on colon irritation produced by intrarectal administration of fatty acids (5). Oral administration of a free bile acid, cholic acid, also induced hyperproliferation of colon epithelium in mice. This mitogenic response to oral cholic acid was alleviated by increased dietary calcium (20).

In rat colon carcinogenesis initiated by 1,2-dimethylhydrazine and promoted by a high dietary intake of fat, supplemental calcium or vitamin D<sub>3</sub> reduced tumor incidence and tumor burden per rat (21). In rats with jejuno-ileal resection as a model of humans with ileal resection, hyperproliferation of colon epithelium was inhibited by supplemental dietary calcium (22), as was colon carcinogenesis induced by azoxymethane (23).

Hyperproliferation in rats induced by colon carcinogen N-methyl-N'-nitro-N-nitrosoguanidine was reduced by a calcium-enriched diet with low or high fat (24). In a study of colon cancer induced in rats by azoxymethane and promoted by high dietary fat, similar to that in the human diet in the United States, calcium supplementation inhibited colon neoplasia, particularly at the promotional phase (25). Using three levels of dietary calcium in rats, another group found that azoxymethane-induced neoplasia decreased as dietary calcium increased (26).

The polyamines (putrescine, spermidine, and spermine) and their key biosynthetic enzyme ornithine decarboxylase are important in cell proliferation in normal colon epithelial cells and are often elevated in neoplasia. In an azoxymethane rodent colon-carcinogenesis model, increased dietary calcium inhibited rat colon mucosal elevation of ornithine decarboxylase (27). Dietary calcium also suppressed rectal mucosal ornithine decarboxylase activities in human subjects with adenomatous polyps (28). Raising dietary calcium or vitamin D<sub>3</sub> inhibited ornithine decarboxylase elevation in rat colon epithelium induced by cholic acid (29).

In recent rodent studies, a nutritional stress diet was designed based on the AIN-76 semisynthetic diet, modified to contain four suggested risk factors of the human Western-style diet, increased dietary fat (40% of calories) and phosphate (0.8 mg/kcal) and decreased calcium (0.1 mg/kcal) and vitamin D (0.1 IU/kcal), on the principle of nutrient density (30, 31). The control and nutritional stress diets, without any chemical carcinogen, were fed to mice and rats for 12 weeks. Hyperproliferation and hyperplasia developed in the colons of both mice and rats, suggesting that the stress diet, as a mimic of Western-style diet, induced changes in colon mucosa that occur in carcinogen-induced rodent models and in humans at increased risk for colon cancer. With elevation of calcium intake to the median level of human intake, hyperproliferation still developed in mice and rats, but with a high calcium intake hyperproliferation was reduced almost to control levels (31).

**Calcium and Proliferation of Colon Epithelial Cells in Humans**

A study was carried out to test whether calcium has a similar effect in humans (32). Previous work had shown that increased proliferation of colon epithelial cells is accompanied by an increased risk for colon cancer. The frequency distributions of tritiated thymidine-labeled proliferative cells in the colon mucosa revealed significant differences between populations at high risk and at low risk for colon cancer. In subjects at high risk for familial colon cancer, the frequency and distribution of proliferating cells lining the colonic crypt were studied before and after oral supplementation of their conventional diets with 1250 mg calcium (about 1.5 times the United States recommended dietary allowance), in the form of calcium carbonate, each day for 2–3 months. Before dietary supplementation with calcium, the proliferation of epithelial cells in the colonic crypts was elevated, comparable to that previously observed in other individuals affected by familial colon cancer. After dietary calcium supplementation, proliferation was significantly reduced, yielding an altered colonic crypt profile approaching that previously observed in subjects at low risk for colon cancer, e.g., Seventh Day Adventists. Oral calcium supplementation induced a more quiescent proliferation equilibrium in the mucosa of these high-risk individuals, similar to that observed in subjects at low risk. This appeared to be the first demonstration in humans of reversal of an abnormal proliferation site in colon epithelial cells known to be associated with increased risk for colon cancer and also appeared to support the hypothesis that low calcium intake may play a role in human colon cancer. The results of the original report were confirmed in another population, where 1.25 g of calcium p.o. daily also suppressed elevated colon mucosa proliferation in 21 subjects at risk for colorectal neoplasia (33).

**Epidemiology of Calcium and Colon Cancer**

In epidemiological studies of human populations, several investigators reported inverse correlations between levels of dietary calcium intake and the incidence of colon cancer. In a 19-year prospective study of 1965 men in Chicago, Garland et al. (34) found that the risk of colorectal cancer correlated inversely with dietary intake of calcium and vitamin D. A dietary intake of 150 IU or more of vitamin D was associated...
CALCIUM, VITAMIN D, AND COLON CANCER

with a 50% reduction in incidence of colorectal cancer, whereas an intake of 1200 mg or more of calcium was associated with a 75% risk reduction.

In a case-control study in Utah, Slattery et al. (35) found that increased dietary calcium intake reduced the risk of colon cancer, particularly in males. Colon-cancer risk reduction was also associated with increased intake of milk and dairy products, Sorenson et al. (36) reviewed six epidemiological studies with a major source of dietary calcium in the United States diet. 

The concept that increased dietary intake of calcium could reduce the hyperproliferative and cancer-promoting effect of the high dietary-fat intake characteristic of Western-style diets. Laboratory data in vitro and in vivo in rodents support this hypothesis. In human subjects at risk for colon cancer, hyperproliferation of colon epithelium was reduced towards normal by dietary calcium supplementation. Epidemiological studies generally find that increased dietary calcium is correlated with a lower incidence of colon cancer. Increasing dietary intake of calcium and vitamin D may be a prudent method of reducing the risk of colon cancer.

The concept that increased dietary intake of calcium could reduce the risk of colorectal cancer in human subjects on a Western-style diet is currently being tested in several randomized clinical trials in the United States, Europe, and Israel, to evaluate recurrence rates of colon adenoma after supplemental dietary calcium. A workshop on calcium, vitamin D, and colon cancer, sponsored by the Chemoprevention Branch of the National Cancer Institute, was held in Vail, CO, July 8–10, 1990, to review these findings (38).

Summary

It has been hypothesized that increased dietary calcium intake reduces the hyperproliferative and cancer-promoting effect of the high dietary-fat intake characteristic of Western-style diets. Laboratory data in vitro and in vivo in rodents support this hypothesis. In human subjects at risk for colon cancer, hyperproliferation of colon epithelium was reduced towards normal by dietary calcium supplementation. Epidemiological studies generally find that increased dietary calcium is correlated with a lower incidence of colon cancer. Increasing dietary intake of calcium and vitamin D may be a prudent method of reducing the risk of colon cancer.

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