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WJG, 1999 December; 5(6):461-464
World Journal of Gastroenterology
 ISSN 1007-9327

Editorial

Update of preclinical and human studies of calcium and colon cancer prevention

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Subject headings calcium/metabolism; colonic neoplasms/prevent ion and control; vitamin D

Many articles on the subject of colon cancer begin by noting that the disease continues to be a major cause of tumor mortality in the United States and other countries. Despite attempts at reduction, the incidence of this disease is still high in Western populations and is increasing in some Eastern countries. Chemoprevention of this disease therefore continues to be an important public health objective. Among recent chemopreventive approaches, an increased intake of calcium and vitamin D continues to be evaluated in both preclinical and clinical studies. Many experimental findings, as described below, have indicated real associatio ns between high calcium and vitamin D intake and decreased risk for colorectal c ancer.

BASIC STUDIES OF CALCIUM METABOLISM

Calcium is both an essential structural body component and a critical functional element in living cells. It is a key component for maintaining cell structure, membrane viscosity or rigidity, and the related membrane permeability is partly dependent on local calcium concentration. Calcium is also a pivotal regulator of a wide variety of cell functions in its role as a major second messenger[1].

Among the numerous cell properties modulated by calcium, its participation in cell division and the regulation of cell proliferation and differentiation are particularly important[2]. Low levels of intracellular ionized calcium contribute to cell proliferation, and increasing calcium concentration in cell and organ culture media decreased cell proliferation; and induced cell differentiation in rat esophageal epithelial cells[3], murine epidermal cells[4], mammary cells[5,6], and colon cells[7].

The absorption and metabolism of calcium are carefully regulated, 1, 25-dihydroxyvitamin D_3 is an important calcium modulator that can become deficient as a consequence of inappropriate diet or inadequate exposure to sunlight. Therefore vitamin D_3 also may have a role in the regulation of cell proliferation and differentiation while modulating calcium metabolism. It has also been shown to directly inhibit the proliferation of several malignant cell lines *in vitro*[8-10], and to induce the differentiation of human colonic cells[11], human myeloid leukemia cells[12], and other cell lines *in vitro*[13,14]. A role of vitamin D as a chemopreventive agent has also been studied in rodent models[15-19], and the tumor growth and promotional stag e of chemical carcinogenesis have been inhibited by vitamin D. On the other hand, vitamin D_3 enhanced chemically-induced transformation of cultured cells *in vitro*[20,21] and promoted skin tumor formation in mice[22].

PRECLINICAL AND EARLY HUMAN INTERVENTION STUDIES OF EFFECTS OF INCREASED DIETARY CALCIUM

Preclinical studies

The results of direct experimental studies of calcium intake and colon cancer development are summarized in Table 1. The results of many individual studies are further described in Tables 2-5. In many preclinical experimental models, calcium effects on colon cancer development and on cellular processes associated with colon cancer have been remarkably consistent: ① decreasing and normalizing excessive proliferation of colonic epithelial cells, reducing the susceptibility of proliferating epithelial cells to accumulate abnormalities in their DNA; ② reducing the cytotoxicity of fecal water; ③ increasing the differentiation and maturation of colonic epithelial cells; and ④ decreasing the end-stage development of colon cancer itself.

In animal models (Tables 2,3), oral calcium supplementation decreased epithelial cell hyperproliferation when it was induced by several factors that stimulate tumor promotion: the administration of bile acids and fatty acids, dietary fat, a Western-style diet, and partial enteric resection. Of further importance colonic

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Received 1999-09-28

carcinogenesis itself, when induced by chemical carcinogens, decreased with increasing dietary calcium intake, with almost all studies showing a decrease in the number of tumors induced, the percent of invasive carcinomas, or the number of animals with multiple tumors.

Thus, a wide variety of rodent studies (Tables 1-3 with references) demonstrated that increasing dietary calcium intake reduced colonic tumor formation: mechanisms involved included decreased epithelial cell hyperproliferation; decreased or nithine decarboxylase activity; decreased ras mutations in colonic epithelial cells; and calcium-binding of bile acids, fatty acids and phosphate into insoluble complexes, reducing their direct irritant and hyperproliferative effects on colonic epithelial cells and reducing the cytotoxicity of fecal water.

Two recent series of studies also have evaluated the effects of calcium and vitamin D on colonic tumor development when these nutrients were fed to rodents on Western-style diets. The first group of studies utilized preclinical models of n ormal mice. In the colonic crypts of these normal mice hyperproliferation, hyper plasia, abnormal differentiation and maturation of colonic crypt epithelial cells, and the late-stage preneoplastic lesion of whole-colonic-crypt dysplasia developed when the mice were fed- Western-style diets containing low calcium and vitamin D[26,33,34].

The second series of studies utilized mice having targeted mutations that are relevant to human colon cancer, the targeted mutation causing adenomas and carcinomas to develop in the mice[35,36]. Recent studies demonstrated the Western-style diets increased the development of the neoplastic colonic lesions that were initiated by those mutations; and the neoplasms together with carcinomas were decreased by: @ increasing calcium and vitamin D together with lowering fat content of diet[37], or increasing dietary calcium and vitamin D alone (Yang *et al*, unpublished data).

In other organs, Western-style diets also have induced epithelial cell hyperproliferation and hyperplasia in mammary gland[38,39], and hyperproliferati on in pancreas[40] and prostate gland[41] in short-term studies; increasing dietary calcium and vitamin D alone also inhibited the development of those lesions[25].

EARLY HUMAN CLINICAL TRIALS OF CALCIUM AND COLON CANCER CHEMOPREVENTION

Prior to most of the preclinical studies noted above, a first human study was carried out[28]which began to evaluate calcium's chemopreventive effects on the human colon. That first study, and a majority of the human studies that followed demonstrated that increased dietary calcium could decrease hyperprolife ration of colonic epithelial cells in human subjects; and several studies further demonstrated calcium's binding of bile acids and fatty acids into insoluble complexes in the colon, decreasing the cytotoxicity of fecal water, the latter co ntributing to the decreased colonic epithelial cell hyperproliferation observed in human subjects (Tables 4,5).

The first pilot study in this human series noted above, and several other that followed, demonstrated significant reduction of excessive colonic epithelial cell proliferation, or reduced size of the proliferative compartment in colonic crypts. However, other human studies of supplemental calcium administration did not show this effect[42]. Several of those studies were accompanied by experimental techniques that included extremely low initial baseline levels of colonic cell proliferation measured before calcium administration, very high amounts of calcium intake by subjects before calcium was given, and enemas given prior to colonic biopsies that likely perturbed the mucosa[42]. Because early positive results were found in humans where calcium reduced colonic epithelial cell proliferation[28], a further large randomized adenoma-recurrence clinical trial was developed and carried out, recently verifying that increased calcium intake caused a significant reduction in the development of actual tumors (recurrent adenomas) in the human colon[32]. A further human study was recently carried out increasing dietary calcium intake through low fat dairy foods: this caused increased maturation and decreased proliferation of colonic epithelial cells following the increased dietary calcium intake[43].

Table 1 Summary of studies on calcium and colon cancer

·Majority of epidemiologic studies suggest protective effect
·*In vitro* studies: decreased proliferation and increased differentiation and maturation of many types of epithelial cells
·In vivo rodent studies: numerous studies demonstrated inhibition of colonic tumor development preceded by decreased hyperproliferation, ODC and ras mutations, binding of bile and fatty acids into insoluble complexes reducing irritant and hyperproliferative effects, reduced cytotoxicity of fecal water
·Human studies: decreased hyperproliferation in most studies, increased differentiation and maturation of colonic epithelial cells, binding of bile and fatty acids into insoluble complexes, decreased cytotoxicity of fecal water
·Decreased recurrence of human adenomas

Table 2 Dietary calcium effects on epithelial cells in the colon and other organs of rodents

Cell proliferation	References*
Calcium decreased hyperproliferation Gover *et al*, 1994	
Calcium: decreased hyperproliferation when induced by doxycholic acid	Wargovich *et al*, 1983
Decreased hyperproliferation when induced by fatty acids	Wargovich *et al*, 1984
Decreased hyperproliferation when induced by cholic acid	Bird *et al*, 1986
Decreased hyperproliferation induced by partial enteric resection	Appleton *et al*, 1986
Decreased deoxycholic acid-induced hyperproliferation	Hu *et al*, 1989
Decreased MNNG-induced hyperproliferation on diet low in fat and calcium	Reshef *et al*, 1990
Decreased hyperproliferation induced by Western-style diet	Newmark *et al*, 1991
Decreased AOM-induced ODC and Tyr K	Arlow *et al*, 1989
Decreased ODC induced by bile acids	Baer *et al*, 1989
Decreased hyperproliferation when induced by Western-style diet	Richter *et al*, 1995[24]
Decreased hyperproliferation in other organs when induced by Western-style diet	Xue *et al*, 1999[25]

*Studies without reference numbers are found in[23].

Table 3 Dietary calcium effects on colonic epithelial cells of rodents

Tumor development	References*
Calcium: decreased tumors induced by partial enteric resection and carcinogen	Appleton *et al*, 1987
Decreased proliferation and tumor formation induced by dietary fat and carcinogen	Pence *et al*, 1988
Decreased intestinal tumors after AMO	Skrypec *et al*, 1988
Decreased colonic tumors induced by AMO	Wargovich *et al*, 1990
Decreased the number of invasive carcinomas after MNU and cholic acid	McSherr y *et al*, 1989
Decreased the number of rats with multiple tumors after DMH	Sitrin *et al*, 1991
Decreased K-ras mutations	Llor *et al*, 1990
Unchanged tumor incidence after DMH	Karkara *et al*, 1989
Unchanged tumor incidence after DMH	Kaup *et al*, 1989
Decreased late-stage precancerous lesion of whole colonic crypt dysplasia	Ri sio *et al*, 1996[26]

*Studies without reference numbers are found in [23].

Table 4 Calcium effects on colonic cell proliferation, differentia tion and cytotoxicity in human subjects

In vitro	References*
Decreased proliferation (2mM)	Buset *et al*, 1986
Decreased proliferation (2 4mM)	Appleton *et al*, 1988
Decreased proliferation (2mM)	Arlow *et al*, 1988
Decreased proliferation (2mM)	Buset *et al*, 1987
Decreased proliferation (2mM)	Friedman *et al*, 1989
Protected colonic cells against toxicity of bile acids and fatty acids (5mM)	Buset *et al*, 1989
Decreased growth of human colon cancer cell lines	Guo *et al*, 1990[27]
Increased histone acetylation: cell differentiation (1-2mM)	Boffa *et al*, 1989

*Studies without reference numbers are found in [23].

Table 5 Calcium effects on colonic cell proliferation, differentiati on and cytotoxicity of fecal water in human subjects

In vivo	References*
Decreased hyperproliferation	Lipkin *et al*, 1985[28]
Decreased hyperproliferation	Lipkin *et al*, 1989
Decreased hyperproliferation	Rozen *et al*, 1989
Decreased proliferation	Lynch *et al*, 1991
Decreased proliferation	Berger *et al*, 1991
Decreased proliferation	Wargovich *et al*, 1992
Decreased proliferation	Barsoum *et al*, 1992
Decreased proliferation	O'Sullivan *et al*, 1993
Decreased proliferation	Bostick *et al*, 1995
Unchanged proliferation	Gregoire *et al*, 1989
Unchanged proliferation	Cats *et al*, 1995
Decreased ODC	Lans *et al*, 1991[29]
Normalized differentiation-associated lectin binding	Yang *et al*, 1991[30]
Decreased cytotoxicity of fecal water	Govers *et al*, 1996[31]
Increased maturation of colonic epithelial cells	Holt *et al*, 1998[43]
Decreased adenoma recurrence	Baron *et al*, 1999[32]

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Edited by MA Jing-Yun
Proofread by MIAO Qi-Hong