Journal of Cellular Biochemistry, Supplement 22:65–73 (1995)

Calcium and the Prevention of Colon Cancer

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Abstract Chemoprevention studies utilizing calcium have now progressed from basic measurements to clinical trials. Calcium's effects on epithelial cells have demonstrated decreased proliferation and induced cell differentiation with increasing levels of calcium *in vitro*, similar *in vivo* effects in rodent and human colon, and decreased carcinogen-induced colonic tumor formation in rodents. Current studies are attempting to inhibit colonic adenoma formation in human subjects. Most but not all epidemiologic studies also link increased dietary calcium with a decreased risk of colon cancer.

In animal models, supplemental dietary calcium has decreased mammary epithelial cell hyperplasia and hyperproliferation and colonic cell hyperproliferation when the latter was induced by bile acids, fatty acids, and partial resection of the small intestine. Supplemental dietary calcium also decreased carcinogen-induced colonic tumors in several rodent models. In normal mice, and in mice carrying a targeted *apc* gene mutation, we recently increased colonic polypoid hyperplasias by a Western-style diet containing low calcium and vitamin D.

In human subjects at increased risk for colon cancer, oral calcium supplementation significantly reduced colonic epithelial cell proliferation in most of the studies, including four randomized clinical trials. These studies have now progressed to short-term human clinical trials, including trials that measure the regrowth of transformed adenoma cells. Short-term adenoma-regrowth clinical trials, however, are limited in their ability to measure whether chemopreventive agents inhibit early genotoxic events, abnormal cellular metabolic activities involved in tumor promotion over many years, or the progression of adenoma cells to carcinoma.

Key words: Calcium, chemoprevention, clinical trials, colon cancer

Colorectal cancer continues to be a major cause of tumor mortality in the United States and other countries. Despite attempts to improve screening of high-risk populations, the incidence of this disease is still very high; as a result, chemoprevention continues to be an important goal for the primary prevention of colorectal cancer. Among recent chemopreventive approaches, the administration of calcium and vitamin D are being evaluated in both preclinical and clinical studies. Many epidemiologic and experimental findings have indicated associations between high calcium and vitamin D intake and decreased risk for colorectal cancer.

EPIDEMIOLOGIC STUDIES

Most of the studies that have analyzed calcium intake and colon cancer incidence in human populations have shown an inverse relationship between the consumption of calcium and colon cancer risk (Table I) in both cohort and case-control studies [1–13]. Among these studies, a 19-year prospective study of 1,965 men in the Chicago area by Garland *et al.* [1] demonstrated a significant protective effect of dietary calcium

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and vitamin D_3 against colorectal cancer, with a daily intake of 1,200 mg or more of calcium associated with a 75% reduction of risk [1]. Slattery [9] also found that increased dietary calcium intake reduced the risk of colonic cancer, particularly in men. In a further review of the role of dietary calcium as a protective factor against colonic cancer, Sorenson [6] found general support for a protective effect, noting that calcium intake above 1,200 mg daily appeared to alter an individual's risk for developing the disease. An inverse geographic relationship between latitude and colon cancer incidence also has been noted; findings have suggested that increased exposure to sunlight reduced the risk of colorectal cancer presumably through mechanisms that involve vitamin D utilization and calcium [14]. A dietary intake of 150 I.U. or more of vitamin D was asso-

TABLE I. Epidemiologic Studies of Dietary Calcium and Colorectal Cancer Risk

A. Cohort Studies

Investigators	Calorie or Fat Adjustment	Relative Risk	Method of Evaluation	References
Garland	None	0.3	Quartile	1
Stemmerman	Fat	0.6	Tertile (Sigmoid)	2
Wu	None	0.9	Tertile	3
Rosen	None	0.94 (M) 0.84 (F)	Milk Intake	4
Bostick	Calories	0.5 (F)	Quintile (age adjusted)	5
Sorenson	None	Strong Trend	Intercountry Comparison	6

B. Case Control Studies

Investigators	Calorie or Fat Adjustment	Relative Risk	Method of Evaluation	References
Marquart-Moulin	Calories	0.7	Quartile	7
Lee	None	0.8	Tertile	8
Slattery	Calories	0.4 (M), 0.5 (F)	Quartile	9
Peters	Calories	0.4	Quintile	10
Kune	None	0.8	Quintile	11
Arbman	Calories	0.33		12
Phillips	None	0.3 (Odds Ratio)	Tertile	13
Heilbrun	Yes	0.7 to 1.4 (Odds	High-low	20
Tuyns	None	1.3 (Colon), 1.0	Quartile	15
Freudenheim	None	1.5 (M)	Quartile	16
		1.6 (F)	Tertile	
Benito	Calories	1.5	Quartile	17
Meyer	Calories	1.1 (M), 0.7 (F)	Quartile	18
Kampman	None	1.1 (M), 1.2 (F)	Quintile	19

ciated with a 50% reduction in the incidence of colorectal cancer. Other studies have not shown positive correlations between dietary calcium intake and colon cancer risk [15–20].

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 [21].

Among the numerous cell properties modulated by calcium, its participation in cell division and the regulation of cell proliferation and differentiation are particularly important [22]. Low levels of intracellular ionized calcium contribute to cell proliferation. Increasing calcium concen-

TABLE II. Effects of Supplemental Dietary Calcium on Proliferation and Differentiation of Colonic Epithelial Cells and on Chemical Carcinogenesis in Rodents [54]

Cell Type	Calcium Effect	References (by incorporation)
Colonic	Decreased hyperproliferation induced by deoxycholic acid	Wargovich *et al.*, 1983
	Decreased hyperproliferation induced by fatty acids	Wargovich *et al.*, 1984
	Decreased hyperproliferation induced by cholic acid	Bird *et al.*, 1986
	Decreased hyperproliferation induced by partial enteric resection	Appleton *et al.*, 1986
	Decreased deoxycholic acid-induced hyperproliferation (calcium effect blocked by phosphate)	Hu *et al.*, 1989
	Decreased MNNG-induced hyperproliferation on diet low in fat and calcium	Rashef *et al.*, 1990
	Decreased hyperproliferation induced by nutritional stress diet (low Ca^{+2}, vit. D; high fat, P)	Newmark *et al.*, 1991
	Decreased ODC and Tyr K induced by AOM	Arlow *et al.*, 1989
	Decreased ODC induced by bile acids	Baer *et al.*, 1989
	Decreased cholic acid-induced mortality	Cohen *et al.*, 1989
	Decreased tumor formation 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 AOM	Skrypec *et al.*, 1988
	Decreased colonic tumors induced by AOM	Wargovich *et al.*, 1990
	Decreased the number of invasive carcinomas after MNU and cholic acid	McSherry *et al.*, 1989
	Decreased the number of rats with multiple tumors after DMH	Sitrin *et al.*, 1991
	Unchanged tumor incidence after DMH	Karkara *et al.*, 1989
	Unchanged tumor incidence after DMH	Kaup *et al.*, 1989

tration in cell and organ culture media decreases cell proliferation and induces cell differentiation in rat esophageal epithelial cells [23], murine epidermal cells [24], mammary cells [25,26], and colon cells [27].

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* [28–30] and to induce the differentiation of human colonic cells [31], human myeloid leukemia cells [32], and other cell lines in vitro [33,34] A role for vitamin D as a chemopreventive agent has also been studied in rodent models [35–40] and the tumor growth and promotional stage of chemical carcinogenesis has been inhibited by vitamin D. On the other hand, vitamin D_3 enhanced chemically induced transformation of cultured cells *in vitro* [40,41] and promoted skin tumor formation in mice [42].

A hypothesis linking calcium with colon cancer risk [43] proposed that calcium inhibits the promoting role of high dietary fat by binding soluble fatty acids and bile acids in the colonic lumen, forming insoluble calcium complexes. Experiments with bile acids and fatty acids in

TABLE III. Effects of Supplemental Calcium on Proliferation and Differentiation of Colonic Cells in Human Subjects [48,54]

Cell Type	Calcium Effect	References (by incorporation)
	Dietary *in vivo*:	
Colonic	Decreased hyperproliferation	Lipkin *et al.*, 1985
	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
	Unchanged proliferation	Gregoire *et al.*, 1989
	Unchanged proliferation	Bostick *et al.*, 1993
	Increased proliferation	Cats *et al.*, 1990
	***In vitro*:**	
Colonic	Decreased proliferation (mM)	Buset *et al.*, 1986
	Decreased proliferation (2–4 mM)	Appleton *et al.*, 1988
	Decreased proliferation (2 mM)	Arlow *et al.*, 1988
	Decreased proliferation (2 mM)	Buset *et al.*, 1987
	Decreased proliferation (2 mM)	Friedman *et al.*, 1989
	Protected colonic cells against toxicity of bile acids and fatty acids (5 mM)	Buset *et al.*, 1989
	Increased histone acetylation: Cell differentiation (1–2 mM)	Boffa *et al.*, 1989

rodents (Table II) and in human cells (Table III) provide evidence to support this hypothesis, showing a reduction in bile-acid and fatty-acid toxicity on colonic mucosa following oral and *in vitro* calcium supplementation.

Synergistic interactions between vitamin D_3 and calcium have been shown, with vitamin D playing a permissive role for the expression of a chemopreventive function of calcium [44–46]. However, the combination of both supplemental calcium and vitamin D_3 at very high levels appeared to antagonize the inhibitory effect of each supplement alone on fat-promoted DMH-induced carcinogenesis in rodent colon [47]. Calcium and vitamin D may exert chemopreventive roles through different pathways: calcium binding bile acids and fatty acids and directly inhibiting colonic cell proliferation, and 1,25-dihydroxyvitamin D_3 inducing cell differentiation. Thus, if vitamin D_3 increased calcium absorption, it could inhibit calcium's protective effect in the colonic lumen, or supplemental calcium could inhibit the hydroxylation of the inactive form of vitamin D_3, thereby decreasing the concentration of the dihydroxy active differentiating form [47]. Apparently, achieving maximum colon cancer chemoprevention requires proper biologically balanced intake of calcium and vitamin D_3; large excesses may be counterproductive.

STUDIES OF CALCIUM IN RODENT MODELS

In animal models (Table II), oral calcium supplementation decreased colonic epithelial cell hyperproliferation when it was induced by several factors including bile acids and fatty acids, partial enteric resection, dietary fat, and a nutritional stress diet, all of which are known to increase cell proliferation.

Chemical carcinogenesis in rodents has also been modified by calcium intake. Most studies have shown decreased numbers of tumors induced, percent of invasive carcinomas, or numbers of animals with multiple tumors. On the other hand, several studies failed to show any calcium influence on DMH-induced carcinogenesis (Table II).

STUDIES OF CALCIUM SUPPLEMENTATION IN HUMAN SUBJECTS

Many studies in humans have shown the proliferative compartment of epithelial cells to increase in size in colonic crypts of humans at increased risk for colorectal cancer [48]. In most studies carried out, calcium supplementation was effective in decreasing epithelial cell hyperproliferation and correcting abnormal patterns of cell proliferation in colonic mucosa at increased risk for colorectal cancer (Table III); the proliferative compartment size was decreased when oral calcium intake was increased. Changes in cell proliferation were absent or less pronounced when initially low proliferation levels were present. Thus, in most of the human studies both *in vivo* and *in vitro*, increasing calcium intake slightly above the upper end of the current human RDA level decreased colonic epithelial cell proliferation. These studies now include five randomized clinical trials [49–52] in which hyperproliferation decreased in four of the randomized trials [49–51] after oral calcium supplementation. The randomized clinical trials in which supplemental dietary calcium decreased cell proliferation include measurements based on [^{3}H]dThd incorporated into proliferating cells [49]; BrdU incorporated into proliferating cells [50]; and measurements of mitotic figures in colonic crypt epithelial cells (crypt cell production rate) [51].

A recent NIH (June, 1994) conference on the adequacy of dietary calcium recommended increasing dietary calcium to 1,500 mg/day, higher than the current RDA for adolescents and several adult groups, to reduce the eventual risk of osteoporosis. Most of the epidemiologic, rodent and human studies of calcium intake also support this level as a possible means to reduce colon cancer risk. However, it was recommended that the relationship of calcium intake to colon cancer risk be further studied in clinical trials.

THE DEVELOPMENT OF CLINICAL TRIALS TO MEASURE ADENOMA RECURRENCE AND PROBLEMS TO BE CONSIDERED IN THESE TRIALS

Because of the findings noted above, further clinical trials are currently being planned and are underway to evaluate the possible chemopreventive efficacy of calcium and a variety of other putative chemopreventive agents. In clinical trials that measure biomarkers in flat colonic mucosa from abnormal cell proliferation to dysplasia, investigators are attempting to improve the standardization of the biomarkers to facilitate

their use in large studies [53]. In further clinical trials, investigators also are beginning to measure the effects of chemopreventive regimens on colonic adenoma recurrence; the latter clinical trials will attempt to measure whether the regrowth of adenomas is affected by the chemopreventive agent(s) being tested. Clinical trials of this type have great potential for evaluating the efficacy of chemopreventive agents; however, various limitations are now recognized in clinical adenoma trials and in interpreting their results.

A major problem in the design of a clinical adenoma trial is whether it is capable of actually measuring the activity and effect of the chemo-

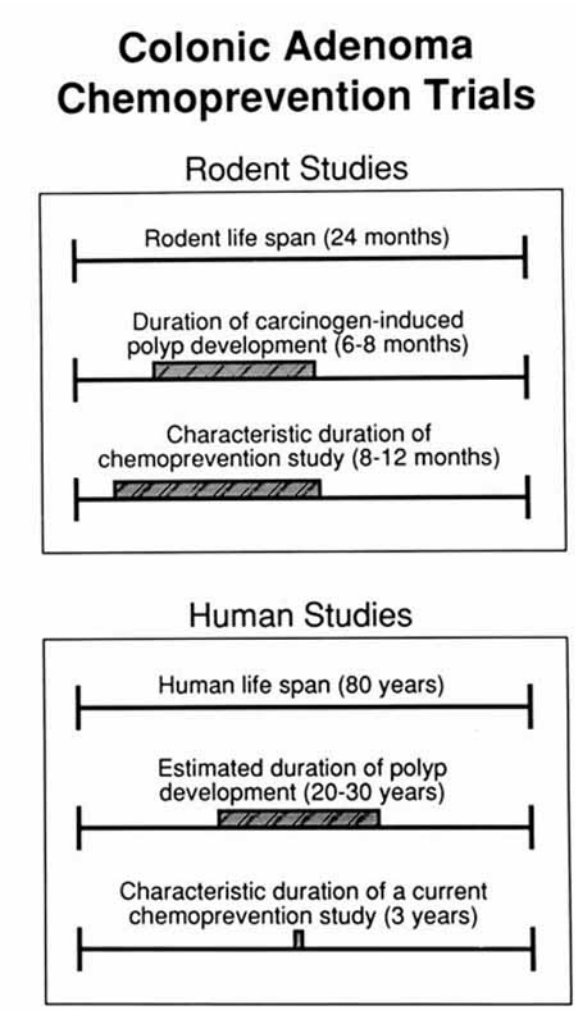

Fig. 1. Diagram of rodent life span, with the typical duration during which carcinogen-induced colonic tumors develop and during which chemoprevention studies are carried out. Previous chemoprevention studies in rodents have characteristically administered the agent to be tested over a large part of the rodent's life span, beginning at an early age.

In humans, adenomas develop over a long duration, evolving through multiple stages of abnormal cell development from normal cells to cells that progressively accumulate multiple genetic and metabolic defects involved in genotoxicity, and in the initiation, promotion and progression of the cells to tumors. However, current clinical adenoma trials are only able to measure the regrowth of small adenomatous tumors during a late stage of abnormal cell development as the transformed cells accumulate above the surface of the colonic mucosa.

preventive agent being tested. Colonic adenomas develop in the human colon over a 20–30 year duration, evolving and progressing through multiple stages of abnormal cell development from normal cells, to cells that progressively accumulate multiple genetic and metabolic abnormalities resulting from genotoxic events, to the initiation, promotion, and progression of these cells to tumors. Current clinical trials are now only able to measure the regrowth of small adenomatous tumors which arise from previously transformed adenomatous cells; currently those measurements can only be carried out during a short three- or four-year period, through a small window of observation that measures the late stage of regrowth of transformed cells (Fig. 1).

Current clinical adenoma trials thus do not measure whether a chemopreventive agent can prevent genotoxic events that occur in the early stages of abnormal cell development, nor whether the agent can inhibit metabolic abnormalities that develop over many years during early and mid-stages of adenoma development, nor during the progression of adenomas to carcinomas. Thus, since adenomas develop over decades, a clinical trial that briefly measures the regrowth of small adenomas over a few years can only measure the possible utility of a chemopreventive agent on late-stage events involved in the rapid, short-term regrowth of transformed adenoma cells.

Among the numerous classes of chemopreventive substances, naturally occurring compounds generally have weaker activities compared to pharmaceutical agents, but are generally safer to administer to large populations. Many naturally occurring substances such as calcium characteristically have their activity in cells that are normal or near-normal. Therefore, chemoprevention studies that use a 3- to 4-year window of observation of adenoma cell regrowth, measuring transformed cells accumulating above the mucosal surface, are likely to require potent chemopreventive agents with potentially higher levels of toxicity targeted at mechanisms affecting cells in later stages of abnormal development in order to achieve a rapid inhibitory effect on regrowth of the transformed adenoma cells. To design clinical trials that can accurately test the utility of diverse classes of chemopreventive agents, chemoprevention studies should incorporate into the clinical trial design the following information: (1) the specific stage of abnormal cell development that is being measured in the clinical trial; (2) whether or not the known activity of the agent, previously observed to have utility in preclinical studies, is properly assayed in cells during the stage of adenoma development investigated; and (3) whether this activity corresponds to mechanisms that are being measured in the proposed adenoma clinical trial.

Other errors in adenoma studies include an unfortunate miss-rate of adenomas occurring in examinations, and poor compliance that can occur after several years. Thus, in a single colon oscopy, 10% or more of small adenomas may not be detected. With multiple colonoscopies during a chemoprevention trial, a higher percentage of adenomas can remain undetected, thus introducing a further error into the analysis of results. These considerations, as well as others in large-scale clinical trials not addressed here, will need to be considered when planning and carrying out human adenoma trials with chemopreventive agents.

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