

EDITORIAL

Development of Clinical Chemoprevention Trials

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Measuring the effects of chemopreventive agents in human populations has now become one of the most important objectives of cancer research. The potential for inhibiting tumor development in targeted high-risk populations and in the general population has greatly increased in recent years. Accordingly, many new classes of chemopreventive agents are beginning to be studied in human subjects.

Among the earliest potential chemopreventive agents to be evaluated in human subjects is the naturally occurring, safe dietary component calcium, for its possible protective role against colon cancer. Many studies [reviewed in (1)] have now demonstrated inhibitory effects of calcium on colonic epithelial cell proliferation in both rodents and humans in vitro and in vivo, and oral calcium supplementation has reduced colonic tumor formation in rodents.

In this issue of the Journal, two groups of investigators, Bostick et al. (2) and Baron et al. (3) report the results of randomized clinical trials evaluating the effects of dietary calcium supplementation on colonic epithelial cell proliferation, one of the earliest intermediate end points that has been associated with colon cancer risk (4). Both studies were carried out on patients at increased risk of colon cancer because of previous occurrence of sporadic adenomas. In both studies, the overall proliferative whole-crypt labeling indices in the colon did not decrease after calcium administration. However, Bostick et al. found that calcium supplementation decreased the size of the proliferative compartment in the colonic crypts. Both of these measurements, an increased size of the proliferative compartment in colonic crypts, and increased whole-crypt labeling index have been previously found in human subjects at greater risk for colonic cancer in many studies (4). In human studies, an increased size of the proliferative compartment has occurred more frequently as a more sensitive indicator of subjects at increased colon cancer risk; in rodent studies, it has been the earliest and most sensitive proliferative indicator of increased risk following the administration of chemical carcinogens.

In attempting to interpret the reports of Bostick et al. (2) and Baron et al. (3) on the effects of calcium, several questions should be considered, including the following. (a) What have previous studies of calcium supplementation shown? (b) What are characteristics of the Bostick et al. and Baron et al. studies that might contribute to the differences found?

In studies carried out in rodent models, virtually all have demonstrated that oral calcium supplementation decreased excessive colonic cell proliferation, including the whole-crypt labeling index, the size of the proliferative compartment, and subsequent colonic tumor formation. These decreases occurred when excessive proliferation and tumor formation were induced by bile acids (5-8), fatty acids (9,10), partial enteric resection (11,12), western-style nutritional diets (13,14), and chemical carcinogens (15-19).

In studies of human colonic epithelial cells, direct in vitro exposure of the colonic cells to physiologic levels of calcium has uniformly decreased cell proliferation (20-25), and the decrease has similarly been demonstrated in vitro with exposures of normal cutaneous, esophageal, mammary, and other epithelial cells to physiologic levels of calcium (26-29).

When oral calcium supplementation has been given to human subjects in vivo, however, results have been less consistent, and thus far it has not been possible to carry out human studies as well controlled as the animal studies. The human clinical trials of oral calcium supplementation now include five randomized clinical trials in which one or both of the proliferative parameters noted above decreased after calcium administration compared with placebo administration (2,30-32) and other randomized clinical trials, where decreased cell proliferation has not been observed when compared with placebo treatment (3,33-36). Contributing to this variability are many methodologic problems that have now been recognized, and attempts are being made to improve the standardization of the populations studied and the methods with which the assay end points are measured (37).

Authors of previous human studies that did not demonstrate differences between calcium-treated and placebo-treated groups have discussed various difficulties that developed in their studies that might have affected their results, including the following (35,36). (a) Part of the study populations had low cell proliferation values at the beginning of the study, making it less

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likely that cell proliferation could decrease further below endogenous base-line levels. (b) Cell proliferation values in the placebo group were not level or uniform during the study but drifted toward lower endogenous base-line levels, making it less likely that a small effect of calcium could be detected. (c) Individuals in the placebo group might have increased their calcium intake during the study, contributing to the drift of proliferation values to lower levels. (d) Study subjects were ingesting high levels of calcium in their diets both before and during the study, decreasing the ability of calcium to further lower the end points measured [an effect that has been demonstrated to occur in animal models (38)]. (e) Some study subjects, including those taking placebos, had adenomas removed before the study began, which may lower cell proliferation (39), and other study subjects were developing recurrent adenomas that may increase cell proliferation.

With regard to the second question noted above, it appears that in the specific studies of Bostick et al. (2) and Baron et al. (3), major differences were present in study design and methodology. The Bostick et al. study was carried out in a single facility where rectal biopsy specimens were taken, without prior enema preparation, before and after calcium administration. The Baron et al. study was carried out in multiple facilities where biopsy specimens were taken during colonoscopy after multiple types of bowel-cleansing preparations and where the duration of the study period was variable.

Perhaps the major difference between the two studies reported in this issue of the Journal is that Bostick et al. studied base-line proliferation values at zero time, and also at three additional time points, to obtain data with which to evaluate any calcium intervention effect over time. The Baron et al. study only took biopsy specimens at the end of the study period and was not able to measure any change that might have occurred from the beginning to the end of the study. The Bostick et al. approach is a far more effective design with which to evaluate small changes that might occur during any chemopreventive intervention trial.

In the two studies (2) and (3), at the end of the study period the resting level of proliferating cells in the upper crypt differed markedly, including those in the placebo group. In the Baron et al. study, the upper crypt cell proliferation values were much lower than those of the Bostick et al. study, leaving less room for any further decrease to occur. Since the Baron et al. study was carried out after a resting period following enemas and the Bostick et al. study was carried out without enemas to perturb the mucosa, perhaps this difference contributed to the results observed. These various considerations indicate the advisability of continuing to attempt to standardize the methods of experimental procedure and the study populations participating in this type of clinical trial (37).

At the present time, further clinical trials also are being planned and are under way to evaluate the possible chemopreventive efficacy of calcium and a wide variety of other putative chemopreventive agents. Some of these trials have progressed to the stage of measuring the effects of various chemopreventive agents on the recurrence of colonic adenomas, attempting to measure whether the regrowth of adenomas is affected by the chemopreventive agents tested. Clinical trials of this type have

great potential for evaluating the efficacy of chemopreventive agents and, at first glance, would appear to be the best way to approach this topic. However, major limitations are now also known to be present in carrying out current human colonic adenoma trials and in interpreting their results.

A major problem in the design of the emerging clinical adenoma trials is whether they are capable of actually measuring the activities and the effects of the chemopreventive agents they are testing (40). Colonic adenomas develop in the colons of humans over a duration of 20-30 years, evolving and progressing through multiple stages of abnormal cell development: from normal cells to cells that progressively accumulate multiple genetic and metabolic abnormalities during the initiation, promotion, and progression of the cells to tumors. Current clinical adenoma trials are now only able to measure the regrowth of small adenomatous tumors that arise above the mucosal surface from previously transformed adenomatous cells, and measurements are made during a short 3- or 4-year period through a small window of observation that measures the late stage of regrowth of transformed cells.

Current clinical adenoma trials thus do not measure whether a chemopreventive agent can prevent genotoxic events occurring in the early stages of abnormal cell development. They also do not measure whether the agent can inhibit metabolic abnormalities developing over many years during early and mid stages of adenoma development or 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 with pharmaceutical agents but are generally safer to administer to large populations over long periods. 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 higher levels of potential toxicity to achieve the rapid late-stage adenoma inhibitory effect required (40).

To design clinical trials that can accurately test the many classes of chemopreventive agents now available, it will likely be necessary to carry out studies (a) of longer duration, (b) beginning earlier in life, and (c) that focus on the mechanisms and specific stage of abnormal cell development that preclinical studies show are modulated by the agent being tested. Thus, various questions arise in the development of clinical trials for evaluating the many types of chemopreventive agents now available. By addressing these questions and by designing clinical trials that are appropriate for testing the known activities of specific agents, progress can continue to be made in this field.

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