

Interaction of Calcium Supplementation and Nonsteroidal Anti-inflammatory Drugs and the Risk of Colorectal Adenomas

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Abstract

Background: Calcium and aspirin have both been found to be chemopreventive against colorectal neoplasia. However, the joint effect of the two agents has not been well investigated. **Methods:** To explore the separate and joint effects of calcium and aspirin/nonsteroidal anti-inflammatory drugs (NSAID), we used data from two large randomized clinical trials among patients with a recent history of colorectal adenomas. In the Calcium Polyp Prevention Study, 930 eligible subjects were randomized to receive placebo or 1,200 mg of elemental calcium daily for 4 years. In the Aspirin/Folate Polyp Prevention Study, 1,121 eligible subjects were assigned to take placebo, 81 mg of aspirin, or 325 mg of aspirin daily for 3 years. In each study, subjects completed a validated food frequency questionnaire at enrollment and were asked periodically about medications and supplements used. Recurrent adenomas and advanced adenomas were the end points considered. We used generalized linear models to assess the separate and combined effects of aspirin (or NSAIDs) and calcium supplementation (or dietary calcium) and the interactions between these exposures.

Results: In the Calcium Trial, subjects randomized to calcium who also were frequent users of NSAIDs had a reduction of risk for advanced adenomas of 65% [adjusted risk ratio (RR), 0.35; 95% confidence interval (95% CI), 0.13-0.96], and there was a highly significant statistical interaction between calcium treatment and frequent NSAID use ($P_{\text{interaction}} = 0.01$). Similarly, in the Aspirin Trial, 81 mg aspirin and calcium supplement use together conferred a risk reduction of 80% for advanced adenomas (adjusted RR, 0.20; 95% CI, 0.05-0.81); there was a borderline significant statistical interaction between the two treatments ($P_{\text{interaction}} = 0.09$). In this trial, we found similar trends when we considered baseline dietary calcium intake instead of calcium supplements. For all adenomas considered together, the interactive patterns were not consistent.

Conclusion: Data from two different randomized clinical trials suggest that calcium and NSAIDs may act synergistically to lower the risk of advanced colorectal neoplastic polyps. (Cancer Epidemiol Biomarkers Prev 2005; 14(10):2353-8)

Introduction

Human clinical trials have shown a protective effect of calcium supplementation on colorectal neoplasia (1, 2), and epidemiologic data are broadly in agreement (3-6). Even more extensive human data show that nonsteroidal anti-inflammatory drugs (NSAID) also reduce the risk of colorectal cancer and adenomas (7-10). Combinations of chemopreventive agents might be more effective than individual agents; however, there has been little research on the joint effects of calcium and NSAIDs.

To clarify this issue, we used data from two randomized controlled trials, which studied the chemopreventive effects of calcium and aspirin in the large bowel (2, 11). In each of the two studies, we conducted an analysis directed at the individual and joint effects of these interventions, taking advantage of the fact that in each analysis, one of these two factors had been assigned at random.

Materials and Methods

The design and findings of the two studies have been previously described (2, 11). These are two large, multi-

centered, randomized controlled trials of potential chemopreventive agents against recurrence of colorectal adenomas. In the Calcium Polyp Prevention Study, 930 subjects from six clinical centers were randomized to placebo or 1,200 mg of calcium (3 g of calcium carbonate) daily. In the Aspirin/Folate Polyp Prevention Study trial, 1,121 participants from nine clinical centers were randomized to placebo, low-dose aspirin (81 mg), or higher-dose aspirin (325 mg). Soon after the study began, the study was expanded to also investigate folate (1 mg versus placebo) in a 3×2 factorial design. The Human Subjects Committees at each participating institution approved the research, and all subjects provided written informed consent.

In both studies, eligible patients had a recent history of a histologically confirmed colorectal adenoma and had had complete colonoscopy within 3 months before enrollment with no known polyps left in the bowel. In the Calcium Trial, follow-up colonoscopies were scheduled 1 and 4 years after study entry; in the Aspirin Study, there was one follow-up examination about 3 years after randomization. The follow-up colonoscopy schedules were in accord with the recommended schedules in place at the time the studies were conducted. In addition to routine pathologic examination at the clinical centers, slides of each lesion removed from the bowel after randomization were sent to a study pathologist for uniform review. Information about medical history and lifestyle habits was recorded at enrollment, along with a semiquantitative food frequency questionnaire (12). In both trials, subjects periodically completed interval questionnaires about health problems and use of over-the-counter products and prescribed

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medications. In the Calcium Study, questionnaires were mailed every 6 months. Subjects were asked about their current use of medicines at the time they completed the questionnaire. Details regarding dose and frequency of use were not requested. There were no specific questions regarding use of NSAIDs or aspirin. In the Aspirin Study, interval questionnaires were submitted every 4 months. There were explicit questions regarding use of calcium supplements and vitamins and minerals during the previous 4 months, requesting detailed information regarding doses and frequency of use. Recruitment in the Calcium Trial began in 1988 and randomized treatment ended in 1996. For the Aspirin Trial, recruitment began in 1994 and randomized aspirin treatment ended in 2001.

Statistical Analysis. In the Calcium Trial, 832 subjects completed the follow-up colonoscopies. Of those, 825 provided interval information about use of aspirin and other NSAIDs and are included in the present analysis. In the Aspirin Trial, 1,084 subjects completed a follow-up colonoscopy. Of those, 1,080 provided interval information about use of calcium supplements and are included here. Only 6% of the subjects included from each trial (51 from the Calcium Trial and 66 from the Aspirin Trial) completed less than six questionnaires during the study period under analysis.

Our primary end point was adenoma recurrence during the 3-year on-treatment study period between years 1 and 4 of surveillance examinations in the Calcium Study and during the 3-year treatment period in the Aspirin Study. We also evaluated the occurrence of advanced lesions (as assessed by the study pathologist): tubulovillous adenomas (25-74% villous component), villous adenomas ($\geq 75\%$ villous), advanced dysplasia or invasive cancer, and large adenomas (at least 1 cm in diameter).

About 62% of subjects in the Calcium Trial reported taking NSAIDs (including aspirin) during the main study period and about 30% regularly (reported in more than half of the interval questionnaires). Given the lack of information regarding dose or frequency of use, we quantified NSAID or aspirin use by the percentage of interval questionnaires reporting use: non-users (no questionnaires indicating use), sporadic users ($\leq 50\%$ indicating use), and frequent users ($>50\%$ indicating use). As we did not expect sporadic NSAID or aspirin use to modify the effects of calcium supplementation on adenoma recurrence (13), we assessed the joint and separate effects of calcium treatment and frequent use of NSAIDs or aspirin, using placebo/non-frequent users as a reference. We also assessed modification of the protective calcium effect by frequent use of NSAIDs or aspirin. This was a stratified analysis, with relative risks for calcium treatment computed separately among frequent NSAID users and non-frequent users. The same approach was applied for aspirin use only.

In the Aspirin Study, we found a chemoprotective effect of 81 mg of aspirin but not for 325 mg. Therefore, we focused the analysis on the separate and joint effects of calcium supplement use and randomization to 81 mg of aspirin. Only 25% of participants reported any use of calcium supplements during the trial; around 15% reported use in $>50\%$ of interval questionnaires. Given this low rate, we simply defined a dichotomous variable for calcium use: ever users of calcium supplements (one or more questionnaires indicating use) and nonusers. The analyses were similar to those for the Calcium Study, following the same methodology to explore the joint effects and separate effects of low-dose aspirin and use of calcium supplements. We also assessed the joint and combined effects of 81 mg of aspirin use with baseline dietary calcium intake. In this analysis, we computed the residual of the regression of the log of calcium intake on the log of caloric intake and coded baseline intake as a binary variable for levels above and below the median of the

residuals. Finally, we replicated all these analyses for 325 mg of aspirin. All the regression models included a covariate for assignment to the aspirin dose that was not the main exposure in that model.

Overdispersed generalized linear models for the Poisson distribution as an approximation to the binomial family were used to compute relative risks of at least one adenoma. Covariates were age, sex, center, duration of follow-up, and number of adenomas before study entry. Because of the low number of end points, computed crude estimates might be preferred for advanced adenomas. However, unadjusted and adjusted findings were very similar, and for consistency with the presentation for all adenomas, the latter are presented in the tables. Models for aspirin treatment and dietary calcium also included the log of caloric intake as covariate. Interactions were evaluated using product interaction terms between treatment assignment and the use of NSAIDs or aspirin in the Calcium Study and the use of calcium supplements or baseline dietary calcium intake in the Aspirin Study. Wald tests were used to test the significance of these terms. Results did not change when we restricted our analysis to subjects with at least six completed interval questionnaires (94% in each trial).

Results

Baseline characteristics of the subjects are summarized in Table 1. Treatment groups in each trial did not differ in demographic characteristics, history of adenomas (2, 11), baseline use of calcium supplements and NSAIDs, or baseline dietary calcium intake. In the Calcium Study, 30% of subjects reported taking NSAIDs in the year before study entry; in the Aspirin Trial, this proportion was 80%. Use of calcium supplements in the year before study entry was 3% and 14%, respectively. Baseline dietary calcium intake was significantly higher in the Calcium Study, with an average intake of 879 mg versus an average of 756 mg in the Aspirin Trial.

About 62% of subjects in each treatment arm of the Calcium Study reported use of NSAIDs at some time after randomization. Similarly, post-randomization use of calcium supplements was equally common among the three treatment arms in the Aspirin Study; however, $<30\%$ of subjects reported use (data not shown).

Calcium/NSAID Interaction in the Calcium Polyp Prevention Study. In the Calcium Trial, frequent NSAID use alone (i.e., in the placebo group) was associated with essentially no reduction in risk of adenomas, and randomized calcium treatment alone conferred a modest reduction in risk (Table 2). Calcium subjects who used NSAIDs frequently experienced a significant 29% reduction of risk [adjusted risk ratio (RR), 0.71; 95% confidence interval (95% CI), 0.52-0.98], but this was not statistically different from the reduction in risk among subjects with only one exposure ($P_{\text{interaction}} = 0.96$). However, for advanced adenomas, a marked pattern emerged. Calcium alone and frequent NSAID use alone each conferred no reduction in risk, but both exposures together conferred a $>60\%$ reduction (adjusted RR, 0.35; 95% CI, 0.13-0.96), and this was statistically different from having either exposure alone ($P_{\text{interaction}} = 0.01$). These patterns were broadly similar when aspirin was considered instead of all NSAIDs, although the differences were somewhat less marked (Table 2).

Underlying this pattern was clear evidence that NSAIDs modified the effect of calcium supplementation. Among frequent NSAID users, calcium conferred a striking reduction in risk of advanced adenomas (adjusted RR, 0.26; 95% CI, 0.08-0.81), whereas among those who did not use NSAIDs frequently, there was no calcium effect (adjusted RR, 1.05; 95% CI, 0.64-1.71; $P_{\text{interaction}} = 0.01$). There was a similar interaction with aspirin use (data not shown).

Table 1. Baseline characteristics of the participants in the Calcium Polyp Prevention Study and the Aspirin Folate Polyp Prevention Trial

Baseline characteristics	Calcium trial (n = 825)	Aspirin trial (n = 1,080)
Age* (y)	60.8 (8.9)	57.5 (9.6)
Male sex, n (%)	594 (72.0)	687 (63.6)
Race, n (%)		
White	703 (85.2)	925 (85.7)
African American	65 (7.9)	62 (5.7)
Hispanic	25 (3.0)	60 (5.6)
Other	32 (3.9) [†]	33 (3.1) [†]
No. lifetime adenomas before study entry*	2.5 (2.7) [†]	2.4 (2.2) [†]
No. subjects reporting use of NSAIDs [‡] (%)	249 (30.2)	861 (79.7)
No. subjects reporting use of calcium supplements [‡]	24 (2.7)	151 (14.0)
Baseline dietary calcium intake* (mg)	876.6 (434.7) [§]	756.4 (423.2) [§]

*Mean (SD).

[†]Four subjects have missing information.[‡]Percentage of subjects that reported use of NSAIDs/calcium in the baseline questionnaire (before randomization).[§]Twenty subjects have missing information in the Calcium Trial and 48 in the Aspirin Trial.

Calcium/Aspirin Interaction in the Aspirin/Folate Polyp Prevention Study. In the Aspirin Trial, use of calcium supplements alone (i.e., in the placebo group) did not affect the risk of all adenomas and there was a nonsignificant, 12% reduction in risk from 81 mg of aspirin alone. In subjects with both exposures, there was a 31% reduction in risk in comparison with those with neither (adjusted RR, 0.69; 95% CI, 0.50-0.96; $P_{\text{interaction}} = 0.17$; Table 3). As in the Calcium Trial, results were more striking for advanced adenomas. Aspirin (81 mg) and use of calcium supplements together brought an 80% reduction in risk (adjusted RR, 0.20; 95% CI, 0.05-0.81), and the interaction between low dose of aspirin and calcium supplements use was borderline significant ($P_{\text{interaction}} = 0.09$).

There was corresponding evidence that calcium modified the effect of 81 mg of aspirin on advanced adenomas. Among subjects who did not take calcium supplements, the RR for 81 mg of aspirin was 0.69 (95% CI, 0.43-1.11); whereas among those who did use calcium, it was 0.21 (95% CI, 0.05-0.87;

$P_{\text{interaction}} = 0.09$). Calcium did not alter the null effects of 325 mg of aspirin (data not shown).

There were suggestions that dietary calcium also modified the effect of 81 mg of aspirin on risk of advanced adenomas. Subjects randomized to 81 mg of aspirin who also had a high calcium intake (levels above the median of the residuals) had a 67% reduction in risk of advanced adenomas (adjusted RR, 0.33; 95% CI, 0.16-0.68; Table 3). Although the aspirin effect was greater among subjects with higher calcium intake, the interaction was compatible with chance ($P_{\text{interaction}} = 0.18$).

Discussion

Our analyses of two chemoprevention trials involved both observational and randomized exposures. In both trials, there were clear indications that calcium and NSAIDs act together to reduce risk of advanced colorectal adenomas, yielding a reduction in risk of >60% with the combination of these agents. Results for all adenomas followed the same trend but were not as striking.

There has been relatively little investigation of the potential interaction between calcium supplementation and NSAIDs. Experimental studies in rodents have not been clear on this point (14, 15), although in one of the studies, there was a suggestion of a negative interaction (15). To date, only one observational study (16) has addressed this issue. In contrast to our findings, the authors found a significant protective effect of total calcium intake only among nonusers of NSAIDs. Associations with advanced adenomas were not reported.

Anticarcinogenic effects of calcium in the large bowel have been consistently found in experimental studies (17-20), and the association of calcium with a reduced risk of colorectal neoplasia is supported by many but not all (21-24), epidemiologic studies (5, 6, 25, 26), and two clinical trials (1, 2). It has been hypothesized that calcium protects the colorectal mucosa by binding and precipitating soluble fatty acids and bile acids, preventing irritation and inflammation of the mucosa (20, 27-32). Recent studies also suggest a direct effect of extracellular calcium on cellular proliferation and differentiation through the calcium sensing receptor (18, 28, 33-35). The anticarcinogenic properties of NSAIDs in the colorectum are well documented by experimental studies (36-41), epidemiologic investigations (7, 8, 42-46), and randomized trials (11, 47-49).

Table 2. Relative risk for calcium supplementation by NSAID use in the Calcium Polyp Prevention Study

	N_{total}	All adenomas		Advanced adenomas	
		Cases (n)	Adjusted relative risk* (95% CI)	Cases (n)	Adjusted relative risk* (95% CI)
Overall effect of calcium treatment					
Placebo	423	159	1.00 (reference)	44	1.00 (reference)
Calcium	409	127	0.81 (0.67-0.99)	32	0.72 (0.47-1.11)
Effects of calcium treatment and frequent NSAID use [†]					
Calcium treatment					
Frequent NSAID use					
Placebo	293	114	1.00 (reference)	27	1.00 (reference)
Placebo	126	43	0.90 (0.67-1.19)	17	1.53 (0.86-2.73)
Calcium	283	90	0.80 (0.64-1.00)	28	1.03 (0.62-1.70)
Calcium	123	34	0.71 (0.52-0.98)	4	0.35 (0.13-0.96)
$P_{\text{interaction}}$			0.96		0.01
Effects of calcium treatment and frequent aspirin use [†]					
Calcium treatment					
Frequent aspirin use					
Placebo	329	123	1.00 (reference)	31	1.00 (reference)
Placebo	90	34	0.98 (0.71-1.34)	13	1.42 (0.77-2.64)
Calcium	99	99	0.79 (0.63-0.98)	29	0.87 (0.54-1.42)
Calcium	25	25	0.85 (0.60-1.23)	3	0.40 (0.13-1.23)
$P_{\text{interaction}}$			0.67		0.08

*RRs adjusted for age, sex, center, interval of follow-up and number of lifetime adenomas before study entry.

[†]NSAID and aspirin use defined by the percentage of interval questionnaires reporting use.[‡] P for multiplicative interaction between treatment assignment and frequent use of NSAIDs/aspirin (Wald test).

Table 3. Relative risk for aspirin by calcium supplements use and baseline dietary calcium intake in the Aspirin/Folate Polyp Prevention Study

	<i>N</i> _{total}	All adenomas		Advanced adenomas	
		Cases (<i>n</i>)	Adjusted relative risk* (95% CI)	Cases (<i>n</i>)	Adjusted relative risk* (95% CI)
Overall effect of aspirin treatment					
Placebo	363	171	1.00 (reference)	47	1.00 (reference)
ASA 81	366	140	0.83 (0.70-0.98)	28	0.58 (0.37-0.90)
ASA 325	355	160	0.95 (0.80-1.12)	38	0.83 (0.55-1.23)
Effects of aspirin treatment and calcium supplement use ^{†,‡}					
Aspirin treatment		Calcium supplement use			
Placebo		No	528	247	1.00 (reference)
Placebo		Yes	187	83	1.01 (0.82-1.25)
ASA 81		No	275	114	0.88 (0.73-1.06)
ASA 81		Yes	90	26	0.69 (0.50-0.96)
<i>P</i> _{interaction} ASA 81/calcium supplements [§]			0.17		0.09
Effects of aspirin treatment and baseline dietary calcium intake ^{,‡}					
Aspirin treatment		Dietary calcium			
Placebo		Low	346	172	1.00 (reference)
Placebo		High	341	148	0.88 (0.75-1.05)
ASA 81		Low	175	70	0.82 (0.65-1.03)
ASA 81		High	174	62	0.71 (0.56-0.90)
<i>P</i> _{interaction} ASA 81/baseline intake of dietary calcium [§]			0.62		0.18

*RRs adjusted for aspirin dose, age, sex, center, interval of follow-up, and number of lifetime adenomas before study entry.

[†]Calcium use defined as any interval questionnaire reporting calcium supplement use.

[‡]Aspirin (325 mg) was not associated with a reduced risk of colorectal neoplasia overall, or in conjunction with either calcium supplementation or high baseline dietary calcium intake. For simplicity, these detailed analyses for aspirin (325 mg) and calcium are not included in the table.

[§]*P* for multiplicative interaction between aspirin (81 mg) and calcium supplements use/baseline dietary calcium at the median (Wald test).

^{||}Median based on log-log residuals from regression of dietary calcium intake on caloric intake; RRs also adjusted for log-calories. Cut point for dietary calcium intake for the median of the log-log residuals (assuming 2,000 kcal intake): 676.4 mg. Forty-eight subjects with a follow-up colonoscopy did not have dietary information and are not included in this analysis.

Inhibition of cyclooxygenase and mechanisms independent of this enzyme seem involved (13, 38, 50-55). The mechanisms underlying a synergy between the two agents have not been studied experimentally and are not clear.

We cannot explain why our results for advanced adenomas and all adenomas differ. In both the Calcium and the Aspirin Studies, the overall effect of the agents was stronger for advanced adenomas, suggesting that larger, villous, and severely dysplastic adenomas differ in some fundamental way from their smaller, tubular, and moderately dysplastic counterparts, or that the agents work on later stages of the pathway from adenoma to carcinoma (11). In this regard, several studies have found differences in the extent to which early adenomas, advanced adenomas, and carcinomas are associated with various lifestyle factors (56-58). However, the mechanisms underlying these findings remain unknown. Experimental studies regarding cellular growth and apoptosis have not consistently reported differences between early adenomas and advanced adenomas (59-65). There are suggestions that advanced adenomas have more genetic changes than the earlier lesions, but differences in the expression and mutation status of p53, bcl-2, K-ras, and others have also been inconsistent (66-68). Cyclooxygenase-2 expression may be another relevant difference between early and more advanced adenomas, as it seems more extensive in large and villous polyps (69-71), and levels of cyclooxygenase-2 protein increase with degree of dysplasia (69, 72, 73). These factors could explain a greater sensitivity of advanced adenomas to aspirin and/or calcium, but they do not explain a positive interaction between the two factors specifically for advanced lesions, and we cannot give a convincing explanation for the differences seen in our findings for advanced and nonadvanced adenomas.

Three advantages of our analyses include the large sample size, the randomization of one factor in each interaction

analysis, and the separate consideration of advanced adenomas. These factors could easily explain the difference between our findings and the previous epidemiologic study on the topic. However, there was an observational element in each of our analyses as well, and the statistical interactions we observed did not consistently achieve statistical significance. Moreover, these were all secondary analyses for our studies, and as in all analyses of interactions, modeling assumptions may be important. Furthermore, we did not have information about doses of NSAIDs (in the Calcium Study) or of calcium (in the Aspirin Study). In the latter study, use was too infrequent to allow us to incorporate frequency of use into the analysis. Nevertheless, the similar results obtained in the Aspirin Trial when we grouped the subjects by baseline dietary calcium intake support our approach.

Overall, in this study, we found suggestions of a synergistic effect between calcium supplementation and NSAIDs for the reduction of risk of advanced colorectal adenomas. Obviously, this possibility needs confirmation before clinical recommendations can be made. The attenuated results for smaller tubular adenomas suggest that investigation into the biological and molecular differences between these early lesions and more advanced adenomas would be valuable. However, our findings, if confirmed, would indicate a way to greatly increase the effectiveness of colorectal chemoprevention.

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