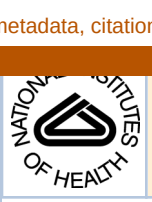

Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2011 April ; 20(4): 679–682. doi:10.1158/1055-9965.EPI-10-1135.

Serum Salicylate Levels and Risk of Recurrent of Colorectal Adenomas

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Abstract

Background—Intake of aspirin is associated with reduction in risk of colorectal adenoma and carcinoma. Some plants contain salicylates, and individuals not taking aspirin may have measurable salicylate levels. However, the association between serum salicylate level and recurrence of adenoma in non-users of aspirin has not been studied.

Methods—We measured serum salicylate levels in participants in a randomized controlled trial with calcium supplementation for the prevention of colorectal adenomas. Generalized linear models were used to assess the association between serum levels and adenoma risk during the follow-up period of the trial.

Results—We did not find an association with recurrence of adenomas or advanced adenomas with serum salicylate levels at year 1 among non-users of aspirin. There was no effect modification of the chemopreventive effect of calcium supplementation in reducing risk of recurrent adenomas or advanced adenomas.

Conclusions—Among non-users of ASA, serum salicylate levels are not associated with risk of recurrence of adenomas.

Impact—Serum salicylate levels can be detected in individuals not taking aspirin, but the levels may be too low to confer protection from risk of recurrent adenomas.

Introduction

Aspirin use has been shown to reduce the risk of colorectal neoplasia in randomized controlled trials and cohort studies (1-6), presumably through inhibition of COX-1 and COX-2 (7, 8) but also through other non-COX mechanisms (9-11). Salicylic acid (SA), the deacetylated metabolite of aspirin (acetylsalicylic acid; ASA), is readily detected in serum and urine of humans and is a measure of salicylates, whether from ASA use or from diet and endogenous sources. Salicylate levels are also measurable in individuals not taking aspirin or salicylate medications (9, 11-14). Diet is known to be a source of salicylates, including fruits, vegetables and meat. The salicylate content of blood and urine was shown to increase

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following consumption of a variety of meals, both vegetarian and meat-based, ranging from southern Indian dishes to European ones (15, 16).

In plants, SA is synthesized through metabolism of benzoic acid. A synthetic form of benzoic acid, sodium benzoate, is a widely used a food preservative, and also contributes to SA levels after ingestion. In individuals not taking aspirin, it is estimated that 20% of the variability in serum SA may derive be from dietary sources (17). Understanding various endogenous sources of salicylates is an ongoing area of research (18, 19). These include certain colonic bacteria species that may have the ability to synthesize SA, and synthesis of SA via metabolism of hippuric acid, a metabolite of benzoic acid demonstrated to be formed endogenously in man (18) The associations of serum salicylate levels, in particular among non-users of aspirin, in reducing risk of colorectal adenoma are not known.

The aim of our study was to determine whether serum salicylate levels among individuals with history of prior adenoma are associated with risk of colorectal adenoma in individuals not taking aspirin

Methods

We performed a secondary observational analysis on data from Calcium Polyp Prevention Study, a randomized controlled clinical trial of supplemental calcium for the prevention of colorectal adenomas. The design and findings of the trial have been previously described (20, 21). In the trial, 930 subjects from six clinical centers, all with at least one histologically confirmed colorectal adenoma and no known polyps remaining in the large bowel, were randomized to receive placebo or 1200 mg elemental calcium as 3 grams of calcium carbonate for 4 years. Recruitment for the trial began in 1988 and randomized treatment ended in 1996.All subjects completed several baseline questionnaires, including a food frequency questionnaire. Intakes of aspirin or other NSAIDs were not restricted. Although reports of Aspirin (ASA) use were collected, the specific dose and frequency was not recorded. About one year after randomization all subjects underwent blood draws and serum salicylic acid levels were measured as described below. All subjects underwent colonoscopy at year 1 and 4 after randomization. While on treatment, questionnaires were sent to participants every 6 months regarding adherence to study treatment and use of medications, over the counter drugs and nutritional supplements.

Serum SA measurements were performed on stored serum samples collected at the year 1 blood draw using a highly sensitive HPLC method described previously (9, 22). All measurements were performed blindly at the same laboratory facility. ASA use during treatment was summarized by percentage of interval questionnaires reporting use. Individuals were considered non-users if none of the questionnaires completed before/up to the time of blood draw at year 1 reported ASA use AND there was no reported use, or reported use in 25% or fewer of questionnaires, between year 1 and year 4. Subjects who reported ASA in more than 25% of questionnaires after the blood draw were considered ASA users.

Statistical Analysis

Of the 930 randomized subjects, 832 completed follow-up colonoscopies between year1 and year 4 of the trial. Eight hundred and twenty five subjects provided information on ASA use in periodic questionnaires. Adequate samples for SA measurements were available for 691 of these subjects, of which 459 were non-ASA users and were included in the analysis. To assess selection bias, we compared individuals with and without specimens available with regard to differences in baseline or outcome variables. All analysis was carried out using STATA version 11.0 (College Station, TX)

The primary endpoint of the study was adenoma recurrence after the year 1 examination up to and including the year 4 exam. We also evaluated occurrence of advanced lesions, defined as any lesion with the following features: tubulovillous components (25-74% villous), villous adenoma (≥ 75% villous), advanced dysplasia or invasive cancer, and large adenomas (≥1cm in diameter).

We evaluated the association of serum SA with risk of recurrent adenoma and advanced lesions among non-users of ASA during the main risk period, and also conducted analyses stratified by calcium treatment assignment. We evaluated the interaction between calcium intake and serum SA level on risk of recurrent adenomas and advanced lesions using standardized serum SA values as a continuous variable (SA/sd(SA)), as a 4-level variable based on quartiles, and as a dichotomous variable above versus below the median. We used generalized linear models for binomial distribution with a log link function to compute risk ratios, adjusted for potential confounders. Variables considered in the model included age, sex, center, follow-up time and treatment group. Interactions were assessed using product interaction terms between treatment assignment and serum SA levels at year 1. Wald's tests were used to assess the statistical significance of association of serum SA and adenoma risk

Results

Baseline characteristics of the trial participants included in the analysis are summarized in Table 1. Treatment groups were similar in demographic characteristics. Of the individuals with adequate samples for SA measurement, 459 (61%) were non-users of aspirin. Overall, the median SA level at that time was 1.05 μmol/L (IQR 0.21 – 2.21 μmol/L).

In generalized regression models, among non-users of ASA, there was no association between serum SA and risk of adenoma or advanced adenoma over the next three years in crude or adjusted models (adjusted RR per standard deviation = 1.01 (95% CI 0.97-1.04) and 1.05 (95% CI 1.00-1.10) for all adenomas and advanced adenomas. Results were unchanged when serum SA was entered in the model as a 4-level variable based on quartiles (Table 2), However the point estimates associated with quartile 2 and higher suggest that there may be a threshold protective effect. There was no interaction between calcium supplementation and serum SA levels on risk of adenoma or advanced adenoma (p for interaction 0.60 and 0.58 for all adenomas and advanced adenomas respectively).

Discussion

Our study is the first effort to evaluate the relationship between serum salicylate levels and risk of recurrent adenoma. In this regard we had several notable findings. First, we are able to confirm that salicylic acid levels are detectable in human serum, in individuals with little or no reported regular intake of ASA-containing compounds. This is consistent with growing literature on dietary and endogenous sources of SA (9, 12, 22). Benzoic acid is a natural constituent of plants and found in large amounts in fruits such as berries. (17) Patterson et al have reported the conversion of benzoic acid to SA in humans (12). One of the main metabolites of benzoic acid, hippuric acid, which is metabolized to salicylate, may be also formed endogenously in man (18). Several factors likely contribute to serum SA levels, such as differences in dietary intakes of fruits, vegetables, benzoic acid, and red meats, variation in endogenous cellular production through metabolism of hippuric acid, or from de novo synthesis by colonic bacteria. There is evidence that the SA content of certain food dishes, such as vindaloo curry is comparable to low dose (75 mg) aspirin (11). Other factors such as genetic factors, polymorphisms affecting enzyme activities, and the interaction of genetic, dietary and environmental factors are currently being investigated. Recent evidence suggests that chronic consumption of low dose aspirin (75 mg) reduces

long-term colorectal cancer incidence and mortality (23), Whether comparable chemoprotection can be achieved from SA derived from non-ASA sources is an important question. We found no association between serum SA and risk of adenomas or advanced adenomas on colonoscopy performed 3 years later (or in a few instances in the interval between), and no modification by SA level of the effect of calcium in reducing risk of all recurrent adenomas or advanced adenomas among non-users of ASA. The findings of a possible threshold effect when quartiles of serum SA were used in the model should be interpreted with caution, particularly since we did not see similar trend for advanced adenoma. We chose not to specifically test for the threshold effect, because post hoc nature of the test would invalidate it.

There is ample literature on ASA intake and reduction in risk of adenomas and colorectal cancer from observational studies (5, 24) and ASA supplementation as a chemopreventive agent for adenomas and advanced adenomas from randomized clinical trials (2, 6). However, in the absence of ASA use, our results suggest that serum SA levels, derived from dietary and endogenous sources may be insufficient to confer protection from risk of subsequent adenomas, or to demonstrate an interaction with calcium supplementation.

Our study has several limitations. There is a possibility of type II error, as the the risk estimates do suggest a threshold effect. Serum SA levels were not available in all 930 subjects, and the 691 subjects included in this analysis, although they did not differ in baseline or outcomes variables of interest, they may differ in other characteristics that could affect adenoma risk. We had no information regarding aspirin use specifically in the day before the blood draw, and it is possible some subjects thought to be off aspirin had actually taken the drug. We measured serum SA onetime only, although the literature supports that the measurements are reproducible (12). We measured SA in serum only, whereas the literature reports measurement of urinary salicylate levels. Urine salicylate levels may be a more useful measure of the body's SA exposure than serum SA because they reflect use over a longer time period. We did not have information on the timing of the blood draws in relation to the participants' last meals, which may influence serum SA levels. Strengths of our study include large sample size, randomization of calcium supplementation and separate analysis of advanced adenomas.

Our data suggest a lack of an association of risk of adenoma occurrence with serum SA among non-users of ASA. Further research would be desirable to confirm this, ideally with prospective, long-term designs and careful correlation of serum levels with proximate measurement of ASA intake.

Acknowledgments

Supported in part by grants:

NCI (CA37287 and CA23108) And VA Minneapolis Career Development Award (CDA-2) (A.S.)

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Table 1

Baseline characteristics of study participants (N= 459)

Baseline Characteristics	Calcium group (n=228)	Placebo group (n=231)
Mean Age in years ±SD	59.8 ±9.1	59.7±9.4
Male sex (%)	164 (71.9%)	149 (64.5%)
Race, n (%)		
White	185 (81.1%)	199 (86.2%)
African American	21 (9.23%)	17 (7.4%)
Hispanic	9 (4.0%)	6 (2.6%)
Other	13 (5.7%)	9 (3.9%)
Median serum salicylic acid level at year 1 (mmol/L)	0.86	0.87

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2013 July 11.

Table 2

Relative risk for all adenomas and advanced adenomas by quartiles of baseline serum SA levels and by standard deviation in serum SA, adjusted for age, sex, center, treatment group and follow-up time among non-users of ASA (n=459):

	N	All adenomas		Advanced adenomas	
		Cases (n)	Adjusted Relative Risk (95% CI)	Cases (n)	Adjusted Relative Risk (95% CI)
Serum SA quartile					
1	125	52	1.00 (reference)	12	1.00 (reference)
2	117	40	0.77 (0.55-1.08)	10	0.85 (0.38-1.88)
3	114	40	0.79 (0.56-1.12)	16	1.14 (0.54-2.39)
4	103	33	0.79 (0.54-1.14)	8	0.73 (0.30-1.77)
Effect of change of 1 SD in serum SA					
Overall			1.01 (0.97-1.04) *		1.05 (1.00-1.10) *
placebo group #			1.01 (0.98-1.04)		1.05 (1.01-1.10)
calcium group #			0.97 (0.87-1.09)		1.00 (0.85-1.18)

Cut-off values for Serum SA quartiles: 1: 0.001-0.21mmol/L, 2: 0.211-0.99mmol/L, 3: 1.00-2.18 mmol/L, 4: >2.18 mmol/L

* p trend all adenomas 0.245, advanced adenomas 0.649;

p for interaction for calcium with serum SA= 0.60 for adenoma, 0.58 for advanced adenoma