Short Communication

Fecal and Rectal Mucosal Diacylglycerol Concentrations and Epithelial Proliferative Kinetics[1]

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Abstract

Fecal diacylglycerol (DAG) concentrations have been suggested as biomarkers for colonic neoplasia because of their potential to be absorbed in the colon and to stimulate epithelial cell proliferation. The interrelationships among nutrient intake, fecal and mucosal DAG, and colonic proliferative markers have not previously been studied. We designed a pilot study to evaluate the feasibility of evaluating these interrelationships in 12 volunteers who had a history of colonic adenomatous polyposis. Total mucosal DAG concentrations were not related to fecal DAG concentrations, but mucosal DAG correlated inversely with the whole crypt labeling index. Dietary intake did not alter fecal DAG concentrations. However, the percentage of calories from dietary fat correlated positively with the whole crypt labeling index. Fiber and calcium intake showed a positive correlation with the labeling index in the upper 40% of the crypt. The present pilot study failed to demonstrate a correlation between dietary components and fecal and total mucosal DAG. Additional studies relating fecal DAG with mucosal proliferation will require the evaluation of DAG concentrations in subcellular compartments of mucosal cells and/or measurement of fecal DAG fatty acid composition.

Introduction

DAGs,[3] released by the hydrolysis of cellular membrane phospholipids under the influence of growth factors (1), are known to stimulate cellular proliferation in colon cancer cells (2) and rabbit colon *in vivo* (3). DAGs are found in colonic contents and the feces of normal humans in a wide range of concentrations (4). These DAGs have been shown to be formed by specific fecal bacteria (4). Luminal DAGs can be taken up by the colon mucosa (5) and have been postulated to stimulate colonic cellular proliferation. Thus, colonic and fecal DAG may be an important lipid component contributing to colonic neoplasia (6) and may also be a biomarker of risk for neoplasia.

The present pilot study was designed to evaluate whether the concentration of DAG in the feces correlated with rectal mucosal DAG concentrations and indices of rectal cell proliferation *in vivo*. The purpose of this study was to test the hypothesis that DAGs in the colonic lumen stimulate the rate of mucosal epithelial cell proliferation and contribute to shifting the position of proliferating cells in the colonic crypt toward the luminal surface, both biomarkers associated with neoplastic risk (7). In addition, several nutritional risk factors have been implicated in the etiology of colon cancer: total energy; fat and protein intake or percentage of calories; and dietary fiber, calcium, and/or vitamin D. Relationships among these dietary components, fecal and mucosal DAG, and rectal proliferative indices were also studied.

Materials and Methods

Twelve consecutive volunteers were studied undergoing rectal biopsies for baseline studies of proliferative kinetics before randomization for a chemoprevention study. Volunteers ranged from 59–84 years of age (mean age ± SE, 65.6 ± 2.0). Three were women, and 9 were men, and all had a previous history of colonic adenomas. Subjects reporting current chronic use of aspirin and nonsteroidal anti-inflammatory agents were excluded. Also excluded were subjects with chronic gastrointestinal disorders and gastrointestinal surgery other than appendectomy. The dietary total energy intake and daily consumption of fat, protein, carbohydrate, fiber, calcium, and alcohol were determined from 3-day food diaries on two occasions through in-person interviews by a trained nutritionist. The amounts consumed were determined using household measures or estimated using food pictures and models. Nutrient components were analyzed using the Minnesota Nutrition Data System (version 2.6). The dietary intake of total energy, macronutrients, and selected micronutrient components of the subjects is shown in Table 1. Heights and weights were obtained, and BMI (Kg/m^2) was calculated. The study was approved by the Institutional Review Board of St. Luke's-Roosevelt Hospital Center on June 24, 1993.

On the day of the rectal biopsies, a stool was passed into a special container that was brought to the laboratory within 60 min. The stools were weighed, then they were immediately snap-frozen and kept for up to 1 month at −80°C before analysis. Before further testing, the stools were lyophilized to constant weight. The water content of the stools is shown in Table 2.

Biopsies were taken at a standard time between 8 and 10 a.m., after a 60-cc tap water enema. A fibersigmoidoscope was passed to a point between 10 and 15 cm from the anus, and mucosal biopsies were taken in 4 quadrants using 2.8-mm

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[3] The abbreviations used are: DAG, diacylglycerol; BMI, body mass index.

Table 1 Daily nutrient intake of volunteer subjects[a]

	n	BMI (kg/m²)	Total energy (kcal)	Carbohydrate (g)	Protein (g)	Fat (g)	Calcium (mg)	Fiber (g)	Fiber (g)	Alcohol (% of total energy)
M Mean ± SE	9	32.4	2055	228	83	77	683	16.2	10.3	7.7
		1.4	147	14	5.5	10	54	0.8	0.9	2.0
F Mean ± SE	3	29.0	1777	252	86	49	600	16.0	12.0	4.9
		1.2	194	32	12	5	72	3.7	4.8	4.9
Total Mean ± SE	12	31.6	1985	234	84	70	662	16.1	10.8	7.0
		1.2	122	13	5	9	44	1.0	1.0	1.8

[a] Daily nutrient intake was determined from a 3-day food diary and calculated using Minnesota Nutrition Data System (version 2.6). *n*, number of subjects; *M*, male; *F*, female.

Table 2 Fecal and mucosal DAG content[a]

	n	Fecal water (%)	Fecal DAG conc. (μmol)		Mucosal DAG conc. (μmol)	
			Wet wt	Dry wt	per mg	per g of PL
M Mean ± SE	8	76.0	0.49	2.23	0.29	29.7
		0.9	0.10	0.36	0.03	2.1
F Mean ± SE	3	78.1	0.37	1.80	0.36	37.5
		1.6	0.05	0.24	0.05	2.3
Total Mean ± SE	11	76.1	0.39	1.78	0.31	32.0
		1.3	0.22	0.96	0.10	7.6

[a] Fecal components were determined on rapidly frozen fecal samples. Fecal DAG concentrations were calculated as a function of both wet weight and after lyophilization. Mucosal DAG concentrations were calculated as a function of mucosal weight and mucosal phospholipid content. PL, phospholipid; conc., concentration; *M*, male; *F*, female.

forceps. Two to three biopsies were obtained for determination of mucosal DAG and total phospholipid content and were immediately transferred into liquid nitrogen. Two to three biopsies were placed into a pregassed transport medium (Eagle's Minimal Essential Medium; Life Technologies, Inc., Grand Island, NY) containing antibiotics, orientated, and then incubated within 15 min in a shaking chamber under positive pressure for 90 min in MEM containing 10% calf serum and 5 μCi/ml of tritiated thymidine (8). After incubation, biopsies were washed, placed in 95% ethanol, and then processed as for routine histopathology. Four-μm sections were stained with H&E, and adjacent sections were microautoradiographed as described previously. The biopsies were read by a trained technologist who was unaware of the details of the study. A mean of 26.8 ± 1.6 crypts were read. (8). Briefly, the number of unlabeled and labeled crypt cells and the position of tritiated thymidine-labeled cells in one side of well-orientated, longitudinally sectioned, colonic crypt columns were counted microscopically for each subject, and the height of the ^{3}H-labeled epithelial cells in colonic crypts measured. For measurements of the distribution of labeled cells, each cell was assigned a crypt coordinate value (between 0 and 1) equal to the localization of the cell above the base of the crypt (9), and labeling indices were calculated for each compartment, from compartment 1 (the basal one-fifth of the column) through compartment 5 (the top one-fifth of the column).

Fecal DAG concentrations were analyzed as both wet and lyophilized dry feces (10). Briefly, lyophilized stools were reconstituted with 4 volumes of 0.1 M potassium phosphate buffer, fecal lipids were extracted by the method of Bligh and Dyer (11), and DAG content was determined using *Escherichia coli* DAG kinase (10). Mucosal phospholipid content was determined by measuring total lipid phosphorus (12) and multiplying by a factor of 6.4. Mucosal DAG was determined per milligram of mucosal tissue weight and as a proportion of mucosal phospholipid content.

Statistics. Pierson product-moment correlation coefficients were calculated among the following variables, using the SAS ProcCorr procedure (13): DAG measures (fecal dry and wet concentrations, mucosal concentration/mg and as a proportion of phospholipid); proliferation (labeled cells/column, total crypt labeling index, and labeling index in compartments 4 and 5); and nutritional parameters (energy, fat, protein, carbohydrate, calcium, total, soluble and insoluble fiber, pectin, and alcohol).

Results

The daily nutrient intake and BMI of volunteer subjects are shown in Table 1. Mean energy intake of the total group was 1985 kcal, with a caloric distribution of macronutrients similar to that of many Americans (32% of kcal as fat, 17% as protein). Mean calcium intake was found to be quite low at 662 ± 44 mg/day, and the mean fiber intake was 16.1 ± 1.0 g/day. The female volunteers tended to have a lower total caloric intake and ate somewhat less fat than the male subjects. The intake of the remaining components did not differ between the genders.

Fecal DAG concentrations varied considerably among individual subjects, ranging from 0.33–3.16 mmol/g dry weight (Table 2). The data for mucosal DAG are presented for only 11 volunteers because there was insufficient biopsy tissue to measure mucosal DAG in 1 subject. Neither wet nor dry fecal DAG concentration correlated with mucosal DAG concentrations calculated as a function of mucosal weight or phospholipid content.

Rectal crypt proliferative indices are shown in Table 3. The number of total cells/crypt column was 50.2 ± 0.9 and did not differ with the sex of the volunteers. Data for labeling indices were determined for the whole crypt and separately for compartment 4 and compartments 4 + 5. It is of interest that the whole crypt labeling index was less in the 3 female volunteers than the 9 male volunteers, and the compartment 4 labeling index was also 40% less.

Univariate associations between dry or wet fecal DAG concentration showed no correlations with any of the crypt labeling index parameters studied. However, mucosal DAG concentration correlated inversely with the number of labeled

Table 3 Crypt proliferative kinetic in the rectal mucosa[a]

	n	Crypt cell no. (no. of cells/column)	Labeled epithelial cells (no. of cells/column)	LI (%)		
				Whole crypt	Compartment 4	Compartment 4 + 5
M Mean ± SE	9	51.05	2.93	5.72	5.07	3.0
		1.0	0.3	0.5	1.2	0.7
F Mean ± SE	3	48.36	2.09	4.4	2.57	1.29
		1.6	0.6	1.3	1.2	0.6
Total Mean ± SE	12	50.23	2.70	5.36	4.32	2.46
		0.9	0.3	0.5	0.3	0.3

[a] The data were determined by counting a mean of 26.8 ± 1.6 well-oriented crypts in each subject. *n*, number of subjects; LI, labeling index; *M*, male; *F*, female.

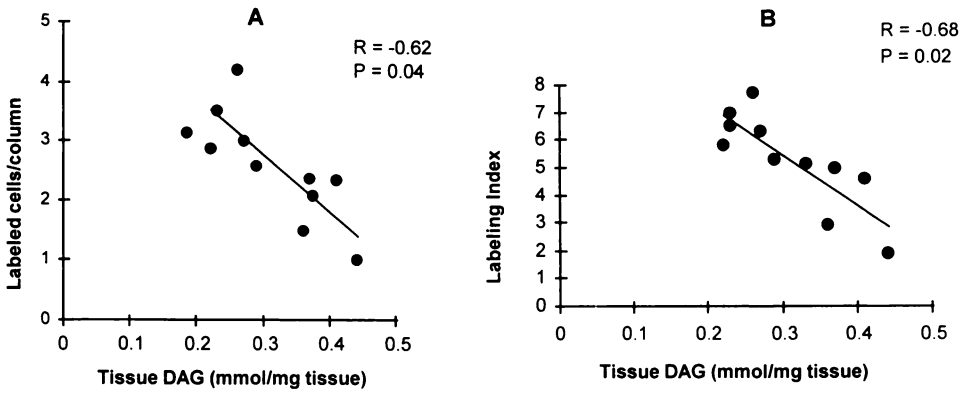

Fig. 1. Correlation between tissue DAG concentration and (*A*) the number of labeled cells/crypt column or (*B*) the whole crypt labeling index data derived from 11 subjects in whom both determinations were available.

Table 4 Linear regression of associations between DAG, nutrient intake, and labeling indices[a]

		Total crypt labeling index		Compartment 4 + 5 labeling index	
		Regression	*p*	Regression	*p*
DAG conc.	Fecal	+0.1487	>0.1	+0.2493	>0.1
(μmol)	Mucosal	−0.6635	<0.03	−0.0201	>0.1
Diet	Energy (cal)	+0.3453	>0.1	+0.4040	>0.1
	Fat (% of cal)	+0.6643	<0.03	−0.0976	>0.1
	Total fiber (g/day)	−0.7025	<0.02	+0.6486	<0.05
	Insoluble fiber (g/day)	−0.2304	<0.1	+0.1507	>0.1
	Calcium (g/day)	−0.1201	>0.1	+0.8491	<0.005

[a] Mean linear regression and statistical (Pierson product-moment) correlations were calculated. Conc., concentration.

cells/column (Fig. 1*A*) and the whole crypt labeling index ($P < 0.03$; Fig. 1*B*) but not with the labeling indices in compartments 4 and 5. There were no correlations between the patients' mean BMI or the daily dietary intake of calories, carbohydrate, fat, protein, calcium, or fiber with either fecal or mucosal DAG concentrations. However, the percentage of calories from fat correlated positively with the labeled cells/crypt column (+0.712; $P < 0.02$) and total labeling index (+0.664; $P < 0.03$; Table 4). The total fiber intake correlated negatively with the total crypt labeling index (*p*) but positively with labeling in compartments 4 and 5 (Table 4). When total fiber intake was divided into soluble and insoluble fiber and pectin, the statistical correlations were restricted to the insoluble fiber content only. Overall, the total fiber intake in our subjects was not large, ranging from 12.0–23.4 g/day. Calcium intake also correlated positively with labeling in the upper 40% of the crypt (Table 4).

Discussion

The primary aims of the present study were to determine whether fecal DAG concentration would contribute to proliferation kinetics in the rectal mucosa and, if so, to evaluate the relationship of fecal DAG and mucosal DAG concentration. Secondarily, we aimed to evaluate the influence of dietary components upon fecal and mucosal DAG as well as proliferation.

The study confirms that DAG is excreted in the stool in widely ranging concentrations by different individuals. The women volunteers had somewhat lower fecal DAG concentrations than did the men, but the difference in this small population study was not significant. However, total mucosal DAG concentrations calculated as a function of mucosal weight or phospholipid content did not correlate with fecal DAG concentrations. Mucosal DAG also did not correlate with the crypt cell number or upper crypt labeling indices, although labeling in the upper crypt has been used as a predictive index of risk for colon neoplasia (6). These observations cast some doubt on the hypothesis that DAG from the colon might contribute to mucosal DAG and activate PKC, thereby stimulating mucosal proliferation (7). However, it is possible that only a fraction of mucosal DAG (for example, the cytoplasmic or membrane fraction)

functions to stimulate proliferation. Mucosal cell DAG was not fractionated into subcellular components in our study. It is also possible that only DAGs containing specific fatty acids interact with cellular signal transduction pathways and proliferation (14). In this regard, it is of interest that dietary lipid fatty acid composition may alter the fatty acid composition of fecal DAGs (15).

We showed previously that calcium administration (2.4–3.6 g/day) to patients after jejunal-ileal bypass led to a reduction of fecal DAG (16) and in rectal proliferation (17). Furthermore, in normal volunteers, calcium (1.8 g/day) showed a trend to lower fecal DAG concentrations (18). Therefore, we analyzed the intake of calcium as well as energy, fat, protein, and fiber. There were no correlations between fecal DAG concentrations or mucosal DAG concentrations and the intake of energy, carbohydrate, protein, fat, fiber, or alcohol. There was also no correlation with calcium intake. However, it must be emphasized that calcium intake in these volunteer subjects was quite low (mean of 662 ± 44 mg/day), and the range (476–939 mgs) was also narrow. For these reasons, an effect of calcium upon fecal DAG may have been missed. Crypt cell proliferation kinetics and dietary intakes were also measured. The data revealed that the percentage of calories from fat (although not fat intake in grams) correlated positively with the crypt labeling index. These data are consistent with some studies in rodents (19) that point to the effect of dietary fat intake on colonic proliferation.

Previous studies of the effect of fiber intake upon colorectal cell proliferation have been very confusing because both enhanced (20, 21) and reduced (22) proliferation or upper crypt labeling has been described. The present study is also confusing because although the total crypt labeling was less with increased fiber intake, labeling in the upper crypt was higher. This effect was associated with the insoluble fiber content of the diet. Calcium intake also correlated positively with labeling in the upper 40% of the crypt, however, the range and levels of calcium consumed were very limited. Proliferation kinetics did not correlate with BMI.

In summary, the present study has demonstrated no correlation between fecal and total mucosal DAG concentrations. In future investigations of the hypothesis that colonic or fecal DAG directly contributes to mucosal DAG and thereby might stimulate proliferation, mucosal DAG content and fatty acid composition should be measured in subcellular compartments. This study also represented a pilot study to explore correlations of proliferation with the ingestion of differing food components. The previous studies correlating diet with changes in proliferation have added fat, fiber, or calcium to the diet. Whether the results of such intervention studies can be compared to individual dietary components within lower ranges of consumption in the United States population remains to be determined. Studies are underway in our laboratory to address this possibility.

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