Calcium Reduces the Increased Fecal 1,2-sn-Diacylglycerol Content in Intestinal Bypass Patients: A Possible Mechanism for Altering Colonic Hyperproliferation

Gideon Steinbach, Masami Morotomi, Koji Nomoto, Joanne Lupton, I. Bernard Weinstein, and Peter R. Holt

ABSTRACT

Diacylglycerol (DAG) is a second messenger for protein kinase C, an enzyme with a key role in cellular signal transduction and growth control. In previous studies, it was demonstrated that DAG is produced by intestinal microflora. Bacterial DAG production is increased by bile acids and phospholipids, both of which may be precipitated by calcium. We have demonstrated that fecal total lipids, bile acids, and rectal epithelial proliferation are increased in intestinal bypass (IB) patients. Calcium was shown to alter fecal lipid composition and to reduce cell proliferation. In the present study, fecal DAG content and 14C-labeled DAG, 14C-phosphatidylcholine, and 14C-phosphatidylinositol metabolism were measured in 24-h stool collections in 15 stable IB patients before and after 3-month therapy with oral elemental calcium, 2.4 or 3.6 g/day. Fecal DAG concentration and output in IB patients were >25- and >200-fold greater than in normal controls. Oral calcium markedly reduced fecal DAG concentration and output and increased DAG, phosphatidylcholine, and phosphatidylinositol metabolism without enhancing DAG production. We conclude that fecal DAG content is markedly elevated post-IB and that calcium supplementation in these patients reduces fecal DAG and accelerates bacterial metabolism of DAG and its precursors. In separate studies, we have found that calcium supplementation also decreases rectal hyperproliferation in IB patients. Taken together, these findings suggest that a high luminal level of DAG enhances colonic cell proliferation and that calcium reduces cell proliferation in part by decreasing the level of DAG.

INTRODUCTION

The enzyme PKC, a calcium-dependent protein serine/threonine kinase, is important in signal transduction and growth control (1–3). PKC function is associated with the action of growth factors via activation of phospholipase C. This action results in hydrolysis of phosphatidylinositol 4,5-bisphosphate, producing inositol 1,4,5-triphosphate, which can stimulate the release of calcium from intracellular storage sites, and 1,2-sn-diacylglycerol, which binds to PKC and activates the enzyme. Photorbl ester tumor promoters may also activate PKC by binding to the same regulatory region of PKC as DAGs, thus substituting their function. PKC belongs to a multigene family consisting of at least 10 isoforms (2), although the specific functions of the individual isoforms are not known. Several laboratories have been interested in the possibility that DAGs and the PKC pathway may be important in human colon cancer. DAG has been detected in normal human fecal samples (4, 5) and is derived at least in part from the action of intestinal bacteria (5). Friedman et al. (4) have shown that DAG can enhance proliferation of colonic adenomas and carcinomas in cell culture.

MATERIALS AND METHODS

Patients. Fifteen volunteers whose weight and clinical condition were stable at least 2 years after IB surgery for morbid obesity were studied. The majority of subjects had undergone a duodenal-ileal bypass (12). Excluded were patients with a personal history of colon cancer or inflammatory bowel disease, a family history of familial colon cancer, calcium supplementation of over 500 mg per day, a serum calcium concentration of under 8.4 mg/dl, or serum 1,25-dihydroxyvitamin D concentration of under 20 pg/ml. Controls were 10 normal volunteers previously reported (5).

Study Design. The subjects were treated with calcium carbonate (Caltrate 600; Lederle) containing 2400 mg (n = 4) or 3600 mg (n = 11) of elemental calcium per day in 2 divided doses for a period of 12 weeks. Twenty-four-h stools were collected on a portable toilet at the beginning of the study and after 12 weeks of calcium supplementation. Individual stools were placed in plastic bags on dry ice immediately after being passed. Control volunteers provided stools but were not treated with calcium. The frozen stools were weighed and stored at -80°C. Aliquots from each bowel movement were kept frozen, and the remainder was lyophilized to constant weight. The study was approved by the St. Luke's-Roosevelt Hospital Center institutional review board.

Preparation of Fecal Homogenates. Fecal specimens were thawed and homogenized with 4 volumes (w/v) of 0.1 M K2HPO4 buffer (pH 7.0) by shaking vigorously with glass beads. Debris was removed by centrifugation at 1000 x g for 1 min. Lyophilized stools were reconstituted with K2HPO4 buffer prior to analysis.

Quantitation of Total DAG in Fecal Specimens. Lipids were extracted by the method of Bligh and Dyer (13) from fecal homogenates (10 mg equivalent wet weight). The DAG content of fecal extracts was analyzed using Escherichia coli DAG kinase (Lipidex, Middleton, WI) by a previously described procedure (14, 15) and was expressed as nmol per g wet or dry weight of original feces. Preliminary studies demonstrated that the extraction procedure recovered DAG quantitatively (mean recovery, 98.6%) and that the enzymatic method used was linear to 60 min of incubation. The fecal content of DAG was measured in frozen and lyophilized stool in parallel with similar results. The protein concentration of wet stool was determined by a modified procedure of Lowry et al. (16) using bovine serum albumin as a standard. Fecal protein concentrations were 0.45 ± 0.07 mg/100 g in the stools of controls and 0.19 ± 0.05 mg/100 g in patients.

Assays of DAG and Phospholipid Metabolism. Substrates used were: 1-stearoyl-2-archidonyl-phosphatidylcholine (PC), 1-stearoyl-2-arachidonyl-phosphatidylinositol (PI), 1-stearoyl-2-archidonyl-DAG, and 1-stearoyl-2-[14C]archidonyl-PC. -PI or -DAG, 110 mCi/mmol (Amersham).
Stock solutions were prepared by mixing labeled and unlabeled substrates with deoxycholic acid (100 mM methanolic solution) and taking the mixture to dryness at room temperature under nitrogen. To the dried residue, distilled water was added, and the solution was sonicated for 1 min in a Bransonic bath sonicator so that the aqueous suspensions were adequately dispersed. Fifty µl of the sonicated substrate were incubated with 25 µl of fecal homogenate in a shaking water bath at 37°C. The final concentrations of substrates in the reaction mixture were 1.6 mM and 10³ cpm/assay. The incubation was terminated by adding 670 µl of chloroform:methanol (1:2, v/v) and 100 µl of 2 M KCl. After mixing, the chloroform layer was separated by low speed centrifugation and dried under nitrogen. The dried lipid residue was dissolved in a small amount of chloroform:methanol (2:1, v/v), chromatographed on silica gel G plates using hexane:diethylether:acetic acid (50:50:3 by volume), and subjected to autoradiography. The major metabolites MAG, FFA, and DAG were separated, and the radioactive spots were counted in a scintillation counter.

Fecal fat content was measured gravimetrically after extraction of the stool with chloroform:methanol (2:1, v/v). To quantify long chain FFA, fecal lipids were extracted according to the procedure of Folch et al. (17), and FFA was methylated by the boron method (18). FFA were quantified by gas chromatography comparing to methyl esters to known standards (19).

Statistical differences between groups were analyzed by unpaired t tests. Relationships between fecal parameters were analyzed by regression analysis. All IB patients who provided baseline and follow-up stools (n = 15) were included in the analysis.

RESULTS

Fifteen IB subjects completed the 12 weeks of calcium supplementation and collected 24-h stools at the beginning and end of the study. Fecal dry weight, 282 ± 25 g/day (mean ± SE), and lipid output, 131.9 ± 13 g/day, in IB patients were elevated more than 5- and 10-fold the normal values (normal laboratory values < 45 and <12 g/day, respectively). Baseline fecal lipid concentration in dry stool was 471 ± 26 mg/g (normal < 225 mg/g), and fecal unesterified long chain fatty acid concentration was 294 ± 29 mg/g (normal < 94.5 mg/g). Calcium supplementation reduced fecal dry weight by 36% (P < 0.001) without altering total fecal lipid or FFA concentration (Table 1).

In IB patients, fecal DAG concentration was elevated over 25-fold compared to controls (22.4 versus 0.7 µmol/g dry weight) and daily DAG output, 7.18 ± 29 mmol/day, was elevated over 200-fold (normal < 30 mmol/day; Fig. 1). Calcium supplementation reduced the DAG concentration in dry stool by 63% (P < 0.05) and in wet stool by 58% (P < 0.05; Fig. 1).

DAG was actively metabolized in vitro by fecal bacteria obtained from control and IB subjects (Table 2). In specimens from controls, 30% of DAG was degraded principally to FFA, whereas in specimens from IB subjects 65.6% of DAG was metabolized both to FFA and MAG. With calcium supplementation, DAG metabolism was accelerated significantly to over 71% with production of FFA and MAG. In control subjects, 82.2% of PC was metabolized, principally to FFA (80.6%) and to DAG (2.2%). In contrast, fecal specimens from IB subjects metabolized significantly less PC with formation of only a negligible amount of DAG (Table 3). Calcium supplementation in IB subjects significantly increased fecal PC metabolism (to 52.1%; P < 0.01), again with the production of only FFA. FFA production was weakly related to fecal calcium concentration (r = 0.425). PI in control subjects was extensively metabolized (65.1%), essentially only to FFA, whereas in IB subjects not treated with calcium only 19.8% of the substrate was metabolized, principally to FFA (Table 4). During calcium supplementation, PI metabolism increased significantly to 33.1% without formation of DAG. The amount of FFA produced was positively correlated to fecal calcium concentration (r = 0.636).

Calcium induced opposite changes in fecal DAG and FFA concentrations. After calcium supplementation, the ratio of fecal FFA to total lipid increased, whereas the ratios of fecal DAG to total lipid and to MAG decreased (P < 0.01). This in vivo finding paralleled observations.

| Table 1 Effect of calcium administration upon fecal weight, and dry fecal concentration of calcium, lipid, unesterified fatty acid and 1,2-sn-diacylglycerol in IB subjects |
|------------------|------------------|------------------|------------------|------------------|
| Wet wt. (g/day) | Dry wt. (g/day)  | Calcium (µmol/g)* | Total Lipid (mg/g)* | FFA (mg/g)* | DAG (µmol/g)* |
| IB (-Ca)        | IB (+Ca)         | Control           | IB (-Ca)          | IB (+Ca)      | Control       |
| 1291 ± 109*     | 282 ± 25*        | 130 ± 15*        | 471 ± 26          | 294 ± 29      | 22.4 ± 8*    |
| 646 ± 94        | 179 ± 2          | 510 ± 88         | 447 ± 24          | 311 ± 20      | 8.3 ± 3      |

* P < 0.001.  
** P < 0.05.
of fecal metabolism in vitro that suggest that calcium supplementation accelerates the metabolism of fecal DAG, PC, and PI to FFA.

**DISCUSSION**

Fecal bile acid and lipid output are greatly elevated in patients after IB. Rectal epithelial proliferation, an intermediate biomarker of colon cancer risk, increases in parallel (11). Colonie epithelial hyperproliferation and increased incidence of carcinoigen-induced colon tumors have been demonstrated in rodents following experimental IB (6, 7). Calcium supplementation reduced colonie epithelial proliferation in humans post-IB (11) and in rodents post-intestinal resection (9) and also lowered the incidence of carcinoigen-induced tumors in experimental animals (8). Administration of calcium has been shown to sequester bile acids and lipids in vivo (20, 21) and in vitro (22), and the beneficial effects of calcium are postulated to be mediated in part by its effect on fecal DAG.

Increased colonie DAG concentrations may contribute to the elevated rectal epithelial proliferation found in these subjects (11). The beneficial effect of calcium on rectal epithelial proliferation may be mediated in part by its effect on fecal DAG.

Calcium supplementation accelerated fecal PC, PI, and DAG degradation in vitro, thereby speeding elimination of DAG and its precursors. Calcium also increased the ratio of fecal FFA to total lipid and decreased DAG relative to total lipid in vivo. The mechanism by which calcium increases PC, PI, and DAG metabolism and reduces DAG content remains to be studied. It is possible that the binding of FFA to calcium may be a contributing factor.

**REFERENCES**


Calcium Reduces the Increased Fecal 1,2-sn-Diacylglycerol Content in Intestinal Bypass Patients: A Possible Mechanism for Altering Colonic Hyperproliferation

Gideon Steinbach, Masami Morotomi, Koji Nomoto, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/54/5/1216

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.