Effect of dietary calcium on the colonic luminal environment

G V N Appleton, R W Owen, E E Wheeler, D N Challacombe, R C N Williamson

Abstract

Dietary supplementation with calcium may prevent the development of colorectal cancer. This mechanism may be related to fatty acid and bile salt chelation in the small bowel forming non-toxic calcium-soap compounds. Calcium may also act locally or systemically on the colonic mucosa. Faecal concentrations of free fatty acids and free bile acids were measured in 17 Sprague-Dawley rats (weighing 472 (39 g)) whose daily calcium intake had been trebled by enriching the chow and adding calcium lactate (24 g/l) to the drinking water. Mean (SEM) faecal concentrations of free bile acids were 33% less than in 19 controls (1.23 (0.15) v 1.82 (0.20) mg/g; $p < 0.001$), whereas free fatty acid concentrations were 117% higher (14.68 (3.59) v 6.76 (2.41) mg/g; $p < 0.02$). The 'direct' effect of calcium was assessed by organ culture of rat colonic explants in three different concentrations of calcium. Crypt cell production rate (measured by a stathmokinetic technique), which was (mean (SEM)) 4.80 (0.23) cells/crypt/h in control medium ($\text{Ca}^{2+} = 2.14 \text{ mmol/l}$), fell by 43% when calcium concentration was doubled ($p < 0.05$) and by a further 43% when the concentration was trebled ($p < 0.02$). Calcium binds free fatty acids but not free bile acids intraluminally. Calcium has a direct antitropic action on colonic crypts.

Interest in calcium as a chemopreventive agent in colorectal carcinoma began in 1982 when a Scandinavian study showed that the cancer incidence was least in populations consuming a diet rich in dairy products despite a high fat intake.¹ Three years later, Garland's 19 year prospective dietary study on 1954 men from Chicago concluded that a high calcium intake ($> 1.5 \text{ g/day}$) was associated with a 65% reduction in cancer incidence.² It seems that populations consuming high calcium diets may enjoy a relative freedom from colon cancer.

Experimental work in our laboratory has reinforced some of these findings. Doubling the calcium intake in rats by adding 24 g/l to the drinking water halves the yield of colorectal tumours induced by azoxymethane.³ There is a corresponding fall in crypt cell production rate in both right and left colon.⁴ Recent data from elsewhere show a similar effect.⁵ Calcium can also modify the behaviour of cell lines and small explants in vitro. The extracellular concentration of calcium is critical in the regulation of proliferation and differentiation of keratinocytes⁶ while calcium reduces proliferation rates in cultured mammary, oesophageal, bronchial, and urothelial cells.^{7,8}

Newmark *et al* have proposed that potentially toxic bile acids and fatty acids are bound by calcium in the enteric lumen and rendered harmless by the formation of insoluble, non-irritant calcium soaps.⁹ In support of this, the locally damaging effects of both bile acids and fatty acids instilled intrarectally are reversed by giving concomitant calcium gluconate by mouth.^{10,11} The present two part study was designed to test Newmark's intuitive hypothesis and to investigate the 'direct' effect of calcium. Faecal concentrations of free bile acids and free fatty acids were measured in rats fed different dietary concentrations of calcium. Colonic biopsy specimens were cultured in varying concentrations of calcium, and cytokinetic changes were determined by measuring crypt cell production rate.

Methods

Adult male Sprague-Dawley rats ($n=42$) weighing (mean (SD)) 472 (39) g were used in a two part study on the direct and indirect effects of calcium.

STUDY 1: FAECAL FATS AND BILE ACIDS

Rats either acted as controls ($n=19$) and received normal chow and tap water, or they had calcium lactate 24 g/l added to the drinking water and ate a calcium enriched chow ($n=17$) thereby trebling their total daily calcium intake from 0.44% w/w to 1.32% w/w. After two weeks all animals were sacrificed between 1000 and 1200 hours. A blood specimen was taken by cardiac puncture for measurement of serum calcium. At laparotomy the distal half of the colon was opened, and all faeces were collected. Faecal specimens were immediately deep frozen to -40°C . Later, samples were analysed by gas-liquid chromatography for concentrations of free bile acids and free fatty acids according to the method of Alme modified by Owen.¹²

STUDY 2: COLONIC ADAPTATION

Rats ($n=6$) were lightly anaesthetised with ether, and a small segment of bowel (1-2 cm) was excised from the lower descending colon. Each specimen was divided into 15-20 explants (2 mm^2) which were established in organ culture in three different concentrations of calcium. Control explants were cultured in standard medium containing calcium 2.14 mmol/l . In the other two groups, calcium chloride was added to the medium to double and triple the final concentrations of calcium, which were 4.28 mmol/l and 6.42 mmol/l . A rocking method of organ culture was used, as previously described.¹³ Vincristine

University Department of Surgery, Southmead Hospital, Bristol
G V N Appleton

Public Health Laboratory Service, Porton Down, Wiltshire
R W Owen

Somerset Children's Research Unit, Musgrove Park Hospital, Taunton
E E Wheeler
D N Challacombe

Royal Postgraduate Medical School, London
R C N Williamson

Correspondence to: Professor R C N Williamson, Department of Surgery, Royal Postgraduate Medical School, Hammersmith Hospital, Duane Road, London W12 0HS.

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Concentrations of faecal bile acids and free fatty acids (mg/g dry wt faeces) (mean (SEM))

	Control	Added calcium
Total bile acids	1.82 ± (0.20)	1.23 ± (0.15)†
Total free fatty acids	6.76 ± (2.41)	14.68 ± (3.59)*
Saturated fatty acids	3.09 ± (1.45)	6.50 ± (2.47)*
Unsaturated fatty acids	2.63 ± (0.93)	6.07 ± (2.38)*
Other fatty acids	1.04 ± (0.34)	2.09 ± (0.88)

†p<0.001, *p<0.02.

0.5 µg/ml was added to the medium after an 18 hour culture, and explants were removed at 30 minute intervals from 60–180 minutes thereafter. The number of vincristine-arrested metaphase figures were counted in 15 crypts per specimen and plotted against time after adding vincristine. Crypt cell production rate was calculated by least squares regression analysis of the slope of the line so obtained.¹⁴

STATISTICAL ANALYSIS

All results quoted are mean (SEM). Faecal concentrations of fats and bile acids and blood calcium were analysed by the Kruskal-Wallis test and subsequently compared by the Mann-Whitney U test. Regression lines were compared by Student's *t* test.

Results

SERUM CALCIUM

The threefold difference in calcium intake had no effect on serum calcium concentration. The level was (mean (SEM)) 2.90 (0.21) mmol/l in controls and 2.79 (0.15) mmol/l in rats with the enriched diet.

FAECAL BILE ACIDS AND FATS (Table)

The faecal content of free (unconjugated) bile acids was 33% lower in rats receiving the calcium enriched diet than in control rats. By contrast, concentrations of free fatty acids were 117%

higher in the calcium group. This increase reflected greater excretion of both saturated and unsaturated fats (110–131%).

ADAPTATION

After 18 hours of organ culture and a further stathmokinetic period (1–3 hours), colonic mucosal explants were generally well preserved (Fig 1). In 5% of explants crypt necrosis led to those samples being excluded from the analysis. Overall, counts were made on 29 control explants, 33 from the group with added calcium 2 mmol/l and 35 from the 4 mmol/l calcium group. Crypt cell production rate for normal colonic mucosa in the standard medium with no additives was 4.80 (0.23) cells/crypt/h (Fig 2), which compares well with values in our previous studies.^{4,15} Doubling calcium concentrations caused a 43% reduction in crypt cell production rate to 2.75 (0.31) cells/crypt/h. Trebling calcium concentrations caused a further 43% fall to a level (1.56 (0.39) cells/crypt/h) that was only 32% of control values.

Discussion

Dietary fat, especially unsaturated fat, is a potent co-carcinogen in most animal studies of experimental colorectal cancer.^{16,17} Our finding that supplemental calcium more than doubled the output of free fatty acids indicates intraluminal binding of fats. Calcium is a powerful chelator of fats; it binds to them even more strongly than it does to the powerful calcium binding agent ethylenediamine tetra-acetate, especially in a basic pH environment.¹⁸ Calcium soaps of fatty acids are known to be relatively insoluble and non-toxic to the colorectal mucosa.¹⁹ Interaction between supplemental calcium and dietary fat to form calcium-fatty acid soaps in the small bowel may thus explain the lower colorectal tumour yields seen in our study³ and in rats on high fat diets,⁵ and the reduction of the colonic proliferative response to bolus delivered corn oil.²⁰ Moreover, the increased numbers of mammary neoplasms seen in rats fed high fat diets²¹ is partially reversed by enriching diets with calcium (or vitamin D).²²

Intracolonic bile acids are also important promoters of experimental carcinogenesis.^{23,24} In humans higher faecal concentrations of free bile acids closely correlate with the malignant potential of adenomas²⁵ and the incidence of colorectal carcinoma.²⁶ Our important finding that calcium lowers potentially toxic colonic concentrations of free bile acids may be explained (as for free fatty acids) by the formation of calcium-bile acid soap complexes. As we found no such compounds in faecal residues, perhaps bile acid soaps that had precipitated in the small bowel did not escape into the colon owing to enteric absorption. Alternatively, chelation of fat by calcium could prevent fat induced stimulation of primary bile acid secretion, with a concomitant reduction in colonic concentrations of secondary bile acids. Few data concerning bile acid soaps are available, but it is known that calcium soaps of fatty acids, especially unsaturated fatty acids such as oleic acid, can be absorbed in the small bowel.²⁷

Figure 1: Histological section of colonic mucosa after 21 hour organ culture (three hours after addition of vincristine). Good preservation. Many metaphase figures can be seen arrowed. (Haematoxylin & eosin, original magnification ×200.)

Figure 2: Crypt cell production rate in organ culture. * $p < 0.05$ v $Ca^{2+} = 2.14$, $p < 0.02$ v $Ca^{2+} = 4.28$.

Two recent studies in which colorectal carcinogenesis was stimulated by dietary augmentation with bile acids and fats are in concordance with our data. A threefold increase in dietary calcium in rats receiving parenteral *N*-methyl-*N*-nitrosourea failed to reverse the tumour enhancing effect of oral cholic acid.²⁸ By contrast, the tumour promoting effect of a high fat diet was completely reversed by doubling oral calcium intake.⁵ It thus seems probable that calcium does not bind free bile acids in the small bowel or that there is preferential binding of free fatty acids. Some of the data of McSherry *et al*²⁸ are controversial, as raised dietary calcium actually enhanced tumour yields in some groups. However, the dose of *N*-methyl-*N*-nitrosourea (and the yield of colorectal tumours) was small in this experiment, and calcium concentrations in the control diet were higher than in ours (0.56%). Wargovich emphasises the need for calcium concentrations in control rats to match 'human nutrient density'.²⁹ Most commercially available animal feeds have calcium concentrations up to tenfold those in normal human diet.³⁰

It is interesting that calcium lowers the birth rate of colonic mucosa in organ culture. A recent (autoradiographic) study of cultured rectal biopsy specimens from 'high risk' patients showed a similar effect: the high labelling indices usually found in these patients were reduced to normal when the concentration of calcium in the culture medium was doubled.³¹ Higher concentrations of calcium (and vitamin D₃) lead to increased differentiation in a cell line derived from a human colorectal carcinoma,³² although malignant epidermal cells do not respond to calcium fluctuations.⁶ It is proposed that calcium prevents loss of cell to cell contact by preserving tight junctions, thus inhibiting cell proliferation.²⁹ Any 'direct' effect of calcium is unlikely to be mediated systemically, since serum concentrations were unchanged in this and previous studies in our laboratory.³

Although the relative contributions of 'direct' and 'indirect' effects of intraluminal calcium on colonic adaptation and carcinogenesis are unknown, evidence for a direct antitropic effect of calcium on colorectal mucosa (and many other epithelia) is now substantial. Indeed, sufficient animal data are available to justify more trials on human subjects. There are no obvious contra-

indications to oral calcium supplements of 1–2 g/day. Urinary lithiasis is not provoked; in fact it is actually inhibited in patients with a tendency to form oxalate stones.³³ Calcium may have the added benefit of reducing blood pressure in some hypertensive patients.³⁴ The evaluation of colonic cytokinetics has great potential in the identification of 'high risk' patients. Lipkin and Newmark's encouraging results indicating a possible prophylactic role for calcium³⁵ may have profound implications for general dietary advice.

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