Protective role of faecal pH in experimental colon carcinogenesis[1]

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Summary: There is epidemiological evidence that populations with alkaline stool pH are at greater risk for colon cancer than populations with acid stool pH. This association was investigated in the laboratory using the rat-dimethylhydrazine colon carcinogenesis model. Rats with acid stool pH, produced by consumption of lactulose or sodium sulphate or both, had significantly fewer colon tumours after injections of dimethylhydrazine (DMH) than rats treated with DMH alone. The results confirm the hypothesis that acidification of the stool can protect against the induction of colon cancer.

Introduction

It has been hypothesized that alkaline faecal pH increases the risk of colon cancer (Thornton 1981, Burkitt 1981). We have used the rat-dimethylhydrazine colon carcinogenesis model to test this hypothesis. In rats, the injection of 1, 2-dimethylhydrazine (DMH) induces colon cancers and polyps identical to those found in humans. Stool pH can be lowered directly, without first altering bile flow, bile composition or colonic bacterial flora, through the feeding of lactulose, sodium sulphate or a combination of the two. We therefore set out to determine if fewer tumours were induced in rats given dietary supplements of lactulose or sodium sulphate or both and injections of DMH than were induced in rats treated with DMH alone.

Methods

Twenty-five male Sprague-Dawley rats 4–6 weeks of age were assigned to each of four dietary regimens: rat chow alone, lactulose-supplemented rat chow, sodium sulphate-supplemented rat chow, and rat chow with both additives. On an intake-for-body-weight basis, the additives were provided in concentrations similar to those used in humans: 1.5 ml lactulose syrup or 50 mg sodium sulphate per 20 g pellet. After four weeks of acclimation to this diet, a series of 16 weekly subcutaneous injections of DMH base, 15 mg/kg, were given to all rats.

Eight weeks after the last injection, all rats were killed with an overdose of ether. The number, location and size of colon tumours were recorded, as was the presence of extracolonic tumours or metastases.

Faecal pH was monitored with a Beckman model 41 pH meter and glass electrodes. Individual samples were emulsified with 1 cm^3 of neutral 0.9% sodium chloride. In a pilot study, rats were killed and faecal pH was measured in the right and left colon. In the DMH study groups, freshly passed stool specimens were used.

Statistical tests used included randomized analysis of variance, with the revised least significant difference method to discriminate between individual group means. The mean, variance and confidence limits were calculated for each group. Variables investigated included total number of tumours per rat, tumours per anatomic region of the colon, and a crude estimate of total tumour burden.

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Table 1. Tumour incidence

	Group 1 DMH alone	Group 2 DMH + lactulose	Group 3 DMH + Na_2SO_4	Group 4 DMH + lactulose + Na_2SO_4
No. of rats	22	23	23	23
Mean tumours/rat ± s.e. (mean)●	3.5 ± 0.4	2.4 ± 0.2	2.3 ± 0.2	2.6 ± 0.3
% tumours right colon	19	13	32	19
% tumours left colon	81	87	68	81
Total small bowel tumours	17	16	19	18
Total rats with metastases	2	0	0	0
% tumours > 4 mm	18	24	17	12

●$P < 0.05$ (Group 1 *v.* 2, 3 or 4 by ANOVA)

Table 2. Stool pH

	DMH alone	DMH + lactulose	DMH + Na_2SO_4	DMH + lactulose + Na_2SO_4
Pilot study				
Faeces in right colon	6.8	5.9	6.6	6.1
Left colon	6.9	6.3	6.1	6.3
Excreted faeces				
Month 0 ± s.e. (mean)	6.9 ± 0.06	6.1 ± 0.06	5.9 ± 0.06	5.9 ± 0.06
Month 3 ± s.e. (mean)	6.8 ± 0.03	6.2 ± 0.03	5.8 ± 0.06	5.9 ± 0.05

Results

The final groups varied in size from 21 to 23 rats. The total number of tumours per group was: regular diet alone without DMH, zero; DMH alone, 77; DMH plus lactulose, 55; DMH plus sodium sulphate, 53; and DMH plus both lactulose and sodium sulphate, 59. Colon cancer developed in all rats that received DMH. The data are summarized in Table 1. Results of pH measurements can be seen in Table 2.

The total number of tumours was statistically different ($P < 0.05$) between the DMH-alone group and all diet-supplemented groups. There was no statistical difference in tumour location, burden or metastases. Also the lactulose and sodium sulphate supplemented groups were statistically indistinguishable for all the variables measured.

Discussion

The epidemiological association of high colon cancer risk with low consumption of crude fibre and high consumption of animal fat, animal protein and beer is well known. Other major risk factors include a substantial genetic component, even when patients with known familial syndromes such as familial polyposis are excluded, and alkaline faecal pH. Details of the three reports summarizing the latter association are shown in Table 3 (MacDonald *et al.* 1978, Malhotra 1982, Pietroiosti *et al.* 1983).

In our experiment we wished to determine if primary pharmacological alteration of stool pH would alter the number of colon tumours induced in rats. The results showed that acidification of the stool using either lactulose or sodium sulphate significantly reduced the number of colon tumours induced. It is worth stressing that acidification only of the faeces of these rats was demonstrated (throughout the colon as well as in the expelled faeces). It is not known if

Table 3. Population studies of stool pH and colon cancer risk

Reference	Population	Mean stool pH	*P*
MacDonald *et al.* 1978	Seventh Day Adventists (low risk)	6.5±0.6	—
	Control	6.7±0.6	NS
	Colon cancer patients	7.0±0.6	<0.03
Malhotra 1982	Northern Indians (low risk)	6.5±1.0	—
	Southern Indians (high risk)	7.8±1.1	<0.001
Pietroiosti *et al.* 1983	Patients without cancer	6.6±0.44	—
	Colon cancer patients	8.0±0.44	<0.01

the surface of the colonic mucosa was also acidified or if the mucous barrier prevented this acidification. The mechanism by which an acid pH protects against colon cancer is unknown, though it may be decreased 7-alpha-dehydroxlation of bile salts by bacterial enzymes in an acid environment (Thornton 1981).

Two means were chosen by which to lower stool pH – lactulose and sodium sulphate. Lactulose is a synthetic, nonabsorbable disaccharide which is metabolized into organic acids by colonic bacteria. These acids reduce the pH of the right colon to as low as 4.5. Lactulose has been shown to have relatively little effect on the pH of the contents of the left colon. Sodium sulphate is a stimulant laxative that decreases stool pH by a mechanism different from that of lactulose. Its greatest effect is in the left colon. In humans, the best results of acidification of the contents of the entire colon were achieved when the two drugs were given together (Bown *et al.* 1974). In our experiment, similar degrees of acidification and protection were found regardless of the substance used to lower stool pH (Tables 1 and 2.)

One objection that might be raised to the conclusions drawn from this experiment is that, since lactulose and sodium sulphate are both laxatives, the decreased induction of tumours was caused by decreased faecal transit time rather than reduced stool pH. There are several reasons why this may not be so. First, though dietary fibre was believed to exert its protective effect in colon cancer by decreasing faecal transit time, epidemiological studies that have measured transit time have not supported this theory. Japanese residents of Hawaii, who have a risk of colon cancer similar to that of Americans, maintain the shorter faecal transit time of the Japanese (Glober *et al.* 1977). In the rat-DMH model, supplementation in the diet of cellulose fibre did not confer any protection against colon tumour induction (Ward *et al.* 1973), nor did supplementation of psyllium seeds, a bulk laxative (Castleden 1977), nor magnesium sulphate, a stimulant laxative that does not lower stool pH (Cleveland & Cole 1969). Last, interposition of a short segment of colon in the rat into the proximal jejunum, where transit time would be extremely rapid, again provided no protection in the transposed segment against tumours induced by DMH (Celik *et al.* 1981). Therefore, the decreased induction of colon tumours in the rats given dietary supplements that reduced stool pH is most likely to have resulted from colon acidification and not from decreased faecal transit time.

A second point to address is whether or not alteration of stool pH might have resulted in altered bile flow or bacterial flora, since changes in either of these factors are known to have an effect on colon cancer risk (Nelson 1983). Acidification of the stool in humans has been shown to alter the chemical composition of the bile and the degree of saturation of the bile with cholesterol (Thornton 1981, Thornton & Heaton 1981). Some factors that relate hepatic metabolism to colon cancer risk include epidemiological evidence that patients who have had a cholecystectomy have an increased risk for colon cancer (Linos *et al.* 1981). Cholecystectomy has been shown to increase the incidence of tumours of the colon in mice treated with DMH (Werner *et al.* 1977). It is certainly important when studying the complex relationship of hepatic and colonic metabolism to remember that the liver and the colon are connected by a two-way interaction. Once again, we do not know the complete mechanism by which our

dietary supplement decreased the number of tumours induced, only that the first step of the mechanism was acidification of the contents of the rats' colons. The fact that both supplements had equivalent effectiveness in acidification and tumour reduction by different mechanisms strengthens this relationship.

How might stool pH be lowered in humans? Wheat bran is quite similar to lactulose in that it is metabolized by colonic bacteria to short-chain fatty acids and thereby acidifies the stool (Cummings *et al.* 1976, Thornton 1981). Bran also has an effect similar to that of lactulose on the chemical composition of bile (Pomare *et al.* 1976). It is probably by these mechanisms that bran consumption decreases colon cancer risk rather than the alteration of faecal transit time (Burkitt 1981). Another means of lowering stool pH for individuals who are lactase-deficient (most Orientals, 70% of Blacks, and 30% of Caucasians) is the consumption of dairy products. Undigested lactose in the colon will act just as lactulose does and so, at the risk of some flatulence, diarrhoea and occasional upset stomach, may provide protection against colon cancer.

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