Prevention of colon cancer: role of short chain fatty acids produced by intestinal flora

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Any polysaccharide, whether starch or fibre (ie non-starch polysaccharides) may be fermented in the large bowel by resident microflora (anaerobic bacteria). Amongst other substances, the short chain fatty acid butyrate is produced during fermentation. Butyrate is important in the maintenance of normal epithelial biology; it is probably the means by which dietary fibre prevents colonic epithelial atrophy. Starch which escapes digestion in the small intestine (resistant starch) also prevents colonic epithelial atrophy. Dietary fibres differ greatly in their physicochemistry and also in their biological effects. As a general rule, resistant starch (especially of type 2) tend to behave more like soluble then insoluble nonstarch polysaccharides. In humans, resistant starch results in substantial production of butyrate in the colon. Butyrate can be shown to have "antitumour" effects at various levels (cell and molecular), and this could explain the important inverse association between starch intake and colon cancer incidence (on a country by country basis). The nature of the variables affecting butyrate production from dietary polysaccharides by resident microflora need to be explored with a view to better understanding the practical application of this to cancer prevention.

Purpose

The purpose of this article is to describe how polysaccharides reaching the colon might interact with tumorigenesis, focusing on fermentation-dependent events and various ways of manipulating these. The status of studies defining the effects in animal models and in humans will also be outlined.

Fermentation of polysaccharides

Any polysaccharides, starch or non-starch polysaccharides (NSP), are subject to fermentation in the lumen of the large bowel by anaerobic bacteria[1]. The result of this process is the breakdown of the polysaccharide substrate and the generation of gases (hydrogen, carbon dioxide, methane), short chain fatty acids (butyrate, acetate and propionate) and other organic acids (such as lactate). There is a resultant reduction in pH and provision of energy for bacteria. Butyrate is especially interesting as it is the principal energy source for colonic epithelium, and as such, is a key to epithelial behaviour. For instance, it stimulates epithelial proliferation and has a variety of effects on colonocyte DNA which are not shared by acetate or propionate[2].

Epidemiological studies and fibre

There have been over 50 case-control and cohort studies of dietary associations with colorectal cancer[3]. Of 10 substantial and well designed studies addressing vegetable intake, 9 have shown an inverse association with colorectal cancer. Of 12 substantial studies addressing "dietary fibre", 7 have shown an inverse association and 5 no obvious association. Those with the highest level of fibre intake have about half the risk for colorectal cancer of those with the lowest level.

A number of issues are raised by these studies. The food sources of dietary fibre are highly variable in their composition and it is conceivable that the protection is conferred not simply by the fibre content therein but the associated substances as well. Furthermore, there is a considerable variation in the chemistry of fibre in these foods. The majority of dietary fibre is comprised of various NSP. As a broad rule, those which are relatively soluble are highly fermentable in the colon, whereas those which are relatively insoluble are slowly fermented.

The issue of whether soluble or insoluble non-starch polysaccharides are the most effective at prevention are not yet clear from epidemiological studies. A study by Freudenheim *et al*[4] examined 428 males with colorectal cancer in a case-control study. Overall fibre intake did not confer significant protection. Based on an odds ratio of 1 for those with lowest intake, those with the highest intake of fruit and vegetable fibre had an odds ratio of 0.59 (significantly different) indicating protection. The odds ratio for overall grain fibre consumption was 0.30 (also significant). Odds ratios for insoluble fibre (0.41) and soluble fibre (0.79) were also significantly different in those with a high intake and bordered on being significantly different from each other. The epidemiological tools to fully resolve this question are not available

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and other types of studies need to be considered.

Starch polysaccharides

It may be that starch polysaccharides are also important. It is now known that some dietary starch does reach the colon. This is referred to as "resistant" starch and amounts to 25-50g a day in a typical westernised diet[5]. These starch polysaccharides can be fermented to produce short chain fatty acids in the same way as non-starch polysaccharides and it has recently been shown that they stimulate epithelial proliferation and promote colonic cell mass in similar fashion to NSP (Young et al, submitted). That is, like NSP, they prevent colonic mucosal atrophy. The potential for starch to be associated with protection against colorectal cancer was highlighted in 1994 by Cassidy *et al*[6] when they examined starch intake in grams per day over a number of different populations. They found an inverse correlation with a coefficient of -0.76. Countries such as Australia and the USA had the lowest starch intake and the highest colon cancer incidence. It is conceivable that resistant starch in these diets was responsible for the protection.

Mechanisms of protection

The mechanisms by which dietary polysaccharides could protect against colorectal cancer are multiple. Possibly all are important in certain settings. They certainly dilute stools, generally hasten transit, absorb mutagens, alter luminal bacterial metabolism of dietary mutagens and bile salts (secondary bile salts act as promoters), lower the luminal pH (which is felt to be protective) and increase concentrations of luminal short-chain fatty acids, especially butyrate (also considered protective)[7,8].

The evidence that fermentable substrates protect

Generally speaking, the evidence that dietary fibre protects falls into three categories. The first type is epidemiological and has been discussed above. It establishes only an association and not a cause and effect relationship. The second type of evidence concerns mechanisms. That is, ingestion of dietary polysaccharides influences in a seemingly beneficial way, some of the putative protective mechanisms described above. This type of evidence demonstrates that an agent creates favourable conditions without providing the case. The third type of evidence is intervention. It proves cause-effect relationships, may clarify the particular dietary factor responsible and can be targeted at mechanisms, intermediate biomarkers (see below) or tumour endpoints such as adenoma and cancer (depending on whether one is studying animal models or humans).

Animal studies

The rat carcinogen model, especially using dimethyl-hydrazine or azoxymethane, has been used in many studies. The tumours produced are similar to those produced in humans, especially in their localisation to the more distal colorectum. The stages of tumorigenesis are similar in that they involve the hyperproliferation-dysplasia-carcinoma sequence. There are also certain genetic parallels, especially in relationship to mutations in *ras* and *p53*. Mutations of the *apc* gene also cause colorectal cancer in rodents. Studies in the animal model allow direct comparison of agents, evaluation of mechanisms and determination of the stage at which certain dietary factors act.

Generation of butyrate in the colon

We have used the rat model to examine the influence of fermentation-dependent events on colonic tumorigenesis. We developed the butyrate hypothesis[8] as a result of the following: the demonstration that butyrate *in vitro* slows growth of cultured colon cancer cells; the fact that it is generated in mM concentrations in the colon as a result of fermentation; and the fact that it induces expression of differentiation markers in colon cancer cells. This hypothesis states that butyrate is a diet-regulated, "natural", anti-tumour compound at least partly responsible for the anti-tumour effect of dietary fibre. As an initial test of this hypothesis, we needed to define the type of fibre in the rat which effectively delivered high butyrate concentrations to the distal colon, the site at which bowel cancer is most common. A series of experiments demonstrated that this was best achieved by feeding coarse wheat bran (a source of insoluble NSP) rather than guar gum or oat bran (soluble NSP)[9]. Two factors accounted for this: wheat bran pushed fermentation from the caecum further down into the colon, and it was more slowly fermented. In other words, substrate was still available for fermentation in the distal colon when wheat bran was consumed but not when guar gum or oat bran were consumed.

Butyrate generation and carcinogenesis

We then fed and compared diets containing these fibres to rats in which we induced colonic tumours with dimethylhydrazine. These studies demonstrated that rats given wheat bran had significantly fewer tumours than those fed guar gum or oat bran. Importantly, when we used multiple regression analysis to relate fermentation dependent events in the lumen such as butyrate concentration, pH and propionate concentration, we showed a significant negative relationship for butyrate which accounted for 32% of the variance[10]. In other words, the higher the butyrate concentration the lower the number of tumours and the smaller the size. This was the first evidence that *in vivo* butyrate production might be significant in suppressing colorectal tumorigenesis. Subsequently, we have used the animal model to examine the stages of turnorigenesis at which wheat bran exerts its protective effect. This effect is not mediated at the diffuse hyperproliferative phase, the first phenotypically recognisable step, but becomes manifest at the time of formation of focal areas of dysplasia (aberrant crypts)[11].

Butyrate has a range of effects on gene expression which is relevant to colorectal tumorigenesis[8]. It causes hypermethylation of DNA, an important early event. Various oncogenes (*ras, myb, myc*) are down-regulated by butyrate in colorectal cancer cell lines. One of the tumour suppressor genes (*p53*) is also affected. Phenotypic and molecular (alkaline phosphatase) markers of differentiation are also induced. It seems likely then, that butyrate

suppresses certain key molecular events responsible for the formation of clones of dysplastic cells.

Human studies

Of course, it is more difficult to conduct interventional studies in humans which use cancers as an endpoint. Thus, a number of studies have been conducted to examine the effect of wheat bran on adenomas. DeCosse *et al*[12] studied the effect of daily supplementation with 22.5g of wheat fibre from AllBran (Kelloggs) on rectal adenoma formation in 58 patients with familial adenomatous polyposis and rectum intact. They found that fibre consumption reduced adenoma formation by 50% in those who complied with the diet.

A more recent study, The Australian Polyp Prevention Project[13], has examined the effects of wheat bran in patients with sporadic adenomas. This was a randomised trial of low fat (less than 25% of energy), high fibre (25g of wheat bran) and betacarotene (20 mg) in a 2x2x2 factorial design. Four hundred and twenty-four patients with adenomas removed were entered into the study. They commenced the relevant diets and were re-evaluated by colonoscopy at 2 and 4 years. To summarise a large volume of data, the low fat intervention was shown to bring about a significant reduction in the chance of large adenomas occurring (odds ratio = 0.3). The betacarotene produced a trend to increased large adenomas with an odds ratio of 3.0 (confidence interval just overlapping 1). Wheat bran itself produced a trend to reduction of dysplasia in the adenomas (odds ratio = 0.6). Combining the effect of low fat and wheat bran consumption, subjects in this group did not develop any large adenomas at all (P = 0.03). In other words, this dietary lifestyle would appear to be a powerful factor in reducing recurrence of significant adenomas. How much of it is due to butyrate production, is, of course, speculative.

Shortcuts

Studies such as these are difficult, take a long time to achieve an answer, are very expensive, and essentially allow the evaluation of only a few factors. It would be helpful to find more expedient, surrogate markers which allow one to evaluate a range of potential protective approaches. These fall into two categories. They may be mechanistic in the sense that one may conduct interventions and then look to see if the intervention has changed the milieu in such a way that would favour protection. Examples would be faecal water toxicity, luminal or faecal butyrate concentrations and pH. The other approach is to measure effect of interventions on intermediate biomarkers or endpoints, that is, events in tumorigenesis occurring prior to development of adenomas or cancers. The most obvious example is the rate of epithelial proliferation. Diffuse hyperproliferation has been shown to be the first recognisable phenotypic step in colon carcinogenesis. It is present throughout the colon of the majority of patients with cancer and large adenomas. Theoretically, one can administer an agent or a diet and examine to see if the rate of proliferation is reduced. The theory would be that if such a hyperproliferation were reduced, then the agent would be of benefit. Actual proof that this is the case is still lacking.

Mechanistic studies

An example of the mechanistic approach has been provided by Kashtan *et al*[14] who compared the effect of wheat bran versus oat bran consumption on faecal butyrate concentrations in humans. They found that the faecal butyrate concentrations of those on wheat bran were double those on oat bran (P = 0.01). Of course, one must be careful not to over-interpret this finding.

There is a need to find ways of manipulating the colonic milieu using readily acceptable substances. There is an obvious problem with fibre in terms of the food sources and its texture. Various forms of resistant starch can be readily incorporated into foods in a way which tends to disguise them. To determine if resistant starch could alter the colonic milieu in an apparently beneficial way, we conducted a randomised crossover study of high- and low-resistant starch diets for 21 days in 11 volunteers[5]. Volunteers consumed 2g per 420kj of resistant starch, using partly milled wheat, high amylose maize starch and banana flour as sources. Diets were monitored carefully and stools collected for the last 3 days of each dietary period. We found that the high resistant starch diet increased faecal bulk by 25-30%, decreased faecal pH by 0.6 units, increased faecal starch output substantially, and doubled faecal butyrate concentrations and faecal butyrate output. This at least shows potential at a mechanistic level for resistant starch to be useful for protection against colorectal cancer in humans.

Intermediate biomarkers

The only study examining the effect of different dietary fibres on the intermediate biomarker rectal epithelial proliferation, has not yet been published (Macrae et al, submitted). In this study, healthy volunteers were assigned to 6 week periods in which they supplemented their diet with 11g of fibre from either oat bran, wheat bran or AllBran. Rectal biopsies were taken at the end of each dietary period and rectal epithelial proliferation measured by immunohistochemistry for PCNA. None of these diets significantly altered either labelling index or the percentage of labelled cells in the top two-fifths of the crypt. Clearly, further studies are needed in this respect and the predictive value of influences of diet on hyperproliferation remains uncertain. Van Munster *et al*[15] have demonstrated that feeding resistant starch to healthy humans tends to reduce the rate of rectal epithelial proliferation, and does reduce faecal water cytotoxicity.

Summary and conclusions

In summary, the evidence is accumulating that fermentative production of butyrate is a significant mechanism by which dietary polysaccharides protect against colorectal cancer. Clearly, different dietary fibres differ in their ability to generate high concentrations of butyrate in the distal colon where tumorigenesis is most common. As a general rule, insoluble fibres are better than soluble fibres but this does not negate the beneficial effect of soluble fibres. Certainly, resistant starch in humans also

elevates distal colonic butyrate concentrations. Animal studies of tumorigenesis confirm a protective effect by insoluble fibre and indicate that this protection occurs at the stage of formation of focal areas of dysplasia Unfortunately, there are few human interventional studies, design is difficult, and confirmation generally lacking. As a generalisation, there appears to be rather little difference between insoluble and soluble fibres in humans in terms of their effect on the luminal milieu or on epithelial proliferation. Certainly, insoluble fibres decrease adenoma recurrence in patients with familial adenomatous polyposis, and consumption of insoluble fibre in adenoma patients who also reduce their fat intake, appear to be at very low risk for developing recurrence of large (and thus significant) adenomas.

The potential for benefit from resistant starch needs to be pursued and it is important that more research funding be provided for interventional studies aimed at examining questions such as these. The actual role of bacteria in generating a favourable luminal environment needs to be understood. Furthermore, more studies on mechanistic epidemiology are required. In other words, attempts to measure events in the faeces which may be related to high or low incidence of colorectal cancer at a population level are lacking.

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結腸癌的預防：由腸道菌群製造的短鏈脂肪酸的作用

摘要

任何多糖，不管是澱粉還是纖維（如非澱粉多糖）可能被大腸中的微生物區系（厭氧細菌）發酵。短鏈脂肪酸丁酸鹽是在發酵過程中產生的許多產物之一。丁酸鹽在維持正常上皮的生物活性上很重要，這大概是食物纖維預防結腸上皮萎縮的方式，沒有被小腸消化的澱粉（抗消化澱粉 Resistant starch）也預防結腸上皮萎縮。食物纖維的生理化學及生物效應差別甚大。一般來說，抗消化澱粉（Resistant starch）（特別 2 類）的活動傾向像可溶性的而不像不可溶性非澱粉多糖。在人體，抗消化澱粉（Resistant starch）在結腸中產生大量的丁酸鹽，丁酸鹽被顯示在不同的水平（細胞和分子）有「抗腫瘤」作用，這可以解釋澱粉的攝入和結腸癌的發生率之間重要的反比關系（以不同國家為基礎），影響腸道菌群由食物多糖製造丁酸鹽的多種因素的特性需進一步探索，以利其在結腸癌預防上的應用。

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