Effect of different dietary carbohydrates on colon function. Design of healthier foods

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Abstract

The overall objective of the project was to develop a scientific basis for recommending new dietary guidelines and for implementing the production of novel industrially-produced foods containing carbohydrates that have a protective effect on the colonic mucosa.

In particular the project addressed the following aims:

- To investigate the patterns of SCFA formed by fermentation of a variety of complex carbohydrates by the human gut microflora in vitro.
- To assess whether a shift in the composition of the diet from simple sugars (e.g. sucrose) to complex carbohydrates (e.g. starch) has the potential to block steps leading to neoplastic transformation in the colon.
- To investigate, using human volunteers, animal models and in vitro methods, the possible mechanisms (including faecal concentration of short chain fatty acids, long chain fatty acids, bile acids, calcium, bacterial species) underlying the protective effects of selected complex carbohydrates, so that more effective carbohydrate sources can be developed.

The work of the project fell under four main headings:

1. In vitro studies of carbohydrate fermentation by the human gut microflora
2. Animal studies designed to evaluate critically the potential protective role of complex carbohydrates, especially starches, in colon cancer
3. Dietary intervention studies in human volunteers to provide more definitive information on the protective effects of complex carbohydrates on colon damage in man.
4. Development and use of in vitro systems based on isolated colon cells in culture to investigate mechanisms involved and to identify gut luminal factors, particularly gut flora metabolites, with modulating activity towards cytotoxic and genotoxic damage in the gut.
1 Introduction

Epidemiological data suggest that diets rich in lipid and low in starch and fibre may increase the incidence of colon cancer (Cassidy et al 1994). It has been proposed that these correlations of dietary habit and cancer risk are a consequence of some dietary components acting as tumour promoters and others as protective factors. It has been demonstrated that diets deficient in starch, cellulose and calcium increase colon mucosa proliferation, considered to be a marker for increased risk of colon cancer (Caderni et al, 1991; Lipkin, 1988). Furthermore, rats given boluses of sucrose exhibit a burst of cell proliferation in the colon (Stamp et al, 1993). Such observations may have implications for cancer risk in man since most western diets incorporate relatively high amounts of sucrose and processed foods are often supplemented with sucrose and consumed on an empty stomach.

There is therefore, preliminary evidence that the type of carbohydrate in the diet, particularly whether it is a simple sugar or a complex carbohydrate, can have marked effects on the colonic mucosa function possibly influencing colon cancer. Dietary carbohydrates likely to have the greatest effect on the colon are those that are poorly digested in the small intestine and hence pass intact into the large bowel. There are three main types of such carbohydrates (Englyst et al, 1992):

1. **Non-starch polysaccharides (dietary fibre).** These encompass a wide range of polysaccharides including soluble and insoluble fibres (e.g. cellulose, hemicellulose, gums) and pectins and are largely derived from plant cell walls or algae.

2. **Resistant starch.** Most dietary starch is broken down by amylase in the small intestine, but some starches resist digestion as a consequence of their inaccessibility to amylase (e.g. starch in intact seeds and grains), chemical resistance to the enzyme (e.g. raw potato starch and amylomaize starches such as Hylon VII), or because of retrogradation of the amylose polymer caused by successive heating and cooling (e.g. heated and cooled potatoes, and the processed starch CrystaLean used in many of the present studies).

3. **Non-digestible oligosaccharides (NDO).** Some low molecular weight carbohydrates comprising 3 - 10 sugar moieties, such as stachyose raffinose, fructo-oligosaccharides and xylo-oligosaccharides, possess particular glycosidic linkages that are not susceptible to the hydrolytic enzymes in the small bowel, and so pass into the colon (Rumney and Rowland 1995). The xylo-oligosaccharide used in the present project is produced by from xylan by enzymic hydrolysis and is comprised of 2 - 5 xylose units.

It has been proposed that many of the effects of starch and non-starch polysaccharides and NDO on the colon are a consequence of the fermentative activities of the gut microflora. Fermentation of carbohydrates yields short chain fatty acids (SCFA), primarily acetic, propionic and butyric acids which may directly influence the colonic mucosa resulting in changes in cell proliferation rates, apoptosis (programmed cell death of damaged cells) and differentiation (Roediger, 1996; Cummings, 1997). In addition, carbohydrates may alter directly, or indirectly via SCFA formation, the physico-chemical conditions in the gut lumen, e.g. pH, and modify the composition of the microflora and the bacterial synthesis of carcinogens and promoters (Rowland, 1996). Since the various SCFA have very different metabolic fates (e.g. acetate is absorbed and reaches the liver and muscles where it is used as an energy source, whereas butyrate is a preferred energy source of colonocytes and induces cellular differentiation in colon cell lines), the extent of fermentation and pattern of SCFA is likely to be of crucial importance in determining the physiological effects of a particular carbohydrate.
2 Aims and objectives
The overall objective of the project was to develop a scientific basis for recommending new dietary guidelines and for implementing the production of novel industrially-produced foods containing carbohydrates that have a protective effect on the colonic mucosa.

In particular the project addressed the following aims:

- To investigate the patterns of SCFA formed by fermentation of a variety of complex carbohydrates by the human gut microflora \textit{in vitro}.
- To assess whether a shift in the composition of the diet from simple sugars (e.g. sucrose) to complex carbohydrates (e.g. starch) has the potential to block steps leading to neoplastic transformation in the colon.
- To investigate, using human volunteers, animal models and \textit{in vitro} methods, the possible mechanisms (including faecal concentration of short chain fatty acids, long chain fatty acids, bile acids, calcium, bacterial species) underlying the protective effects of selected complex carbohydrates, so that more effective carbohydrate sources can be developed.

3 Results
The work and results of the project can be grouped under four sub-headings:

1. \textit{In vitro} studies of carbohydrate fermentation by the human gut microflora

2. Animal studies designed to evaluate critically the potential protective role of complex carbohydrates, especially starches, in colon cancer

3. Dietary intervention studies in human volunteers to provide more definitive information on the protective effects of complex carbohydrates on colon damage in man.

4. Use of animal models, human volunteers and \textit{in vitro} systems based on isolated colon cells in culture, to investigate mechanisms involved and to identify gut luminal factors, particularly gut flora metabolites, with modulating activity towards cytotoxic and genotoxic damage in the gut.

3.1 \textit{In vitro} studies of carbohydrate fermentation by the human gut microflora
The work on the project has shown that gut bacteria found mainly in the colon, ferment different carbohydrates to varying extents. For example starches resistant to breakdown in the upper gut were less readily metabolized by bacteria than digestible corn starch, although there were considerable differences between individual human volunteers in fermentation capacity. More importantly, the products formed varied depending on the type of carbohydrate, with non-starch polysaccharides such as soy fibre, or apple fibre being fermented mainly to acetate, while substantial amounts of butyrate are produced from starches.

3.2 Animal studies on colon functions related to cancer
The animal studies provided valuable information on the influence of carbohydrate type on various gut microfloras and gut mucosal changes related to carcinogenesis in the colon. Our results demonstrated that boluses of sucrose increased cell proliferation in the colon, but had an irregular influence on aberrant crypt foci (ACF) and did not increase the incidence of tumours in the colon.
ACF are early alterations of the colonic mucosa which appear after the administration of carcinogens to rodents.

In rats colonized with a human gut microflora (HFA rats), both digestible and resistant starches (RS) modified gut physiology and gut microbial metabolism in a potentially beneficial manner and usually the effects were more pronounced in the RS-fed rats. The RS used was the retrograded, high amylose starch ‘CrystaLean’. However, an increase in mucosal cell proliferation was observed in the rats fed RS.

CrystaLean starch-fed rats exhibited less carcinogen-induced DNA damage in the colon than those fed sucrose, digestible starch or soy fibre. Overall, the results suggested therefore that CrystaLean may suppress the initiation phase of carcinogenesis (genotoxicity) but increase cell proliferation.

Other studies conducted as part of the project indicated protective effects of starchy foods. For example, a long-term colon cancer bioassay in rats provided evidence that pasta reduced the incidence of colon tumours by comparison to sucrose-fed animals.

3.3 Dietary intervention studies in human volunteers

A dietary intervention study in human volunteers indicated that over a long term, a diet rich in starch reduced colon mucosa proliferation. The effect of a high intake of starch on proliferation was not dependent on the contemporary reduction of simple carbohydrates. The study also demonstrated that a short-term (three weeks) reduction in dietary intake of sucrose was ineffectual in reducing colon mucosa proliferation. There were however changes in the faecal microbial flora associated with the low sucrose diet, in particular an increase in lactobacilli and a decrease in the anaerobe/aerobe ratio. The study also identified \textit{L. acidophilus} and \textit{Bifidobacterium breve} as marker organisms characteristic of subjects on the low sucrose diet.

3.4 Investigations of mechanisms involved in protective effects of carbohydrates.

It seemed likely that the influence of carbohydrates, particularly starches and dietary fibres, on the colon mucosa proliferation and damage, was mediated by SCFA. This hypothesis was tested in this project in a series of \textit{in vitro} experiments, studies in animal models and in patients with intestinal disease.

\textit{In vitro} studies into the modulating effect of SCFA on induction of DNA damage in colon cells, demonstrated that n-butyrate and acetate reduced the level of damage, induced by \textit{H}_2\textit{O}_2. In contrast, propionate and i-butyrate had no protective effects. It should be noted that n-butyrate and acetate are utilised best as energy sources by the colon cells. It was also found that protection against DNA damage was apparent in cells exposed to mixtures of SCFA characteristic of starch fermentation, but not with SCFA mixtures typical of soy or apple fibre fermentation.

There was evidence from \textit{in vitro} studies in human colon cell lines that n-butyrate could induce apoptosis in the cells. Apoptosis is a mechanism by which damaged cells are removed from a tissue and is considered to play a protective role in colon cancer by eliminating cells which might otherwise develop into tumours.

A further protective mechanism that seemed feasible from our studies was the induction in colon cells by SCFA of the protective enzyme glutathione transferanse (GST) which detoxifies foreign compounds.
Evidence that SCFA can modulate cell proliferation in the human colon came from studies in ulcerative colitis (UC) patients who have an elevated colon cancer risk. The high rate of proliferative activity in these patients was decreased after SCFA enemas. In contrast, familial polyposis (FAP) patients, who also exhibit increased cancer risk, seemed refractory to these treatments. Slow-release butyrate pellets, which could provide a more acceptable means for delivering SCFA via the oral route were tested in a rat model and found not to affect colonic proliferation and ACF formation and progression. It may still however be worth testing the pellets in UC patients, in which a beneficial effect of SCFA has been demonstrated.

Overall, the results do not support the hypothesis that sucrose is a risk factor in colon carcinogenesis. However, starchy foods, as opposed to sucrose, appear to be protective. The increase of the content of complex carbohydrates in the diet is, potentially, an effective and cheap dietary variation that may assist in the reduction of colon cancer risk. Caution needs to be exercised however in relation to resistant starch, which exhibited both beneficial and adverse effects on parameters associated with colon carcinogenesis.

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References