Evidence for the Putative Health Benefits of Prebiotic Oligosaccharides Involved in the Prophylactic Management of Gut Disorder: Mechanisms and Human Data

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In recent times, the activities of the human gut microbiota have been more fully elucidated. Whilst it is apparent that certain species may be involved in gut disorders like ulcerative colitis, bowel cancer, acute enteritis and pseudomembranous colitis (1), there has been much momentum for dietary approaches that modulate the gut flora composition towards improved health (2). Mainly historical associations have been made between probiotics and gastrointestinal improvements, but there is a very rapidly developing functional food sector in Europe based on food ingredients containing prebiotics. These are non-viable food components that are selectively fermented in the colon (by a 'beneficial' but not detrimental flora). In many cases, literature on the effects of these ingredients in human studies is sparse, and our mechanistic understanding inadequate. However, the prebiotic approach may be a very straightforward route for preventative management of gut disease. The aim of this article is to take a critical view of the proposed mechanisms for the health promoting effects of prebiotics and to overview recent human studies in the area. It is not our aim to provide a comprehensive review of the literature, but rather to focus on relevant recent research reports, concentrating on human trials and mechanisms of effect.

Background to the prebiotic concept

The human colon is an immensely complex microbial ecosystem containing several hundred bacterial species (3). Within this diversity it is possible to recognise bacteria with potentially positive or negative effects upon human health (Fig. 1). The concept that diet can shift the balance away from undesirable micro-organisms towards physiologically positive species is not new (Fig. 1). To this end, there is a thriving

functional food industry based around the concept of including lactic acid bacteria (probiotics) into foods such as yoghurt and other fermented milks. There are, however, concerns about the survival of such micro-organisms during processing, storage and passage through the alimentary system. For example, gastric acid, bile salts and pancreatic secretions are all barriers towards long-term probiotic persistence in the gut. Moreover, in the colon they then face a huge indigenous micro-flora with which they have to effectively compete if any advantageous properties are to ensue.

Whilst certain probiotic strains may be robust enough to overcome some or all of these confrontations, they are bound to be few in number and perhaps compromised in terms of activity. An alternative approach towards improved microflora modulation is therefore to intake carbohydrates that resist digestion in the stomach and small intestine, reach the colon intact and are then selectively metabolised by (beneficial) bifidobacteria and/or lactobacilli. This is known as the prebiotic approach (4). In Europe, there are four prebiotics commonly used in foods: inulin, fructo-oligosaccharides (FOS), lactulose and galacto-oligosaccharides (GOS). In Japan, the situation is more widespread with many oligosaccharides being incorporated into foods for their prebiotic effects (5). For a summary of *in vitro* and *in vivo* trials with prebiotics see (6). It can be said with some confidence that good prebiotics exist, and are being developed, that have selective effects on the gut microbiota composition. A key question remains on the health bonuses however. Four promising areas of importance have arisen:

The barrier effect against GI pathogens

One of the most mechanistically strong health benefits proposed for prebiotics is the barrier function against invading gastrointestinal pathogens. If particular, this may be a successful way of prophylactically addressing the burden of microbial food safety through tackling problems such as campylobacters, salmonellae and *E. coli*.

Proposed mechanisms. There are several prospective mechanisms for the inhibition of pathogens by bifidobacteria and lactobacilli (Fig.2). Fermentation of carbohydrates results in the production of short-chain fatty acids (SCFA) (4). These reduce lumenal pH in the colon to below levels below those at which pathogens such as *E. coli* can effectively multiply. In addition, increased populations of bifidobacteria and lactobacilli can compete with other organisms for nutrients and receptors on the gut wall (7). Probiotics (the target microorganisms for prebiotic intake) can also inhibit pathogens via a more direct mechanism. They are known to produce antimicrobial agents active against a range of pathogens (8). Whilst it is not recognised whether such metabolites function effectively in the human gut, their powerful effects have led to their widespread as food preservatives (9).

Probiotics are also reputed to modulate the activities of the immune system, resulting in a non-specific enhancement of immune function. In particular, encouraging results have been obtained with both lactobacilli (10) and bifidobacteria (11) against rotaviral infection in infants. Fortification of indigenous probiotics, by efficient prebiotic use, should have similar effects.

Human studies. It is, of course, impossible to collect human data on the probiotic barrier against infection with pathogens through challenge-type testing. Most fermentation studies in this area are therefore carried out using *in vitro* models of the human gut (12) or animals (13). Both have limitations, but do generate useful mechanistic data relevant to the situation for humans. Whilst bacterial pathogenesis in humans is difficult to predict, certain situations like antibiotic associated diarrhoea and gastrointestinal problems suffered, by frequent travellers, seem good avenues for prebiotic use. Moreover, our own recent data have exploited the use of a rhesus monkey colony to infect animals with enteropathogenic *E. coli* (14). The experiments were carried out using placebo and blind control, with genotypic probes for the bacteriology. In essence, some protection against diarrhoea was seen in the presence of bifidogenic substrates. These type of studies need to be taken further through human trials which apply sound genomic principles to the bacteriology (15).

Protection against colon cancer

Possible mechanisms. The most likely means by which prebiotics could influence the development of bowel cancer is by modulation of the colonic flora. Prebiotics are fermented to organic acids, and in some cases this includes butyrate (4, 16). Butyrate inhibits apoptosis (17) and is thought to be protective against colon cancer.

Many faecal micro-organisms produce carcinogens and tumour promoters from dietary and other components entering the colon (Fig. 3). In addition, several enzymic activities, associated with faecal bacteria, produce toxic or carcinogenic products from substrates entering the colon (Table 1). The microbial species responsible for these

activities have not been unambiguously identified beyond certain *Bacteroides* spp. and *Clostridium* spp. It is known, however, that bifidobacteria and lactobacilli do not have such capabilities and a reduction in these activities can be demonstrated in faeces from humans or rats fed lactic acid bacteria or prebiotics (18,19).

Human studies. Much *in vivo* data on the protective effects of prebiotics on colon cancer come from animal studies where, for example, inulin has been shown to inhibit formation of aberrant crypt foci (20). Human studies are few in number and tend to focus on faecal markers of carcinogenesis rather than being epidemiological in nature. Human data from healthy volunteers are summarised in Table 2. In three out of the four trials, a decrease in several markers of carcinogenesis was seen. In two of these, significant increases in bifidobacteria were also observed, with no microbiology being carried out in the third.

A recent study however (24), found no significant changes in bifidobacteria or in markers of carcingenesis. These results might, at first sight seem anomalous, as the test substrate (GOS) have been found to be prebiotic in many studies in the past (25). However, starting populations of bifidobacteria in the volunteers was rather high (9.2-9.4 log). It has been noted before (26, 27) that the magnitude of the response to prebiotics by bifidobacteria depends upon the starting levels. It appears that there is a maximum level of bifidobacteria (about 10 log values) achievable in the human gut. If populations are already at or near this level, then little or no further increase in numbers is generally seen. This is an important point and is currently the subject of further research. The implication is that a healthy diet supporting a balanced

gastrointestinal microflora may not further benefit from prebiotic functional foods (24).

Improved calcium absorption

There has been increasing interest in recent years in the possibility of increasing mineral (particularly calcium) absorption through the consumption of prebiotics. Although the small intestine is the principle site of calcium absorption in humans, it is thought that significant amounts are also absorbed throughout the length of the gut, consequently, a maximising of colonic effects is desirable.

Possible mechanisms. Several mechanisms have been postulated for increased calcium absorption as induced by prebiotics (28, Fig. 4), although it is far from clear at the present time, which (if any) actually operate *in vivo*.

i) Fermentation of prebiotics such as inulin results in a significant production of SCFA leading towards a reduction in lumenal colonic pH. This is likely to increase calcium solubility and overall levels in the gut.

ii) Phytate (myoinositol hexaphosphate) is a component of plants that reaches the colon largely intact (29). It also forms stable, insoluble complexes with divalent cations, like calcium, rendering them unavailable for transport. Fermentation results in the bacterial metabolism of phytate thereby liberating calcium.

iii) It is postulated that a calcium exchange mechanisms operates in the colon. In this system, SCFA enter the colon in a protonated form and then dissociate in the

intracellular environment. The liberated proton is then secreted into the lumen in exchange for a calcium ion.

Human studies. Numerous animal studies have indicated that prebiotics increase absorption of calcium from the colon thereby decreasing losses from bone tissue (30). Very few human studies have been carried out, however. In one such study, the feeding of 40 g inulin day^{-1} for 28 days to nine healthy subjects resulted in a significant increase in calcium absorption (31). A more realistic 15 g inulin, FOS or GOS day^{-1}, when fed to 12 healthy subjects for 21 days resulted in no significant effect on the absorption of calcium or iron (32).

In a more recent study, 12 adolescent boys (aged 14-16) were fed 15 g FOS day^{-1} for nine days in a placebo controlled trial against sucrose (33). The data showed a 10.8% increase in calcium balance with no significant effect on urinary excretion.

Effects on blood lipids

There is intense interest in the food industry in developing functional foods to modulate blood lipids such as cholesterol and triglycerides.

Possible mechanisms. The mechanisms suggested by which prebiotics may influence blood lipids are summarised in Fig. 5. There is evidence that FOS decreasesthe *de novo* synthesis of triglycerides by the liver. The means by which this occurs is not fully understood but the effect appears be exerted at the transcriptional level. It is also

possible that prebiotics (such as inulin) can modulate insulin-induced inhibition of triglyceride synthesis (34).

Effects on serum cholesterol levels have also been postulated for prebiotics, although the mechanism is more difficult to envisage. It has been suggested (for a review, see 34 and 35) that propionate produced by the bacterial fermentation of prebiotics inhibits the formation of serum LDL cholesterol. The difficulty with this hypothesis is that bacterial fermentation of prebiotics generally produces much more acetate than propionate. Moreover, acetate is a metabolic precursor of cholesterol and may therefore tend to increase, not decrease, serum levels. A more likely role for the gut microflora in the reduction of cholesterol is direct metabolism of cholesterol by colonic bacteria, or its conversion to other metabolites such as coprostanol (36). The evidence for this is not presently well clarified however.

Human studies. Human studies on the lipid lowering properties of prebiotics when consumed at a realistic (tolerable) dose are not clear-cut (37). These can be divided into trials carried out on subjects with hyperlipidaemia and on normal subjects (Table 3). Hitherto, data indicate that there may be a significant effect on blood triglycerides but not cholesterol in normal subjects, although three of the five studies did not show any effect. For subjects with elevated lipid levels, however, there does seem to be a useful decrease in cholesterol levels. It would seem to be of high priority to carry out more research in this area – especially now that reliable methods for tracking microbiota changes through molecular procedures are available (45).

Concluding remarks

Prebiotics have a long history of use in Japan, but the market for prebiotics as food ingredients in Europe is also now established (46). There has been a tendency in the past for unsubstantiated health claims to be made for functional foods and it is essential, if we are to have confidence in the protective effects of prebiotics, for any claims to be based on rigorous science - preferably carried out in humans. To date, there have been a few well-designed volunteer studies, and these have sometimes given contradictory conclusions. One problem with evaluation of the effects of prebiotics lies is the difficulty of identifying faecal micro-organisms using conventional culture-based approaches. It is estimated that a large percentage of the total faecal microflora has yet to be described and is probably unculturable (47). The advent of molecular methods of bacterial identification have undoubtedly improved this situation (45).

Given the significance of the human gut microbiota and its activities (the colon is the body's most metabolically active organ, 48), it seems a very reasonable approach to advocate dietary modulation by prebiotics. At present, we are at the stage where efficacious forms exist and can be made to operate in the food matrix (49). The health benefits that have been suggested are varied but also very important. In addition to good human volunteer studies we also need to enhance our mechanistic understanding of the health effects of prebiotics. Progress is being made in this area and it is to be expected that the prebiotic approach to prevention of disease will be have a much stronger foundation. This will lead to better informed decisions by clinicians, nutritionists and consumers.

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Table 1. Faecal bacterial enzymes producing carcinogenic or toxic products

ENZYME	SUBSTRATE
β-glucosidase	Plant glycosides (e.g. rutin, cycasin)
Azoreductase	Azo compounds (e.g. benzidines)
Nitroreductase	Nitro compounds (e.g. nitrochrysene)
β-glucuronidase	Biliary glucuronides (e.g. benzidine)
IQ hydratase-dehydrogenase	2-amino-3-methyl-3H-imidazo (4,5-*f*) quinoline (IQ)
Nitrate/nitrite reductase	Nitrate, nitrite

Table 2. Human studies investigating the anti-cancer effects of prebiotics

Prebiotic	Subjects and study design	Dose (g day^{-1})	Increase in bifidobacteria	Markers of carcinogenesis	Reference
FOS	20, placebo-controlled, 12 days	12.5	1.2 log	No change in bile acids, neutral sterols, nitroreductase, azoreductase, or β-glucuronidase	21
FOS	12, controlled diet, control and treatment periods, 26 days	4	0.5 log	Significant decreases in β-glucuronidase (75%) and glycocholic acid hydroxylase (90%)	22
Resistant starch (RS)	12, controlled diet, high and low RS periods, 28 days	55.2 ± 3.5 and 7.7 ± 0.3	Not investigated	Significant decreases in neutral sterols (30%), 4-cholesten-3-one (36%), total bile acids (30%), secondary bile acids (32%) and β-glucosidase activity (26%) in high RS phase compared to low RS phase.	23
GOS	40, placebo-controlled, 21 days	7.5 or 15	No significant increase	No significant changes in SCFA, bile acids, ammonia or skatoles.	24

Table 3. Effects of prebiotics on blood lipids

Reference	Date	Prebiotic	Dose	Duration	Effect		
					TG	LDL-Ch	Ch
Hyperlipidaemic subjects							
38	1984	FOS	8 g/day	14 days	NS	-10%	-8%
39	1998	Inulin	18 g/day	6 weeks	NS	-14%	-8.7%
Normal subjects							
40	1995	Inulin	9 g/day	4 weeks	-27%	-7%	-5%
41	1996	FOS	20 g/day	4 weeks	NS	NS	NS
42	1997	Inulin	14 g/day	4 weeks	NS	NS	NS
43	1998	Inulin	10 g/day	8 weeks	-19%	NS	NS
44	1999	Inulin	15 g/day	3 weeks	NS	NS	NS
44	1999	FOS	15 g/day	3 weeks	NS	NS	NS
44	1999	GOS	15 g/day	3 weeks	NS	NS	NS

NS: no significant; TG: triglycerides; LDL-Ch: LDL-cholesterol; Ch: cholesterol