The SYNCAN project: goals, set-up, first results and settings of the human intervention study

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Experimental evidence on the anticancer properties of dietary prebiotics such as chicory inulin and oligofructose and dietary probiotics has accumulated in recent years. Various experimental models ranging from chemoprevention studies, tumour implantation models to genetically modified mice models, etc. have systematically shown the protective effects of these food ingredients. In some studies it appeared that synbiotics (combination of pre- and probiotics) exerted synergistic activity against processes of carcinogenesis. The logical next step in research was to find out if these observations also would be valid for human volunteers. This was the principal goal of the EU-sponsored SYNCAN project (QLK1-1999-346) which involved the integration of an *in vitro* study to select the most suitable synbiotic preparation, the application of this synbiotic in an *in vivo* rat model of chemically induced colon cancer, and, as the heart of the project, the investigation of the synbiotic effects in a human intervention study. The *in vitro* tests consisted of fermentation studies where the interaction of pre- and probiotics was studied. Cell-free supernatants were generated from various synbiotic combinations fermented by faecal slurry, which were then used to optimise a series of bioassays. In the rat study the anticarcinogenic effect of prebiotics and synbiotics but not of probiotics was demonstrated. Using tissue samples generated in this model, attempts were made to gain a better insight into the mechanisms underlying cancer development. The human intervention study consisted of two groups of volunteers. One group was composed of people at high risk (polypectomised subjects) for colon cancer and the other of volunteers (colon cancer subjects) who had previously undergone 'curative resection' for colon cancer but were not currently receiving treatment. The present paper describes the experimental design of the SYNCAN study, and demonstrates a functional effect of the synbiotic preparation (probiotic survival during gastrointestinal transit and modification of the intestinal flora). Detailed experimental outcome of the human intervention study will be reported elsewhere.

Colon cancer: *Lactobacillus*: *Bifidobacterium*: Inulin: Oligofructose: Man

Prebiotics are food ingredients that cannot be degraded by intestinal and pancreatic digestive enzymes, and as such are completely available for fermentation by the intestinal flora. An additional requirement for a product to be a prebiotic is that it has to be selectively fermented, so that the composition of the intestinal flora improves (a relative increase of saccharolytic flora often accompanied by a decrease in proteolytic fermentation; bifidobacteria and lactic acid bacteria are indicator organisms). The modification in both the composition and the metabolic activity of the intestinal flora is the basis of the nutritional benefits of prebiotics. These beneficial effects include improving bowel habit and mineral absorption, modulating lipid metabolism and the immune system, and retarding the process of carcinogenesis.

Salminen *et al.* (1999) have defined probiotics as 'microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well-being of the host.' Although not always essential, it may be desirable that probiotics are viable in order to exert their beneficial nutritional effects during their passage through the intestinal tract. Additionally, they need to be able to survive exposure to gastric juice and bile acids in the upper gastrointestinal tract. Some of the beneficial activities associated with the consumption of probiotics include immune stimulation, reduced incidence of intestinal infection and in some cases an inhibitory effect on carcinogenesis.

Over the past decade, a solid basis of evidence for the anticarcinogenic effects of prebiotics and probiotics has been built up. The anticancer properties of prebiotics or probiotics have been shown by means of various models.

In chemoprevention models, laboratory animals are challenged by injection of a carcinogenic chemical such as azoxymethane (AOM) or 1,2-dimethylhydrazine to induce colon cancer, or 4-methyl nitrosourea to induce breast cancer in female test animals. Typically, animals are administered either a control diet or a diet containing the prebiotic substances (e.g. inulin, oligofructose 5–10 % added to the diet), or the probiotic bacteria (10^9–10^{10} cfu/d) or a combination of the two, described as a synbiotic. The main end point of these experiments is the formation of preneoplastic lesions (after 2 months) or tumours (after 35–52 weeks). In other cases, the growth of previously implanted tumours can be monitored as a function of time (volume is measured with a Vernier calliper). From such experiments there is consistent evidence that inulin-type fructans (Reddy *et al.* 1997; Rao, 2001; Verghese *et al.* 2002), some probiotics (Kulkarni & Reddy, 1994; Gallaher *et al.* 1996) and certain synbiotic combinations (Rowland *et al.* 1998; Bolognani *et al.* 2001) are able to significantly reduce (up to 80 %) the number of preneoplastic lesions (aberrant crypt foci or mucin-depleted foci), as well as the number of tumours. Synbiotics are not the only combinations to have shown improved

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anticancer activity as it was also shown that a combination of prebiotics which differently influence colonic fermentation (i.e. oligofructose and long-chain inulin known as oligofructose-enriched inulin or Synergy1®) showed a stronger anticancer potential (Verghese *et al.* 2003; Van Loo, 2004). A significant retardation of the development of chemically induced mammary tumours has also been reported with inulin or oligofructose (Taper & Roberfroid, 1999).

Another carcinogenesis model is the tumour implantation model where aggressive and invasive tumour cells are implanted either in the muscle or in the peritoneum of mice. The animals are either fed control or prebiotic inulin-type fructans supplemented food 1–2 weeks before the tumour implantation and mortality curves are monitored. Histological evaluation is used to determine the metastatic potential and the size and type of tumour. Dietary inulin and oligofructose were shown to significantly reduce the development of the tumours, with, as a consequence, an increase in life span (Taper *et al.* 1998). The tumours that still developed were less invasive in the prebiotic fed group (Taper & Roberfroid, 2000).

Genetically predetermined models (APC^{min} mice) spontaneously develop tumours, mainly in the small intestine but also in the colon. Experiments with this model have shown that consumption of oligofructose reduces the number of colonic tumours (Pierre *et al.* 1997). A new Western style diet designed by Lipkin and supplemented with oligofructose and inulin was fed to APC^{min} mice and it has been reported that the mice developed significantly less tumours in both the small intestine and in the colon (M Lipkin, unpublished results). Probiotics have also been shown to reduce tumour incidence in genetic models. Consumption of *Lactobacillus salivarius* ssp. *salivarius* UCC118 in IL-10 knockout mice (colitis model) was associated with a trend towards reduced tumour development and attenuation of gastrointestinal inflammation. Gastrointestinal neoplastic transformation was evident in 50 % of mice from the placebo group while only 10 % of the probiotic group demonstrated any evidence of neoplastic change (O'Mahony *et al.* 2001).

These and other similar experiments show an interesting potential for prebiotics and/or probiotics. As it is difficult to extrapolate results from experimental rodent models to man, it was necessary to verify if these food ingredients also have a potential to reduce risk for colon cancer in human volunteers. The SYNCAN project was designed to investigate whether or not this is the case (Van Loo & Jonkers, 2001). In the present paper, the strategy of the SYNCAN project is laid out. Published data from experimental models are summarised and the setting of the human intervention study is explained. The experimental outcome and interpretation of the human intervention study will be published elsewhere.

Materials and methods

Prebiotics and probiotics

The prebiotic used in the SYNCAN project was a combination of fructan fractions with different fermentation characteristics known as oligofructose-enriched inulin or 'Synergy1' (Raftilose®Synergy1 from Orafti, Belgium). One fraction was composed of linear β(2-1) fructans with a degree of polymerisation (DP) ranging between 10 and 65 (average DP was 25) and the other fraction contained similar molecules but with chain length varying between 2 and 8 (average DP is 4). The latter molecules are rapidly fermented by the colonic flora

leading to modification of the composition of the intestinal flora in a proximal part of the colon. The longer chains are typically fermented at a slower rate, but still in a selective way. This longer chain fraction has the ability to maintain the metabolic activity of the improved flora for a longer period of time, or spatially, in more distal parts of the intestine. In the experimental models it was shown that this mixture of inulin-type fructans could reduce the incidence of aberrant crypt foci significantly more than its individual components alone (Verghese *et al.* 2003). It was also thought that a cocktail of probiotics would be more efficient than a single strain. From a practical point of view it was decided to take existing commercial strains with a history of anticancer properties in experimental models. The selected strains were *Lactobacillus rhamnosus* GG called 'LGG' (from Valio, Finland) and *Bifidobacterium lactis* Bb12 called 'Bb12' (from Christian Hansen Labs, Sweden).

Results

In vitro *fermentation studies*

The *in vitro* studies included fermentation of various combinations of the prebiotics, probiotics and synbiotics either alone (pure culture of bacteria) or in the presence of faecal slurry as inoculum. Control fermentations (prebiotics replaced by glucose and no added probiotics) were included. Those fermentations were performed either as batch fermentations or as three-stage continuous fermentations mimicking the three compartments of the colon (McBain & Macfarlane, 1997). All fermentations were carried out in anaerobic conditions and with controlled pH and temperature.

Cell-free supernatants of such fermentation broths were applied to two colon cancer cell lines (HT29 and CaCo-2) and cellular parameters of survival, differentiation, tumour progression and invasive growth were monitored. Interestingly, samples that originated from fermentations with prebiotics alone or synbiotics were significantly less cytotoxic than control fermentation supernatant (based on glucose as C source). The observed lower toxicity could be correlated with relative higher butyrate concentration in the prebiotic fermentations. Samples originating from the three-stage gut model showed that the third compartment, which had the highest concentration of butyrate, also had the lowest cytotoxic potential. These data thus support the hypothesis that effects of oligofructose-enriched inulin on colon cells could be due to an altered microflora, producing higher amounts of butyrate. However, it is likely that this is not the only parameter that can explain the observations. Further research involving bacteria–host interactions might give more insight in these matters. This *in vitro* part of the SYNCAN project gives a rational support to the prebiotic and/or synbiotic approach to reduce the risk of colon cancer development (Klinder *et al.* 2003).

In vivo *animal study*

The *in vivo* animal study was designed to compare the effects of the probiotics, the prebiotics and the synbiotic combination in a model of carcinogenesis. As intestinal and immunological tissues are more readily available than in human volunteers, this experimental approach was also taken to help elucidate the possible mechanisms of action of prebiotics, probiotics and synbiotics.

Four groups of twenty-five rats were fed control diet, prebiotic diet (control diet + 10 % Synergy1), probiotic diet

(control diet + 10^8 cfu/g Bb12 and LGG) or the synbiotic diet (Fig. 1). After 10 d of adaptation to the diets the rats were injected twice with 15 mg AOM/kg body weight with 1-week interval. Tumour incidence was determined after 31 weeks. The results show that the prebiotic and the synbiotic diets, but not the probiotic diet, significantly reduced the tumour incidence in the colon. The synbiotic-fed group had fewer tumour-bearing rats than the two other groups (prebiotic and probiotic). In the prebiotic- and synbiotic-fed groups, the concentration of SCFA was almost twice that in the control and the probiotic groups; the molar ratio of butyrate also was significantly higher in the prebiotic- and synbiotic-fed groups (Femia *et al.* 2002). This was consistent with the possible protective effect of butyrate observed in the *in vitro* part of the project.

Faecal water (FW), which is the aqueous phase of faeces, contains most of the free reactive and soluble factors thought to interact with the mucosa *in vivo*. The cytotoxic and genotoxic potential of FW is considered a measure of the potential of the dissolved factors to induce damage in the colonic mucosa. FW was extracted from the faecal matter of the rats and was applied *in vitro* to cell lines in culture. FW derived from prebiotic- or synbiotic-treated animals had a significantly lower genotoxic potential in human cell lines when compared to the FW from the control group. The genotoxic potential of FW was determined using the single cell gel electrophoresis or COMET assay (Klinder *et al.* 2004). The overall genotoxic potential of tumour-bearing rats was significantly higher than that of non-tumour-bearing rats, independent of the diet that was used. The correlation between genotoxic activity in faeces and tumour incidence indicates that reducing the genotoxicity of the faecal or caecal water might be another mechanism of chemoprevention of carcinogenesis. Prebiotic- and synbiotic-induced colonic fermentation reduced the exposure of the gut mucosa to genotoxins.

In healthy rats (not treated with carcinogenic AOM), oligo-fructose-enriched inulin mainly interacted with the gut-associated lymphatic tissue. IL-10 production in the Peyer's patches was enhanced and the secretory IgA production was stimulated in the caecum as compared to the control group that received a standard, non-sup'plemented diet. Immune markers were not significantly modified in probiotic (LGG and Bb12)-fed animals. The synbiotic combination, however, increased secretory IgA production in the ileum and natural killer cell activity in peripheral blood, while it reduced the oxidative burst activity of blood neutrophils as compared to the probiotic-fed rats. The synbiotic combination of probiotics and prebiotics does not simply result in an additive effect (Roller *et al.* 2004*a*).

In a model in which carcinogenesis was induced with AOM, the functioning of the immune system was suppressed. When AOM-treated rats were fed a prebiotic (oligofructose-enriched inulin) or a synbiotic (oligofructose-enriched inulin + LGG and Bb12) the immunosuppressive effect of AOM in spleen, mesenteric lymph nodes and Peyer's patches was counterbalanced (levels became similar as in control group). No such effects were observed with the probiotic combination used in this study.

Prebiotic and synbiotic treatment reduced the tumour incidence (Femia *et al.* 2002). This anticarcinogenic effect could be correlated with a stimulation of natural killer cytotoxicity and increased IL-10 production in the intestine's Peyer's patches (Roller *et al.* 2004*b*).

Human intervention study

Design

The third and ultimate part of the SYNCAN project was the human intervention study – pilot study.

A common criticism of probiotic research is that the fate of the introduced bacterium in the gastrointestinal tract is not always investigated. It is important to confirm that introduced probiotic strains transit the gastrointestinal tract so that any observed effect may be associated with probiotic consumption. In the human intervention study subjects consumed wild-type probiotic
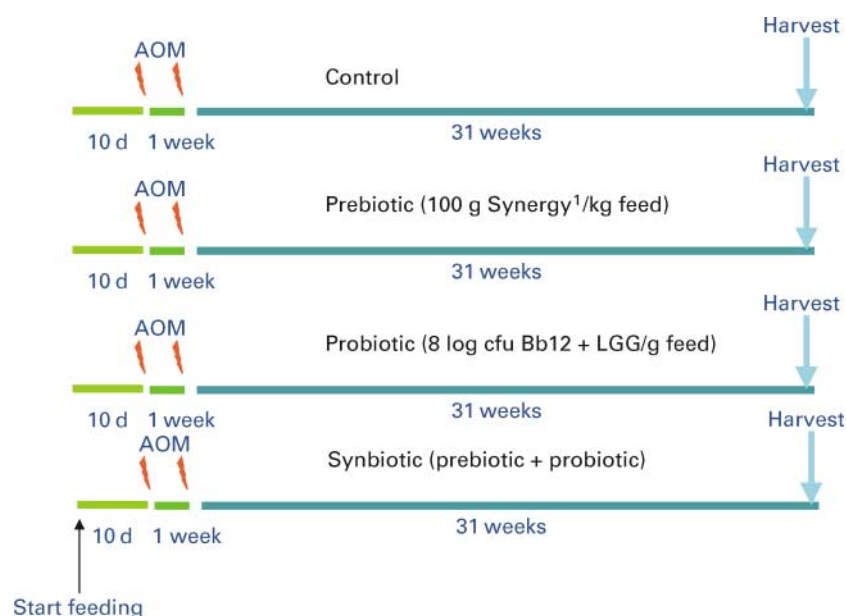
Fig. 1. Experimental design of the long-term chemoprevention anticancer model where rats (*n* 25) were injected twice with azoxymethane (AOM) with 1-week after a 10 d adaptation period to the diets. Colon tumour incidence is counted 31 weeks later.

strains, which cannot be selectively isolated from the faeces. In order to confirm that LGG and Bb12 strains could survive the transit through the human gastrointestinal tract we performed a pilot study with antibiotic-resistant strains in three healthy volunteers.

Spontaneously occurring rifampicin-resistant (rif^R) mutants were selected from Bb12 and LGG. Previous experience in humans demonstrated that such rif^R strains were not present in stool samples, and that rifampicin resistance is a suitable marker to allow selective isolation and enumeration of introduced probiotic strains from faeces (Dunne *et al.* 1999, 2001). The rifampicin-resistant strains were freeze-dried and the resultant freeze-dried product was checked for purity.

Briefly, a 1·5 l fermenter containing modified MRS broth [0·4 % yeast extract, 2 % glucose, 0·2 % K$_2$HPO$_4$, 0·5 % Na-acetate, 0·2 % (NH$_4$)$_3$ citrate, 0·02 % MgSO$_4$, 0·0046 % MnSO$_4$ and 1·8 % peptone] for LGG rif^R or modified MRS broth containing 0·05 % cysteine for Bb12 rif^R was inoculated overnight with cultures of rif^R strains. Fermentation was carried out for 16 h at 37°C and constant pH with stirring. The suspension was centrifuged, the pelleted bacteria resuspended in 10 % skim milk, 2 % sucrose and ascorbic acid and frozen at −80°C for at least 1 h. The bacteria were freeze-dried for 48 h and viable bacteria per gram of freeze-dried product were enumerated by spread plating appropriate dilutions on MRS agar (LGG) and modified raffinose bifidobacterium agar (Hartemink *et al.* 1996), where raffinose was replaced with lactose, for Bb12. The number of viable bacteria in the freeze-dried product was found to be 5·9 × 10^{10} cfu/g in the case of Bb12 rif^R and 4·7 × 10^{10} cfu/g in the case of LGG rif^R. The freeze-dried rif^R strains and the wild-type parent strains were found to be identical using API (a rapid biochemical test).

In the SYNCAN project, it was considered desirable that the probiotics reach the colon in a viable state in order to exert possible effects. As the acidity in the stomach is a main reason for bacteria not to survive passage through the upper intestinal tract, the probiotics were administered in a coated capsule. The coating resists the acid conditions in the stomach, and only opens up in the small intestine. The selected probiotic strains have previously been shown to resist the bile acids that are secreted in the small intestine. The freeze-dried strains were therefore encapsulated and three healthy volunteers consumed one capsule containing 10^{10} cfu of both LGG rif^R and Bb12 rif^R with a sachet of oligofructose-enriched inulin (10 g) for a

7 d period. The volunteers provided faecal samples before feeding and at the end of feeding. The number of rif^R bacteria present in faeces before and after 7 d of feeding was enumerated using standard plate count techniques.

Total stool from one passage was collected in a plastic container and mixed using a sterile wooden spatula. An aliquot was used to make a faecal slurry with sterile 60 % PBS/40 % glycerol/0·05 % cysteine which was serially diluted 10-fold in sterile PBS/0·05 % cysteine. Appropriate dilutions were spread-plated on the media described previously.

The goal was to recover at least 10^5 cfu of each strain/g of faeces, a level that was presumed to be the lowest active dose and to correspond to an acceptable 0·1 % survival (oro-anal transit) of the bacteria based on the calculation that in volunteers receiving 10^{10} cfu/d and producing on average 100 g stool/d, 10^8 cfu would have to be recovered if the viability had been 100 %. In this study on average 4·17 × 10^6 and 2·65 × 10^7 cfu of rif^R LGG and rif^R Bb12, respectively, were recovered per g of faeces (Fig. 2) indicating that 1–10 % of the probiotics survived passage through the gastrointestinal tract, which is considered a high survival rate. This experiment was carried out in the presence of the prebiotic. These results indicate that the probiotics when administered as a synbiotic combination are able to transit the human gastrointestinal tract and reach the colon in a viable state and in sufficient numbers to potentially exert a beneficial effect.

Human intervention study

A 12-week randomised, double-blind, placebo-controlled trial of a synbiotic food supplement for reduction in cancer risk biomarkers was carried out in The Mercy University Hospital, Cork, Ireland. The synbiotic product contained a probiotic mixture of *L. rhamnosus* GG (LGG) and *B. lactis* Bb12 (Bb12) and the prebiotic oligofructose-enriched inulin. This study was evaluated and approved by the Cork University Hospitals Ethics Committee and the subjects gave their informed consent.

A group of volunteers at increased risk for colon cancer (polypectomised) and (colon cancer subjects who had previously undergone 'curative resection' for colon cancer) were selected. A total of eighty subjects (forty-three polyp, thirty-seven cancer) who fulfilled the inclusion and exclusion criteria (Table 1)
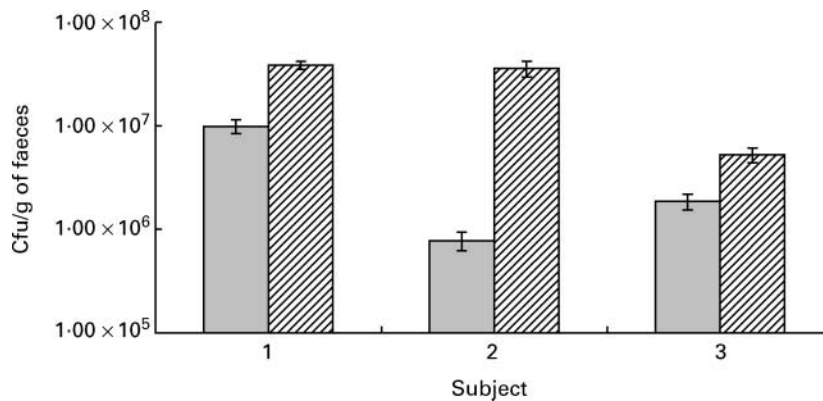

Fig. 2. Demonstration of the high viability of rifampicin-resistant *Lactobacillus rhamnosus* LGG (■) and *Bifidobacterium lactis* Bb12 (▨) recovered from faeces in three healthy volunteers after daily consumption of 10^{10} cfu of these bacteria for 7 d. Mean values with standard error of the mean represented by veritcal bars.

were recruited for the intervention. Overall nineteen cancer and twenty-one polyp volunteers received placebo (maltodextrin), while eighteen cancer and twenty-two polyp volunteers received the synbiotic combination. The outcome of the various mucosal, immunological and FW parameters will be reported elsewhere.

The outline of the study was as follows:
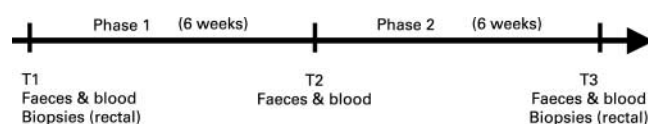
The subjects attended appointments with the study nurse at T1 (before intervention), T2 (after 6 weeks intervention) and T3 (after 12 weeks intervention). Blood and faecal samples were obtained at T1, T2 and T3. Rectal biopsies were obtained by sigmoidoscopy performed by surgical registrars at T1 and T3. Subjects were randomly assigned to two groups. One group received the synbiotic product [encapsulated probiotic bacteria (10^{10} cfu LGG and 10^{10} cfu Bb12) and a 10 g sachet of prebiotic oligofructose-enriched inulin]. The other group received the placebo product (encapsulated maltodextrin and a 10 g sachet of maltodextrin). Subjects kept a 6-week diary for each phase of the intervention. At T1 and T2 the subjects received a box containing sufficient product for 6 weeks. At T2 and T3 subjects were interviewed by the study nurse, and reactions to the product, medications taken and any adverse events, which had occurred in each 6-week period, were recorded.

Subjects provided samples at three time points. Faecal samples were provided at the beginning of intervention (week 0), midway through intervention (week 6) and at the end of intervention (week 12) for assessment of biomarkers and faecal flora. For the latter, total stool from one passage was collected in a plastic container and mixed using a sterile wooden spatula. An aliquot was used to make a faecal slurry with sterile 60 % PBS/40 % glycerol/0·05 % cysteine. Faecal slurries were stored at −80°C until analysis, thawed, and serially diluted 10-fold

in sterile PBS/0·05 % cysteine and either spread-plated or pour-plated in appropriate dilutions on suitable media. Colony identities were confirmed by Gram stain. Microbial numbers were calculated as colony forming units per gram of wet weight of faeces.

Blood samples were provided at the beginning of intervention (week 0), midway through intervention (week 6) and at the end of intervention (week 12) for assessment of immune markers and cholesterol measurements.

Rectal biopsy samples were taken at the beginning of intervention (week 0) and at the end of intervention (week 12) for investigation of genotoxic damage, mucosal cell proliferation and gene expression.
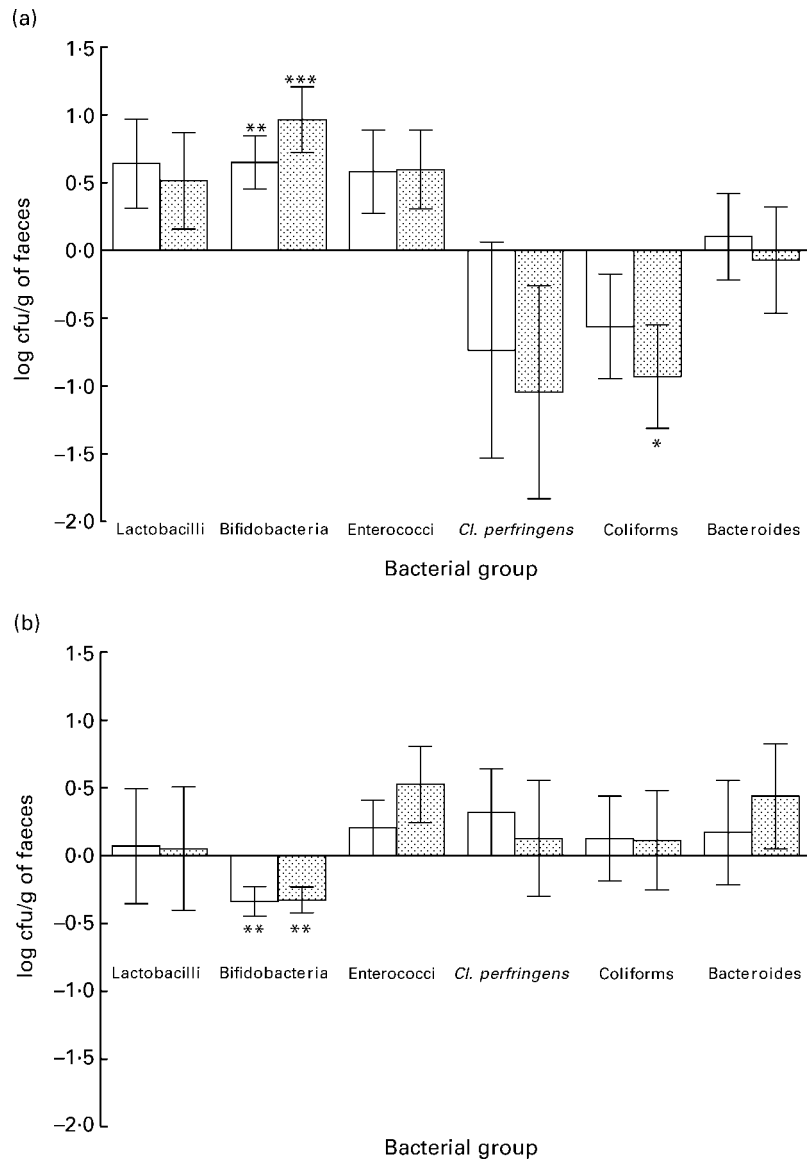
The study was carried out in a double-blinded manner. Subjects were coded from Syn-01 to Syn-80 and each time point was coded X, Y or Z. Coded samples were processed and transported to partners involved in biomarker assessment in batches as subjects completed intervention to ensure that analysis of samples was also carried out in a blinded manner. For the duration of the study the code was stored by one individual and following completion of all analyses was distributed to all partners.

Results

Faecal flora.   The ability of the synbiotic combination to modulate the gut flora was demonstrated in both the cancer and the polypectomised subjects (Fig. 4). The prebiotic effect was also confirmed as prebiotics have been reported to enhance the growth of bifidobacteria and other Gram-positive bacteria, while remaining unfermented by the majority of Gram-negative bacteria in the colon. The consumption of probiotics may also have contributed to the increase in bifidobacteria and lactobacilli. The viable faecal counts of the ingested probiotics (5–7 log cfu), however, were up to three orders of magnitude lower than the total *Bifidobacterium* and *Lactobacillus* count in the faeces of the volunteers (8–10 log cfu).

Bifidobacterium in both polypectomised and cancer patients consuming synbiotic significantly increased, as did the *Lactobacillus* in the polypectomised patients (*Lactobacillus* was increased at T2 in the cancer group although not significantly $P = 0.067$), after a period of 6 weeks and of 12 weeks intervention (compared to T0 of each group). Previously, Kruse *et al.* (1999) reported that the prebiotic effect of inulin-type fructans lasted for 2 months (9 weeks). In the control groups the bifidobacteria and lactobacilli either remained unaffected, or even decreased (cancer patients) during the intervention period. In the groups consuming synbiotics the benefit of consuming prebiotics and probiotics together was proven. The selectivity of fermentation, which is the main characteristic of the prebiotic effect, was shown by the bifidogenic effect, which was accompanied by a decrease of coliforms in both polypectomised and cancer patient groups and a decrease of (potentially pathogenic) *Clostridium perfringens* in the polypectomised group. The selectivity of the interaction of synbiotics is further demonstrated by the lack of effect on *Bacteroides* populations.

These results thus demonstrate that the synbiotic preparation administered to volunteers modulated the flora. Thus, it can be hypothesised that the eventual changes in biomarkers monitored in the human anticancer study in test groups *v.* control group

Table 1. Inclusion and exclusion criteria for human volunteers in the SYNCAN dietary intervention study

Inclusion criteria
  Subjects must, within the last 5 years, have
    had biopsy- and histologically confirmed adenomatous polyps, or undergone resection for histologically confirmed colon cancer
  Subjects with polypectomy and resected colon carcinoma may be enrolled both retrospectively and prospectively
Exclusion criteria
  Subjects over 75 years of age.
  Pregnancy or a desire to become pregnant during the study period
  Subjects with known lactose intolerance and subjects with clinically significant immunodeficiency
  Subjects who are considered to be poor clinic attendees or unlikely for any reason to be able to comply with the trial
Concomitant drug usage
  Subjects will be excluded if they require cyclooxygenase-2 inhibitors or other selected non-steroidal anti-inflammatory drugs
  Subjects who have been on antibiotics within the previous month or are likely to require antibiotics during the trial period will be excluded
  Subjects with additional gastrointestinal disorders, (e.g. Crohn's disease or ulcerative colitis)
  Malignancy or any concomitant end-stage organ disease

Fig. 3. Demonstration of the efficacy of the synbiotic mixture to modulate the gut flora (a), compared to placebo (b), in cancer patients after 6 weeks (□) and 12 weeks (▨) of intervention compared to week 0. Mean values with standard error of the mean represented by vertical bars. *Bifidobacterium* and *Lactobacillus* increased, *Clostridium* and coliforms decreased and *Bacteroides* were unaffected. Mean values were significantly different compared to week 0: (*$P = 0.01$ to 0.05, **$P = 0.001$ to 0.01, ***$P < 0.0001$.

Discussion and conclusions

The *in vitro* and *in vivo* animal studies have shown that the chosen synbiotic (Synergy1/LGG/Bb12) can modulate the genotoxic and cytotoxic potential of cell-free fermentation supernatants and reduce tumour incidence in AOM-induced carcinogenesis in rats. Moreover, the analysis of various markers showed reduced genotoxicity, increased caecal SCFA particularly butyrate, priming of the immune system to be more efficient to fight cancer cells and to counteract inflammation, and a reduction in DNA damage. These concurred with earlier reported anticancer activities of prebiotics, probiotics and synbiotics, and confirmed that the hypothesis 'are synbiotics

are likely to be the consequence of the administration of an active synbiotic preparation.

relevant to fight cancer in human volunteers' could be investigated using the selected synbiotic preparation in a human dietary intervention study.

The description of the experimental design of the human study in the SYNCAN project shows that all conditions were met to generate objective results, in optimal conditions for the synbiotics (both probiotics and prebiotics were shown to be functional) to exert their possible anticancer effects. The results and the discussion of the results of the project are not fully completed at the time of publication of present paper. They will be published in the near future.

However, if the synbiotic approach is proven to suppress the carcinogenic process, then future research should focus on elucidating the effect of the prebiotic (oligofructose-enriched inulin or Synergy1) and the probiotics separately. Understanding these mechanisms will help in optimising the use of prebiotic, probiotic
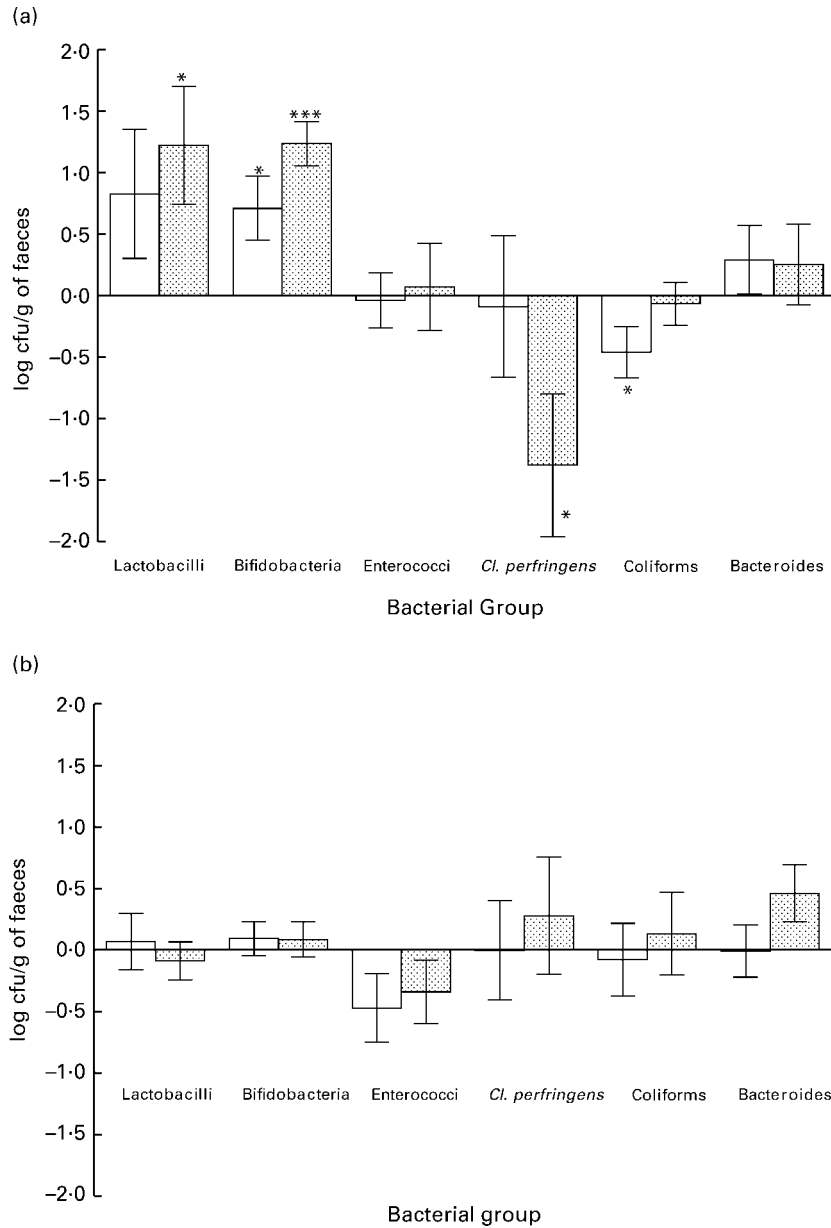
Fig. 4. Demonstration of the efficacy of the synbiotic mixture to modulate the gut flora (a), compared to placebo (b), in polypectomised patients after 6 weeks (□) and 12 weeks (▨) of intervention compared to week 0. Mean values with standard error of the mean represented by vertical bars. *Bifidobacterium* and *Lactobacillus* increased, *Clostridium* and coliforms decreased and *Bacteroides* were unaffected. Mean values were significantly different compared to week 0: *$P = 0.01$ to 0.05; **$P = 0.001$ to 0.01; ***$P < 0.0001$.

or synbiotic approaches to fight against cancer in early phases. To prevent is better than to heal.

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