Gut Fermentation Products of Inulin-type Fructans Modulate the Expression of Xenobiotic-metabolising Enzymes in Human Colonic Tumour Cells

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Abstract. *Epidemiological studies suggest that nutrition plays an important role in colonic cancer prevention. A possible mechanism of this prevention may be the modulation of carcinogen metabolism and scavenging of reactive intermediates. In particular, dietary fibres are discussed as potentially protective food ingredients. Therefore, the aim of this study was to analyse if the expression of genes related to biotransformation is modulated by fermentation samples of dietary fibres. HT29 (human colonic carcinoma) and LT97 (human adenoma) cells were incubated with fermentation supernatant (SFS), produced by in vitro fermentation of inulin enriched with oligofructose. Possible mechanisms of detoxification were investigated by analysing the expression of catalase and glutathione-S-transferase (GST) A4 mRNA and the enzyme activity of catalase and GST. In addition, protection of both cell lines against DNA damage, induced by H_2O_2 or 4-hydroxynonenal (HNE), was analysed using the comet assay. Incubation with SFS resulted in significantly increased GSTA4 mRNA expression, significantly enhanced catalase activity and a significant reduction in the amount of H_2O_2-induced DNA damage in HT29 cells. Our results show that complex fermentation samples of inulin-type fructans favourably modulate expression of genes related to biotransformation in carcinoma cells, which in turn supports the important role of dietary fibres in primary chemoprevention.*

There is increasing evidence that human cancer can be prevented not only by avoiding exposure to carcinogens but also by favouring the intake of protective factors that can modulate defence mechanisms of the host organism. This preventive strategy, referred to as chemoprevention, can be pursued either by means of pharmacological agents or by dietary factors (1). One of the cancer forms that could be successfully prevented, especially by dietary ingredients, is colorectal cancer (CRC), because there is evidence for an inverse association of CRC with a high intake of dietary fibres (2, 3). One hypothesis explaining this inverse-association states that dietary fibres are fermented by gut flora to yield short-chain fatty acids (SCFA) which induce glutathione-S-transferases (GSTs), and protect the cells from the genotoxic activity of compounds such as 4-hydroxynonenal (HNE) and hydrogen peroxide (H_2O_2) (4).

An imbalance caused by overproduction of oxidants leads to oxidative stress, resulting in damage to large biomolecules such as lipids, DNA and proteins (5), which in turn increases the risk of degenerative diseases such as cancer. Free radicals such as H_2O_2, organo-peroxides, superoxide anions (O_2^-) and hydroxyl radicals (OH•) are generated in biological systems, not only by extrinsic factors, for example, ionising radiation, but also by intrinsic factors such as by-products, emerging during oxygen metabolism or cellular redox reactions (6). According to generally-accepted mechanisms, major deleterious effects are caused by OH radicals generated from H_2O_2 and by O_2^- in the presence of redox active transition metals (7). A favourable balance of biotransformation enzymes, including a lower level of various phase I enzymes, *e.g.* cytochrome *P450* monooxygenases, and reinforced activity of phase II enzymes, such as glutathione-S-transferases (GSTs) or UDP-glucronosyl transferases, might protect target cells from mutations (8). According to Wattenberg, this mechanism of action has been defined as "blocking agent activity" (9).

Since Burkitt's pioneering research that pointed to an inverse relationship between colonic cancer risk and consumption of fibre-rich foods, many studies have tested this hypothesis (10, 11). It has been proposed that amongst other possible mechanisms, dietary fibres protect against colon cancer by stimulating the growth of *Bifidobacterium* spp. and

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Key Words: Dietary fibres, HT29, LT97, colonic cancer, biotransformation.

lactobacilli which may reduce the survival of pathogens by enhancing competition for nutrients and production of SCFA (12, 13). Butyrate, a major SCFA, is considered to be beneficial due to its trophic effects as an essential nutrient for the colonic epithelium (14). Previous studies have also shown that pre-incubation of colonic cells with butyrate significantly reduced the genotoxic effects of H_2O_2 (15).

Among various dietary fibres, inulin and oligofructose have been subjected to extensive research (2, 16). Inulin-type fructans are the natural constituents of a wide range of common vegetables and fruits, such as onion and garlic (17). Structurally, inulin-type fructans are polydisperse carbohydrates consisting mainly of β-(2-1)-fructosyl-fructose links (16). This linkage cannot be hydrolysed by pancreatic or brush-border digestive enzymes. Therefore these non-digestible dietary fibres reach the colon where they are fermented by *Bifidobacterium* spp. and other lactic acid-producing bacteria (16). Fermentation products of inulin-type fructans, mainly SCFA, have been found to alter the expression of genes related to drug metabolism and stress response (8, 18), but overall, little is known about the response of tumour cells to genotoxic agents and the modulation of this response through complex fermentation samples. Therefore, we investigated a fermentation sample of Synergy1® (inulin enriched with oligofructose) using human adenoma and carcinoma cells representing early and late stages of carcinogenesis. The analysed markers of chemoprotection included modulation of mRNA expression of *catalase* and *GSTA4*, which are phase II enzymes of biotransformation and de-toxify many carcinogens in the body (19, 20). In addition, the aim was to understand the functional consequences of modulated gene expression by analysing protective effects of SFS against H_2O_2- or HNE-induced DNA damage by the comet assay. H_2O_2 and HNE were used as model substances and have high reactivity towards large biomolecules such as DNA and lipids and are substrates for various enzymes, including catalase and GSTs, in the body (14, 21). Therefore, the inhibition of DNA damage may reflect activities of SFS related to inhibition of tumour initiation.

Materials and Methods

In vitro fermentation of Synergy1®. The carbohydrate source used in the fermentation experiments was the fructan Synergy1®, a commercially-available 1:1 mixture of inulin (22) enriched with oligofructose (ORAFTI, Tienen, Belgium). The fermentation of this inulin-type fructan mixture was conducted *in vitro* under anaerobic conditions (80% N_2, 10% CO_2, 10% H_2) at 37˚C in a batch-culture system with faecal inocula from three different donors (23). Fermentation was performed at 37˚C for 24 h, generating an aqueous fermentation supernatant which was centrifuged at 6000 ×g for 30 min and sterilized by filtration and stored at –80˚C until use. A negative control containing only the faecal samples was prepared as fermentation blank. Based on the no-effect concentration in cell proliferation assays and present knowledge of the physiological detectable range (24), a 5% solution of fermentation supernatant (SFS) and fermentation blank (FB) in DMEM was prepared immediately before use.

Cell lines and culture conditions. Passages 20-30 of HT29 (obtained from American Tissue; Rockville, MD, USA) culture collection and 25-35 of LT97 cells (obtained from Institute of Cancer Research, University of Vienna, Austria), were used for the experiments (25, 26). HT29 cells were maintained in (DMEM) (Invitrogen, Darmstadt, Germany) supplemented with 10% (FCS) whereas LT97 cells were maintained in MCDB 302 (Biochrom, Berlin, Germany) containing 20% L15 Leibovitz medium, 2% FCS, 0.2 nM triiodo-L-thyronine, 1 μg/ml hydrocortisone, 10 μg/ml insulin, 2 μg/ml transferrin, 5 nM sodium selenite, 30 ng/ml epidermal growth factor (EGF) and 50 μg/ml gentamicin. The medium was changed at regular intervals of 2-3 days and the cells were cultivated in a humidified incubator (95% O_2 and 5% CO_2) at 37˚C. At regular intervals, a mycoplasma test based on quantifying the luminescence of ATP produced by conversion of ADP to ATP catalysed by mycoplasma enzymes (MycoAlert™ Detection Kit) was performed and contamination with mycoplasma was excluded.

Real-time qPCR analysis. mRNA expression of GSTA4 and Catalase were determined by real-time qPCR by incubating HT29 and LT97 cells for 24 h with 5% of SFS and FB, respectively, in 6-well plates. Total RNA was isolated from HT29 and LT97 cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, then dissolved in 30 μl RNase-free water and stored at –80˚C. The integrity of the RNA was checked using an Agilent Bioanalyser 2100 (Agilent Technologies, Waldbronn, Germany). The RNA integrity number (RIN) values ranged from 9-10 in all the samples. Purity and concentration of RNA was additionally assessed photometrically using a Nanodrop® ND-1000 instrument (Peqlab Biotechnology, Erlangen, Germany). The expression of genes was analysed by real-time qPCR using an iCycler IQ thermocycler (Biorad GmbH, München, Germany). Two microgrammes of total RNA were subjected to reverse-transcription (Superscript II, First strand cDNA Synthesis System; Invitrogen, Karlsruhe, Germany) with oligo (dT) 12-18 primers, according to the manufacturer's instructions. The cDNA was diluted 1:50 and used in 25 μl PCR amplification reaction containing 2×iQ SYBR green super Mix (Bio-Rad Biotechnologies) (100 mM KCl, 40 mM Tris HCl (pH 8.4), 0.4 mM each dNTP, 50 U/ml Taq DNA polymerase, 6 mM $MgCl_2$, SYBR Green I, 20 nM fluorescein, stabilizers) and 10 pmol-gene specific primers for the target genes and reference gene Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The following primer sequences were used to amplify a region of *CAT*, *GSTA4* and *GAPDH* mRNA: iCAT_F: 5' TGG ACA AGT ACA ATG CTG AG 3', iCAT_R: 5' TTA GGA TGA ACG CTA AG 3', iGSTA4_F: 5'CCG GAT GGA GTC CGT GAG ATG G 3', iGSTA4_R: 5'CCA TGG GCA CTT GTT GGA ACA GC 3', iGAPDH_F: 5'CCA CCC ATG GCA AAT TCC ATG GC 3', iGAPDH_R: 5'AGT GGA CTC CAC GAC GTA CTC AG 3'.

PCR cycles included 1 cycle at 95˚C for 2 min, followed by 40 cycles each at 95˚C for 30 s, 60˚C for 30 s and 72˚C for 40 s and a final extension step of 72˚C for 10 min. Product-specific amplification was confirmed by melting curve analysis. All

experiments were performed in triplicates. The fluorescence threshold value (CT) was calculated by the iCycler iQ optical system software. The relative quantification of the target mRNA expression was calculated with the comparative $\Delta\Delta C_T$ ($\Delta\Delta C_T = \Delta C_{T\ control} - \Delta C_{T\ experiment}$) method. For normalization, ΔC_T values were calculated by subtracting the average of the C_T value for the reference gene in the control from the average of the C_T value for the target gene and subtracting the average of the C_T value of the reference gene in the treated sample from that of the target gene. The difference between the ΔC_T values of the control and treatment ($\Delta\Delta C_T$) was then calculated. The fold change was calculated according to the efficiency method, where it is assumed that the PCR efficiency is 100% (E=2; fold change $= E^{difference}$) (27).

Determination of enzyme activity. GST activity: For the determination of total GST activity, cytosols were prepared as described elsewhere (28). In brief, the cell suspension was homogenised for 1 min at approximately 40 W with ultrasound (Bandelin Electronics, Berlin, Germany). The cytosols were obtained by centrifugation (16000 ×*g* for 60 min and 4˚C). The GST activity was measured photometrically at 340 nm using 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 1 mM glutathione as substrates according to Habig *et al*. (29). The values are expressed as nmol/10^6 cells since this was considered to be more accurate than nmol/μg protein, because environmental factors can enhance general protein content in parallel with induction of GSTs (30), thereby falsifying the results.

Catalase activity: Catalase activity was assayed photometrically at 25˚C by following the extinction of H_2O_2 at 240 nm, as described elsewhere (31). Assay mixtures contained 10 mM H_2O_2 and 100 μl of cytosol in 50 mM potassium phosphate buffer at pH 7.0. Enzyme activities were calculated using 0.0394 mM^{-1} cm^{-1}, as absorption coefficient of H_2O_2 at 240 nm.

Determination of DNA damage using the comet assay. To analyse effects on DNA damage, the cells were seeded in 6-well plates and were incubated with 5% of SFS and FB for 24 h at 37˚C after reaching a confluency of 50-60%. The pre-treated cells were washed with Phosphate Buffer Saline (PBS), trypsinised, and subsequently incubated with 75 μM H_2O_2 (diluted in PBS) for 5 min at 4˚C or with 300 μM HNE for 30 min at 37˚C, respectively. Cell viabilities were determined by CASY® cell counter (Roche Innovatis AG, Mannheim, Germany), and the remaining cells were mixed with low-melting agarose and distributed on frosted microscopical slides followed by distribution of another layer of agarose. Further steps were carried out as described elsewhere (4). In short, the cells were lysed for 60 min at 4˚C and subjected to alkaline conditions for 20 min then electrophoresis was carried out at 25 V and 300 mA. Subsequently the slides were neutralised with PBS and stained with SYBR Green (Sigma-Aldrich GmbH, Steinheim, Germany). Tail intensities were quantified using an image analysis system and the software Comet Assay IV of Perceptive Instruments (Halstead, UK) evaluating 150 cells per sample. Data are presented as the means±SD of three independently reproduced experiments.

Statistical evaluation. Means and standard deviations were calculated from at least three independently-reproduced experiments. Differences were calculated by two-sided unpaired *t*-test.

Results

Effect of SFS incubation on mRNA expression of GSTA4 & Catalase. LT97 and HT29 cells were incubated with 5% SFS and FB to determine the effects on gene expression of *GSTA4* and *CAT* using real-time qPCR. The effects on gene expression are shown in Figure 1 A and B (LT97) and C and D (HT29), respectively. In LT97 cells, the expression level of *CAT* was increased (fold-change=1.33), whereas the expression of *GSTA4* decreased insignificantly (fold change=0.65), in comparison to the medium control. In HT29 cells, the expression of *CAT* was also increased without reaching significance (fold-change=1.85). However, the expression of *GSTA4* was significantly enhanced after incubation of HT29 cells with SFS and FB (fold-change of 2.44 and 3.49, respectively). Thus, the effects of fermentation products were stronger for modulating *GSTA4* expression, and the changes were more distinct in HT29 carcinoma cells. Interestingly, the FB also significantly increased *GSTA4* gene expression in HT29 cells when compared to SFS (unpaired *t*-test).

Effects on GST activity. One possible mechanism by which SFS could reduce the genotoxic effects of carcinogens is an enhancement of GST activity. Therefore the effect of 5% SFS on GST enzyme activity was measured. The basal activity (in medium control) of GSTs in LT97 cells was much lower than that in HT29 cells. However, there was only a small trend ($p>0.05$) for an increased GST activity resulting from incubation of both cells lines for 24 h (Figure 2) with SFS. Additionally, FB had no impact on GST activity.

Effects on catalase activity. Another possible mechanism by which SFS might reduce genotoxicity could be an enhancement of catalase activity. Compared to medium control and FB, SFS treatment resulted in a significant two-fold induction of catalase activity ($p<0.05$) in HT29 cells (Figure 3). Although there was a trend ($p>0.05$) for an increased catalase activity in LT97 cells, this effect was not significant. The incubation with FB control, however, had no effect at all in either of the cell lines.

Protection against carcinogen-induced DNA damage. We determined the anti-genotoxic effects by pre-treating the cells with 5% SFS for 24 h and subsequently challenging with two genotoxic agents, namely H_2O_2 and HNE (substrates for catalase and GSTA4 enzymes). Cells pre-incubated with medium were used as untreated controls. Figures 4 and 5 show that both HNE and H_2O_2 were clearly genotoxic without any indication of concomitant cytotoxicity (cell viability determined by CASY cell counter was always >80%).

Pre-incubation of cells with 5% SFS resulted in a substantially reduced level of H_2O_2-induced DNA strand-breaks (tail intensity of 36% in medium control and 27% in

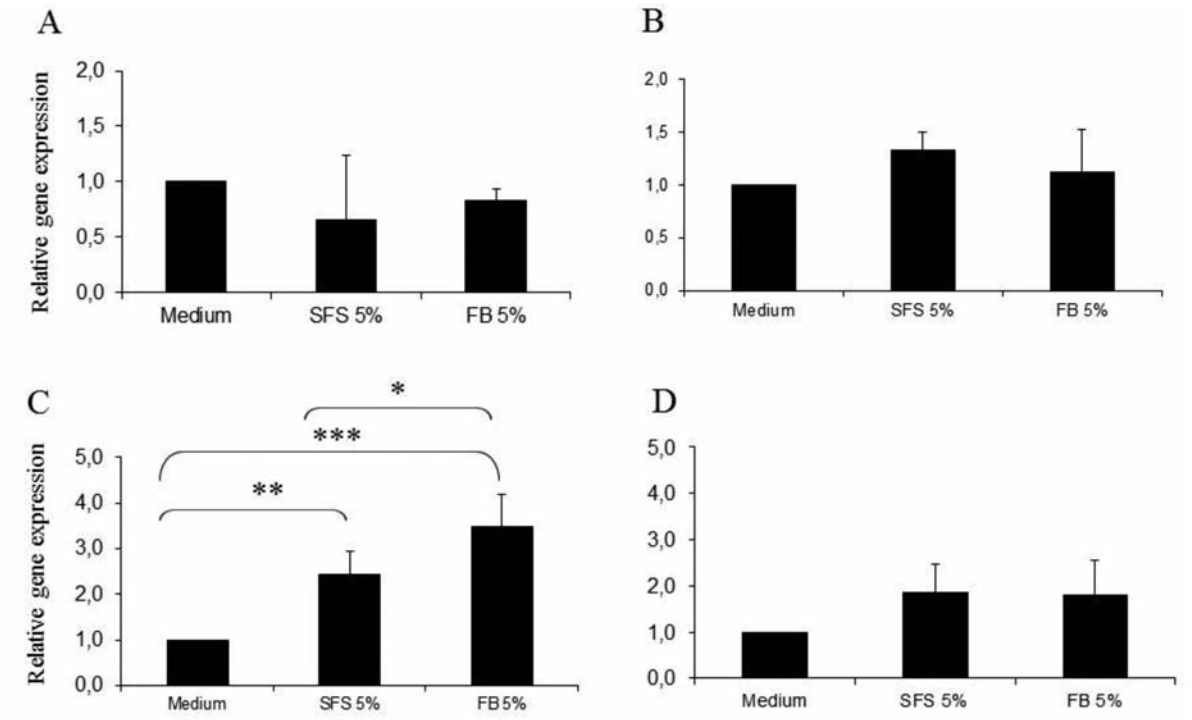

Figure 1. *Expression of (GSTA4) (A, C) and (CAT) (B, D) genes in LT97 (A, B) and HT29 (C, D) after incubation with Synergy fermentation supernatant (SFS) and fermentation blank (FB) analysed with real-time qPCR. The means and SD of three independently-reproduced experiments are shown. Statistical variation was calculated by t-tests. *p<0.05, **p<0.01, ***p<0.001.*

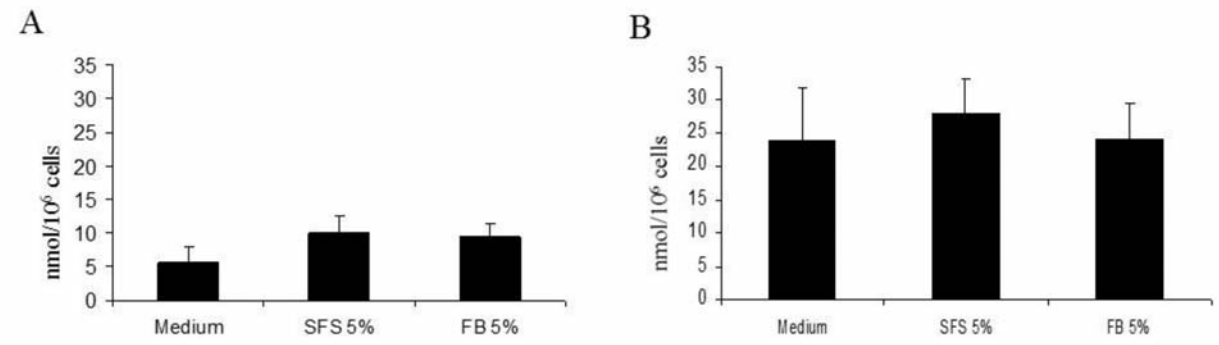

Figure 2. *GST activity/106 cells for LT97 (A) and HT29 (B) cells after incubation with Synergy fermentation supernatant (SFS) and fermentation blank (FB) for 24 h. The means and SD of three independently-reproduced experiments are shown. Statistical variation was calculated by t-test.*

FS-treated cells) in HT29 cells when compared to medium control and FB control, whereas this effect was not significant in LT97 cells (Figure 4). In contrast to the results with H_2O_2, incubation with 5% SFS was unable to modulate the genotoxic effects of HNE (Figure 5).

Discussion

The progression of CRC is a complex multistep process involving specific molecular genetic alterations in tumour suppressor genes, proto-oncogenes, and genes encoding proteins for DNA repair (32). The colon is especially susceptible to dietary oxidants and antioxidants that can be liberated after fermentation by the gut flora (33). It has been shown that gut flora-mediated fermentation of dietary fibres can protect colonic cells from genotoxic insults by elevating phase II de-toxification (blocking agent activity) (18). These 'blocking activities' lead to reduced exposure to genotoxic risk factors, either by inhibiting the formation of harmful compounds by scavenging reactive intermediates, or by

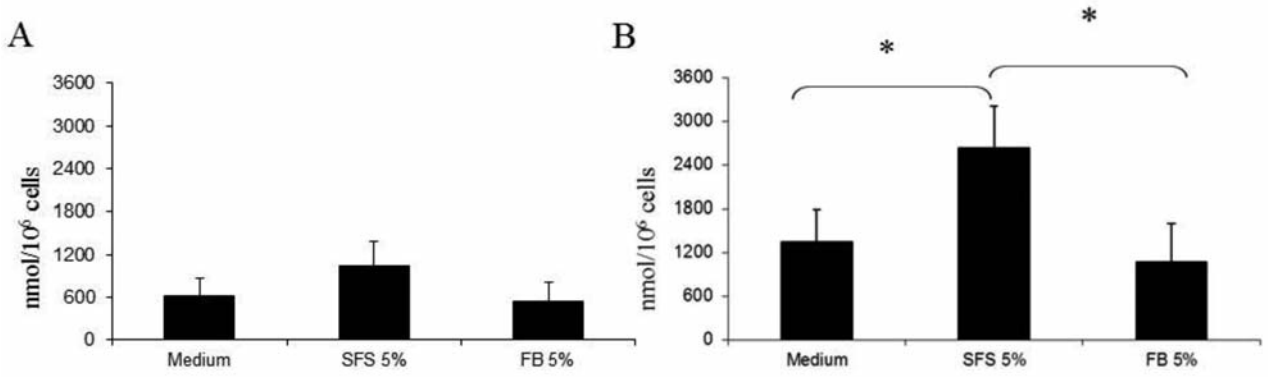

Figure 3. *Catalase activity of LT97 (A) and HT29 (B) cells after incubation with Synergy fermentation supernatant (SFS) and fermentation blank (FB) for 24 h. The means and SD of three independently reproduced experiments are shown. Statistical variation was calculated by t-tests *p<0.05.*

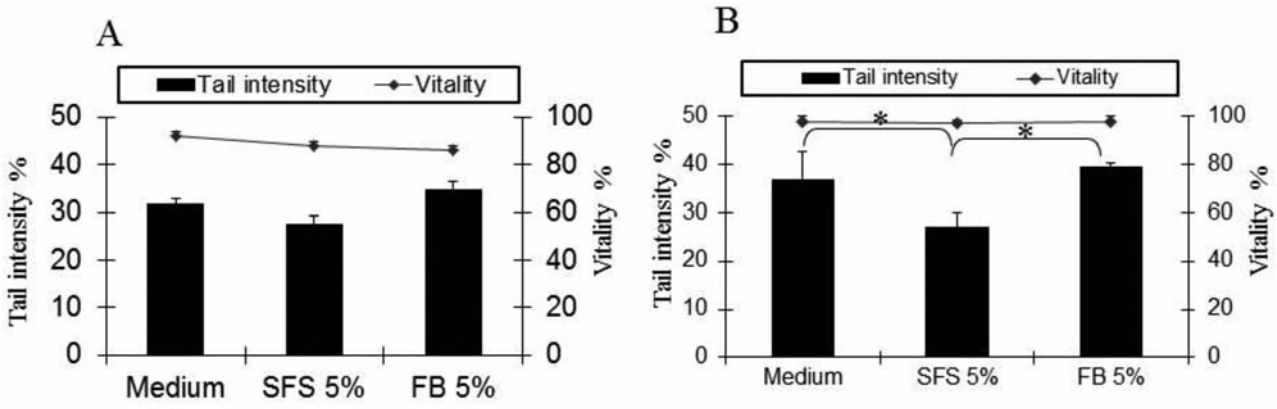

Figure 4. *H_2O_2-induced DNA damage (75 μM H_2O_2) after pre-treatment of LT97 (A) and HT29 cells (B) with Synergy fermentation supernatant (SFS) and fermentation blank (FB) for 24 h. The means and SD of three independently-reproduced experiments are shown. Statistical variation was calculated by t-tests *p<0.05.*

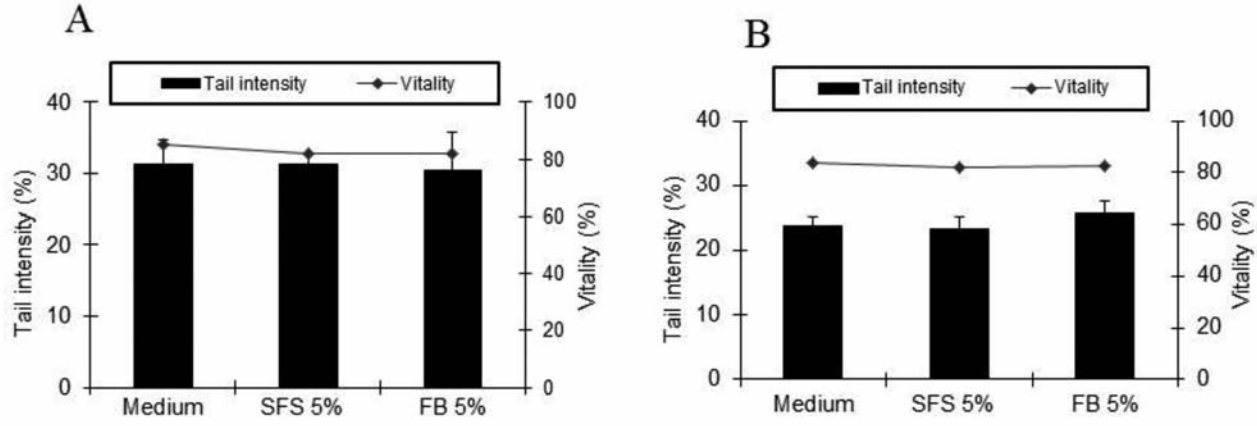

Figure 5. *HNE-induced DNA damage (300 μM HNE) after pretreatment of LT97 (A) and HT29 cells (B) with Synergy fermentation supernatant (SFS) and fermentation blank (FB) for 24 h. The means and SD of three independently-reproduced experiments are shown. Statistical variation was calculated by t-tests.*

modulating the balance of metabolising systems in cells in favour of de-activation of carcinogens. Studies on inulin-type fructans, pre-biotic dietary fibres have shown a consistent protective role of fibre consumption in reduced risk of colorectal cancer (34, 35). Both colonic adenoma and carcinoma cells were investigated in this study since there was a need to understand how cell models from these two different stages (pre-malignant adenoma cells and malignant tumour cells) would respond to gut lumen-specific environmental factors.

In the present study, modulation of mRNA expression of *CAT* and *GSTA4* by SFS was thought to be an important mechanism leading to chemoresistance of cells. It has been shown that treatment with the SCFA butyrate resulted in an up-regulation of GSTA4 in both HT29 and LT97 cells (8). According to available databases, the product of this gene not only inactivates endogenous aldehydes, quinones, epoxides and hydroperoxides, formed as secondary metabolites, during oxidative stress, but also protects from food contaminants, such as polycyclic aromatic hydrocarbons (36). In the present study a significant increase in *GSTA4* mRNA expression in HT29 cells after incubation with SFS was observed. Possible mechanisms by which products of fermentation, mainly butyrate, may influence gene expression in human colon tumour cells, are by activation of the mitogen-activated protein kinase signalling transduction pathway and by modifying the acetylation of histones at the *N*-terminal lysine-rich tails (8). Additionally, there was a significant induction of *GSTA4* mRNA after pre-treatment of cells with FB, which is in line with previous studies (18). Since SFS and FB differ in their composition, *e.g.* in SCFA and bile acids (24), it is possible that different mechanisms are responsible for *GSTA4* mRNA up-regulation by the two samples. Moreover, the involvement of the products of bacterial metabolism of non-digested food residues and excretable metabolites which may have their origin in faecal samples needs to be investigated in detail, which in turn could lead to a better understanding of the underlying mechanisms.

Next, the modulation of GSTs and catalase was studied after incubation with SFS at the enzymatic level. The analysis of GST and catalase activity was of particular interest because it is likely that an induced expression of these enzymes will result in protection of cells from genotoxic insults (1). However, we found no change in GST activity after 24 h of treatment in either cell line. These results are in line with some previous *in vitro* studies in carcinoma and adenoma cell lines, which in turn were explained by very low butyrate concentrations in the fermentation supernatants (1.3 mM and 0.3 mM in SFS and FB) measured by HPLC (24), or by the presence of other inhibitory substances in the faeces (37). Moreover, the induction of GSTA isoforms which have only a moderate affinity for 1-chloro-2,4-dinitrobenzene (39), and which are not abundant in colonic cells, would probably not be detected by the GST activity measurement. In addition to GSTs, catalase is another a phase II enzyme which represents a key defence against oxidative stress because it detoxifies H_2O_2 to oxygen and water (19). A previous study showed a significant increase in the mRNA expression of the *CAT* gene (2.9-fold) and a 65% increase in catalase enzymatic activity after incubation of human primary colonic cells with 10 mM butyrate for 2 h (14). In the present study, a significant two-fold increase in catalase enzyme activity after incubation of carcinoma cells with SFS for 24 h was observed. This significant up-regulation of activity suggests that it may not be regulated on the transcriptional level but by some other, as yet unknown, post-translational mechanisms.

Furthermore, we investigated possible anti-genotoxic effects of SFS against H_2O_2 and HNE to determine for functional consequences of cell-treatment. H_2O_2 was used as a model substance for free radical intermediates of oxidative stress, whereas HNE is an important lipid peroxidation product (21). Pre-treating HT29 cells with SFS for 24 h resulted in a significant decrease in DNA damage, induced by subsequent H_2O_2 challenge. This could be a reflection of enhanced cellular metabolism, including stimulation of DNA repair and antioxidant defence sytems *e.g.* up-regulation of catalase which in turn is responsible for decomposing H_2O_2 (12). Although a significant increase in the mRNA expression of *GSTA4* was observed, this was not be confirmed for the parameter of prevention of HNE- induced genotoxicity. This may be either due to post transcriptional modification of the gene responsible for detoxification of HNE or because a greater amount of SCFA, especially butyrate, and/or a longer incubation time may be required to induce this functional effect.

Conclusion

The results of the present study suggest that fermentation products of prebiotic dietary fibres increase the gene expression of *GSTA4* and inhibit the oxidative stress induced by H_2O_2 in carcinoma cells under experimental conditions. This could be linked to butyrate, the main fermentation product of dietary fibres. Because our approach is based on an *in vitro* model system, human intervention studies are necessary to more precisely determine the relevance of these findings for the *in vivo* situation.

Conflicts of Interest

None.

Acknowledgements

This work was supported by a grant from Deutscher Akademischer Ausstausch Dienst (DAAD), Triple Plus Bundesministerium für Forschung und Technologie, Germany (BMBF Nr. 0313829A),

Deutsche Forschungsgemeinschaft, Germany (DFG Nr. 284/8-3). Additionally, we thank Professor Marian, Institute of Cancer Research, University of Vienna, Austria for the generous gift of LT97 adenoma cells.

References

1 Pool-Zobel B, Veeriah S and Bohmer FD: Modulation of xenobiotic metabolising enzymes by anticarcinogens-focus on glutathione-*S*-transferases and their role as targets of dietary chemoprevention in colorectal carcinogenesis. Mutat Res *591*: 74-92, 2005.

2 Pool-Zobel BL: Inulin-type fructans and reduction in colon cancer risk: Review of experimental and human data. Br J Nutr *93(Suppl 1)*: S73-S90, 2005.

3 Reddy BS, Hirose Y, Cohen LA, Simi B, Cooma I and Rao CV: Preventive potential of wheat bran fractions against experimental colon carcinogenesis: Implications for human colon cancer prevention. Cancer Res *60*: 4792-4797, 2000.

4 Glei M, Hofmann T, Kuster K, Hollmann J, Lindhauer MG and Pool-Zobel BL: Both wheat (*Triticum aestivum*) bran arabinoxylans and gut flora-mediated fermentation products protect human colon cells from genotoxic activities of 4-hydroxynonenal and hydrogen peroxide. J Agric Food Chem *54*: 2088-2095, 2006.

5 Adom KK and Liu RH: Antioxidant activity of grains. J Agric Food Chem *50*: 6182-6187, 2002.

6 Oberreuther-Moschner DL, Rechkemmer G and Pool-Zobel BL: Basal colon crypt cells are more sensitive than surface cells toward hydrogen peroxide, a factor of oxidative stress. Toxicol Lett *159*: 212-218, 2005.

7 Ajila CM and Prasada Rao UJ: Protection against hydrogen peroxide induced oxidative damage in rat erythrocytes by *Mangifera indica* L. peel extract. Food Chem Toxicol *46*: 303-309, 2008.

8 Pool-Zobel BL, Selvaraju V, Sauer J, Kautenburger T, Kiefer J, Richter KK, Soom M and Wolfl S: Butyrate may enhance toxicological defence in primary, adenoma and tumor human colon cells by favourably modulating expression of glutathione-S-transferases genes, an approach in nutrigenomics. Carcinogenesis *26*: 1064-1076, 2005b.

9 Wattenberg LW: Inhibition of carcinogenesis by minor dietary constituents. Cancer Res *52*: 2085s-2091s, 1992.

10 Bingham SA: Epidemiology and mechanisms relating diet to risk of colorectal cancer. Nutr Res Rev 9: 197-239, 1996.

11 Burkitt DP: Epidemiology of cancer of the colon and rectum Cancer *28*: 3-13, 1971.

12 Abrahamse SL, Pool-Zobel BL and Rechkemmer G: Potential of short chain fatty acids to modulate the induction of DNA damage and changes in the intracellular calcium concentration by oxidative stress in isolated rat distal colon cells. Carcinogenesis *20*: 629-634, 1999.

13 Ten Bruggencate SJ, Bovee-Oudenhoven IM, Lettink-Wissink ML, Katan MB and van der MR: Dietary fructo-oligosaccharides and inulin decrease resistance of rats to salmonella: Protective role of calcium. Gut *53*: 530-535, 2004.

14 Sauer J, Richter KK and Pool-Zobel BL: Physiological concentrations of butyrate favorably modulate genes of oxidative and metabolic stress in primary human colon cells. J Nutr Biochem *18*: 736-745, 2007.

15 Rosignoli P, Fabiani R, De BA, Spinozzi F, Agea E, Pelli MA and Morozzi G: Protective activity of butyrate on hydrogen peroxide-induced DNA damage in isolated human colonocytes and HT29 tumour cells. Carcinogenesis *22*: 1675-1680, 2001.

16 Roberfroid MB: Introducing inulin-type fructans. Br J Nutr *93*: 13-25, 2005.

17 Coussement PA: Inulin and oligofructose: Safe intakes and legal status. J Nutr *129*: 1412S-1417S, 1999.

18 Sauer J, Richter KK and Pool-Zobel BL: Products formed during fermentation of the prebiotic inulin with human gut flora enhance expression of biotransformation genes in human primary colon cells. Br J Nutr *97*: 928-937, 2007b.

19 Chelikani P, Fita I and Loewen PC: Diversity of structures and properties among catalases. Cell Mol Life Sci *61*: 192-208, 2004.

20 Scharlau D, Borowicki A, Habermann N, Hofmann T, Klenow S, Miene C, Munjal U, Stein K and Glei M: Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. Mutat Res *682*: 39-53, 2009.

21 Knoll N, Ruhe C, Veeriah S, Sauer J, Glei M, Gallagher EP and Pool-Zobel BL: Genotoxicity of 4-hydroxy-2-nonenal in human colon tumor cells is associated with cellular levels of glutathione and the modulation of glutathione-S-transferase A4 expression by butyrate. Toxicol Sci *86(1)*: 27-35, 2005.

22 Femia AP, Luceri C, Dolara P, Giannini A, Biggeri A, Salvadori M, Clune Y, Collins KJ, Paglierani M and Caderni G: Antitumorigenic activity of the prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium* lactis on azoxymethane-induced colon carcinogenesis in rats. Carcinogenesis *23*: 1953-60, 2002.

23 Barry JL, Hoebler C, Macfarlane GT, Macfarlane S, Mathers JC, Reed KA, Mortensen PB, Nordgaard I, Rowland IR and Rumney CJ: Estimation of the fermentability of dietary fibre *in vitro*: A European interlaboratory study. Br J Nutr *74*: 303-322, 1995.

24 Munjal U, Glei M, Pool-Zobel BL and Scharlau D: Fermentation products of inulin-type fructans reduce proliferation and induce apoptosis in human colon tumour cells of different stages of carcinogenesis. Br J Nutr *102*: 663-671, 2009.

25 Fogh J: Human tumor lines for cancer research. Cancer Invest *4*: 157-184,1986.

26 Richter M, Jurek D, Wrba F, Kaserer K, Wurzer G, Karner-Hanusch J and Marian B: Cells obtained from colorectal microadenomas mirror early premalignant growth patterns *in vitro*. Eur J Cancer *38*: 1937-1945, 2002.

27 Pfaffl MW, Horgan, GW and Dempfle L: Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res *30*: e36, 2002.

28 Ebert MN, Beyer-Sehlmeyer G, Liegibel UM, Kautenburger T, Becker TW and Pool-Zobel BL: Butyrate induces glutathione-*S*-transferase in human colon cells and protects from genetic damage by 4-hydroxy-2-nonenal. Nutr Cancer *41*: 156-164, 2001.

29 Habig WH, Pabst MJ and Jakoby WB: Glutathione-*S*-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem *249*: 7130-7139, 1974.

30 Treptow-van LS, Rechkemmer G, Rowland I, Dolara P and Pool-Zobel BL: The carbohydrate crystalean and colonic microflora modulate expression of glutathione-S-transferase subunits in colon of rats. Eur J Nutr *38*: 76-83, 1999.

31 Aebi H: Catalase *in vitro*. Meth Enzymol *105*: 121-126, 1984.
32 Fearon ER and Vogelstein B: A genetic model for colorectal tumorigenesis. Cell *61*: 759-767, 1990.
33 Ferguson LR and Harris PJ: The dietary fibre debate: More food for thought. Lancet *361*: 1487-1488, 2003.
34 Klinder A, Forster A, Caderni G, Femia AP and Pool-Zobel BL: Fecal water genotoxicity is predictive of tumor-preventive activities by inulin-like oligofructoses, probiotics (*Lactobacillus rhamnosus* and *Bifidobacterium* lactis), and their symbiotic combination. Nutr Cancer *49*: 44-155, 2004.
35 Pool-Zobel B and Cherbut C: Diets enriched with cereal brans or inulin modulate protein kinase C activity and isozyme expression in rat colonic mucosa. Br J Nutr *89*: 283-284, 2003.
36 Hayes JD, Flanagan J U and Jowsey IR: Glutathione transferases. Annu Rev Pharmacol Toxicol *45*: 51-88, 2005.
37 Beyer-Sehlmeyer G, Glei M, Hartmann E, Hughes R, Persin C, Bohm V, Rowland I, Schubert R, Jahreis G and Pool-Zobel BL: Butyrate is only one of several growth inhibitors produced during gut flora-mediated fermentation of dietary fibre sources. Br J Nutr *90*: 1057-1070, 2003.
38 Mates JM and Sanchez-Jimenez FM: Role of reactive oxygen species in apoptosis: implications for cancer therapy. Int J Biochem Cell Biol *32*: 157-170, 2000.
39 Eaton DL and Bammler TK. Concise review of the glutathione-S-transferases and their significance to toxicology. Toxicol Sci *49(2)*: 156-64, 1999.
40 Kiefer J, Sehlmeyer GB and Pool-Zobel BL: Mixtures of SCFA, composed according to physiologically available concentrations in the gut lumen, modulate histone acetylation in human HT29 colon cancer cells. Br J Nutr *96*: 803-810, 2006.

Received August 30, 2012
Revised October 12, 2012
Accepted October 14, 2012