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Original article

Fructooligosaccharide associated with celecoxib reduces the number of aberrant crypt foci in the colon of rats

Bruno BUECHER[a], Cécile THOUMINOT[a], Jean MENANTEAU[b], Christian BONNET[c], Anne JARRY[a], Marie-Françoise HEYMANN[a], Christine CHERBUT[c], Jean-Paul GALMICHE[a], Hervé M. BLOTTIÈRE[a,c,d]*

[a] Centre de Recherche en Nutrition Humaine de Nantes, INSERM U539, CHU Hôtel-Dieu, 44035 Nantes, France
[b] Centre de Recherche en Nutrition Humaine de Nantes, INSERM U419, CHU Hôtel-Dieu, 44035 Nantes, France
[c] Centre de Recherche en Nutrition Humaine de Nantes, INRA-UFDNH, CHU Hôtel-Dieu, 44035 Nantes, France
[d] Present address: UNSA-INRA, Domaine de Vilvert, 78352 Jouy-en-Josas Cedex, France

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Abstract — According to Burkitt's hypothesis, dietary fibres may protect against the development of colorectal cancer. In rats, studies have shown that only butyrate-producing fibres are protective. In parallel, in humans, non-steroidal anti-inflammatory drugs, which target cyclooxygenases, have been shown to display a protective effect against colorectal cancer. Among them, COX-2-selective inhibitors which present less side effects than non-selective agents, are promising as chemopreventive agents. Our aim was to analyse the effect of an association between butyrate-producing fibres and the COX-2 inhibitor on the development of aberrant crypt foci (ACF) in rats. Fisher F344 rats were fed with (1) a standard low fibre control diet; (2) the standard diet supplemented with 1500 ppm celecoxib; (3) a diet supplemented with 6% fructo-oligosaccharide (FOS); and (4) a diet with both celecoxib and FOS. Three weeks later, the rats were injected twice with azoxymethane and the number of ACF was determined 15 weeks later. In the control group, 43.8 ± 6.4 ACF were found. This number was not significantly modified by the addition of FOS or celecoxib alone to the diet. However, the association of FOS and celecoxib resulted in a 61% reduction in the number of ACF ($P < 0.01$). The number of aberrant crypt per foci was also reduced. Thus, although no significant effect of celecoxib or FOS alone was identified, the association of butyrate-producing fibre and celecoxib was effective in preventing the development of ACF. This preliminary study argues for a strong protective effect of such an association which deserves further studies.

COX-2 / butyrate / colorectal cancer / NSAID

* Corresponding author: hblot@jouy.inra.fr

1. INTRODUCTION

Colorectal cancer is the second leading cause of death by cancer in Europe and America, and is thus a major concern of public health. Several strategies have been proposed in order to reduce its incidence [1]. Among them, the use of non-steroidal anti-inflammatory drugs (NSAID) has shown promising development [2].

In fact, epidemiological studies have revealed that regular consumption of aspirin or other NSAID reduces the risk of colorectal cancer [3–5]. These drugs target enzymes involved in the metabolism of arachidonic acid called cyclooxygenases (COX), and catalyse its conversion to prostaglandins (PG) and other eicosanoids [6]. Two isoforms have been identified and well characterised. COX-1 has been shown to be constitutively expressed in a variety of tissues including the gastro-intestinal epithelium, and has been shown to participate in several physiological processes [7]. COX-2 is a highly inducible gene that is turned on by several stimuli including pro-inflammatory cytokines and growth factors [8]. An over expression of COX-2 has been described in most human colorectal carcinomas and in about 50% adenomas [9–11]. This overexpression has also been observed in azoxymethane-induced colonic tumours in rats [12] and in intestinal tumours occurring naturally in APC mutant mice [13]. It is associated with increased PG levels in the tumours, particularly PGE2. [14]. The high expression observed in premalignant adenomas in humans and in polyps in APC mutant mice argue for an implication in the early event of carcinogenesis. This high expression leads to phenotypic alterations including the modulation of cell adhesion and the inhibition of apoptosis, as demonstrated in vitro after transfection of intestinal epithelial cells with a COX-2 expression vector [15]. These phenotypic changes result in an enhanced tumorigenic potential of these cells, which is reversed by NSAID. Moreover, in a transgenic murine model, the overexpression of COX-2 in the mammary glands is sufficient to induce mammary carcinoma [16]. Consequently, the targeting of COX-2 with more or less selective inhibitors has been proposed as a new protective or therapeutic approach for colorectal cancer. Two families of compounds have been generated with different selectivity towards the two COX isoforms [17]). Drugs of the first family which include aspirin, sulindac sulphide or ibuprofen, act either on the two isoforms or are more selective against COX-1. The members of the second family including celecoxib are very selective against COX-2. In patients with familial adenomatous polyposis, the administration of sulindac or celecoxib results in a reduction in the number and size of polyps [18–21]. In the azoxymethane-induced tumour model in rats, NSAID have also been demonstrated to reduce the number of either aberrant crypt foci (ACF) or tumours [1, 22, 23].

Another approach to chemoprevention has also been proposed and involves a control of the diet. Indeed, epidemiological studies have revealed a strong association between colorectal cancer and diet habits [24]. The hypothesis of a protective effect of dietary fibre was suggested by Burkitt [25], based on the observation of a low incidence of colorectal cancer in African countries with high-fibre diets. Several reports either based on cohort or intervention studies have provided a debatable lack of a prevention effect of dietary fibre [26–29], however two recent papers, also based on a large number of cases, have shown an association between a high intake of dietary fibre and a decreased risk of either colorectal adenomas or colorectal cancer [30, 31]. It is noteworthy that, in most studies, dietary fibres are considered as an all, but individually their interaction with colon microflora and thus their fermentation properties and the production of short-chain fatty acids varies. Among them, fibres producing large amounts of butyrate are of particular

interest. Indeed, butyrate is a key regulator of the colon epithelium homeostasis. It is the major fuel for colonic epithelial cells, but also affects several cellular functions including proliferation and apoptosis [32–34]. It is noteworthy that the other short chain fatty acids present less or no cellular effects [35]. Butyrate has also been shown to modulate the immune system by acting on particular transcription factors [36]. In animal models of colorectal cancer, butyrate-producing fibres exert a protective effect [37, 38]. Interestingly, wheat bran, the fibre used in intervention studies, does not produce large amounts of butyrate in rats after starch removal and is devoid of an effect on aberrant crypt foci development [39].

None of these strategies is able to abolish the development of ACF or tumours. Thus, the aim of our study was to analyse the effect of the association of a COX-2 selective inhibitor, celecoxib (Celebrex™, Pharmacia) with a diet supplemented with butyrate-producing fibre on the development of aberrant crypt foci (ACF) in rats.

2. MATERIALS AND METHODS

2.1. Animals and experimental design

Sixty male Fischer F344 rats (Charles River, L'Arbresle, France) aged 4 weeks were randomly divided into 4 groups. After 2 weeks of adaptation to the animal facility, each group received one of the four experimental diets ad libitum for 15 weeks. Rats were weighed once a week until sacrifice. Three weeks later, the animals were injected sub-cutaneously with 15 $mg \cdot kg^{-1}$ azoxymethane (AOM; Sigma, St Quentin Fallavier, France) twice at a one week interval [38]. Eleven weeks after the second injections, the animals were killed and the tissues were collected. All animal handling procedures were done in accordance with the rules of the French Ministry of Agriculture (agreement No. A44565).

2.2. Experimental diet

Diets were prepared by the INRA animal diet service (Jouy-en-Josas, France). The four experimental diets were standard low fibre control diets: standard diet supplemented with short-chain fructooligosaccharide (FOS); standard diet supplemented with celecoxib; and standard diet supplemented with both, FOS and celecoxib (Tab. I). Short chain fructo-oligosaccharides (Actilight P, Beghin-Meiji Industries, Neuilly-sur-Seine, France) were used as previously described [38]. This diet was shown to induce a stable butyrate producing colonic ecosystem. Celecoxib

Table I. The composition of the experimental diets (in $g \cdot 100\ g^{-1}$).

	Control	FOS	Celecoxib	FOS + Celecoxib
Casein	20.0	20.0	20.0	20.0
Cellulose	2.00	2.00	2.00	2.00
DL-Methionine	0.40	0.40	0.40	0.40
Corn oil	2.00	2.00	2.00	2.00
Lard	6.33	6.33	6.33	6.33
Minerals*	4.50	4.50	4.50	4.50
Vitamins*	0.50	0.50	0.50	0.50
Pregelatinised corn starch	64.27	61.12	64.27	61.12
FOS		6.00		6.00
Celebrex™			0.15	0.15

* 102 mixed formula.

(Celebrex™, Pharmacia, St Quentin-en-Yvelines, France) was used at 1500 ppm. This dosage was shown to generate a plasmatic concentration of 3.5 $\mu g \cdot mL^{-1}$ [22].

2.3. Tissue collection and ACF counting

The large intestine was first isolated, removed and opened longitudinally. The colon was then cut into two parts following the longitudinal axis. Half of the colon was used for ACF counting. Briefly, the half-colons were fixed in formaldehyde 10%, and then stained with methylene blue (1%) as described [39]. ACF were scored blindly, by two independent observers unaware of the diet received. Aberrant crypt (AC) were distinguished by their slit-like opening, increased staining, and size as described [39–41]. The number of AC per foci, ranging from 1 to 10, was enumerated. The number of large ACF (≥ 4 aberrant crypt per foci) was also considered, it was described as an intermediate biomarker for tumour incidence in rats [40, 41].

2.4. Statistical analyses

The data were examined by the Kruskal-Wallis analysis of variance (ANOVA) on ranks followed by the Dunn Test.

3. RESULTS

As shown in Figure 1, no difference was noticed between the weight of the rats whatever the diet was. Considering the ACF count, the two observers gave very homogenous results without any statistically significant difference between the observers. Whatever the diet was, all proximal colons were healthy without any aberrant crypt. On the contrary, the distal colons were all sensitive to azoxymethane-induced carcinogenesis.

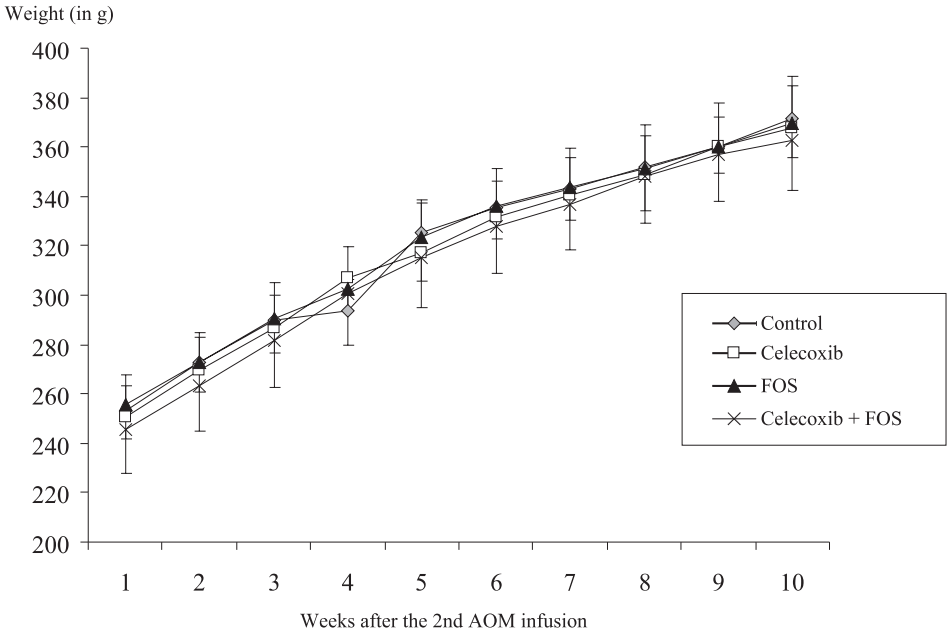

Figure 1. The weight of the rats during the experiments depending on the diet. Data correspond to mean ± SEM of 15 rats. No statistically different value was found between each group of animals.

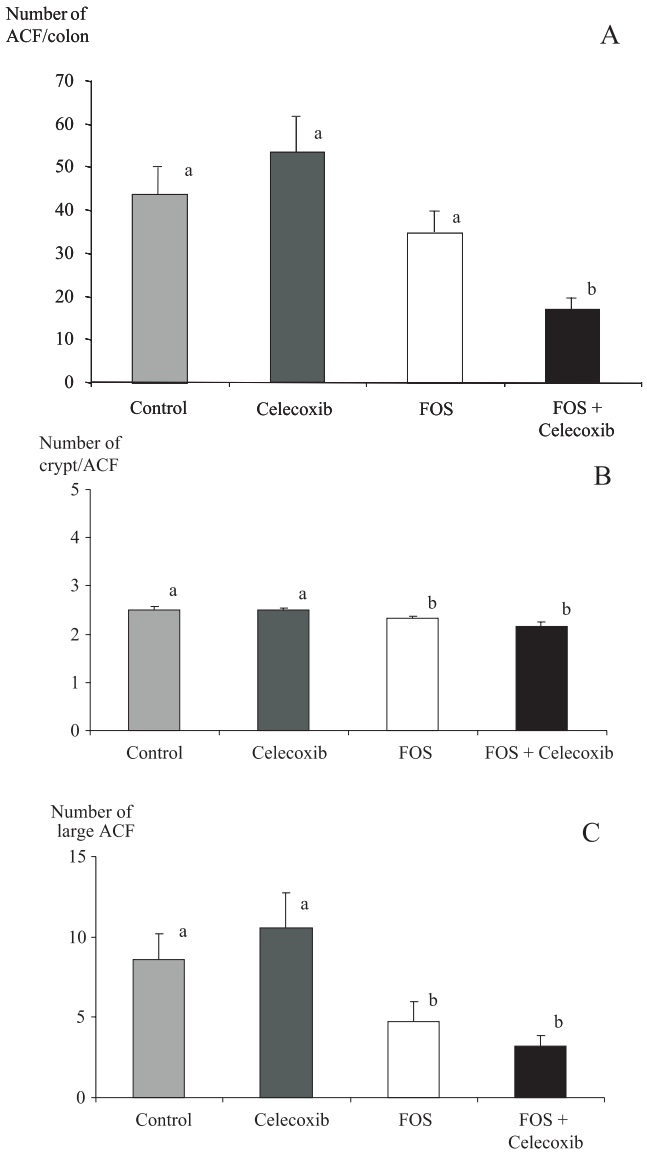

Figure 2. (**A**) Number of aberrant crypt foci (ACF) in the half colon of rats after azoxymethane injection depending on the experimental diet. Data are mean ± SEM of 15 rats (mean indicated). a,b: different letters correspond to statistically different data as tested by ANOVA. $P < 0.01$. (**B**) Number of aberrant crypts per foci. $P < 0.05$. (**C**) Number of large aberrant crypts per foci (≥ 4 aberrant crypts per foci). $P < 0.05$.

An average of 43.8 ± 6.4 ACF was enumerated by the half-colon of rats in the standard low fibre control diet. The multiplicity of crypts per ACF was also considered. An average of 2.51 ± 0.05 aberrant crypts was found, and up to 10 crypts per foci were observed.

In the celecoxib group (Fig. 2A), an average number of 53.5 ± 8.0 ACF was counted. Although the number of ACF was

increased, this was not statistically different from the control group of rats ($P > 0.05$). The number of crypts per foci was not modified (2.53 ± 0.05). In the group fed with FOS, the number of ACF was diminished (from less than 20%) as compared to the control group (34.8 ± 4.9) in a statistically non-significant manner. However, as shown in Figure 2B, a significant reduction in the number of crypts per foci was noticed (2.33 ± 0.05) in this group, with the highest number of crypts per foci being 7. Finally, the combination of FOS and celecoxib yielded in a major reduction in the number of ACF. Indeed, a 61% reduction was noticed in this group of rats and it was highly significant ($P < 0.001$). The mean number of ACF was 16.9 ± 2.6. The number of crypts per foci was also affected (2.15 ± 0.09), since the maximal number of crypts per foci was 6. Another parameter studied was the number of ACF formed by at least 4 aberrant crypts (Fig. 2C). It was found that diet supplementation by FOS as well as the combination of FOS and celecoxib resulted in a statistically significant reduction of the number of large ACF (≥ 4 aberrant crypts per foci).

4. DISCUSSION

Several strategies have been proposed in an attempt to protect against the development of colon carcinoma. Here, we evaluated the impact of a nutritional intervention using butyrate-producing dietary fibres combined with a chemo-intervention using a COX-2 selective inhibitor. This study demonstrates that the administration of celecoxib together with a diet enriched with FOS significantly suppressed AOM-induced colonic ACF formation. To our knowledge, this is the first report of a successful association between a COX-2 selective inhibitor and dietary fibre in vivo. The three parameters studied, i.e. the number of ACF, number of crypts per ACF, and number of large ACF (≥ 4 aberrant crypts per foci) were reduced by the combination of FOS and celecoxib. It is noteworthy that the latter parameter was validated as an intermediate biomarker for tumour incidence in the rat model [40, 41].

Celecoxib was selected because it is representative of the new class of NSAID which selectively target COX-2 and present less gastro-intestinal side effects than non selective NSAID [2, 42]. Indeed several studies were performed with aspirin or sulindac sulphide which more selectively target COX-1, or with piroxicam which is much less selective and acts on both COX isoforms [17]. Surprisingly, in our hands, celecoxib did not significantly influence AOM-induced ACF. The concentration used (1500 ppm) has been successfully used in the same model by others [22, 23]. In fact, celecoxib reduced the number of ACF in Fischer F344 male rats by around 50%. Similar results were found by others [43]. At this concentration, celecoxib has also been shown to reduce the size and the number of tumours induced by AOM [23, 44]. However, in another rat strain, celecoxib was shown less efficient (22% reduction in the number of ACF) [45]. The reason why celecoxib was found to be inefficient is difficult to understand. The concentration used, the rat strain and the induction protocol were exactly identical to the one described by Reddy and colleagues [22]. The only important difference is the composition of the diet and particularly the % of cellulose in the diet. Indeed, we used a low residue diet (2% cellulose), whereas 5% was used by Reddy et al. It is questionable whether such a difference can explain why celecoxib was found to be ineffective, however the results of our study strongly suggest that the diet could be a key determinant in the celecoxib effect on colon carcinogenesis. The presence of residue in the colon is important for its physiology especially for the distal colon [46].

A low fibre diet was also compared to a 6% enriched FOS diet. This diet was

previously shown to induce a high and stable butyrate concentration in the colon [38, 47]. It was also shown to reduce the number of ACF in rats [38]. Depending on the batch, a 32 to 46% reduction in the number of ACF was found. In our experiments, FOS reduced the number of ACF by almost 20%, but this reduction was not statistically significant. However, it significantly affected the number of aberrant crypts per ACF and the number of large ACF. The only difference between our study and the previous study is the rat strain. Indeed, we used Fischer F344 male rats, whereas BDIX rats were used in the aforementioned study [38]. In this respect, others using 10% FOS and Fischer F344 rats obtained a 24% reduction in the number of ACF [48]. Thus, the rat strain may explain such a discrepancy. In Wistar rats, a moderate intake in galacto-oligosaccharide fibres (8.88%) was found without an effect on the development of colonic tumours [49].

The major finding of our study is that the association of celecoxib to butyrate-producing fibres leads to a strong reduction in the number of ACF. A similar association has been proposed previously in an APC mutant mouse model, in which aspirin was associated to resistant starches [50]. This model mainly yielded small intestine tumours. It was found that aspirin alone is inefficient and that high amounts of resistant starches (12.5% raw potato starch and 12.5% Hylon VII) increase the number of tumours that is reversed by aspirin. In our study, the amount of fibre (6%) better fitted the recommendation for human consumption and the physiological tolerance to such fermentable fibres. Moreover, selective COX-2 inhibitors, which present less gastro-intestinal side effects, are now to be considered. The mechanisms involved in such additive protective effects remain elusive. The direct effect of butyrate on colon cancer is now well documented [33, 34]. It has been shown to inhibit the proliferation of colon cancer cells by acting on cell cycle regulatory proteins, especially $p21^{CIP1}$ [32, 33]. Considering celecoxib, the mechanisms by which NSAID and selective COX-2 inhibitors are acting are now relatively well characterised [2, 42, 51]. In vitro, it was shown that butyrate may directly increase COX-2 expression (52), and that aspirin or NS-398, another COX-2 selective inhibitor, can exert synergistic anti-proliferative and pro-apoptotic effects [52, 53]. However, by using six different colon carcinoma cell lines expressing different levels of COX-2, we found no synergistic effects of celecoxib and butyrate on cell proliferation and apoptosis (unpublished data). Thus, the importance of colonic fermentation products on COX-2 expression and on the celecoxib effect has to be further investigated in vivo.

In conclusion, although we observed a protective effect of the association between celecoxib and butyrate-producing fibres on colonic ACF formation in rats, this is just a preliminary study. It remains to be understood why celecoxib had no significant effect in our hands and to analyse if the association is also active on tumour formation. Moreover, the precise mechanism of action of such a synergistic effect has to be elucidated. In this regard, the analysis of the rat colon at the molecular level has to be performed, especially concerning the expression of COX-2. Finally, the efficiency of this association remains to be tested in humans. In this regard, the results of the concerted action polyp prevention studies (CAPP-1 and CAPP-2) in which aspirin and resistant starch are tested alone or in synergy in patients with Familial Adenomatous Polyposis or in Hereditary Non-Polyposis Colon Cancer syndrome, will provide critical information on the feasibility and the relevance of this kind of study [54].

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REFERENCES

[1] Corpet DE, Tache S. Most effective colon cancer chemopreventive agents in rats: a systematic review of aberrant crypt foci and tumor data, ranked by potency. Nutr Cancer 2002, 43: 1–421.

[2] Buecher B, Heymann MF, Blottiere HM. Rationale and prospects for the use of cyclooxygenase-2 (COX-2) inhibitors in colorectal cancer. Gastroenterol Clin Biol 2001, 25: 967–978.

[3] Rosenberg L, Palmer JR, Zauber AG, Warshauer ME, Stolley PD, Shapiro S. A hypothesis: nonsteroidal anti-inflammatory drugs reduce the incidence of large-bowel cancer. J Natl Cancer Inst 1991, 83: 355–358.

[4] DuBois RN, Giardiello FM, Smalley WE. Nonsteroidal anti-inflammatory drugs, eicosanoids, and colorectal cancer prevention. Gastroenterol Clin North Am 1996, 25: 773–791.

[5] Sandler RS, Galanko JC, Murray SC, Helm JF, Woosley JT. Aspirin and nonsteroidal anti-inflammatory agents and risk for colorectal adenomas. Gastroenterology 1998, 114: 441–447.

[6] DuBois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE. Cyclooxygenase in biology and disease. FASEB J 1998, 12: 1063–1073.

[7] Smith WL, Langenbach R. Why there are two cyclooxygenase isozymes. J Clin Invest 2001, 107: 1491–1495.

[8] Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000, 69: 145–182.

[9] Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology 1994, 107: 1183–1188.

[10] Sano H, Kawahito Y, Wilder RL, Hashiramoto A, Mukai S, Asai K, Kimura S, Kato H, Kondo M, Hla T. Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res 1995, 55: 3785–3789.

[11] Karnes WE Jr, Shattuck-Brandt R, Burgart LJ, DuBois RN, Tester DJ, Cunningham JM, Kim CY, McDonnell SK, Schaid DJ, Thibodeau SN. Reduced COX-2 protein in colorectal cancer with defective mismatch repair. Cancer Res 1998, 58: 5473–5477.

[12] Shao J, Sheng H, Aramandla R, Pereira MA, Lubet RA, Hawk E, Grogan L, Kirsch IR, Washington MK, Beauchamp RD, DuBois RN. Coordinate regulation of cyclooxygenase-2 and TGF-beta1 in replication error-positive colon cancer and azoxymethane-induced rat colonic tumors. Carcinogenesis 1999, 20: 185–191.

[13] Boolbol SK, Dannenberg AJ, Chadburn A, Martucci C, Guo XJ, Ramonetti JT, Abreu-Goris M, Newmark HL, Lipkin ML, DeCosse JJ, Bertagnolli MM. Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res 1996, 56: 2556–2560.

[14] Rigas B, Goldman IS, Levine L. Altered eicosanoid levels in human colon cancer. J Lab Clin Med 1993, 122: 518–523.

[15] Tsujii M, DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995, 83: 493–501.

[16] Liu CH, Chang SH, Narko K, Trifan OC, Wu MT, Smith E, Haudenschild C, Lane TF, Hla T. Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J Biol Chem 2001, 276: 18563–18569.

[17] Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proc Natl Acad Sci USA 1999, 96: 7563–7568.

[18] Debinski HS, Trojan J, Nugent KP, Spigelman AD, Phillips RK. Effect of sulindac on small polyps in familial adenomatous polyposis. Lancet 1995, 345: 855–856.

[19] Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK, Levin B. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000, 342: 1946–1952.

[20] Phillips RK, Wallace MH, Lynch PM, Hawk E, Gordon GB, Saunders BP, Wakabayashi N, Shen Y, Zimmerman S, Godio L, Rodrigues-Bigas M, Su LK, Sherman J, Kelloff G, Levin B, Steinbach G. A randomised, double blind, placebo controlled study of celecoxib, a selective cyclooxygenase 2 inhibitor, on duodenal polyposis in familial adenomatous polyposis. Gut 2002, 50: 857–860.

[21] Cruz-Correa M, Hylind LM, Romans KE, Booker SV, Giardiello FM. Long term treatment with sulindac in familial adenomatous polyposis: a prospective cohort study. Gastroenterology 2002, 122: 641–645.

[22] Reddy BS, Rao CV, Seibert K. Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. Cancer Res 1996, 56: 4566–4569.

[23] Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a

specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res 1998, 58: 409–412.

[24] Sandler RS. Epidemiology and risk factors for colorectal cancer. Gastroenterol Clin North Am 1996, 25: 717–735.

[25] Burkitt DP. Possible relationships between bowel cancer and dietary habits. Proc R Soc Med 1971, 64: 964–965.

[26] Fuchs CS, Giovannucci EL, Colditz GA, Hunter DJ, Stampfer MJ, Rosner B, Speizer FE, Willett WC. Dietary fiber and the risk of colorectal cancer and adenoma in women. N Engl J Med 1999, 340: 169–176.

[27] Alberts DS, Martinez ME, Roe DJ, Guillen-Rodriguez JM, Marshall JR, van Leeuwen JB, Reid ME, Ritenbaugh C, Vargas PA, Bhattacharyya AB, Earnest DL, Sampliner RE. Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. Phoenix Colon Cancer Prevention Physicians' Network. N Engl J Med 2000, 342: 1156–1162.

[28] Bonithon-Kopp C, Kronborg O, Giacosa A, Rath U, Faivre J. Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. European Cancer Prevention Organisation Study Group. Lancet 2000, 356: 1300–1306.

[29] Schatzkin A, Lanza E, Corle D, Lance P, Iber F, Caan B, Shike M, Weissfeld J, Burt R, Cooper MR, Kikendall JW, Cahill J. Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. Polyp Prevention Trial Study Group. N Engl J Med 2000, 342: 1149–1155.

[30] Bingham SA, Day NE, Luben R, Ferrari P, Slimani N, Norat T, Clavel-Chapelon F, Kesse E, Nieters A, Boeing H, Tjonneland A, Overvad K, Martinez C, Dorronsoro M, Gonzalez CA, Key TJ, Trichopoulou A, Naska A, Vineis P, Tumino R, Krogh V, Bueno-de-Mesquita HB, Peeters PH, Berglund G, Hallmans G, Lund E, Skeie G, Kaaks R, Riboli E. Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 2003, 361: 1496–1501.

[31] Peters U, Sinha R, Chatterjee N, Subar AF, Ziegler RG, Kulldorff M, Bresalier R, Weissfeld JL, Flood A, Schatzkin A, Hayes RB. Dietary fibre and colorectal adenoma in a colorectal cancer early detection programme. Lancet 2003, 361: 1491–1495.

[32] Siavoshian S, Segain JP, Kornprobst M, Bonnet C, Cherbut C, Galmiche JP, Blottiere HM. Butyrate and trichostatin A effects on the proliferation/differentiation of human intestinal epithelial cells: induction of cyclin D3 and p21 expression. Gut 2000, 46: 507–514.

[33] Augenlicht LH, Mariadason JM, Wilson A, Arango D, Yang W, Heerdt BG, Velcich A. Short chain fatty acids and colon cancer. J Nutr 2002, 132: 3804S–3808S.

[34] Blottière HM, Buecher B, Galmiche JP, Cherbut C. Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation. Proc Nutr Soc 2003, 62: 101–106.

[35] Siavoshian S, Blottière HM, Le Foll E, Kaeffer B, Cherbut C, Galmiche JP. Comparison of the effect of different short chain fatty acids on the growth and differentiation of human colonic carcinoma cell lines in vitro. Cell Biol Int 1997, 21: 281–287.

[36] Segain JP, Raingeard de la Bletiere D, Bourreille A, Leray V, Gervois N, Rosales C, Ferrier L, Bonnet C, Blottière HM, Galmiche JP. Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. Gut 2000, 47: 397–403.

[37] McIntyre A, Gibson PR, Young GP. Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. Gut 1993, 34: 386–391.

[38] Perrin P, Pierre F, Patry Y, Champ M, Berreur M, Pradal G, Bornet F, Meflah K, Menanteau J. Only fibres promoting a stable butyrate producing colonic ecosystem decrease the rate of aberrant crypt foci in rats. Gut 2001, 48: 53–61.

[39] Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. Cancer Lett 1987, 37: 147–151.

[40] Pretlow TP, O'Riordan MA, Somich GA, Amini SB, Pretlow TG. Aberrant crypts correlate with tumor incidence in F344 rats treated with azoxymethane and phytate. Carcinogenesis 1992, 13: 1509–1512.

[41] Caderni G, Giannini A, Lancioni L, Luceri C, Biggeri A, Dolara P. Characterisation of aberrant crypt foci in carcinogen-treated rats: association with intestinal carcinogenesis. Br J Cancer 1995, 71: 763–769.

[42] Gupta RA, Dubois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer 2001, 1: 11–21.

[43] Yamada Y, Yoshimi N, Hirose Y, Hara A, Shimizu M, Kuno T, Katayama M, Qiao Z, Mori H. Suppression of occurrence and advancement of beta-catenin-accumulated crypts, possible premalignant lesions of colon cancer, by selective cyclooxygenase-2 inhibitor, celecoxib. Jpn J Cancer Res 2001, 92: 617–623.

[44] Reddy BS, Hirose Y, Lubet R, Steele V, Kelloff G, Paulson S, Seibert K, Rao CV. Chemoprevention of colon cancer by specific cyclooxygenase-2 inhibitor, celecoxib, administered

during different stages of carcinogenesis. Cancer Res 2000, 60: 293–297.

[45] Brown WA, Skinner SA, Malcontenti-Wilson C, Misajon A, DeJong T, Vogiagis D, O'Brien PE. Non-steroidal anti-inflammatory drugs with different cyclooxygenase inhibitory profiles that prevent aberrant crypt foci formation but vary in acute gastrotoxicity in a rat model. J Gastroenterol Hepatol 2000, 15: 1386–1392.

[46] Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev 2001, 81: 1031–1064.

[47] Le Blay G, Michel C, Blottière HM, Cherbut C. Prolonged intake of fructo-oligosaccharides induces a short-term elevation of lactic acid-producing bacteria and a persistent increase in cecal butyrate in rats. J Nutr. 1999, 129: 2231–2235.

[48] Reddy BS, Hamid R, Rao CV. Effect of dietary oligofructose and inulin on colonic preneoplastic aberrant crypt foci inhibition. Carcinogenesis 1997, 18: 1371–1374.

[49] Wijnands MV, Appel MJ, Hollanders VM, Woutersen RA. A comparison of the effects of dietary cellulose and fermentable galacto-oligosaccharide, in a rat model of colorectal carcinogenesis: fermentable fibre confers greater protection than non-fermentable fibre in both high and low fat backgrounds. Carcinogenesis 1999, 20: 651–656.

[50] Williamson SL, Kartheuser A, Coaker J, Kooshkghazi MD, Fodde R, Burn J, Mathers JC. Intestinal tumorigenesis in the Apc1638N mouse treated with aspirin and resistant starch for up to 5 months. Carcinogenesis 1999, 20: 805–810.

[51] Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst 2002, 94: 252–266.

[52] Crew TE, Elder DJ, Paraskeva C. A cyclooxygenase-2 (COX-2) selective non-steroidal anti-inflammatory drug enhances the growth inhibitory effect of butyrate in colorectal carcinoma cells expressing COX-2 protein: regulation of COX-2 by butyrate. Carcinogenesis 2000, 21: 69–77.

[53] Menzel T, Schauber J, Kreth F, Kudlich T, Melcher R, Gostner A, Scheppach W, Luhrs H. Butyrate and aspirin in combination have an enhanced effect on apoptosis in human colorectal cancer cells. Eur J Cancer Prev 2002, 11: 271–281.

[54] Burn J, Chapman PD, Bishop DT, Mathers J. Diet and cancer prevention: the concerted action polyp prevention (CAPP) studies. Proc Nutr Soc 1998, 57: 183–186.