Effect of dietary fibre of barley variety 'Rihane' on azoxymethane-induced aberrant crypt foci development and on colonic microbiota diversity in rats

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Abstract

Many epidemiological and experimental studies have suggested an important role for dietary fibre (DF) of cereals in the prevention of colon cancer. The objective of the present study was to explain the effects of the DF of barley Rihane (BR) on azoxymethane (AOM)-induced aberrant crypt foci (ACF) and colonic bacterial diversity in rats. Following an acclimatisation period, rats were divided into four groups and fed a control (C) diet or experimental diet containing 30 % of BR. DF content in the experimental diet was twice that of the C diet (total DF was 8·69 % in the C diet and 15·24 % in the BR diet). At 7 and 8 weeks of age, rats received two successive subcutaneous injections of AOM at 20 mg/kg body weight. At 12 weeks after the first injection, ten animals from each group were killed. The BR diet decreased colonic pH ($P < 0.05$) compared with the C diet. The total number of ACF observed decreased considerably in the BR/AOM group compared with the C/AOM group ($P < 0.05$). Comparison of similarity coefficients showed variability of colonic microbiota species between the different groups. In addition, we showed inter-individual variability within the same group. This similarity was affected by BR and AOM. The present results show that bifidobacteria numbers were lower in rats fed the BR diet compared with those fed the C diet. However, the number of enterobacteria in colonic content was increased ($P < 0.05$) in the BR group compared with the C group. The results from the present study show that the DF of BR reduced the incidence of AOM-induced ACF and increased microbiota biodiversity.

Key words: Barley variety 'Rihane': Aberrant crypt foci: Colonic microbiota: Temporal temperature gradient gel electrophoresis: Real-time PCR

The potential for whole grains to lower the risk of colorectal cancer is recognised. This effect could be attributed to dietary fibre (DF)[1]. Barley (*Hordeum vulgare* L.) is an interesting source of DF. It is an ancient cereal grain, which upon domestication has evolved from largely a food grain to a feed and malting grain[2]. However, barley food use today remains important in some cultures around the world, particularly in Tunisia, which is the second centre of diversity for barley. In Tunisia, barley is used for both feed (85 %) and food (15 %)[3]. Barley soup, barley bread and 'malthouth' are probably the main endemic barley-based food processed from barley grain in the region. Rihane, a Tunisian barley variety, officially registered in 1987, has contributed significantly to the increase in barley national production in Tunisia, and it

is cultivated in Morocco, Algeria, Libya, Lebanon, Iraq, Iran, Afghanistan, Cyprus and China[3]. Animal models of chemically induced colon cancer may provide a useful tool in identifying potentially preventive dietary strategies and in helping to better understand the mechanisms by which protection might be achieved. The effect of DF on the reduction in colon cancer has been attributed to increased faecal bulk, dilution of potential carcinogenic compounds and fermentation by colonic anaerobic bacteria to produce SCFA[4,5]. The rat azoxymethane (AOM) model of colon cancer is used for this purpose[6]. AOM is a metabolite of the procarcinogen 1,2-dimethylhydrazine, and is one metabolic step closer to the proximate carcinogen capable of inducing colonic aberrant crypt and colon tumours (adenomas and adenocarcinomas)[7].

Aberrant crypt foci (ACF) are early pre-neoplastic lesions of adenocarcinoma that appear on the surface of rodents after subsequent treatment with chemically induced colon carcinogens such as AOM. ACF appear as single focuses that are characterised by more than one crypt, they possess thickened epithelia with altered luminal openings and are more elevated than normal crypts when viewed under a microscope[8]. The colonic microbiota is a complex ecosystem, which plays a key role in gut health. The composition and metabolism of the gut microbiota are strongly diet dependent. Until recently, this ecosystem has been identified using cultures of specific medium and phenotypic characteristics. A better assessment of microbiota composition and dominant species complexity could be achieved using molecular techniques. These techniques are based on the amplification of 16S ribosomal DNA sequences and coupled with separation by denaturing gel electrophoresis[9]. The presence of any fermentable DF is likely to lead to an increase in microfloral bifidobacteria and enterobacteria levels, as these bacteria are dominant flora that ferment carbohydrates.

The aim of the present study was to investigate the effect of the DF of the barley variety 'Rihane' (BR) on the development of AOM-induced ACF. In addition, the effects of these compounds on biodiversity microbiota were studied.

Materials and methods

Barley Rihane

The 'Rihane' variety, a six-rowed barley, was provided by the Field Crop Laboratory of INRAT. This variety was grown in 1·5/50 m head-row plots at the Agricultural Experimental Station of Béja, 100 km north-west of Tunisia. Table 1 summarises the chemical composition of barley 'Rihane' samples[10].

Animal and diets

Adult male Wistar rats were purchased from the Central Pharmacy (Pharmaceutical Industries Society). The rats were housed two per cage in a temperature-controlled room (22–24°C) with a 12 h light–dark cycle (lights on 08.00–20.00 hours) and humidity rate (70 ± 4%). They were allocated

Table 1. Chemical composition of whole barley Rihane (% DM)[10]

(Mean values with their standard errors)

Composition	BR	
	Mean	SEM
Protein	11·37	0·09
Lipids	3·82	0·03
Carbohydrates	70·02	0·12
Insoluble dietary fibre	19·62	1·02
Soluble dietary fibre	12·58	0·17
Total dietary fibre	32·20	1·01
Ash	3·6	0·05
Total phenolic content*	220·11	0·32

* Expressed as mg gallic acid equivalents/100 g fresh weight.

Table 2. Composition of the experimental diets (% DM)

	C	BR
Diet ingredients (%)		
Maize starch	68	40
Soyabean meal	27	25
Barley	–	30
Mineral mix*	3·5	3·5
Vitamin mix*	1·0	1·0
DL-Met	0·5	0·5
Chemical composition (% DM)		
Crude protein	17·64	17·58
Total dietary fibre	8·69	15·24
Total energy (kJ/kg DM)	11984·06	11704

C, control; BR, barley Rihane.
* AIN-76 vitamin and mineral mixtures[11]. Vitamin mixture composition: thiamin mononitrate (6 mg/kg), riboflavin (6 mg/kg), pyridoxine hydrochloride (7 mg/kg), nicotinic acid (30 mg/kg), D-calcium pantothenate (16 mg/kg), folic acid (2 mg/kg), D-biotin (0·2 mg/kg), cyanocobalamin (vitamin B_{12}) (10 μg/kg), retinyl palmitate (vitamin A) (4 IU/g; 2·2 μg/g), tocopheryl acetate (vitamin E) (50 IU/kg; 50 mg/g), cholecalciferol (vitamin D_3) (1 IU/g; 0·025 μg/g), menaquinone (vitamin K) (0·05 mg/kg). Mineral mixture composition: Ca (0·52%), P (0·40%), Na (0·102%), K (0·36%), Mg (0·05%), Mn (54 mg/kg), Fe (35 mg/kg), Cu (6 mg/kg), Zn (30 mg/kg), I (0·2 mg/kg), Se (0·1 mg/kg), Cr (2 mg/kg), chloride (0·156%), sulfate (0·10%).

randomly into four groups (ten animals per group) with approximately equal body weights and given free access to water and diet throughout the study. The diet composition is shown in Table 2. Diet was prepared as pellets (Provital). The control (C) diet was a modified form of the American Institute of Nutrition (AIN)-76 diet[11]. The experimental diet containing 30% of BR was prepared by a partial substitution of maize and a soyabean meal, which were used in the C diet. The diets were isonitrogenous and isoenergetic. However, DF content in the experimental diet was twice that of the C diet (total DF was 8·69% in the C diet and 15·24% in the BR diet).

Experimental design

The design of the study is outlined in Fig. 1. After a 2-week adaptation period, animals were divided into four groups. The rats were fed the experimental diet for 1 week, and then received two injections of AOM (Sigma Chemicals) at 7 and 8 weeks of age, according to the standard protocol established by Martha et al.[12]. Carcinogen was administered subcutaneously in saline at 20 mg/kg body weight. The negative controls were injected with saline. Body weights and food intake were measured twice per week. At 12 weeks after the first injection, ten animals from each group were killed, their colons removed and examined for ACF as outlined below. Colonic pH was measured. A sample of the colonic content was frozen at −80°C for further bacterial analysis. All procedures were performed as per Tunisian guidelines for the care and use of experimental animals.

Aberrant crypt foci assay and histological classification of aberrant crypt foci

ACF were identified using the method of Norazalina et al.[13]. The colon was cut in 2 cm intervals from the anal side, labelled
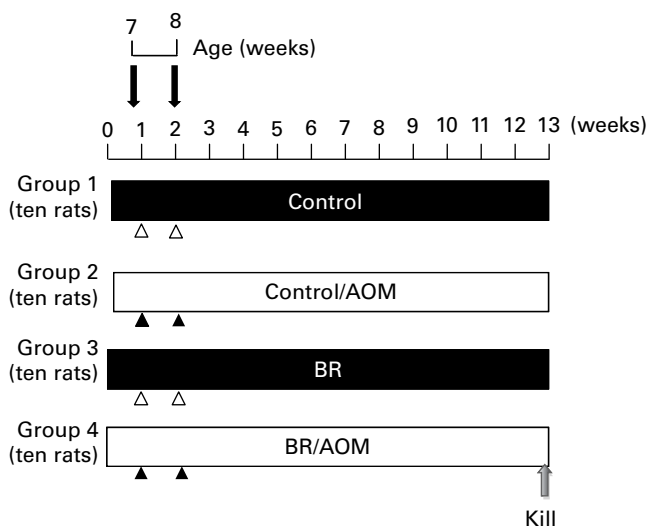
Fig. 1. Experimental design. Δ, Saline; ▲, subcutaneous injection of azoxymethane (AOM) at 20 mg/kg body weight. BR, barley Rihane.

and fixed in 10 % formalin (v/v) overnight, and prepared for haematoxylin and eosin staining. The number of ACF in each group was quantified. ACF were identified as rounded and defined clusters of crypts, where the crypt sizes are polymorph and greater than normal and the luminal sizes are also polymorph.

The classification of ACF from each group was also assessed for the presence and degree of dysplasia according to the criteria described previously by Papanikolaou *et al.*[14]. The criteria for the assessment of the degree of dysplasia are described as follows:

(1) Mild to moderate dysplastic foci:

  (a) Crypts have greater increased crypt height and more dilation of the crypt.
  (b) Cells exhibit moderately enlarged nuclei with irregular location, orientation, shape and darker colour with some focal nuclear stratification and slight vesiculation with no mitotic bodies observable.
  (c) There is a greater loss of the cytoplasm.
  (d) Crypts are moderately basophilic and more hypercellular.

(2) Moderate to severe dysplastic foci:

  (a) Crypts have increased height with severe dilation.
  (b) Cells exhibit enlarged nuclei with irregular location, loss of cell polarity, abnormal colour and shapes, some pleomorphism, extensive stratification and more vesiculation with prominent or multiple nucleoli.
  (c) Crypts are strongly basophilic and very hypercellular.

Extraction and purification of total DNA

DNA was extracted from colon samples (125 mg) using a bead-beating method adapted from Godon *et al.*[15]. The

protocol has been described in detail in a previous study[16]. The amount and integrity of DNA were estimated using 2 % (w/v) agarose gel electrophoresis containing ethidium bromide (0·1 ng/ml), in 1 × Tris−Borate−EDTA (Sigma Chemical Company).

Bacterial temporal temperature gradient gel electrophoresis

The colonic microbiota of rats were analysed using temporal temperature gradient gel electrophoresis (TTGE). The primers S-D-Bact-339-a-S-20 (50-CTC TAC CGG GAG GCA GCA GT-30) and S-D-Bact 788-a-A-19 (50-GGA CTA CCA GGG TAT CTA A-30) were used to amplify the variable regions 3 and 4 of the bacterial 16S ribosomal RNA genes. A GC-rich sequence (50-CCC CCC CCC CCC CGC CCC CCG CCC CCC GCC CCC GCC GCC C-30) was added to the 5′ end of the primer S-D-Bact-788-a-A-19, as described previously by Magne *et al.*[16]. The DCode universal mutation detection system (Bio-Rad) was used for sequence-specific separation of amplicons. Additionally, known bacterial strains were used to standardise band migration and gel curvature among different gels. This ladder consisted of the following organisms listed in their migration order: *Bacteroides* sp., *Prevotella* sp., *Enterococcus faecium*, *Staphylococcus epidermidis*, *Escherichia coli* and *Bifidobacterium longum*[17].

TTGE analysis was based on the method of Deplancke *et al.*[18]. Gel patterns were analysed using Diversity Database 2.1 Discovery Series (Bio-Rad).

Bands were detected automatically. Each band was defined through its relative intensity and relative front (RF). Relative intensity was the intensity of a particular band in a lane expressed as a percentage of the total intensity data in the lane. The RF was the distance from the top of a defined lane to the band. In order to compare several gels, a normalised RF derived from the RF was used to classify the bands. This normalised RF was based on the migration of a marker. A band set stored classified the bands defined as unique band types according to their normalised RF.

Comparisons of TTGE profiles were performed using Dice's similarity coefficient (D_{sc}) analysis based entirely on the results of band classification. D_{sc} values were compared, based on the presence or absence of bands. Dice's coefficient is defined as follows:

$$D_{sc} = (2j/(a + b)),$$

where j is the number of common bands between samples A and B; a and b are the total number of bands in samples A and B, respectively. This coefficient ranges from 0 (no common bands) to 1 (identical band patterns). Consequently, the distance between two TTGE profiles was as follows[17]: distance $= 1 - D_{sc}$.

Real-time PCR for bacterial quantification

Reactions were performed in duplicate in a fixed volume (25 μl) within ninety-six-well twin-tech PCR plates, using the Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen).

The forward and reverse primers used were Bif164f and Bif662r for *Bifidobacterium* [19], Ent1113f and Ent1418r for enterobacteria and Bia339f and Bia788r for bacteria[17]. Amplifications were performed in a Mastercycler ep Realplex[4] (excitation source 470 nm, emission 520/550 nm; Eppendorf AG) with the following temperature profile: one cycle at 52°C (2 min), one cycle at 96°C (2 min), forty cycles of denaturation at 96°C (15 s), primer annealing at 62°C (1 min) for bifidobacteria and for enterobacteria and at 55°C (1 min) for total bacteria, and elongation step at 68°C (2 min). The melting curve was obtained by slow heating at temperatures from 60 to 96°C at a rate of 0·2°C/s with continuous fluorescence collection. A negative control and a positive control were included on each plate. Each assay was performed in duplicate in the same run. The cycle threshold (C_t) was calculated as the cycle number at which the reaction became exponential. Standard curves were constructed using plasmid containing 16S ribosomal DNA fragments amplified with corresponding primers. The plasmid concentration was measured using a QubitTM fluorimeter (Invitrogen) according to the manufacturer's instructions.

Statistical analyses

Data are presented as mean values with their standard errors. Statistical analysis was performed using STATVIEW 4.0. Two-way ANOVA was used to determine the effects of the DF of BR, AOM and their interaction. A mean difference is considered significant when $P < 0.05$.

Results

Effect of the diet on food intake and weight body in azoxymethane-treated rats

The diet was well accepted by the rats. No mortality was caused by the diet or AOM. The final body weights and food intake for each group are shown in Table 3. The final body weight was lower ($P < 0.05$) in the BR group compared with the C group. The growth rate of rats was slower

($P < 0.05$) in the BR and BR/AOM groups compared with the C group. Food intake was not affected by dietary treatments.

Effect of the diet on colon, colon content, colonic wall weights and colonic pH in azoxymethane-treated rats

The colon, colon digesta and colonic wall weights for each group are shown in Table 3. BR induced a significant increase in colon weight ($P < 0.05$) compared with the C group. In the same way, this variety significantly increased colon weight even in the presence of AOM. The colon digesta was ($P < 0.05$) lower in the C/AOM group than in the C group, whereas BR induced an increase ($P < 0.05$) in colon digesta compared with the C group. The colonic wall was thickened ($P = 0.03$) in rats fed the BR diet than in the C group, while it was thinner in the C/AOM group ($P < 0.05$). BR increased the colonic wall ($P < 0.05$) even in the presence of AOM.

Colonic pH was ($P < 0.05$) lower in the BR group than in the C group, whereas AOM alone caused a slight decrease in colonic pH. BR significantly reduced colonic pH with or without AOM.

Effect of barley Rihane on the incidence of aberrant crypt foci in the rat colon induced by azoxymethane

The effect of BR on AOM-induced ACF development in rats is summarised in Table 4. The results showed that there were no ACF in the colon of normal rats. However, colonic ACF appeared in all rats that received AOM. In the C/AOM and BR/AOM groups, the incidence of ACF was 100 %. The results showed that the number of ACF/colon was lower ($P < 0.05$) in rats fed the BR/AOM diet compared with rats fed the C/AOM diet. Indeed, BR gave an important reduction (44 %) in the total number of ACF/colon.

Histological classification of aberrant crypt foci

The incidence of dysplastic crypt formation in each group is shown in Table 4. The results of the carcinogenesis

Table 3. Final body weight, body-weight gain, food intake, colon weight and colon content in the different groups (control (C), C/azoxymethane (AOM), barley Rihane (BR) and BR/AOM)

(Mean values with their standard errors, n 10)

Parameters	C* Mean	SEM	C/AOM† Mean	SEM	BR‡ Mean	SEM	BR/AOM§ Mean	SEM
Final body weight (g)	241·0a	6·00	231·0a	6·19	217·0b	6·44	221·0a	5·99
Body-weight gain (%)	60·09a	3·99	53·92a	4·51	45·04b	4·93	47·43b	3·50
Food intake (g/d)	17·0	1·03	17·0	0·79	19·0	1·40	18·0	0·57
Weights (g)								
Colon	8·23a	0·03	6·69b	0·04	8·97c	0·06	10·82d	0·03
Colon digesta	4·87a	0·04	4·11b	0·06	5·49c	0·06	6·03d	0·06
Colonic wall	3·35a	0·02	2·57b	0·05	3·48c	0·01	4·78d	0·05
Colonic pH	6·84a	0·01	6·75a	0·03	5·76c	0·04	6·38d	0·03

a,b,c,d Mean values with unlike superscript letters were significantly different ($P < 0.05$).
* Rats fed the C diet.
† Rats fed the C diet and treated with AOM.
‡ Rats fed the BR diet.
§ Rats fed the BR diet and treated with AOM.

Table 4. The effect of barley Rihane (BR) on the incidence of aberrant crypt foci (ACF) in the rat colon induced by azoxymethane (AOM)

(Mean values with their standard errors, *n* 10)

Group	Treatment	No. of rats	Incidence (%)	No. of ACF/colon	SEM	Morphology	P
1	C*	10	0	0	0	0	0
2	C/AOM†	10	10/10 (100)	188.7[a]	3.57	Severe dysplasia	0.05
3	BR‡	10	0	0	0	0	0
4	BR/AOM§	10	10/10 (100)	83.46[b]	7.86	Moderate dysplasia	0.05

C, control.
[a,b] Mean values with unlike superscript letters were significantly different ($P<0.05$).
* Rats fed the C diet.
† Rats fed the C diet and treated with AOM.
‡ Rats fed the BR diet.
§ Rats fed the BR diet and treated with AOM.

experiment, 12 weeks after the AOM treatment, demonstrated that in rats fed the BR diet and treated with AOM, there was a significant reduction ($P<0.05$) in the severity of dysplasia compared with the C/AOM group (Fig. 2(D) and (B)). Colon sections from these rats showed a slight or moderate dysplasia, whereas those from rats of the C/AOM group showed severe dysplasia or carcinoma *in situ*. However, rats in the C and BR groups showed a normal morphology (Fig. 2(A) and (C)).

Bacterial diversity

The gels resulting from PCR amplification of conserved bacteria domain sequences followed by TTGE (evaluation of total bacterial diversity) were examined for bands at seventy-five possible positions (Fig. 3). The occurrence of bands in the four groups was as follows: fifteen bands in the C group, nineteen bands in the C/AOM group, twenty-three bands in the BR group and seventeen bands in the BR/AOM
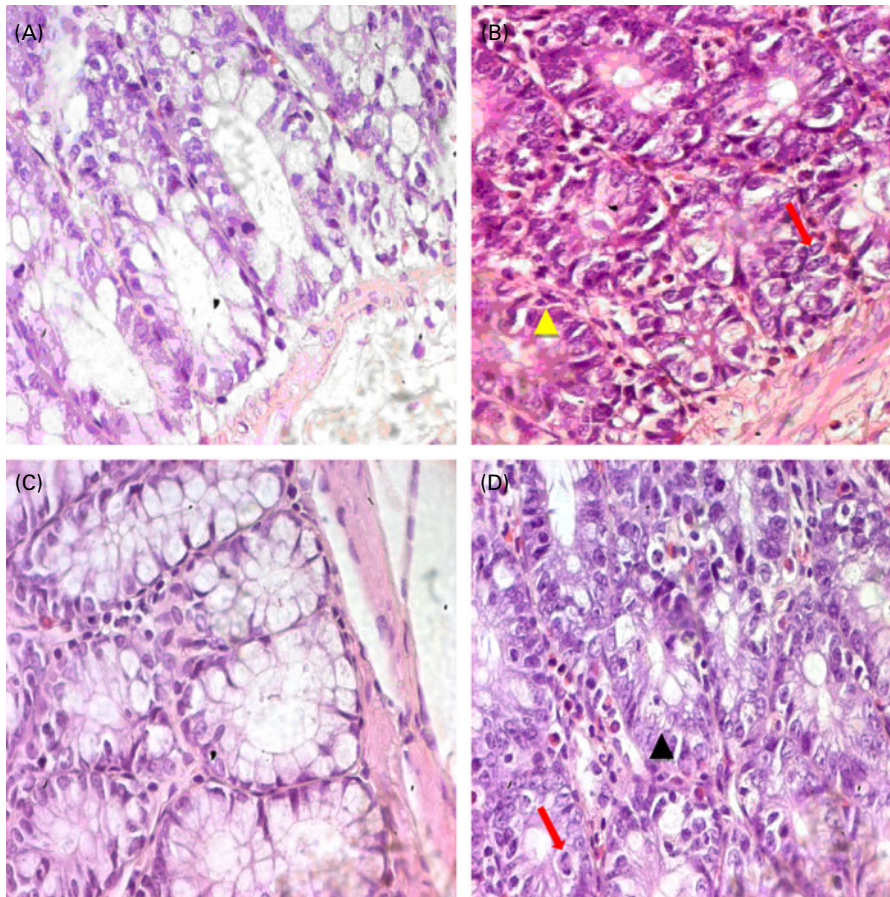
Fig. 2. Morphological evaluations of aberrant crypt foci in different groups of rats for the presence and degree of dysplasia (haematoxylin and eosin staining, 400 ×): (A) histological section from the control (C) group indicating normal morphology, (B) from the C/azoxymethane (AOM) group indicating severe dysplasia or carcinoma *in situ* (the wall of the colon has thickened mucosa and proliferated glands lined by the cells with hyperchromatic, pseudostratified nuclei), (C) from the barley Rihane (BR) group indicating normal architecture and (D) from the BR/AOM group indicating slight or moderate dysplasia. ➡, Increased number of mitoses; ▲, crypts being moderately basophilic and more hypercellular, ▲, crypts being strongly basophilic and very hypercellular. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn)
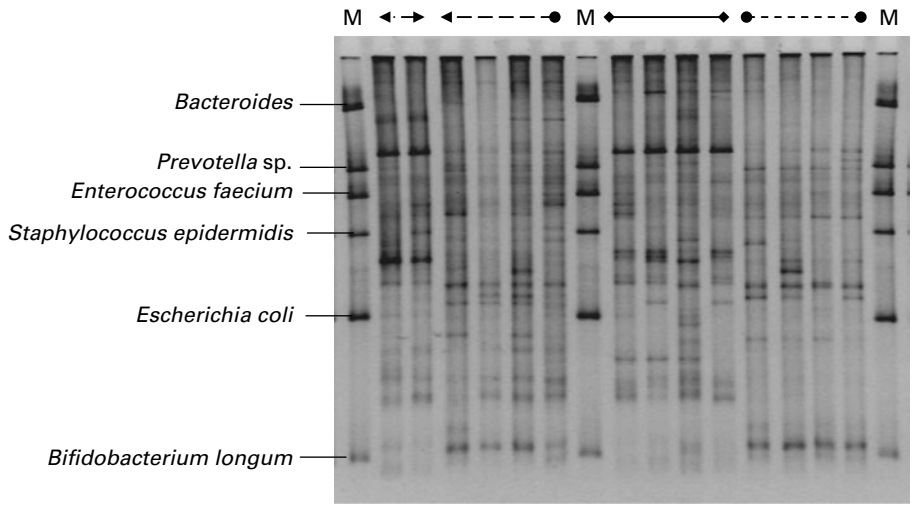
Fig. 3. Temporal temperature gradient gel electrophoresis gel showing bands obtained by the electrophoresis of PCR products from rat colonic content using primers targeted at conserved bacterial 16S ribosomal DNA sequences representative of domain bacteria. Lanes at the left and right extremes represent DNA marker (M). Bands 63 (*Escherichia*) and 95 (*Bifidobacterium*) are marked. C, Control; AOM, azoxymethane; BR, barley Rihane. C (●‑‑‑‑●), rats fed the C diet; C/AOM (◆——◆), rats fed the C diet and treated with AOM; BR (◄— — —●), rats fed the BR diet; BR/AOM (◄·—►), rats fed the BR diet and treated with AOM.

group. The TTGE profiles of the BR group showed that bacterial biodiversity was higher than that in C group. Several bands corresponding to the *Bifidobacterium* genus (band 95) were found in the BR profile. This result showed that BR favoured the *Bifidobacterium* biodiversity. However, several bands corresponding to *E. coli* (band 63) were found in the C/AOM profile. This result showed the AOM favoured the dominance of *E. coli*.

All PCR–TTGE profiles obtained from each gel were compared and the results were plotted as a dendrogram after calculating the Dice coefficient to assess the similarities between them (Fig. 4). We noted a strong similarity of 74% between the species of the BR/AOM group compared with the other groups, but this percentage did not reach the threshold of positive similarity (>98%). Comparison of similarity coefficients showed a species variability of colonic microbiota between the different groups. In addition, we showed inter-individual variability within the same group. This similarity is affected by the diet BR and AOM.

Bacteria, bifidobacteria and enterobacteria quantification

Results for total bacteria, bifidobacteria and enterobacteria in colonic samples using real-time PCR are shown in Fig. 5. The number of total bacteria in all the experimental groups (BR, C/AOM and BR/AOM) increased ($P<0.05$) compared with the C group. The number of bifidobacteria decreased in the BR group compared with the C group. However, the enterobacteria number in colonic content was significantly increased ($P<0.05$) in the BR group compared with the C group.

Discussion

We tested the hypothesis that DF would reduce AOM-induced formation of colonic ACF in rats. ACF are considered to be

possible precursor lesions for colon cancer and are regarded as a short-term marker of colon carcinogenesis in rodents and human subjects[20]. In the present study, colons were examined for ACF 12 weeks after the first injection of AOM. Data from ACF incidence indicated that all rats treated with AOM developed ACF. The results demonstrated that the DF of BR may decrease AOM-induced ACF development. The reduction in the incidence of ACF in rats in the BR group may be due to the induction of apoptosis in the colonic crypts. These results support those of previous investigation by Alabaster *et al.*[21] which reported that the DF of wheat reduced the total number of ACF in the rat colon. The finding of a reduction in the largest lesions may be most pertinent in terms of eventual development of colon tumours. Besides, the decrease in the numbers of ACF suggests that DF may inhibit the growth of ACF. The protective effects of DF on ACF development depend on the nature and source of the fibre,
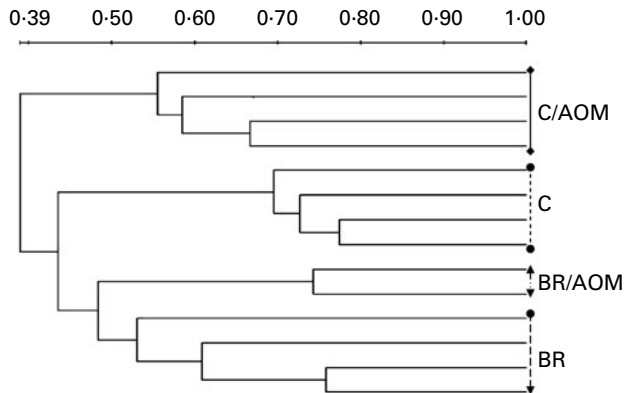

Fig. 4. Dendrogram representing the degree of relatedness among the 16S ribosomal DNA banding patterns of the different groups. C, Control; AOM, azoxymethane; BR, barley Rihane. C (●‑‑‑‑●), rats fed the C diet; C/AOM (◆——◆), rats fed the C diet and treated with AOM; BR (◄— — —●), rats fed the BR diet; BR/AOM (◄·—►), rats fed the BR diet and treated with AOM.
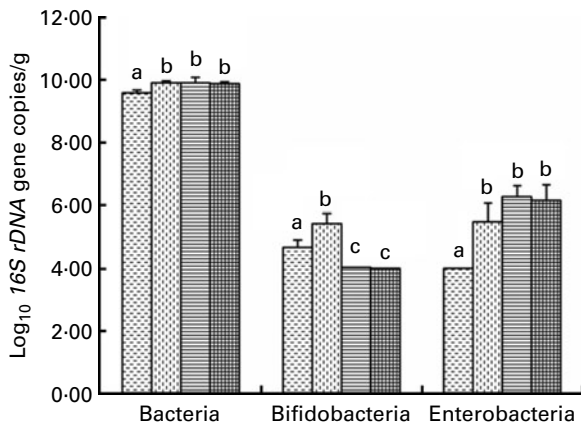
Fig. 5. Effect of the diet supplemented with barley Rihane (BR) on numbers of bacteria, bifidobacteria and enterobacteria in azoxymethane (AOM)-treated rats compared with the control (C). Values are means of ten rats, with standard errors represented by vertical bars. [a,b,c] Mean values with unlike letters were significantly different ($P<0.05$). C (▨), rats fed the C diet; C/AOM (▨), rats fed the C diet and treated with AOM; BR (▨), rats fed the BR diet; BR/AOM (▨), rats fed the BR diet and treated with AOM.

as studied by Reddy *et al.*[22]. After the quantification of ACF was made, the same tissue was used for ACF characterisation. According to Pretlow *et al.*[23] and McLellan *et al.*[24], evaluation of haematoxylin and eosin-stained sections of ACF from rodents reveals a wide range of histology from minor atypia to severe dysplasia. According to Shpitz *et al.*[25], dysplasia is considered as a feature of neoplasia, although it is not sufficient to define cancer. ACF have been classified as dysplastic, based primarily on morphological characteristics[26]. Nuclear atypia and/or dysplasia increases as a function of time following a dose of carcinogen, but, again, there is a great heterogeneity[26]. In the present study, we found an overall significant reduction in dysplastic changes in the BR/AOM group. The DF of BR probably slowed down the development of abnormality and consequently the advancement towards neoplasticity for the ACF observed. Therefore, it suggests that the barley variety used may potentially modulate colon cancer risk from an early stage.

The indigestible fibre components of barley, especially β-glucan, as well as resistant starch, progress through the digestive tract into the large intestine. Fermentation of this material by microflora then occurs, resulting in the formation of SCFA, especially butyrate and propionate[27]. The benefits of these fatty acids in the large intestine are healthy colonic mucosa and provision of an energy source for epithelial cells[28]. Dongowski *et al.*[29] investigated the effects of a high-amylose barley compared with maize starch, a high-resistant starch commercial product and a C diet with no barley. These materials were extruded to prepare experimental diets for rats. All of the animals fed barley diets thrived better and had greater intestinal mass than controls. SCFA were higher in caecal and colon contents of animals fed the experimental diets. The more acid conditions indicated a smaller proportion of secondary bile acids, believed to be promoting factors in colon cancer. Bird *et al.*[30] fed stabilised whole-grain barley flour from Himalaya 292, the high-amylose

barley cited previously[31], with two other barleys, and either wheat or oat bran to rats, to determine the effects of these feeds on intestinal SCFA. Although there were independent differences between animals fed different diets, colonic SCFA were consistently higher and pH was lower in those fed the Himalaya 292 barley than all other diets. Results were attributed to the greater RS content in this barley cultivar, due to its reduced amylopectin and increased amylose content as well as having a high β-glucan content. Biomarkers of bowel health in healthy subjects (faecal weight, faecal concentration of butyrate and SCFA excretion) were all significantly different between the groups and indicative of improved bowel health in subjects who consumed the barley diets. The beneficial health effects were attributed to the presence of resistant starch[30].

These results showed that the BR diet decreased the colonic pH ($P<0.05$) compared with the C diet. Similar results were reported by Zoran *et al.*[32] after feeding diets containing wheat bran. It was suggested that consumption of DF was associated with potentially beneficial changes in colon physiology after colonic pH reduction, which are in relation to the incidence of preneoplastic lesions and tumour risk in the colon. Dongowski *et al.*[29] suggested that a reduction in colonic pH is a possible factor in the suppression of colon tumorigenesis. The present data also confirm the findings of other studies that intake of resistant starch decreases colonic pH, which can be interpreted as improving bowel health and reducing the risk of developing colon cancer[33]. Although lowering of the colonic pH is also usually considered to be protective, Thornton[34] suggested that a very low pH may be a risk factor in carcinogenesis. DF produces high concentrations of SCFA. These reduce the pH in the colon, and values below 6·5 may result in a stimulation of epithelial cell proliferation with the subsequent enhancement of chemically initiated carcinogenesis. A high amount of cell proliferation is known to be a risk factor for colon cancer, presumably because it enhances the fixation of mutations.

Recent studies have revealed that the role of the microbiota was strongly related to the regulation of the incidence and progression of colon cancer. The colon is different from the other digestive organs, because it harbours an enormous number of coexisting microbiota[4]. The analysis of the colonic microbiota of forty rats using bacterial TTGE revealed a high inter-individual variability in the dominant microbiota, which increased with the BR diet. The TTGE profiles of the BR group showed that bacterial biodiversity was higher in the BR group than in C group. The present results suggest that the DF of BR can have long-term beneficial effects on the composition of colonic microbiota. The BR diet increased the biodiversity and number of total bacteria after 12 weeks. According to Venketeshwer *et al.*[35], intestinal microbiota was altered with the intake of DF after 2 weeks. Dongowski *et al.*[29] demonstrated that barley-rich diets providing 7·0–12 g/100 g β-glucan in rats induced no significant changes in total aerobic micro-organisms and significant increases in total anaerobes only after 6 weeks of β-glucan consumption at doses 11–12 g/100 g. These authors showed that the effects of DF on colonic microbiota depend on the type, structure and concentration of the fibre. Abell *et al.*[36] showed that

the supplementation with specific dietary resistant starch leads to changes in faecal microbiota profiles that may be associated with improved bowel health.

Bifidobacteria numbers are depleted in rats fed BR. However, this variety has promoted biodiversity of this species. This is confirmed by Evdokia et al.[37] who indicated that feeding a barley β-glucan diet induced a non-significant bifidogenic effect in Greek healthy adult volunteers. The DF of BR increased the total number of bacteria, but did not alter the composition of the microbiota in the colon. The presence of dietetic components in BR, especially β-glucan, could explain the decreased bifidogenic effect. This decrease could be due to factors intrinsic to the β-glucan composition and to their availability for bacterial attack.

AOM has favoured the dominance of E. coli. The present data also confirm the findings of other studies showing that E. coli are specifically involved in the activation of pro-carcinogens[38].

In conclusion, the results of the present experiments suggest that the DF of BR exert a preventive effect against AOM-induced rat ACF. The present study indicates that these compounds are potent factors to reduce colon cancer risk. More detailed BR studies concerning the mechanism of the onset of colon cancer, particularly regarding the changes in the microbiota (bacterial species identification by sequencing) and its metabolites on mucosal barrier function, are still required.

Acknowledgements

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