Restoration of the integrity of rat caeco-colonic mucosa by resistant starch, but not by fructo-oligosaccharides, in dextran sulfate sodium-induced experimental colitis

Noëlle M. Moreau1,3, Lucile J. Martin1,3*, Claire S. Toquet3,4,5, Christian L. Laboisse3,4,5, Patrick G. Nguyen1,3, Brigitte S. Siliart1,3, Henri J. Dumon1,3 and Martine M. J. Champ2,3

1 Unité de Nutrition et d’Endocrinologie, Ecole Nationale Vétérinaire, Nantes, France
2 Unité des Fonctions Digestives et Nutrition Humaine, Institut National de la Recherche Agronomique, Nantes, France
3 Centre de Recherche en Nutrition Humaine, Nantes, France
4 INSERM U539, Faculté de Médecine, Nantes, France
5 Service d’Anatomie Pathologique, CHU, Nantes, France

(Received 15 April 2002 – Revised 17 February 2003 – Accepted 20 February 2003)

Butyrate is recognised as efficient in healing colonic inflammation, but cannot be used as a long-term treatment. Dietary fibre that produces a high-butyrate level when fermented represents a promising alternative. We hypothesised that different types of dietary fibre do not have the same efficiency of healing and that this could be correlated to their fermentation characteristics. We compared short-chain fructo-oligosaccharides (FOS) and type 3 resistant starch (RS) in a previously described dextran sulfate sodium (DSS)-induced colitis model. Seventy-two Sprague–Dawley rats received water (control rats) or DSS (50 g DSS/l for 7 d then 30 g DSS/l for 7 (day 7) or 14 (day 14) d). The rats were fed a basal diet (BD), or a FOS or RS diet creating six groups: BD-control, BD-DSS, FOS-control, FOS-DSS, RS-control and RS-DSS. Caeco-colonic inflammatory injuries were assessed macroscopically and histologically. Short-chain fatty acids (SCFA) were quantified in caeco-colon, portal vein and abdominal aorta. At days 7 and 14, caecal and distal macroscopic and histological observations were improved in RS-DSS compared with BD-DSS and also with FOS-DSS rats. Caeco-colonic SCFA were reduced in FOS-DSS and RS-DSS groups compared with healthy controls. The amount of butyrate was higher in the caecum of the RS-DSS rats than in the BD-DSS and FOS-DSS rats, whereas distal butyrate was higher in FOS-DSS rats. Partially explained by higher luminal levels of SCFA, especially butyrate, the healing effect of RS confirms the involvement of some types of dietary fibre in inflammatory bowel disease. Moreover, the ineffectiveness of FOS underlines the importance of the type of dietary substrate.

Fructo-oligosaccharide: Resistant starch: Dextran sulfate sodium: Short-chain fatty acids: Butyrate

Acetate, propionate and butyrate, the three major short-chain fatty acids (SCFA) are the endproducts of the colonic anaerobic fermentation of dietary fibre. Luminal SCFA are oxidation substrates for the colonocyte (Clausen & Mortensen, 1994) and have trophic effect on healthy gut epithelial cells (Sakata, 1987). Systemic SCFA also influence the intestinal structure (Tappenden & McBurney, 1998). Among SCFA, butyrate is known to represent the major fuel source for the colonocyte (Roediger, 1980a, 1982; Fleming et al. 1991) and to be essential for mucosa health (Basson & Sgambati, 1998). Indeed, it was shown that a lack of butyrate has deleterious effects on the colonic mucosa (Firmansyah et al. 1989; Roediger, 1993).

Previous studies have demonstrated that SCFA, especially butyrate, have a beneficial trophic effect on inflamed caeco-colonic mucosa of human subjects (Scheppach et al. 1992, 1997; Frankel et al. 1994) or experimental animal colitis (Butzner et al. 1996). Many studies have shown that inflamed rat (Ahmad et al. 2000) or human (Roediger, 1980b; Chapman et al. 1994; Den Hord et al. 1998) colonocytes showed impairment of butyrate oxidation. Butyrate enemas have shown their benefit for mucosal health (Breuer et al. 1991; Steinhart et al. 1994) in patients suffering from Crohn’s disease or ulcerative colitis, which encompass the two major forms of inflammatory bowel disease. However, this positive effect disappears when administered per os (Kanauchi et al. 1999). Hence, among therapeutic alternatives, dietary fibre that produces a high butyrate proportion when fermented in the large intestine, and which could be ingested as a functional food, seems a promising and a more realistic treatment.
for patients. However, no results are available on what the characteristics of the best dietary substrates to heal inflammatory bowel disease are. In the present paper, in order to assess their healing efficiency, two substrates, well known to yield a large amount of butyrate after fermentation, were tested on a previously described model of colitis based on a dextran sulfate sodium (DSS) administration. In a few days, this continuous DSS treatment was able to induce stable chronic inflammation, predominantly with caecal and distal injuries (Moreau et al. 2002). The two fibres were selected according to their fermentation kinetics. When considering the caeco-colon, fructo-oligosaccharides (FOS) are known to be rapidly fermented, whereas resistant starch (RS) is more slowly degraded and can reach the distal part of the colon (Le Blay et al. 1999a,b).

The objective of the present study was to examine which substrate induced the best recovery of mucosa by looking at morphological macroscopic and histological criteria. Indeed, we made the hypothesis that these fibres, which have different fermentation patterns, would not have the same capacity to restore the inflamed colonic mucosa and that this effect could be partly caused by their ability to be fermented to produce SCFA, especially butyrate.

Methods

Animals and housing

Seventy-two male Sprague–Dawley rats (Harlan, Gannat, France), weighing 310 (SEM 10) g at arrival, were used for the present experiment. They were housed individually in cages designed to avoid coprophagy, and a light–dark cycle (light on 08.00–20.00 hours) was used. The animals were allowed free access to diet and water until killing. The experiment was carried out according to the French animal welfare rules (Decree no. 2002/464, 29 May 2001) and approved by the Nantes Ethics Committee for Laboratory Animals.

Diets

Three experimental diets with different fibre contents (Table 1) were specifically formulated for the experiment (INRA, Jouy-en-Josas, France). The basal diet (BD) was a low-fibre diet containing 20 g cellulose/kg. The FOS diet was enriched with short-chain FOS (Actilight P; Beghin-Meiji Industries, Paris, France) and the RS diet with RS type 3 (retrograded Hylon 7, high amylo maize starch, Novelose 330; National Starch and Chemical Ltd, Manchester, UK). They were formulated to provide 60 g indigestible carbohydrate + cellulose/kg DM. The metabolisable energy contents (kJ/g DM) were 17.30, 16.76 and 16.34 for the BD and FOS and RS diets respectively. Water, food intake and body weight were recorded daily.

Colitis induction

At the beginning of the experiment, after a 7 d adaptation to the diet and housing conditions, the animals were randomly divided into two groups. The first group received 50 g DSS/l in water (DSS group) (DSS molecular mass 36–44 kDa; ICN Pharmaceuticals, Orsay, France) for 7 d (day 0). Then, the concentration of the DSS solution was reduced to 30 g/l for 7 d (day 7) or 14 d (day 14) in order to induce a stable chronic active colitis as previously described (Moreau et al. 2002). The second group was free from DSS throughout the experiment and received drinking water (control group). As shown in Fig. 1, during the adaptation period and the 50 g DSS/l period, all the animals (control and DSS groups) received the BD. The rats were then randomly assigned to one of the three experimental diets (BD, FOS and RS diets). The six groups were identified as: BD-control and BD-DSS, FOS-control and FOS-DSS, RS-control and RS-DSS.

Six rats from each of the six groups were killed at day 7 (water or 50 g DSS/l for 7 d then 30 g DSS/l for 7 d), and six rats at day 14 (water or 50 g DSS/l for 7 d then 30 g DSS/l for 14 d).

Collection of digestive tissues and contents

After being anaesthetised by intraperitoneal injection of 15 mg xylazine/kg (Bayer Pharma, Puteaux, France) and intramuscular injection of 55 mg ketamine/kg (Mérial, Lyon, France), the rats were laparotomised and blood samples were collected from the portal vein and abdominal aorta with an infusion set. The animals were then killed by an intra-cardiac injection of sodium pentobarbital (Vétouquinol, Lure, France). The caecum and the colon were resected after ligature of the ileo-caecal junction up to the rectal ampoule. The entire colon length was measured and the three segments (caecum, proximal and distal colon) were then removed and weighed to assess inflammation. Afterwards, each was cut longitudinally and tissues and digestive contents were recovered. The whole procedure occurred between 09.30 and 10.30 hours for all rats.

Macroscopic and histological scores

Caecum, proximal and distal colon were gently cleaned with isotonic solution (9 g NaCl/l; Aguettant, Lyon, France), and colitis activity was scored by visual examination of the mucosa (Le Blay et al. 1999a). The severity was scored on a scale of 0 (no lesions) to 5 (extensive ulcerations). For histological analysis, samples were fixed in 10% formaldehyde, paraffin embedded and cut into 4–5 µm sections. Sections were stained with haematoxylin and eosin (H&E) and scored as described (Le Blay et al. 1999a). The scoring was performed on at least 10 fields per colon.

Table 1. Composition of the three experimental diets (g/kg)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Basal</th>
<th>FOS</th>
<th>RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-chain FOS*</td>
<td>0</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td>RS (type 3)†</td>
<td>0</td>
<td>0</td>
<td>115</td>
</tr>
<tr>
<td>Pregelatinised maize starch‡</td>
<td>650</td>
<td>587</td>
<td>535</td>
</tr>
<tr>
<td>Casein</td>
<td>204</td>
<td>204</td>
<td>204</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Refined maize oil</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Lard</td>
<td>58</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Cellulose§</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Minerals</td>
<td>44</td>
<td>44</td>
<td>44</td>
</tr>
<tr>
<td>Vitamins</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

FOS, fructo-oligosaccharide; RS, resistant starch.
* Actilight P; Beghin-Meiji Industries, Paris, France.
† Novelose 330; National Starch and Chemical Ltd, Manchester, UK.
‡ Cerestar SF 12018; Cerestar, Vilvoorde, Belgium.
§ Arbocel BOO; Arbocel, Marne-la-Vallée, France.
assessment (assessors were blinded to the treatment group), using a scale described by Appleyard & Wallace (1995) with slight modifications: tissues adhesions (0, none; 1, minor; 2, severe), caecal mucosa (0, normal; 1, hyperaemia; 2, blood in contents; 3, ulcerations), diarrhoea (0, none; 1, mild; 2, severe), blood in the contents (0, none; 1, in one-third of the colon; 2, in two-thirds of the colon; 3, in the entire colon), colonic mucosa thickening (0, normal; 1, moderate; 2, severe), colonic ulcerations (0, any site; 1, focal hyperaemia; 2, limited ulcerations; 3, ulcerations extending more than 10 mm along the length of the colon). The total macroscopic score corresponded to the sum of these partial scores.

Tissue was fixed in neutral buffered formalin (100 ml/l), dehydrated, and paraffin-embedded. Sections (5 μm) were stained with haematoxylin and eosin, and scored by considering the loss of mucosal architecture (scored 0–3), cellular infiltration (0–3), muscle thickening (0–3), crypt abscesses (0–1) and goblet cell depletion (0–1) (maximum score 11) (Appleyard & Wallace, 1995; Moreau et al. 2002).

Short-chain fatty acid quantification in caeco-colonic contents

A sample of the contents of three gut segments was removed and homogenised in 2 ml mercuric chloride (1 g/l; Merck, Darmstadt, Germany). After centrifugation, the supernatant fraction was frozen immediately at −80°C and stored until later analysis. SCFA concentrations were quantitated by GC (GC-6890; Hewlett Packard, les Ulis, France) using a 1 μm FFAP column (30 m × 0.53 mm) (Hewlett Packard) and flame ionisation detector, using the procedure described by Brighenti (1997), slightly modified, with 2-ethylbutyric acid as internal standard. SCFA were expressed as molar ratios, μmol per caeco-colonic compartment (‘amount’ of SCFA per compartment) and μmol/g wet digestive contents (‘concentrations’ of SCFA in each compartment). In order to verify if DSS inhibits the fermentative intensity, SCFA were expressed as μmol/g food DM intake (mean value of the last 3 d) to take into account the possible differences in food intake between DSS and control rats and between the different diets.

Short-chain fatty acid measurements in blood samples

Total arterial or venous blood was collected in a heparinised tube, centrifuged at 2100 g for 10 min, and plasma frozen at −80°C. Plasma SCFA measurements were performed using isovaleric acid (Sigma-Aldrich, St Quentin-Fallavier, France) as internal standard with the procedure described by Brighenti (1997).

Calculations and statistics

Levels of SCFA appearing in the portal blood were calculated by arterio-venous difference (Δ) between concentrations obtained in the portal vein and in the abdominal aorta (Demigné & Rémésy, 1982). The objective of calculating this difference was to subtract the endogenous part of SCFA (which is not of digestive origin) from the portal concentrations, in order to consider only SCFA that a priori came solely from caeco-colonic fermentation. Portal SCFA were also expressed as molar ratio (ΔC/ΔSCFA).

Results were expressed as mean values with their standard errors, and n refers to the number of rats in each of the six groups. Macroscopic and histological scores were compared using the non-parametric Kruskal–Wallis test. When a
significant effect ($P<0.05$) was observed, diets were compared separately using a Mann–Whitney test. The other variables described in the present paper were analysed by ANOVA with treatment (DSS v. control), diet and time (day 7 and day 14) as main factors. When there was no significant interaction effect in the two-way (treatment $\times$ diet) or three-way (treatment $\times$ diet $\times$ time of killing) comparisons, we conducted a one-way comparison, whereas two-way or three-way comparisons were maintained when the $P$ value of two-way or three-way interaction effect first was $<0.05$. When the tested effect was significant, the differences between mean values were assessed by contrasts analysis using Super ANOVA V 1.11 (Abacus Concepts Inc., Berkeley, CA, USA). For all calculations, the accepted level of statistical significance was $P<0.05$.

Results

Daily consumption

No significant difference either in the DSS consumption (Table 2) or in the water:diet consumption ratio was found among BD, FOS and RS groups. In the control group, daily food and water intake were not affected by the type of diet (BD, FOS or RS diet). Diet and DSS consumption of rats fed BD, FOS and RS between days 0 and 7 and between days 7 and 14 (for rats killed at day 14) (see p. 76 and Fig. 1) are indicated in Table 2. In the DSS rats, no difference in food intake appeared between days 0 and 7. Conversely, the FOS-DSS rats had a lower food intake between days 7 and 14 compared with the BD-DSS rats (NS) and RS-DSS rats ($P=0.047$).

Body-weight gain

In the control group, body-weight gain was not influenced by the type of diet (results not shown). In the DSS group, the greatest weight loss was observed in the FOS-DSS group (Table 2). Nevertheless, body weight was stable between days 7 and 14 among the groups.

Length and weight of the colon and weight of the caecum

Macroscopic variables differed significantly according to the treatment DSS v. control (length of the colon $P=0.0001$, weight of the caecum $P=0.0002$, weight of the colon $P=0.0001$). Neither the type of diet nor the time of killing (day 7 or 14) had a significant effect. However, at day 14 the colons of RS-DSS rats tended to be longer than FOS-DSS (NS) and BD-DSS rats ($P=0.061$) (Table 3). While the FOS-DSS and BD-DSS rats had significantly heavier caeca and colons than the corresponding

Table 2. Diet intake, dextran sulfate sodium (DSS) intake and body-weight gain of rats fed basal, fructo-oligosaccharide (FOS) or resistant starch (RS) diet for 7 (day 7) or 14 (day 14) d and treated with 50 g DSS/l (7 d) then 30 g DSS/l for 7 (day 7) or 14 (day 14) d$^*$

<table>
<thead>
<tr>
<th>Day…</th>
<th>Diet intake (g/d)</th>
<th>DSS intake (g/d)</th>
<th>Body-weight gain†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td></td>
<td>0–7</td>
<td>0–14</td>
<td>0–7</td>
</tr>
<tr>
<td>Diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>12.0$^a$</td>
<td>1.1</td>
<td>14.5$^{ab}$</td>
</tr>
<tr>
<td>FOS</td>
<td>10.3$^a$</td>
<td>0.5</td>
<td>12.5$^{ab}$</td>
</tr>
<tr>
<td>RS</td>
<td>11.9$^a$</td>
<td>1.1</td>
<td>14.8$^{ab}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group…</th>
<th>Length of the colon (mm)</th>
<th>Weight of the caecum (g)</th>
<th>Weight of the colon (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>DSS</td>
<td>Statistical</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>Diet</td>
<td>significance</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Basal</td>
<td>178$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FOS</td>
<td>168$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS</td>
<td>181$^a$</td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Basal</td>
<td>176$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FOS</td>
<td>176$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RS</td>
<td>187$^a$</td>
</tr>
</tbody>
</table>

For details of diets and procedures, see Table 1, Fig. 1 and p. 76. For details of diets and procedures, see Table 1, Fig. 1 and p. 76.
Resistant starch restores inflamed rat mucosa

control rats, there was no difference between the RS-DSS and RS-control rats. Moreover, FOS-DSS colons were therefore heavier than RS-DSS ($P=0.0511$) (Table 3).

Macroscopic score

Whatever the time of killing, the control rats’ score values were zero. In DSS-treated rats, there was no difference in the score between diets at day 7: 6·6 (SEM 1·1), 7·7 (SEM 1·1) and 6·8 (SEM 1·2) for the BD-, FOS- and RS-DSS groups respectively. However, at day 14 the macroscopic score of the RS-DSS rats (2·7 (SEM 0·6)) was significantly lower than the BD-DSS (6·7 (SEM 0·9), $P=0.0082$) and FOS-DSS rats (6·1 (SEM 1·5), $P=0.0060$). Total score and all the variables included in the macroscopic score at day 14 are detailed in Fig. 2.

Histological assessments

Histological scores of control groups were zero. At day 7 (Fig. 3(a)) and at day 14 (Fig. 3(b)), histological scores measured in the caecum were significantly ($P<0.05$) lower in RS-DSS than in BD-DSS rats and in FOS-DSS rats (at day 7). There was no difference between diets in the proximal colon. In the distal colon, the RS diet significantly ($P<0.05$) decreased the histological score compared with the BD at days 7 and 14.

The total histological score (caecum+proximal+distal scores) was significantly lower for RS-DSS than BD-DSS ($P=0.0008$ at day 7, $P=0.0124$ at day 14) and than FOS-DSS ($P=0.0195$ at day 7, $P=0.0406$ at day 14).

Caeco-colonic fermentations

Effect of dextran sulfate sodium on the caeco-colonic measurements of short-chain fatty acids ($\mu$mol or $\mu$mol/g wet contents). Whatever the diet, the treatment and the time of killing, the amount of SCFA ($\mu$mol) was considerably higher in the caecum than in proximal and distal colon (Table 4). In the caecum, SCFA contents were lower when the rats received the DSS treatment compared with healthy controls. Conversely, proximal and distal amount of SCFA did not vary with DSS (except for RS at day 7).

When results were expressed as concentrations ($\mu$mol/g wet contents), except in the caecum at day 7, digestive SCFA of rats fed BD were not modified by the DSS treatment. Conversely, caeco-colonic SCFA concentrations of rats fed the RS- or FOS-enriched diets were significantly ($P<0.05$) reduced with DSS compared with healthy controls (Table 4).

Effect of dextran sulfate sodium on the intensity of fermentation ($\mu$mol/g DM intake). The consequences of a DSS-induced inflammation on the intensity of fermentation (SCFA measurements expressed as $\mu$mol/g DM intake) differed among diets. DSS treatment did not modify proximal and distal SCFA measurements expressed as $\mu$mol/g DM intake. As previously shown (Moreau et al. 2002), DSS reduced the fermentative abilities of rats fed for 7 d (day 7) with a low-fibre diet (BD) ($P=0.0249$), but this inhibition disappeared at day 14. In contrast, when DSS-treated rats were fed the diet supplemented with FOS, caecal fermentation was strongly inhibited at day 7 (8·1 (SEM 1·8)) v. 25·0 (SEM 2·5) $\mu$mol SCFA/g DM intake for control, $P=0.0004$) and at day 14 (12·9 (SEM 2·1) v. 30·6 (SEM 4·2) $\mu$mol/g DM intake for controls, $P=0.0003$). Conversely, for RS rats, no change appeared at day 7 between control (25·4 (SEM 2·6) $\mu$mol/g DM intake) and DSS groups (18·9 (SEM 3·6) $\mu$mol/g DM intake). At day 14, there was a trend of decrease in caecal SCFA by DSS treatment (13·5 (SEM 4·1) v. 22·6 (SEM 2·1) $\mu$mol/g DM intake for controls, $P=0.0578$).

Effect of dextran sulfate sodium on short-chain fatty acid molar ratio. Table 4 also summarises the qualitative disturbances of caeco-colonic fermentation induced by DSS administration. For the BD-DSS rats, the caecal butyrate molar ratio was higher at day 7 compared with control groups. With FOS diet, the acetate molar ratio was reduced in DSS groups at day 7 in the caecum and the proximal colon, whereas the propionate molar ratio of DSS rats

---

Fig. 2. Changes in the total and detailed macroscopic score of rats fed basal (BD), fructo-oligosaccharide (FOS) or resistant starch (RS) diet for 14 d and treated with 50 g dextran sulfate sodium (DSS)/l (7 d) then 30 g DSS/l for 14 d. □, BD-DSS; □, FOS-DSS; ■, RS-DSS. For details of diets, procedures and scoring system, see Table 1, Fig. 1 and p. 77. Values are means for six rats per group with their standard errors shown by vertical bars. a,bMean values with unlike superscript letters were significantly different: $P<0.05$. 
was higher compared with control rats. No molar ratio modifications were found in the RS-DSS rats compared with the RS-control rats. Concerning butyrate, it was interesting to note that its molar ratios in caecal, proximal and distal contents were not modified by the introduction of DSS when the animals were fed a fibre-enriched diet.

Effect of dextran sulfate sodium on the amount of butyrate. Because of the lack of time effect (and time × diet interaction, two-way ANOVA, $P=0.79$) on the amount of digestive butyrate ($\mu$mol), results obtained at days 7 and 14 were pooled. The amount of caecal butyrate observed in RS-DSS (38.8 (SEM 8.2) $\mu$mol) rats was significantly higher than in BD-DSS (9.6 (SEM 1.1) $\mu$mol, $P=0.0002$) and in FOS-DSS (12.5 (SEM 2.5) $\mu$mol, $P=0.0006$). No difference among diets was observed in the proximal colon. Conversely, in the distal colon, the greatest amount of butyrate was measured in FOS-DSS rats (4.4 (SEM 0.8) $\mu$mol) compared with the RS-DSS (3.9 (SEM 0.9) $\mu$mol) (NS) and BD-DSS animals (2.3 (SEM 0.4) $\mu$mol, $P=0.0198$).

Short-chain fatty acids in portal blood

Arterio–venous differences in SCFA are detailed in Table 5. Whatever the diet, DSS rats had a lower $\Delta$acetate than controls, whereas $\Delta$propionate and $\Delta$butyrate
Table 4. Short-chain fatty acid (SCFA) measurements, weight of the wet contents and amounts of the major SCFA in the caecum, proximal and distal colon of rats fed basal, fructo-oligosaccharide (FOS) or resistant starch (RS) diet for 7 (day 7) or 14 (day 14) d and treated with water (control group) or 50 g dextran sulfate sodium (DSS)/l for 7 d then 30 g DSS/l for 7 (day 7) or 14 (day 14) d‡

(Mean values with their standard errors for six rats per group)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Day 7</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>DSS</td>
</tr>
<tr>
<td>Group</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Caecum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCFA (µmol)</td>
<td>193.7</td>
<td>28.5</td>
</tr>
<tr>
<td>SCFA (µmol/g wet contents)</td>
<td>80.1</td>
<td>6.0</td>
</tr>
<tr>
<td>wet content (g)</td>
<td>2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>SC2 (%)</td>
<td>76</td>
<td>1</td>
</tr>
<tr>
<td>SC3 (%)</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>SC4 (%)</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Proximal colon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCFA (µmol)</td>
<td>19.2</td>
<td>5.6</td>
</tr>
<tr>
<td>SCFA (µmol/g wet contents)</td>
<td>37.7</td>
<td>5.0</td>
</tr>
<tr>
<td>wet content (g)</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>SC2 (%)</td>
<td>79</td>
<td>1</td>
</tr>
<tr>
<td>SC3 (%)</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>SC4 (%)</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Distal colon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCFA (µmol)</td>
<td>10.4</td>
<td>1.3</td>
</tr>
<tr>
<td>SCFA (µmol/g wet contents)</td>
<td>29.6</td>
<td>9.0</td>
</tr>
<tr>
<td>wet content (g)</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>SC2 (%)</td>
<td>80</td>
<td>1</td>
</tr>
<tr>
<td>SC3 (%)</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>SC4 (%)</td>
<td>11</td>
<td>1</td>
</tr>
</tbody>
</table>

C2, acetate; C3, propionate; C4, butyrate.

Mean values were significantly different from those of the control group at days 7 and 14: *P<0.05, **P<0.01, ***P<0.001.

Interactions of diet x treatment were significantly different for each variable (two-way ANOVA): †P<0.05.

‡ For details of diets and procedures, see Table 1, Fig. 1 and p. 77.
Day 14

Interactions of diet

Mean values were significantly different from those of the control group for each diet: * (Breuer et al. 2002), lymphocytes (Chapman, 2001), neutrophils (Liu et al. 2002), arterio–venous difference for acetate; ** (Segain et al. 2000) or nuclear factor-κB (Segain et al. 1997) or in animals (Butzner et al. 1996). But, interestingly, there was no effect when butyrate was given to control animals or rats treated with water (control group). The effect of butyrate on the health of the mucosa is well known and its role appears to be multi-factorial. Indeed, its presence is known and its role appears to be increasingly well documented (for example via interactions with cytokines (Nancey et al. 1994) or in animals (Breuer et al. 1999; Araki et al. 1999) and pectin (Fernandez-Banares et al. 1999) and germinated-barley foodstuff in this DSS model concern the distal colon and predominantly the caecal and distal mucosa. This seemed to appear as soon as day 7 nor at 14 compared with the BD. Conversely, fermentation of short-chain FOS (Le Blay et al. 1999b) and RS (Le Blay et al. 1999a) are known to yield high levels of butyrate. Results obtained in models of inflammatory bowel disease (trinitrobenzene sulfonic acid-induced) indicated that FOS (Cherbut et al. 2003) and RS (granulated pea-starch) (Jacobasch et al. 1999) are good candidates for the treatment of the inflamed epithelial mucosa. FOS (Gallagher et al. 1996; Pierre et al. 1997; Perrin et al. 2001) and RS (Perrin et al. 2001) have also shown beneficial properties against colon carcinogenesis, even if the impact of RS seems controversial (Sakamoto et al. 1996; Pierre et al. 1997).

Our results presented here focus on the effect of FOS and RS on the digestive mucosa inflamed by DSS. Histopathological characteristics of injuries observed in this model concern the distal colon and predominantly the caecum (Moreau et al. 2002). Macroscopic and histological results showed no caeco-colonic mucosal repair with FOS neither at day 7 nor at 14 compared with the BD. Conversely, macroscopic and histological results indicated that RS diet can provide beneficial effects on the chronic inflamed caecal and distal mucosa. This seemed to appear as soon as 7 d (day 7) and persisted throughout the experiment. This RS healing effect against DSS damage was significant compared with the fibre-free BD, but also compared with the FOS-supplemented diet. As proximal injuries induced in this DSS model were few (Moreau et al. 2002), it is not surprising that dietary manipulations had a minor effect on the proximal colon.

The differing histopathological consequences of a FOS or RS supplementation could be suspected to be linked with some possible bias, such as food consumption differences. It appeared that this effect cannot be explained by the trophic action of butyrate.
differences either in diet or in DSS consumption. We observed identical food consumption for the three DSS-treated groups between days 0 and 7, whereas food consumption of FOS-DSS rats was lower than the RS-DSS group between days 7 and 14 (Table 2). Simultaneously, histological analysis indicated that the first signs of mucosal healing appeared at day 7 for the RS-DSS groups, whereas the inflammation observed in FOS-DSS rats was always more severe. This suggests that the lower food consumption observed in FOS-DSS rats could be a consequence of the changes in the inflammation process compared with RS-DSS rats. It is probably not a causal event explaining the lack of healing effect of FOS diet on the injured mucosa. Moreover, the water: diet intake ratio remained constant among groups.

Many hypotheses could be proposed to explain, at least in part, the difference of effect between FOS and RS on the inflamed mucosa. Among them, differences in caecocolonic SCFA profiles and concentrations yielded after fermentation of the tested diets could be proposed. Considering only DSS-treated rats, our present results underline discrepancies in SCFA measurements between diets. The amount of caecal SCFA (µmol) measured at day 7 in FOS-DSS rats was considerably lower than in RS-DSS rats (results were equivalent at day 14). Moreover, at day 7 as well as 14, there was proportionally less butyrate in the caecum of FOS-DSS rats than in RS-DSS rats, which could partially explain the lack of benefit of FOS observed in the caecal mucosa. Nevertheless, it is interesting to note that in FOS-DSS rats, despite a higher level of butyrate in the distal colon compared with the other diets, no improvement of distal histological scores was observed. Consequently, the caecal fermentation appears to be essential for the healing process.

After 7 and 14 d of chronic colitis, our present results indicated that the DSS treatment inhibits the fermentation process, SCFA measurements (amount or concentrations) being lower in DSS rats than in controls (except for BD rats at day 14). Consequently, to be efficient, dietary fibre-containing diets must yield sufficient butyrate to counteract the inhibition of fermentation caused by DSS inflammation. These results underline the fact that the efficiency of dietary fibre against DSS injuries seems to be partly related to their fermentation characteristics.

Another possible explanation of the difference between FOS and RS could concern the luminal composition and particularly sulfate-reducing bacteria. They have been reported to colonise the large intestine in ulcerative colitis (Pitcher et al. 1995; Roediger et al. 1997) and to generate high levels of sulfides, sulfites and mercaptans (Roediger & Nance, 1990; Roediger et al. 1997). Some of these compounds may inhibit the butyrate uptake by the colonocytes (Stein et al. 1995; Jacobasch et al. 1999) and the colonic butyrate oxidation (Roediger et al. 1993a,b, 1996; Pitcher & Cummings, 1996) leading to an alteration of the mucosal integrity (Roediger, 1995).

Sulfate-reducing bacteria prefer neutral pH conditions and sulfate reduction is optimal at alkaline pH (Widdel, 1988). By decreasing caeco-colonic pH with the production of SCFA, FOS and RS could inhibit the action of sulfate-reducing bacteria. Moreover, RS (Brown et al. 1997; Kleessen et al. 1997; Cresci et al. 1999) and FOS (Djouzi & Andrieux, 1997; Le Blay et al. 1999b; Marx et al. 2000) are available to stimulate the growth of lactic-acid bacteria such as Bifidobacteria and Lactobacilli. On one hand, they contribute to a decrease in digestive pH, and on the other hand, Lactobacilli have been reported to inhibit sulfate-reducing bacteria. These reasons may indicate that both RS and FOS may be of great interest for the inflamed mucosa. However, our present results showed that only the RS-supplemented diet was effective, suggesting that this effect cannot be exclusively attributable to its interaction with the luminal microflora. To explain the RS effect, it could be hypothesised that the digestive pH obtained after RS ingestion could rapidly reach a steady state, while pH obtained after FOS ingestion would be more unstable. Moreover, it was previously shown that colonic mucin synthesis increased with butyrate (Finnie et al. 1995) and lower pH may also stimulate mucin production (Bustos-Fernandez et al. 1978). As the amount of butyrate in the caecum with the RS diet is higher than with FOS, the higher production of mucus should be one of the hypothetical factors of mucosa protection.

In conclusion, a dietary RS supply induced significant improvement in macroscopic and histological characteristics of caeco-colonic inflammatory lesions due to DSS in rats. This could therefore confirm the interest in substrates generating high levels of butyrate during their colonic fermentation in inflammatory bowel disease. According to our present results, the effect of RS could be partially explained by higher amounts of caecal SCFA and especially butyrate. The ineffectiveness of the FOS-supplemented diet underlines the importance of the type of dietary substrate. Obviously, all the substrates susceptible to be fermented producing a high proportion of butyrate are not equivalent as far as their healing properties on DSS inflammation are concerned.

Acknowledgements

The authors acknowledge National Starch and Chemical Ltd for providing the Novelose 330 and the competent technical assistance of Thérèse Fréger, Sigrid Parois and Philippe Bleis during the study.

References


Resistant starch restores inflamed rat mucosa 83


