The faecal flora of two patients with food-related irritable bowel syndrome during challenge with symptom-provoking foods

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Summary. The faecal microbial flora of two patients with food-related irritable bowel syndrome was examined while they were on a diet excluding symptom-provoking foods, and then on a diet including such a food. The patients reacted differently to the challenge diet but some changes in faecal output, flora and short chain fatty acid content were seen.

Introduction

Irritable bowel syndrome (IBS) is a condition in which abdominal pain, diarrhoea, constipation or variability of bowel habit may be experienced. The involvement in some patients of non-immunological intolerance of specific foods has been demonstrated and symptoms may be controlled by diet management (Hunter and Alun Jones, 1985; Smith *et al.*, 1985). It has been suggested that the bacterial gut flora are involved in this condition and changes in faecal flora of IBS patients have been shown previously (Balsari *et al.*, 1982; Bayliss *et al.*, 1986; Bradley *et al.*, 1987) but not linked directly to specific foods.

Detailed comparisons of the faecal flora between IBS sufferers and healthy subjects are difficult to make because of the complexity of the normal flora and the marked differences found between those of different normal individuals (Holdeman *et al.*, 1976). For a given individual, however, the rank order of species is usually stable (Moore *et al.*, 1981), therefore any changes in flora due to symptom-provoking foodstuffs might be revealed by challenge with that food. In the pilot study reported here, two patients were hospitalised and maintained on a diet free of their symptom-causing food until given a blind challenge of that food. The bacterial flora of the faeces and other faecal parameters were analysed during the different dietary periods.

Patients and methods

Case histories

Patient P3. A 25-year-old man presented 9 months before the study with a 5-year history of recurrent abdominal pain and a 6-month history of sporadic diarrhoea. At presentation he complained of alternating diarrhoea and constipation, abdominal bloating, flatulence and lack of energy. Blood count, urea, electrolytes and liver function tests, and jejunal biopsy were normal. No pathogens were found in his faeces. An elimination diet at Addenbrookes Hospital identified bread and coffee as symptom-provoking foods. No antibiotics had been taken for at least 3 months before the study.

Patient P7. A 25-year-old woman presented one year previously with a 5-year history of abdominal bloating and pain, vomiting, and diarrhoea. These had become much worse since a bout of *Campylobacter* gastroenteritis about 4 months previously. Radiographs of stomach, gall bladder and colon, rectal biopsy, urea, electrolytes and liver function tests were normal; no pathogens were found in her faeces. A psychiatrist found no psychopathology. An elimination diet identified several symptom-provoking foods, including rice, wheat, onion and eggs.

For both patients, double blind challenges confirmed their food intolerances and both were well whilst on exclusion diets.

Diets during the studies

Whilst in hospital, the patients ate a diet free of known symptom-provoking foods, except when challenged as detailed below.

Patient P3. The normal diet included two servings per day of a soup thickened with rice flour (100 g/day). On days 3–6 of the study, soup thickened with wheat flour was substituted. The patient was not aware of the change.

Table I. Symptoms present in two patients during challenge with symptom-provoking foods

Patient P3			Patient P7			
Day	Headache	Bloating	Day	General condition	Headache	Bloating
1	0	0	1	0.0	3.3	0.0
2	0	0	2	2.0	0.7	0.0
3*	0	0	3*	3.4	5.4	5.0
4†	++	0	4†	0.6	2.2	1.1
5	++	+	5	6.4	0.0	10.0
6†	0	0	6†	2.4	0.0	5.0
7	0	+++	7	5.7	5.0	3.4
8§	+	0	8§	1.3	0.0	0.0
9	0	0	9	8.3	10.0	0.0
10	0	0				

Symptoms scored as follows—

Patient P3: 0 = not present + = mild ++ = moderate +++ = severe

Patient P7: score is position on a scale of 0–10, from normal to extremely unwell (general condition), or from absent to severe (headache and abdominal bloating); mean of scores for two 12-h periods per day.

* First meal of challenge diet.

† Last meal of challenge diet.

‡ First challenge markers present in faeces.

§ First post-challenge markers present in faeces.

Radio-opaque markers were fed in opaque capsules (2 × 10 markers per day) employing different shapes before, during and after feeding the challenge diet.

Patient P7. The normal diet during the study included two portions of rye flakes (100 g/day), served as a breakfast cereal with orange juice instead of milk as the patient was also intolerant of milk. During days 3–6 of the study, wheat flakes were substituted for the rye flakes; the two flakes were visually indistinguishable. Radio-opaque markers were fed as above.

Each patient recorded symptoms experienced, and in the case of patient P7, filled in a questionnaire which included marking a 10 cm bar for severity of symptoms (table I).

Measurement of stool parameters

All stools passed were collected by the patients in plastic bags, weighed and placed in an anaerobic cabinet containing an atmosphere of N₂ 85%, H₂ 10% and CO₂ 5% within 5–15 min of being passed. Viable counts of anaerobes and aerobes in the faeces were performed as previously described (Bradley *et al.*, 1987). For selected stools, all of the colonies from the highest dilution showing growth on both the aerobic and anaerobic plates were characterised as previously described (Bradley *et al.*, 1987).

Samples of faeces were dried to a constant weight at 100°C to determine moisture content. The remainder of each stool was frozen at –20°C for subsequent analysis

of short chain fatty acid (SCFA) content by the method of Bayliss and Houston (1985). The presence of markers was determined by radiographic examination of the stool samples. The samples between the first to contain challenge markers and the last before the appearance of the post-challenge markers (inclusive) were taken to represent the challenge diet stools.

Results

Both patients displayed symptoms during the studies (table I). The non-specific symptoms of headache or general condition did not correlate well with diet; however, the specific symptom of abdominal bloating, with which both patients had presented, was markedly associated with the period of the challenge diet. Patient P3 passed 14 stool samples and patient P7 passed 20 stool samples during the study period. Total faecal weight was much greater for patient P7. Simple linear regression lines were fitted to the cumulative faecal output by the computerised statistical package MINITAB (Pennsylvania State University).

For both patients there was a slight increase in faecal output whilst on the challenge diet (figure). The stool frequency (mean number of stools/24 h) was higher during the period of the challenge diet for both patients (normal diet P3 1.1, P7 2.0; challenge diet P3 2.0, P7 2.5). Neither patient had

Figure. Cumulative faecal output of the patients during the study. Time zero was taken as the time of passage of the first stool. Simple regression lines are fitted to the data. ● = patient P3; ▲ = patient P7.

diarrhoea and there was no difference in moisture content of the faeces between normal and challenge diet periods (table II).

For patient P3, the first challenge diet markers appeared in stool sample 5 (day 4) and the first post-challenge diet markers in sample 12 (day 8); for patient P7, the first challenge markers appeared in stool sample 8 (day 4) and the first post-challenge markers in sample 18 (day 8).

No significant diet-related differences in bacterial viable counts were seen in either patient (table

II); there was little change with diet in the major species present in the faeces, although in patient P7 the anaerobic gram-positive lactic acid bacteria (*Bifido-bacterium* and *Lactobacillus* spp.) increased from 21% to 43% of the flora during the challenge diet (table III). The major SCFAs present in both patients were acetic, propionic and *n*-butyric acids; for patient P3 the concentrations of all three increased markedly during the challenge diet and this difference was significant for acetic and propionic acids (table II).

Table II. Changes in faecal parameters in two patients during challenge with symptom-provoking food

Patient	Diet	Number of samples tested	Viable counts (mean (SE) log ₁₀ cfu/g*)		Anaerobe: aerobe ratio	Short chain fatty acids (mean (SE) μmol/g*)			Moisture content (mean (SE)%)
			aerobes	anaerobes†		acetic	propionic	<i>n</i> -butyric	
P3	Normal	7	9.16 (0.27)	11.38 (0.11)	166:1	374 (62.1)	15.0 (2.5)	18.1 (3.4)	71.3 (1.8)
	Challenge	7	NS	NS		p < 0.05	p < 0.05	NS	NS
			8.42 (0.15)	11.25 (0.05)	676:1	653 (75.6)	30.9 (4.5)	33.4 (6.8)	74.7 (2.6)
P7	Normal	10	8.7‡ (0.08)	11.27‡ (0.06)	372:1	289 (33.6)	42.2 (5.8)	69.2 (15.9)	79.6 (0.71)
	Challenge	10	NS	NS		NS	NS	NS	NS
			8.98§ (0.13)	11.38§ (0.04)	251:1	292 (21.3)	38.5 (4.6)	89.2 (13.9)	80.1 (0.71)

NS = no significant difference; values were compared by Student's *t* test.

* Dry weight. † includes facultative anaerobes. ‡ 9 samples tested. § 7 samples tested. || 6 samples tested.

Table III. Approximate composition of faecal flora in two patients during challenge with symptom-provoking foods

Principal genera present	Percentage* of faecal flora			
	Patient P3		Patient P7	
	Normal diet	Challenge diet	Normal diet	Challenge diet
AEROBES	(7)†	(7)†	(8)†	(7)†
<i>Streptococcus</i> spp.	24	12	75	80
<i>Escherichia coli</i>	54	53	8	12
<i>Micrococcus</i> spp.	0	16	3	0
<i>Enterobacter</i> spp.	12	1	5	0
Others	10	18	9	8
ANAEROBES	(2)†	(2)†	(2)†	(3)†
<i>Bacteroides</i> spp.	20	26	28	36
<i>Bifidobacterium</i> spp.	3	13	8	18
<i>Eubacterium</i> spp.	19	23	4	2
<i>Lactobacillus</i> spp.	19	12	13	25
gram-negative cocci	12	5	0	0
gram-positive cocci	0	0	9	0
others (not identified)	20	21	38	19
facultative aerobes	7	0	0	0

* Figures represent mean percentages of the total numbers of colonies isolated from either aerobic or anaerobic cultures.

† Number of faecal samples examined.

Discussion

The patients in this study responded differently to blind challenge with symptom-provoking foods, patient P3 reacting more strongly than patient P7. The increased faecal output and stool frequency during challenge compares with the work of Eastwood *et al.* (1984), who found higher mean daily stool weights and frequencies in a group of IBS patients than in age- and sex-matched controls. Although no differences in the density of viable bacteria in the faeces were detectable during our studies, the increased output of faeces whilst on the challenge diet increased the total number of bacteria, and hence bacterial activity, in the colonic lumen. Furthermore, although only small differences in the balance of bacterial species present were seen, with each patient tending to maintain their own particular rank order of species, significant differences in SCFA content of faeces in patient P3 suggest either that bacterial metabolism altered during the challenge diet or that the processes of absorption by the colonic mucosa changed.

Both patients had a lower than usual ratio of anaerobic:aerobic bacteria in their faeces. This is generally >1000:1 (Mitsuoka, 1982) in normal

subjects, and in our experience >10 000:1 (Bradley *et al.*, 1987); this higher level of aerobes seems to be characteristic of some IBS patients (Bayliss *et al.*, 1984, 1986; Bradley *et al.*, 1987) and was found to correlate positively with a score of symptoms in IBS patients in whom the condition arose after abdominal surgery (Bayliss *et al.*, 1986). Balsari *et al.* (1982) noted the appearance of aerobic genera (*Pseudomonas*, *Enterobacter*) in IBS patients not encountered in normal subjects, although in those patients the syndrome was not necessarily food-related. In our work, two of the seven stool specimens from patient P3 during the challenge diet period contained *Micrococcus* spp., in one stool constituting >80% of isolates; this organism is normally only a minor component of faecal flora (Finigold *et al.*, 1974).

It is clear from this pilot study and other work that changes in some aspects of the gut flora are associated with food-related IBS, particularly in the metabolic activity of the flora or the presence of increased numbers of aerobic species. These changes may move in different directions in different individuals, but it is not certain whether they are the cause of the symptoms or just reflect other biological alterations. Further studies with larger numbers of patients are needed. The possi-

bility that pharmacologically active metabolites, derived from food residues by an altered flora in the colon of IBS patients, are the cause of the symptoms should be also investigated.

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