Exclusive elemental diet impacts on the gastrointestinal microbiota and improves symptoms in patients with chronic pouchitis

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Abstract

Background: Treatment resistant chronic pouchitis causes significant morbidity. Elemental diet is effective treatment for Crohn's disease. Since pouchitis shares some similarities to Crohn's disease we hypothesised that elemental diet may be an effective treatment.

Method: Seven pouchitis patients (with ulcerative colitis) were studied. All had active pouchitis with a pouch disease activity index (PDAI) ≥ 7. Exclusion criteria were recent NSAIDs, antibiotics or probiotics. Sufficient elemental diet to achieve energy requirements was provided. Flexible-pouchoscopy was performed, and the Cleveland Global Quality of Life score (CGQoL), Pouch Disease Activity Index (PDAI) and BMI were recorded at baseline and following 28 days of elemental diet. Faecal samples were also collected at these time points and analysed for major bacterial groups using culture independent fluorescence in situ hybridisation. Data were analysed using Wilcoxon’s signed-rank test.

Results: Following 28 days of exclusive elemental diet, median stool frequency decreased from 12 to 6 per day (p = 0.028), median clinical PDAI decreased from 4 to 1 (p = 0.039). There was no significant difference in quality of life scores or PDAI before and following treatment. There was a trend towards an increase in the concentration of Clostridium coccoides–Eubacterium rectale (median 7.9 to 8.5 log10/g, p = 0.08) following exclusive elemental diet.

Conclusion: Treatment with four weeks elemental diet appeared to improve the symptoms of chronic pouchitis in some patients but is not an effective strategy for inducing remission.

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1. Introduction

Ulcerative colitis that is refractory to medical therapy or is complicated by dysplasia can be treated by surgery. Restorative proctocolectomy with ileal pouch-anal anastomosis (RPC) is the surgical procedure of choice. During this procedure the colon and rectum are removed and a reservoir ('pouch') is fashioned from the distal 40 cm of small bowel, which is then anastomosed to the anal canal. The major advantage of this procedure is that it maintains normal anal function and avoids the need for a permanent stoma. Most patients have an excellent functional outcome, but unfortunately up to 50% of patients will develop pouch inflammation, termed pouchitis.1

The symptoms of pouchitis are similar to ulcerative colitis with increased stool frequency, urgency and abdominal cramping. In most cases treatment with antibiotics such as metronidazole or ciprofloxacin is successful.2 Unfortunately some patients will go on to suffer from frequent episodes of pouchitis which remain unresponsive to antibiotics, whilst others will require continuous antibiotic or probiotic treatment to maintain a remission.3,4 A proportion will not respond to these approaches and in this group of patients with chronic pouchitis there are few evidenced-based treatments.1 For example, topical budesonide is occasionally used but is no more effective than metronidazole,5 whilst mesalazines, bismuth carbomer enemas, systemic steroids and immunosuppressants have not been of proven benefit.6 Recent data suggest that biologics may be an effective treatment strategy in some patients with chronic pouchitis. Infliximab was beneficial in one study of patients with pouchitis and extensive pre-pouch ileitis (which may be suggestive of Crohn's disease).7 A later retrospective uncontrolled study of patients treated with infliximab (and thiopurine in the majority) for refractory pouchitis and pre-pouch ileitis demonstrated a sustained clinical response in 56% of patients at a median follow up of 20 months.8 Unfortunately in the UK biologic agents are not funded for patients with pouchitis.

The only proven treatment for these individuals is conversion to a permanent ileostomy; indeed pouchitis accounts for 10% of pouch failures.9

Meta-analyses have shown that treatment with elemental diet is effective in inducing remission in both children and adults with Crohn's disease.10 The mechanism of action of elemental diet is unknown, but there is evidence that it may be effective by modulating gut flora and gut immunological function.11 Certainly its effect on reducing gut inflammation is well demonstrated12–14 and it has also been shown to reduce bacterial load in small bowel bacterial overgrowth.15

Although there is no evidence that treatment with elemental diet is effective in ulcerative colitis,16 a number of similarities between pouchitis and Crohn's disease suggest that treatment with an elemental diet may be effective in pouchitis. Both conditions affect the small bowel, both respond to treatment with antibiotics, and both improve with diversion of the faecal stream. Furthermore at least one study has shown that elemental diet can modify gut microbiota and therefore may be therapeutic in pouchitis.17

We aimed to establish whether an elemental diet could serve as an effective treatment strategy for patients with chronic pouchitis and whether this impacts on the gut microbiota.

2. Method

This prospective study describes the novel use of exclusive elemental diet in a case series of seven patients with chronic pouchitis measuring clinical, nutritional, microbiological and patient-centred outcomes. Ethical permission was granted by the Brent and Harrow Research Ethics Committee.

Inclusion criteria were symptomatic pouchitis with a pouch disease activity index (PDAI) ≥7.18 In addition patients had to have continued symptoms of pouchitis despite four weeks treatment with a combination of ciprofloxacin and metronidazole19 or have chronic antibiotic dependent pouchitis defined as four or more episodes of pouchitis per year that responded to antibiotic treatment but recurred on withdrawal.20 Patients had to be willing and well motivated to comply with the restrictions of an elemental diet regime. Exclusion criteria included a diagnosis of indeterminate colitis or Crohn's disease, treatment with antibiotics or probiotics with the preceding...
2 weeks, concurrent non-steroidal anti-inflammatory drugs, corticosteroids or immunosuppressants within the preceding three months (Fig. 2).

A flexible-pouchoscopy was performed and the PDAI calculated. The Cleveland Global Quality of Life score (CGQoL), a validated tool to measure quality of life in patients with ulcerative colitis undergoing restorative proctocolectomy, was measured. C-reactive protein (CRP), haemoglobin weight, height and body mass index (BMI) were also recorded. All of these were performed at baseline and following 28 days of exclusive elemental diet.

All patients were assessed by a dietitian and were prescribed elemental diet (E028, SHS, UK) as exclusive nutrition for 28 days according to their nutritional requirements. The only other food or liquids allowed were water, black tea and black coffee, in line with normal clinical practice.

Throughout, patients were asked to keep a diary to include symptoms of stool frequency, bleeding, abdominal cramps, and the volume of elemental diet consumed.

2.1. Faecal microbiology

A fresh stool sample was collected at baseline and *Clostridium difficile* toxin testing was performed. Further stool samples were taken at baseline and following 28 days of exclusive elemental diet for quantification of the predominant components of the gastrointestinal microbiota using culture-independent fluorescence *in situ* hybridisation (FISH). Briefly, faecal samples were collected within 1 h of voiding, homogenised in a stomacher, then faecal bacteria were harvested in phosphate-buffered saline (PBS). Bacteria were fixed by incubation in 4% paraformaldehyde (wt:vol) for a minimum of 4 h at 4 °C, then washed and resuspended in a 1:1 solution of PBS and 96% ethanol (vol:vol) and stored at −20 °C until hybridisation.

Quantification of bacterial groups was undertaken using hybridisation with Cy3 labelled oligonucleotide probes targeting specific regions of bacterial 16S rRNA (Microsynth, Switzerland) (Table 1). Bacteria were serially diluted in PBS, mounted on 3-aminopropyltriethoxysilane treated 8-well microscope slides, then hybridised with the probe (50 ng/μL) in hybridisation buffer (0.9 mol/L NaCl; 0.2 mol/L Tris/HCl; 0.01% wt:vol sodium dodecyl sulphate) and incubated overnight at 46 °C. Following incubation excess probe was washed away, then unbound and non-specifically bound probe was removed by incubation in hybridisation buffer at a specific wash temperature for 20 min (Table 1). Slides were rinsed in deionised water then mounted in 50% PBS:glycerol. Total bacteria were quantified using the nucleic acid stain 4,6-diamidino-2-phenylindole. Bacteria were visualised and enumerated using epifluorescence microscopy on a Zeiss Axioskop 2 microscope (Zeiss, Germany) fitted with a fluorescent bulb. The two wells corresponding to the most appropriate dilution (i.e. 20–50 cells per field) were quantified by counting 15 systematically chosen microscope fields per well. Hybridisation and quantification were undertaken by the same researcher who was blinded to all patient information and to whether samples were from baseline or follow-up. All bacterial concentrations were log transformed.

2.2. Statistical analysis

SPSS (v. 15, SPSS inc. Chicago, Illinois, USA) was used for all statistical analysis. For the description of data, raw data is presented together with the median (IQR). Wilcoxon’s signed-rank test was used to compare continuous data before and

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**Figure 2** Flow chart, outlining patient enrolment, exclusion, and follow up.
after treatment with elemental diet. A two-tailed p-value <0.05 was considered significant.

3. Results

Nine patients were recruited to the study, two patients were excluded; one because initial assessment suggested a diagnosis of Crohn’s disease which was subsequently confirmed following further investigation and one patient withdrew shortly after starting the intervention. Therefore seven patients were studied and completed 28 days of exclusive elemental diet. Of these seven patients, four were male, and their median age was 41 (min 30–max 63) and the median number of years since RPC was 10 (min 4–max 22).

Clinical data for each patient before and following treatment with elemental diet are recorded in Table 2.

Treatment with elemental diet resulted in a significant reduction in stool frequency (from median 12 to 6, p = 0.028) and the PDAI symptom score (from 4 to 1, p = 0.039) (Fig. 1). There was a non-significant trend towards an improvement in the ability to defer defaecation (from 25 to 60 min, p = 0.078). However, there was no change in the CGQoL score between baseline and following 28 days of exclusive elemental diet, and indeed, there was a trend towards an increase in CRP (median 10.5 to 16, p = 0.063).

Following 28 days of exclusive elemental diet there were no significant changes in the concentration of bacteria. There was, however, a trend toward an increase in Clostridium coccoides–Eubacterium rectale, which increased from 7.9 to 8.5 log_{10}/g faeces (p = 0.08). Total bacterial concentrations were in the region of 10^8 cells per g faeces both before and after exclusive elemental diets. However, many samples were found to have concentrations of specific bacterial groups that were below the limit of detection for FISH (6 log_{10}/g faeces) (Table 3). Due to the number of participants and the number of ‘undetectable’ samples all raw data have been presented.

4. Discussion

Patients with chronic pouchitis are difficult to manage. Antibiotics remain the mainstay of treatment, however some patients develop side-effects, others do not respond and antibiotic resistance remains a problem of frequent prescription.22 Elemental diet is safe, free of side effects and if effective it could offer an alternative treatment for patients who develop antibiotic resistance or experience side effects to antibiotic treatment.

Although polymeric diets are more palatable,10 we chose to treat patients with elemental diet because this formulation is antigen free whereas polymeric formulations contain whole proteins which may provoke immune mediated damage to pouch mucosa. In addition although two week treatment has been reported to be effective for inducing remission in Crohn’s disease23 others have described a four week treatment regimen.24 We opted to treat patients for four weeks in order...
concerns regarding the high osmolality of the elemental diet five of the seven patients were able to tolerate the diet.

To the best of our knowledge this is the first study reporting the outcome of patients treated with elemental diet for pouchitis. Despite concerns regarding the high osmolality of the elemental diet five of the seven patients (71%) reported a reduction in stool frequency and there was also a significant reduction in the PDAI symptom score in these patients. In addition these five patients reported an increase in their quality of life, but overall this did not reach statistical significance. This is important and may be related to the restrictive nature of elemental diet and emphasises the importance of measuring quality of life in studies where the treatment or intervention may have an independent impact.

All but one patient experienced a reduction in BMI following treatment with elemental diet, both this and the quality of life scores almost certainly reflect difficulty tolerating sufficient quantities of the elemental formula. Indeed previous studies comparing enteral nutrition therapy and treatment with corticosteroids in adult patients report withdrawal rates of over 40% due to intolerance of the liquid diet.24,25

Despite symptomatic improvement there was no reduction in endoscopic or histological signs of inflammation suggesting that the reduction in stool frequency and improvement in symptoms in some patients may be due the effect of merely consuming a low residue diet and not as a result of modifying the underlying inflammatory process. However it should be appreciated that a significant proportion of patients who achieve clinical remission following antibiotic treatment for chronic pouchitis do not achieve endoscopic remission, with less than 50% of patients who achieved clinical remission also achieved endoscopic remission. This has also been reported by others following both antibiotic and probiotic treatment.20,26

The healthy pouch microbiota largely consists of the same bacterial groups that predominate in the healthy colon.27 However, 16S gene sequencing has demonstrated lower species diversity in pouches formed for UC, and even lower diversity in those with pouchitis,28 highlighting a distinct dysbiosis of the microbiota in pouchitis.29 To date, neither a specific causative microbial agent nor a distinctive pouchitis microbiota has been identified.29,30 However, some studies have identified lower Bacteroidetes28,31 and increased Clostridiaceae31 in the mucosa of pouches formed for UC, and increased Clostridiaceae32 in chronic Vs single-episode pouchitis. Information on microbial concentrations in pouchitis is scarce due to the use of non-quantitative analysis (e.g. gene sequencing). However, our study found concentrations of approximately $8 \log_{10}/g$, compared with typical concentrations of $6-7 \ log_{10}/g$ in the ileum and $10-11 \ log_{10}/g$ in the colon.33 This suggests that whilst the predominant bacterial groups of the pouch generally adopt a modified colonic profile, rather than an ileal profile, in pouchitis at least, total bacterial numbers remain lower by several orders of magnitude.

In the current study we saw evidence of modification of the pouchitis microbiota following 28 days of exclusive elemental diet, with a trend towards increased concentrations of the *C. coccoides–E. rectale* group (*Clostridium* Clusters XIVa and XIVb, probe EREC482).34 Importantly, this group includes many of the major producers of butyrate.35 Butyrate is the preferred metabolic substrate for enterocytes, it can inhibit pro-inflammatory cytokine production,36 and can regulate gene expression, differentiation and apoptosis.37,38 Butyrate

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<th>Patient</th>
<th>Total bacteria</th>
<th>Bacteroides-Prevotella</th>
<th>Bifidobacteria</th>
<th>Clostridium coccoides–Eubacterium rectale</th>
<th>F. prausnitzii</th>
<th>Lactobacillus, Enterococcus</th>
<th><em>D. infantis</em></th>
<th><em>C. coccoides–E. rectale</em></th>
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Table 3: Bacterial concentrations pre and post 28 days of elemental diet (log10 cells/g faeces).

- Median (IQR) 8.0 (1.1) 8.8 (0.6) 8.3 (N/A) 8.1 (1.6) 7.8 (N/A) 0.109 0.116 0.016 0.116
- p value = 0.116 0.109 0.180 0.080 N/A N/A
- concentration of bacterial cells below the detection limit for FISH (10^6 cells per g faeces)
also promotes epithelial water absorption\(^{38}\) and this may be another mechanism for the reduced stool frequency observed in the current study. High levels of mucosal Clostridiaceae and TLR2 and TLR4 have recently been identified in the pouch mucosa of patients with chronic pouchitis.\(^{32}\) If these are causal, then if elemental diet really does increase numbers of \(C.\) coccoides–\(E.\) rectale then this raises the possibility of subsequent increased TLR2 activation.

Exclusive enteral formula is known to significantly reduce the total gastrointestinal microbiota in healthy people, without increasing any specific bacterial groups.\(^{39,40}\) Therefore, that 6 out of 7 patients with pouches had evidence of increasing any specific bacterial groups.\(^{39,40}\) Therefore, that subsequent increased TLR2 activation. \(C.\) coccoides causal, then if elemental diet really does increase numbers of the pouch microbiota (e.g. bifidobacteria) to resist the increased colonisation.\(^{41}\)

The elemental formula provided 110 g sucrose per 2000 kcal, which is substantially higher than typical sugar intakes in the UK (51–79 g/d).\(^{42}\) A pilot study has indicated that 6 out of 7 patients with pouches had evidence of fructose malabsorption.\(^{43}\) Therefore, in the current study, not only was there higher delivery of sucrose (fructose and glucose) to the small intestine, but also following disaccharide digestion, the fructose monosaccharides may be malabsorbed, and thus delivered to the pouch microbiota for fermentation. Interestingly, genome sequencing of two members of the \(C.\) coccoides–\(E.\) rectale group (\(E.\) rectale, \(E.\) eligens) has revealed over-representation of genes for monosaccharide transport\(^{44}\) indicating that these may be better equipped to transport monosaccharides. However, whether the \(C.\) coccoides–\(E.\) rectale group is able to outcompete other bacteria for fructose utilisation is unclear.

Despite the interesting findings of this study, the data are clearly limited by a lack of statistical power. Treatment with exclusive elemental diet is restrictive and consequently recruitment to this study was difficult. In addition, as this was a novel treatment, only patients who had failed or were poorly tolerant to antibiotic treatment were eligible for ethical reasons. Therefore the numbers included in this study were small and it is likely that the study lacks sufficient power to identify significant differences in the variables recorded. In addition, the microbiological data is underpowered due to missing data points for some bacteria that were present at concentrations below the detection limit for FISH (6 log\(_{10}\)/g faeces). Although this is clearly a limitation of this technique, it is likely that bacteria present at concentrations below this value will have comparatively little impact on pouch fermentation. Despite these limitations, these results contribute to the emerging picture of dysbiosis in pouchitis after UC and contribute valuable information on the concentration of the pouch microbiota.

In conclusion treatment with 28 days of exclusive elemental diet reduced stool frequency and improved symptoms of chronic pouchitis in some patients but is unlikely to be an effective strategy for inducing remission. This small study does not provide evidence that elemental diet can be recommended for the routine treatment of pouchitis but it could be considered in a selected group of patients to offer temporary symptomatic relief.

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