Modification of enteral diets in inflammatory bowel disease

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The provision of food is thought to promote the maintenance of gut integrity. Nutrients are able to elicit and affect both systemic and mucosal immune responses. Enteral diet therapy has long been known to be efficacious in inflammatory bowel disease (IBD), particularly in childhood Crohn's disease. However, the mechanisms of action of these diets are not clear. Nutritional repletion, direct effects on the gut mucosa or decreased intestinal permeability have all been postulated as being important in nutritional therapy. There is some evidence that the enteral diet has a direct effect on the gut mucosa by reducing cytokine production and the accompanying inflammation, thus leading to decreased intestinal permeability. Modifications of enteral diet composition have been evaluated in many studies. Such modifications include fat and/or protein content and the addition of bioactive peptides. The fatty acid composition of the enteral diet seems to have a much greater impact on its efficacy than modification of the N source. As specific fatty acids are precursors of inflammatory mediators derived from arachidonic acid, the reduction in these components may be beneficial in nutritional therapy for IBD. Addition of bioactive peptides to enteral diet formulas may also have a role; such peptides may have specific growth factor or anti-inflammatory actions. There is still much work to be done to define disease-specific enteral diet formulas that are effective as therapies for both Crohn's disease and ulcerative colitis.

Enteral diet formulas: Crohn's disease: Nutritional therapy: Inflammation

There is plenty of evidence to suggest that food and nutritional factors affect immune responses in the gut. Nutritional status, both at the macro- and micronutrient level, affects both systemic and mucosal immunity. Early work in our laboratory, under the auspices of the late Professor Anne Ferguson, showed that in protein-deprived mice there was a profound reduction in the induction of oral tolerance (Lamont *et al*. 1987*a*,*b*, 1988). It follows, therefore, that immunological functions of the gut and nutrient intake would be particularly important in gut disease (Ferguson, 1994). A breakdown of oral tolerance to gut bacteria has been hypothesised as a mechanism by which patients develop inflammatory bowel disease (IBD). Thus, if such immune responses are profoundly affected by nutrition, then nutritional therapy may have a role in the treatment of IBD.

IBD can be divided into two disease patterns, Crohn's disease (CD) and ulcerative colitis (UC), with distinct clinical, histological and immunological features. Both disease patterns show dysregulated cytokine balance; CD tends to be shifted towards a T-helper 1 (pro-inflammatory) phenotype and UC towards a T-helper 2 (anti-inflammatory) phenotype. This distinction in cytokine phenotype between the two disease states is not totally consistent with a T-helper 1–T-helper 2 split. Both diseases show an increase in inflammation of the gut mucosa, together with increased production of the pro-inflammatory cytokine interleukin (IL)-1β and a decrease in the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra). Indeed, in both CD and UC a decrease in IL-1ra:IL-1β production has been described in isolated colonic biopsies compared with those from normal controls or patients with infectious colitis (Dionne *et al*. 1998). This imbalance has been proposed as being of pathogenic importance in IBD (Casini-Raggi *et al*. 1995).

The intestine is a metabolically active organ which maintains gut function through various mechanisms, e.g. peristalsis, secretory immunoglobulin A, mucin secretion. Starvation, and possibly parenteral feeding, predisposes the gut to metabolic and immunological deterioration of barrier function, leading to the release of pro-inflammatory cytokines and decreased gut permeability, allowing other macromolecules to cross. Gut contents stimulate immunological mechanisms such as intestinal immunoglobulin A

production, which blocks the binding of bacteria to epithelial cells. Enteral feeding, as opposed to parenteral feeding, appears to maintain these mechanisms by providing food (Donnell *et al.* 1998; Minard & Kudsk, 1998; Rowlands & Gardiner, 1998).

Enteral diets in inflammatory bowel disease

There is little doubt that an enteral diet (ED) is effective in CD, particularly in children, although there is some question as to the clinical efficacy of ED in UC. ED are important in re-establishing nutritional repletion in malnourished patients. There is also some evidence that ED have some effect on the disease activity itself, comparable with steroid therapy, although this factor has been debated widely. A meta-analysis (Griffiths *et al.* 1995) of trials comparing ED with steroids showed that although ED was effective in CD, it was still less effective than steroid treatment. However, other studies have shown that ED are equally as effective (Zoli *et al.* 1997). Either way, it is known that ED is a possible alternative in those patients who may have contra-indications for steroid therapy in CD.

Mechanism of action

What really is not clear is the mechanism by which these diets have their action. There is still much discussion as to the best approach for trying to investigate such mechanisms. Although *in vivo* studies are the most relevant, as regards clinical efficacy and side effects, the actual mechanisms by which ED have their effect are hard to deduce, mainly because the gut is a difficult organ to study directly. Such studies may require repeated invasive or unpleasant procedures for patients in order to define a 'before and after' treatment effect, and subtle or transient changes may not be detected. Thus, knowing the best way to modify ED in order to increase clinical efficacy and reduce side effects becomes more difficult. *In vitro* studies are simpler to perform but, by their very nature of being *in vitro*, do not reproduce the many factors that affect gut function, and can only investigate defined and measurable effects such as cytokine production or changes in expression of particular molecules. However, *in vitro* studies of ED, looking at such specific mechanisms of action, may be useful in defining macro- and micronutrient effects on gut tissue, which can then be assessed further by *in vivo* studies.

Various hypotheses as to the action of ED in IBD have been proposed. There is still some discussion as to whether ED has a direct effect on the gut, or whether the efficacy of ED is due to indirect effects such as improvement in nutritional status. In one study of ED in CD, both nourished and malnourished patients responded equally (Gorard *et al.* 1993). Other researchers have found that clinical improvement in disease activity occurred before changes in nutritional status were detected (Teahon *et al.* 1995; Croft *et al.* 2001), suggesting that ED has a direct effect on the gut.

Decreased intestinal permeability has also been proposed as a mechanism through which ED therapy may act. Clinical improvement of CD has been accompanied by decreases in urinary ^{51}CrEDTA excretion and faecal excretion of

^{111}In-labelled leucocytes (Teahon *et al.* 1991), or a decrease in quantitative leucocyte scintigraphy (Mansfield *et al.* 1995). Zoli *et al.* (1997), in a study comparing ED with steroids, showed a significant decrease in intestinal permeability with ED ($P < 0.05$), but not with steroids, even though both treatment groups went into clinical remission. Studies from our own laboratory have also indicated a decrease in intestinal permeability in CD patients, through decreased protein (immunoglobulin G) loss in whole-gut lavage fluid (Ferguson *et al.* 1998), after treatment with an elemental ED for 2 weeks. This decrease in intestinal permeability was accompanied by a concomitant decrease in IL-1β levels. These results provide further evidence that ED have a direct effect on the gut, by changing cytokine levels, thus resulting in decreased inflammation, which in turn leads to decreased permeability.

Elemental or polymeric enteral diets?

More recent studies in our laboratory have begun to investigate the possible direct anti-inflammatory effect of ED, in an *in vitro* organ culture model, by monitoring changes in cytokine production (Shand *et al.* 2000). Ileal or colonic biopsies from patients with CD and UC were incubated with ED or medium alone for 24 h. Supernatant fractions were collected and cytokines (IL-ra, IL-1β and IL-10) were measured by ELISA. Initial studies used an elemental formula ED (E028; Scientific Hospital Supplies, Liverpool, UK), in which the N source was free amino acids, as opposed to polymeric ED, in which the N source was whole proteins or oligopeptides. We established that the biopsies maintained viability in culture, and we looked for changes in IL-1ra:IL-1β; an increase in the ratio would indicate a decrease in IL-1β or an increase in IL-1ra production, i.e. an anti-inflammatory effect of ED in these experiments. Results showed that an increase in IL-1ra:IL-1β was obtained in biopsies from CD patients incubated with ED, but no changes in cytokine production were seen in biopsies from UC patients. Further studies used ED with oligopeptides (whey) or whole protein (casein) as the N source and found that the increased IL-1ra:IL-1β was not abolished. Indeed, if anything, for casein the effect was more pronounced. These results agree with those from clinical studies of elemental, oligopeptide or polymeric ED, which showed that there appears to be little difference between diet efficacy and the N source (Griffiths *et al.* 1995; Mansfield *et al.* 1995; Verma *et al.* 2000).

It has been proposed that elemental ED are effective as they provide gut rest or a low antigen load, through decreased protein content. However, the efficacy of polymeric ED and maintenance of remission through the supplementation of a normal diet with ED (Wilschanski *et al.* 1996) bring these theories into dispute. Indeed, in animal models too much 'gut rest', through use of parenteral nutrition or long-term treatment with an elemental ED, increases the likelihood of bacterial translocation or gut atrophy (Evers *et al.* 1990; Serizawa *et al.* 1994; Xu *et al.* 1998). Many polymeric ED are based on milk proteins and there is some evidence that casein and whey themselves have direct immune modulatory functions (Wong *et al.* 1996; Cross & Gill, 1999).

Modification of fat composition of enteral diets

ED, particularly those of an elemental formula, often have a low fat content, which is another possible mechanism of efficacy. However, studies comparing high- and low-fat ED have found no differences in achieving clinical remission of CD (Royall *et al.* 1994; Leiper *et al.* 2001). Although there may be little effect by changing the amount of fat in ED, there may be some benefit from modification of the fat composition. We have carried out experiments in which colonic tissues from IBD patients were incubated with ED containing specific oils. Preliminary results indicated that incubation of tissue from UC patients with ED containing fish oil led to a significant increase in IL-ra:IL-1β ($P < 0.05$). However, for tissue derived from CD patients, incubation with ED containing fish oil had little effect on cytokine production profiles (Meister *et al.* 2001*b*).

There has been much interest in dietary supplementation with different fats in inflammatory conditions (de Pablo & de Cienfuegos, 2000; James *et al.* 2000). Previous studies of the use of *n*-3 dietary fats in IBD have given conflicting results (for review, see Beluzzi *et al.* 2000). In UC several studies have indicated that supplementation of ED with fish oil may be beneficial, particularly as concomitant decreases were seen in markers of inflammation (e.g. leukotriene B$_4$ levels) in rectal mucosa. However, not all studies showed significant clinical differences, possibly due to small numbers (Lorenz *et al.* 1989; Aslan & Triadafilopoulos, 1992; Hawthorne *et al.* 1992; Stenson *et al.* 1992). In animal models of UC administration of *n*-3 fatty acids by oral and topical enema routes indicated that reduced pathology and decreases in leukotriene levels could be achieved (Yuceyar *et al.* 1999). Moreover, there may also be a decrease in the colonic antioxidant defence systems, which may promote oxidative injury at the site of inflammation (Nieto *et al.* 1998), and thus explain the clinical results which showed decreases in pathology or inflammation but less effect on clinical remission rates. Although some *in vivo* studies have indicated little effect of fish oil in CD (Lorenz *et al.* 1989; Lorenz-Meyer *et al.* 1996), other studies have indicated that fish oil may be beneficial in CD (Beluzzi *et al.* 1996). The discrepancy between our *in vitro* results for fish oil-supplemented ED on CD and UC may be due to the fact that, as already mentioned, *in vitro* studies use a reductionist model, which may not parallel effects that occur *in vivo*. Our studies have only monitored one mechanism, i.e. alterations in cytokine production, albeit an important pathogenic mechanism in IBD (Casini-Raggi *et al.* 1995). It has been shown that fish oil reduced production of inflammatory cytokines from mononuclear cells (Endres *et al.* 1989). However, other inflammatory mechanisms which are altered by dietary fat intake and composition may be differentially important in CD and UC. For example, macrophage cytotoxic function has been shown to be affected by dietary fat composition (Wallace *et al.* 2000), as has the antigen-presenting function of monocytes (Hughes & Pinder, 2000), the production of inflammatory mediators (for review, see James *et al.* 2000) and cellular immune functions (for review, see Calder, 1998).

We have also cultured colonic tissues from IBD patients with ED containing sunflower oil, which gave significant increases in IL-1ra:IL-1β in both CD and UC tissues ($P < 0.05$ in both cases) but was not seen when tissues were incubated with ED containing safflower oil (Meister *et al.* 2001*a*). These results indicate that substitution by other fats in ED may be important for CD and UC therapy. Although in these ED preparations safflower oil-substituted ED contains more polyunsaturated fatty acids in total, the main difference in the fatty acid composition is that the sunflower oil-substituted ED used here contained no linoleic acid. Linoleic acid is a precursor of arachidonic acid, which in turn is a precursor of the inflammatory eicosanoid mediators leukotriene B$_2$, prostaglandin E$_2$ and thromboxane A$_2$, all of which have been implicated in IBD pathogenesis. Mucosal phospholipids derived from polyunsaturated fatty acids have been shown to be increased in the plasma membranes of active CD, possibly contributing to eicosanoid synthesis and inflammation (Pereira *et al.* 1996). Studies from Japan have implicated that dietary intake of *n*-6 polyunsaturated fatty acids, compared with *n*-3 polyunsaturated fatty acids, was a risk factor for development of CD (Shoda *et al.* 1996). However, a case–control study of CD patients, both newly diagnosed and with longstanding disease, indicated that intake of fat (both quality and quantity) was not different between patients and controls, but that fat metabolism in CD patients was changed, so that different fatty acids were present in plasma membrane phospholipids of CD patients compared with controls (Geerling *et al.* 1999). In addition, increased lipid oxidation has also been reported in CD (Mingrone *et al.* 1996), indicating that increased lipid intake may be beneficial in CD.

Bioactive peptides

Most studies of ED in IBD have concentrated on the macronutrients, i.e. protein and fat content or composition. It is possible that bioactive peptides may be important, and should perhaps be included in ED formulas. There are growth factors and peptides that could be very important in the maintenance of gut integrity in inflammatory diseases, many of which are present in colostrum (Playford *et al.* 2000). One such peptide is epidermal growth factor, which stimulates intestinal epithelial cell growth. A recent study in which patients with UC were treated with enemas containing epidermal growth factor indicated the efficacy of such growth factors in IBD (Sinha *et al.* 2001). Another bioactive peptide is transforming growth factor-β, which also occurs in colostrum and is known to have an anti-inflammatory effect on intestinal cell lines (Donnet-Hughes *et al.* 2000). Its potential benefit has been borne out in a study by Fell *et al.* (2000) who used a commercially available casein-based diet, which also contained transforming growth factor-β_2 in the treatment of paediatric CD and found a decrease in pro-inflammatory cytokine mRNA in the gut mucosa.

Conclusion

In summary, many studies have shown that ED is an effective therapy in patients with CD. Modification of ED with different fatty acids, bioactive peptides and, to a lesser extent, N source may have a direct effect on the

inflammatory mediators produced in both CD and UC, thus increasing the efficacy of ED in both forms of IBD. Further studies are required to ascertain how ED formulas should be modified in ways that are disease-specific for CD and UC.

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