

## Beneficial effect of a polymeric feed, rich in TGF- $\beta$ , on adult patients with active Crohn's disease: a pilot study

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### SUMMARY

So far, nutritional support with a polymeric diet rich in TGF- $\beta$  has been studied only in children with Crohn's disease and produced satisfactory results. There are no data concerning the effect of this kind of diet in adult patients with Crohn's disease. The aim of this pilot study was to present our initial experience on the use of a polymeric diet rich in TGF- $\beta$  in patients with mild or moderately active Crohn's disease. **Patients and Methods:** Twenty nine patients with active Crohn's disease received Modulen IBD as an exclusive diet for 4 weeks (50gx5/d). Patients continued to be on their regular conservative treatment (if so).

Activity of the disease was assessed at the beginning of the therapeutic trial and after 4 weeks by the use of CDAI. Various anthropometric parameters and serum estimations, including ESR, CRP, platelets, albumin, vitamin B12 and folic acid were carried out at the beginning and after 4 weeks of application of the special diet.

**Results:** Clinical improvement was noticed in 69% (20 out of 29 patients). No change of the situation or worsening was noticed in 9 patients (31%). The main alterations on anthropometric, hematological and biochemical parameters estimated before and after the application of the special diet are shown in the table.

Parameter	Before treatment	After treatment	P-value
Body weight	61.7+/-13.8	63.2+/-13.6	0.003
Body Mass Index	21.2+/-4.5	21.7+/-4.0	0.002
Skin Fold Thickness	13.9+/-7.5	15.1+/-8.7	0.039
Mid Arm Circumference	26.7+/-4.5	28.1+/-4.8	0.004
Albumin	3.14+/-0.62	3.6+/-0.41	0.049
Fibrinogen	494.5+/-105.4	424.3+/-92.6	0.002
Folic acid	4.9+/-3.1	6.7+/-3.9	0.038
CRP	30.6+/-26.2	11.3+/-14.6	0.011
Immunoglobulin B	126.8+/-68.2	151.4+/-103.4	0.017

(Mean +/-1SD)

**Conclusion:** Dietetic intervention with polymeric diet rich in TGF- $\beta$ 2 represents a quite satisfactory therapeutic modality in adult patients with mild to moderately active Crohn's disease. However, these results must be confirmed in larger, randomized, placebo controlled clinical trials.

**Key words:** Crohn's disease, Inflammatory bowel disease, Inflammation, Transforming Growth Factor- $\beta$ , Milk

### INTRODUCTION

Various genetic, immunological and environmental factors have been implicated in the etiology of Inflammatory bowel disease (IBD), based on epidemiological and basic research observations. Most studies suggest an immunological and genetic pathogenic background, whereas the inflammatory response is influenced by environmental factors and by the organism itself.

Inflammatory cytokines, released locally, play a significant role in IBD. In Crohn's disease there is a selec-

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tive activation of Th-1 lymphocytes which induces massive production of pro-inflammatory cytokines, such as IL-1, IL-6, IL-12 and TNF- $\alpha$ .

The contribution of the immune system to the development of IBD implies new therapeutic perspectives, in which nutrition can play a primary role. In patients with IBD the goal of nutritional treatment is not only to improve nutritional status, but also to modify the inflammatory immunological response in order to decrease disease activity and induce clinical remission.<sup>1</sup>

It is well known that several epidermal growth factor (EGF)-like peptides (EGF, transforming growth factor [TGF]- $\alpha$ , heparin-binding EGF-like peptide, amphiregulin, and betacellulin) are present in the gut.<sup>2</sup> Among them TGF- $\beta$ , a multifunctional polypeptide (cytokine) present in human and bovine milk, plays a critical role in the development of tolerance, the prevention of autoimmunity, and in anti-inflammatory responses. It is a potent inhibitor of intestinal epithelial cell growth and stimulates intestinal epithelial cell differentiation. It also seems to play a significant role in promoting healing of inflammatory lesions both in human and animal studies. TGF- $\beta$  has a gradient of expression along the crypt-villus axis, with maximum production at the villus tip.

On the other hand it is well established that intestinal adaptation is highly dependent on enteral nutrition, and it is likely that growth factors are involved in adaptation. Little is known, however, about interactions between nutrients and growth factors. Milk contains a range of potentially important growth factors. Polymeric diets seem to be helpful in adult patients with Crohn's disease, although are less efficacious compared with corticosteroids and have the advantage to contain whole protein they are more tolerable than elemental diets and have lower cost.

Nutritional treatment with a polymeric rich in TGF- $\beta$  has been applied in children with terminal ileum Crohn's disease with satisfactory results. Also, recent data referring to experimental colitis in rats suggest that IL-10-/- mice fed a TGF- $\beta$  containing diet gained more weight, did not develop diarrhoea or prolapse, and had lower pathological scores, thus further supporting the use of TGF- $\beta$  containing enteral diets as one mode of therapy for Crohn's disease.<sup>3,4</sup>

The present study was designed as a pilot one, to investigate the effectiveness of a polymeric diet rich in TGF- $\beta$ , in reducing disease activity in adult patients with mild to moderately active Crohn's disease.

## PATIENTS AND METHODS

### *Patients*

Twenty-nine patients with Crohn's disease in the acute phase were recruited for this study after obtaining informed consent and approval of our hospital ethical committee. The main clinical and demographic characteristics of the patients are shown in table 1.

### *Design and procedures*

All patients were managed as usual with conventional drug treatment, and all other usual therapeutic interventions (e.g. fluid and electrolyte replacement) were used as appropriate for each case. In addition patients received Modulen IBD 50gx5 per day as an exclusive diet for 4 weeks. Modulen IBD is a polymeric diet with casein as its protein source, which is rich in TGF $\beta$  (>24 p.p.m.). The protein content is 14%, the carbohydrate content 44% and the fat content 42%. It is lactose free with glucose polymer and sucrose as the carbohydrate source. Its lipid content is made up of milk fat (55.6%) corn oil (13.9%) and medium chain triglycerides (26.1%). The calorie density of the feed is 312mosm/L. It has been formulated to contain adequate amounts of vitamins, minerals and trace elements.<sup>5</sup> The formulation of the final solution is very easy. The patient must dilute 50grams of Modulen IBD powder in 210 ml of fresh water and to consume the final product in about half an hour. Each meal offers more than 210 Kcals. Table 1 shows the main ingredients of Modulen IBD powder.

Drug treatment was left unchanged during the period of enteral nutrition. So, 17 out of 29 patients continued to receive mesalazine (7 patients), metronidazole (3 patients), mesalazine plus azathioprine (4 patients), butesonide (1 patient) and infliximab maintenance treatment (2 patients).

### *Anthropometry*

Height, weight, mid-arm circumference, triceps and subscapular skin-fold thickness, were measured using standard anthropometry techniques. Anthropometric parameters were estimated before and at the end of the trial.

### *Assessment of disease activity and severity and clinical response*

Clinical disease activity was studied at baseline and 4 weeks after initiation of treatment by using Crohn's Disease Activity Index (CDAI). Patients were categorized as suffering from mild severity disease (CDAI: 150-200,

**Table 1.** Constituents of MODULEN IBD polymeric diet

	Units	Per 100 ml
Energy	Kcal	100
Protein (14% TEI)	g	3.6
Carbohydrate (44% TEI)	g	11
Lipid: 42% TEI includingo		
<i>Essential fatty acids</i>	g	0.47
<i>Linoleic acids</i>	g	0.43
<i>A linoleic acids</i>	g	0.04
<i>Medium chain triglycerides</i>	g	1.2
Vitamin A	IU	250
Vitamin E	IU	1.5
Vitamin D3	IU	20
Vitamin K1	µg	4
Vitamin C	mg	7.9
Vitamin B1	mg	0.08
Vitamin B2	mg	0.13
Vitamin B5	mg	0.5
Vitamin B6	mg	0.1
Niacin	mg	1
Folic acid	µg	20
Biotin	µg	15
Vitamin B12	µg	0.4
Choline	mg	7.3
Inositol	mg	4.3
Na	mg	35
K	mg	120
Cl	mg	73
Ca	mg	91
P	mg	60
Mg	mg	20
Fe	mg	1.2
Cu	mg	0.1
Zn	mg	0.78
Mn	mg	0.1
Iodine	µg	8
Se	µg	3.5
Mo	µg	7
Cr	µg	4

TEI= total energy intake

17 patients) or moderate severity disease (CDAI: 201-400, 12 patients).

Response to treatment was categorized as a) remission (CDAI<150), b) satisfactory (reduction of CDAI of at least 50 points), c) no change and d) worsening.

### Laboratory measurements

Various blood parameters were estimated at baseline and at the end of trial. These parameters included estimation of inflammation markers (ESR, CRP), vita-

**Table 2.** Clinicoepidemiological characteristics of the patients studied.

Men:	20
Women:	9
Total:	29
Age (Years – range):	41+/-16 (17-70)
Presence of fistulas:	8 patients
Duration of disease:	6.2+/-3.9 years
Location of disease: oSmall bowel: 21oLarge bowel: 2	
Small & large bowel: 6	

min levels and trace elements, as well as estimation of other biochemical measurements (electrolytes, hemoglobin, albumin etc).

### Statistical analysis

For statistical analysis a paired t-test was used. Results were considered significant if P was less than 0.05.

## RESULTS

### Clinical response

Clinical remission and satisfactory response was achieved in 11/29 (38%) and 9/29(31%) patients, respectively. The overall improvement reached the level of 69% (20 out of 29 patients). No change was seen in 6 out of 29 patients (21%) and worsening was noticed in 3 out of 29 patients (10%).

CDAI was significantly reduced compared to pre-treatment values (266+/-63 vs 176+/-43) (P<0.05)

### Anthropometry

Statistically significant improvement in all anthropometric parameters examined compared to pre-treatment values were noticed (Table 3).

**Table 3.** Alterations in anthropometric parameters after introduction of the specific diet.

Parameter	Before feeding	After feeding	P-value
Body weight (Kg)	61.7+/-13.8	63.2+/-13.6	0.003
Body Mass Index	21.2+/-4.1	21.7+/-3.9	0.0001
Skin Fold Thickness (cm)	14.8+/-8.6	16.2+/-8.8	0.001
Mid Arm Circumference (cm)	27.4+/-5	28.4+/-4.9	0.001

(mean +/-ISD)

### ***Nutritional and biochemical parameters***

Significant improvement of many of the nutritional and biochemical parameters studied was noticed at the end of 4 weeks (Table 4). An interesting finding was the improvement in the serum levels of HDL and the reduction in the serum levels of LDL.

### ***Inflammatory and immunological indices***

Significant reduction in the serum levels of CRP and fibrinogen and significant reduction in the value of ESR was noticed after 4 weeks of the application of the special diet (Table 5).

### ***Patients with fistulas***

In 4 out of 8 patients with enterocutaneous fistulas reduction (at least 50%) in the secretion through the fistula was noticed after 4 weeks. In one more patient specific diet resulted in almost complete closure of fistula (Figure 1).

### ***Side-effects***

Some minor symptoms attributed to nutritional treatment, were reported (mainly some kind of nausea in one patient and diarrhea in one more patient). Otherwise the food was well tolerated by all patients.

**Table 4.** Alterations in nutritional and biochemical parameters after introduction of the specific diet.

Parameter	Before feeding	After feeding	P-value
Albumin (g/dl)	3.14+/-0.62	3.6+/-0.41	0.049
Ferritin (ng/ml)	52.6+/-59	61+/-55	0.019
Folic acid (ng/ml)	4.9+/-3.1	6.7+/-3.9	0.038
HDL (mg/dl)	45.5+/-11.7	47.7+/-11.3	0.003
LDL (mg/dl)	83.6+/-31	75+/-31	0.002

(mean +/- ISD)

**Table 5.** Alterations in inflammatory and immunological parameters after introduction of the specific diet.

Parameter	Before feeding	After feeding	P-value
CRP (mg/L)	30.6+/-26.2	11.3+/-14.6	0.011
ESR (mm/h)	46+/-13	23+/-9	0.01
Fibrinogen (mg/dl)	494.5+/-105.4	424.3+/-92.6	0.002
Immunoglobulin A (mg/dl)	497+/-240	512+/-242	0.001
Immunoglobulin G (mg/dl)	1458+/-360	1377+/-514	0.002
Immunoglobulin M (mg/dl)	114+/-80	150+/-81	0.031
Complement 4 (mg/dl)	19+/-8	49+/-93	0.007

(mean +/- ISD)



**Fig. 1.** Influence of immunomodulating diet (Modulen IBD) on abdominal fistula: Almost complete closure after 6 weeks application of the special diet.

### ***Long-term follow-up***

Ten patients continued to be on 100g of the special diet (two doses of MODULEN IBD per day, plus two regular meals per day) for more than 12 months. All are in extremely good clinical situation. However, during the first six months three other patients (belonging to the group of non-responders) were submitted to operation (enterectomy plus end-to-end anastomosis).

## **DISCUSSION**

This prospective, pilot, uncontrolled clinical study has shown that oral administration of a specific polymeric diet containing TGF- $\beta$  is effective in inducing remission in a significant proportion of adult patients with mild or moderately active Crohn's disease. Moreover, clinical response was associated with reduction in the serum levels of some inflammatory indices including CRP and ESR. Finally, all nutritional parameters examined, also

improved significantly at the end of treatment.

The remission rate or improvement of almost 70% after 4 weeks is an encouraging preliminary result, bearing in mind that powerful medical treatments including administration of corticosteroids, antibiotics, immunosuppressants and/or immunomodulating factors produce only slightly better results.<sup>6-8</sup> On the other hand the almost complete absence of side-effects makes this therapeutic modality quite attractive in patients suffering from mild to moderate flare-up of Crohn's disease.

It is of interest that all anthropometric parameters examined were improved. Patients stopped losing weight and the score of general well being improved. Many other biochemical parameters improved (including serum calcium), although differences did not reach statistical significance.

Two other studies have been so far published.<sup>5,9</sup> The first one was a small study of patients with Crohn's disease mainly affecting the small bowel while the second one included patients with different location of the disease. In both studies, patients received Modulen IBD for 8 weeks as an exclusive diet. The TGF- $\beta$  diet was effective in inducing remission and mucosal healing. Biochemical markers of inflammation such as ESR and CRP were normalized while the serum levels of albumin were increased. Histology of the large bowel was improved significantly. It is of interest that there was a reduction in the mRNA levels for IL-1, IL-8 and IFN- $\gamma$  with an increase in endogenous TGF- $\beta$ .

Several nutrients, hormones, growth factors and immunoactive molecules are present in both human and bovine milk, although some protective factors are reduced in bovine milk, thus leading to some susceptibility to infections and allergic reactions in children fed with bovine milk formulas.<sup>10</sup> However, efforts of the industry aiming to preserve the biological activity of some bioactive molecules in end products resulted in the production of this kind of food containing TGF- $\beta$ , a polypeptide present in both human and bovine milk. TGF- $\beta$  plays a critical role in the development of tolerance, the prevention of autoimmunity, and in anti-inflammatory responses. It is also a potent inhibitor of intestinal epithelial cell growth and stimulator of intestinal epithelial cell differentiation.

Enteral nutrition is a widely used treatment in adult patients with Crohn's disease, although its efficacy in children is more prominent.<sup>11</sup> However, there is considerable speculation concerning the mode of its action on enteral nutrition including the mode of action of Modu-

len IBD. In Crohn's disease there is marked over-expression of pro-inflammatory cytokines such as TNF- $\alpha$  and increased production of matrix degrading enzymes by fibroblasts and macrophages. Endogenous healing pathways mediated by TGF- $\beta$  are inhibited because mucosal inflammatory cells express Smad7, the endogenous intracellular inhibitor of TGF- $\beta$  signalling.<sup>12</sup> Concerning the mode of action of Modulen IBD, the above mentioned cascade of events makes it unlikely that enteral feeds containing TGF- $\beta$  are therapeutic by means of direct anti-inflammatory effects, although TGF- $\beta$  may still be involved because it is a well known epithelial mitogen and may promote mucosal healing in synergy with changes in mucosal bacterial populations as a result of the change in the diet.

Among the possible mechanisms, antigen exclusion, changes in bacterial flora and bowel rest seem to be the most important.<sup>13</sup> However, the traditional hypothesis claiming that enteral nutrition works by the exclusion of dietary antigens seems unlikely since specific disease-associated foods have only rarely been identified. Recently, possible mechanisms of action of enteral nutrition proposed are both low residue and prebiotic properties of the polymeric liquid formula.<sup>14</sup> It has been suggested that the clinical remission achieved is a result of a reduction in inflammation, rather than a consequence of some other nutrition effect.<sup>15</sup> It is of interest that the clinical response to Modulen IBD is associated with mucosal healing and a down-regulation of mucosal pro-inflammatory cytokine mRNA in both the terminal ileum and the colon.<sup>4</sup> Moreover, recent observations suggest that when administered before and during methotrexate treatment, Modulen IBD supplementation provided statistically significant protection against weight loss, hypoalbuminemia, acidosis, and GI damage in a rat model.<sup>16</sup>

An important question is related to the ability of TGF- $\beta$  to pass the whole digestive tract without degradation by the powerful digestive enzymes. According to Beattie et al<sup>9</sup> a survey of the TGF- $\beta$  content of twenty milk-based preparations demonstrated that its presence is dependent upon the source of milk protein and processing conditions. It seems that the presence of casein is critical as it may inhibit the enzymatic degradation of TGF- $\beta$  by the duodenal and enteric juice.<sup>17</sup>

There is no information in the literature concerning long-term results of this special diet. However, the continuous administration of Modulen IBD in the form of two meals per day (2X50 g) plus two regular meals, in 10 patients with good initial response, resulted in sustained

remission. However, this observation needs confirmation in larger prospective studies.

**In conclusion**, polymeric diet rich in TGF- $\beta$  could be a significant part of our therapeutic armamentarium in adult patients with mild to moderately active Crohn's disease. However, more randomized studies, using larger number of patients must be contacted in order to prove the efficacy of this interesting therapeutic modality.

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