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Original article

Maintenance treatment of Crohn's disease with a polymeric feed rich in TGF-β

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SUMMARY

Rationale: Dietetic intervention with polymeric diet rich in transforming growth factor-β2 (TGF-β2) represents a relatively satisfactory therapeutic modality in adult patients with mild to moderately active Crohn's disease. However, there is no information concerning long-term results of this special diet. The aim of this study was to compare the results of the administration of a special diet (Modulen IBD) rich in TGF-β2 with those of the administration of mesalamine, in maintaining remission in patients with Crohn's disease. Methods: Of the 83 patients initially included in the study, 76 (91.5%) [36 in the Modulen and 30 in the mesalamine arm] completed the trial. Patients were randomly assigned to receive either two meals (2X50g) of Modulen IBD plus two regular meals per day (43 patients) or mesalamine (800mg three times a day) (40 patients) for six months. Patients were assessed at sixth months after initiation of the trial. Relapse was defined as a Crohn's Disease Activity Index of greater than 150 points or at least 60 points over baseline. Various anthropometric parameters and serum estimations including ESR, CRP, platelets, albumin, vitamin B12 and folic acid were carried out at the beginning of the trial and after six months. Results: Twenty-five patients in the Modulen arm (69%) continued to be in remission after six months compared with 18(60%) receiving mesalamine (no significant differences). Among patients with relapse the mean time to relapse was 103 days for those treated with mesalamine and 123 days for those treated with Modulen (no significant differences). Conclusion: Modulen-IBD treatment reduced relapse rate to a better degree compared with mesalamine treatment, although statistical significance was not achieved.

Key words: Crohn's disease, inflammatory bowel disease, Immunomodulation, Transforming Growth Factor-β, Milk

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INTRODUCTION

Various genetic, immunological and environmental factors have been implicated in the etiology of inflammatory bowel disease (IBD). Inflammatory cytokines, released locally, play a significant role in IBD. In Crohn's disease (CD) there is a selective activation of Th-1 lymphocytes which induces massive production of pro-inflammatory cytokines, such as IL-1, IL-6, IL-12 and TNF-α.[1]

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.[2]

Several epidermal growth factor-like peptides (EGF, transforming growth factor [TGF]-α, heparin-binding EGF-like peptide, amphiregulin, and betacellulin) are present in the gut[3]. Among them TGF-β, 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-β has a gradient of expression along the crypt villus axis, with maximum production at the villus tip.[3]

On the other hand intestinal adaptation is highly dependent on enteral nutrition, and it is likely that growth factors

are involved in this adaptation. Data referring to experimental colitis in rats suggest that IL-10-/- mice fed a TGF-β containing diet, gained more weight, did not develop diarrhoea, and had lower pathological scores,[4,5] thus supporting the use of TGF-β containing enteral diets as a possible therapeutic modality for CD patients. Nutritional treatment with a polymeric diet rich in TGF-β has been applied in children with terminal ileum CD with satisfactory results[6-8] and the same was described in adult patients with CD.[9]

Drugs used for maintenance treatment in CD include butezonide[10] mesalamine,[11] and immunosuppressives[12] and the recently introduced biologic agents (infliximab and adalimumab).[13] So far there are no data concerning the use of a diet rich in EGF-beta as a maintenance treatment in patients with CD.

The aim of this study was to compare the results of the administration of a special diet rich in TGF-β2 (Modulen IBD) with those of mesalamine, in maintaining remission in patients with quiescent CD.

PATIENTS AND METHODS

Patients

Eighty-three patients with CD in remission (CDAI <150points) participated in the study after obtaining informed consent and approval of our hospital ethical committee. However, of these 83 patients initially included in the study, 76 (91.5%), 36 in the Modulen and 30 in the mesalamine arm, completed the trial. The main clinical and demographic characteristics of these 76 patients are shown in table 1.

Design and procedures

Modulen IBD is a polymeric diet with casein as its protein source, which is rich in TGFβ (>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.[6,7] The formulation of the final solution is very easy. The patient must dilute 50g 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 2 shows the main ingredients of Modulen IBD powder.

Patients received two meals (2X50g) of Modulen IBD plus two regular meals per day (36 patients) or mesalamine (800mg three times a day, 30 patients) for six months. No other medications were allowed during the trial period.

Assessment of disease activity and severity and clinical response

Clinical disease activity was studied at baseline and 3 and 6 months after initiation of treatment using Crohn's Disease Activity Index (CDAI). Relapse was defined as a CDAI greater than 150 points or at least 60 points over baseline.

Anthropometry

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

Laboratory measurements

Various blood parameters were estimated at baseline and at the end of the sixth month. These parameters included inflammation markers (ESR, CRP) and other biochemical measurements (hemoglobin, WBC, platelets, 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

Relapse rate

At the end of the third month, 6 out of 36 (16.7%) of patients in the Modulen group and 7 out of 30 (23.3%) patients in the mesalamine group had suffered from relapse, while at the end of the sixth month 11(30.5%) patients in the Modulen arm and 12 out of 30 (40%) patients in the mesalamine group suffered a relapse (no significant differences.

Among those with relapse the mean time to relapse was 103 days for those treated with mesalamine and 123 days for those treated with Modulen (no significant differences).

Anthropometry, nutritional and biochemical parameters

Significant improvement in all anthropometric parameters compared to pre-treatment values was noticed in the group of patients receiving Modulen IBD (Table 3). However, no significant alterations in the anthropometric parameters in the group of patients receiving mesalamine were noticed.

Table 1. Clinicoepidemiological characteristics of the patients studied.

	Modulen arm (36 pts)	**Mesalamine arm (30 pts)**
Parameter	Value	Value
Men	22	19
Women	14	11
Total	36	30
Age (Years – range)	41+/-16 (17-70)	39.8+/-19 (23-66)
Inactive fistulas	4 patients	3 patients
Duration of disease (years)	6.2+/-3.9	6.9+/-4.6
Location of disease		
Small bowel	22	16
Small & large bowel	12	9
Large bowel	2	5

Again in the group receiving Modulen IBD, significant improvement of many of the nutritional and biochemical parameters studied was noticed (Table 3). No significant differences in the group of patients receiving mesalamine were observed.

In the group of patients receiving Modulen IBD an improvement in the serum levels of High Density Lipoproteins (HDL) and a reduction in the serum levels of Low Density Lipoproteins (LDL) at the end of trial was noticed.

Patients with fistulas

In the group of Modulen, 4 patients with inactive enterocutaneous fistulas at the beginning of the trial continued to be inactive and the same was noticed in the 3 patients with enterocutaneous fistulas in the mesalamine group.

Side-effects

Some minor symptoms attributed to nutritional treatment were reported, including nausea in two patients and diarrhea in four. Otherwise the food was well tolerated by all patients.

Patients submitted to operation

During the trial, three patients belonging to the group of non-responders (one in Modulen arm and two in mesalamine group were submitted to operation (enterectomy plus end-to-end anastomosis).

DISCUSSION

The maintenance treatment of those patients with CD in whom remission was achieved either surgically or using conservative treatment remains problematic, as many of the drugs used in induction of remission such as mesalamine and immunosuppressives offer little benefit. Biologic agents are a satisfactory alternative solution although data available for patients undergoing surgical enterectomy plus end-to-end anastomosis are very few at the moment.

To the best of our knowledge there are no studies using Modulen IBD as a maintenance treatment in patients with CD. So far, only studies in pediatric patients have been published[6-8] but again no results of the administration of Modulen IBD as a maintenance treatment are available. In this study we have shown that by substituting two meals with a polymeric, rich in TGF-β, diet (Modulen IBD) for six months, we can achieve better results (although not statistically significant) compared to mesalamine administration (800mg three times a day) in maintaining remission in patients with quiescent CD. At the end of the sixth month, all nutritional parameters examined were significantly improved in the Modulen arm while no significant improvement in the group of mesalamine was noticed. Taking into account the almost complete absence of side-effects, this therapeutic modality sounds quite attractive in patients with CD in remission.

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 leading to some susceptibility to infections and allergic reactions in children fed with bovine milk formulas[14]. However, efforts of the industry aiming to preserve the biological activity of bioactive molecules in end products resulted in the production of food containing TGF-β, a polypeptide present in both human and bovine milk. TGF-β 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

Table 2. Constituents of MODULEN IBD polymeric diet.

	Units	Per 100 ml
Energy	Kcal	100
Protein (14% Total Energy Intake)	g	3.6
Carbohydrate (44% Total Energy Intake)	g	11
Lipid: 42% TEI including		
Essential fatty acids	g	0.47
Linoleic acid	g	0.43
A linoleic acid	g	0.04
Medium chain triglycerides	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

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.[15] However, there is considerable speculation concerning the mode of action of enteral nutrition including Modulen IBD. In CD there is marked overexpression of pro-inflammatory cytokines such as TNF-α and increased production of matrix degrading enzymes by fibroblasts and macrophages. Endogenous healing pathways mediated by TGF-β are inhibited because mucosal inflammatory cells express Smad7, the endogenous intracellular inhibitor of TGF-β signalling.[16]

Among the possible mechanisms of action of enteral nutrition, antigen exclusion, changes in bacterial flora and bowel rest seem to be the most important.[17] 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. Enteral feeds containing TFG-β are therapeutic by means of direct anti-inflammatory effects, although TGF-β may be involved because it is a well known epithe-

Table 3. Alterations observed in anthropometric and nutritional parameters in the two arms at baseline and after six months.

	Modulen arm (36 pts)			Mesalamine arm (30 pts)		
Parameter	At baseline	End of 6th month	P-value	At baseline	End of 6th month	P-value
Body weight	63.2	65.1	0.003	61.7	63.2	ns
	+/-13.5	+/-13.3		+/-13.8	+/-13.6	
Body Mass Index	21.2	21.9	0.002	21.2	21.7	ns
	+/-4.5	+/-4.0		+/-4.5	+/-4.0	
Skin Fold Thickness	15.8	16.9	0.039	13.9	15.1	ns
	+/-7.5	+/-8.7		+/-7.5	+/-8.7	
Mid Arm Circumference	28.0	29.1	0.004	26.7	28.1	ns
	+/-4.4	+/-4.7		+/-4.5	+/-4.8	
Folic acid	6.3	8.2	0.038	5.9	6.3	0.030
	+/-3.1	+/-3.9		+/-3.0	+/-3.4	
Ferritin	60.6	69.9	0.019	52.6	61	0.038
	+/-49	+/-35		+/-59	+/-55	
HDL	47.4	51.1	0.003	49.5	50.1	ns
	+/-10.3	+/-9.8		+/-9.7	+/-10.3	
LDL	77.5	68.1	0.002	81.5	79.7	ns
	+/-30	+/-29		+/-24	+/-26	

(Mean+/-1SD)(Comparisons of values at baseline and after 6 months) (ns: no significant)

lial mitogen and may promote mucosal healing in synergy with changes in mucosal bacterial populations as a result of the change in the diet. Other mechanisms of action of enteral nutrition proposed recently are both the low residue and prebiotic properties of the polymeric liquid formula.[18,19] As mentioned previously, it has been suggested that the clinical remission achieved by Modulen IBD is probably a result of reduction in inflammation, rather than a consequence of some other nutritional effects.[20] Of most interest is the fact that the clinical response to Modulen IBD is associated with mucosal healing and down-regulation of mucosal pro-inflammatory cytokine mRNA in both terminal ileum mucosa and the colon.[4] Moreover, recent observations suggest that when administered before and during methotrexate treatment, Modulen IBD provided statistically significant protection against weight loss, hypoalbuminemia, acidosis, and GI damage in a rat model.[21]

An important question is related to the ability of TGF-β to pass the whole digestive tract without degradation by the digestive enzymes. According to Beattle et al[7] a survey of the TGF-β content of twenty milk-based preparations demonstrated that its presence is dependent upon the source of milk protein and processing conditions. It seems that casein itself may inhibit the enzymatic degradation of TGF-β by the duodenal and enteric juice.[22]

Finally it was of interest to see an increase in the levels of HDL lipoproteins and a decrease in the levels of LDL lipoproteins in the group of patients receiving Modulen IBD. The role of dysfunctional HDL in cytokine induction and inflammation seems to be quite important as HDL can modulate LDL oxidation and LDL-induced cytokine production and inflammation.[23] Moreover, dysfunctional HDL has been identified in animal models and humans with chronic inflammatory diseases. Evidence suggests that the anti-inflammatory properties of HDL may be at least as important as the levels of HDL-cholesterol. The pathophysiological consequences of Modulen IBD administration need further exploration, as the levels of HDL and LDL before and after treatment of IBD patients could be useful as an index of inflammatory activity.

In conclusion, administration of Modulen IBD 100g per day in two doses can maintain remission in patients with quiescent CD in a similar to mesalamine degree. Larger studies and for a longer period of time, especially in conjunction with other drugs including biologic agents, are needed in order to confirm the benefits derived from the administration of this kind of diet.

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