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The Effect of Enzyme Supplementation on Symptoms and Duodenal Histology in Celiac Patients

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Abstract *Background*: The etiology of celiac disease (CD) is related to undigested fragments of gluten and gliadin which damage the small bowel. Mucosal enzyme deficiency is an important factor in CD pathology. A clinical trial has shown that the effects of a gluten challenge to patients with CD could be ameliorated by the use of an enzyme supplement. *Objective*: Enzyme therapy using enterically coated tablets containing caricain (Gluteguard) was investigated as a means of protecting patients with CD against wheat gluten. *Methods*: A randomized placebo-controlled trial was carried out on 20 CD patients in clinical remission. The patients were divided into a group of 14 given Gluteguard and a group of 6 given a placebo daily. Both groups were given a challenge of 1g of gluten daily. Symptoms were graded and recorded over a period of 42 days. Duodenal tissue was taken at the beginning and end of this period, together with blood for assay of tissue transglutaminase (tTG-IgA) antibodies. *Results*: The results showed that oral enzyme therapy based on caricain, was effective in ameliorating the symptoms of CD giving a statistically significant difference between treatment and placebo ($P<0.01$) after 14 days challenge. General well-being was also improved from 6.1 to 8.4 ($P< 0.01$) by the enzyme therapy. Four of the six placebo group patients (67%) and one of the 14 treatment patients (7%) to withdraw from gluten challenge after 14 days due to development of serious symptoms. The difference between the groups was significant ($p < 0.001$). For the per protocol patients on Gluteguard therapy, there were no significant changes in markers of histological damage or biopsy results after 42 days of gluten challenge. *Conclusions*: This study demonstrated that oral anti-gluten enzyme therapy using Gluteguard was able to significantly protect celiac patients from adverse symptoms being induced by gluten challenge. Furthermore, mucosal damage was not exacerbated in patients taking Gluteguard along with their daily gluten challenge, suggesting that the enzyme tablets may also help with the recovery of epithelium in the longer term. Availability of a preventative enzyme treatment like Gluteguard will likely add to the quality of life and well-being of coeliac patients, especially those who have difficulty in strictly adhering to a gluten-free diet.

Keywords: *coeliac disease, gluten, caricain, enzyme therapy, Gluteguard*

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1. Introduction

The prolamin group of proteins in wheat, rye, barley, triticale and oats are causative agents in celiac disease (CD). These proteins cause damage to the small intestine of individuals with CD which results in malabsorption of nutrients [1,2]. The incidence of CD is more than 1 % in the USA and Europe [3]. The symptoms normally observed are abdominal pain, bloating, diarrhea, nausea and cramps, but general malaise and tiredness are also common.

If these symptoms are noticed in childhood the disease is best diagnosed at this time of life because, if left undiagnosed, serious health problems, including osteoporosis, anemia, lymphomas and gastrointestinal cancers can result [4].

Two other studies have suggested a significantly increased risk of mortality over time in patients with undiagnosed CD [5,6]. In order to overcome these problems, the main treatment to date has been the use of a gluten-free diet [7]. However, the gluten-free diet by itself does not seem to be the complete answer. Rubio-Tapia et al [8] studied the rate of mucosal recovery after such treatment. They found that most patients took several years before significant recovery occurred. Poor compliance was strongly associated with persistent mucosal damage but 82% had some clinical response to

the diet. Other workers also found that, although substantial clinical response is observed in most patients with CD after only a few weeks on a gluten-free diet, mucosal recovery does not always occur in a short timeframe [9]. Pekki et al [10] also showed that histological recovery in patients on a gluten-free diet for 1 year was better in those who had better dietary adherence. However, high compliance was still not sufficient to normalize the histology in this period of time.

Clinical studies have indicated that the harm caused by exposure of coeliac patients to gluten could be partly corrected by the use of enzyme supplementation [11,12]. More recently we have suggested that caricain (EC 3.4.22.30) is a useful enzyme for this purpose [13], especially in view of its success in patients with the related disease dermatitis herpetiformis [14] and its ability to reduce immunoreactive gluten in baked goods [15]. Caricain belongs to the cysteine protease group of enzymes [16,17]. The enzyme needs to be able to disrupt certain key sequences in gliadin peptides which have been shown to be toxic *in vivo* to coeliac patients. Caricain appears to function as a prolyl endopeptidase that is able to attack such peptides on the N-terminal side of proline residues. Other workers also have pointed out the difficulty of digesting certain proline-rich peptides that produce immunological reactions leading to intestinal damage [18]. The 33-mer peptide of α-2 gliadin [19] is one such peptide, the antigenicity of which could be reduced by the enzyme prolyl oligopeptidase (EC3.4.21.26). All of this work provides the rationale for enzyme therapy to supplement the gluten-free diet.

The study aimed to: test whether Gluteguard (a caricain preparation) reduced symptoms in coeliac patients challenged with gluten, and whether serological and histological markers of gluten damage could be modified with the use of Gluteguard. Our hypothesis was that taking Gluteguard tablets orally with food containing small amounts of gluten would result in digestion of toxic gluten peptides, thus alleviating the effect of gluten ingestion in celiac patients.

2. Material and Methods

Patients on a gluten-free diet were subjected to a challenge of 1g gluten/day for 6 weeks. Symptoms were recorded in patients on enterically coated tablets of a caricain preparation and compared against those on a placebo. Endoscopy was performed on all patients at the beginning and end of the trial.

2.1. Patients

Twenty adult volunteers [18-70 years of age, 5 male (M) and 15 female (F)] with diagnosed celiac disease, in clinical remission, maintaining their usual gluten-free diet since CD diagnosis were included in the study. The patients were diagnosed at least 4 month to 36 years ago. Diagnosis of CD was established based on earlier clinical presentation, immunological criteria (antibodies against endomysium and against tissue transglutaminase) and results of duodenal histology. The study was conducted according to GCP, study protocol was approved by Ethical Committee of Central Clinical Hospital of Ministry of Interior in Warsaw, Poland. All the patients signed informed consent before entering the study. The volunteers followed their regular gluten-free diet for the duration of the study. Gluten-free diet was introduced immediately after diagnosis. Table 1 gives background information on all patients in the study.

Table 1. Background information of patients involved in the study

Patient No	Sex	Study Group	Age	BMI	Year gluten-free diet applied	Concomitant diseases	Celiac disease in the family	Symptoms before diagnosis
1	F	T	63	27,8	2008	NIL	YES	Abdominal pain, headache, anemia
2	M	T	27	23.8	2014	NIL	NO	Abdominal pain, flatulence
3	F	P	24	22.8	2014	HCV	NO	Abdominal pain, diarrhea, flatulence
4	M	T	39	21.9	2013	NIL	NO	Anemia
5	F	T	28	22.6	2009	Hashimoto	NO	Anemia
6	F	T	51	20.8	2009	NIL	NO	Abdominal pain, flatulence, weight loss
7	F	T	40	21.7	2012	HCV	NO	Diarrhea, flatulence
8	M	P	36	26.1	2014	Wilson disease	YES	Abdominal pain, flatulence
9	F	P	24	17.3	2011	DH	NO	Abdominal pain, anemia
10	M	T	37	25.2	1978	NIL	NO	Weight loss, diarrhea,
11	F	T	39	19.2	2012	NIL	YES	Abdominal pain, anemia
12	F	P	25	21.3	2004	Hashimoto	NO	Abdominal pain, diarrhea, weight loss
13	F	P	18	26.2	2013	Asthma	NO	Abdominal pain,constipation
14	M	T	28	28.7	2013	NIL	NO	Abdominal pain, diarrhea
15	F	T	20	19.2	1996	NIL	NO	Abdominal pain, diarrhea, weight loss
16	F	P	67	24.4	2009	HCV	YES	Adnominal pain, diarrhea
17	F	T	28	19.4	2012	Ulcerative colitis	NO	Abdominal pain, diarrhea
18	F	T	26	27.2	2007	NIL	NO	Abdominal pain, weight loss
19	F	T	62	28.7	2012	RZS, Siogrena	NO	Diarrhea, flatulence, weight loss
20	F	T	54	26.0	2012	Polyneuro-pathy	NO	Abdominal pain, flatulence, weight loss

2.2. Gluten Challenge and Enzyme Administration

Patients were subjected to a modest gluten challenge consisting of about one gram of gluten daily. Gluten was given in a form of commercial wheat biscuits (Holland's-wheat, Jarosław) each containing approximately 0.47 gram of gluten. Two biscuits were taken with breakfast each day. The study design was a randomised double-blind type: twenty patients were divided into 2 groups -

one group (14 patients) received caricain enzyme supplement Gluteguard (The Australian Therapeutic Goods Administration Registration AUST L 154 806, Glutagen Pty Ltd, Melbourne, Australia) and the other six patients received placebo. Patients were assigned to the treatment or placebo groups according to computer generated random numbers. Tablets were taken orally with every meal: 2 tablets with breakfast, one with lunch and one with dinner (four tablets per day). Each tablet of Gluteguard contained 18 000 U of caricain, a natural enzyme derived from papaya fruit (Carica papaya). Each placebo tablet contained 300 mg calcium citrate.

2.3. Clinical Evaluation

Every two weeks, clinical evaluation was performed by the doctor supervising the study and symptoms rated using the same scale where: 0 = symptoms absent, 2 = mild, 4 = moderate, 6 = severe. Patients who developed severe symptoms before the six week end of the study, stopped the gluten challenge and but continued taking their tablets until the end of a six week period and completion of the trial.

2.4. Patient Self-Evaluation

Daily, patients recorded the presence and grade of gastrointestinal symptoms in a symptoms diary. Specific symptoms they were asked to grade were fatigue, nausea/vomiting, stomach pain, cramps and stomach bloat but they were also asked to record and grade any other symptoms they experienced. They were asked to grade symptoms on a scale from 0 to six, where 0 = symptoms absent, 2 = mild, 4 = moderate, 6 = severe. Study participants were also required to score their feeling of well-being each day over the course of the study. Well-being was rated as: 0 = ill, 2 = miserable, 4 = unwell, 6 = reasonable, 8 = good, 10 = excellent.

2.5. Histological Methods

Duodenal endoscopy and biopsy were performed prior to the start of the study and at completion (6 weeks). Duodenal biopsy specimens were evaluated according to the Marsh criteria [20] after completion of the trial and blind to intervention. The following histological parameters were examined: Villus height to crypt depth ratio (VH/CR), Intra-epithelial lymphocytosis (IEL), Epithelial stunting (ES), Vacuolation of epithelium (V/E). See footnotes to Table 5 for scoring system.

2.6. Serological Methods

Investigations included recording of the tTG-IgA antibody titres at the start of the trial and at the end six weeks later. The antibody titres were measured using h-tTG ELISA kit [11].

2.7. Statistical Analysis

The means of total symptom points of patients on placebo ($\bar{X}_1$) were compared with the means of patients on enzyme therapy ($\bar{X}_2$) to determine if there was any significance using a 't' test. The standard deviation(s) based on both samples jointly, and sample numbers (n_1 & n_2), together with the above means were used to estimate 't'. The difference between the means of the scores for well-being was also tested in the same way [21].

3. Results

3.1. Clinical Evaluation

The results of clinical evaluations performed on all patients at the start and during the trial 0, 14, 28 and 42 days are summarized in Table 2. After 14 days of gluten challenge, severe symptoms were observed in five patients, four of whom were on placebo. These five patients discontinued gluten challenge at or prior to the 14 day clinical evaluation and continued with the study treatments until completion of the trial. One other patient from the treatment cohort (#2) did not take their gluten challenge on days 16 to 19 and 33 to 42 due to severe symptoms recorded at the 28 day clinical evaluation. All other patients completed the study as per protocol. There was a significant difference in the proportion of patients who discontinued gluten challenge in the placebo group (4/6 = 67%) and the treatment group (1/14 = 7%, $p< 0.01$). Of the 14 patients who completed the study as per protocol, five (35.7%) had no symptoms at the end of the study and all of these were from the treatment group. The other nine patients, which included the two remaining from the placebo cohort, had only mild symptoms at the end of the study. There was no obvious gender bias but there were not enough males to make a conclusion.

For the five patients who withdrew from gluten challenge at day 14, all continued taking tablets as per study design. At the final clinical evaluation at day 42, three (two placebo and one treatment) had no symptoms and the other two had only mild symptoms.

3.2. Patient Self-Evaluations

Evaluations of symptoms and well-being were recorded daily by the patients themselves and the results subjected to statistical analysis. Grades for well-being were also subjected to the same analysis.

Severe symptoms were recorded by five patients, but almost all the patients suffered from mild to moderate symptoms at some stage during the trial. The results are summarized in Table 3 and show that for the treatment group there were no significant changes in mean symptom or well-being scores over the course of the study, despite daily challenge with gluten. For the placebo group, 4 of the 6 subjects withdrew from gluten challenge by day 14 and as such, mean group data collected post day 14 are of little significance. Analysis of data from the first 14 days of the study, a period when nearly all subjects complied with daily gluten challenge, demonstrated a significant difference in both symptoms and well-being between the study groups. Those taking the Gluteguard enzyme supplement were significantly less likely to report symptoms of celiac disease than the placebo group (28 versus 118; $p<0.01$), and also reported a higher feeling of well-being than the placebo subjects (8.4 versus 6.1; $p<0.01$). Individual well-being data from all patients for the first 14 days of the study are shown in Table 4.

Table 2. Results of Clinical Evaluations

			Clinical evaluation				
Patient No	Sex	Study Group	Start	14 day	28 day	42 day	Days without gluten challenge
1	F	Treatment	0	3	3	3	None
2	M	Treatment	0	3	6	3	16-19, 33-42
3	F	Placebo	0	6	6	3	9-42
4	M	Treatment	0	0	0	0	None
5	F	Treatment	0	3	0	3	None
6	F	Treatment	0	3	3	3	None
7	F	Treatment	0	0	3	0	None
8	M	Placebo	0	3	3	3	None
9	F	Placebo	0	6	3	0	14-42
10	M	Treatment	0	6	0	0	12-42
11	F	Treatment	0	0	0	0	None
12	F	Placebo	0	6	3	0	10-42
13	F	Placebo	0	3	3	3	None
14	M	Treatment	0	0	3	3	None
15	F	Treatment	0	0	0	0	None
16	F	Placebo	0	6	3	3	9-42
17	F	Treatment	0	3	3	3	None
18	F	Treatment	0	3	3	3	None
19	F	Treatment	0	0	3	3	None
20	F	Treatment	0	0	3	0	None

Scale: 0 - symptoms and/or signs absent, 3 - mild, 6 - severe**.**

Footnotes:

- In placebo group average symptoms score was 5.0 over the 3 evaluations on trial; at the end four patients withdrew.
- In treatment group at 14 days average symptoms score was 1.7; at the end one patient withdrew.
- the differences in symptom scores were still obvious after 28 days but were much less after 42 days over the 3 evaluations on trial;
- There was no obvious gender bias but there were not enough males to make a conclusion.

Table 3. Symptom points and well-being of gluten challenged patients on Placebo or Treatment

Period	Intervention	Mean symptom points	Mean well-being
1-14 days	Placebo Treatment	118* 28*	6.1** 8.4**
15-28 days	Placebo Treatment	137 29	4.8 8.3
29-42 days	Placebo Treatment	124 (115) 29 (31)	5.4 (5.4) 8.1 (8.0)

- Footnotes:
- Figures in brackets are after eliminating patients who withdrew from trial due to severity of symptoms (4 on placebo, 1 treatment)
- * means are significantly different ($p<0.01$)
- ** means are significantly different ($p<0.01$).

Table 4. Assessment of well-being (14 days)) of patients on placebo compared with those on treatment

Placebo n=6

Patient No	Total well-being score	Score/day	Comments
3	94	6.7	gluten withdrawn
8	122	8.7	
9	78	5.6	gluten withdrawn
12	96	6.9	gluten withdrawn
13	88	6.3	
16	38	2.7	gluten withdrawn
means		6.1*	
Treatment n = 14			
1	136	9.7	
2	110	7.9	
4	122	8.7	
5	112	8.0	
6	108	7.7	
7	138	9.9	
10	120	8.6	gluten withdrawn
11	126	9.0	
14	120	8.6	
15	140	10.0	
17	94	6.7	
18	87	6.2	
19	140	10.0	
20	86	6.1	
means		8.4*	

*These means are significantly different ($p =< 0.01$).

3.3. Other Symptoms

Other symptoms that patients reported were diarrhea (5 patients = 25%) and headache (4 patients = 20%). There were isolated reports of gastric reflux, dizziness, skin inflammation (potential dermatitis herpetiformis), joint pain and flatulence. With regards to diarrhea, three patients on the placebo suffered from the condition (one patient had traces of blood in the stool for 17 days) compared with two patients on treatment. Statistical analysis was carried out on the results based on the number of patients and days on which diarrhea was recorded by placebo and treatment groups. The results were suggestive of beneficial effect of enzyme therapy but the difference was not statistically significant. Headache was confined to two patients on placebo and two on treatment.

3.4. Histopathology

The results of histopathology are summarized on Table 5. The most useful parameter appeared to be villus height compared with crypt ratio (VH/CR) and is the most informative in regards to actual tissue damage. For scores refer to section 2.5. (Histological Methods).

Regarding the VH/CR ratio, it was seen that six patients showed significant damage (ratio 1:2, score 3) and (ratio

1:4, score 4) at start of the trial. Eleven patients showed no damage or slight damage to tissue at start of the trial. Nine patients showed change in VH/CR over course of the trial. Five on treatment improved, two on treatment became worse and two on placebo became worse. Six patients had no damage at end of trial; all of these were on treatment with Gluteguard. The ratio of changes between treatment and placebo groups were not statistically significant. The majority of patients needed to have a VH/CR score of zero or 1 at the start of the trial in order for detection of significant histological changes caused by gluten challenge. Furthermore, prevention of these changes by Gluteguard is also dependent upon having close to normal villous architecture at the start of investigation. Instead, as it eventuated, we had 30% with significant damage (VH/CR 3 or 4) and only 55% with scores of 0-1. It was noted that 6 patients (Nos 1, 6, 10, 18, 19, 20) when challenged were able to maintain or improve their low VH/CR score on Gluteguard (6/15 = 40%). Only one patient (No. 17) was the reverse of this (score 0 to 1). The other two patients on placebo showed either moderate damage at the start (No. 12) or became moderate (No. 13).

Table 5. Results of histopathology

Patients	Endo-scopy day	Study Group	VH/CR[1]	IEL[2]	ES[3]	LPI[4]	V/E[5]
1	1	Treatment	0	0	3	2	1
	42		0	0	3	2	1
2	1	Treatment	3	3	2	2	2
	42		3	3	2	1	2
3	1	Placebo	1	2	2	2	2
	42		2	2	2	2	2
4	1	Treatment	4	3	0	3	1
	42		4	3	0	3	1
5	1	Treatment	4	3	0	2	1
	42		4	3	1	2	1
6	1	Treatment	0	0	3	1	2
	42		0	0	3	1	2
7	1	Treatment	4	3	0	2	1
	42		4	3	1	2	0
8	1	Placebo	2	2	2	2	1
	42		2	2	2	2	1
9	1	Placebo	1	1	3	1	2
	42		1	2	3	1	2
10	1	Treatment	1	1	2	1	1
	42		0	1	2	1	1
11	1	Treatment	4	3	1	2	1
	42		3	3	2	1	1
12	1	Placebo	2	2	2	1	1
	42		1	3	2	2	1
13	1	Placebo	1	1	2	1	2
	42		2	1	2	1	2
14	1	Treatment	4	3	1	2	0
	42		2	2	2	1	1
15	1	Treatment	2	3	1	2	1
	42		3	3	2	2	1
16	1	Placebo	1	2	1	2	1
	42		0	2	2	1	2
17	1	Treatment	0	1	1	1	2
	42		1	1	2	1	2
18	1	Treatment	0	1	2	2	2
	42		0	1	2	2	2
19	1	Treatment	0	1	2	2	2
	42		0	1	2	2	2
20	1	Treatment	0	0	3	1	2
	42		0	0	3	1	2

1. VH/CR = Villus height to crypt depth ratio. Scores were allocated according to the ratio determined as follows: ratio 5:1-3:1, scored 0; ratio 2:1, scored 1; ratio 1:1-1:2, scored 2; ratio 1:2, scored 3; and ratio 1:4, scored 4.
2. IEL = Intra-epithelial lymphocytosis. Scores were reported on a scale of 0-3, where normal = 0; mild = 1; moderate = 2; severe = 3.
3. ES = Epithelial stunting. Scores were reported on a scale of 0-3 where zero = 0; mild = 1, moderate = 2; severe = 3.
4. LPI = Lamina propria lymphoplasmocytic infiltrate. Excess infiltrate was scored as normal = 0; mild = 1; moderate = 2; severe = 3.
5. V/E = Vacuolation of epithelium. Scores used were zero = 0; mild = 1; severe = 2.

3.5. Other Histological Parameters

Scores for IEL and LPL were also of interest as indicators of potential tissue damage but did not seem to be of such importance as the VH/CR scores. Briefly, IEL scores were between 0 and 3 (nil to severe) with all three of the changes being as expected. The other 17 patients showed no change. LPL scores changed in five patients of whom four showed some improvement on treatment together with one patient (No 16) who was on the placebo but withdrew from gluten challenge. Epithelial stunting (ES) showed that only mild changes occurred in six

patients and none of these improved. Vacuolation of epithelium (VE) showed changes in only three patients. For the five histological parameters, scores equivalent to "severe" were recorded in 17 patients, 13 of which were in patients at the start of the trial. IEL, LPI, ES and VE scores did show a trend but no significant differences between the groups.

3.6. Summary of Histology (VH/CR) Relative to Time on Gluten-free Diet

In summary, IEL, LPS, ES and VE scores each show the trend but there were no statistically significant differences between the two groups.

3.7. Serology

High levels of tTG-IgA antibodies (>30 U/ml) were present in seven patients at the start of the trial indicating that these patients were not adhering to a strict gluten-free diet, however in three of these patients (two on treatment), levels of antibodies were lower after six weeks. Nine patients had moderate titres (>20) and ten patients had low titres (5-20). One patient had recognized total IgA deficiency (patient 12). Extremely high levels of tTG-IgA antibodies (400 U/ml) were present in 1 patient at start and end of trial. Based on differences between the titres at 6 weeks and at the start of the trial, Table 6 shows that there is a trend for the tTG-IgA titers to decrease on therapy with a decrease in eight out of ten patients on therapy compared with two out of four patients on placebo.

Table 6. Changes in titers of tTG- IgA antibodies from start to the end of trial

Patient No	Placebo or Treatment	tTG-IgA - Start U/ml (N<50)	tTG-IgA - End U/ml (N<50)2
1	T	14.9	10.6
2	T	267.9	282.1
3	P	130.4	150.1
4	T	400	400
5	T	18.6	46.3
6	T	22.2	8.4
7	T	386.5	150.4*
8	P	167.9	83 *
9	P	12.1	8
10	T	25.5	7.1
11	T	10.7	21.9
12	P	deficiency IgA: IgA: 1,3; IgG: 8.7	deficiency IgA: IgA-4,4; IgG: 6.0
13	P	137.5	152
14	T	8.2	9,5
15	T	161	86.4 *
16	P	5.5	8.1
17	T	5.7	8.5
18	T	5.1	3
19	T	9.8	5.4
20	T	11.7	0.9

Footnote: *Patients showing notable reduction in high titer.

The placebo group showed a mean reduction in titer 2.8% against 7.9% for the treatment group, but these figures were calculated from the raw data and may not be truly representative because of their geometric nature.

3.8. Relationship between Serology and Histology at Start of Trial.

Several patients had severe mucosal damage at the start of the trial (No's 2, 4, 5, 7, 11 and 14). These patients did not always correspond to those with the highest titres of tTG-IgA antibodies. Sometimes patients with mild damage gave high titres (patients No 3 and 13).

4. Discussion

Results of this clinical study are consistent with that Gluteguard, when used as an oral enzyme supplement, is able to digest significant amounts of ingested gluten in the gut such that gluten's toxicity in individuals with coeliac disease is greatly reduced or even abrogated. Our findings demonstrated that 13 of 14 celiac patients taking Gluteguard tablets showed no detrimental changes in clinical symptoms or feeling of well-being when challenged daily for 6 weeks with 1g of gluten. In contrast, four of six subjects taking placebo developed severe symptoms within the first two weeks of the study and had to withdraw from further gluten challenge. Similarly, previous studies [22] have found that celiac patients when challenged even with very small quantities of gluten (1 mg per day) can develop symptoms and mucosal damage. That Gluteguard was able to significantly protect celiac patients from gluten challenge was anticipated as our previous clinical trial with caricain in an enterically-coated tablet [14] indicated that this approach was successful for patients with dermatitis herpetiformis, where gluten is also the causative agent. Furthermore the recent work of Buddrick et al [15] demonstrated that caricain significantly reduced the amount of immunoactive gliadin in whole-meal bread by allowing the enzyme to react in the dough during proofing.

It is clear that the primary treatment for celiac disease is the strict maintenance of a gluten free diet [1]. However, it is also clear that many celiac patients do from time to time ingest gluten containing foods (probably unknowingly),

and can often have significant symptoms develop as a result. Data from the present study support these observations as high levels of tTG-IgA antibodies (>30 U/ml) were present in seven patients at the start of the trial indicating that these patients were not adhering to a strict gluten-free diet. As such, the value of anti-gluten enzyme therapy may lie in helping coeliac patients cope with the difficulties of maintaining a very strict gluten-free diet, especially in situations where food preparation is out of their control. Moreover, this would also likely apply to those who have other forms of gluten intolerance such as dermatitis herpetiformis [14], a variant of celiac disease.

Damage to the small bowel is a key pathological symptom of celiac disease. Long-term strict maintenance of a gluten free diet has been shown to resolve such damage, but such repair is very time dependent. [8,10] This was evidenced by primary data from the subjects in the present study where at the start of the study, highest VH/CR scores (3 or 4), indicative of mucosal damage, were generally seen in patients who had been on a gluten-free diet for the shortest time (Patients 2, 4, 7, 11, 14). Conversely, the lowest VH/CR scores at start of trial (0 or 1) tended to be those patients who had been maintaining a gluten-free diet for the longest time (Patients 1, 3, 6, 9, 10, 13, 16, 17, 18, 19 and 20). This suggests that the more stringent a celiac patient can be in completely removing gluten from their diet, the more quickly they may resolve mucosal damage. We speculate that use of an anti-gluten enzyme supplement in situations where small amounts of gluten may possibly be ingested may assist in the speed of mucosal recovery. Of interest from our study was that despite daily challenge with gluten for 6 weeks, the mean VH/CR score for the per protocol treatment subjects at the start time 0 was 1.83, not significantly different to that at the end of the study, 1.75. Whilst this finding in itself does not demonstrate that enhanced mucosal recovery is achievable by use of anti-gluten enzyme therapy, it does suggest that further studies to directly investigate this possibility are warranted.

This study had some limitations. Ideally, patients should have had a VH/CR score of zero or 1 at the start of the trial to enable detection of significant histological changes caused by gluten challenge. However, 30% of subjects began the trial with significant damage (VH/CR 3 or 4) and only 55% had scores of 0-1. With the elevated measures at baseline, there was less opportunity to record such changes. Additionally, four of the six placebo patients had to withdraw from gluten challenge after only two weeks of the study due to development of serious clinical symptoms. This meant that direct comparisons between treatment and placebo groups could only be conducted using symptom data collected during the first two weeks of the study.

But Gluteguard along with their daily gluten challenge, suggesting that the enzyme tablets may also help with the recovery of epithelium in the longer term helping to reduce serious health risks for coeliac patients [23].

5. Conclusions

This study demonstrated that oral anti-gluten enzyme therapy using Gluteguard was able to significantly protect celiac patients from adverse symptoms being induced by gluten challenge (one gram per day). Furthermore, mucosal damage was not exacerbated in patients taking Availability of a preventative enzyme treatment like Gluteguard will likely add to the quality of life and well-being of coeliac, especially those who have difficulty in strictly adhering to a gluten-free diet.

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