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Enzyme Therapy for Patients with Celiac Disease - An Update

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Abstract Enzyme therapy as a management tool for patients with celiac disease (CD) or gluten intolerance is gaining acceptance around the world. Gluten has multiple side effects and limitations of a gluten-free diet (GFD) in management of CD are evident and mainly related to the presence of hidden gluten or cross-contamination of meals in restaurants. The present paper discusses enzyme therapy with caricain, namely Gluteguard, in light of various approaches searching for a treatment or an effective management of CD. Regular users of the supplement Gluteguard report a high level of satisfaction with the product. This indicates that enzyme therapy based on caricain has its place as a safeguard when the gluten-free meals are prepared outside the control of the patients.

Keywords: *celiac disease, enzyme therapy, caricain, gluten-free diet, gluten intolerance, dermatitis herpetiformis*

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1. Introduction

Celiac disease (CD) is a form of gluten intolerance in which the small bowel is damaged by proteins present in wheat, rye, barley and some varieties of oats. These proteins cause severe damage to the duodenum and jejunum and can produce a variety of symptoms including abdominal pain and cramps, bloating, diarrhea and nausea and numerous extra-intestinal manifestations [1,2].

If left untreated, severe malabsorption can result in the loss of vital nutrients causing conditions such as osteoporosis and anaemia. The long-term gluten exposure can also lead to increased risk of neurological complications, increased incidence of non-celiac autoimmune diseases and malignancies [3,4,5]. The only treatment for the condition has traditionally been a gluten-free diet (GFD), which needs to be maintained for the rest of life.

However, it has become apparent that a GFD is almost impossible to maintain due to the ubiquity of gluten in the foods, gluten contamination of seemingly gluten free products and many other factors summarized recently [6]. Obviously, there is a great unmet need for a better solution than just a GFD alone. In the last twenty years different ways have been studied to find an effective alternative to GFD [7]. These studies investigated autoimmune reactions [8], gut permeability [9], influence of microbial biota [10], intestinal parasites [11] and even a CD vaccine [12]. Alhassan et al [13] reviews more non-dietary therapies for CD and makes the point that a GFD alone is not sufficient to control symptoms and prevent mucosal damage from unintentional gluten exposure. Other types of interventions such as TG2 inhibitors, HLA DQ2 blockers and cathepsin S inhibitors were also discussed.

None of these studies produced an alternative to the GDF or a likely protection against gluten contamination. So far only the enzyme therapy approach provides a practical solution to the problem of gluten contamination of GFD.

1.1. Enzyme Therapy in a Nut Shell

CD is a gluten dependent enteropathy with a strong genetic influence. It is a multi-genetic disorder associated mainly with major histocompatibility class II HLA DQA and DQB genes and multiple non-HLA genes [14]. The association of CD with gluten ingestion led to an interest in investigation of gluten digestion. The possibility of an enzyme deficiency in CD patients was first suggested by Frazer et al. in 1959 in his work with pre-digestion of gluten with hog mucosa [15]. Those pre-digestion studies have led to the development of the enzyme therapy concept in general and the Gluteguard supplement, in particular.

Further work of Cornell et al led to the discovery of undigested gluten peptides in intestinal mucosa of celiac patients [16,17] confirming the existence of mucosal enzyme deficiency in CD. The inability to fully digest gluten was identified as a principal event leading to the development of clinical CD and spearheaded the development of the current enzyme therapy.

An alternative theory of CD etiology was proposed by Falchuk and Strober [18] and Shuppan [19], who

suggested that immunological responses to gluten peptides are the exclusive cause of CD. This theory in preference to the enzyme deficiency has gained acceptance by a majority of celiac researchers around the world.

However, immunological theory alone has one key flaw. It posits that immunologically active gluten peptides such as 26 or 33-mer somehow permeate through intestinal mucosa and get access to the lymphatic system of the *lamina propria* in only a small proportion of the genetically predisposed individuals. This event initiates development of CD in these individuals but not in the others who are similarly predisposed. The mystery of how large peptides can traverse the mucosal barrier in some HLA DQ2 /DQ8 allele's carriers but not in the others is not explained by the standard immunological theory. Why does it happen in only some cases? The possibility that the changes in permeability of cellular tight junctions may allow small peptides up to 1 kD to pass was demonstrated but there is no evidence that a gluten peptide large enough to elicit immune response can penetrate the mucosal barrier [20]. Moreover, a clinical trial aiming at modulation of permeability of tight junctions in celiac patients by inhibiting the action of zonulin proved clinically disappointing [21].

Furthermore, there is the assumption that mammalian endopeptidases, in general, are not able to digest gluten peptides rich in proline and glutamine residues [22]. This is not accurate. It was shown that mucosal extracts from cow, sheep and pig can efficiently digest gluten into its basic constituents - amino acids and peptides [23]. We are not aware of any manifestation of equivalent form of human CD in farm animals. Undoubtedly, selection of animals for performance quickly eliminates non-thriving individuals from their respective genetic pools. Transient gluten sensitivity was observed in foetal chick [24] and foetal rat [25] and it may be expected to exist in other species. However, once the intestinal system matures, the full complement of enzymes necessary for digestion of gluten becomes operational and the gluten sensitivity disappears. It seems that the acquisition of the HLA DQ 2 / DQ8 alleles is unique to the human genetic pool and a small proportion of these individuals suffer from some form of enzyme deficiency enabling the development of CD. HLA DQ2/DQ8 heterodimers contribute almost 40% of the disease heritability; the remaining 60% is estimated to be shared between an unknown number of non-HLA genes [26]. Some of these non-HLA genes may well be responsible for the observed enzyme deficiency in the affected subset of HLA DQ2/DQ8 individuals.

The vast majority of the general population has no problems with digesting gluten and only about 1% develops CD [14,27,28]. Presence of HLA DQ2/DQ8 allele is necessary but not sufficient for the development of the disease. Approximately30% -40% of Western populations carry these genetic markers but only a small proportion of carriers develop CD [27,28]. Intriguingly, those HLA DQ2 / DQ8 carriers who develop CD exhibit enzyme deficiency and are unable to produce sufficient quantity of brush border endopeptidases to complete gluten digestion [16,17]. We postulate that in such individuals gluten is digested to a level of large peptides, and further digestion to the level of amino acids and di-peptides is deficient or stopped altogether. At this stage there is the accumulation of toxic peptides in sufficient quantity to damage enterocytes and induce mucosal lesions. Those peptides with direct toxicity act by disruption of cellular and cell organelle's membranes. This type of toxicity is best described as direct toxicity because of its directly harmful effects on organelles such as rat liver lysosomes [29] and on enterocytes of the foetal chick intestine [24]. Notably, Riecken et al [30] showed that gluten exposure induces damage to the lysosomal membranes of celiac patients with consequent autolysis of enterocytes and loss of mucosal architecture. Removal of gluten from the diet restored the integrity of mucosa and the lysosome numbers. There is evidence that gluten possesses inflammatory activity independent of classical T-cells and probably acts by damaging enterocytes directly [31]. More so, gluten has other significant side effects. It influences the microbiome, it is pro-oxidative, pro-apoptotic, affects epigenetics, decreases cell variability and differentiation and through inflammatory injury enhances gut permeability [5]. Thus, opening of the gut/blood barrier through lesions allows immunogenic peptides to interact with the *lamina propria* lymphatic tissue and to initiate the cascade of immunological responses resulting in more inflammation and further destruction of enteric mucosa from within.

It is concluded that both theories- the enzyme deficiency and the immunological one - are not mutually exclusive but present the two different phases of the disease development as the unified hypothesis of CD suggests [32]. The existence of gliadin peptides differing in immunogenicity and toxicity attest to their relative significance for the different stages of the disease development [33].

Consistent with the theory implicating direct toxicity is the experience of the Nexvax2 vaccine project. The standard immunological theory of CD postulates that dampening immunological responses to gluten peptides should arrest the damage to the intestinal mucosa and return celiac patients to health. Alas, this was not confirmed in practice. The vaccine was successful in producing antibodies against the gluten peptides but did not improve patient's ability to digest gluten or to make any difference to the intestinal damage [34].

1.2. Non-celiac Gluten Sensitivity (NCGS)

For some time, there have been significant numbers of individuals who claim that their well-being is adversely affected by gluten. A clue to their status is that they are often related to those with diagnosed CD. Siblings of celiac patients in a family commonly say that they feel better when their gluten is restricted because of gluten-free food being purchased of necessity for the celiac patients in that family. Cornell & Rolles [35] showed many years ago that many first-degree relatives of celiac patients have a partial enzyme deficiency, but were not diagnosed with CD. They showed that relatives of diagnosed celiac patients revealed various degrees of difficulty in digesting gluten, even without a full spectrum of CD symptoms. This finding was supported more recently showing that

between 5% [36] and 10% [37] of CD patient's relatives exhibit various grades of the disease symptoms possibly depending on homo or heterologous arrangement of inherited HLA DQ alleles.

Uhde et al [38] revealed a state of systemic immune activation in conjunction with a compromised intestinal epithelium, but in whom the epithelium was not damaged sufficiently for a diagnosis of CD to be made. It would appear that the incidence of this condition is far greater than for diagnosed CD. A partial enzyme deficiency in the small intestine may well be the reason, as previously reported [35]. Again, use of an enzyme supplement is helpful in this condition, as indicated by feedback from users of Gluteguard.

1.3. The Gluten-free Diet in Celiac Disease

The GFD remains the only effective treatment to date for CD, but it faces widespread difficulty, contamination with hidden gluten. It is often assumed that the food in question is gluten-free when it is not [6,39,40]. Common hidden sources of gluten are medications, processed meats, sauces and food contaminated by wheat-containing cereals. About half of Australian celiac patients [41] fail to heal their bowel and suffer persistent symptoms even after many years on a GFD. Lack of healing of small bowel mucosa in celiac patients of GDF is well documented [42,43] leading to increased risk of complications [44,45] and mortality [46].

The present-day problem with GFD exists for two reasons. Firstly, the international standard for gluten-free foods allows 20 ppm of gluten to be present. A number of celiac patients exhibit extreme sensitivity to gluten so even that level of gluten is detrimental [47]. It has been reported that even as little as one mg per day of gluten is sufficient to prevent mucosal recovery [48]. Secondly, gluten-free foods often exceed the recommended level of gluten and the lax restaurant food preparation standards allow for gluten contamination [40,49,50,51]. Studies conducted in various countries indicate that a large proportion of celiac patients maintaining GFD still suffer from intestinal lesions and unpleasant symptoms.

Even where there appears to be a lack of severe symptoms to an accidental minor gluten intake, the small bowel may still be damaged. This is clear from volunteers in our clinical trials who felt they were doing their best to maintain a strict gluten-free diet but in whom small bowel histology was abnormal at the start of the trial [52,53].

People with CD, without the use of an enzyme supplement, run the risk of other more serious health problems, because of constant damage being done to their small bowel [45,46]. There are reports that up to 50% of patients with CD are exposed to hidden gluten and a similar proportion harbour mucosal damage where unrecognised gluten consumption is the most common identified cause of non-responsive CD [43,54].

1.4. Toxic Peptides of Gluten

In 1988, De Ritis et al. identified the motifs PSQQ and QQQP in peptides such as A-gliadin 5-20 as being associated with toxicity [55]. These serine - containing peptides with their direct toxic action, are different from the tyrosine - containing peptides such as A-gliadin 75-86, which are immunogenic and contain a tyrosine motif - PYPQ. The presence of key motifs in undigested residues from remission celiac mucosal digestion, which are in greater amounts than from normal mucosal digestion, is in keeping with our previous findings [16,17] and indicates the need for an enzyme supplement that could compensate for this deficiency.

Our other studies [56,57,58] have indicated that the most suitable detoxifying enzyme is caricain, an enzyme derived from papaya. (International Classification E.C. 3.4.22.30). It is ideal for attacking both the 12-19 and the 77-84 undigested A- gliadin peptides at vital points so that these amino acid motifs are dismantled and detoxification ensues.

1.5. Clinical Trials of Gluteguard

Discovery of undigested peptides in celiac patient's mucosa [16,17] was followed by analysis of these peptides and finding that some of them were toxic to the enterocytes. Research was conducted to identify suitable enzymes which could be used to break down those peptides and complete the digestion of gluten. Further work led to development of formulations suitable for testing in clinical trials, the first containing pig prolidase and the two others the plant enzyme caricain.

In all, three randomised double blind clinical trials exploring the enzyme therapy concept for treatment of CD were led by Prof. Finlay Macrae of the Royal Melbourne Hospital. The world first clinical trial of an enzyme supplement for CD which employed an enterically coated gelatine capsule containing pig intestinal prolidase extract was published in Scandinavian Journal of Gastroenterology in 2005 [52]. The extract was administered orally to adult CD patients in remission. Despite daily challenge with one gram of gluten, an amelioration of symptoms was achieved. The results indicated a good protective value of enzyme treatment against gluten challenge.

Unfortunately, with the advent of mad cow disease (bovine spongiform encephalitis) in 2005, the use of animal enzymes for human treatment faced severe regulatory restrictions. As a consequence, we embarked on a search for an equivalent enzyme of plant origin and identified caricain as a suitable substitute for pig prolidase [57]. Clinical trials in patients with Dermatitis Herpetiformis (DH) and CD followed, using an improved formulation of an enterically coated tablet (Gluteguard) based on the enzyme caricain. Those trials were conducted to test the protective value of this enzyme against the gluten challenge. DH is a skin condition seen in CD which is also triggered by sensitivity to gluten. Like CD, a strict lifelong GFD is essential for managing DH thus, preventing skin inflammation and other complications. Results of both trials were published in the International Journal of Celiac Disease [53,59].

Briefly, the DH trial was carried out first because the symptoms are presented as erythema or rash of the skin and blisters, the area of which can be readily measured. In the DH trial, 20 patients were challenged with six grams of gluten daily for seven days. Read blind to the

intervention (Gluteguard vs placebo), Gluteguard offered a significant 81% protection by reducing the area of skin lesions from 19.5 $cm^2$to 3.7cm^2, (placebo against treatment, p = 0.02), a substantial 71% reduction in the appearance of skin lesions (24 lesions against 7), and a 38% reduction in emergence of troublesome itch (40 against 25 episodes). Of seven DH patients who withdrew from the study due to severe gluten challenge-related symptoms, six were taking placebo.

In the CD trial, 20 volunteers were challenged with one gram of gluten for 42 days with 14 of them taking Gluteguard and six taking a placebo tablet. Patients recorded symptoms and wellbeing daily and small intestinal tissue was examined before and after the study. Thirteen of 14 CD patients (93%) on Gluteguard showed no detrimental changes in clinical symptoms, biopsy results or well-being throughout the 42 days of challenge. Conversely, four of the six taking placebo developed severe CD symptoms and withdrew from further gluten challenge after 14 days. After this period those taking Gluteguard reported milder CD symptoms and averaged higher well-being scores. Tissue damage was less in the Gluteguard group. Even in a small trial such as this, the probability that the results were due to chance was only p <0.01.

2. Value of a Dietary Supplement to the GFD

Lerner [7,60] considered Gluteguard potential as preventive therapy adding to the quality of life, especially those who have difficulty adhering to a strict GFD diet through no fault of their own. He stated: “Degrading of the gliadin (toxic/ immunogenic peptides) is one of the most effective strategies to help cope with small amount of gluten in the diet. The present strategy seems logical since it treats (detoxifies) gluten before it can induce damage to the intestine”.

Macrae [41] suggested that we can avert the immune response by enzyme therapy. He commented: “Many patients find difficulty in following a strict GFD. Dining out, hidden sources of gluten are wheat, barley, rye and some varieties of oats. DH trial: for such a modest trial the results were remarkable. CD trial: attenuation of mucosal injury”. He concluded: “Gluteguard is a useful adjunct to GFD for CD and DH patients. It detoxifies gluten before it is able to induce intestinal damage and stimulation of the immune system. This enzyme therapy will not cure CD or DH but it can help in digesting hidden dietary gluten, which on the existing trial evidence, limits mucosal damage and makes life easier for many patients with celiac or DH”.

In addition, Tanner et al. carried out independent tests at the University Melbourne [61]. He evaluated nine commercial supplements including Gluteguard for their ability to digest gliadin. Gluteguard was superior to eight other commercial preparations for its ability to digest gliadin. All preparations showed that they were capable of digesting gliadin at various rates. However, the results indicated that Gluteguard digested specific gluten epitopes associated with symptoms and intestinal pathology of CD faster than any other preparation tested. It showed cleavage of glutamine to proline residues on C-terminal and N-terminal sides, enough to detoxify peptides inducing CD. None of the 33-mer epitopes (PFPQPQLPY, PYPQPQLPY and PQPQLPYPQ) was detected in samples after digestion by Gluteguard. The speed of digestion is critical in interactions of the toxic residues of gluten with the intestinal mucosa.

3. Other Possible Uses of Gluteguard

Although CD is identified mainly through gastrointestinal symptoms and histology, extra intestinal manifestations also occur [2] i.e. in skin disorders of DH patients. Neurological symptoms related to gluten exposure have also been reported [4,62]. Some of these symptoms may be improved by a GFD. Neuropsychiatric complications of CD such as schizophrenia, depression and anxiety are discussed by Arnone and Conti [63]. There is mounting evidence supporting a role for a GFD in reducing neurological and psychiatric complications [64,65]. Interestingly, as reported recently, several non-celiac autoimmune diseases might also ameliorate on GFD [5,66,67].

It follows that there may be a role for enzyme therapy in management of people who exhibit symptoms that indicate extra-intestinal manifestations of CD and selected non-CD autoimmune diseases. More so, adolescence, stunted growth, osteoporosis, nutritional deficiencies and malignancies can be prevented by adhering to GFD supported by enzymatic supplemental therapy.

Without the use of an enzyme supplement like Gluteguard people with CD, DH, non-celiac gluten sensitivity and gluten ataxia run the risk of developing more serious problems because they have no safeguard and protection against the hidden gluten [68].

4. Enzyme Supplements for CD

There are two enzyme therapies comparable to Gluteguard in the depth of scientific evaluation. Latiglutenase (formerly ALV 003) is a combination of two genetically engineered enzymes derived from a bacterium and barley plant. While being a very impressive achievement in genetic engineering, Latiglutenase failed in clinical trials as a possible treatment for CD [69]. Further work on this supplement is in progress. The second development, AN-PEP, is a manufactured enzyme derived from the fungus *Aspergillus niger*. It was originally developed for clarification of beer but it also showed promise for digestion of gluten. It appears that clinical evaluation of this enzyme for treatment of CD failed to show any benefit for celiac patients in comparison with placebo [70]. AN-PEP enzyme is now being marketed as a GliadinX, a gluten digestion aid for non-celiac applications. GliadinX can degrade gluten in the stomach, although researchers caution the enzyme is not intended to treat or prevent CD.

Gluteguard is the only registered product with assignation as a dietary supplement for people with medically diagnosed gluten sensitivity. Apart from Gluteguard, no other enzyme supplement for gluten digestion has gained official approval of a major

regulatory body such as the Australian Therapeutic Goods Administration (TGA) for this purpose. Gluteguard is a natural product, which is well tolerated and allows patients to enjoy food without concern for their well-being.

5. Summary of Users' Responses to Gluteguard

Even at this early stage of the marketing of Gluteguard, it is obvious that the vast majority of users are satisfied that Gluteguard has value as a supplement to the GFD. Gluteguard provides peace of mind to the users replacing the minefield they have to traverse every day, trying to avoid gluten exposure. Theme 2020 Gluteguard Facebook survey [71] concluded that: out of the 292 survey responders who were Gluteguard users, 98.5% found it helpful, particularly in providing peace of mind against the risk of hidden gluten in meals caused by cross-contamination. About 83% claimed they were following a strict GFD when using Gluteguard. Mostly it was of value when eating out at restaurants (74.4%).

6. Conclusions

Enzyme Therapy is becoming established as a means of protecting people with CD on a GFD from hidden gluten. Two clinical trials, one on patients with CD, the other on patients with DH, where the causative agent is also gluten, have indicated that an enterically coated tablet of caricain protects individuals with either disease from the ill effects of a gluten challenge. Customer's feedback indicates that Gluteguard has shown to be well tolerated, having the advantage that it is based on a natural product. The easy to swallow tablet is enterically coated so as to deliver it intact to the small intestine where it is able to detoxify any remaining gluten. It relieves patients of the worry about whether the food is completely free from gluten and other harmful cereal proteins and reduces the risk of intestinal damage caused by unintentional gluten exposure.

The important point is that small, permissible amounts of gluten in the GFD together with incidental contamination will not allow full recovery of the small bowel mucosa in very sensitive patients. The use of Gluteguard together with the GFD gives a better chance of this happening because it targets the toxic residues of gluten. Gluteguard ensures a selective digestion of those peptides which is not achievable by the majority of other commercial products.

Conflict of Interest

HJC is a scientific consultant to Glutagen Pty Ltd. TS is a former Executive Director of Glutagen Pty Ltd and a shareholder in the company. AL does not have any conflict of interest.

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