Difficulties encountered when treating coeliac disease: from molecule to miracle cure?

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Abstract

Dietary treatment of coeliac disease should be based on a sound theoretical knowledge of the different immunogenicities of various cereal grains, an appreciation of the limitations of the current Codex Alimentarius recommendations and an understanding of the factors that limit dietary compliance in many patients. The expertise of dieticians, nutritional chemists, gastroenterologists and clinical pharmacists should be made readily available to coeliac patients, to coeliac societies and to coeliac self-help groups. Various enzymatic and pharmacological modalities that may be used to treat coeliac disease in the future are highlighted as potential ways to improve quality of life in these patients in whom the coeliac diet often promotes poor compliance or may lead to significant social alienation.

Key words

Coeliac disease, tissue transglutaminase, gluten, villous atrophy

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Know your grains; know your glutens

The seeds of wild grasses eaten by nomadic primitive hunter-gatherers were sown and harvested by primitive urban settlers to produce today's cereal grains. Milling and grinding allowed brewing and baking by releasing starches in the inner "endosperm" from the husk and outer coatings. Wheat endosperm, which is the food store for the embryo in the wheat grain contains oils, carbohydrates and proteins. Cereal proteins include albumin, globulin, alcohol-insoluble glutens and the alcohol-soluble prolamins which may trigger coeliac disease. Glutens are complex polymers with molecular weights of several million that give bread dough the viscoelastic properties that favour baking. The prolamines implicated in coeliac disease (CD) contain a high content of glutamine: namely gliadin in wheat, hordeins in barley and secalins in rye. Oat prolamines called avenins are more distantly related to wheat. The mechanisms by which these prolamines cause immune damage in coeliac patients have been outlined in a previous issue of this Journal.

Treating the patient: where do we start?

A gluten-free diet remains the cornerstone of treatment but presents several theoretical and practical difficulties. Wheat, rye, and barley must be excluded as their prolamines (30-50%) contain many immunogenic peptides. Corn and rice are not immunogenic. Oats are traditionally allowed as their prolamins (5-15% by weight) contain less glutamine and are of low immunogenicity except in occasional individuals. Intolerance to oats is often due to contamination with other prolamins during processing in mills used to grind wheat.

How low should we go?

The minimum amount of gluten allowed in the diet continues to be debated. In 1980 the Codex Alimentarius commission (FAOUN/WHO)[1] stated that food labeled "gluten-free" should contain less that 0.03% of total protein derived from wheat, barely, rye or oats. A stringent limit of 20mg gluten per kg of food rendered gluten-free during processing is being considered. CD patients ingesting less that 1g gluten per day in one study and less that 2.5-5.0g gluten per day in another study showed increased numbers of Intra epithelial lymphocytes (IELs) in their duodenal biopsies but their villous architecture was normal. Collin et al[2] showed that since flour intake rarely exceeds 300g per day in coeliac patients, a limit

of 100mg/kg food (30mg gluten intake) is compatible with normal duodenal histology, is easily achieved by industry, and being less stringent should encourage longer and better compliance. The Codex recommendations are not immune from bias arising from a producer lobby and should be based on more rigorous studies of the clinical effects of various long-term gluten intakes. Enforcement of a Codex standard by a food or medicines regulatory authority is limited by the "sandwich ELISA" method used by Food Standard Laboratories to verity the manufacturer's gluten-free labelling. This assay allows for different gliadins present in gluten and for their susceptibility to heat during manufacture but may not identify many of the toxic gliadin epitopes present inside gliadin fragments.[3]

Administrating the coeliac diet: how do we go about it?

Newly diagnosed coeliac patients require a nutritional assessment before referral to a state-registered dietician for dietary advice and long-term supervision. Children may show poor linear growth while adults may show suboptimal body mass index or irreversible short stature at presentation. Folate and iron deficiency may require specific replacement. The prescribed "gluten-free diet" (GFD) may be low in folate derived from cereals and in energy. Up to 50% of newly diagnosed patients will be osteopenic or osteoporotic on DEXA bone density measurement. Calcium and vitamin D malabsorption may be associated with raised serum bone-derived alkaline phosphatase isoenzyme and elevated serum parathyroid hormone levels. The GFD should include abundant milk products but calcium and vitamin D supplementation may be needed if the patients are milk-intolerant.

The principles of substituting bread, pasta and wheat cereals with alternatives such as rice, corn, millet and buckwheat should be explained. The patient should be encouraged to react to and understand food labels and to consult food lists published by National Coeliac Societies[4], local coeliac self-help groups or web-based support pages[5] when purchasing refined foods which often incorporate wheat based emulsifiers or colouring additives. Further education can be achieved by the dieticians through local support groups.

Defective compliance to a GFD still causes major limitations during long-term care. Compliance is highest in patients diagnosed at a young age and lower in adults (e.g. 17-45%) than in teenagers (typically 56-83%). Resolution of symptoms, particularly abdominal pain occurs in most patients, on GFD even when serological or biopsy response is incomplete and constitutes the main incentive for continued strict compliance. Poor symptom resolution is commonly due to inadvertent gluten consumption (e.g. in medications or fibre additives), persistent lactose intolerance, associated microscopic colitis, refractory sprue, ulcerative jejunitis or emergent T-cell intestinal lymphoma. Compliance is often reduced when gluten-free foods are expensive and only partly subsidised by health services,

have low palatability, unattractive colour or consistency, or are available in a limited range. The availability of gluten-free foods on prescription in Malta has had immediate tangible effects on patient compliance. Communal eating in restaurants or when travelling, reduces availability and exerts a societal pressure to relapse. Unsupervised children often have relapses at school. Absence of symptoms immediately after cheating may worsen poor resolve or sub-conscious procrastination to adhere strictly to a GFD.

High serum antigliadin IgA antibodies return to normal levels in about 75% or patients compliant to a GFD and rise again rapidly after a gluten challenge. Serum tissue transglutaminase (tTG) levels often reflect the level of compliance to GFD in patients whose sera were initially positive for antiendomysial antibody but do not reflect mucosal recovery. Repeat duodenal biopsy may be necessary in compliant patients with persistently raised serum tTG or persistent symptoms.

Even with excellent compliance, a well-supervised diet may cause problems. Berti *et. al*[6] showed that the digestibility of the starch component of various processed gluten-free foods using in-vitro multi-enzyme digestion was higher than that in gluten-rich foods. Gluten-free foods were shown to produce a higher glycaemic response resulting in higher circulating blood glucose concentrations. These findings are of concern given that diabetes mellitus occurs in about 5% of coeliac patients. Some patients fail to gain weight or height despite adequate dietary compliance due to undiagnosed diabetes, thyroid disease or adrenal insufficiency which are known associations of CD. The chromosomal associations of CD, namely Down's syndrome and Turner's syndrome will also cause persistent short stature. Some patients will remain underweight because they become more health-conscious at diagnosis and limit their dietary energy intake while others who fail to compensate for reversed malabsorption develop new-onset obesity.

CD patients require regular follow up by a multidisciplinary team supervised by an adult or paediatric gastroenterologist employing a nutritionist, dietician, clinical pharmacist and a specialist nurse in liaison with a rheumatologist and endocrinologist who should all be accessible to CD patient-support groups. A clinical pharmacist is often consulted regarding the gluten content of various medicinal preparations.

Even with best of care many patients on GFD report a low self-rated level of happiness.[7] The main impact of CD on quality of life in diet-compliant patients does not result from coeliac-related symptoms but from social alienation secondary to a restricted diet when eating out or travelling.

Sliced bread or spliced peptides: a taste of future treatment

A close look at the pathogenetic cascade of events leading to mucosal damage, as described in the previous issue of this Journal identifies several steps which may be amenable to

novel therapeutic approaches thus allowing more gluten to be incorporated in future coeliac diets.

1. The discovery that certain gliadin peptides (e.g. aminoacids 62-75 and 57-68 in A gliadin) are immunodominant[8], i.e. they consistently elicit an immune response from intestinal T-cells, has prompted food research chemists to modify or delete these amino acid sequences in dietary A gliadin to produce a non-toxic genetically modified flour. Alternatively, different varieties of natural wheat can be screened for gliadins with less toxic properties.

2. An alternative approach could incorporate into wheat products, enzymes capable of hydrolysing toxic peptides in the intestinal lumen at multiple peptide bonds so as to disrupt their antigenic sequences. Such enzymes would have to be acid and pepsin-stable, and ideally heat labile to allow baking. A number of prolyl endopeptidases (PREP) from different sources active at intestinal pH (pH 6-7) are being evaluated,[9] but there is concern that the enzyme may not penetrate chyme fast enough to clear all immunogenic peptides. The oral enzymatic approach was prompted by the observation that many coeliac patients exhibit upregulation of the PREP gene suggesting that a defect in the function of PREP allows survival of large uncleaved immunogenic peptides in the intestinal lumen.[10]

3. Since intestinal bacteria may increase exposure of antigen presenting cells to immunogenic peptides by widening the tight junction between enterocytes[11] and because gluten-ingesting bacteria may also be sampled by dendritic cells, probiotics or antibiotics are alternative candidate pharmacological treatments for CD.

4. Once gliadin epitopes have gained access to the submucosa, the next step in the pathogenesis is the tTG-mediated cross-linking of glidadin and the deamidation of glycine residues on gliadin epitopes. Cross-linking increases the availability of toxic epitopes in the submucosa and deamidation facilitates binding of epitopes to DQ2 or DQ8 glycoproteins on antigen presenting cells. Orally administered peptides that inhibit tTG would reduce disease activity by inactivating this key enzyme and auto-antigen. However since the tTG family of enzymes are responsible for regulating apoptosis and stabilizing connective tissue in a number of organs, these oral peptides are unlikely to be safe.[12]

5. Further down the immunological cascade there is interest in designing inhibitors that would block the binding of epitopes to DQ2 and DQ8 molecules on antigen presenting cells. This still represents a targeted approach as it would not disturb the binding of other important antigens.

6. Less specific would be the administration of cytokines such as IL-10 which make the dendritic cell tolerogenic, ultimately leading to active suppression and anergy of T cells.[13] This would avoid stimulation of T-cell receptors which usually results in a Th_1 cytokine-mediated apoptosis and a Th_2 response that promotes B-cells to produce antibodies to gluten and tTG.[14]

7. Treatment with prednisolone is reserved for patients who remain severely symptomatic and whose intestinal biopsies fail to resolve on a strict GFD. Azathioprine has been used as a steroid sparing agent with variable success.[15] Intravenous cyclosporine may be indicated in ill patients who do not respond to steroid treatment.[16] Coeliac disease which is refractory to diet is less likely to respond to steroids and azathioprine when IELs are phenotypically immature and lack characteristic T cell markers. Many of these steroid–resistant cases harbour or eventually develop small intestinal T cell lymphomas.[15]

Conclusion

Life long adherence to a strict gluten-free diet, backed by strategies that maximize dietary compliance remain the most effective modality to manage coeliac disease. Understandably dietary guidelines cannot be refined until the whole spectrum of immunodominant peptides has been unravelled. Maximum safe limits in daily consumption of those dietary peptides that are compatible with normalization of intestinal biopsies still need to be determined. Future dietary treatments may utilize genetically modified cereal proteins or peptides that have been modified by prolylpeptidases during food preparation or during luminal digestion.

The discovery of several key protagonists in the long sequence of antigen processing and presentation of modified antigen to the immune system introduces many possibilities for attenuating immune damage at an early stage in the immune cascade, ideally before amplification by cytokines has taken place. However more basic research is required before pharmacologic therapy can be proposed as an alternative or as an adjunct to dietary management.

Therefore more scientific research is likely to be required before focusing on clinical trials of genetically modified cereal foods in celiac disease.

Acknowledgements

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