Celiac Disease
Gluten free: Not just a fad!
What do pharmacists need to know?

• https://www.youtube.com/watch?v=Oht9AEq1798
Objectives

- Describe the basic pathophysiology of celiac disease
- Explain what is known about history and epidemiology of celiac disease
- Describe current and potential treatments for CD
- List areas where pharmacists can advise CD patients

Genetics or Environment?

- Both
- Immune mediated result of a person who has the genetic predisposition and is exposed to gluten proteins
- Newer strains of wheat may have more t-cell stimulating profile than older strains
- 50% of calories involve wheat products
- Possible infectious triggers
  - Adenovirus and hepatitis virus
  - Interferon α

Digestion

GI protease enzymes in the small intestine brush-border break down peptides from gastric digestion
- First defense vs. toxic dietary proteins
- Intestinal epithelium (with intact intercellular junctions) forms a barrier to passage of macromolecules into lamina propria
Gluten

- Proteins exist in two fractions
  - Gliadins – most likely to cause immune response
  - Glutenins
- Amino acids glutamine and proline withstand digestive processes
  - Normally excreted without prompting immune reaction
  - Small amounts cross through tissue junctions of intestinal epithelium

Proteins in grains

- Wheat- gliadin
- Rye- secalin
- Barley- hordein

These proteins are...

- Rich in glutamine and proline amino acids
- Resistant to degradation by gastric & pancreatic enzymes & epithelial dipeptidases secreted by enterocytes
- Incompletely degraded into immunogenic peptides which initiate immunological cascade in celiac patients
Pathophysiology

• Enterocytes (epithelium) release Zonulin in response to gluten in the intestine
• Protein Zonulin loosens intercellular tight junctions
• Compromised integrity of tissue junctions allow gluten into the lamina propria
• Gluten accumulates under the epithelial cells

Pathophysiology cont.

• Gluten induces enterocytes to secrete interleukin-15 (IL-15)
• Immune response of lymphocytes against enterocytes is induced by IL-15
• Enterocytes become damaged & release transglutaminase (tTg,TG2), which modifies (deamidates) gluten
• Deamidated peptides are more immunostimulatory

Pathophysiology cont.

• Antigen presenting cells join the modified gluten and human leukocyte antigen (HLA-DQ2, HLA-DQ8)
• Helper T cells recognize those complexes & attract immune response
Result

- Villous atrophy
- Hyperplasia
- Antibody producing B cells
- Intestinal and extra-intestinal manifestations
  - Malabsorption
  - Osteoporosis
  - Dental hypoplasia
  - Short stature
  - Delayed puberty
  - Amenorrhea...

History

- Cave man diet: fruits, vegetable, meats
- 10,000 years ago, agricultural changes led to eating grains
- With increase in global consumption of wheat/grains came increase in incidence of CD
- In Finland & U.S., prevalence has increased X4 in the past 50 years

History

- Celiac greek for “hollow” (as in bowels)
- First description 100 AD in Roman Aretaeus
- First published description 1888
- 1952, WK Dick, Dutch pediatrician showed that children improved when wheat unavailable in WWII
Epidemiology

- Most patients have human leukocyte antigen (HLA) DQ2 or DQ8 molecules expressed on surface of antigen-presenting cells
- 30% of people with European ancestry carry predisposing genes
- 95% of these carriers have no intolerance to gluten

Prevalence

- About 1% of population CD
  - Previous thinking European only, but population-based screening shows equal rates in African, South Asian, Latin American, and Middle Eastern countries
- 10% some type of gluten intolerance
- 1st degree relatives of CD patients have 1:22 likelihood
- 2nd degree relatives chance is 1:39
- Down Syndrome 1:11
- Type 1 DM 1:23
- U.S. population CD patients also have
  - 18% thyroid disease
  - 10% dermatitis herpetiform
  - 3.3% type 1 DM
  - 2% Sjogren's

Theories

- More protein in wheat
- More wheat consumption
- Less microbe “protection”
- Changes in popularity of breast feeding
  - Protective?
- Timing of introduction to grains
Finland vs. Russia

• Study of 5,500 subjects genetically related in province on border of Finland and Russia (Karelia)
• 1 in 100 Finnish children had CD
• 1 in 500 Russian children had CD

Finland vs. Russia

• Finland ranks #1 for autoimmune Type 1 diabetes
• Antibodies for autoimmune thyroiditis higher
• 1 in 20 Finnish children had H pylori
• Russia per-capita income 1/15 of Finland’s
• 6X less frequent Type 1 DM
• 3 of 4 Russian Karelian children had H pylori
• House dust & water indicate higher microbe content

Probiotics?

• Rat & in vitro human studies show
  – E. Coli increased gluten-induced inflammation of intestines & “leakage”
  – Bifidobacteria protected intestinal barrier

• Breast fed infants show increased bifidobacteria in gut than formula fed
The Swedish experiment

Accidental experiment where 3 things happened simultaneously
1. Guidelines on infant feeding told parents to delay intro to gluten till 6 month old
2. Breast-feeding mothers stopped at 6 month average
3. Increase in gluten in baby food

3% of Swedes born between 1984-1996 vs. 1% of general population
Theory: breast-feeding AFTER first exposure to gluten may be protective

Studies
• Small studies in Spain and U.S. have shown possible connection between lactobacillus and bifidobacteria levels and decrease in Celiac Disease and other autoimmune diseases
• Breast milk from urban areas contain less microbes than that from farming mothers
  – Theoretically farming mothers carry bifidobacteria in breast milk

Where is gluten?
• Wheat
• Barley
• Rye
• Bran
• Graham flour
• Spelt
• Wheat germ
• Oats??

Oats are distantly related & may contain a few disease-producing proteins OR may be contaminated in manufacturing process
Why is gluten-free so difficult?

- Not just a question of avoiding bread and pasta or “carbs”
- Foods prepared in same kitchen
- Aerosolized particles from breadcrumbs
- Toasters, utensils, etc.
- Lack of understanding in restaurants (fad diet mentality)
- 0.015 mg per day is enough to cause symptoms

Canadian study

- Evidence suggests somewhere between 10-50 mg/day is threshold for causing histological changes to the intestinal mucosa
- Study of patients in Canada consuming “gluten-free” diets using potential contamination of non-gluten containing grains with 10, 20 or 50 ppm
- 50 ppm resulted in over 10 mg/day
- Concluded that maximum allowable contamination is 20 ppm

Obvious sources

- Pastas
- Breads/Pastries
- Noodles
- Breading
- Breakfast foods (pancakes, waffles, etc.)
- Beer
- Crackers
- Tortillas
Must be verified

- Energy/granola bars
- Potato Chips
- Soup
- Candy/candy bars
- Self-basting poultry
- Pre-seasoned meats
- Communion wafers

Hidden sources

- Drugs
- Vitamins
- Lip Balm
- Pickles
- Bleu cheese
- Hot dogs
- Soy sauce
- Frozen veggies
- French fries
- Salad dressing

Gluten is used as a binder to give structure to many products.

Gluten (A) or No gluten (B)?
Food Allergen Labeling and Consumer Protection Act of 2004

• All food products manufactured after 1/1/2006 must be clearly labeled to indicate 8 food allergens
  – Milk
  – Eggs
  – Fish
  – Shellfish
  – Tree nuts
  – Peanuts
  – Soybeans
  – Wheat

FDA definition of Gluten-free

For voluntary use in food labels…
Food must not
• Contain a gluten-containing grain
• Contain an ingredient derived from gluten-containing grain that has not been processed to remove
• Contain greater than 20 ppm or more of gluten

FDA update 8/2014

• Food containing wheat starch can only be labeled gluten-free if
  – It has been processed to remove gluten
  – Tests show below 20 parts per million of gluten
  – An asterisked statement on the label explains that the wheat has been processed to comply with FDA requirements
Cross-contact

- Toasters
- Flour sifters
- Deep fryers (shared oil)
- Condiments (butter, peanut butter, jam, mustard)
- Shared containers, not cleaned well

Airborne flour

- Wheat flour can stay airborne for hours and can land on
  - Exposed preparation surfaces
  - Utensils
  - Uncovered gluten-free products
  - Food being prepared without gluten-containing ingredients
    - Gluten-free pizza made in same area as regular pizza
    - Gluten-free bakery without separate prep area

Diagnosis of Celiac Disease

- Gold standard is small bowel biopsy
- IgA and IgG serum tests for*
  - tTG, EMA, AGA and deamidated gliadin peptide antibodies
  - IgA tTG most reliable and cost-effective
  - IgA EMA and IgA tTG sensitive & greater than 95% specificity
- Genetic testing for HLA susceptibility markers

*must be eating gluten for tests to show positive
4 of 5 criteria

1. Positive history for symptoms
2. Positive serological biomarkers (tTg or EMA)
3. Positive genetic testing for HLA-DQ2 or DQ8 alleles
4. Small intestinal biopsy showing blunting or absence of villi (Marsh III)
5. Improvement of symptoms with gluten-free diet

Other indicators

- Other autoimmune disease
- Dermatitis herpetiformis
- Close relative with CD
- Deficiencies of iron, folic acid, vitamin B₁₂, fat-soluble vitamins (malabsorption)

Current state

- Gluten-free diet only treatment
  - After 6-12 months, 80% will test negative by serology
  - After 5 years, more than 90% will test negative
- Non-responsive CD (NRCD)
  - Persistent symptoms, lab abnormalities despite GFD
  - 7-30% of patients on GFD
  - Complicated by inadvertent consumption (35-50%)
- Refractory CD (RCD)
  - 1-2% of patients
  - Type 1 and Type II
Treatment of RCD

Type I (more common in U.S.)
- Lymphocyte infiltration in small intestine similar to untreated patients
- Avoid inadvertent exposure
- Systemic steroids (prednisone)
- Immunosuppressive agents (azathioprine)
- Recent reports possible effectiveness of budesonide or small-intestine release mesalamine
- 5 year survival 93%

RCD continued

Type II
- Abnormal phenotype in intraepithelial T-cells
- Less favorable prognosis
- Potential to transform to enteropathy-associated T-cell lymphoma
- 5 year survival 44%
- Systemic corticosteroids
- Enteric-coated budesonide
- Azathioprine
- 6-mercaptopurine
- Methotrexate
- Cyclosporine
- Anti-TNF antibodies
- Cladribine

Potential treatments?

- Gluten detoxification (alter gluten-containing foods)
- Gluten digestion (by proteases) to decrease immunogenicity
- Bar/prevent gluten entry
- Decrease intestinal permeability with molecules to enhance tight junctions between enterocytes
- Induction of regulatory suppressor T-cell response (Gluten vaccination)
Types of treatment

• Development of modified wheat/grains
• Enzymes to break down gluten protein to help desensitize
• Drugs to induce body to close junctions between intestinal cells
• Treatment to modify immune reaction
• Vaccines to induce tolerance to gluten
• Drugs to bind gluten & remove from body unchanged via stool

Genetically modified grain

• Amino acid sequences of the α-gliadin derived peptides have been identified
• These amino acids were then tested for their T cell stimulatory capacity
• A proline to serine substitution was found to eliminate the immune response to wheat gluten protein
• Attempts are being made now to introduce the revised SNPs as specific mutations into wheat genes to create non-toxic grain

Enzymatic degradation of gluten

• Family of enzymes called Prolyl endopeptidases (PEP) found to cleave proline residue
• Those PEPs expressed in human small intestine are less efficient in cleaving gliadin peptide than those in certain microbes & fungi
• *Aspergillus niger, Sphingomonas capsulata, Flavobacterium meningosepticum, Myxococcus xanthus*
PEPs

• Proteolytic enzymes are in various stages of study
  • A. Niger PEP (AN-PEP) is resistant to gastric peptides & has been tested in vitro & in vivo
  • 2 complementary peptidases (one from barley EP-B2 & one from F. meningosepticum) combined called ALV003 shown to be safe & well-tolerated in Phase I & effective in Phase II
  • Cocktail of enzymes called STAN-1 not yet published but abstracts say no reduction in disease

Inhibition of intestinal permeability

• Larazotide acetate (AT-1001, Alba Pharmaceuticals) is an octapeptide inhibitor with a structure derived from Vibrio cholerae zonula occludens toxin
  • Subjects in early trials showed intact intestinal barrier function & less GI symptoms than controls
  • However, further studies have not shown significant differences in permeability, despite continued evidence of decreased symptoms

*The toxin impairs epithelial tight-junctional integrity

Modifiers of immune response TG2 inhibitors

• The specific immune reaction that involves the deamidation reaction catalyzed by transglutaminase 2 (TG2) is a feature of celiac disease
  • Studies are being done to look at TG2 inhibitors
  • Cystamine is one TG2 inhibitor that seems to reduce the proliferative response of gluten-reactive T-cells
  • 3 other TG2 inhibitors have been developed & are heading for trials
    • ZED1098, ZED 1219, ZED 1227 (Zedira)
  • TG2 “knock-out” mice have developed abnormal inflammatory responses with age, indicating that highly selective inhibition may not be as well-tolerated as reversible inhibitors
Modifiers of immune response HLA-DQ2 inhibitors

- Theoretically, since the majority of CD patients carry the HLA-DQ2 blocking, blocking the DQ2 binding site by gliadin antagonists could suppress presentation by antigen-presenting cells
- HLA-blocking approaches have been attempted with other autoimmune diseases such as MS, RA, type 1 DM without clinical benefit

Modifiers of immune response CCR9 antagonists

- CCR9 is a chemokine receptor on the surface of lymphocytes, which is responsible for homing them to the small intestine
- Studies have been done in both Celiac and Chrohn’s disease
- Exacerbation of intestinal inflammation in mice have resulted in response to blocking CCR9

Vaccines

- Nexvax2 is a peptide-based therapeutic vaccine developed to modify the pathogenic T-cell response
- Australian company Nexpep analyzed the gluten protein, broke it down into 2,700 distinct fragments, & added them to the blood of 200 CD patients to determine which peptides specifically cause reaction
- Three peptides (gliadin, hordein, secalin) which triggered the response were combined into a vaccine to desensitize patients
- Multiple small doses are given to create tolerance & ultimately prevent T-cells from initiating immune cascade (Phase IIa trials are being planned)
Sequestering gluten without digesting

- Synthetic polymeric compound* sequesters gluten over a wide pH in the stomach
- Forms a high-affinity complex with α-gliadin
- Prevents the GI enzymes from digesting protein into smaller peptides which stimulate the pathogenic process

*Poly-hydroxyethylmethacrylate-co-styrene sulfonate (P(HEMA-co-SS))

Clinical Trials

- Randomized crossover clinical study to establish degradation of gluten in vivo by AN-PEP was conducted (results pending)
  - NCT01395503*
- Phase llb study of ALV003
  - NCT01990885*
- First-in-man safety & exposure trial of P(HEMA-co-SS)
  - NCT01990885*
- Phase llb trial of efficacy & safety of AT-1001 (larazotide acetate)
  - NCT01395503*
- Phase ll study in celiac patients with CCR9 antagonist
  - NCT00540657*

What’s needed?

- Accurate, low-cost diagnostic tests
- Treatment other than lifelong gluten free diet
- Reasonably priced alternative foods
**Pharmacist’s role**

- Identifying drugs
  - Hospital Pharmacy 2013:48(9):736-743
  - AJHP 2015:72: 54-59
  - Package insert
  - Call manufacturer
- Identifying OTC and herbal products
- Discussing OTC "cures"
- Identifying beauty products, etc.

Free CE program from National Foundation for Celiac Awareness
www.celiaclearning.com

**Pharmaceutical excipients**

- DEX
  - Dextrates/dextrins (some type of starch)
  - Dextrose (corn starch)
  - Dextrals (corn starch)
  - Dextrimaltose (barley malt)
  - Caramel coloring (barley malt)
  - Plus cross-contamination…

**RWJUH database for medications**

- Drug information service at Robert Woods Johnson University Hospital determined gluten content of medications & created database:
  1. Package insert search for:
     - Dextrates, dextrins, pregelatinized starch, flour, caramel coloring, wheat, oat, rye, etc.
  2. Contacted manufacturers
**RWJUH Categories**

- **Gluten free**
  - No listed ingredients
  - AND manufacturer confirmed testing
  - OR manufacturer confirmed no gluten in ingredients & no gluten-containing ingredients at the manufacturing site

- **Contains gluten:**
  - Listed ingredients
  - OR manufacturer confirmed cross-contamination in plant

- **Possibly contains gluten:**
  - All others
  - Many medications...

**Cosmetics**

- Gluten not absorbed through skin
- Molecule too large
- Anything that could be ingested
  - Toothpaste
  - Mouthwash
  - Lipstick/lip balm etc.

**On pharmacy shelves**
Untested enzymes

<table>
<thead>
<tr>
<th>Supplement Facts</th>
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<tbody>
<tr>
<td>Serving Size: 1 Capsule</td>
</tr>
<tr>
<td>Servings per Container: 30</td>
</tr>
<tr>
<td>*Daily value not established.</td>
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<tr>
<td>Other Ingredients: Cellulose, rice flour, vegetable magnesium stearate, silica.</td>
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<tr>
<td>Warnings: If you have Celiac Disease, use only under your physician's supervision. Store in a cool, dry place.</td>
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Amount Per % Daily Serving Value

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<tr>
<th>GCX50™ Gluten Blend:</th>
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<tr>
<td>400 mg *</td>
</tr>
<tr>
<td>Protease I, II, III, IV and V; Amylase, Cellulase, Lactase, Alpha Galactosidase, Beta Glucanase, Xylanase, Lipase, Hemicellulase, Pectinase, Phytase, Invertase, Ginger Extract (Root), Dried Peppermint Oil and Deglycyrhizinated Licorice (Root).</td>
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CELIAC

C Consultation with dietician
E Education about the disease
L Lifelong adherence to gluten-free diet
I Identifying & treating nutritional defic.
A Access to advocacy group
C Continuous long-term follow-up by multidisciplinary team
Assessment

1. The percentage of calories that contain gluten in the average American diet is:
   a. 20%
   b. 30%
   c. 50%
   d. 90%

Assessment

2. Celiac disease is:
   a. a combined response to genetic predisposition and environmental factors
   b. an immune mediated disease
   c. increasing in incidence
   d. all of the above
Assessment

3. Airborne gluten can contaminate gluten-free foods prepared without any gluten-containing ingredients.

a. True
b. False

References


Other resources

- http://www.theglutenfreebar.com/