STRUCTURE - ACTIVITY RELATIONSHIPS OF GLIADIN PEPTIDES IN COELIAC DISEASE

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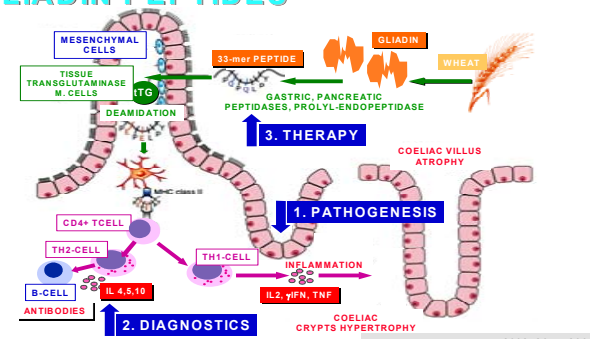
ABSTRACT

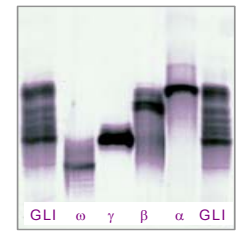
Coeliac disease (CD) is a chronic autoimmune inflammatory intestinal disease with known environmental trigger - gluten and a strong genetic component. Coeliac-toxic gliadin peptides result by proteolytic digestion of gluten proteins and peptides containing the sequences **Pro-Ser-Gln-Gln** and **Gln-Gln-Gln-Pro** and may be involved in the pathogenesis of coeliac disease. We published in 1988 α-gliadin fragments acquired by peptic-tryptic-protease digestion (PTP-aGli). These PTP peptides were analysed by RP-HPLC, MALDI-TOF and BLAST and three important sequences **QPFPQPQLP**, **QLQPFPQPQ** and **RPQQPYPQPQPQ** were detected. In previous papers we demonstrated the opioid activity of PTP-aGli investigated in vitro by the contraction of guinea pig ileum. The tendency to form a β-turn in α-gliadin was estimated using the B-cell determinant prediction program based on the Chou and Fasman probability of β-turn formation. By means of solid-phase synthesis were obtained in 1991 several α-gliadin peptides and their toxicity was tested using the foetal chick duodenum. Our last studies are focused on deamidated gliadin peptides in CD diagnosis. Tissue transglutaminase epitopes in ELISA tests are blocked by PTP-aGli fragments, and antibodies of coeliac patients cannot bind as we published in 2006. We compared ELISA methods of Inova (iDGPA, iDGPG) and Euroimmun kits with deamidated gliadin peptides (**PLQPEQPFP** and **PEQLPQFEE**) to ELISA with purified α-gliadin as antigen. Deamidated gliadin peptides could increase sensitivities, specificities and accuracies of coeliac disease serology screening. Results of this study suggest as optimal screening modality a combination of anti-tissue transglutaminase IgA and Inova iDGPA and iDGPG ELISA antibodies. **Gliadin peptide sequences** have important role in the **ethiopathogenesis, diagnosis and probably in the therapy of coeliac disease** as well.

GLIADIN PEPTIDES



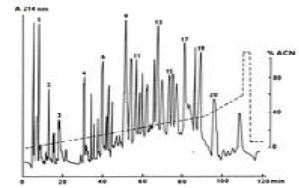
acc: Mowat AT, Lancet 2003, 361: 1290

GLIADIN PEPTIDES ISOLATION



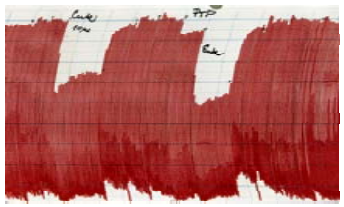
A simple modification of the horizontal starch-gel electrophoresis of gliadin proteins with the use of LKB Multiphor System has been developed.

Peptidic fragments of α-gliadin were obtained by peptic-tryptic-pancreatic (PTP) digestion of the α-gliadin fraction isolated by ion-exchange chromatography on a sulphopropyl Sephadex C-50 column.



The proteolytic digest was fractionated by ultrafiltration and separated using reversed-phase high-performance liquid chromatography with gradient of acetonitrile in 0.1% trifluoroacetic acid and a Separon SGX-C18 sorbent.

GLIADIN PEPTIDES ACTIVITY

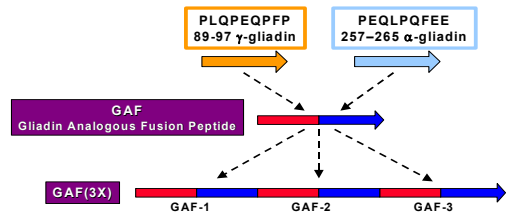


Opioid activity of gliadin and their proteolytic fragments (PTP digest) were studied with a model of electrically stimulated guinea-pig ileum. The myenteric plexus-longitudinal muscle preparation from the guinea-pig was set up in a 5 ml organ bath and fixed to isotonic sensor of Unirecord 7050 (Ugo-Basile, Italy). Electrical rectangular pulses were used of 1 ms duration, 0.1 Hz frequency and 20 - 60 mA current.

The tendency to form a β-turn in α-gliadin was estimated using the B-cell determinant prediction program based on the Chou and Fasman probability of β-turn formation. Six sequences possessing a high probability of β-turn formation were found. A statistically high agreement was found between these six sequences and three areas in α-gliadin with the occurrence of **Pro-Ser-Gln-Gln** sequence which has recently been considered responsible for toxicity in coeliac disease. By means of solid-phase synthesis seven peptides were obtained covering the above-mentioned regions. Their toxicity was tested using the fetal chick duodenum. The results support the suggestion that peptides containing the sequences **Pro-Ser-Gln-Gln** and **Gln-Gln-Gln-Pro** may be involved in the pathogenesis of coeliac disease.

DEAMIDATED GLIADIN PEPTIDES

Our last study was to evaluate two different commercial ELISA kits that are using deamidated gliadin peptides as antigen on the group of 120 serum samples from patients with small bowel biopsy performed (79 florid CD, 35 with normal histology, 6 CD in remission). Presence of α-gliadin antibodies in IgA and IgG classes was tested by "Quanta Lite Gliadin IgA II and IgG II" (iDGPA/G, Inova Diagnostics) and "anti-gliadin ELISA IgA and IgG" (eDGPA/G, Euroimmun) and compared to results of routinely used method (with purified α-gliadin as antigen, AGA/G, Dialab).

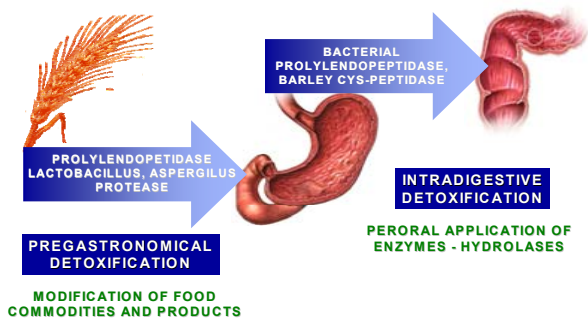


DIAGNOSTIC EFFECTIVITY

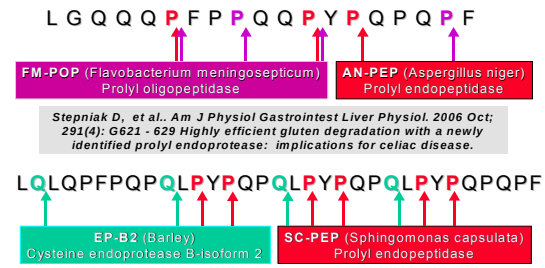
	SN	SP	PPV	NPV	ACC
atTG IgA	86.25	97.14	98.57	75.56	89.85
EmA IgA	88.75	100.00	100.00	79.54	92.17
AGA-A/AGA-G	95.00	94.28	97.43	89.19	94.78
Inova DGP A/G	95.65	97.14	98.51	91.89	96.15
Eurim DGP A/G	82.43	68.57	84.72	64.86	77.98
AGA/G + atTG	97.50	91.43	96.29	94.12	95.65
InoDGP + atTG	97.10	94.29	97.10	94.28	96.15
EurimDGP + atTG	98.75	68.57	87.78	96.00	89.56

Sensitivities, specificities and accuracies of serology screening methods - antigliadine antibodies IgA (AGA-A), IgG (AGA-G), endomysial antibodies IgA (EmA), anti-tissue transglutaminase IgA (atTG) compared with deamidated gliadine peptides Inova and Euroimmun IgA and IgG classes in single tests and in combination of gliadine/DGP with anti-tissue transglutaminase IgA

FUTURE COELIAC THERAPY



GLIADIN PEPTIDES DEGRADATION



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