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-Glu-Glu-Pro and may be involved in the pathogenesis of coeliac disease. We published in 1988 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 PFPQFPQ and QPFPQPQPQPQP were detected. In previous papers we demonstrated the opioid activity of PTP-aGli investigated in vitro by the use of guinea pig ileum. The tendency to form β-turn in a 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 we obtained in 1991 several γ-gluten 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, PEGLPQFE) to ELISA with purified α- gliadin as antigen. Deamidated gliadin peptides could increase sensitivities, specificities and accuracies of coeliac disease serology testing. Results of these study suggest optimal screening modality to be a combination of two screenings (low and high titre) and Euroimmun 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 ACTIVITY

Opioid activity of gliadin and their proteolytic fragments (PTP-aGli) was studied with a model of electrically stimulated guinea pig ileum. The potential opioid activity of gliadin fractions prepared from the duodenum preparation of a guinea-pig was set up in a 5 ml organ bath and loaded to isometric sensor of Uronckord 7500 (Ugo- Basile, Italy). Electrically rectangular pulses were used of 1 ms duration, 0.1 Hz frequency and 20 - 60 mA current.

The tendency to form β-turn in a 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 and three areas in a gliadin with the occurrence of Pro-Ser-Gln-Gln sequence which has recently been considered responsible for gliadin toxicity in coeliac disease. Six more of the above synthetic sequences were prepared covering the above-mentioned regions. Their toxicity was tested using the foetal chick duodenum. The results support the suggestion that peptides containing the sequence Pro-Ser-Gln-Gln and Gln-Glu-Glu-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 utilizing deamidated gliadin peptides as antigen on the group of 120 serum samples from patients with small bowel biopsy performed (15 field CD, 35 with normal histology, 60 in CD in remission). Presence of gliadin antibodies in IgA and IgG classes was tested by "Quantum Lab Gliadin IgA and IgG" (iDGPA, Euroimmun) and "anti-gliadin ELISA IgA and IgG" (iDGPA, Inova) and compared to results of routinely used method (with purified α-gliadin as antigen, AGA-Di Diagnost). The proteolytic digest was fractionated by ultrafiltration with use of LKB Multiphor System has been developed.

GLIADIN PEPTIDES DEGRADATION

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 tested and Euroimmun IgA and IgG classes in single tests and in combination of gliadine/DGP with anti-tissue transglutaminase IgA.

REFERENCES