Gluten Challenge Effect on Duodenal Biopsy Cultivation of Coeliac Patients and Controls.

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Coeliac disease was described 100 years ago and the pathogenetic effect of gluten-gliadin is unquestionable. Nevertheless, although many different gliadin preparations including native gliadins, gliadin fragments after degradation and synthetic peptides have been prepared (1,2) and their biological activity has been tested in the past years, the key peptide has not been isolated and the toxic sequence is not yet known. DeRitis et al. (3) reported the importance of two sequences Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro, based on his experiments with CNBr-cleaved gliadin.

Synthetic gliadin peptides with high tendency to form a β-turn according to computer modelling (4) and covering all occurrences of Pro-Ser-Gln-Gln in α-gliadin have been prepared by a continuous-flow solid-phase multiple peptide technique using Fmoc/t-Bu protection strategy (5). The highest biological effect, tested on fetal chick duodenum, has been expressed by dodecapeptide sequence 8–19 covering both Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro (6).

The goal of this study was to confirm a high biological effect of peptide 8–19 on human intestine and to test the relationship between mucosal enzymes and immunological response in the culture medium and the effect of gluten challenge on coeliac patients in remission. α-Gliadin purified on Sulfopropyl-Sephadex (7), proteolytically degraded fragments (8) (PTP-digest) and peptide 8–19 were added to the culture medium. The short-term (24 hr) cultivation of duodenal biopsies was carried out by the technique reported by Falchuk et al. (9), and the medium composition was prepared according to Jos et al. (10).

Duodenal biopsy samples were obtained by endoscopic technique with Olympus fibroscope GIF Q or JF 1T and biopsy forceps FB-24K from coeliac patients (n=16) in remission before and after one-week of gluten challenge. Control subjects (n=11) were also studied. Enzyme activities (endopeptidase (11)-Suc-Ala_2-Leu-NAp; aminopeptidase-Leu-NAp, sucrase-sucrose, alkaline phosphatase-p-nitrophenylphosphate) were determined in the Tritonized supernatant by kinetic or end-point methods. Immunological parameters (IgG, IgM, secretory IgA — [sIgA], antigliadin IgA [aGIgA], antienterocytic IgA [aEIgA], gamma interferon — [GIF], cytotoxicity as TNF) were evaluated in culture medium, mainly by ELISA techniques (12,13).

Results of all 52 experiments (>900 biopsy samples) were evaluated using the statistical package SPSS-PC+. Correlations were performed between immunological and enzymatic activities. Student's t-test and Duncan's test were used to calculate significance. Two sub-groups were distinguished in the coeliac group in clinical remission according to the endopeptidase activity *in-vivo*. The CS1-group (before gluten challenge) was significantly different ($p<0.05$) from all the other three (CS1+, CS2-, CS2+). All four enzymatic activities behaved in the same manner during culture (correlation $p<0.01$), with the highest value ($r=0.801$) in the case of EP-SA. A high significance ($p<0.01$) was found also for negative correlation between EP-sIgA, EP-aGIgA, EP-IgM, SA-sIgA and SA-IgM, showing a relationship between immunological response determined in culture medium and enzyme activities in biopsy tissue.

The following hypothesis of two mechanisms involved in the pathogenesis of coeliac disease has been based on our results. The peptide 8–19 could have activated an immunological response in the first stage i.e. before the gluten challenge in remission. Another peptide present in the mixture of peptides in the PTP-digest of α-gliadin, but not the peptide 8–19 could have been triggering the second stage — after the gluten challenge. The coeliac patients (CS1-) in remission with high endopeptidase activity, compared with control subjects, are influenced to a greater extent by peptide 8–19. The immunological stimulation caused by gluten challenge changes their response to the presence of gliadin peptides during the cultivation experiment and the highest influence has been determined for PTP-digest of α-gliadin. There was no difference between this CS1+ group and coeliacs with poor remission (CS2-), who did not respond to the gluten challenge (CS2+). Confirmation of this hypothesis will be the aim of our further study.

Table 1.

Activity U/g	**Control**	**PTP-digest**	**α-Gliadin**	**seq.8–19**
CS 1 -	23.71	39.97	22.57	*16.48*
CS 1 +	14.44	*10.47*	11.48	13.86
CS 2 -	8.95	*10.81*	12.81	12.76
CS 2 +	8.66	*8.83*	13.00	9.66

Activity of endopeptidase (u/g) after 24-hr cultivation of duodenal biopsies from two groups of coeliacs (CS1,CS2), before and after one-week (*in vivo*) gluten challenge (-,+) in presence or absence of different gliadin preparations (*in vitro*).

References

1. Kocna P, Fric P, Kasafírek E, Slaby J, Tlaskalová H, Vancíková Z, Hekkens WThJM. *Isolation and properties of lectin-like glycopeptide from wheat gluten.* Lectins-Biology, Biochem. Clin Biochem 1990;7:45–48.
2. Kocna P, Holáková-Kocová M, Sasek A. *Simple starch-gel electrophoresis of gliadin proteins using the LKB-Multiphor system.* Z Lebens Unters Forsch 1983;177:454–456.
3. DeRitis G, Auricchio S, Jones HW, Lew EJL, Bernardin JE, Kasarda DD. *In vitro (organ culture) studies of the toxicity of specific A-gliadin peptides in coeliac disease.* Gastroenterology 1988;94:41–49.
4. Krchnák V, Mach O, Maly A. *Computer prediction of potential immunogenic determinants from protein amino acid sequence.* Analyt Biochem 1987;165:200.
5. Vagner J, Kocna P, Krchnak V. *Continuous-flow synthesis of a-gliadin peptides in an ultrasonic field and assay of their inhibition of intestinal sucrase activity.* Peptide Research 1991;4:284–288.
6. Kocna P, Mothes T, Krchnák V, Fric P. *Relationship between gliadin peptide structure and their effect on the fetal chick duodenum.* Z Lebens Unters Forsch 1991;192:116–119.
7. Charbonnier L, Mossé J. *Large scale isolation of gliadin fractions.* J Sci Food Agric 1980; 31:54–61.
8. Kocna P, Fric P, Kocová-Holáková M, Slaby J, Kasafírek E, Hekkens WThJM. *Isolation and analysis of alpha-gliadin peptidic fragments using reversed-phase high-performance liquid chormatography.* J Chrom Biomed Applic 1988;434:429–438.
9. Falchuk ZM, Gebhard RL, Sessoms C, Strober W. *An in vitro model for gluten sensitive enteropathy. Effect of gliadin on intestinal epithelial cells of patients with gluten-sensitive enteropathy in organ culture.* J Clin Invest 1974;53:487–500.
10. Jos J, Lenoir D, DeRitis G, Rey J. *In vitro pathogenetic studies of coeliac disease. Effects of protein digests on coeliac intestinal biopsy specimens maintained in culture for 48 hours.* Scand J Gastro 1975;10:121–128.
11. Kocna P, Fric P, Slaby J, Kasafírek E. *Endopeptidase of the brush border membrane of rat enterocyte. Separation from aminopeptidase and partial characterization.* Hoppe Seyl Z Phys Chem 1980;361:1401–1412.
12. Kocna P, Fric P, Tlaskalová H, Krchnák V, Kasafírek E, Sírová M, Czerkinski C. *Short-term cultivation of duodenal biopsies in coeliacs. Effect of the gluten challenge and gliadin peptides.* Recent Advances in Mucosal Immunology; 1993 Plenum Press USA (in press).
13. Tlaskalová-Hogenová H, Stepankova R, Fric P, Kolinska J, Vancikova Z, Jilek M, Kocna P. et al. *Lymphocyte activation by gliadin in coeliac disease and in experimentally induced enteropathy of germfree rats.* Advances in Mucosal Immunology 1990;781–784.

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