

AN ATTEMPT OF SPECIFIC DESENSITISING TREATMENT WITH GLIADIN IN CELIAC DISEASE

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Gluten-free diet is the current treatment of celiac disease. We decided to verify the occurrence of histological and serological modification and/or clinical manifestations during a gradual and progressive introduction of gliadin in the diet and if it may induce a tolerance to food, as it occurs in allergic patients. We studied the case of a celiac woman with complete clinical and histological remittance after 10 years of gluten free diet. She took increasing daily doses of gliadin, reaching the final dose of 9 g of gliadin (15 g of gluten) in 6 months. Then she started a free dietary regimen. During the 15-month follow-up period esophago-gastro-duodenoscopy showed normal Kerkring folds and villi. Anti-gliadin, anti-endomysium and anti-tissue-transglutaminase antibodies, as well as the haematological and biochemical parameters remained normal. Our results represent a new approach in the research concerning celiac disease, and could provide a future line of study for its management.

Celiac disease (CD) is a state of intolerance to gluten, characterised by small intestinal inflammation, villous atrophy and crypt hyperplasia. The intestinal mucosa is densely infiltrated with B and T-cells. The number of α/β intraepithelial lymphocytes directly correlates with the degree of villous atrophy. Furthermore there is an increased amount of γ/δ lymphocytes, accounting for 20-30% of all the intraepithelial lymphocytes: they are considered markers of CD latency and specific for potential or overt CD, even when the small intestinal mucosa shows no histological abnormalities (1-2).

A gluten-free diet is recommended as the only therapy to avoid the short and long term risks associated with CD, even though the gliadin content of the standard gluten-free diet is about 1 mg a day (3).

Collin et al studied the gluten content of naturally gluten-free and wheat starch-based gluten-free products and compared the daily intake in patients on gluten-free diet with mucosal histology. They stated that the threshold dose for gluten-contamination was 100 ppm (mg/kg), corresponding to an average daily intake of 30 mg of gluten (4). Many reports are available dealing with the assessment of a threshold dose by gluten challenge in celiac patients. Catassi et al showed that 100 mg/day of gliadin, for at least four weeks, is near the threshold dose for detectable intestinal damage in most CD patients and the effects of chronic ingestion of gliadin have been shown to be clearly dose-dependent. The dose-effect relationship is confirmed by an increased intraepithelial lymphocyte (IELs) count and villous height/crypt depth ratio.

Key words: celiac disease, gluten desensitisation, gliadin

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These two parameters are very sensitive, even though the increased lymphocyte count seems to be the earliest morphometric change appearing 12 hours after gluten ingestion (5). However, the occurrence of intestinal damage from lower gliadin doses can not be excluded.

On the basis of our experience on desensitizing treatment in food allergy and intolerance (6-9), we attempted to use the same method in a patient with CD, to verify what is the minimum dose which causes the occurrence of histological and serological modifications and/or clinical manifestations or if a gradual and progressive introduction of gliadin in the diet may induce a tolerance to food, as it occurs in allergic patients.

MATERIALS AND METHODS

We selected a 39-year-old female with a history of fatigue, abdominal discomfort, diarrhea, anemia with iron deficiency, elevated titres of antibodies to gliadin (AGA) and endomysial antibody (EMA). The esophago-gastro-duodenoscopy (EGDS) confirmed the diagnosis of CD with evidence of Marsh III lesions in small intestinal biopsies.

After a 10-year gluten-free dietary regimen, she did not manifest symptoms, the jejunal biopsy specimens showed a normal intestinal mucosa and a normal intraepithelial lymphocyte count (also for γ/δ intraepithelial T lymphocytes count). Serum levels of AGA and antibodies anti-tissue transglutaminase (tTG) were normal and EMA negative. Laboratory workup (haemoglobin, serum iron, ferritin, folates, B12 vitamin, TSH, FT3, FT4, anti-TPO antibody, anti-TG antibody, total IgA, calcium, aPTT, TP and fibrinogen) was normal. In order to exclude a cellular or humoral gluten allergy, the patient underwent allergological *in vivo* and *in vitro* tests. Prick and patch tests were performed with wheat commercial extract (5000 PNU/ml, MERCK S.p.A, Milan, Italy), wheat flour and gliadin and serum levels of eosinophilic cationic protein, total IgE, specific IgE and IgG4 to bread wheat (*Triticum aestivum* L.), rye, barley, gluten and gliadin were measured. Skin tests resulted negative and serologic parameters were normal.

The clinical trial was conducted in the Department of Allergology of the Policlinico A. Gemelli of Rome in accordance with good clinical practice after being approved by our hospital's ethics Review Board. After giving her informed written consent, the patient received increasing daily doses of gliadin, a final dose of 9 g being reached in 6 months (Table I). Alfa, beta, gamma, and omega gliadin were extracted from bread wheat (*T. aestivum* L.) cv. Bolero with 70% (v/v) ethanol after elimination of albumin-

globulin with 0.5M NaCl. An aliquot of the ethanol solution was frozen and freeze-dried (60% of gliadin). In the first phase (from day 1 to 40) the hydro-alcoholic solution was used, from a 1:10⁶ dilution (0,000001 mg of gliadin) to the pure one (70 mg of gliadin). Increasing doses of freeze-dried undiluted gliadin were used from day 41 to day 150, until the highest dose of 4.23 g. In the 6th month the patient received gradually increasing doses of gliadin as rusks (60 % of gliadin) until a total amount of 9 g of gliadin (corresponding to 15 g of gluten). The patient continued taking the maintenance dose of gliadin (9 g) as rusks during the 7th month, bread and pasta during the 8th maintaining a daily dose of 15 g of gluten, which is the average daily dose of gluten in a standard diet. From the 9th month, she came back to a free gluten-containing dietary regimen.

During the follow-up the patient underwent EGDS before starting the gliadin treatment, and 1, 3, 6, 9, 12 and 15 months afterwards. They were performed using the EG-485ZH video-endoscope (Fuji, Fujinon, Omiya, Japan), which allows high-resolution standard video-endoscopy (using an 850,000 pixel chip) and an adjustable image magnification in a continuous range up to 2.0x. During the duodenum exploration the evaluation of the villous profiles was firstly determined, after a minimal inflation of the duodenum, by simple endoscopic magnification (EM). Subsequently, water (0.4 syringes, 100-200 ml) was rapidly inserted into the duodenum. With this procedure duodenal villi were raised to obtain fingered images, which were particularly well visible tangentially, along the edges of the folds. This reduces the failure to notice a patch lesion (10).

The tissue specimens were obtained from the inferior, posterior and superior duodenal wall, respectively at about 5, 10 and 15 cm of the proximal descending duodenum.

Histological findings were described according to Marsh classification. IELs and γ/δ count were performed on every specimen. On the diagnostic specimen only IELs count was performed (1,11,12). AGA, EMA and tTG antibodies, as well as the haematological and biochemical parameters were determined once a month.

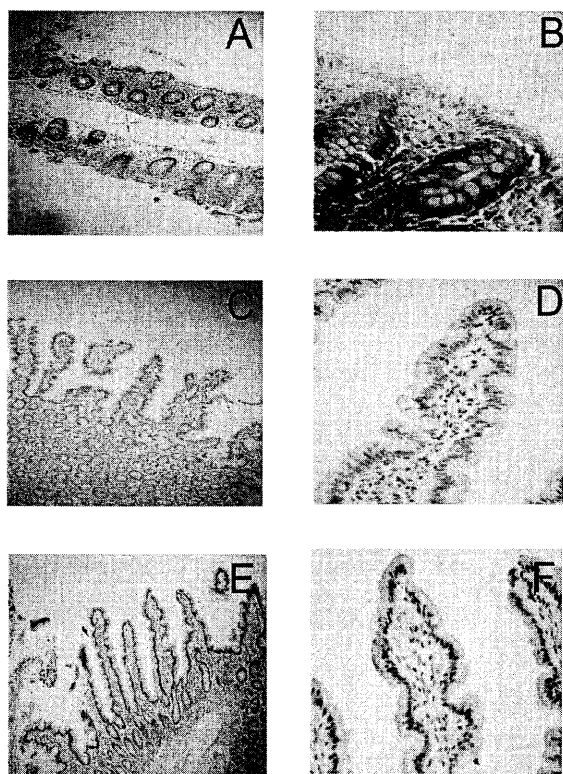
RESULTS

During the follow-up laboratory workup, specific IgE and IgG₄, AGA, EMA and tTG antibodies were found within the normal ranges. All the EGDS, even the last one (15th month), showed normal Kerckring folds. No macroscopic duodenal signs of CD (such as loss of folds, scalloped folds, mosaic or nodular mucosa) was found. IELs count was performed on every specimen: the mean basal ratio was 18 lymphocytes per 100 enterocytes, while it was 15 after 3 months of treatment, 25 after 6 months, 22 after 1

Table I. Protocol of desensitising treatment with gliadin

	GLIADIN FORMULATION	DILUTION	GLIADIN AMOUNT (once a day)
MONTH 1 Days 1-30	Hydroalcoholic solution (6.6 mg/ml)	From: 10^{-6} To: pure solution	From: 0.000001 mg To : 23 mg
MONTH 2 ♦ Days 31-40	Hydroalcoholic solution	Pure solution	From : 27 mg To : 70 mg
♦ Days 41-60	Freeze-dried gliadin (60%)	Undiluted	From : 75 mg To : 170 mg
MONTH 3 Days 61-90	Freeze-dried gliadin (60 %)	Undiluted	From: 175 mg To : 585 mg
MONTH 4 Days 91-120			From: 610 mg To : 2000 mg
MONTH 5 Days 121-150			From: 2070 mg To : 4230 mg
MONTH 6 Days 151-180	Rusks (60 %)		From: 4380 mg To : 9000 mg *

Corresponding to 15 g of gluten, which is the daily average dose of gluten of a standard diet.

**Fig. 1** - 39-year-old female patient.

A and B, biopsy during free dietary regimen: villous atrophy and irregular enterocyte profile with high IELs count (Hemat-Eos, A: 30x, B: 150 x). These bioptic findings are pathognomonic of celiac disease.

C and D, after 10-year gluten-free diet: the epithelium shows no abnormalities, the villous morphology and the IELs count are normal (Hemat-Eos, C: 30x, D: 150 x).

E and F, 1st month of treatment: no modification in terms of epithelium morphology, infiltration of lamina propria and IELs count compared to the previous (Hemat-Eos, E: 30x, F: 150 x).

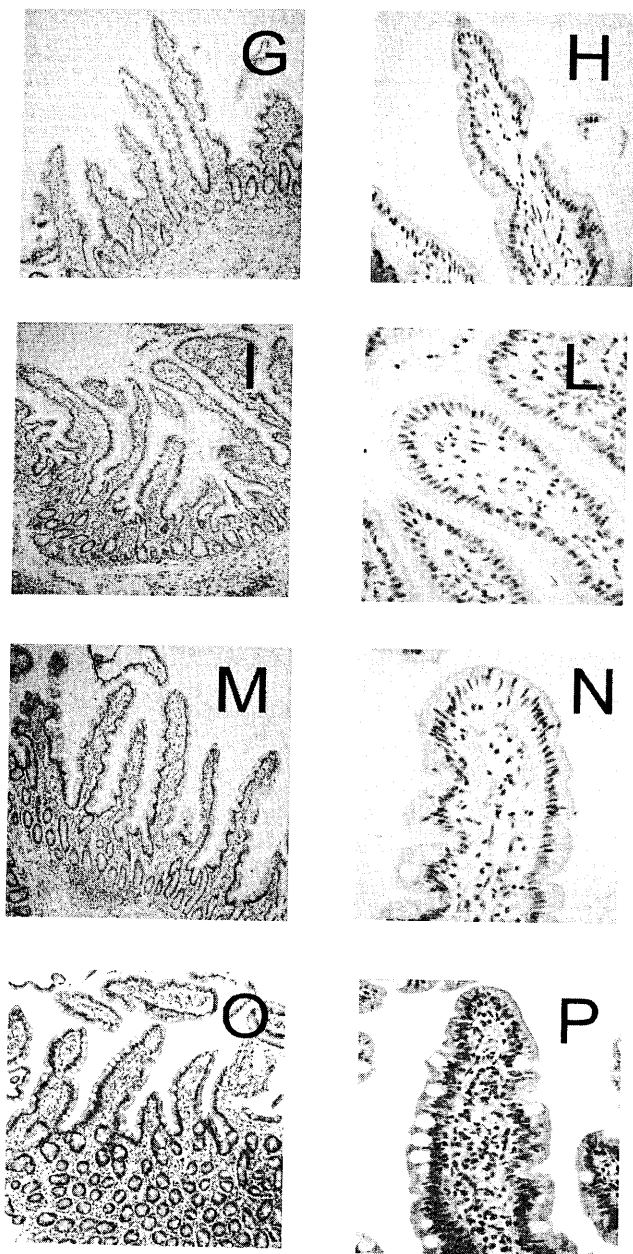


Fig. 2— Same patient as in Figure 1. A and B, 3rd month of treatment (see table 1); C and D: 6th month biopsy (see table 1); E and F: 9th month (see table 1); G and H, biopsy after free (glutencontaining) dietary regimen. All the biopsies show no modification in terms of epithelium morphology, infiltration of lamina propria and IELs count compared to the previous (Hemat-Eos; A, C, E, G: 30x; B, D, F, H: 150 x).

year. γ/δ count was 1 before the treatment and 0 in all the other specimens.

These results show a normal lymphocytes infiltration of the mucosa, according to Marsh criteria, without any significant increase of IELs compared to those observed before the gliadin treatment. (Figures 1-2).

DISCUSSION

The literature reports models of gluten dose-dependent intolerance, in which 100 mg is probably

near the threshold dose, able to cause a detectable intestinal damage in celiac patients. Actually, we did not find a threshold dose in our patient: she reached a maximum of 9 g of gliadin, corresponding to 15 g of gluten (average daily amount in a free diet), without any clinical symptoms, increase of auto-antibody titres and, above all, without histological changes. So our results do not agree with the previous studies, maybe because our results were achieved through a slow and increasing introduction of gliadin. In fact, the patient is back to a free

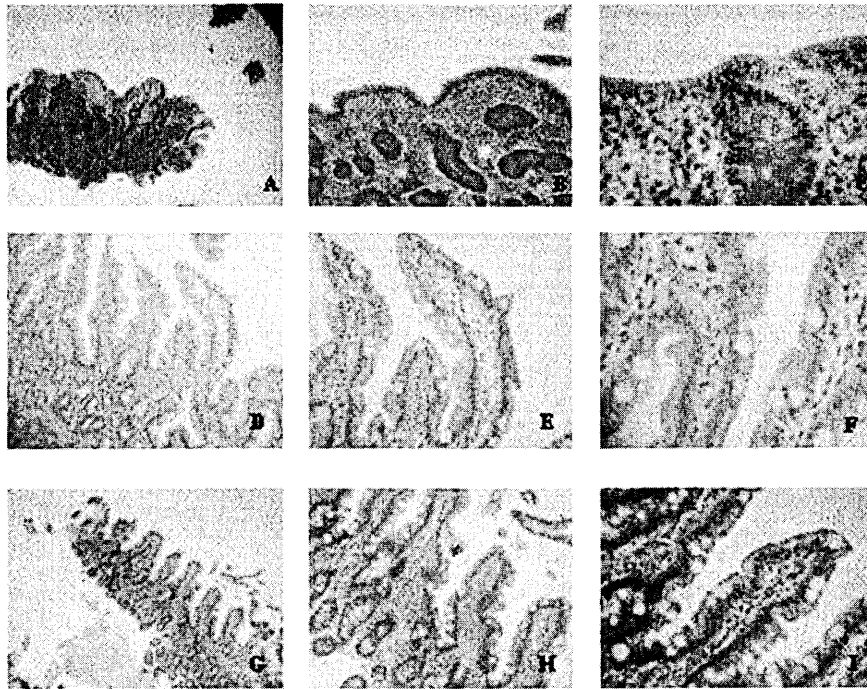


Fig. 3 – Duodenal biopsies of a 46-year-old celiac woman. A, B, C: “active” disease during free gluten-containing diet: villous atrophy and crypt hyperplasia, irregular enterocyte profile, epithelium highly infiltrated by IELs, lymphoplasmacellular infiltration of lamina propria (Hemat-Eos, A: 75 x, B: 150 x, C: 350 x). D, E, F: 1st month biopsy: the mucosa shows no abnormalities, the villous morphology and the IELs count are normal (Hemat-Eos, D: 75 x, E: 150 x, F: 350 x). G, H, I: 3rd month biopsy no modification of epithelium morphology, infiltration of lamina propria and IELs count compared to the previous (Hemat-Eos, G: 75 x, H: 150 x, I: 350 x).

dietary regimen since the 9th month without the occurrence of malabsorption. Currently, 20 months after beginning the study, she eats bread, pasta and other commercial wheat-based products (crackers, rusks, bread-sticks, biscuits, pizza) without restrictions. Chemistry and haematology results are normal. Also histological parameters, such as the IELs count (less than 30 lymphocytes per 100 enterocytes), γ/δ count (0 in all the specimens), the villous morphology and the villous height/crypt depth ratio, which are considered very sensitive diagnostic tools, show no modification if compared with basal specimens.

The mechanism which underlies the tolerance to gluten is unknown and probably different from the one which occurs during desensitisation in food allergy: in fact allergic patients present a switch from a Th2 to Th1 pattern, with consequent increase of specific IgG₄ and decrease of IgE.

Our patient is not allergic and did not show any

modification of specific IgG₄ and IgE and we are not able to explain how tolerance occurred. However, our results may represent a new approach, without previous references, in the research concerning CD.

Additional studies are currently being performed: in fact we are using the same protocol in other two celiac patients: the first has completed the 2nd month of treatment, the second has reached the amount of 4,23 g of gliadin (end of the 5th month) without clinical symptoms and modifications of histological and serological parameters (Fig. 3).

Even though “a swallow doesn’t make a summer”, our results could provide a future line of study for CD management, considering the increasing incidence and prevalence of this disease. Furthermore they could have an impact on the patients’ quality of life, considering that a gluten-free diet requires a high patient compliance, difficult to achieve, especially in teenagers.

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