Immunologic evidence of no harmful effect of oats in celiac disease\textsuperscript{1–3}

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ABSTRACT

Background: It was recently shown that antiendomysial antibodies (EMAs), which are highly sensitive and specific for celiac disease, are produced by intestinal mucosa. Furthermore, EMAs were detected previously in supernatant fluid from cultured duodenal mucosa specimens collected from untreated celiac disease patients and in culture media of biopsy specimens collected from treated celiac disease patients after an in vitro challenge with gliadin. Moreover, it was recently shown in vivo that oats are not toxic to celiac disease patients, suggesting the safety of oats in a gluten-free diet.

Objective: The objective was to better define the controversial role of oats in celiac disease to determine whether oats can be safely included in a gluten-free diet.

Design: We used an in vitro model to test whether oats induce EMA production in supernatant fluid from cultured duodenal mucosa specimens collected from 13 treated celiac disease patients. The biopsy specimens were cultured with and without peptic-tryptic digest (PT) of gliadin and avenin (from oats) and in medium alone. Samples from 5 of the 13 patients were cultured with the C fraction of PT-avenin. Indirect immunofluorescence was used to detect EMAs.

Results: EMAs were detected in specimens from all 13 patients after the challenge with gliadin but not after culture in medium alone. By contrast, no EMAs were detected in any of the specimens cultured with PT-avenin and its C fraction.

Conclusions: Because the in vitro challenge with PT-avenin and its C fraction did not induce EMA production in treated celiac disease patients, it appears that oats have no harmful effect on celiac disease. Therefore, oats can be safely included in a gluten-free diet. Am J Clin Nutr 2001;74:137–40.

KEY WORDS Avenin, celiac disease, oats, intestinal mucosa biopsies, antiendomysial antibodies, gluten-free diet

INTRODUCTION

Celiac disease, defined as permanent intolerance of the small-bowel mucosa to the storage proteins of cereals (1, 2), is one of the most common immunologically mediated gastrointestinal diseases in Europe (3). In this hereditary disease, an abnormal immune response to gliadin, long considered the pivotal event in the pathogenesis of celiac disease, initiates a cascade of as yet undefined events that lead to the tissue damage typical in celiac disease. Celiac disease is usually diagnosed after histologic examination of duodenal mucosa biopsy specimens. The disease is characterized by villous flattening with crypt hyperplasia, defined by a villous height–crypt depth ratio <3:1 (4).

The cereal constituents gliadins, secalins, hordeins, and avenins are deleterious to celiac disease patients. Consequently, these patients have been advised to avoid the consumption of cereals containing these substances in their gluten-free diet (GFD) (5). The consumption of oats in the GFD of patients with celiac disease is still controversial (1, 2, 6, 7). However, recent studies suggest that moderate amounts of oats can be included in the GFD of adult celiac disease patients and of patients with dermatitis herpetiformis without adverse effects (8–11).

Because of their high sensitivity and specificity, antiendomysial antibodies (EMAs) and not antigliadin antibodies (AGAs), although the culprit antigen of celiac disease is gliadins, have emerged as an outstanding tool in the screening and follow-up of celiac disease patients (12, 13). Moreover, our previous investigations showed that EMAs are produced by the duodenal mucosa of celiac disease patients (14, 15). These studies showed that EMAs, which are not present in the culture media of biopsy specimens from treated celiac disease patients, are newly produced in these patients after an in vitro challenge with gliadin or its peptides (14, 15). The aim of the present study was to clarify the immunologic effects of oats to determine whether oats can be safely included in a GFD. We carried out this aim by studying cultured duodenal mucosal biopsies collected from treated celiac disease patients consuming a GFD.

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TABLE 1
Number of patients in whom antiendomysial antibodies were produced in supernatant fluid

<table>
<thead>
<tr>
<th>Condition</th>
<th>Treated celiac disease patients</th>
<th>Control patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium alone</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medium + PT-gliadin</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Medium + PT-avenin</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Intestinal specimens from celiac disease patients in remission and in a control group of patients with gastrointestinal disease other than celiac disease were cultured for ≤72 h with a peptic trypsic digest (PT) of gliadin or avenin. Supernatant fluid from the cultured specimens was collected and used for detection of antiendomysial antibodies by indirect immunofluorescence.

SUBJECTS AND METHODS

Patients

Twenty adult celiac disease patients (9 men and 11 women aged 27.5 y; range: 18–59 y) in clinical remission were enrolled in the study. All of the patients had duodenal villous atrophy, had inflammatory cells in the lamina propria of the duodenal mucosa, and tested positive for serum EMAs while consuming a gluten-containing diet. The same patients, after consuming a well-controlled GFD for 12 mo, had no clinical symptoms of celiac disease and tested negative for serum EMAs. A second duodenal biopsy was performed in these patients before enrollment in the study. Admission criteria included no histologic signs of mucosal atrophy and no EMAs in undiluted supernatant fluid from biopsy specimens cultured with medium alone. Only 13 of the 20 patients met these criteria.

Thirty patients (15 men and 15 women aged 31.7 y; range: 21–60 y) with gastrointestinal diseases other than celiac disease (n = 21 with gastroesophageal reflux and 9 with ulcers) were enrolled in the study as a control group. Biopsies of duodenal mucosa were obtained from these 30 patients for diagnostic purposes. All procedures followed were in accord with the ethical standards of the responsible institutional committee on human experimentation.

Gliadin and avenin peptides

Peptic-tryptic digests of gliadin (PT-gliadin) and of avenin (PT-avenin) were obtained by enzymatic sequential digestion of prolamine fractions extracted from pure varieties of wheat bread (San Pastore) and oats (Astra) (16, 17). The C fraction of avenin was prepared by using affinity chromatography on Sepharose-6-B-mannan of N-acetylglucosamine (18).

Histologic analysis and biopsy culture

Three duodenal biopsy specimens were obtained from each patient by esophagogastroduodenoscopy. One specimen was submitted to hematoxylin-eosin histologic morphometric analysis to measure the villous height-crypt depth ratio, an index of mucosal atrophy, and to assess the presence of inflammatory cells infiltrating the lamina propria of the duodenal mucosa. A villous height–crypt depth ratio <3:1 was considered to suggest celiac disease. Each of the 2 other duodenal mucosa biopsy specimens was divided into 2 parts and cultured (16) for ≤72 h at 37°C as follows: in medium alone, in medium with PT-gliadin (1 g/L), in medium with PT-avenin (2 g/L), and with the C fraction of PT-avenin. Samples from only 5 of the 13 patients were cultured with the C fraction of PT-avenin; samples from the remaining 8 patients were cultured twice with PT-avenin. Supernatant fluid from the cultured biopsy specimens were collected and stored at −70°C until used. Because different samples from the same patient were cultured with and without PT-gliadin or PT-avenin, each subject acted as his or her own internal control.

Time course of in vitro antiendomysial antibody production

At 6, 24, and 48 h, the duodenal biopsy specimens cultured with PT-gliadin were washed in an isotonic sodium chloride solution (0.9%) and then replaced in fresh medium with PT-gliadin.

Viability test

The duodenal biopsy specimens cultured with PT-avenin were subsequently washed in an isotonic sodium chloride solution (0.9%) and then replaced in fresh medium with PT-gliadin (1 g/L) and cultured for an additional 48 h to determine viability, ie, whether the specimens could still synthesize EMAs.

Detection of antiendomysial antibodies

Indirect immunofluorescence analysis was used to determine the presence of EMAs in undiluted culture media and in sera diluted 1:5 with phosphate-buffered saline (16, 19) on cryostat sections of monkey esophagus (Eurospital, Trieste, Italy).

RESULTS

Detection of antiendomysial antibodies in culture supernatant fluids

All 13 celiac disease patients were tested for EMAs in supernatant fluid from the biopsies cultured with PT-gliadin (Table 1). In contrast, no EMAs were detected in supernatant fluid from the biopsies collected from the same patients and cultured with medium alone, PT-avenin, or the C fraction of avenin. Furthermore, no EMAs were detected in supernatant fluid from the biopsies collected from the 30 control patients and cultured with medium alone, PT-gliadin, or PT-avenin.

Time course of in vitro EMA production

EMAs were detected in supernatant fluid from 0 of the 13 patients after 6 h of challenge with PT-gliadin, in 8 of the 13 patients (61.5%) after 24 h of challenge with PT-gliadin, and in all 13 patients after 72 h of challenge with PT-gliadin. In all of the above-mentioned conditions, EMAs were detected in supernatant fluid diluted up to 1:8 with phosphate-buffered saline.

Viability test

In supernatant fluid from all 13 celiac disease patients, no EMAs were detected after 72 h of challenge with PT-avenin, but were newly produced in the same specimens after a subsequent 48-h challenge with PT-gliadin.

DISCUSSION

As is well known, cereals (Gramineae) are divided into 4 major groups: Bambusoideae, Pooideae, Panicoideae, and Chloridiodeae. The Pooideae group comprises 2 subgroups: Triticeae (wheat, rye, and barley) and Avena (oats) (20). The constituents of wheat
(gliadin), rye (secalin), barley (hordein), and oats (avenin) that are injurious to celiac disease patients are the alcohol-soluble protein fractions known as prolamines. The amino acid sequences Pro-Ser-Gln-Gln and Gln-Gln-Gln-Pro are considered the toxic fractions of these prolamines. At least one of these sequences exists in each of the above-mentioned cereals (21).

Although the question of whether to include oats in a GFD has been debated for many years, the effect of oats on the small-intestinal mucosa of celiac disease patients has been analyzed in relatively few studies (Table 2). Early observations were based on a small number of patients, mainly children, and were performed by using methods that are no longer considered acceptable for diagnosing celiac disease.

In some previous studies, a large number of adults with celiac disease in remission received an in vivo challenge with oats (8–11). No changes were observed in the mucosal villi architecture and no inflammatory cells had infiltrated the lamina propria; therefore, it was concluded that moderate amounts of oats can be included in a GFD without adverse effects (8–11). In 2 of these studies, sera negative for EMAs confirmed the histologic findings (9, 10).

Previous studies showed that testing for the presence of EMAs in supernatant fluid from cultured duodenal biopsies collected from treated celiac disease patients is suitable indicator of the immunologic effect of gliadin (14, 15). We used this in vitro culture system to test the specific immunologic effect (ie, EMA production) of oats in celiac disease. We showed that EMAs were not detected in the supernatant fluid after only 6 h of culture; however, EMAs were detected after 24 h in 8 of the 13 samples. It is important to note that EMAs were detected in all 13 samples after 72 h of an in vitro challenge with PT-avenin. Therefore, our data show that the culture time is crucial for EMA production. Moreover, in culture media obtained after the viability test, no EMAs were detected after 72 h of culture with PT-avenin. However, EMAs were produced after an additional challenge of the same specimens with PT-gliadin, suggesting that the EMAs were newly produced at this time and not simply released (14, 15).

Furthermore, our data also suggest that the specimens cultured with PT-avenin were still viable after 72 h and that the absence of EMAs in the supernatant fluid was proof of a non-toxic effect of oats in celiac disease despite the high concentration of oats used (2 g/L) and the long culture time (72 h). The same results were observed after challenge with the C fraction of PT-avenin, which is known to be the toxic fraction of cereal prolamines (18). In agreement with the findings of other studies (8–11), we conclude that oats can be safely included in the GFD of celiac disease patients. In addition, the organ culture system is suitable for testing the toxicity of different cereals in celiac disease patients.

### Table 2

<table>
<thead>
<tr>
<th>Reference and year</th>
<th>Patient group</th>
<th>Method of analysis</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicke et al (1), 1953 (n = 1)</td>
<td>Children with celiac disease</td>
<td>Fecal fat analysis</td>
<td>Steatorrhea</td>
</tr>
<tr>
<td>Dissanayake et al (6), 1974 (n = 4)</td>
<td>Children with celiac disease</td>
<td>Small-bowel biopsy</td>
<td>No histologic signs of celiac disease in 3 patients</td>
</tr>
<tr>
<td>Baker et al (7), 1976 (n = 12)</td>
<td>Children and adults with celiac disease</td>
<td>Xylose test</td>
<td>Positive results of oats toxicity</td>
</tr>
<tr>
<td>Janatuinen et al (8), 1995 (n = 92)</td>
<td>Adults with celiac disease</td>
<td>Small-bowel biopsy</td>
<td>No histologic signs of celiac disease</td>
</tr>
<tr>
<td>Srinivasan et al (9), 1996 (n = 10)</td>
<td>Adults with celiac disease</td>
<td>Small-bowel biopsy; AGA and EMA detection via in vivo challenge with avenin</td>
<td>No positive results of oats toxicity</td>
</tr>
<tr>
<td>Hardman et al (10), 1997 (n = 10)</td>
<td>Adults with dermatitis herpetiformis</td>
<td>Small-bowel and skin biopsies; AGA, ARA, and EMA detection via in vivo challenge with avenin</td>
<td>No positive results of oats toxicity</td>
</tr>
</tbody>
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AGA, antigliadin antibodies; ARA, antireticulin antibodies; EMA, antiendomysial antibodies. AGA, ARA, and EMA were detected in sera after in vivo challenge with avenin.

### References


