ORIGINAL ARTICLE

Oral Immunotherapy in Children With IgE-Mediated Wheat Allergy: Outcome and Molecular Changes

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Abstract

Background: IgE-mediated wheat allergy affects around 0.5% of the population, and current management is based on avoidance. We propose an active intervention to promote tolerance in wheat-allergic children.
Objectives: To investigate the efficacy and safety of an oral immunotherapy (OIT) protocol with wheat to treat IgE-mediated wheat-allergic children.
Methods: Six wheat allergic patients assessed in a double-blind, placebo-controlled food challenge (DBPCFC) underwent wheat OIT with an up-dosing phase until 100 g of wheat was tolerated, followed by a 6-month maintenance phase. Tolerance to rye and oat was evaluated, as were specific IgE (sIgE) to wheat and other cereals and sIgE, sIgG4, and sIgG1 to a panel of wheat proteins (α-amylase and trypsin inhibitors, wheat lipid transfer proteins, gliadins, and glutenins).
Results: Threshold doses in the wheat DBPCFC ranged from 6.6 g to 96.6 g. Five out of 6 (83%) patients successfully finished the up-dosing phase in 3 to 24 days; after a 6-month maintenance phase, all the patients maintained good tolerance of 100 g of wheat daily. Only 6.25% of doses in the up-dosing phase elicited mild adverse reactions. All 5 patients who successfully finished the up-dosing phase tolerated rye after OIT, and all but 1 tolerated oat as well. The median baseline wheat sIgE was 47.5 kU_A/L, increasing to 84.55 kU_A/L after up-dosing and decreasing to 28.75 kU_A/L after 6 months of follow-up. None of the patients showed sIgE to 5-ω-gliadin, but α-amylase inhibitors were recognized by all patients. Specific IgG4 and sIgG1 increased in all patients.
Conclusions: Our wheat OIT protocol was safe, efficient, and rapid. In our population, α-amylase was the major allergen.

Key words: Food allergy. Immunotherapy. Food immunotherapy. Oral immunotherapy. Wheat allergy. Gluten. α-Amylase inhibitors. 5-ω-gliadin. Children. LTP.

Resumen

Introducción: La alergia a trigo mediada por IgE afecta alrededor del 0,5% de la población, y su manejo se basa en la evitación. En este estudio proponemos un tratamiento activo para promover la tolerancia en niños alérgicos a trigo.
Objetivos: Investigar la eficacia y la seguridad de una pauta de inmunoterapia oral (ITO) con trigo para tratar la alergia a trigo mediada por IgE en una población de niños alérgicos a trigo.
Métodos: Seis niños diagnosticados de alergia a trigo mediante una provocación oral doble ciego controlada con placebo (PODCCP) se sometieron a un tratamiento de ITO con trigo con una fase de inducción hasta 100g, seguida de 6 meses de tratamiento en fase de mantenimiento. La tolerancia a avena y centeno también se investigó. Se determinaron la IgE específica (sIgE) a trigo y a otros cereales además de la sIgE, sIgG4 y sIgG1 para un panel de proteínas de trigo (inhibidores de α-amilasa y tripsina, LTP de trigo, gliadinas y gluteninas).
Resultados: Las dosis umbrales en la PODCCP con trigo variaron entre 6,6 y 96,6 g. Cinco de 6 (83%) pacientes finalizaron con éxito la fase de ascenso empleando de 3 a 24 días; después de 6 meses en fase de mantenimiento todos los pacientes mantuvieron buena tolerancia de 100g de trigo a diario. Únicamente un 6,25% de las dosis en la fase de inducción indujeron reacciones adversas leves. Todos los pacientes que finalizaron con éxito el tratamiento toleraron centeno tras la ITO, y todos salvo uno toleraron avena. La mediana de sIgE (kU_A/L) para trigo en el momento basal del estudio fue de 47,5, aumentando hasta 84,55 tras la dosis de ascenso y descendiendo a 28,75 a los 6 meses de la fase de mantenimiento. Ninguno de los pacientes presentaba sIgE para 5-ω-gliadina, pero los inhibidores de la α-amilasa fueron reconocidos por todos los pacientes. Se observó un aumento de la sIgG4 y sIgG1 en todos los sujetos.

Conclusión: Nuestro protocolo de ITO con trigo fue seguro, eficaz y rápido. En nuestra población los inhibidores de α-amilasa fueron el alérgeno mayor.

Palabras clave: Alergia alimentaria. Inmunoterapia. Inmunoterapia con alimentos. Inmunoterapia oral. Alergia a trigo. Gluten. Inhibidores de alfa-amilasa. 5-ω-gliadina. Niños. PTL.

Introduction

Wheat is one of the 10 foods that most frequently produce allergy in childhood [1], and the results of challenge testing show that wheat allergy affects up to 0.5% of the population [2]. Wheat is also a relevant aeroallergen that induces baker's asthma and pollen allergy [3]. Dietary restrictions imposed by wheat allergy may lead to nutritional imbalance. Furthermore, because of the widespread use of wheat in foodstuffs, patients might face avoidance difficulties, despite compulsory labeling of wheat content in Europe [4] and elsewhere, thus leading to accidental exposures and potentially life-threatening allergic reactions.

Wheat (*Triticum aestivum*) is a major cereal of the grass family Poaceae (Gramineae), and over 30 related allergens are involved in wheat allergy. Analysis of wheat proteins was traditionally based on the products of their extraction in a series of solvents, the so-called Osborne fractions [5], which include water-soluble proteins (albumins), saline-soluble proteins (globulins), and alcohol/water-soluble proteins (glutenins).

Management of wheat allergy is based on avoidance and use of rescue medication in accidental reactions. Since only 29% of patients affected by this allergy will outgrow it during the first 4 years of life and around 35% will still be allergic by the age of 12 years [6], interventions that promote tolerance are necessary. Taxonomic relationships mean that other members of the Gramineae family, especially the Triticeae (wheat, barley, and rye) and the Aveneae (oats) subfamilies, are also excluded from the diet of affected individuals owing to the high degree of cross-reactivity between them [7].

We investigated the efficacy and safety of a cluster oral immunotherapy (OIT) protocol to treat children with IgE-mediated wheat allergy, the allergens involved, and the resulting immunological changes. We also assessed the efficacy of wheat OIT in the treatment of allergy to other gluten-containing cereals.

Methods

Patient Recruitment

This study was performed at the Allergy Outpatient Clinic, Hospital Niño Jesús, Madrid, Spain. We searched our patient database to identify individuals diagnosed with IgE-mediated wheat allergy. None of the procedures required hospitalization. The study was approved by the Ethics Committee of Hospital Niño Jesús.

Patient Selection

Patients had to fulfill the following inclusion criteria: history of immediate symptoms (<2 hours) after wheat consumption; age 5 years or older; serum specific IgE (sIgE) to wheat >0.35 kU_A/L; a positive double-blind, placebo-controlled, food challenge (DBPCFC); and signed informed consent.

The exclusion criteria were as follows: non–IgE-mediated symptoms with wheat ingestion; positive antitransglutaminase or antiendomysial antibodies or confirmed celiac disease; malignancy; placebo reaction; and uncontrolled severe asthma.

Study Design

A baseline visit was conducted to update the patient's history, perform a physical examination and skin prick test (SPT), and obtain blood samples. If patients were eligible, the DBPCFC with wheat was performed. After the DBPCFC but before the wheat OIT, patient tolerance to oat was assessed by means of an open food challenge (OFC) with oat. After the up-dosing period, an OFC with rye was also performed. Follow-up visits were scheduled immediately after up-dosing was complete and 6 months later (Figure 1).

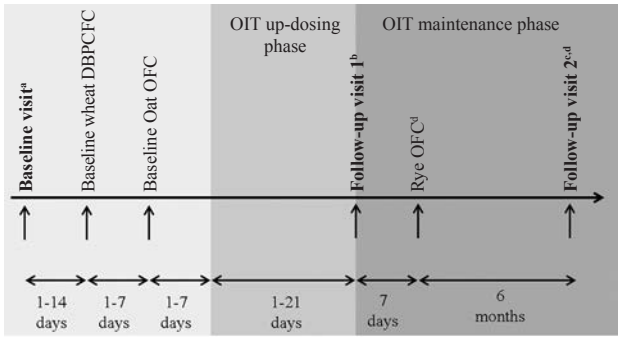

Figure 1. Study timeline. Detailed information about determinations in each visit.
[a]Physical examination; allergy history; informed consent; celiac disease screening; skin prick test for wheat, gluten, rye, and oat; total IgE; specific IgE (sIgE) (CAP, Thermo Fisher) to wheat, gluten, rye, oat, rice, maize, *Phleum*, rPhl p 12, rPru p 3, *Cupressus, Olea*, and *Platanus*. Serum sample for molecular diagnosis.
[b]Wheat skin prick test; total IgE; sIgE (CAP, Thermo Fisher) to wheat, gluten, rye, oat, rice, and maize. Serum sample for molecular diagnosis.
[c]Allergy history; celiac disease screening; wheat skin prick test; total IgE; sIgE (CAP, Thermo Fisher) to wheat, gluten, rye, oat, rice, and maize. Serum sample for molecular diagnosis.
[d]If the oat oral food challenge was previously positive, it was repeated.

Double-Blind Placebo-Controlled Food Challenge

Treatment with antihistamines, oral corticosteroids, and inhaled bronchodilators was forbidden before the DBPCFC. The challenges were performed under direct supervision, with full resuscitation equipment available. The DBPCFC was

performed by administering 9 doses at 20-minute intervals. For the active challenge, the first 5 doses were administered as durum wheat semolina porridge (Holle baby food GmbH, 10.6% protein), and the remaining doses were administered as boiled durum wheat semolina pasta (De Cecco, 13.0% protein) until a cumulative dose of 100 g (12.52 g of wheat protein) of dry wheat semolina was reached. The doses of porridge were 0.001, 0.01, 0.1, 0.5, and 1 g; the doses of pasta were 5, 10, 20, and 60 g.

For the placebo, a gluten-free porridge made of rice and maize was used for the first 5 doses, and boiled rice pasta for the last 4 doses. Wheat semolina and gluten-free porridges looked similar and taste was homogenized by adding sugar. As rice pasta was whiter than semolina, it was crushed and mixed with tomato sauce before serving to blind both taste and appearance.

Oral Immunotherapy Up-dosing Phase

After the DBPCFC, participants were prescribed daily cetirizine throughout the up-dosing phase. The first dose was determined according to the patient's individual threshold in the DBPCFC. Doses were increased twice weekly at our outpatient clinic, and daily maintenance doses were self-administered thereafter at home until the next increment. If up-dosing triggered symptoms, the last tolerated dose was repeated. The up-dosing sequence was as follows: first, semolina porridge at 0.0005, 0.001, 0.01, 0.05, 0.1, 0.5, 1, 2, and 4 g; then, semolina pasta at 8, 16, 30, 55, and 100 g (13 g of wheat protein).

Oral Immunotherapy Maintenance Phase

Once 100 g of wheat was tolerated in a single administration, the maintenance phase was started, and antihistamine treatment was tapered over 1 week. This dose was repeated daily for dinner (pasta, bread, cakes) under parental supervision with avoidance of exercise for 4 hours after ingestion.

Tolerance to Other Gluten-Containing Cereals

An OFC with 100 g of oat (oat flakes, BIOCOP) was performed after the positive wheat DBPCFC and before the OIT was started. The OFC dosage was 10, 20, and 70 g, administered at 20-minute intervals, with 2 hours of observation after the last dose. When the up-dosing phase had been successfully completed, the oat OFC was repeated only if it was previously positive, and an OFC with rye bread (organic whole-grain rye bread, PEMA) was performed with the same dosage as that used for oat.

Protein Extracts and Isolation of Purified Allergens

Protein extracts were obtained using bran and flour from different cereals following previously published methods [8]. Bran was dissolved in Tris HCl 0.1 M EDTA, 10 mM buffer, pH 7.5 at 1:20 wt/vol for 1 hour at 4°C with gentle shaking. The supernatant was then filtered and dialyzed using a 3500-D membrane for 2 days at 4°C with gentle shaking. The extract was frozen in liquid nitrogen and lyophilized overnight.

To obtain the gliadin- and glutenin-enriched extracts, flour was extracted twice with 50% 1-propanol and centrifuged for 10 minutes at 4500*g*. The supernatant was dried to obtain the gliadin-enriched extract. The precipitate was freeze-dried and extracted with 0.4 mL 50% propanol, 25 mM Tris HCl, pH 8.0, and centrifuged as above. The supernatant was dried to obtain the glutenin-enriched extract [9]. The protein extracts were quantified using the Bradford method [10].

In order to isolate the α-amylase inhibitors, the wheat extract was separated by molecular filtration into a monomeric fraction (0.28), dimeric fractions (0.53, 0.19), and tetrameric fractions (CM1, CM2, CM16, CM17). The isolation was completed with reverse-phase high-performance liquid chromatography (Beckman Coulter) using a linear gradient of acetonitrile in 0.1% TFA (0-85% in 120 minutes; 1 mL/min) on a Nucleosin 300 column (5 µm 8 × 250 mm) [11]. Tri a 14 (wheat lipid transfer protein [LTP]) [12] was isolated using a VacRC cartridge column (Waters) in 50 mM ammonium acetate and eluted with 1M NaCl. Protein quantization was carried out using the bicinchoninic acid assay. The purity of the samples was tested using the N-terminus amino acid sequence and mass spectrometry.

Immunological Parameters

Immunological parameters were evaluated at baseline, 1 week after up-dosing was complete, and after a 6-month maintenance phase. Baseline SPTs (Laboratorios LETI) were performed with wheat, gluten, rye, and oat [13]. Baseline total IgE was determined, as were specific IgE (sIgE) (CAP, Thermo Fisher) to wheat, gluten, rye, oat, rice, maize *Phleum*, rPhl p 12 (*Phleum profilin*), rPru p 3 (peach LTP), *Cupressus, Olea*, and *Platanus*. At the follow-up visits, SPT was performed with wheat, and sIgE was determined with wheat, gluten, rye, oat, rice, and maize.

Furthermore, sIgE, sIgG1, and sIgG4 to wheat and a panel of wheat proteins (CM1, CM 2, CM16, CM 17, 0.19, 0.53, 0.28, Tri a 14, peroxidase, gliadins, glutenins, and 5-ω-gliadin) were analyzed using ELISA at each time point [14]. ELISA plates were coated for 2 hours at 37°C with 5 µg/µL of each purified protein and 30 µg/µL of total wheat extract. After blocking, patient sera were incubated (1:20 dilution for IgE, 1:30 dilution for IgG1 and IgG4) overnight at 4°C. Specific IgE was detected by means of incubation with mouse antihuman IgE-peroxidase conjugate antibodies in a 1:3000 dilution for 1 hour at room temperature. Specific IgG1 and sIgG4 were detected by means of incubation with mouse anti-IgG1 or anti-IgG4 antibodies and goat antimouse IgG horseradish peroxidase–conjugated antibodies, both in a 1:10 000 dilution in PBS with 1:4 blocking solution. Binding was revealed with substrate solution, and absorbance was measured at 492 nm. The cutoff was calculated by the mean OD value plus 3 SDs of the healthy control sera (white) for each antigen.

Other Blood Tests

All patients were screened for celiac disease antibodies (IgA antitransglutaminase or antiendomysial antibody) before enrolment and after 6 months on maintenance with 100 g of wheat daily.

Statistical Analysis

Median and range were calculated for continuous variables; absolute and relative frequency (percentage) were used for qualitative variables. Given the small size of the population, only a descriptive analysis was performed.

Results

Baseline Patient Profile

We identified 14 patients with a diagnosis of wheat allergy; 7 were younger than 5 years and were thus excluded. Seven patients met the inclusion criteria, but 1 (a 7-year-old girl) was excluded because of uncontrolled severe asthma. Six patients were enrolled (5 boys, 83%); median age was 5.5 years (range, 5-11 years). Median age at the first episode of wheat allergy was 8.1 months (range, 7-9 months), and all patients had followed a strict gluten-free diet since then, although they tolerated gluten-free cereals.

All patients had atopic dermatitis. One patient had allergic asthma, and 2 had allergic rhinoconjunctivitis. The median greater diameter in the wheat SPT was 6 mm (range, 2-10), and all the patients had a positive SPT result to all the cereals. The results for specific IgE (kU_A/L) were as follows: wheat, median 47.5 (range, 17-481); rye, median 59.9 (range, 10-201); oat, median 14.3 (range, 2-36); maize, median 10.7 (range, 1-34); and rice, median 6.3 (range, 0.4-18). All patients were sensitized to all the pollens tested. Table 1 shows detailed sensitization profiles.

Table 1. Patients Baseline Allergic Profile[a]

Patient	1	2	3	4	5	6
Other food allergies	Fruit	Egg	Egg, Legumes, and Fruits	Milk, Nuts, Legumes, Fruits	Egg	Egg, Milk, Legumes, Fruit, Fish, Nuts
Other allergic diseases	AD, pollen allergic RC and asthma	AD	AD	AD, pollen allergic RC	AD	AD
Total IgE, kUA/L	512	2663	1124	1250	1182	636
Specific IgE, kU_A/L						
Wheat	67.3	481	16.9	25.2	130	28.2
Rye	36.2	201	10.3	13.7	77	21.5
Oat	6.93	34.6	1.44	4.69	36	2.17
Rice	2.47	11.9	0.4	3.41	18	1.81
Maize	8.72	34.5	2.34	6.51	11.1	1.05
Phleum	17.7	7.65	0.78	8.04	41	3.59
rPru p 3	2	37.5	4.41	45.1	3.04	1.35
rPhl p 12	1.56	0.26	0	1.73	0.25	0
Specific IgE, OD[b]						
CM1	0.244	0.440	0	0	NP	0.118
0.19	0.137	0.266	0	0.012	NP	0.037
0.53	0.192	0.432	0	0.05	NP	0.065
CM2	0.210	0.692	0	0	NP	0.014
CM17	0	0.214	0	0	NP	0
Peroxidase	0	0.131	0	0	NP	0
CM16	0.189	0.210	0	0	NP	0.012
ω-5 gliadin	0	0	0	0	NP	0
Tri a 14	0	0	0	0	NP	0
0.28	0.184	0.104	0.04	0.039	NP	0.165
Gliadin	0	0.186	0	0	NP	0
Glutenin	0	0	0	0	NP	0

Abbreviations: Abbreviations: AD, atopic dermatitis; NP, not performed; RC, rhinoconjunctivitis.
[a]Allergic background of each patient and sIgE to other cereals. *Phleum* and the relevant food panallergens recombinant peach LTP (rPru p 3) and recombinant *Phleum* profilin (rPhl p 12).
[b]Specific IgE for wheat proteins was measured by ELISA and is expressed in arbitrary units as optical density (OD) after subtracting the OD cutoff value for each particular determination.

Table 2. Summary of the Main Immunological and Clinical Parameters

Baseline		Double-Blind, Placebo-Controlled Food Challenge[a]					OIT Updosing Phase	
		Dose eliciting symptoms, g						
Patient No. (age), y	Wheat sIgE, kUA/L	Whole durum wheat semolina extract		Cumulative pure wheat protein	Symptoms	Treatment	Initial dose, g	Length, d
		Eliciting	Cumulative					
P1 (11)	67.3	20	36.611	4.72	Cough and generalized urticaria	Adrenaline (IM), corticosteroids, dexchlorpheniramine	8	15
P2 (5)	481	5	6.611	0.82	Cough, rhinitis, and hypotension (68/32 mmHg)	Adrenaline (IM), corticosteroids, dexchlorpheniramine	1	23
P3 (7)	16.9	60	96.611	12.52	8-10 isolated hives on back, face, and chest	Dexchlorpheniramine	55	3
P4 (5)	25.2	10	16.611	2.12	Vomiting	Dexchlorpheniramine	4	NA, failure
P5 (5)	130	5	6.611	0.82	Conjunctivitis, cough, profound malaise	Adrenaline (IM), corticosteroids, dexchlorpheniramine	1	24
P6 (6)	28.2	10	16.611	2.12	Rhinoconjunctivitis, urticaria, abdominal pain	Adrenaline (IM), corticosteroids, dexchlorpheniramine	4	19

Abbreviations: IM, intramuscular; NA, not applicable; NP, not performed; OIT, oral immunotherapy; sIgE: specific Immunoglobulin E.
[a]Doses eliciting symptoms in the double-blind, placebo-controlled food challenge are expressed in grams of whole extract (porridge for the first doses followed by pasta for the last doses), as well as grams of pure wheat protein.

Double-Blind Placebo-Controlled Food Challenge

All patients underwent the DBPCFC with wheat, and none reacted to the placebo. Immediate objective symptoms were recorded at a median dose of 2.12 g (range, 0.82-12.52 g) of wheat protein. No patients showed worsening of their atopic dermatitis, and 4 out of 6 (66.7%) experienced anaphylaxis [15], which was successfully treated with intramuscular adrenaline, oral/intramuscular corticosteroids, and antihistamines (Table 2).

Clinical Efficacy of Oral Immunotherapy

Five out of 6 patients (83%) successfully finished the up-dosing phase. Median duration and number of doses administered at hospital were 16 days (range, 3-24 days) and 6 doses (range, 2-9 doses), respectively. Patient number 4 discontinued the study voluntarily because of persistent adverse reactions during up-dosing.

Safety of Oral Immunotherapy

During up-dosing, 6 mild adverse reactions were recorded from 96 doses administered (6.25%); all occurred at the hospital while the dose was being increased. Five occurred in the patient who discontinued the study (patient 4, parental decision) and consisted of recurrent abdominal pain after wheat ingestion. Patient 2 experienced mild rhinitis with 8 g of wheat, although he successfully completed the treatment.

All the patients who completed the treatment ate wheat daily and were followed up for 6 months. Only patient 2 experienced a reaction during maintenance (generalized urticaria induced by exercise immediately after intake); the reaction was successfully treated with oral antihistamines and corticosteroids. The patient was strictly advised to refrain from exercise after ingestion of wheat and had no further adverse reactions. For the last 5 months, this child has continued to ingest 100 g of wheat daily with no further problems.

Tolerance to Other Gluten-Containing Cereals

Patients 2 to 6 underwent an open food challenge with oat before OIT, patient 1 refused to undergo the challenge, and only patient 6 had a positive result, with abdominal pain and generalized pruritus after eating 10 g of oat flakes. After up-dosing, patients 1 and 6 were challenged with oat; patient 6 tolerated the challenge, whereas patient 1 experienced generalized urticaria, cough, and vomiting 20 minutes after the second dose of oat (cumulative 30-g dose). All patients successfully finished the up-dosing phase and tolerated rye.

Immunologic Changes: SPT, sIgE, sIgG1, and sIgG4

The median greater diameter in the wheat SPT was 6, 4, and 2 mm at the 3 different time points. Median baseline sIgE to wheat was 47.5 kU_A/L (range, 17-481 kU_A/L), which increased immediately after up-dosing (median, 84.55 kU_A/L; range, 19.9-589 kU_A/L) but decreased after 6 months during maintenance therapy (median, 28.75 kU_A/L; range, 14.7-435 kU_A/L). Although these changes follow a trend, the differences were not statistically significant. Specific IgE to gluten, rye, oat, rice, and maize was positive in all patients at all time points with no relevant changes (data not shown).

All patients had sIgE to 0.28, 80% of them to 0.19 and 0.53, and 50% to CM2 and CM16. CM17, peroxidase, 5-ω-gliadin, Tri a 14, gliadin, and glutenin proved to be minor or unrepresented allergens (Figure 2). It is remarkable that ELISA revealed a lack of sIgE to 5-ω-gliadin at baseline and showed how it became positive in patient 2, who experienced an adverse reaction due to exercise during the maintenance phase.

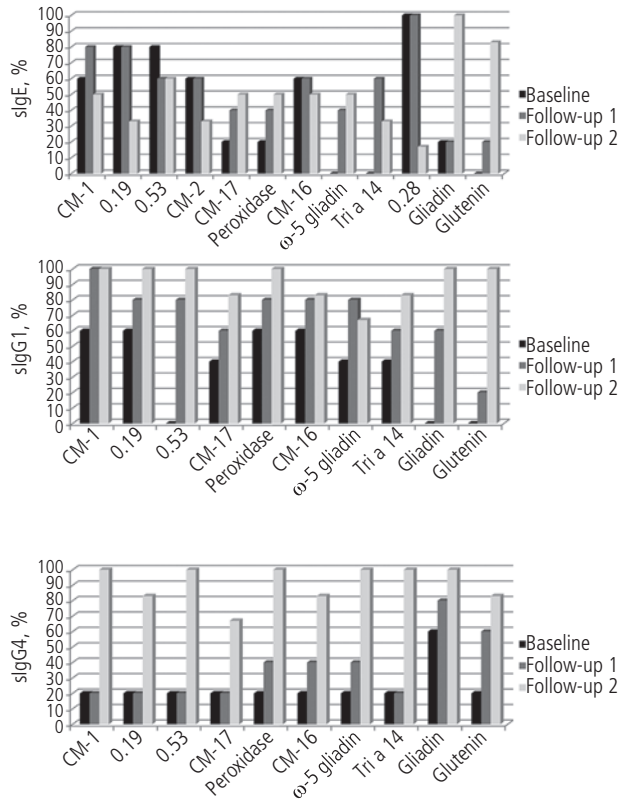

Figure 2. Percentage of patients showing sIgE, sIgG1 and sIgG4 to each protein at each time point. The left column represents the percentage of patients with a positive determination to each of the proteins specified.

Concentrations of sIgG1 to wheat and its proteins, with the exception of glutenins, became positive immediately after the up-dosing phase for more than half of the patients. Wheat and wheat protein sIgG4 were negative (except for gliadin) in all patients at baseline, except for patient number 2. After 6 months in the maintenance phase, most patients showed sIgG1 and sIgG4 to all proteins (Figure 2).

Celiac screening was negative for all patients at all stages.

Discussion

To our knowledge, this is the first cohort of patients with IgE-mediated wheat allergy treated with wheat OIT. Since the sensitivity of sIgE for the diagnosis of symptomatic wheat allergy is low [16-18], food challenges are mandatory. Several reports describe methods for wheat DBPCFC [17], although very few are specifically designed for a pediatric population. In our study, wheat allergy was assessed using a DBPCFC, which enabled us to confirm the diagnosis of wheat allergy and thus justify the use of OIT. Traditional recommendations for wheat challenges proposed starting the challenge with a dose of 100 mg of protein [17,18]; however, current guidelines aim for a lower dose of 3 mg [19]. We started with 0.13 mg of protein because, in our methodology, determining the lowest dose able to trigger symptoms was an essential step in shortening the up-dosing phase. Given that reported trigger doses in wheat allergy are high [18], we explored larger cumulative doses of protein than previously suggested (4.032 g [18] or 4.443 g [20]) in order to minimize false negatives and found that 2 out of 6 patients had symptoms with higher doses. Therefore, we propose that higher doses of wheat should be considered in wheat challenges.

Food-based OIT is now one of the best allergen-specific treatment options for active induction of tolerance in persistent food allergy [21]. The literature contains numerous reports with milk [22-25], egg [26-28], and peanut [29-31], as well as some scattered trials with other foods, such as apple [32]. However, despite the high prevalence of wheat allergy, we could only find 2 reports of cases treated with wheat OIT. Patriarca et al [33] and Nucera et al [34] described 2 patients who successfully tolerated 49 g of pasta 3 times/day after a 7-month up-dosing phase. Fujino et al [35] induced tolerance to 25 g of pasta in 2 pediatric inpatients within 8 and 10 days. In contrast, we induced tolerance to a larger amount of pasta (100 g) ingested as a single dose. Our protocol was shorter than those of Patriarca et al [33] and Nucera et al [34], and only a few days longer than that of Fujino et al [35], although it had the advantage that it could be administered in an outpatient regimen. Our protocol was safe, with few mild reactions (6.25%) affecting only one-third of the sample, and pragmatic, with dose escalations performed on an outpatient basis. It was also rapid, as shown by the short up-dosing phase, not only compared with other wheat OITs, but also with most of the aforementioned OIT trials.

Clinical tolerance to oat in wheat-allergic patients has not been extensively studied. Data on patients with wheat-dependent, exercise-induced anaphylaxis (WDEIA) showed low cross-reactivity between 5-ω-gliadin from wheat and oat allergens [36]. The patients in our study did not have WDEIA and were not sensitized to 5-ω-gliadin; however, it is remarkable that 4 out of 6 patients tolerated oat before treatment. In view of this finding, we propose that every wheat-allergic child should be challenged with oat despite the presence of sIgE, not only because of the rich nutritional properties of oat, but also to expand the patient's food choices. The differences observed between patient 1, who did not tolerate oat after treatment, and patient 6, who eventually became tolerant after treatment, should be studied in greater depth. In our population, once wheat allergy was proved in the DBPCFC, patients were

considered to be allergic to rye, again because its allergen profile is similar to that of wheat [7]; therefore, we can assume that wheat OIT induced tolerance to this closely related cereal.

The wheat allergen profile is complex, comprising soluble albumins and globulins, prolamins, α-amylase inhibitors, peroxidase, nonspecific LTPs, and profilins, all of which contribute to immediate respiratory hypersensitivity reactions to wheat [3]. Tri a 14 [8] has been described as a major allergen in baker's asthma syndrome, and ω-5-gliadin has been implicated as a marker of anaphylaxis to wheat in children [37] and of WDEIA [36]. However, food allergy to wheat seems most likely to be the result of sensitization to several allergens rather than sensitization to a single allergen.

In our population, the baseline sIgE recognition profile revealed a high prevalence of sensitization to 3 members of the α-amylase inhibitors family—0.28 (monomeric), 0.19 and 0.53 (both dimeric)—that play a key role in baker's asthma [3] and, less frequently, in wheat food allergy [38]. However, given the early onset of wheat allergy in the patients we report, exposure to either wheat pollen or flour is unlikely, suggesting that in this type I allergic population, sensitization to α-amylase inhibitors could have occurred through a route other than the respiratory tract. We could not demonstrate sIgE to 5-ω-gliadin, which is considered an effective marker not only of WDEIA, but also of wheat allergy [37,39], thus supporting the role of α-amylase inhibitors as the main allergens. It is noteworthy that all patients recognized Pru p 3 (peach LTP), but none showed sIgE to Tri a 14 (wheat LTP), which has been reported to have low cross-reactivity with LTPs from other families [12].

The immunologic changes observed are consistent with findings from other OIT studies with various foods [24,28,29,33] and provide further support that our wheat OIT protocol is immunomodulatory. Although increased sIgG4 has been reported [29] from 3-6 months after treatment, we observed an early increase in sIgG1 that has not been previously described in OIT. This increase in sIgG1 has been described in the disease modification induced by pollen-specific immunotherapy, although its role has not been fully elucidated [40]. It also remains to be determined whether this is merely a marker of exposure or functionally related to induction of tolerance.

Our study is limited by its small sample size and the absence of a placebo group. Nevertheless, we believe that our data provide a preliminary indication that this wheat OIT protocol may be safe and efficacious, even in a highly allergic population. As such, our study provides proof of concept for larger controlled trials to assess the therapeutic benefit of this approach to a major clinical problem.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Previous Presentation

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