Allergology International 68 (2019) 112–113

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Letter to the Editor

Quantification of the ω5- and γ-gliadin content in wheat flour and rat plasma with an enzyme-linked immunosorbent assay using antibodies specific to their IgE-binding epitopes

Dear Editor,

ω5-Gliadin and γ-gliadin are known as causative allergens for conventional and hydrolyzed wheat protein-type of wheat-dependent exercise-induced anaphylaxis (WDEIA), respectively.[1] As a wheat-eliminating diet is crucial for patients with WDEIA, measuring the amounts of ω5- and γ-gliadins, in processed foods or hypoallergenic wheat is helpful to ensure the safety of patients.[2–4] WDEIA is typically diagnosed based on the results of a positive-oral challenge test to exercise and/or aspirin intake in combination with wheat ingestion. Previous reports have shown that serum gliadin monitoring in combination with oral challenge tests was useful to exclude the false-negative results from diagnosis for WDEIA.[5,6] Thus, monitoring of ω5- and γ-gliadins content in plasma can further improve the precision of diagnosis for WDEIA using oral challenge tests. We previously produced rabbit polyclonal antibodies (Abs) specifically recognizing the IgE-binding epitope sequences (KQQSPEQQQFPQQQIPQQQ) of ω5-gliadin.[7] In this study, we also produced rabbit polyclonal Abs specifically recognizing the IgE-binding epitope sequences (QFLQPQQPFPQQPQ) of γ-gliadin.[4,8] Furthermore, we determined the content of ω5- and γ-gliadin in wheat cultivars and rat plasma after gluten ingestion using enzyme-linked immunosorbent assay (ELISA) with the derived Abs.

Polyclonal Abs (IgGs) against IgE-epitope peptides of γ-gliadin were produced using New Zealand white rabbits and affinity purified using allergen peptide. IgGs against the ω5-gliadin epitope peptide produced in a previous study was used.[7] To determine the contents of total ω5- and γ-gliadin in samples, sandwich ELISA were performed using each anti-gliadin Ab. This study was approved by the animal ethics committee of Hiroshima University (approval No. A16-138). All details are shown in Supplementary Methods.

We first confirmed the specific detection of wheat proteins with sandwich ELISA using the IgE-binding epitope Abs. The ELISA using the anti-gliadin Ab could detect total gliadin (Tokyo Chemical Industry, Tokyo, Japan) but not each recombinant gliadin component produced in *Escherichia coli* (Supplementary Fig. 1A).[4] The ELISA using anti-ω5-gliadin Ab specifically detected ω5-gliadin alone whereas the ELISA using anti-γ-gliadin Ab reacted with ω1,2-gliadin more strongly rather than γ-gliadins (Supplementary Fig. 1B, C). This might be reasonable because the repeating numbers of the QPQQPFPQ sequence in the epitope domains on ω1,2-gliadin (14 sites) were reportedly more than those on γ-gliadin (two sites) (Supplementary Fig. 2).[4] Therefore, detection of ω1,2-gliadin in wheat products can result in the overestimation of γ-gliadin content though the γ-gliadin content in wheat gluten is ≈ 5-fold higher than that of ω1,2-gliadin.[9]

Next, we determined the contents of total, ω5- and γ-gliadins in glutens from Hokushin, ω5-gliadin-deficient Hokushin presented by Kohno *et al.*[7] and Norin 61 as well as reagent gluten (Tokyo Chemical Industry) and hydrolyzed gluten (Glupearl 19S®, Katayama Chemical, Osaka, Japan). The concentrations of total, ω5-, and γ-gliadins in each sample were calculated using reagent, recombinant ω5- and γ-gliadins as a standard, respectively. The cut-off value was defined as the two-fold mean value of the standard deviation for each gliadin. The calibration curves for absorbance against gliadin content each fit the four-parameter logistic analysis. The detection limit of total, ω5-, and γ-gliadin using the ELISA with each Ab were obtained as 1.6, 0.2 and 3.1 ng/ml, respectively. The total gliadin content in gluten from Hokushin and ω5-gliadin-deficient Hokushin and the γ-gliadin content in all types of gluten exceeded 1 g/g gluten (Table 1). Wheats are polyploids with four or six sets of chromosomes and gliadin is encoded by plural genes, indicating that amino-acid sequences of native gliadin components can be slightly different among wheat cultivars. Thus, the results might be explained by differences in the affinities of anti-gliadin and anti-γ-gliadin Abs against each gliadin component between each sample and the recombinant protein used as standards. The total gliadin content in gluten from Norin 61 was slightly lower than those of Hokushin and ω5-gliadin-deficient Hokushin. Additionally, total gliadin from reagent gluten was significantly lower than those from all Japanese cultivars. The content of ω5-gliadin from ω5-gliadin-deficient Hokushin decreased to 20% of that from Hokushin, though no significant difference was observed between Hokushin and Norin 61. Our preliminary immunoblot assay showed that the anti-ω5-gliadin Abs slightly cross-reacted with other gliadin components except for ω5-gliadin. Thus, the slight detection by anti-ω5-gliadin Ab in the ω5-gliadin-deficient wheat may be due to the cross-reactivity to gliadin components except for ω5-gliadin. These results indicate that the ELISA using the anti-ω5-gliadin Ab is suitable to detect ω5-gliadin in wheat cultivars to a similar extent as using it to detect recombinant ω5-gliadin dissolved in solution. The γ-gliadin amount did not significantly differ among the three Japanese cultivars and reagent gluten. In hydrolyzed gluten, total gliadin was minimally detected but ω5- and γ-gliadins were not detected due to its degradation and deamidation.

Peer review under responsibility of Japanese Society of Allergology.

https://doi.org/10.1016/j.alit.2018.04.012

Table 1
Contents of total, ω5- and γ-gliadins in gluten from three wheat cultivars, reagent gluten and hydrolyzed wheat gluten.

Gliadin component	Total (g/g gluten)	ω5 (mg/g gluten)	γ (g/g gluten)
Hokushin	1.15 ± 0.13	5.17 ± 0.51	1.66 ± 0.38
ω5-gliadin-deficient Hokushin	1.25 ± 0.15	1.21 ± 0.10**	1.86 ± 0.62
Norin 61	0.61 ± 0.03	5.65 ± 0.90	1.51 ± 0.27
Reagent	0.17 ± 0.02**	3.49 ± 0.57	1.04 ± 0.13
Hydrolyzed wheat gluten	0.06 ± 0.00**	ND	ND

Reagent gluten was obtained from Tokyo Chemical Industry. Hydrolyzed wheat protein, glupearl 19S; ND, Not detected. Each value represents the mean ± SEM for three to six trials. **$p < 0.01$: significantly different from Hokushin.

Table 2
Plasma concentrations of total and ω5-gliadins 1 h after gluten ingestion.

Gliadin component	Total (ng/ml)	ω5 (ng/ml)
Gluten (−)	ND	ND
Gluten (+)		
Rat #1	361 ± 43*	19 ± 12
Rat #2	869 ± 218**	87 ± 28*

ND, Not detected. Aspirin was administered at a dose of 30 mg/kg 20 min before oral administration of gluten (50 mg/body). Blood was collected via the abdominal aorta 1 h after vehicle [Gluten (−)] or gluten (Rat #1 and Rat #2) administration. Each value represents the mean ± SEM for three to five trials. *$p < 0.05$, **$p < 0.01$: significantly different from control [Gluten (−)].

Finally, we attempted to measure the rat plasma concentrations of total, ω5- and γ-gliadins after gluten ingestion. Rat plasma was collected 1 h after ingestion of vehicle alone or gluten (50 mg) following oral aspirin administration (30 mg/kg). Gliadins were extracted from rat plasma with 70% ethanol and dissolved with Can Get Signal® solution-1 for the ELISA assay. Calibration curves were produced using rat plasma containing standard gliadins. The detection limit of total gliadin and ω5-gliadin were 100 and 3.1 ng/ml, respectively. However, we could not obtain the calibration curve for absorbance against γ-gliadin content because some impurities in plasma may interfere with the measurement of γ-gliadin by ELISA.

Total and ω5-gliadins could not be detected in the plasma from control rats administered vehicle with aspirin. In contrast, increased plasma concentrations of both gliadins were detected 1 h after gluten ingestion (Table 2), indicating that the total and ω5-gliadins that have multiple IgE-binding epitopes can be detected by ELISA in plasma using our epitope-specific Abs. According to the estimation suggested by Food and Drug Administration, 50 mg of gluten dose in rats corresponds to 1.3 g in humans.[10] The challenge tests for patients with WDEIA were performed by ingestion of wheat products containing 60–135 g of wheat flour, which includes 5.1–11.5 g of gluten.[5,9] Thus, total and ω5-gliadins that have multiple IgE-binding epitopes in plasma can be measured by ELISA using our epitope-specific Abs even in clinical challenge tests.

In conclusion, the ELISA using our epitope-specific Abs is useful to determine the content of each gliadin component in wheat cultivars and plasma from WDEIA patients. Gliadins detected in our ELISA have multiple IgE-binding epitopes. However, all detected gliadin may not necessarily exhibit the allergenicity due to their reduced molecular sizes by intestinal digestion. Further study is necessary to clarify the allergenicity of gliadins detected in our ELISA.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (No. 16K08371).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.alit.2018.04.012.

Conflict of interest
The authors have no conflict of interest to declare.

Tomoharu Yokooji [a,*], Hitomi Nouma [a], Ryohei Ogino [a,b], Takanori Taogoshi [a], Eishin Morita [b], Hiroaki Matsuo [a]

[a] *Department of Pharmaceutical Services, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan*
[b] *Department of Dermatology, Faculty of Medicine, Shimane University, Izumo, Japan*

* Corresponding author. Department of Pharmaceutical Services, Graduate School of Biomedical and Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8553, Japan.
E-mail address: yokooji@hiroshima-u.ac.jp (T. Yokooji).

References

1. Matsuo H, Yokooji T, Taogoshi T. Common food allergens and their IgE-binding epitopes. *Allergol Int* 2015;**64**:332–43.
2. Tatham AS, Morimoto K, Horikawa T, Osuna H, Ikezawa Z, Kaneko S, et al. Identification of the IgE-binding epitope in omega-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *J Biol Chem* 2004;**279**:12135–40.
3. Matsuo H, Kohno K, Morita E. Molecular cloning, recombinant expression and IgE-binding epitope of omega-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *FEBS J* 2005;**272**:4431–8.
4. Yokooji T, Kurihara S, Murakami T, Chinuki Y, Takahashi H, Morita E, et al. Characterization of causative allergens for wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat proteins in facial soap. *Allergol Int* 2013;**62**:435–45.
5. Kohno K, Matsuo H, Takahashi H, Niihara H, Chinuki Y, Kaneko S, et al. Serum gliadin monitoring extracts patients with false negative results in challenge tests for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergol Int* 2013;**62**:229–38.
6. Brockow K, Kneissl D, Valentini L, Zelger O, Grosber M, Kugler C, et al. Using a gluten oral food challenge protocol to improve diagnosis of wheat-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 2015;**135**:977–84.
7. Kohno K, Takahashi H, Endo TR, Matsuo H, Shiwaku K, Morita E. Characterization of a hypoallergenic wheat line lacking ω-5 gliadin. *Allergol Int* 2016;**65**:400–5.
8. Yokooji T, Okamura Y, Chinuki Y, Morita E, Harada S, Hiragun M, et al. Prevalences of specific IgE to wheat gliadin components in patients with wheat-dependent exercise-induced anaphylaxis. *Allergol Int* 2015;**64**:206–8.
9. Wieser H. Chemistry of gluten proteins. *Food Microbiol* 2007;**24**:115–9.
10. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). *Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*; 2005.

Received 12 March 2018
Received in revised form 25 April 2018
Accepted 26 April 2018
Available online 19 May 2018