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ARTICLE

Comparison of blood glucose levels and allergic responses on treatment with six wheat cultivars

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Abstract Allergies and indigestion problems have increased in our society with increasing wheat consumption. Among wheat proteins, omega-5 gliadins (ω5-gliadins) and high molecular weight (HMW)-glutenin are known to be some of the major factors responsible for an allergic response and indigestion. Certain low molecular weight (LMW)-glutenin subunits are also able to promote local inflammation, and share common epitopes with ω5-gliadins. It is known that different wheat cultivars have different combinations of HMW- and LMW-glutenins. We investigated the relationship between the composition of LMW-glutenins and the degree of allergic response or indigestion. The extent of allergic reaction and indigestion characteristics in response to six different wheat cultivars were compared. Patterns of the change of blood glucose level with time, which were employed to measure the indigestion, were quite different, depending on the type of wheat cultivars. The extent of allergic response, as measured with the histamine and IgE levels, was also quite different, depending on the type of wheat cultivars. In addition, the extents were not correlated with patterns of LMW-glutenin DNA and HMW/LMW glutenin protein of various wheat cultivars. The results from the present study suggest that some more other factors, together with factors studied above, need to be considered to better explain the physiological phenomena of variation in blood glucose level and allergic response.

Keywords Allergic response · Blood glucose level · Glutenin · Histamine · IgE · Wheat cultivar

Introduction

Wheat cultivation began about 10,000 years ago (Shewry 2009). Nowadays, it is one of the most popular cereals. According to the Food and Agriculture Organization of United Nations (FAO), 703.6 million tons of wheat was consumed in 2014, globally accounting for 41% of cereal calories (Shiferaw et al. 2013). Although wheat is the staple food in many countries, it is known to cause allergic hypersensitivity in some people. For example, the key factor of wheat-dependent exercise-induced anaphylaxis (WDEIA) was identified as omega-5 gliadin in wheat (Kati et al. 1999; Morita et al. 2003, 2009). Wheat proteins are mainly divided into salt-soluble proteins and salt-insoluble glutens, which include glutenins and gliadins, including omega-5 gliadin (Morita et al. 2009).

Wheat allergy is one of the common causes of food allergies (Joo et al. 2016). Continued and intensive research has revealed that a variety of protein factors in wheat is involved in the allergic reaction. It is known that two of the major factors among wheat proteins that are responsible for an allergic response and indigestion are ω5-gliadins and HMW-glutenin (Morita et al. 2009). LMW-glutenin is also

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able to promote local inflammation and shares common epitopes with ω5-gliadins (Wan et al. 2014). Histamine release is triggered when allergens of wheat proteins bind to mast-cell-bound IgE antibodies. When higher amount of free IgE is produced by external factors, there are higher chances of mast cells triggering more histamine release (Galli and Tsai 2012). Thus, we suspected that the pattern of exposure to LMW-glutenins subunits might be correlated with the extent of blood glucose release and secretion of histamine and IgE, manifesting as indigestion and allergic response, respectively. Various wheat cultivars have different combinations of whole-glutenins (including HMW and LMW). Therefore, treatment with various wheat cultivars would affect the levels of blood glucose and histamine/IgE in blood. In this study, we collected six different wheat cultivars to study this relationship. We have tried to understand the extent and factors of influence of different wheat cultivars on the blood glucose levels and secretion of histamine/IgE.

Materials and methods

Animals

Specific pathogen-free 4-week-old male C3H/HeJ mice and 7-week-old SD rats were purchased from Shizuoka Laboratory Center (Seoul, Korea) and housed in an air-conditioned animal room with a 12-h light/dark cycle at 22 °C and 50% humidity. Mice were maintained on a standard laboratory diet and water. The research was approved by the Korea University Institutional Animal Care and Use Committee (IACUC). All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85-23, revision at 1996).

Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed after fasting the animals for 18 h. Positive control group was administrated with 2 g glucose per kg of body weight, while negative control group received no treatment. Experimental groups were administrated with 2.86 g wheat flour per kg of body weight. Blood glucose level was measured with blood from caudal vein at 0, 30, 60, 90, 120, 150, and 180 min after glucose or wheat flour administration using a glucose meter. Positive control received glucose treatment.

Measurement of allergic response by intra-gastric sensitization with wheat flour

C3H/HeJ mice were sensitized intragastrically with 5 mg flour (wheat samples) and 1 mg aluminum at days 1, 2, 3, 7, and 21 (Marrack et al. 2009). Positive control group was sensitized with water and 1 mg aluminum. After 28 days, the C3H/HeJ mice were sacrificed, and blood samples were collected for further analyses. Blood samples of C3H/HeJ mice were centrifuged at 12,000×*g* for 30 min at 4 °C, and the resulting serum was collected. Total concentration of histamine and IgE in serum was quantified using histamine ELISA kit (Enzo Life Sciences, Farmingdale, NY, USA) and IgE ELISA kit (Affymetrix, Santa Clara, CA, USA), respectively.

Genomic DNA extraction

Genomic DNA was isolated using a plant DNA extraction kit (Exgene^{TM}, Geneall, Seoul, Korea) from wheat samples that were ground into powders. The concentration of DNA samples was measured using NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). All samples had A260/A280 absorbance ratios of >1.8 and A234/A260 ratios of 0.5–0.8.

Multiplex PCR for loci of LMW-glutenin

PCR was performed using an automated thermal cycler (Biometra, Gottingen, Germany). All reactions were performed in 25-μl microtubes containing distilled water, 2× PCR master mix (Biosesang, Seongnam, Korea), corresponding primers of multiplex PCR, and 1 μl of genomic DNA (Table 1). Total multiplex PCR sets were chosen for the following loci of LMW-glutenin: *Glu-1*, *Glu-A3*, and *Glu-B3*. Multiplex PCR patterns were amplified by three pairs of primers for each loci of LMW-glutenin: *Glu-Alc*/*Glu-Blbcf*/*Glu-Dld, Glu-A3ac*/*Glu-A3d*/*Glu-A3e*, and *Glu-B3d*/*Glu-B3 fg*/*Glu-B3h* for *Glu-1*, *Glu-A3*, and *Glu-B3*, respectively (Lei et al. 2006). Sequence of primers for identification of individual *Glu-1* were as follows: *Glu-Alc* forward, 5′-AAGACAAGGGGAGCAAGGT-3′; *Glu-Alc* reverse, 5′-GTGCTCCGCGCTAACATG-3′ (Lafiandra et al. 1997); *Glu-Blbcf* forward, 5′-TTCTCTGCATCA GTCAGGA-3′; *Glu-Blbcf* reverse, 5′-AGAGAAGCTG TGTAATGCC-3′ (Lei et al. 2006); *Glu-Dld* forward, 5′-GCCTAGCAACCTTCACAATC-3′; and *Glu-Dld* reverse, 5′-GAAACCTGCTGCGGACAAG-3′ (Smith et al. 1994). Sequence of primers for identification of individual *Glu-A3* were as follows: *Glu-A3ac* forward, 5′-AACAGAATTATT AAAGCCGG-3′; *Glu-A3ac* reverse, 5′-CTGTGCTTGGA TGATACTCTA-3′; *Glu-A3d* forward: 5′-TTCAGAT GCAGCCAAACAA-3′; *Glu-A3d* reverse, 5′-TGGGGTTG GGAGACACATA-3′; *Glu-A3e* forward, 5′-AAACAGAA TTATTAAAGCCGG-3′; and *Glu-A3e* reverse, 5′-GGCA CAGACGAGGAAGGTT-3′ (Wang et al. 2010). Sequence of primers for identification of individual *Glu-B3* were as follows: *Glu-B3d* forward, 5′-CACCATGAAGACCTTC

Table 1 Conditions of multiplex PCR

Multiplex PCR	*Glu-1*	*Glu-A3*	*Glu-B3*
Allele	*Glu-Alc/Glu-Blbcf/Glu-Dld*	*Glu-A3ac/Glu-A3d/Glu-A3e*	*Glu-B3d/Glu-B3 fg/Glu-B3h*
PCR reaction components			
Taq DNA polymerase (U)	2	2	2
Each dNTP (mmol/l)	2	2	2
One gene (μmol/l)			
Concentration of DNA (ng)	100	100	100
PCR amplification conditions			
Pre-denature	94 °C, 15 min	94 °C, 5 min	94 °C, 8 min
Denature	94 °C, 40 s	94 °C, 1 min	94 °C, 1 min
Annealing	60 °C, 1 min	60 °C, 1 min	58.5 °C, 1 min
Extension	72 °C, 1 min	72 °C, 1 min	72 °C, 2 min
No. of cycles	30	35	40
Final extension	72 °C, 5 min	72 °C, 5 min	72 °C, 10 min
Gel electrophoresis			
Agarose (%)	1.0	1.0	1.0
Voltage (V)	100	100	100
Time (min)	40	40	40

CTCA-3′; *Glu-B3d* reverse, 5′-GTTGTTGCAGTAGAAC TGGA-3′; *Glu-B3fg* forward, 5′-TATAGCTAGTGCAA CCTACCAT-3′; *Glu-B3fg* reverse, 5′-CAACTACTCTG CCACAACG-3′; *Glu-B3h* forward, 5′-CCACCACAACA AACATTAA-3′, and *Glu-B3h* reverse, 5′-GTGGTGG TTCTATACAACGA-3′ (Wang et al. 2009). *Glu-1, A3*, and *B3* in six wheat samples were amplified with PCR using primers mentioned above.

Analysis of glutenin composition using SDS-PAGE

We extracted wheat glutenins of six wheat samples using the method of Singh et al. (1991). Briefly, 125 μl of 50% 1-propanol and 0.08 M Tris–HCl (pH 8.0) including 1% of 1 M DTT (v/v) were added to 25 mg of ground wheat, which was then incubated for 30 min at 65 °C. After incubation, the samples were centrifuged at 10,000×*g* for 5 min. To alkylate the proteins, supernatants were transferred to another tube, and 125 μl of 50% 1-propanol and 0.08 M Tris–HCl (pH 8.0) containing 1% 4-vinylpyridine (v/v) were added. The samples were further incubated at 65 °C for 30 min and centrifuged at 10,000×*g* for 2 min. The supernatant was used for SDS-PAGE analysis. Bradford assay was used to quantify extracted protein. Samples were mixed with 2× laemmli sample buffer (BioRad, Hercules, CA, USA) at a ratio of 1:1 and denatured at 99 °C for 10 min, followed by loading onto a 10% polyacrylamide gel. Each sample containing 8 μg of protein was quantified using Bradford assay. Tris–glycine buffer (pH 8.3) was used as running buffer. For the first 30 min, the gel was run at 80 V, and for the next 120 min, the gel was run at 120 V. Finally, the gels were stained with Coomassie brilliant blue dye.

Results and discussion

It is known that a variety of wheat cultivars have unique proteins patterns, mainly due to the species-specific glutenin subunit patterns (Long et al. 2005). In the present study, six wheat cultivars were chosen, differing in their origins, wheat types, and cultivated year. Hard wheat type contains high extent of protein (10–14%) and soft wheat type contains low percentage of protein (8–12%) (Delcour et al. 2012). The six wheat chosen cultivars were as follows: hard red winter wheat (HRW), American origin, hard wheat type, year 2014; Australian hard wheat (AH), Australian origin, hard wheat, year 2014; Iksan Goso soft wheat (IGSW), Korean origin, soft wheat, year 2014; Gyeongnam Jokyung soft wheat (GSW), Korean origin, soft wheat, year 2014; Australian standard white wheat (ASW), Australian origin, medium wheat type, year 2015; Gwangju Kumkang wheat (GKW), Korean origin, medium wheat, year 2015. The extent of allergic responses and digestive problems with the wheat flours manufactured with the six wheat samples have been measured in this study.

Oral glucose tolerance test (OGTT) was performed after administration of six kinds of wheat flour. Figure 1A shows the change in the blood glucose level with time. In the first 30 min, blood glucose levels of all samples showed similar increasing pattern, except that of the negative control. Blood glucose level with ASW (E) dropped at 60 min,

while those of the other wheat flour samples increased further to variable extents. After 90 min, the levels generally decreased with different degrees. The average values of blood glucose levels at 90 min were in the following order: HRW (A) ≒ AH (B) ≒ IGSW (C) ≒ GSW (D) > GSW (F) > positive control > ASW (E) > negative control. At 180 min, the average values of blood glucose levels were in the following order: IGSW (C) > AH (B) > GSW (D) ≒ ASW (E) > GKW (F) > HRW (A) > positive control > negative control. Thus, patterns of changes in blood glucose levels over time differed, depending on the kind of wheat cultivar. We hypothesized that the trend of blood glucose level with each cultivar is related with varying combinations of glutenins from each cultivar, as discussed below. This is because glutenins are known to cause indigestion as well as wheat-dependent exercise-induced anaphylaxis (WDEIA) (Kozai et al. 2006).

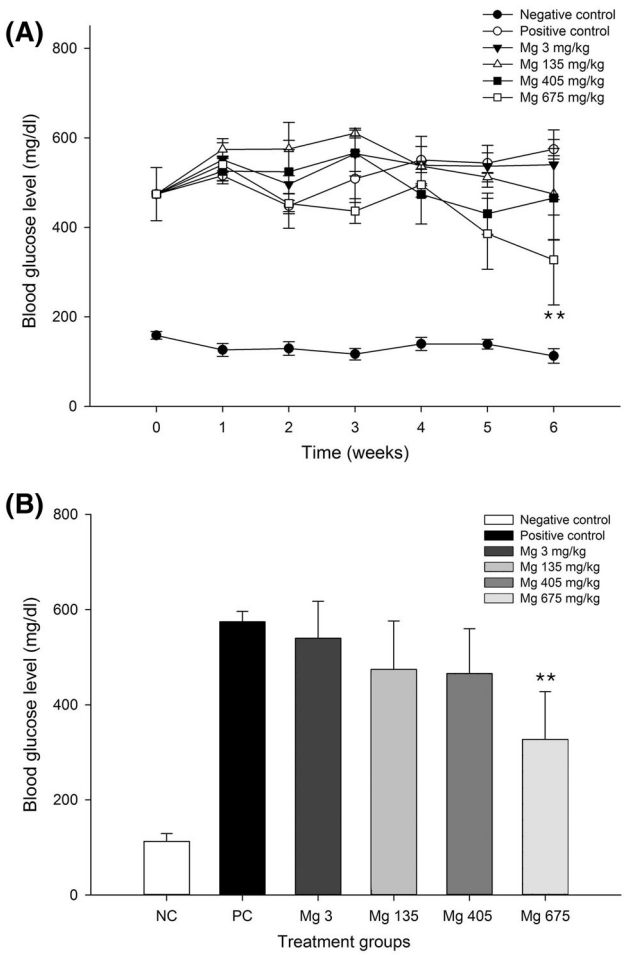

Fig. 1 Changes in blood glucose levels after administration of wheat flours from six wheat cultivars. *NC* negative control, and *PC* positive control. The wheat cultivars: *A* HRW, *B* AH, *C* IGSW, *D* GSW, *E* ASW, *F* GKW. Data are represented as mean ± SD ($n = 5$) at every 30 min (**A**). The blood glucose levels at 120 min after wheat flour administration. *$p < 0.05$, **$p < 0.01$, ***$p < 0.00$ (*t* test) (**B**)

The concentrations of histamine and IgE after administration of six kinds of wheat flours are shown in Fig. 2. The average histamine concentrations were in the following order: ASW (E) > GKW (F) > IGSW (C) > GSW (D) > AH (B) > HRW (A). The average concentrations of histamine were lower with HRW (A), AH (B), and GSW (D) treatment than those with positive and negative controls. The average concentrations of IgE were in the order of GKW (F) > ASW (E) > GSW (D) > HRW (A) ≒ AH (B) ≒ IGSW (C). The average concentrations were lower with HRW (A), AH (B), and IGSW (C) treatment than those treated with the other kinds of wheat flours. The extent and patterns of histamine and IgE concentrations did not coincide, but the three kinds of wheat flours, HRW (A), AH (B), GSW (D), induced much lower concentrations of

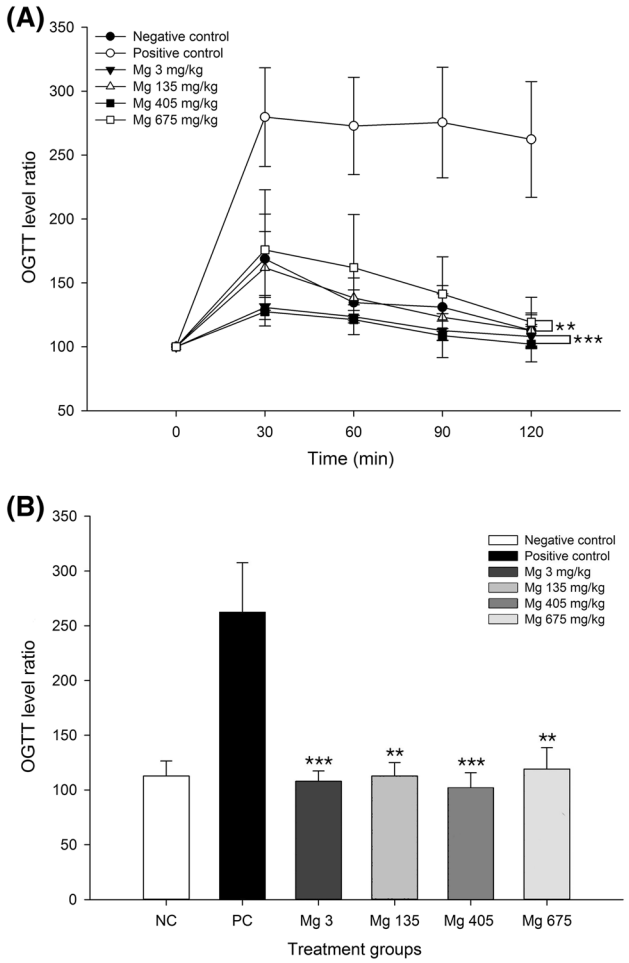

Fig. 2 Allergic responses observed as secretion levels of histamine and IgE in C3H/HeJ mice after administration of wheat flour from six wheat cultivars. Variation in the secretion levels of histamine (**A**) and IgE (**B**) according to wheat cultivars is shown. Data are the mean ± SD from triplicate experiments. *NC* negative control, and *PC* positive control. The wheat cultivars: *A* HRW, *B* AH, *C* IGSW, *D* GSW, *E* ASW, and *F* GKW. Data are the mean ± SD from triplicate experiments

histamine and IgE than the other kinds of wheat flours. This suggests that the extent of allergic response depends on the types of wheat cultivars.

We suspect that the results obtained above might be related to different combinations of glutenins from various wheat cultivars (D'Ovidio and Masci 2004). LMW subunits of glutenin are coded by genes at three genetically unlinked loci, *Glu-1*, *Glu-A3*, and *Glu-B3* (Shin et al. 2012). Thus, the different wheat cultivars could be endowed with variable combination of genes for glutenins, resulting in expression of characteristic patterns of glutenins. As shown in Fig. 3A, each gene group was amplified and visualized by multiplex PCR and agarose gel electrophoresis. Combination of glutenin genes was differed with each type of wheat cultivar. For example, the existence of *Glu-Alc*, *Glu-A3d*, *Glu-B3h*, and *Glu-B3d* genes was dependent on the kind of cultivar. Each cultivar could have distinctive characteristics for their LMW subunits of glutenin. We expected that ASW (E) and GKW (F) may have very similar LMW-glutenin patterns, as they showed higher histamine and IgE levels than the other wheat cultivars. However, their LMW-glutenin patterns were quite different, as observed from the selected alleles of *Glu-Did*, *Glu-A3d*, *Glu-B3h*, and *Glu-B3d*. The LMW-glutenin pattern of GKW (F) was more similar to those of IGSW (C) and GSW (D) than the other wheat cultivars. IGSW (C) and GSW (D) have shown lower histamine and IgE levels than that shown by GKW (F). This kind of phenomenon was also observed with SDS-PAGE patterns of proteins, including HMW/LMW glutenins from six wheat cultivars (Fig. 3B). The present results suggest that the allergic response, observed with the secretion extents of histamine and IgE, might not be directly correlated with HMW/LMW-glutenin patterns of various wheat cultivars, and some different factors, other than HMW/LMW-glutenin, could be involved in the same.

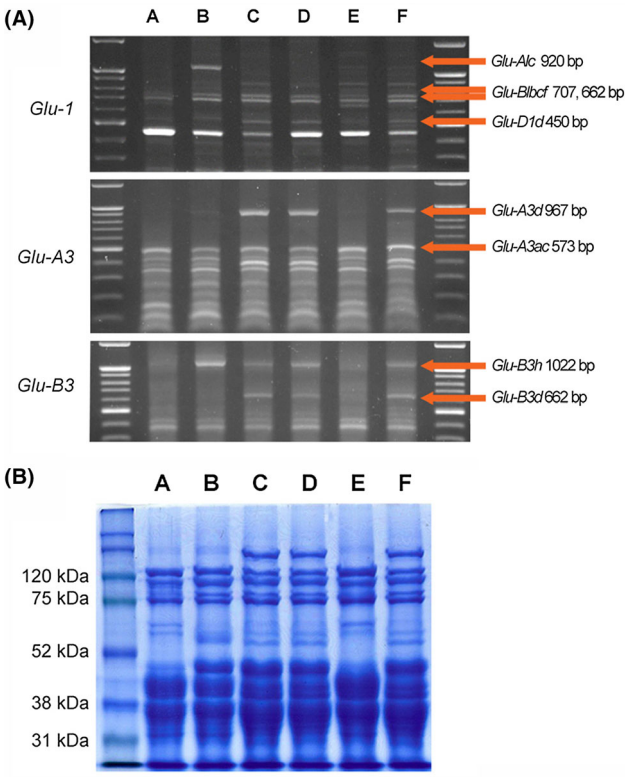

Fig. 3 Pattern analyses for alleles and proteins of HMW/LMW-glutenin of wheat flours from six wheat cultivars. Agarose gel electrophoresis after multiplex PCR amplification of *Glu-1*, *A3*, and *B3* (**A**). SDS-PAGE gel electrophoresis patterns for HMW/LMW-glutenin proteins of six wheat cultivars (**B**). The wheat cultivars: *A* HRW, *B* AH, *C* IGSW, *D* GSW, *E* ASW, and *F* GKW

LMW-glutenin was known to be one of the main factors involved in indigestion and allergic response (Pastorello et al. 2007). Figures 2 and 3 show our attempt to correlate the patterns of blood glucose levels/indigestion and allergic response with HMW/LMW-glutenin patterns of various wheat cultivars. However, no correlation was observed. The results of the present study indicate that physiological symptoms, such as variation in blood glucose levels and allergic response (i.e., histamine and IgE secretion), may not be directly explained by HMW/LMW-glutenin patterns of different wheat cultivars, but some other factors may be involved together with factors studied above. Further studies are necessary to better explain the causes underlying these phenomena.

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