Nutrition 58 (2019) 30–35

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Pilot study

Influence of polyphenolic content on the in vitro allergenicity of old and new apple cultivars: A pilot study

Josephine Kschonsek M.Sc. [a], Cornelia Wiegand Dr. [b], Uta-Christina Hipler PD, Dr. [b], Volker Böhm PD, Dr. [a,*]

[a] Institute of Nutritional Sciences, Friedrich Schiller University Jena, Jena, Germany
[b] Department of Dermatology, University Medical Center Jena, Jena, Germany

ARTICLE INFO

Article History:
Received 31 May 2018
Received in revised form 3 July 2018
Accepted 4 July 2018

Keywords:
Apple polyphenols
HPLC
Enzymatic browning
Birch pollen allergy
Mal d 1
CAST
BAT
IgE

ABSTRACT

Objectives: More than 70% of birch pollen-allergic individuals are affected by a cross-allergy from apples. The aim of this study was to determine if an increased polyphenolic content of apples is inversely related to clinical allergic reactions in sufferers.
Methods: The polyphenolic content of two old and two new apple cultivars was analyzed using high performance liquid chromatography. The in vitro concentration of sulfidoleukotrienes and the CD63 basophil activation of 27 birch pollen sufferers with cross-reactivity to apples were determined with cellular antigen stimulation and basophil activation tests after incubation with different apple cultivars.
Results: The flesh of old cultivars was characterized by significantly higher total polyphenolic content (86.1 ± 5.5 μg/g) than that of new cultivars (24.7 ± 7.2 μg/g). The concentration of sulfidoleukotrienes and the CD63 basophil activation of old apple cultivars was up to 62% lower than new ones and decreased as the degree of enzymatic browning increased.
Conclusion: Old apples cultivars are better tolerated than new ones by birch pollen-allergic individuals. The in vitro allergenicity (activation of effector cells) of apples depends on the total polyphenolic content and the degree of enzymatic browning.

Introduction

Birch (*Betula verrucosa*) pollen-allergic individuals often develop intolerance to apples (*Malus domestica*). The birch pollen-related apple allergy (BP-RAA) is a result of primary sensitiszation to Bet v 1 (*Betula verrucosa* 1) followed by immunoglobulin (Ig)E cross-reaction to homologous allergens in apples [1–3]. In northern and central Europe >70% of birch pollen-allergic individuals are affected by this cross-allergy because of the major apple allergen Mal d 1 (*Malus domestica* 1) [4,5]. Mal d 1 is an 18 kDA protein of the pathogenesis-related protein family 10 [6]. It is induced in peel and flesh by pathogen attack, abiotic stress factors, and fungal elicitors [7–9].

Over the past decades, BP-RAA has become the most important fruit allergy in Germany with about 4 million individuals showing clinical reactions to apples. This increasing number of allergic individuals is assumed to be associated with a higher consumption of new apple cultivars like Braeburn, Elstar, Golden Delicious, Granny Smith, and Jonagold. The allergen level differs between cultivars as demonstrated by enzyme-linked immunosorbent assay (ELISA) and immunoblotting [7,10–12]. Allergenic differences between apple cultivars are mainly related to expression levels of Mal d 1 [13]. Studies further showed that other internal factors like polyphenolic content, polyphenol oxidase activity, and antioxidant capacity affect apple allergenicity [8,13]. New apple cultivars are characterized by being bred from the same apple strains (Golden Delicious, McIntosh, Jonathan, Cox Orange, Red Delicious, and James Grieve) [14]. It is asserted that new apple cultivars have lower polyphenolic content than old cultivars as polyphenolic compounds were largely bred out, reducing the astringent taste and rapid enzymatic browning. It is thought that the lower polyphenolic content of new apple cultivars is responsible for their increased allergenicity.

Polyphenols are a class of bioactive compounds characterized by aromatic ring(s) with one or more hydroxyl moieties [15,16]. Flavanols, hydroxycinnamic acids, dihydrochalcones, flavonols, and anthocyanins are major classes of polyphenols that are commonly found in apples. Polyphenols are able to influence the development of allergic immune responses on two critical phases: during allergic sensitization and after re-exposure to the allergen [17]. The interaction of polyphenols with allergens can influence the sensitization by forming insoluble complexes. Furthermore,

* Corresponding author: Tel.: +49 3641 949633; Fax: +49 3641 94970.
E-mail address: volker.boehm@uni-jena.de (V. Böhm).

https://doi.org/10.1016/j.nut.2018.07.001
0899-9007/

polyphenols have an inhibitory effect on the secretion of mediators by effector cells (mast cells, basophils) [18]. The most studied group of polyphenols are phenolic acids and flavonoids, which are known to possess an anti-inflammatory and antiallergic potential [19].

It has been suggested that old apple cultivars are more tolerated than new ones, presumably because of higher polyphenolic content [20]. To our knowledge, this statement has not yet been scientifically confirmed. Thus, the present study focused on the investigation of the relation between the polyphenolic content and allergenic reactions to old and new apple cultivars. In a pilot study, in vitro allergenicity (activation of effector cells) of four different apple cultivars was determined in birch pollen-allergic individuals with a cross-allergy to apples using the cellular antigen stimulation test (CAST) and the basophil activation test (BAT). Apple polyphenols were quantified using high performance liquid chromatography with diode array detector, and the degree of enzymatic browning was determined spectrophotometrically.

Materials and methods

Chemicals

All solvents used were of HPLC grade and reagents of analytical grade. Chlorogenic acid, caffeic acid, and (+)-catechin were purchased from Carl Roth (Karlsruhe, Germany). Procyanidin B1, procyanidin B2, (-)-epicatechin, phloretin-2′-*O*-glucoside (phloridzin) dihydrate, and phloretin were from Sigma-Aldrich (Taufkirchen, Germany). Dulbecco's phosphate-buffered saline (PBS) was purchased from Pelobiotech (Planegg, Germany) and ethylenediaminetetraacetic acid-free Protease Inhibitor Cocktail from Roche (Basel, Switzerland). Water for solutions was obtained from a Milli-Q Purification System (Millipore, Merck, Darmstadt, Germany).

Selection of apple cultivars and preparation

Four different apple cultivars were chosen, including two old apple cultivars (Dülmener Rosenapfel and Ontario) and two new cultivars (Braeburn and Granny Smith). The apple cultivars were selected according to the reporting list of the BUND Lemgo from the year 2015, which is based on individual reports of allergic individuals about the tolerance of different apple cultivars. After peeling and coring, the flesh of each apple cultivar was homogenized using a mill (Retsch, Haan, Germany) and stored at −25°C until use. A fresh weight of 20 ± 0.1 g of apple flesh was weighed into a 100-mL Erlenmeyer flask. Samples were extracted by addition of 20-mL Dulbecco's PBS and two tablets of ethylenediaminetetraacetic acid-free Protease Inhibitor Cocktail and a shaking process overnight (4°C) [21]. The resulting extracts were filtrated and centrifuged (5 min, 16 000*g*). The supernatants were used for all following analyses.

Protein and Mal d 1 determination

Total protein concentration was determined with the Pierce BCA Protein Assay Kit from Thermo Fisher Scientific (Darmstadt, Germany). Color absorption was measured in a microplate reader (Fluostar Galaxy, BMG Labtech, Offenburg, Germany) at 595 nm. Bovine serum albumin was used as standard protein (25–2000 μg/mL).

Mal d 1 concentration was determined by competitive ELISA using a previously described method [3]. Human sera from birch pollen-allergic individuals for competition were provided by the Department of Dermatology, University Medical Center Jena.

Pilot study

Participants

The study involved 34 patients with allergy due to birch pollen with cross-reactivity to apples. Patients were recruited on the basis of their clinical history by completing a questionnaire and demonstration of specific IgE to rBet v 1 and rMal d 1 (ImmunoCAp, Phadia GmbH, Freiburg, Germany). Those patients who had undergone specific immunotherapy 4 wk before recruitment were excluded from the study. The other exclusion criteria were the use of drugs (antihistamines, antiallergic drugs, corticosteroids) and multiple sensitizations that might affect the study. Blood sampling for the investigation of in vitro allergenicity took place at the Department of Dermatology Jena. The study protocol including justification and details on blood sampling procedures was reviewed and approved by the Ethical Committee of the Friedrich-Schiller-Universität Jena at the Medical Faculty (Jena, Germany).

Sample preparation

Extracts of apple samples were diluted in PBS depending on the total protein content. Final concentrations of 0.1 mg/mL (CAST) and 1 mg/mL (BAT) were used, which was determined in preliminary experiments (data not shown). Each approach consisted of a negative control, stimulation control(s), and rMal d 1 (CAST: 200 ng/mL; BAT: 225 ng/mL) as positive control.

Cellular antigen stimulation test

The concentration of sulfidoleukotrienes ($sLTC_4$, $sLTD_4$, $sLTE_4$) was determined by ELISA with the CAST assay kit from Bühlmann (Schönenbuch, Switzerland). Color absorbance was measured spectrophotometrically at 405 nm. $sLTD_4$ was used for four-parameter fit regression (50–3200 pg/mL). Allergen stimulation has been considered positive at ≥200 pg sLT/mL after subtraction of the negative control.

Basophil activation test

Determination of CD63 basophil activation was carried out by flow cytometry with the Flow CAST assay kit from Bühlmann (Schönenbuch, Switzerland). A flow cytometer (BD FACSCanto^{M}, BD Biosciences, Heidelberg, Germany) was used with a 488-nm argon laser diode (blue green excitation light) and the following four parameters: forward scatter, side scatter, channels for the fluorochromes fluorescein (FITC), and phycoerythrin. Individual cell populations in a sample were identified depending on size and granularity due to scattered light. Basophils were selected from the cell population by anti-CCR3-phycoerythrin. Subsequently, activated basophils were determined by anti-CD63-FITC. The upregulation of the activation marker CD63 was calculated by the percentage of the CD63-positive cells compared with the total identified basophilic cells. Activation has been considered as positive at a percentage of ≥15%.

HPLC analysis of polyphenols

Polyphenolic compounds were analyzed using a previously described method [22] with slight modifications in the elution conditions (A: 0.5% acetic acid, B: methanol): 0–2 min (100% A), 2–6 min (100–85% A), 6–12 min (85% A), 12–17 min (85–80% A), 17–35 min (80% A), 35–70 min (80–65% A), 70–110 min (65% A), 110–125 min (65–20% A), 125–135 min (20–100% A). A reversed-phase Kinetex C18 column (250 × 4.6 mm, particle size 5 μm) (Phenomenex, Aschaffenburg, Germany) was used for separation at 20°C with a flow rate of 0.8 mL/min. Samples integration was set at 254 nm (flavonols), 280 nm (flavanols), and 320 nm (hydroxycinnamic acids).

Influence of enzymatic browning

The old apple cultivars (Ontario and Dülmener Rosenapfel) were used to analyze enzymatic browning. The apple cultivars were frozen at −80°C for 48 h. The frozen apples were peeled and the flesh was treated with liquid nitrogen. Subsequently, the homogenized flesh samples were subjected to a time-dependent browning. Extracts were made after 5, 10, and 15 min of enzymatic browning. The preparation of the extracts was carried out as described in section "Selection of apple cultivars and preparation". The degree of enzymatic browning was evaluated by using a previously described method [23]. The absorbance was measured at 420 nm using a spectrophotometer (V 530, Jasco, Groß-Umstadt, Germany). Six patients with BP-RAA were recruited using the criteria described previously to study the influence of enzymatic browning. CAST and BAT were performed with extracts without enzymatic browning and with time-dependent enzymatic browning (5, 10, and 15 min).

Statistical analysis

All analyses were done in duplicate. The data are expressed as mean ± standard deviation and were analyzed using the statistical program SPSS 22.0 (Statistical Package for the Social Sciences, Chicago, IL, USA). Values $P < 0.05$ were considered statistically significant. The homogeneity of variances for all data was assumed by Levene's test. Data without homogeneity of variances were transformed. The one-way factorial analysis of variance was used followed by the Student Newmann-Keuls procedure for assessing differences between all four apple cultivars. Differences between the average of the two old and the average of the two new apple cultivars were analyzed using the unpaired *t* test. For evaluating correlations, either the Pearson procedure (normally distributed data) or the