Note
Effects of Soybean Isoflavones on the Release of Chemical Mediators from Rat Peritoneal Exudate Cells by Allergic Reaction in Vitro

Mikako Takasugi¹, Kazuko Shimada², Koji Yamada³ and Hirofumi Arai⁴*

¹Department of Applied Chemistry and Biochemistry, Faculty of Engineering, Kyushu Sangyo University, 2-3-1 Matsukadai, Higashi-ku, Fukuoka 813-8503, Japan
²Department of Nutrition, Faculty of Nursing and Nutrition, Yamaguchi Prefectural University, 3-2-1 Sakurabatake, Yamaguchi 753-8502, Japan
³Division of Applied Biological Chemistry, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki Higashi-ku, Fukuoka 812-8581, Japan
⁴Department of Biotechnology and Environmental Chemistry, Kitami Institute of Technology, 165 Koencho, Kitami 090-8507, Japan

Received December 9, 2013; Accepted February 18, 2014

Soybean isoflavones are expected to reduce the risks of various diseases. In this study, anti-allergic effects of soybean isoflavones such as genistein and daidzein, and equol, a metabolite of daidzein, were investigated by measuring the chemical mediators, leukotriene B₄ (LTB₄) and histamine, released from rat peritoneal exudate cells (PEC) in vitro. Genistein and equol significantly suppressed the release of LTB₄ from PEC stimulated by calcium ionophore without cytotoxicity, whereas the inhibitory effect of daidzein was weak. In contrast, they had no effect on the release of histamine from calcium ionophore-stimulated PEC. These data suggest that the soybean isoflavones and metabolite may contribute to allergy symptom relief by inhibiting leukotriene production, but not histamine release.

Keywords: soybean, isoflavone, equol, leukotriene, allergy

Introduction

The number of patients with allergic diseases shows an increasing trend in Japan. It is known that mast cells play a key role in immediate-type hypersensitivity such as food allergies and hay fever. The cell signaling in mast cells is triggered by the cross-linking of high-affinity IgE receptors (FceRI) via IgE–antigen complexes on the cell surface, which induces protein phosphorylation and Ca²⁺ influx (Siraganian, 2003). This in turn promotes the hydrolysis of arachidonic acids from phospholipids of the cell membrane by phospholipase A₂. Leukotrienes (LTs) are produced by lipoxygenase (LOX) reactions with arachidonic acids and are then released into the extracellular space. Meanwhile, the cell signaling also leads to histamine release from mast cells by degranulation. LT and histamine are the chemical mediators that facilitate mucus secretion, smooth muscle contraction, and leukocyte chemotaxis, which cause immediate-type hypersensitivity.

Abbreviations

BSA; bovine serum albumin, FcεRI; high-affinity IgE receptors, HPLC; high-performance liquid chromatography, LOX; lipoxygenases, LT; leukotriene, PEC; peritoneal exudate cells

*To whom correspondence should be addressed. E-mail: araihrfm@mail.kitami-it.ac.jp
hypersensitivity (Amin, 2012; Galli et al., 2008). To date, anti-allergy drugs such as LT receptor antagonists, 5-LOX inhibitors (Scow et al., 2007), and histamine H1 receptor antagonists have been generally used for symptomatic treatment. In recent years, it has been expected that food components could improve allergy symptoms without side effects.

Isoflavones are a class of flavonoids that are abundant in legumes, generally as glycosides. Genistein and daidzein (Fig. 1) are the major soybean isoflavone aglycones generated because of intestinal digestion of their glycosides, genistin and daidzin, respectively. Furthermore, a part of daidzein is enzymatically metabolized to equol (Fig. 1) by intestinal bacteria. It has been suggested that genistein, daidzein, and equol are remarkable functional food components that may reduce the risks of diseases such as osteoporosis, cancer, and cardiovascular diseases (Ishimi et al., 1999; Mathey et al., 2007; Ren et al., 2001; Yamakoshi et al., 2000). Genistein, daidzein, and equol are known to behave as phytoestrogens and antioxidants by binding to estrogen receptors and G protein-coupled receptor 30 (Setchell et al., 2002; Kuiper et al., 1998; Thomas and Dong, 2006). Anti-inflammatory or anti-allergy effects of soybean isoflavones were reported by some research groups using allergic murine models (Bao et al., 2011; Masilamani et al., 2011). Genistein and daidzein also regulated mucosal immune responses; and the effect of genistein was stronger than that of daidzein (Wei et al., 2012). Our research group has reported that various polyphenols in foods can suppress the release of chemical mediators from rat peritoneal exudate cells (PEC) containing mast cells, rat basophilic leukemia cells (RBL−2H3), and human basophilic cells (KU812) in vitro (Matsuou et al., 1996; 1997; Tachibana et al., 2000). These papers imply that soybean isoflavones have the potential to modulate inflammation or the allergic system. However, the anti-allergic effects of soybean isoflavones have not been sufficiently addressed. In the present study, we examined the effects of genistein, daidzein, and equol on the release of LTB₄ and histamine from rat PEC in vitro.

Materials and Methods

Materials Genistein and daidzein were purchased from Funakoshi (Tokyo, Japan). (+) Equol was obtained from Extrasynthese (Genay, France).

Preparation of PEC Rat PEC were prepared according to the method provided by Matsuou et al. (1996). Tyrode buffer (20 mL/rat) containing 0.1% bovine serum albumin (BSA) was injected into the peritoneal cavity of 8-week-old male Sprague–Dawley rats (Kyudo, Saga, Japan), followed by gentle massaging of the abdomen for 2 min. After abdominal incision, Tyrode buffer containing PEC was collected and centrifuged at 200 × g for 10 min at 4°C. The precipitated cells were suspended in a modified ammonium chloride buffer (150 mmol/L NH₄Cl, 10 mmol/L KHCO₃, 10 mmol/L EDTA–2Na, pH 7.4) and incubated for 5 min at 4°C to remove contaminating erythrocytes.

LTB₄ release assay The induction of chemical mediators release from PEC and their determination were performed by Matsuou’s method with some modifications (Matsuou et al., 1996). PEC were re-suspended in 50 µL of Tyrode buffer (2.0 × 10⁶ cells/mL) containing 0.1% BSA, 0.9 mmol/L CaCl₂, and various concentrations of isoflavones dissolved in ethanol, and stimulated with 5 µmol/L calcium ionophore (A23187) at 37°C for 20 min. The stimulation was terminated by the addition of 50 µL of acetonitrile:methanol (30:25, v/v) containing 250 ng of prostaglandin B₂ as an internal standard. The cell lysate was centrifuged at 10,000 × g for 10 min, and the supernatant was subjected to high-performance liquid chromatography (HPLC) on an ODS-A column (150 × 6.0 mm I.D.; YMC, Kyoto, Japan) at room temperature. The samples were then eluted with 5 mmol/L ammonium acetate aqueous solution:acetonitrile:methanol: (9:6:5, v/v/v) at a flow rate of 1.0 mL/min and LTB₄ was monitored at 280 nm.

Histamine release assay PEC were re-suspended in 2 mL of Tyrode buffer (1.0 × 10⁶ cells/mL) containing 0.1% BSA, 0.9 mmol/L CaCl₂, and 100 µmol/L isoflavones, and stimulated with 5 µmol/L A23187 at 37°C for 20 min. The stimulation was terminated by cooling at 4°C for 15 min. The cell suspension was centrifuged at 300 × g for 10 min, and the histamine content of the supernatant was determined by fluorescence photometry as follows (Shore et al., 1959). One milliliter of the supernatant was mixed with 1 mL of Tyrode buffer, 0.75 g of NaCl, 0.5 mL of 5 mol/L NaOH, and 5 mL of n-butanol:chloroform (3:2, v/v) for 2 min. After centrifugation at 270 × g for 5 min, 4 mL of the organic solvent layer was recovered and mixed with 1.5 mL of n-heptane and 1.5 mL of 0.1 mol/L HCl for 2 min. After centrifugation at 270 × g for 5 min, 1 mL of the HCI layer was recovered and mixed with 0.15 mL of 1 mol/L NaOH and 0.1 mL of 0.2% o-phthalaldehyde. After incubation at room temperature for 5 min, the reaction was terminated by the addition of 0.14 mL of 0.25 mol/L H₂SO₄, and fluorescence intensity was then measured with excitation at 360 nm and emission at 450 nm. The percentage of histamine release was calculated as follows: histamine release (%)
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= (test − negative control)/(total − negative control) × 100. The supernatant from the unstimulated cells was used as the negative control, while the supernatant from the treatment with 5% Triton-X 100 was used as total.

**Statistical analysis**  Data are expressed as mean ± standard error. The statistical significance of differences was analyzed by the Tukey-Kramer test. Differences with \( p \) values of less than 0.01 were considered statistically significant.

**Results and Discussion**

Figure 2 shows the effect of genistein (A), daidzein (B), and equol (C) on the release of LTB\(_4\) from rat peritoneal exudate cells (PEC). Genistein (A), daidzein (B), or equol (C) were added to PEC at 1 – 100 μmol/L and then LTB\(_4\) release was induced by calcium ionophore (A23187). LTB\(_4\) was determined by HPLC with UV detection. Each value represents mean ± SE (n = 3). *^d Values not sharing a common letter are significantly different at \( p < 0.01. \) N.D.: not detected.

Fig. 2. Effect of genistein, daidzein, and equol on LTB\(_4\) release from rat peritoneal exudate cells (PEC).

Genistein (A), daidzein (B), or equol (C) were added to PEC at 1 – 100 μmol/L and then LTB\(_4\) release was induced by calcium ionophore (A23187). LTB\(_4\) was determined by HPLC with UV detection. Each value represents mean ± SE (n = 3). *^d Values not sharing a common letter are significantly different at \( p < 0.01. \) N.D.: not detected.

We have shown that flavonoids such as epigallocatechin gallate and myricetin can inhibit histamine release from rat PEC (Matsuo et al., 1996; Yamada et al., 1999). Several research groups have reported that eriodictyol, luteolin, and quercetin inhibited mast cell degranulation (Yoo et al., 2012; Kimata et al., 2000; Matsuda et al., 2002). Herein, we investigated the effect of genistein, daidzein, and equol on histamine release, another chemical mediator, from calcium ionophore-stimulated rat PEC. As shown in Fig. 3, 100 μmol/L of genistein, daidzein, and equol did not suppress histamine release from PEC by calcium ionophore stimulation. For this reason, genistein and equol may inhibit only LOX reaction generating LTs, while they may not suppress the degranulation resulting histamine release. It is known that PEC includes not only
mast cells, but also macrophages and neutrophils. LTB₄ produced by neutrophils are also important in allergic skin inflammation (Oyoshi et al., 2012). The cell type that is the target of isoflavones for anti-allergic effects will be clarified using cell lines in future studies.

In conclusion, the present data suggest that soybean isoflavones may have beneficial effects on allergy symptom relief by inhibiting LT production in mast cells. To clarify the mechanism of the anti-allergic effects of soybean isoflavones, further experiments on the cell signaling pathways induced by IgE–antigen cross-linking in vitro are needed.

Acknowledgements This work was supported by JSPS KAKENHI Grant Number15700475 and Fuji Foundation for protein research.

References


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