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Inhibitory Effect of Caffeine on Degranulation of RBL-2H3 Cells

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Abstract: It is well known that caffeine can have positive health effects. Caffeine consumption may reduce some of the risks of chronic diseases, diabetes, liver disease, and cancer, as well as improve immune function. Although caffeine has been shown to provide several benefits to human health, there are no reports on anti-allergic activity. In this study, we focused on the inhibitory effect of caffeine on the degranulation of basophils that may indicate the inhibition of type I allergy. Effect of caffeine on antigen-induced degranulation by rat basophil cell line, RBL-2H3 cells was evaluated. As a result, caffeine significantly inhibited the release of β-hexosaminidase from RBL-2H3 cells in a dose-dependent manner without cytotoxicity. Besides, caffeine suppressed the elevation of intracellular Ca2+ concentration induced by antigen.

Keywords: caffeine; degranulation; mast cell; anti-allergy

1. Introduction

Allergic disorders increase dramatically in some developed countries. Allergy is a global healthcare problem that significantly affects daily activities, work productivity, learning, sleep, and quality of life in people of all ages [1]. Allergic reactions represent an immune response to allergens entering into our body from the environment. Allergens may be house dust, pollen, and also foods such as milk, eggs, and wheat [2].

Allergy is classified into four types. Among them, the type I allergy is the most common allergic reaction associated with asthma, hives, hay fever, and allergic dermatitis. During the initiation of type I allergic reaction, the allergen binds to IgE molecules binding to high-affinity IgE receptors (FcεRI) on cell surfaces of mast cells and basophils. The cross-linking between the cell-bound IgE–FcεRI complex and specific antigen causes the aggregation of FcεRI, which induces a variety of cellular responses, including the release of chemical mediators such as histamine, β-hexosaminidase, leukotrienes, prostaglandins, pro-inflammatory cytokines, and chemokines [3-5]. The process of releasing such chemical mediators is defined as degranulation process which induces allergic reactions.

Caffeine (1,3,7-trimethylxanthine, $C_8H_{10}N_4O_2$) is a natural alkaloid or xanthine alkaloid found in coffee beans, tea leaves, cocoa beans, cola nuts, and other plants. It is one of the most widely used pharmacological substances in the world. Caffeine consumption in the world today is quite high. More than eighty percent of the world's population consumes caffeine every day both for stimulants, drug combinations and reducing jetlag [6-10]. This active compound can provide health benefits, such as a source of antioxidants, anti-cancer, anti-bacterial, and anti-inflammatory activities. This study aims to determine the inhibitory effect of caffeine on degranulation of RBL-2H3 cells.

2. Materials and Methods

2.1. Materials

The equipments used in this study were 15 mL and 50 mL centrifuge tubes, pH-meter, 5 mL syringe, 0.22 μm filters, 1.5 mL sample tubes, 50 mL glass beaker, blade grinder, electronic balance, autoclave, rotary mixer, high-speed centrifuge, cell counter, 96-well plates, and plate reader.

While the materials used in this study were caffeine, sterilized distilled water (DW), rat basophilic leukemia RBL-2H3 cells, sterilized phosphate buffered saline (PBS), fetal bovine serum (FBS), Dulbecco's Modified Eagle's medium (DMEM), anti DNP-IgE, DNP-HSA, substrate solution, TritonX-100, trypsin-EDTA (0.25% trypsin-0.02% EDTA-PBS), tyrode's buffer, glycine buffer, loading buffer for blank and sample, loading medium, WST-8 Solution, PVDF membrane, Towbin buffer, running buffer, filter papers, TBS-t (0.1%), 5% skim milk-TBS-t, 1st antibodies, actin, 5% BSA-TBS-t, goat anti-rabbit IgG, HRP-linked antibody, donkey anti-goat IgG, HRP-linked antibody, MeOH, and distilled water (DW).

2.2. Methods

2.2.1. Sample preparation

Caffeine was prepared with a concentration of 20 mM. Firstly, caffeine was weighed as much as 19.5 mg. A total of 3 mL of DW was added to dissolve caffeine. The pH of the sample was adjusted to 7.4 (±0.1), then DW was added to fill up to 5 mL. The caffeine solution was filtered using a 0.22 μm filter, and then it was stored in the freezer.

2.2.2. Cell seeding

RBL-2H3 cells were torn off by sterilized 0.05% EDTA-trypsin, stored in 15 mL centrifuge tube and centrifuged at 1,000 rpm for 5 min. This supernatant was removed, and the precipitated cells were suspended with 10% FBS-DMEM medium, and it was centrifuged at 1,000 rpm for 5 min. This supernatant was removed, and cell density was adjusted to 2.0×10^5 cells/mL. Then, 200 μL/well of suspension was added to 96-well culture plate and incubated for 18 h.

2.2.3. Degranulation assay

First steps of degranulation assay were conducted on the clean bench. Eleven milliliters of 5% FBS-DMEM medium was prepared in centrifuge tube. One milliliter of the solution was taken out and put into sample tube (for blank). Anti-DNP IgE solution was prepared. Then the 96-well plate seeded with RBL-2H3 cells was washed with 200 μL of PBS. After that, 120 μL of anti-DNP IgE solution was added to the 96-well plate and incubated for 2 h. The sample solution was prepared. After 2 h, the 96-well plate was observed under a microscope, and anti-DNP IgE was removed from the 96-well plate. The cells were washed twice with 200 μL of 1x Tyrode's buffer. Then 120 μL of sample solution was added to the 96-well plate and incubated for 10 min. The sample solution was removed, and 120 μL of 1x Tyrode's buffer was added to the plate. Then 10 μL/well of DNP-HSA solution was added to the 96-well plate and incubated for 30 min. During the incubation, 0.1% TritonX-100 solution and substrate solution were prepared outside the clean bench. After 30 min, the 96-well plate was taken out from the incubator and put on ice for 10 min. The supernatant was moved into the other 96-well plate on ice. Then 130 μL/well of 0.1% Triton X-100 solution was added to the cell lysate and crushed it using ultrasonic disintegrator for 5 sec/well on ice. After that, 50 μL/well of supernatant and cell lysate were moved into the other 96-well plate. The 96-well plate was incubated at 37°C for 5 min. After the incubation, 100 μL/wells of substrate solution and 100 μL/ well of 2 M glycine buffer were added to the wells alternately and incubated at 37°C for 25 min. After the incubation, 100 μL/well of substrate solution and 2 M glycine buffer were added to the wells on the reverse. Then the solutions in the 96-well plate were measured by optical absorbance at 405 nm.

2.2.4. Viability assay

After collection of culture supernatant, wells were washed with PBS. One hundred μL of 10% FBS-DMEM medium and 10 μL of WST-8 were added to each well and plate was incubated in the dark. The absorbance was measured at 450 nm and the reference at 655 nm by a plate reader.

2.2.5. Intracellular Ca^{2+} assay

Intracellular calcium assay was carried out after the cell seeding was conducted. Anti-DNP IgE was diluted with 10 mL of 5% FBS-DMEM at 10,000 folds. After 18 h of incubation (cell seeding), RBL-2H3 cells were taken out from the incubator, and the medium was removed from each well. The cells were washed with sterilized PBS, and 120 μL of anti-DNP IgE solution was added to each well, then the plate was incubated for 2 h. During the incubation, loading buffers and loading medium were prepared. After the incubation, the cells were taken out from the incubator, and then anti DNP-IgE solution was removed. The cells were washed with PBS twice, and 100 μL loading buffers (for blank and sample) were added to each well, then it was incubated for 1 h. During incubation, DNP-HSA solution was diluted at 16,000 folds. Then loading buffers were removed, and the cells were washed with PBS twice. After that, 100 μL of recording medium was added to each well and then incubated for 10 min. After incubation, the plate was measured by fluorescence plate reader (at 0 min). Ten μL of DNP-HSA solution was added to each well (outside the clean bench), and then fluorescence intensity was measured by fluorescence plate reader (at 1-30 min).

2.2.6. Statistical analysis

Data were represented as the mean ± SD. Statistical analysis was performed using Dunnett's test to identify significant differences between groups.

3. Results and Discussion

3.1. Effects of caffeine on the antigen-mediated degranulation of RBL-2H3 cells

We examined the effects of caffeine on the antigen-mediated degranulation of RBL-2H3 cells. This study was aimed to examine the inhibitory effect of caffeine on β-hexosaminidase release along with degranulation by RBL-2H3 cells. As shown in Fig. 1, caffeine inhibited the release of the β-hexosaminidase from antigen-induced RBL-2H3 cells, suggesting that caffeine can suppress the release of histamine. Caffeine concentrations used in this experiment were 0.16, 0.62, 2.5 and 10 mM, and caffeine at these concentrations inhibited the release of β-hexosaminidase by 2.8%, 18.1%, 51.7%, and 67.6%, respectively. We found that caffeine at higher concentration had a higher inhibitory effect on degranulation. In addition, caffeine had no cytotoxic effects on RBL-2H3 cells (Fig. 2).

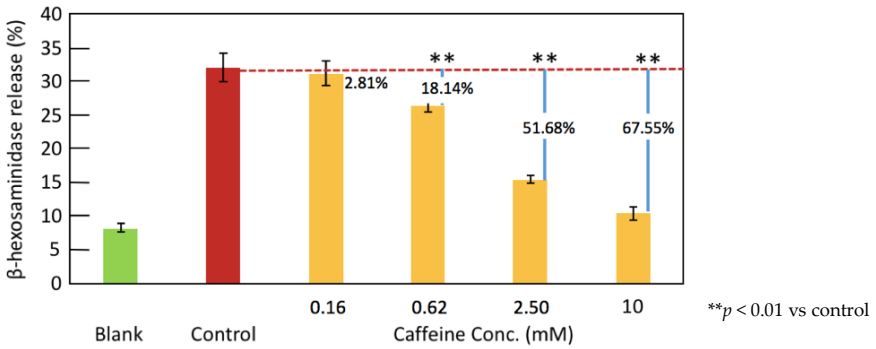

Figure 1. Effect of caffeine on degranulation by RBL-2H3 cells

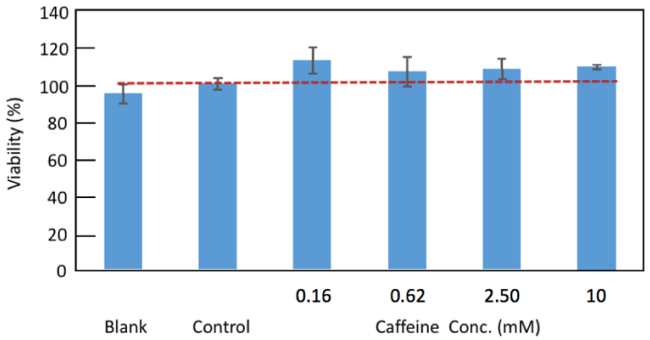

Figure 2. Cytotoxicity of caffeine on RBL-2H3 cells

3.2. Effects of caffeine on Ca^{2+} concentration in RBL-2H3 cells

To clarify the mechanisms that underlie the inhibitory effects of caffeine on antigen-stimulated degranulation, we determined the degranulation-mediated Ca^{2+} influx uptream of degranulation response. Ca^{2+} concentration in RBL-2H3 cells is increased by the antigen-antobody induction. In this experiment, we examined the effect of caffeine on antigen-antobody induced increase in Ca^{2+} concentration in RBL-2H3 cells. As a result, caffeine suppressed increase in Ca^{2+} concentration induced by antigen in a dose-dependent manner.

4. Conclusions

Caffeine was found to be capable of inhibiting antigen-stimulated degranulation in RBL-2H3 cells. The inhibitory effects of caffeine on antigen-stimulated degranulation in RBL-2H3 cells were demonstrated, and this effect was related to the suppression of Ca^{2+} influx.

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