Note

Effects of Tea Infusions of Various Varieties or Different Manufacturing Types on Inhibition of Mouse Mast Cell Activation

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We investigated effects of various tea infusions on mast cell activation using mouse mast cells. Among various tea extracts, infusions from cultivar 'Benihomare' and Taiwan lineage strongly inhibited histamine release after FcεRI cross-linking. Among three types of tea (from cultivar 'Benihomare'), extract from oolong tea or black tea inhibited histamine release more strongly than green tea extract. Furthermore, 'Benihomare' oolong tea extract suppressed tyrosine phosphorylation of cellular proteins after FcεRI cross-linking, but polyvinyl polypyrrolidone treatment of the extract to remove phenolic compounds, weakened the suppressive effect.

Key words: mouse mast cells; 'Benihomare' oolong tea extract; histamine release; PY protein; anti-allergic action

It is well known that tea (Camellia sinensis) has various pharmacological effects, such as anti-allergic action, carciogenesis inhibitory effect, anti-cancer metastasis action, antioxidative activity, anti-dental caries action, and anti-bacterial action. Allergy is an excessive immune response, and mast cells trigger immediate hypersensitivity-type allergic diseases. Mast cells are activated by cross-linking of surface bound to IgE, release chemical mediators (such as histamine and leukotrienes), and secrete several cytokines that are responsible for late-phase allergic reactions. With a great deal of the recently accumulated knowledge on mast cell activation, it will be worth trying to find pharmacological agents to prevent or treat allergic diseases. So, we examined possible anti-allergic effects of tea. This manuscript describes the effect of various type of tea extract on histamine release, and tyrosine phosphorylation of cellular protein in mouse mast cells after FcεRI cross-linking.

Tea extracts in hot water (tea infusion) were prepared from various varieties of microwave-dried tea leaves (first-cropped in 1997) as follows; 'Yabukita', 'Asahi', 'Takachiho', and 'Hatsumomiji' for green tea, 'Taiwan keitou #1', 'Taiwan keitou #2', 'Sansashirae', 'Seishini taipan', 'Seishin oolong', 'Obba oolong', and 'Koukan' for oolong tea, 'Benihomare', 'Cd70', 'Inatsu 131', and 'IND113' for black tea. Ten grams of dry tea leaves were boiled for 30 min in 100 ml of distilled water, filtered, lyophilized, and dissolved in phosphate buffered saline (PBS) for use. Azelastine hydrochloride, which was used as positive control, was kindly provided by Eisai Co. Ltd. Fresh tea leaves (cultivar 'Benihomare') were manufactured into green tea, oolong tea, and black tea.

WEHI-3b cells (IFO 50296), mouse myelomonocytic cells were purchased from Institute for Fermentation (Osaka, Japan) to obtain supernatants containing murine interleukin-3 (IL-3). IL-3-dependent mouse mast cell line MC/9 cells (ATCC CRL1649), and MCP-5 cells (Gibco BRL, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco), 10% WEHI-3b supernatant, 2 mM glutamine, and 50 μM 2-mercaptoethanol in humidified 95% air/5% CO₂ at 37°C. Mouse mast cells were passively sensitized at a density of 2×10⁶ cells/ml with 1 μg/ml anti-dinitrophenyl (DNP) mouse monoclonal IgE (Sigma) at 37°C overnight. After these cells were washed in Tyrode buffer, resuspended in Tyrode buffer at a density of 1×10⁶ cells/ml, and incubated for 10 min with samples at 37°C, they were incubated with 300 ng/ml of DNP-bovine serum albumin (BSA) (LSI Cosmo Bio, Tokyo, Japan) for 10 min at 37°C. Cells were cooled on ice to stop the reaction and centrifuged at 15,000×g for 5 min at 4°C. To the supernatant, an equivalent volume of 0.1 N hydrochloric acid was added. Histamine in the supernatant was measured by on-column HPLC. HPLC was done with a Shimadzu LC-10A pump coupled with a RF detector (ex. 340 nm, em. 450 nm) using a reverse-phase Asahipak-ODP-4E column (7.5 mm i.d. × 150 mm, Shodex, Tokyo, Japan) which was eluted 50 mM borate/acetonitrile.

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Abbreviations: IgE, immunoglobulin E; FcεRI, high affinity receptor for IgE; IL, interleukin; PBS, phosphate buffered saline; PVPP, polyvinyl polypyrrolidone; DNP-BSA, dinitrophenyl conjugates of bovine serum albumin; PY protein, phosphotyrosine-containing protein, SDS-PAGE; sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Fig. 1. Effects of Tea Varieties on Histamine Release From a Mouse Mast Cell Line, MC/9 Cells.

Content of released histamine without tea extract was taken as 100%. Each extract was added to cultured medium of MC/9 cells (final conc.: 75 µg/ml). Values are means±SE of number of triplicated experiments. Values not sharing a common superscripts letter are significantly different between the groups (p<0.05) by Duncan’s multiple range test.

Fig. 2. Effects of Three Types of Tea (Cultivar ‘Benihomare’) on Histamine Release From MC/9 Cells.

Manufacturing processes of three types of tea were as follows; green tea: steaming (60 s)-primary drying-rolling-secondary drying-final drying, oolong tea: withering under the sunlight-panning-rolling-drying, black tea: withering overnight-rolling-oxidation in fermentation room (25°C, 4 h)-drying. Contents of released histamine without tea extract was taken as 100%. Values are means±SE of number in triplicated experiments. Values not sharing a common superscripts letter are significantly different between the groups (p<0.05) by Duncan’s multiple range test.

trile (80/20) buffer containing 1 mM o-phthalaldehyde (Nacalai tesque, Kyoto, Japan), and 1 mM N-acetylcyesteine (Wako, Osaka, Japan), at a flow rate of 0.5 ml/min. at 37°C.

Figure 1 shows effects of various varieties of tea extracts on histamine release from IgE/antigen-stimulated mouse mast cell line MC/9 cells. Among tea extracts from various varieties tested, cultivars ‘Sanssashian’, ‘Taiwan keitou #1’, and ‘Benihomare’ inhibited histamine release by approximately 60%. Extracts of cultivar ‘Asahi’, ‘Seishin oolong’, ‘Koukan’, and ‘IND113’ also had inhibitory effects. The extracts did not affect cell viability of MC/9 cells at 75 µg/ml (data not shown). These results suggest that these tea varieties contain some inhibitory principle(s) against mast cell activation.

To examine the effects of manufacturing methods on histamine release, we manufactured three types of tea from the cultivar ‘Benihomare’ first cropped fresh tea leaves. Among three types of tea, extracts from oolong tea or black tea inhibited histamine release more strongly than green tea extract, as shown in Fig. 2. These results suggested that some principle(s) related to the inhibition of mast cell activation was produced during oxidative reaction (fermentation) in the manufacturing process. It was reported that tea polyphenols, such as tea catechins and 3,3′-di-O-galloylprodelphinidine B-2′ inhibited histamine release from rat peritoneal mast cells, and that phenolic compounds were absorbed with polyvinyl polypirrolidione (PVPP), preventing histamine release or hyaluronidase activity. So we examined effects of major tea catechins in green tea on histamine release. EC, EGC, and EGCg had little or particularly no effects while EGc slightly inhibited histamine release (data not shown). These results suggest that the four major catechins contributed little to the inhibition of histamine release from mouse mast cells. However, other phenolic compounds, which can be produced during oxidative processes, may have inhibitory effects on mast cell activation. The inhibitory effect of histamine release of oolong tea extract was weakened by PVPP (Sigma, USA) treatment (data not shown). FcεRI mediated tyrosine phosphorylation plays an important part in mast cell activation. To clarify the effects of phenolic compounds in tea on mast cell activation, we investigated the effects of oolong tea extract (cultivar ‘Benihomare’), and phenolic compounds-free oolong tea extract (cultivar ‘Benihomare’) treated with PVPP on FcεRI-mediated tyrosine phosphorylation of cellular proteins. To remove phenolic compounds, 20 volumes (v/v) of PVPP was added to ‘Benihomare’ oolong tea extract. The mixture was incubated for 30 min with gentle shaking at room temperature. After centrifugation at 1,800 x g for 15 min, the supernatant was used for experiments. For investigation of tyrosine phosphorylation of protein in MCP-5 cells, cell precipitation after antigen stimulation was used. After FcεRI cross-linking, MCP-5 cells were lysed in 1% Nonidet P-40 buffer with protease inhibitors on ice for 30 min. Proteins in cell lysates were analyzed by SDS-PAGE and blotted onto polyvinylidene difluoride (PVDF) membranes (immobil-1-P, Millipore, USA). The membranes were blocked with 2% gelatin in PBS-T buffer (0.2% Tween-20 in PBS, pH 7.4) and incubated with anti-phosphotyrosine monoclonal antibody 4G10 (UBI, USA). After they were washed, the membranes were incubated with horse-radish peroxidase-conjugated antibody (Bio Source Tago, USA), and detected with an increased chemiluminescence kit (ECLI; Amersham, UK). The results were obtained through densitometric analysis of bands (50–135 kDa) of phosphotyrosine-containing protein (PY protein) of each lanes. As shown in Fig. 3, tyrosine phosphorylation was strongly prevented by the presence of 100 µg/ml extract from oolong tea upon
Fig. 3. Effects of Oolong Tea Extract and PVPP Treated Oolong Tea Extract on PY Protein of MCP-5 Cells After FcεRI Cross-linking.

Antigen (DNP-BSA) stimulated MCP-5 cells were lysed with 1% NP-40-containing buffer. PY proteins in the lysates in the presence or absence of 'Benihomare' oolong tea extract were analyzed by SDS-PAGE. Proteins were blotted on PVDF membranes, and probed with polyclonal anti-phosphorysine antibodies. Data are shown as relative area of PY proteins bands (50-135 kDa) comparing to the area without sample. Values are means ±SE (n = 3).

FcεRI cross-linking on MCP-5 cells. The inhibition was dose-dependent in the tested concentration range of 20 μg/ml to 500 μg/ml, but PVPP treated oolong tea extract had much less effect. These results suggest that some anti-allergic principle(s) was produced during the oxidative reaction (fermentation) in the manufacturing process and a phenolic compound which can be absorbed with PVPP in oolong tea extract, but not the four major catechins, may be such a principle. Since tea has been drunk for many centuries without noticeable adverse effects on human health, the identification of the principle in tea extract, which inhibits mast cell activation, may eventually lead to a new anti-allergic drug without serious side effects. 'Benihomare' oolong tea extract prevented histamine release and PY protein expressions, so we suggested that the principle(s) in 'Benihomare' oolong tea extract were useful as anti-allergic effectors. Now, we are trying to isolate and purify the principle(s) in 'Benihomare' oolong tea extract.

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