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Preventive Effects of Black Tea Theaflavins on Mouse Type I Allergy Induced by Ovalbumin

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Abstract

The preventive effects of black tea theaflavins, theaflavin-3-gallate (3-TF) and theaflavin-3,3'-digallate (TFDG), on mouse type I allergic models induced by ovalbumin were investigated. The antiallergic activities on mouse type I allergy were determined using the abdominal wall method. The oral administration of 3-TF and TFDG at 10 and 50 mg/kg body weight tended to prevent type I allergic symptoms in a dose-dependent manner. Treatment with TFDG at 50 mg/kg body weight exhibited a significant antiallergic effect. Therefore, the theaflavins would contribute to the antiallergic effects of black tea.

Keywords: Black tea, Theaflavin, Type I allergy, Mouse

1. Introduction

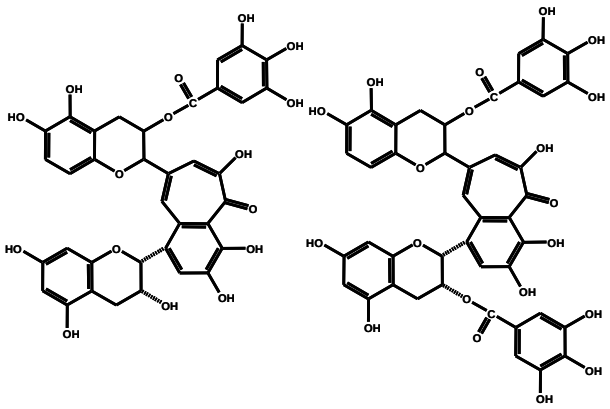
Black tea (*Camellia sinensis* L.) is the most popular tea beverage in the world. Some extracts prepared from black tea as well as green tea and oolong tea have been reported to prevent type I allergy in rats, such as passive cutaneous anaphylaxis[1,2], and type IV allergy in mice, such as picryl chloride-induced contact dermatitis, sheep red blood cells-induced hind paw edema, and oxazolone-induced edema[3,4]. The active components of green tea involved in the preventive effects against type I allergy were suggested to be catechins, including (-)-epigallocatechin-3-*O*-gallate (EGCG)[5–7]. EGCG was reported to suppress the production of interleukin-10 (IL-10) from type 2 T helper (Th2) cells and to inhibit the activation of B cells secreting immunoglobulin E (IgE)[8], release of histamine from mast cells[1,9,10], expression of the high-affinity IgE receptor in human basophils and mast cells degranulation[11,12]. The triphenol structures in EGCG are important for these actions. EGCG also suppressed the vascular permeability in guinea pigs by inhibiting the activity of Df-protease[13]. The extracts prepared from black tea leaves were also shown to inhibit the release of histamine from mast cells[1]. However, the amounts of total catechins in black tea leaves are *ca*. 30% – 50% (w/w) of green tea leaves[14]. During the fermentation process involved

in the production of black tea, catechins are oxidized enzymatically and their dimers, such as theaflavins, and polymers, such as thearubigin, are produced. These oxidized products are related to the red – brown color of black tea infusions[15]. The contents of theaflavins and thearubigin in black tea leaves are 0.7% – 1.5% (w/w)[14] and 10% – 20% (w/w)[16], respectively. In the present study, we investigated the preventive effects of two theaflavins with triphenol structures on mouse type I allergy.

2. Materials and Methods

Two theaflavins from black tea leaves, theaflavin-3-gallate (3-TF) and theaflavin-3,3'- digallate (TFDG), were supplied by Unilever Japan KK (Tokyo, Japan). The purities of both compounds were higher than 95%. Their chemical structures are shown in Figure 1. The allergen ovalbumin (OVA) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and Freund's incomplete adjuvant (FIA) and diphenhydramine hydrochloride were purchased from Wako Pure Chemical Co. Ltd. (Osaka, Japan). Four-week-old male ddY mice were purchased from Japan SLC, Inc. (Shizuoka, Japan).

The antiallergic activities of 3-TF and TFDG in mouse type I allergy were examined using the mouse abdominal wall method reported previously[17], with slight modifications. Briefly, mice were sensitized intraperitoneally with a 1:1 mixture of OVA (2 mg/mL N-saline) and FIA. Theaflavins were administered orally to mice 9 days after initial exposure to OVA. In the allergy group, distilled water was administered instead of theaflavins. The doses of theaflavins were 10 or 50 mg/kg body weight. Diphenhydramine hydrochloride, a known antihistamine, was used at a dose of 1 mg/kg body weight as a positive control. Sixty minutes after administration of the sample, 0.1 mL of Evans blue dye solution (10 mg/mL N-saline) was administered intravenously. Within 5 min after injection of the dye, the abdominal skin of the mice was detached under ether anesthesia, without injury to the abdominal wall. Five minutes after injection of the dye, 50 μL of OVA solution (5 μg/site) was injected into the exposed abdominal wall. The mice were killed by cervical dislocation 7 min after challenge, and the abdominal wall was removed. The area of the abdominal wall permeated by blue dye was measured by densitography with spot image processing software (AE-6920; Atto, Tokyo, Japan). Throughout the experiment, the animals were handled in accordance with "*The Guide for the Animal Experiments in Numazu National College of Technology*."



Theaflavin-3-gallate (3-TF) **Theaflavin-3,3'-digallate (TFDG)**

Figure 1 Chemical structures of black tea theaflavins.

Statistical analyses were performed with the nonparametric Mann–Whitney U test to determine the significance of differences between the appropriate experimental groups. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

3. Results and Discussion

The effects of theaflavins on mouse type I allergy are shown in Figure 2. The oral administration of 3-TF and TFDG at 10 and 50 mg/kg body weight tended to

prevent the allergic dye-infusion in a dose-dependent manner. TFDG at 50 mg/kg body weight showed a significant antiallergic effect. TFDG contains two triphenol structures, whereas 3-TF contains one triphenol structure. These results suggested that the triphenol structure is important for their antiallergic functions. The antiallergic effects of theaflavins were lower than those of diphenhydramine, a well-known H1 blocker.

As shown in Figure 3, type I allergy involves a humoral immune response under the control of cytokines secreted by Th2 cells. The cytokines, such as IL-4, could enhance the production and/or release of the allergic antibodies, IgE, from B lymphocytes. Binding of IgE and the antigens to the surface receptors of the mast cells causes the degranulation and some allergic symptoms such as vascular permeability induced by histamine. It was reported that tea extracts including 10% theaflavins suppressed the production of IL-4 and IL-5 from mouse Th2 cells[18]. This function of theaflavins may contribute to the inhibitory effects of the extracts prepared from black tea leaves on the release of histamine from mast cells. Type I allergy is also known as immediate hypersensitivity and may be accompanied by an inflammatory reaction, such as atopic dermatitis. The oxidative metabolites of arachidonic acid, such as leukotrienes and prostaglandins, are proinflammatory chemical mediators, and these compounds are produced by lipoxygenase (LOX) and cyclooxygenase (COX). Tea catechins were reported to inhibit the activities of LOX and COX[19]. A mixture of theaflavins and catechins and a mixture of theaflavins prepared from black tea also exerted antiinflammatory effects mediated by inhibition of LOX and COX activities[19,20]. Theaflavins[21,22], along with catechins[23,24], showed antioxidative activities. These antioxidative effects of theaflavins may help to inhibit the enzymatic oxidation of arachidonic acid. In addition, the excess production of active oxygen species by phagocytes is thought to provoke inflammation in the neighboring tissues[25,26]. The antioxidative activities of theaflavins may also contribute to the preventive effects against inflammation.

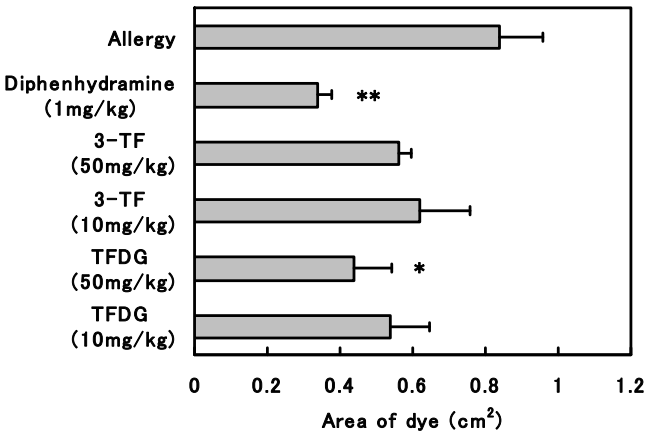


Figure 2 Preventive effects of oral administration of black tea theaflavins against mouse type I allergy.
Mean ± SEM. n = 5. Significant differences from "Allergy" group; * $P < 0.05$, ** $P < 0.01$. The abdominal wall method was used for the determination of the antiallergic activities as described in the Materials and Methods section.

A possible scheme of the preventive effects of theaflavins against type I allergy is presented in Figure 3. Theaflavins are thought to play a prominent role in the antiallergic effects at least *via* the following three functions: (1) inhibition of production and/or release of cytokines secreted from Th2 cells, (2) inhibition of the activities of LOX and COX, and (3) suppression of oxidative stress induced by active oxygen species. Recently, the increase in incidence rates of allergic diseases has become an important medical problem around the world. Daily intake of black tea as a beverage may be beneficial in the prevention of allergic disorders.

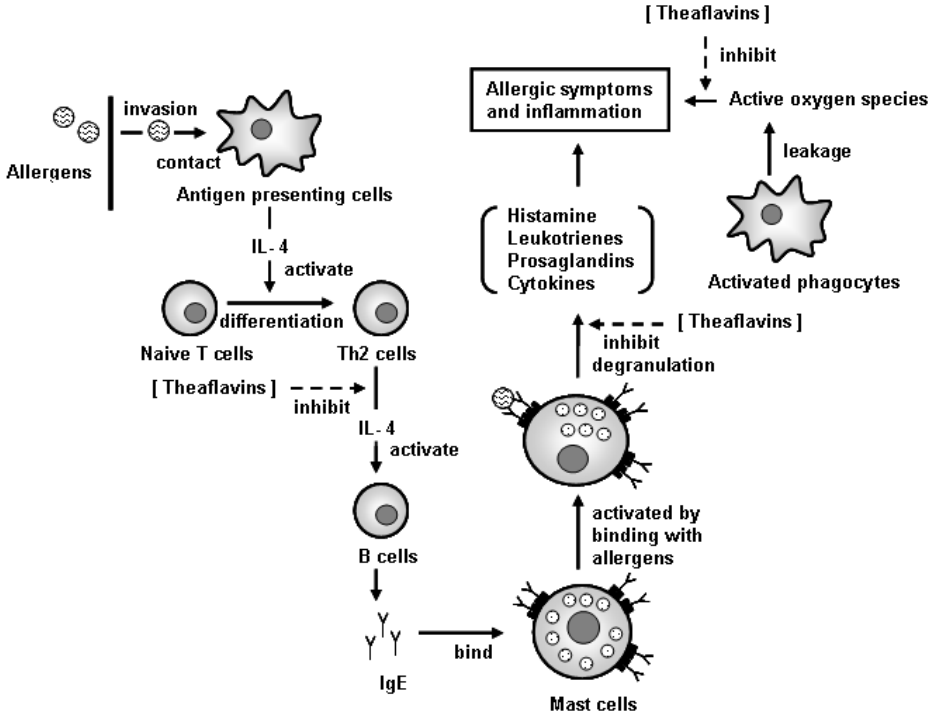


Figure 3 Summary of preventive roles of theaflavins against type I allergy.

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紅茶テアフラビン類の卵アルブミン誘発マウスⅠ型アレルギー抑制作用

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要旨

紅茶テアフラビン類のマウスにおける、卵アルブミン誘発Ⅰ型アレルギーに対する抑制作用について検討した。テアフラビン類としては、テアフラビン-3-ガレート(3-TF)およびテアフラビン-3,3'-ジガレート (TFDG)を用いた。マウスⅠ型アレルギーに対する抑制作用の検定には、マウス腹壁法を用いた。3-TF および TFDG の 10 あるいは 50 mg/kg 体重での経口投与は、用量依存的にⅠ型アレルギーの症状を抑制する傾向が見られた。TFDG の 50 mg/kg 体重での投与で、有意な抗アレルギー作用が認められた。そのため、テアフラビン類は紅茶の抗アレルギー作用に寄与しているものと考えられる。

キーワード：紅茶，テアフラビン，Ⅰ型アレルギー，マウス