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Nobiletin and tangeretin ameliorate scratching behavior in mice by inhibiting the action of histamine and the activation of NF-κB, AP-1 and p38

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ABSTRACT

Nobiletin and tangeretin are polymethoxy flavonoids that are abundantly present in the pericarp of *Citrus unshiu* (family Rutaceae) and the fruit of *Citrus depressa* (family Rutaceae). They exhibit various biological activities, including anti-inflammatory and anti-asthmatic effects. To evaluate the anti-allergic effects of nobiletin and tangeretin, we measured their inhibitory effects in histamine- or compound 48/80-induced scratching behavioral mice. Nobiletin and tangeretin potently inhibited scratching behavior, as well as histamine-induced vascular permeability. Furthermore, they inhibited the expression of the allergic cytokines, IL-4 and TNF-α as well as the activation of their transcription factors NF-κB, AP-1 and p38 in histamine-stimulated skin tissues. They also inhibited the expression of IL-4 and TNF-α and the activation of NF-κB and c-jun in PMA-stimulated RBL-2H3 cells. Furthermore, nobiletin and tangeretin inhibited protein kinase C (PKC) activity and the IgE-induced degranulation of RBL-2H3 cells. These agents showed potent anti-histamine effect through the Magnus test when guinea pig ileum was used. Based on these results, nobiletin and tangeretin may ameliorate scratching behavioral reactions by inhibiting the action of histamine as well as the activation of the transcription factors NF-κB and AP-1 via PKC.

1. Introduction

Pruritus, which is an unpleasant cutaneous sensation that provokes the desire or reflex to scratch, can be local or widespread and is associated with many diseases, such as atopic dermatitis, urticaria, cholestasis, and uremia. Many endogenous amines, proteases, growth factors, neuropeptides, opioids, eicosanoids, and cytokines act as a pruritogen [1–3]. For example, histamine, substance P or compound 48/80 significantly induces scratching in mice [1,4]. Therefore, histamine, substance P, or compound 48/80 is used as a pruritogen for pruritic animal models. Pruritus can cause skin lesions and contribute to severe psychological disturbances [5]. Thus, inhibiting pruritic responses is beneficial for improving the quality of life. However, no specific remedy is available for this common symptom.

Nobiletin (5,6,7,8,3′,4′-hexamethoxy flavone) and tangeretin (5,6,7,8,4′-pentamethoxy flavone) are polymethoxy flavonoids (PMFs) that are abundantly present in the pericarp of *Citrus unshiu* and the fruit of *Citrus depressa* (Taiwan tangerine or Shiikuwasa, family Rutaceae) [6,7]. They exhibit several biological activities, including anticancer [8], anti-inflammatory [9,10], neuroprotective [11], hypolipidemic [12,13] and anti-obesity effects [14]. PMFs also suppress scavenger receptor expression in monocytes [15]. Furthermore, nobiletin attenuates ovalbumin-induced eosinophilic airway inflammation in asthmatic rats [16] and Type II collagen-induced arthritis [17]. To our knowledge, the ability of these PMFs to inhibit scratching behavior has not been studied. Therefore, we evaluated the inhibitory effects of nobiletin and tangeretin in histamine- or compound K-induced scratching behavior in mice.

2. Materials and methods

2.1. Materials

Histamine, compound 48/80, phorbol 12'-myristate 13'-acetate (PMA), Evans blue and azelastine hydrochloride were purchased from Sigma Co. (St. Louis, MO, U.S.A.). Antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Enzyme-linked immunosorbent assay (ELISA) kits for cytokines were purchased from R&D Systems (Minneapolis, MN, U.S.A.). ELISA kit for protein kinase C

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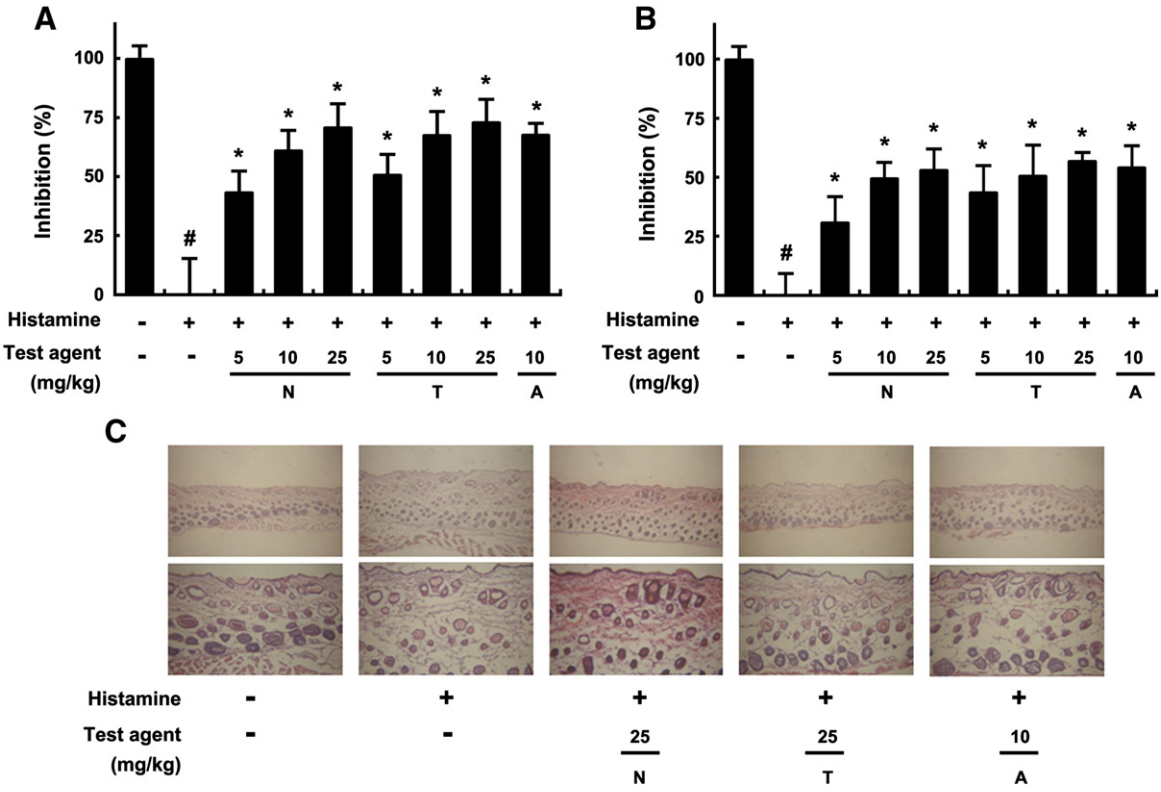

Fig. 1. Inhibitory effects of nobiletin and tangeretin on histamine- or compound 48/80-induced scratching behavior in mice. (A) Effect on histamine-induced scratching behavior. (B) Effect on compound 48/80-induced scratching behavior. The scratching behavior frequency of a normal control treated with saline alone, and histamine- or compound 48/80-induced control group for 1 h was 2 ± 1, 82 ± 5 and 321 ± 27, respectively. (C) Histological exams. The skin of mice that were treated with histamine in the absence or presence of test agents was stained using hematoxylin–eosin and viewed under a light microscope. The test agents (nobiletin at 5, 10, or 25 mg/kg; tangeretin at 5, 10, or 25 mg/kg; and azelastine at 10 mg/kg, dissolved in 2% cremophor) were orally administered 1 h before treatment with the scratching agent. Normal control mice were treated with the vehicle alone instead of test agents or histamine. The values indicate the mean ± SD ($n = 6$). #Significantly different from the normal control group ($P < 0.05$). *Significantly different from the group treated with histamine alone ($P < 0.05$).

(PKC) activity was purchased from Enzo Life Sciences Inc. (Farmingdale, NY, U.S.A.)

2.2. Isolation of nobiletin and tangeretin

Nobiletin and tangeretin were isolated from the fruit of *Citrus depressa* (family Rutaceae) according to the previously reported methods [6,7]. The isolated nobiletin and tangeretin were identified by comparing these spectral data and physical properties of standard nobiletin and tangeretin (Wako Pure Chemical Industries, Japan), respectively.

Nobiletin - colorless needles; mp 137–138 °C; EI-MS, *m/z* 402 (M^+).
Tangeretin - light yellow needles; mp 153–154 °C; EI-MS, *m/z* 372 (M^+).

2.3. Animals

Male ICR mice (5 weeks-old, 20–25 g) and male Hartley guinea pigs (270–330 g) were supplied from Orient Experimental Animal Breeding Center (Seoul, Korea). All animals were housed in wire cages at 20 – 22 °C and 50 ± 10% humidity, fed standard laboratory chow (Orient Experimental Animal Breeding Center) and allowed water ad libitum. All experiments were performed in accordance with the NIH and Kyung Hee University guidelines for Laboratory Animals Care and Use and approved by the Committee for the Care and Use of Laboratory Animals in the College of Pharmacy, Kyung Hee University.

2.4. Assay of scratching behavioral frequency

The behavioral experiments were all performed as previously described [18]. Before the experiments, mice were acclimated in the acrylic cages (22 × 22 × 24 cm) for about 10 min and then divided into groups of 6 mice. The rostral part of the skin on the back of each mouse was clipped, and histamine (300 μg/50 μL) or compound 48/80 (50 μg/50 μL) for each mouse was intradermally injected with the use of a 29 gauge needle. The scratching agents were dissolved in saline. Normal control mice received a saline injection in the place of the scratching agent. Immediately after the intradermal injection, the mice (one animal/cage) were put back into the same cage and, for the observation of scratching, their behaviors recorded using an 8-mm video camera (SV-K80, Samsung, Seoul, Korea) under unmanned conditions. Scratching of the injected site by the hind paws was counted and compared with that of other sites, such as the ears. Each mouse was used for only one experiment. The mice generally showed several scratches for 1 s, and a series of these behaviors were counted as one incident of scratching for 60 min. The test agent, nobiletin, tangeretin or azelastine (5, 10 or 25 mg/kg, dissolved in 2% cremophor), was orally administered 1 h before treatment with the scratching agent in mice.

2.5. Assay of vascular permeability

The increase in vascular permeability caused by histamine was assessed as reported previously [18]. After the intradermal injection of histamine (300 μg/50 μL) into the rostral part of the back of each mouse, the injected site (1 × 1 cm) was outlined with an indelible red marker and 0.2 mL of 1% Evans blue solution in saline was injected