Allergy-Preventive Effects of Chlorogenic Acid and Iridoid Derivatives from Flower Buds of *Lonicera japonica*

Hisae Oku,† Yuko Ogawa,§ Emiko Iwao,∥ and Kyoko Ishiguro*,*

*School of Pharmacy and Pharmaceutical Sciences, Mukogawa Women’s University; 11–68 Koshien Kyuban-cho, Nishinomiya 663–8179, Japan; †Faculty of Pharmaceutical Sciences, Doshisha Women’s College of Liberal Arts; Kyotanabe, Kyoto 610–0395, Japan; and *Department of Pharmacy, Hyogo University of Health Sciences; Minatojima, Chuo-ku, Kobe 650–8530, Japan. Received June 30, 2010; accepted March 1, 2011

Allergy-Preventive activity of flower buds of *Lonicera japonica* THUNB. was found in the 35% EtOH extract (LJ) using an *in vivo* assay. The assay system uses monitoring of a decrease in blood flow (BF) in the tail vein of mice subjected to sensitization with hen-egg white lysozyme (HEL). Bioassay-guided fractionation of the 35% EtOH extract led to isolation of chlorogenic acid (1) and three known iridoid derivatives, loganin (2), secoxyloganin (3) and sweroside (4), all of which inhibited the BF decrease. This suggested that the flower buds of *L. japonica* and compounds isolated from them have allergy-preventive properties. The structure–activity relationship of iridoid derivatives, morroniside (5), geniposide (6), asperuloside (7), aucubin (8) and catalpol (9), were also tested using the same bioassay method. Compounds 2—5 and 9 having the sp³ atom at C-8 showed an allergy-preventive effect, while compounds 6, 7 and 8 having a double bond at C-7, C-8 did not.

Key words *Lonicera japonica*; allergy-preventive effect; blood flow; chlorogenic acid; iridoid derivative; hen-egg white lysozyme

Flower buds of *Lonicera japonica* THUNB. (Caprifoliaceae), one of the most common traditional Chinese medicines, are used to treat various diseases including arthritis, diabetes mellitus, fever, infections, sores and swelling. Pharmacological studies have indicated that the extract of these flower buds have a broad spectrum of biological activities, including antibacterial, anti-inflammatory, antioxidant, antipyretic, antiviral and hepato-protective effects. A number of chemical constituents including flavonoids, iridoids and saponins have been obtained from this plant.

In our continuing search for allergy-preventive substances from natural sources, we have been using our previously developed *in vivo* assay method to estimate effects on complex allergies. This time, we found that the 35% EtOH extract of flower buds of *L. japonica* exhibited allergy-preventive activity. Our *in vivo* assay method monitors the decrease in blood flow (BF) in the tail vein of mice subjected to hen egg-white lysozyme (HEL) sensitization alone without the HEL-challenge as a guide. The BF in HEL-sensitized mice (control group) gradually and significantly decreased to about 70% of that in normal mice on day 9. Thus, the induction phase (promotion stage) of allergy caused by xenobiota can be dynamically and easily measured using BF monitoring. This BF decrease is considered to be due to the contraction of peripheral blood vessels and an increase in blood viscosity, because no relationship with blood pressure was observed. Although anti-HEL immunoglobulin E (IgE) antibody significantly increased after HEL sensitization, there was no significant increase in the number of leukocytes. Thus, a decrease in BF reflects the promoter process of an allergic reaction. The BF decrease is regulated by various factors such as nitric oxide (NO), thromboxane (TX) A₂, prosta-cyclic (PG₂), and endothelin (ET)-1, together with granulocytic elastase (GE), cyclooxygenase (COX)-1 and -2, inducible nitric oxide synthase (iNOS), and constitutive nitric oxide synthase (cNOS). In addition, the BF decrease occurs via both pathways of iNOS-independent and -dependent responses. Therefore this monitoring system should be useful when searching for substances that can prevent complicated inflammatory allergies involving NO, TXA₂, PGI₂, ET-1, GE, COX-1, and COX-2.

This paper describes the evaluation of the allergy-preventive effects of a 35% EtOH extract (LJ) of flower buds of *L. japonica* and the following compounds isolated from LJ: chlorogenic acid (1) and three iridoid derivatives, loganin (2), secoxyloganin (3) and sweroside (4).

The structure–activity relationships of iridoid derivatives, morroniside (5), geniposide (6), asperuloside (7), aucubin (8) and catalpol (9) were also examined, as iridoid glucosides (2—4) isolated from LJ showed allergy-preventive effects.

MATERIALS AND METHODS

General Experimental Procedures Melting points were determined on a Yanagimoto micro-point apparatus. IR spectra were recorded on a Shimadzu 435 spectrometer and ¹H and ¹³C NMR spectra were recorded with a JEOL JEM-GSX 500 spectrometer (tetramethylsilane (TMS) as internal reference). FAB-MS were performed on a JMS-700 double-focusing mass spectrometer having a kinetic energy equivalent to 6kV at an ion-acceleration voltage of 3 kV. The mass marker was calibrated with perfluoroalkylphosphazene (ultra marker), and 3-nitrobenzyl alcohol (NBA) and glycerin (GLY) were used as the matrix.

Plant Materials Flower buds of *L. japonica* (common name: Kinginka) were purchased from Tochimoto Tenkaido Co., Ltd. A voucher specimen is kept at our university. Compounds 5—9 had been previously isolated in our laboratory.

Extraction and Isolation Procedures Flower buds of *L. japonica* (500 g) were extracted with 35% EtOH at room temperature and evaporated in vacuo to obtain the 35% EtOH extract (ext.) (LJ, 135 g). LJ was suspended in distilled water and successively extracted with AcOEt and n-BuOH to obtain the AcOEt ext. (3.7 g), n-BuOH ext. (19.4 g), and H₂O ext. (108 g). Part (2.5 g) of the H₂O ext. was subjected to polyamide column chromatography using H₂O and MeOH as
eluted. The fraction eluted by MeOH was subjected to gel filtration by Sephadex LH-20 column chromatography using H₂O to obtain compound 1 (20 mg, 0.173%). The rest (60 g) of the H₂O ext. was subjected to activated charcoal column chromatography using H₂O and EtOH as eluents. The fraction eluted by EtOH (8.7 g) was subjected to silica gel chromatography using a CHCl₃–MeOH step-gradient system to give four fractions (Fr. Ia—d). Fr. Ib (3.0 g) was subjected to flash column chromatography using a CHCl₃–MeOH gradient to give four fractions (Fr. Ia—d). Fr. IIb (258 mg) was chromatographed on a Cosmosil 75C18-OPN using a H₂O–EtOH step gradient to obtain compound

**RESULTS**

**Isolation and Identification of Compounds** Compounds 1—4 were isolated from LJ by successive chromatography. The structures of these known compounds were identified as chlorogenic acid (1), loganin (2), secoxyloganine (3), and seversonide (4) by comparison of their physical and spectroscopic data (MS, UV, IR, ¹H- and ¹³C-NMR) with the literature values. These compounds were subjected to the bioassay.

**Effects of Extracts and Isolated Compounds on BF Decrease in Response to HEL** The BF in the tail vein of HEL-sensitized mice (control group) gradually and significantly decreased to ca. 70% of the BF of normal mice at day 9, as shown in Fig. 1. On the other hand, oral administration (per os (p.o.)) of LJ (200 mg/kg) significantly (p<0.05) inhibited the BF decrease compared with the control group after day 6 of HEL-sensitization.

Figure 2 shows the effects of AcOEt, n-BuOH and H₂O ext. fractionated from LJ. The H₂O ext. at 200 mg/kg (p.o.) significantly inhibited the BF decrease after day 8 of HEL-sensitization, but AcOEt and n-BuOH ext. did not.

Figure 3 shows the effects of Fr. Ia—d fractionated from the most active H₂O ext. at 100 mg/kg (p.o.). Fr. Ib (–△–), c (–△–) and d (–□–) significantly (p<0.05) inhibited the BF decrease compared with the control group after day 7 of HEL-sensitization.

Figure 4 shows the effects of compounds 1—4 isolated from Fr. IIb and IIc fractionated from the active Fr. Ib. All
compounds significantly (p<0.05) inhibited the BF decrease at 10 mg/kg (p.o.) compared with the control group after day 6 or 7 of HEL-sensitization. The molecular weight of these compounds 1—4 are about the same, and 10 mg/kg is equivalent to 1: 28, 2: 26, 3: 26 and 4: 28 μmol/kg.

Figure 5 shows the effects of the major compound 1 at 20 mg/kg (p.o.) and epinastine hydrochloride, which is used clinically as an anti-allergic medicine, at 5 mg/kg (p.o.) as a positive control. Both compounds showed almost the same activity and significantly (p<0.05) inhibited the BF decrease after day 6 of HEL-sensitization.

Platelet Aggregation Effects of Isolated Compounds

To examine the inhibition mechanism of the BF decrease, the platelet aggregation effects of Fr. 1a—d at 100 mg/kg (p.o.) and compounds 1—4 at 10 mg/kg (p.o.) were examined. All fractions and compounds showed no inhibition of platelet aggregation when compared with the control group (data not shown).

Structure–Activity Relationships of Iridoid Glucoside Derivatives

The results of Fig. 4 indicate the possibility that other iridoid derivatives may also show allergy-preventive effects. Therefore, we also tested five other iridoid glucosides, morroniside (5), geniposide (7), acteoside (6), asperuloside (8), and catalpol (9), to examine the structure–activity relationship, using the same methods.

Significant inhibition of BF was noted with 10 mg/kg of compounds 5 and 9 (Figs. 6a, c), but none with compounds 6, 7, 8, which have a double bond at C-7, C-8 (Figs. 6b, c). The molecular weights of iridoid glucosides 2—9 are about the same.

DISCUSSION

In this study, we investigated the allergy-preventive effects of LJ and isolated compounds using our previously developed in vivo assay method which can be used to estimate complex allergy effects.10,11) LJ significantly inhibited the BF decrease compared with the control group (Fig. 1), suggesting that it possessed allergy-preventive effects.

Bioassay-guided fractionation led to four known compounds Ia, Ib, Ic, or Id from H2O ext. at 0 (starting day), 3, 6, 9 d from sensitization.

Fraction Ia, Ib, Ic or Id from H2O ext. at 0 (starting day), 3, 6, 9 d from sensitization. Each value presents the mean±S.E. (n=5). *p<0.05 as compared with the control group (Dunnett’s test with Bonferroni).

Compounds signifying (p<0.05) inhibited the BF decrease at 10 mg/kg (p.o.) compared with the control group after day 6 or 7 of HEL-sensitization. The molecular weight of these compounds 1—4 are about the same, and 10 mg/kg is equivalent to 1: 28, 2: 26, 3: 26 and 4: 28 μmol/kg.

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pounds 1—4, which significantly inhibited the BF decrease, from H2O extract of LJ (Fig. 4). These compounds have been isolated from many plants, and many of their bioactivities, including anti-inflammatory, anti-allergic, and antiasthmatic effects, have been reported. [16—19] However, this is the first report of their allergy-preventive effects in the induction phase of an allergy. Among them, chlorogenic acid (1) is regarded as an active substance of LJ, since 1 is a major constituent of the extract. These results suggested that 1 inhibits the BF decrease by inhibitory mechanisms on (i) expression of nuclear factor kappa B (NF-κB), iNOS, and COX-2, [20—24] (ii) enzyme activity of iNOS and elastase, [24,25] (iii) increased platelet aggregation, and (iv) expression of TXA2. [26] It is interesting that secoiridoids 2 and 3 showed allergy-preventive activity, because there has been only one report on the anti-allergic activity of secoiridoids, which showed an inhibitory effect on histamine release from rat mast cells. [27]

In a previous study, we found that some flavonones and chalcones from Xanthorrhoea hastilis significantly inhibited BF decrease, with inhibitory mechanisms involving platelet aggregation inhibition. [28] However, iridoid compounds 2—4 did not inhibit platelet aggregation, indicating a different inhibition mechanism on the BF decrease. There have been many reports on the anti-allergic effects of iridoid derivatives which inhibit the production of COX-2 [28] or NO [29] or the expression of iNOS and COX-2. [30] Inhibition of the BF decrease by iridoid derivatives may occur in a similar manner. Details of the mode of action of LJ and the isolated compounds are under investigation.

We also examined the structure–activity relationship of iridoid derivatives with other iridoids 5—8. Compounds 5 and 9 showed significant inhibition of the BF decrease, whereas compounds 6, 7, and 8 did not. Thus, compounds 2—5 and 9 having the sp² atom at C-8 are active, while compounds having a double bond at C-7, C-8 do not show the allergy-preventive effect. Compound 9, the most active, has been reported as an anti-inflammatory medicine which possesses therapeutic potential against LPS-induced acute systemic inflammation by attenuating NF-κB activation. [32]

Further results will be reported elsewhere.

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