Antiallergic Herbal Composition from *Scutellaria baicalensis* and *Phyllostachys edulis*

Authors Dong-Seon Kim[1], Eun-Jung Son[1], Miran Kim[1], Young-Moon Heo[1], Jeong-Bum Nam[1], Jai Youl Ro[2], Sung-Sick Woo[1]

Affiliations
[1] Unigen Inc., Cheonan, Chungnam, Korea
[2] Department of Pharmacology, Sungkyunkwan University School of Medicine, Suwon, Korea

Key words
- mast cell
- standardized herbal composition
- Lamiaceae
- Poaceae
- *Scutellaria baicalensis*
- *Phyllostachys edulis*

received February 20, 2009
revised October 28, 2009
accepted Nov. 1, 2009

Bibliography
DOI http://dx.doi.org/10.1055/s-0029-1240649
Published online December 3, 2009
Planta Med 2010; 76: 678–682
© Georg Thieme Verlag KG Stuttgart · New York ·
ISSN 0032-0943

Correspondence
Sung-Sick Woo, Ph.D.
Unigen, Inc.
200–1 Songjung-Ri
Byeongcheon-Myeon
Cheonan-Si
330–863 Chungnam
Korea
Phone: + 82 4 15 29 15 10
Fax: + 82 4 15 29 15 99
sswoo@unigen.net

Correspondence
Jai Youl Ro, Ph.D.
Department of Pharmacology
Sungkyunkwan University
School of Medicine
300 Chunchun-dong Jangan-Ku
440–746 Suwon
Korea
Phone: + 82 3 12 99 61 91
Fax: + 82 3 12 99 62 09
jyro426@med.skku.ac.kr

Abstract

We aimed to find antiallergic agents from natural sources using mast cells activated during allergic reaction. We screened over 2000 plants for blockade of histamine release and identified two of them, *S. baicalenesis* and *P. edulis*. Bioassay-guided fractionation led to two main constituent flavonoids, baicalin from *S. baicalenesis* roots and isoorientin from *P. edulis* leaves. Based on these two compounds, two standardized extracts (SSBE and SPEE) and a combined standardized herb composition (SHC) were developed. SSBE, SPEE, and SHC remarkably inhibited histamine and leukotriene release from mast cells activated by anti-OVA/OVA binding, and SHC showed a stronger inhibition than either extract alone. SHC also showed greater inhibition potency than either aspirin or cromolyn, which are known antiallergic agents. Our results suggest that SHC reduce degranulation during mast cell activation and could be a promising candidate for the treatment of immune/allergic diseases related to mast cells.

Abbreviations

SSBE:	standardized *Scutellaria baicalensis* root extract
SPEE:	standardized *Phyllostachys edulis* leaf extract
SHC:	standardized herb composition

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

Allergic diseases affect more than 20% of the U.S. population and are the sixth leading cause of chronic disease in the United States [1]. Ocular signs and symptoms include itching, redness, chemosis, tearing, and eyelid swelling, while nasal symptoms include rhinorrhea, sneezing, congestion, and pruritus; these signs and symptoms adversely affect millions of individuals [2].

Allergic reactions are primarily caused by mediator release during the activation of mast cells, which are normal residents of the connective and mucosal tissues, and are found in highest numbers in areas of the body that interface with the environment, such as the skin, lung, and gastrointestinal tract [3]. When mast cell membrane receptors are activated by the cross-linking of antigen-antibody complexes, enzyme systems in the cell membranes become activated. These reactions are the consequences of the release of granular mediators (e.g., histamine, tryptase, and others), newly synthesized mediators (e.g., leukotrienes, prostaglandins, platelet-activating factor, and others), and cytokines such as interleukins [e.g., (IL)-4, IL-5, and IL-3], as well as the tumor necrosis factor [4, 5].

This study was to find antiallergic agents from natural sources using mast cells activated during allergic reaction. To find such agents, we screened 2300 plants for blockade of histamine release, which is a known consequence of mast cell activation during allergic reaction. We discovered two plants, *S. baicalenesis* and *P. edulis*, and isolated two active compounds from their roots and leaves, respectively.

S. baicalensis (Lamiaceae) is a traditional Chinese medicinal plant and has been used as an anti-inflammatory, anticancer, and antiviral agent as well as acting as a detoxifying agent in the treatment of toxicosis and as a modulator of total cholesterol levels and blood pressure. It contains a variety of flavones, phenylethanoids, amino acids, sterols, and essential oils besides over 30 kinds of

Downloaded by: University of Arizona Library. Copyrighted material.

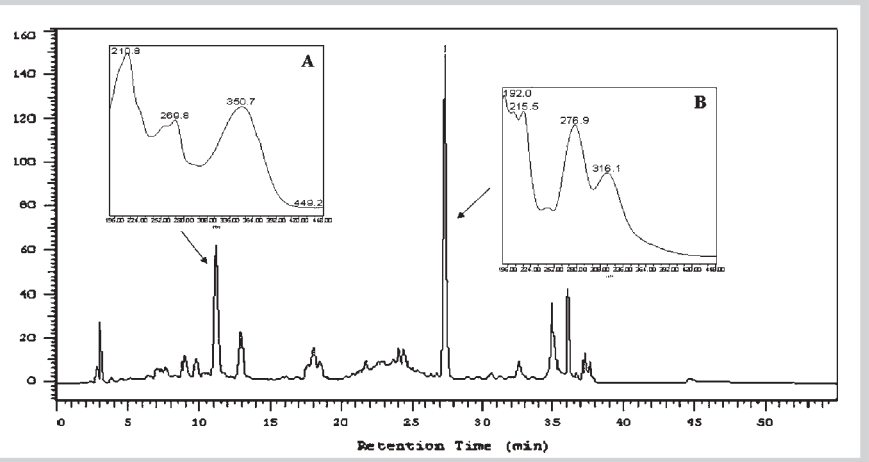

Fig. 1 HPLC chromatogram of SHC. HPLC was recorded at 330 nm as described in Materials and Methods. **A** isoorientin; **B** baicalin.

flavonoids, such as baicalin, baicalein, wogonin, wogonin 7-*O*-glucuronide, oroxylin A, and oroxylin A 7-*O*-glucuronide [6].

P. edulis (Poaceae) is a type of bamboo that has been used both as food and as a traditional medicine for over 1000 years. Bamboo leaves primarily contain flavone glycosides, phenolic acids, coumarin lactones, anthraquinones, and amino acids [7]. The goal of this study was to find antiallergic agents from natural sources that would result in a decrease in the preparation costs of and an increase in the blocking efficacy of ovalbumin (OVA)/anti-OVA reactions in activated guinea pig lung mast cells. We observed that a standardized herb composition (SHC) combined with two standardized extracts, prepared with two active compounds using bioassay-guided fractionation, had antiallergic effects.

Materials and Methods

Plant materials

Plant extracts were provided by the Plant Diversity Research Center in Korea. *S. baicalenesis* roots and *P. edulis* leaves were respectively collected from Goheung and Damyang in Korea in October 2005. Identification of these plants was confirmed by Prof. K.H. Bae, College of Pharmacy, Chungnam National University (Chungnam, Korea). A voucher of the dried *S. baicalensis* roots (#050201) was deposited at the Unigen herbarium as well as the voucher of dried *P. edulis* leaves (#040601).

Extraction and isolation

Dried *S. baicalensis* roots (50 g) were cut into small pieces and extracted with MeOH (500 mL) at room temperature for 24 h. After filtration, the residue was extracted a second time using the same method, and the two fractions were pooled, evaporated under vacuum, resuspended in water (200 mL), and successively partitioned with CH_2Cl_2 (200 mL) and BuOH (200 mL). A portion of the BuOH fraction was evaporated, yielding a dried powder (approximately 2.0 g), and was applied to a silica gel column (60 g; Merck, $CHCl_3$:MeOH, 10 : 1–1 : 1, *v/v*). Among the seven crude fractions obtained, fraction 5 (approximately 400 mg) was separated again on a silica gel column (20 g, BuOH : AcOH : water, 8 : 2 : 3, *v/v/v*), yielding 90.1% baicalin (approximately 230 mg); mp 204–206; UV max (log ε) 277 (4.48), 213 (4.59) nm; *m/z* 445.1 $[M\text{-}H]^-$. ^1H- and ^{13}C-NMR measurements were carried out in DMSO-d_6 using a Bruker DMX^{600} at 400 and 100 MHz (**Table 1S** and **Fig. 1S**, Supporting Information) [8].

The dried *P. edulis* leaves (50 g) were ground and extracted with MeOH (600 mL) at room temperature for 24 h. After filtration, the residue was extracted a second time using the same method, and the two fractions were pooled. The extract was evaporated under vacuum, resuspended in water (200 mL), and successively partitioned with CH_2Cl_2 (200 mL) and BuOH (200 mL). The BuOH fraction was evaporated, yielding a dried powder (approximately 2.4 g), and was applied to a silica gel column (60 g, $CHCl_3$:MeOH, 10 : 1–1 : 1, *v/v*).

Five crude fractions were obtained. Fraction 4 (approximately 400 mg) was separated again on a silica gel column (20 g, $CHCl_3$: MeOH : water, 7 : 3 : 1, *v/v/v*) and a Sephadex LH20 column (Amersham Pharmacia, MeOH), yielding 96.5% isoorientin (approximately 89 mg); mp 217–219; UV max (log ε) 350 (3.20), 213 (4.45) nm; *m/z* 430.3 $[M\text{-}H_2O]^-$. ^1H- and ^{13}C- NMR measurements were carried out in $CDCl_3$ using a Bruker DMX^{600} at 400 and 100 MHz (**Table 2S** and **Fig. 2S**, Supporting Information) [9]. Copies of the original spectra are available from the corresponding author.

HPLC analysis of baicalin and isoorientin

A Waters 600 controller, 717 plus autosampler, 2996 photodiode array (PDA) detector, and 660 pump system were used for the HPLC analysis, performed with a C18 column (Luna, 5 µm, 250 × 4.6 mm; Phenomenex). The column was equilibrated with a 15 : 85 mixture of acetonitrile and 0.1% phosphoric acid at a flow rate of 1.0 mL/min. The fractions were eluted according to the following administration: 0–15 min, 15% acetonitrile; 15–20 min, 15–28% acetonitrile; 20–30 min, 28% acetonitrile; 30–35 min, 28–100% acetonitrile. Baicalin and isoorientin were detected at 330 nm. The corresponding HPLC profiles are shown in **Fig. 1**.

Isoorientin and baicalin are identified by comparing their retention times with those of reference standards. The retention time of isoorientin is approximately 11 minutes and the one of baicalin is approximately 27 minutes.

The concentrations (% Wt) of isoorientin and baicalin were calculated using the following formula:

Downloaded by: University of Arizona Library. Copyrighted material.

$$\text{Wt}\,(\%) = \frac{A_{sample} \times \text{Dilution factor}}{(W_{sample}/V_{sample}) \times [1 - (\text{LOD}/100)]}$$

A_{sample} = average peak area of all injections of the sample
W_{sample} = actual weight of the sample (mg)
V_{sample} = actual volume of the sample (mL)
LOD = sample loss on drying (%)

Preparation of standardized composition (SHC)

The dried *S. baicalensis* roots (1 kg) were cut into small pieces and extracted with water (8 L) at 80 °C for 2 h under conditions to yield baicalin. The extract was filtered, evaporated, lyophilized, yielding approximately 350 g, and referred to as SSBE, which contained 21.8% baicalin.

The dried *P. edulis* leaves (2 kg) were extracted with 70% EtOH (30 L at 80 °C for 4 h) under conditions to yield baicalin. The extract was evaporated to dryness under vacuum and resuspended in 95% EtOH (1.1 L) at 60 °C for 2 h. After cooling, the extract solution was filtered and evaporated under vacuum, yielding a dried supernatant fraction (approximately 65.1 g). A portion of the fraction (10 g) was redissolved in 20% EtOH and adsorbed onto a D101 column (100 g; Shanghai Hualing Co., Ltd.). Elution with a gradient of H_2O : EtOH (80 : 20–0 : 100, *v/v*) yielded 8 fractions (Fr. 1–8), of which fractions 3–6 were combined, evaporated, lyophilized, yielding a powder, and referred to as SPEE, which contained 3.7% isoorientin.

We developed a standardized composition with SSBE and SPEE at a 1 : 2 (*w/w*) ratio by bioassay-guided fractionation, called SHC, which showed the strongest activity against mediator release (e.g., SSBE : SPEE = 1 : 1, 1 : 2, 1 : 3, 2 : 1, or 3 : 1 ratio). At this time, the percentages of baicalin and isoorientin were 7.3% and 2.4%, respectively.

Animals

Hartley albino female guinea pigs (200–250 g; Samtako Com.) were used, and all experiments involving animals were approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and by an Ethical Committee of Sungkynkwan University.

Anti-OVA antibody production in guinea pig

Twenty outbred female guinea pigs were first immunized by foot pad injection with a mixture of 50 µg ovalbumin (OVA) and complete Freund's adjuvant. One week later, the animals received intradermal injections of 100 µg OVA on one side of the back and 200 µg OVA on the other side of the back. After 1 week, sera were collected and stored at −70 °C. Serum IgG_1 antibody was separated by anti-IgG_2 affinity column (0.1 M citric acid, pH 2.1). IgG_1 was passed through the column; absorbed IgG_2 antibody was washed with 0.2 M sodium carbonate (pH 11.3) [10]. Antibody-containing sera were titered by the passive cutaneous anaphylaxis method [11] and found to be 1600–3200.

Guinea pig lung mast cell preparations

Guinea pig lung mast cells were isolated and purified as previously described [10]. Briefly, lungs obtained from 16 unsensitized guinea pigs (3 g per each lung) were minced using a Mcllwain tissue chopper (The Mickle Laboratory Engineering Co., Ltd.). Pooled tissue was treated three times with 125 U/g tissue and 5 U/g tissue of collagenase and elastase for 15, 15, and 25 min, respectively. Cells were filtered, washed with Tyrode buffer without $CaCl_2$ and $MgCl_2$-containing 1% gelatin (TG), layered onto gradients consisting of 10 mL percoll (1.045 g/mL), and centrifuged at 1400 rpm for 20 min. Pelleted cells were resuspended in TG buffer and reapplied onto a discontinuous percoll density gradient (consisting of densities 1.06, 1.07, 1.08, 1.09, and 1.10 g/mL) at 1400 rpm for 20 min. The obtained cell band between the 1.09 and 1.10 g/mL densities contained the highest purity and number ($4–5 \times 10^7$) of mast cells. This gradient band was removed, washed with TGCM buffer (Tyrode buffer containing both $CaCl_2$ and $MgCl_2$) and stained with alcian blue (Sigma). The purity of the mast cells was approximately 80–90%.

Measurement of mediator release from mast cells

The partially purified mast cells (4×10^5 cells/mL TGCM) were passively sensitized with anti-OVA antibody (10^6 cells/mL) in a shaking water bath for 45 min at 37 °C, washed, resuspended in TGCM buffer and challenged with 0.1 µg/mL OVA for 10 min. The reactions were terminated by placement on ice [10]. Each flavonoid, acetylsalicylic acid (purity > 99.0%), and cromolyn (purity > 95%; both from Sigma), as positive controls, were added to the mast cells 5 min before OVA challenge. Pretreatment with each flavonoid and with the positive control alone did not affect either cell viability (using trypan blue exclusion test) or mediator release when added to mast cells sensitized with anti-OVA antibody or in unstimulated mast cells.

Histamine present in the supernatants was analyzed by the fluorometric method [10]. The amount of released histamine was expressed as the amount (ng) released from 4×10^5 cells. The leukotriene content in the supernatant was determined using a Cysteinyl-Leukotriene ELISA kit (Cayman Chemical) [10].

Statistical analysis

Experimental data are shown as mean ± S. E. M. Significance analyses between controls and experimental groups were carried out using the Student's t-test from unpaired data. P values less than 0.05 were considered significant.

Supporting information

The bioassay-guided fractionation of baicalin and isoorientin is available in the Supporting Information.

Results and Discussion

We sought to find agents from natural sources to block histamine release from mast cells activated by an OVA/anti-OVA reaction [10]. From 2300 plant sources, *S. baicalensis* roots and *P. edulis* leaves showed the strongest inhibition of histamine release. Bioassay-guided fractionation lead to the separation of two flavonoid compounds, baicalin from *S. baicalensis* roots and isoorientin from *P. edulis* leaves, with purities of 90.1% and 96.5%, respectively.

To evaluate the antiallergic activities of baicalin and isoorientin, mast cells were first sensitized by treatment with anti-OVA antibody and then challenged with 0.1 µg/mL OVA (OVA/anti-OVA antibody reaction). Subsequently, mast cells were pretreated with varying concentrations (10, 30, or 60 µg) of either 90.1% baicalin or 96.5% isoorientin. Histamine release was inhibited by 47.1%, 59.4%, and 61.5% after baicalin pretreatment and by 21.2%, 47.1%, and 53.8% after isoorientin pretreatment, respectively, compared with the OVA/anti-OVA antibody reaction, which elicited a histamine release of 32.5 ± 0.9% (**Table 1**).

Downloaded by: University of Arizona Library. Copyrighted material.

Table 1 The effects of active components on the release of mediators from passively sensitized guinea pig lung mast cells activated by OVA[a].

Treatment	Histamine (%)[b]	Leukotrienes (pg/4 × 10^5 cells/mL)[c]
OVA	32.5 ± 0.9	674.7 ± 12.5
Baicalin (μg/mL)[d]		
10	17.2 ± 0.8 (47.1) e***	419.7 ± 29.8 (37.9) e***
30	13.2 ± 1.6 (59.4) e***	356.1 ± 85.2 (47.3) e***
60	12.5 ± 1.4 (61.5) e***	335.0 ± 25.1 (50.4) e***
Isoorientin (μg/mL)[d]		
10	25.6 ± 1.5 (21.2) e**	574.2 ± 33.6 (15.0) e*
30	17.2 ± 1.1 (47.1) e***	534.9 ± 67.6 (20.8) e**
60	15.0 ± 0.5 (53.8) e***	358.3 ± 25.8 (47.0) e***

[a] Guinea pig lung mast cells (4 × 10^5 cells/mL) were passively sensitized by anti-ovalbumin (OVA) antibody and challenged with 0.1 μg/mL OVA for 10 min. Mast cells were pretreated with either baicalin or isoorientin for 5 min before OVA challenge. Amounts of spontaneous histamine release and total histamine were 89.3 ± 7.8 and 2074.8 ± 45.6 ng/4 × 10^5 cells, respectively. [b] Amounts of histamine release were already subtracted by amounts of spontaneous histamine release. [c] The concentrations of leukotrienes were calculated using standard curves generated with specific leukotriene standards. [d] The purity of baicalin and isoorientin was 90.1% and 96.5%, respectively. [e] Parentheses represent the decreased percentage compared with the released amount when activated with OVA anti-OVA antibody reaction. * P < 0.05; ** p < 0.01; *** p < 0.001 compared with OVA/anti-OVA antibody reaction

Table 2 The effects of SSBE and SPEE on the release of mediators from passively sensitized guinea pig lung mast cells activated by OVA[a].

Treatment	Histamine (%)[b]	Leukotrienes (pg/4 × 10^5 cells)[c]
OVA	32.5 ± 0.1	679.0 ± 54.2
SSBE (μg/mL)[d]		
10	30.1 ± 2.1 (7.4) [e]	650.9 ± 54.6 (4.1) [e]
30	26.9 ± 0.1 (17.2) e*	569.4 ± 32.9 (16.1) e*
60	9.6 ± 0.4 (70.4) e***	149.5 ± 8.3 (78.0) e**
SPEE (μg/mL)[d]		
10	31.6 ± 1.1 (02.8) [e]	449.0 ± 40.5 (33.9) e**
30	22.4 ± 0.1 (31.1) e***	282.1 ± 47.6 (58.5) e***
60	10.1 ± 0.3 (68.9) e***	137.2 ± 8.9 (79.8) e***

[a] Guinea pig lung mast cells (4 × 10^5 cells/mL) were passively sensitized by anti-OVA antibody and challenged with 0.1 μg/mL OVA for 10 min. Mast cells were pretreated with either SSBE or SPEE for 5 min before OVA challenge. Amounts of spontaneous histamine release and total histamine were 76.3 ± 8.3 and 2137.6 ± 62.7 ng/4 × 10^5 cells, respectively. [b,c] Histamine release and leukotriene production were determined as described in **Table 1**. [d] The amount of baicalin in SSBE or isoorientin in SPEE was 21.8% and 3.7%, respectively. SSBE is a standardized *S. baicalensis* extract and SPEE is a standardized *P. edulis* extract. [e] Parentheses represent the decreased percentage compared with the released amount when activated with OVA/anti-OVA antibody reaction. * P < 0.05; ** p < 0.01; *** p < 0.001 compared with OVA/anti-OVA antibody reaction

Leukotrienes released from mast cell activation after pretreatment with baicalin or isoorientin were inhibited similarly to histamine (**Table 1**).

The purification of baicalin and isoorientin used organic solvents and columns, which would be harmful to humans besides not being economical. Two standardized extracts that include these two active ingredients, SSBE from *S. baicalensis* roots and SPEE from *P. edulis* leaves, were prepared using only ethanol and water, considering safety and cost. The amounts of baicalin in SSBE and isoorientin in SPEE were 21.8% and 3.7%, respectively. Therefore, we prepared a combined extract, SHC, which contained 7.3% baicalin and 2.4% isoorientin.

To examine the effects of SSBE and SPEE on histamine and leukotriene release, mast cells were first sensitized by anti-OVA antibody and challenged with 0.1 μg/mL OVA. Cells were then pretreated with varying concentrations (10, 30, or 60 μg) of SSBE or SPEE. Histamine release was inhibited by 7.4%, 17.2%, and 70.4% after SSBE pretreatment and 2.8%, 31.1%, and 68.9% after SPEE pretreatment, respectively, compared with the OVA/anti-OVA antibody reaction, which elicited a histamine release of 32.5 ± 0.1% (**Table 2**).

Leukotriene levels released from mast cells after pretreatment with the same concentrations of SSBE or SPEE were inhibited by 4.1%, 16.1%, and 78.0% and by 33.9%, 58.5%, and 79.8%, respectively, compared with the OVA/anti-OVA antibody reaction, which elicited leukotriene release of 679.0 ± 54.2 pg/4 × 10^5 cells (**Table 2**).

Overall, SSBE and SPEE were 6–9 times less effective at inhibiting histamine and leukotriene release at low doses (10 and 30 μg/mL) than baicalin and isoorientin, except for the inhibition of leukotriene release by isoorientin. At a high dose (60 μg/mL), both SSBE and SPEE inhibited histamine and leukotriene release to a greater extent than baicalin and isoorientin. We assume that this is due to the fact that pure baicalin and isoorientin are barely soluble in water, whereas the extracts are more soluble. The extracts are emulsified or suspended in water by fatty acids or saponins, which generally exist in plant extracts. However, this hypothesis needs further study, and additional compounds from *S. baicalensis* roots and *P. edulis* leaves must be isolated.

Spontaneous histamine and leukotriene release (negative controls) were 87.2 ± 8.7 ng/4 × 10^5 cells and 170.5 ± 19.0 pg/4 × 10^5 cells, respectively, and within the range of standard error in all experiments. However, both spontaneous histamine and total histamine levels are shown in each table for the calculation of the percentage of histamine release after treatment with the OVA/anti-OVA antibody reaction.

SHC (10, 30, or 60 μg) pretreatment inhibited histamine release by 40.2%, 53.6%, and 69.8%, respectively, compared with the challenge by the OVA/anti-OVA antibody reaction (32.1 ± 0.1 ng/4 × 10^5 cells). SHC (10, 30, or 60 μg) pretreatment inhibited leukotriene release from mast cells by 41.2%, 50.1%, and 77.5%, respectively, compared with 669.1 ± 29.2 pg/4 × 10^5 cells after the OVA/anti-OVA antibody reaction (**Table 3**).

Both acetylsalicylic acid and cromolyn sodium (60 μg) used as positive controls showed less than 5% and 15% inhibitory activity on histamine and leukotriene release, respectively, but a high concentration of these agents (500 μg) significantly inhibited histamine release by 45.8% and 31.5%, respectively, and leukotriene release by 44.1% and 29.5%, respectively, during mast cell activation (**Table 3**). Therefore, SHC was a more potent inhibitor than aspirin [11] and cromolyn [12,13], which at high concentrations were reported to significantly inhibit histamine or leukotriene release, but at low concentrations weakly inhibited the release of these mediators.

Our results show that low concentrations (10 and 30 μg) of SHC inhibit histamine and leukotriene release after mast cell activation to a greater extent than either plant extract individually, and that at a high concentration (60 μg), SHC (**Table 3**) showed similar results to those of each standardized extract (**Table 2**). These data suggest that a high concentration of SHC induces maximum efficacy and that other compounds contained in SHC may also inhibit mediator release. The data also indicate that the other

Downloaded by: University of Arizona Library. Copyrighted material.

Table 3 The effects of SHC on release of mediators from passively sensitized guinea pig lung mast cells activated by OVA[a].

Treatment	Histamine (%)[b]	Leukotrienes (pg/4 × 10^5cells)[c]
OVA	32.1 ± 0.1	669.1 ± 29.2
SHC (μg/mL)[d]		
10	19.2 ± 0.3 (40.2) [e***]	393.4 ± 33.3 (41.2) [e***]
30	14.9 ± 1.0 (53.6) [e***]	333.9 ± 23.3 (50.1) [e***]
60	9.7 ± 0.9 (69.8) [e***]	150.5 ± 21.7 (77.5) [e***]
Acetylsalicylate (μg/mL)		
60	30.9 ± 1.2 (03.7)	656.4 ± 29.0 (01.9)
500	17.4 ± 5.1 (45.8) [e***]	374.0 ± 34.9 (44.1) [e***]
Cromolyn (μg/mL)		
60	27.7 ± 1.3 (13.7) [e*]	576.1 ± 23.7 (13.9) [e*]
500	22.0 ± 1.4 (31.5) [e***]	471.7 ± 26.2 (29.5) [e***]

[a] Guinea pig lung mast cells (4 × 10^5 cells/mL) were passively sensitized by anti-OVA antibody and challenged with 0.1 μg/mL OVA for 10 min. Mast cells were pretreated with SHC for 5 min before OVA challenge. Amounts of spontaneous histamine release and total histamine were 76.3 ± 8.3 and 2137.6 ± 62.7 ng/4 × 10^5 cells, respectively. [b,c] Histamine release and leukotriene production were determined as described in ▶ **Table 1**. [d] The amounts of baicalin and isoorientin in SHC were 7.3% and 2.4%, respectively. [e] Parentheses represent the decreased percentage compared with the released amount when activated with OVA/anti-OVA antibody reaction. * $P < 0.05$; *** $p < 0.001$ compared with OVA/anti-OVA antibody reaction

compounds contained in SHC are present at insufficient concentrations to affect mediator release at lower doses.

In conclusion, we suggest that a low concentration of SHC may have strong potential to reduce several allergic inflammation by suppressing histamine and leukotriene release from mast cells, and that SHC may be clinically useful due to its antiallergic effects.

References

1 *Schatz M, Leung D, Goldstein S.* Consultation and referral guidelines citing the evidence: how the allergist-immunologist can help. J Allergy Clin Immunol 2006; 117: S495–S523
2 *Bielory L.* Allergic and immunologic disorders of the eye. Part II: Ocular allergy. J Allergy Clin Immunol 2000; 106: 1019–1032
3 *Foreman JC.* Introduction to mast cells and basophils, 3rd edition. Immunopharmacology of mast cells and basophils. London: Academic Press; 1993: 1–4
4 *Brown JM, Wilson TM, Metcalfe DD.* The mast cell and allergic diseases: role in pathogenesis and implications for therapy. Clin Exp Allergy 2008; 38: 4–18
5 *Metcalfe DD.* Mast cells and mastocytosis. Blood 2008; 112: 946–956
6 *Li HB, Jiang Y, Chen F.* Separation methods used for *Scutellaria baicalensis* active components. J Chromatogr B Anal Technol Biomed Life Sci 2004; 812: 277–290
7 *Li HY, Sun JW, Dai SW.* Study on chemical components of bamboo leaf. Chin Med Mater 2003; 26: 562–563
8 *Wu S, Sun A, Liu R.* Separation and purification of baicalin and wogonoside from the Chinese medicinal plant *Sutellaria baicalensis* Georgi by high-speed counter-current chromatography. J Chromatogr A 2005; 1066: 243–247
9 *Orhan DD, Aslan M, Aktay G, Ergun E, Yesilada E, Ergun F.* Evaluation of hepatoprotective effect of *Gentiana olivieri* herbs on subacute administration and isolation of active principle. Life Sci 2003; 72: 2273–2283
10 *Ro JY, Lee BC, Kim JY, Chung YJ, Chung MH, Lee SK, Jo TH, Kim KH, Park YI.* Inhibitory mechanism of aloe single component (Alprogen) on mediator release in guinea pig lung mast cells activate with specific antigen-antibody reactions. J Pharmacol Exp Ther 2000; 292: 114–121
11 *Grosman N.* Comparison of the influence of NSAIDs with different COX-selectivity on histamine release from mast cells isolated from naïve and sensitized rats. Int Immunopharmacol 2007; 7: 532–540
12 *Wells E, Jackson CG, Harper ST, Mann J, Eady RP.* Characterization of primate bronchoalveolar mast cells. II. Inhibition of histamine, LTC4, and PGD2 release from primate bronchoalveolar mast cells and a comparison with rat peritoneal mast cells. J Immunol 1986; 137: 3941–3945
13 *Wang XS, Lau HYA.* Histamine release from human buff coat-derived mast cells. Int Immunopharmacol 2007; 7: 541–546

Downloaded by: University of Arizona Library. Copyrighted material.