- Research Letter -

Identification of Dimethylmatairesinol as an Immunoglobulin E-suppressing Component of the Leaves of *Cinnamomum camphora*

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(Received November 4, 2010; Accepted December 20, 2010; Published online December 27, 2010)

Immunoglobulin E (IgE) plays an important role in allergic diseases. In this study, we found that a methanol extract of leaves of the camphor tree *Cinnamomum camphora* (*C. camphora*) reduced the amount of IgE secreted by human myeloma U266 cells. When the methanol extract was fractionated by extraction with organic solvents, the ethyl acetate fraction showed the highest activity. The fraction was further separated into several subfractions by preparative TLC. We identified the component of one of the active subfractions as dimethylmatairesinol. Authentic dimethylmatairesinol exhibited similar activity. Thus, the extract of *C. camphora* and its components including dimethylmatairesinol have potential as an anti-allergic agent.

Key words — dimethylmatairesinol, U266, immunoglobulin E, camphor tree, *Cinnamomum camphora*, lignan

INTRODUCTION

The recent epidemic of allergies has heightened interest in immunoglobulin E (IgE) which plays an important role in the allergic response. It is important to search for a new compound which reduces excess production or secretion of IgE for patients with allergic diseases.

The human myeloma cell line U266 produces IgE and secretes it into the culture medium spontaneously.¹⁻⁶⁾ We have used these cells to evaluate suppressive effect of IgE production or secretion and examined methanol extracts from more than 200 species of plant. As a result, we found that a methanol extract from leaves of the camphor tree Cinnamomum camphora (C. camphora Lauraceae) decreased the amount of IgE in the culture medium of U266 cells dose-dependently. Although C. camphora has long been prescribed in traditional medicine for the treatment of inflammation-related diseases,⁷⁾ no report has described that an extract from C. camphora suppresses IgE production or secretion by U266 cells. In the present study, we identified dimethylmatairesinol as one of the active components in the C. camphora extract which has a suppressive effect on IgE production/secretion in U266 cells.

MATERIALS AND METHODS

Cells and Chemicals — U266 cells were obtained from Dainippon-Sumitomo Pharmaceutical Co. Ltd. (Osaka, Japan) and maintained in RPMI1640 medium supplemented with 10% fetal bovine serum, 50 U/ml penicillin, 50 µg/ml streptomycin, and 2.5 µg/ml amphotericin B at 37°C under a 5% CO₂ atmosphere. An IgE assay kit (MESACUP IgE Test) was purchased from Medical and Biological Laboratories Co. Ltd. (Nagoya, Japan). Dimethylmatairesinol and matairesinol were obtained from APIN Chemicals Ltd. (Oxon, U.K.) and Cayman Chemical Company (Ann Arbor, MI, U.S.A.), respectively.

Plant Material and Extraction — Leaves of *C. camphora* were collected in Shizuoka, Japan, in August 2009. The washed leaves were dried at 60° C for 24 hr. Crushed dry leaves (20 g) were extracted at 25°C with absolute methanol (100 ml) for 1 week with occasional shaking. The methanol extract thus obtained was filtered by filter paper and the filtrate was dried under reduced pressure to yield 303 mg. The extract (273 mg) was then extracted with *n*-hexane, ethyl acetate, *n*-butanol, and water to yield 53.6, 46.8, 50.9, and 70.1 mg of dry mate-

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rial, respectively. These materials were dissolved in dimethyl sulfoxide for use.

TLC — Samples dissolved in methanol were spotted on TLC Plate Silica Gel 60 F_{254} (Merck Ltd., Tokyo, Japan) and developed in chloroformethyl acetate (95:5) or (9:1). Spots were visualized by immersing in 5% ethanolic phosphomolybdic acid,⁸⁾ followed by heating. Preparative TLC was performed similarly using a solvent mixture of chloroform-ethyl acetate.

Enzyme-linked Immunosorbent Assay (ELISA) of IgE — U266 cells were plated in flat-bottomed 96-well plates at 3.5×10^4 cells per well and treated with test samples. After incubation for 4 hr, the supernatants were collected by centrifugation at $1400 \times g$ for 10 min and IgE concentrations were determined using the MESACUP IgE Test according to the manufacturer's instructions.

Cytotoxicity —— Cell viability was determined by the Trypan blue dye exclusion test as reported pre-viously.⁹⁾

Time-of-flight Mass Spectrometry (TOF-MS) and NMR Analysis — High-resolution TOF-MS was performed using a Shimadzu system (MALDI-TOFMS-AXIMA, Shimadzu, Kyoto, Japan). ¹H NMR (400 MHz), ¹³C NMR (100 MHz), HH-correlation spectroscopy (COSY), heteronuclear single-quantum coherence, and heteronuclear multiple-bond correlation spectra (HMBC) were recorded in CDCl₃ at room temperature with a JEOL JNM-A400 system (JEOL Ltd., Tokyo, Japan). Samples (3 mg) were dissolved in 0.5 ml of chloroform-d (ISOTEC Ltd., Tokyo, Japan). Chemical shifts were expressed in parts per million relative to tetramethylsilane as an internal standard.

Statistical Analysis — Data were analyzed by one-way analysis of variance (ANOVA), and post hoc Tukey-Kramer tests. Differences were considered significant at p < 0.05. Data are expressed as means \pm S.E. The statistical calculations were carried out using Stat View 5.0 computer software (SAS Institute, Tokyo, Japan).

RESULTS AND DISCUSSION

When U266 cells, human IgE-bearing B cells, were incubated with the methanol extract from *C*. *camphora* at a final concentration of 10, 30, and $120 \,\mu$ g/ml, the results of ELISA showed that the amount of IgE in the culture medium decreased dose-dependently as compared with the control



Fig. 1. Effect of the Methanol Extract from *C. camphora* on the IgE Concentration in the Culture Medium of U266 Cells

The IgE concentrations in the presence of the methanol extract at different concentrations are expressed as the mean \pm S.E. from 3 determinations. The data with different alphabetic letters are different with statistical significance from each other.

(vehicle-treated cells, Fig. 1). The results of the Trypan blue assay indicated that cell viability was more than 90% in each case (data not shown). These results suggested that the methanol extract contained some compound(s) which suppressed IgE production/secretion in U266 cells and did not cause significant cytotoxicity at least up to a concentration of $120 \mu g/ml$.

The methanol extract from *C. camphora* was then fractionated by successive extraction with organic solvents and the effects of the different fractions on U266 cells were examined by ELISA. The amount of IgE in the culture medium of the cells treated with the hexane, ethyl acetate, butanol, and water fractions at $120 \,\mu$ g/ml for 4 hr was 50.5 ± 2.2 , 25.3 ± 2.1 , 71.5 ± 3.2 and $77.7 \pm 1.2\%$, respectively, of the control value ($100 \pm 7.4\%$).

TLC of the ethyl acetate fraction, which exhibited the strongest inhibitory activity, indicated that several compounds were present (Fig. 2A). We separated this fraction into 9 fractions by preparative TLC (Fig. 2B) and examined their effects on U266 cells (data not shown). We found that fraction 6 exhibited the IgE-reducing activity and one of three subfractions (6b, Fig. 2B) contained the active substance (Fig. 2C) which was named as compound 1. The yield of compound 1 was about 1 mg from 100 mg of the methanol extract from *C. camphora*.

Compound 1 was identified as dimethylmatairesinol (Fig. 3) based on¹H NMR spectrum,





Fig. 2. Fractionation of the Ethyl Acetate Fraction by TLC and the Activity of the Subfractions

The ethyl acetate fraction (EA) was separated by preparative TLC using a developing solvent of chloroform-ethyl acetate (95:5) to give fractions 1–9 (A). The fraction 6 was further separated by preparative TLC using developing solvent of chloroform-ethyl acetate (9:1) to give 6a, 6b, and 6c (B) and the culture medium IgE concentrations after incubation for 4 hr in the presence of these subfractions at 60 μ g/ml are expressed relative to that for the control cells (370 IU/ml, 100%, C).

¹³C NMR spectrum, and TOF-MS. ¹H-NMR $(CDCl_3, 400 \text{ MHz}) \delta 2.50 \text{ (dd, } J = 13.4, 8.0 \text{ Hz},$ H-7b), 2.51 (ddddd, J = 9.1, 8.2, 8.0, 7.3, 5.8 Hz, H-8), 2.60 (ddd, J = 9.1, 6.3, 5.6 Hz, H-8'), 2.62 (dd, J = 13.4, 5.8 Hz, H-7a), 2.91 (dd, J = 14.0,5.6 Hz, H-7'b), 2.95 (dd, J = 14.0, 6.3 Hz, H-7'a), 3.83 (s, H-12), 3.84 (s, H-11), 3.86 (s, H-13), 3.87(s, H-10), 3.89 (dd, J = 8.8, 8.2 Hz, H-9b), 4.12 (dd, J = 8.8, 7.3 Hz, H-9a), 6.49 (d, J = 2.1 Hz, H-2), 6.55 (dd, J = 8.1, 2.1 Hz, H-6), 6.66 (dd, J = 8.3, 2.0 Hz, H-6'), 6.69 (d, J = 2.0 Hz, H-2'), 6.76 (d, J = 8.3 Hz, H-5', 6.78 (d, J = 8.1 Hz, H-5). ¹³C-NMR (CDCl₃, 100 MHz) δ 34.49 (C-7'), 38.19 (C-7), 41.07 (C-8), 46.57 (C-8'), 55.82 (C-12), 55.86 (C-11), 55.86 (C-13), 55.90 (C-10), 71.23 (C-9), 111.06 (C-5), 111.30 (C-5'), 111.81 (C-2), 112.33 (C-2'), 120.55 (C-6), 121.34 (C-6'), 130.18 (C-1'), 130.42 (C-1), 147.86 (C-3'), 147.94 (C-3), 149.02



Fig. 3. Chemical Structure of Compound 1 Identified as Dimethylmatairesinol

A, COSY and HMBC correlations. B, Deduced chemical structure.

(C-4'), 149.04 (C-4), and 178.69 (C-9'). TOF-MS (ESI⁺) m/z 387.1807 [39%, M+H]⁺ [Calculated for $C_{22}H_{27}O_6$ (M+H)⁺ 387.1808] and 405.1879 [88%, M+H₂O+H]⁺ [Calculated for $C_{22}H_{29}O_7$ (M+H)⁺ 387.1808].

We then examined a commercially available dimethylmatairesinol preparation for IgE-reducing activity. The results showed that the IgE concentration in the culture medium of U266 cells decreased dose-dependently (Fig. 4). Cell viability was more than 90% in each case. The concentrations higher than 100 μ g/ml resulted in cytotoxicity, preventing evaluations of IgE production. It should be noted that authentic matairesinol had no IgE-suppressing activity up to 100 μ g/ml.

Thus, we found that the methanol extract of *C. camphora* showed activity to reduce the concentration of IgE in the culture medium of U266 cells. One of the active principles was identified as dimethylmatairesinol. Many natural extracts have been shown to suppress IgE production by U266 cells,²⁻⁶⁾ but these studies failed to identify the active principle. For example, green tea extract has been shown to suppress IgE production in U266 cells, but the structure of the active component(s) remains to be determined.⁶⁾ Therefore, dimethyl-





The culture medium IgE concentrations in dimethylmatairesinol at different concentrations are expressed as the mean \pm S.E. from 3 determinations. The data with different alphabetic letters are different with statistical significance from each other.

matairesinol represents one of the few natural products identified as having such activity.

The methanol extract of *C. camphora* seemed to contain other active compounds, since the whole activity of the methanol extract cannot be accounted for by dimethylmatairesinol alone, judging from its content and since the activity was also detected in fractions 4, 5, and 7–9 in Fig. 2 (data not shown). Thus, the extract of *C. camphora* and its components including dimethylmatairesinol have potential as an anti-allergic agent. More studies are needed to know if these fractions exhibit anti-allergic activities *in vivo*.

REFERENCES

 Nilsson, K., Bennich, H., Johansson, S. G. O. and Ponten, J. (1970) Established immunoglobulin producing myeloma (IgE) and lymphoblastoid (IgG) cell lines from an IgE myeloma patient. *Clin. Exp. Immunol.*, **7**, 477–489.

- Kim, H. M. and Moon, Y. S. (1999) Asiasari radix inhibits immunoglobulin E production on experimental models in vitro and in vivo. *Immunopharmacol. Immunotoxicol.*, 21, 469–481.
- Kim, H. M., Kim, H. J. and Park, S. T. (1999) Inhibition of immunoglobulin E production by *Poncirus trifoliata* fruit extract. *J. Ethnopharmacol.*, **66**, 283–288.
- 4) Kim, H. M., Lee, J. H., Won, J. H., Park, E. J., Chae, H. J., Kim, H. R., Kim C. H. and Baek, S. H. (2001) Inhibitory effect on immunoglobulin E production *in vivo* and *in vitro* by *Siegesbeckia glabrescens*. *Phytother. Res.*, **15**, 572–576.
- Sugahara, T., Nishimoto, S., Morioka, Y., Nakano, K. and Nakano, K. (2009) White sorghum [Sorghum bicolor (L.) Moench] bran extracts suppressed IgE production by U266 cells. Biosci. Biotechnol. Biochem., 73, 2043–2047.
- Hassanain, E., Silverberg, J. I., Norowitz, K. B., Chice, S., Bluth, M. H., Brody, N., Joks, R., Durkin, H. G. and Smith-Norowitz, T. A. (2010) Green tea (*Camelia sinensis*) suppresses B cell production of IgE without inducing apoptosis. *Ann. Clin. Lab. Sci.*, 40, 135–143.
- Lee, H. J., Hyun, E. A., Yoon, W. J., Kim, B. H., Rhee, M. H., Kang, H. K., Cho, J. Y. and Yoo, E. S. (2006) In vitro anti-inflammatory and antioxidative effect of *Cinnamonum camphora* extracts. *J. Ethnopharmacol.*, **103**, 208–216.
- Peterson, B. L. and Cummings, B. S. (2006) A review of chromatographic methods for the assessment of phospholipids in biological samples. *Biomed. Chromatogr.*, 20, 227–243.
- Ogata, K., Mukae, N., Suzuki, Y., Satoh, K., Narumi, K., Nukiwa, T. and Isemura, M. (1995) Effects of catechins on the mouse tumor cell adhesion to fibronectin. *Planta Med.*, **61**, 472–474.