Histamine Release Inhibitory Activity of Piper nigrum Leaf

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Oral administration of a methanolic extract of Piper nigrum leaf (PN-ext, 50, 200 and 500 mg/kg) showed a potent dose-dependent inhibition of dinitrofluorobenzene (DNFB)-induced cutaneous reaction at 1 h [immediate phase response (IPR)] after and 24 h [late phase response (LPR)] after DNFB challenge in mice which were passively sensitized with anti-dinitrophenyl (DNP) IgE antibody. Ear swelling inhibitory effect of PN-ext (50, 200 and 500 mg/kg, per os (p.o.)) on very late phase response (vLPR) in the model mice was significant but weaker than that on IPR. Oral administration of PN-ext (50, 200 and 500 mg/kg for 7 d) inhibited pieryl chloride (PC)-induced ear swelling in PC sensitized mice. PN-ext exhibited in vitro inhibitory effect on compound 48/80-induced histamine release from rat peritoneal mast cells. Two lignans of PN-ext, (−)-cubebin (1) and (−)-3,4-dimethoxy-3,4-dimethylenedioxy cumberbin (2), were identified as major active principles having histamine release inhibitory activity.

Key words Piper nigrum; anti-allergic effect; cubebin; histamine release inhibitory activity

During the course of our search for useful ingredients of cosmetics from natural resource, we have previously reported that Piper nigrum leaf extract showed both melanogenesis stimulation activity and testosterone 5α-reductase inhibitory activity and that (−)-cubebin (1) and (−)-3,4-dimethoxy-3,4-dimethylenedioxy cumberbin (2) were isolated as active lignan constituents.1—4 It was suggested that these substances may be useful ingredients of hair-care cosmetics for prevention of gray hair and alopecia. As a part of our continuous study on P. nigrum leaf, we examined anti-allergic effect of the leaf, because hair care cosmetics having anti-allergic activity may be desirable. It has been reported that anti-allergic effect of a botanical formulation5 in which P. nigrum fruit was included. Anti-allergic activity of piperine,6 a major alkaloid amide of P. nigrum fruit have been reported, but HPLC analysis revealed that the piperine content in the leaf was 0.00007%.7 Moreover, we have not found any reports on anti-allergic effect of the leaf. In this paper, we describe in vivo anti-allergic effects of P. nigrum leaf extract against type I allergy model and type IV allergy model in mice. 2,4-Dinitrofluorobenzene (DNFB) has been shown to induce triphasic increase in ear thickness of mouse by the challenge after a passive sensitization with monoclonal anti-dinitrophenyl (DNP) IgE antibody.8,9 This model serves as an animal model for type I allergy.8,9 In addition, we used pieryl chloride (PC)-induced contact dermatitis in mice as an animal model for type IV allergy.10 Inhibitory effects of Piper nigrum leaf extract, on histamine release were also examined by in vitro assay using compound 48/80-induced histamine release from rat peritoneal mast cells.

MATERIALS AND METHODS

Plant Materials Piper nigrum leaf was the same sample described in the previous paper.1—4

Reagents 2,4-Dinitrofluorobenzene (DNFB), Ficoll, Hanks’ balanced salt solution, olive oil, 0-phthalaldehyde, pieryl chloride (PC), and sodium carboxymethyl cellulose (CMC·Na) were purchased from Nacalai Tesque (Tokyo, Japan). Sodium cromoglycate (SCG) was purchased from Funakoshi (Tokyo, Japan). Compound 48/80, dimethyl sodium salt (DMSO), prednisolone, monoclonal anti-DNP antibody and heparin sodium salt were purchased from Sigma (St. Louis, MO, U.S.A.). Other chemical reagents were reagent grade and were purchased from Wako Pure Chemical Industries, Ltd. unless otherwise noted.

Preparation of PN-ext and Fractionation Methanolic extract of Piper nigrum leaf (PN-ext) was obtained according to the previous paper in yield of 14.0%. The powdered dry leaves (500 g) of P. nigrum were extracted with MeOH (51×3 times) for 1 h under reflux. Combined extracts were evaporated under reduced pressure to give a methanolic extract (PN-ext, 71.1 g). A part of PN-ext (69.6 g) was extracted with hexane (400 ml×3), and the organic layer was evaporated to give a hexane soluble fraction (17.3 g). The hexane insoluble part was extracted with EtOAc (300 ml×2). The EtOAc insoluble part was suspended in H2O (500 ml) and extracted with EtOAc (300 ml×3). The EtOAc extracts were combined and evaporated to afford an EtOAc soluble fraction (20.5 g). The aqueous layer was evaporated followed by lyophilization to give a water soluble fraction (21 g). Natural (−)-cubebin (1) and (−)-3,4-dimethoxy-3,4-dimethylenedioxy cumberbin (2) were isolated from the EtOAc soluble fraction.1—4

Animals Female BALB/c strain mice (14—19 g), female ICR strain mice (30—32 g) and male Wistar strain rats (160—180 g) were provided by SLC (Japan SLC, Hamamatsu, Japan). They were maintained in an air-conditioned room with lighting from 7 a.m. to 7 p.m. The room temperature (about 23 °C) and humidity (about 60%) were controlled automatically. Laboratory pellet chow (Labo MR Stock, Nihon Nosen Kogyo Co., Ltd., Tokyo, Japan) and water were freely available.

IgE-Mediated Cutaneous Reaction in Mice IgE-mediated cutaneous reaction was induced according to the methods of Yamaguchi et al.8 and Tahara et al.9 with minor modifications. Female BALB/c strain mice weighing 14—19 g (n=10 to 11 per group) were passively sensitized with intra-
venous injection of 10 μg of monoclonal anti-DNP IgE antibody dissolved in 0.5 ml of saline. Twenty-four hours after the sensitization, the mice were challenged by painting of 25 μl of 0.15% DNFBS solution in acetone : olive oil (3:1) to each side of right and left ears. The control mice received intravenous injection of saline (0.5 ml) instead of IgE antibody and painting of 25 μl of 0.15% DNFBS solution. The vehicle control mice received intravenous injection of IgE antibody and painting of 25 μl of 0.15% DNFBS solution. Prednisolone was suspended in 0.2% CMC·Na. Appropriate amount of PN-ext was suspended in 0.2% CMC·Na. The suspension of test sample was administered orally (0.2 ml/10 g body weight of mouse/d) at 1 h before DNFB-challenge, 24 h after DNFB-challenge, and every day for successive 7 d. To control and vehicle control mice, 0.2% CMC·Na was administered (0.2 ml/10 g body weight of mouse/d). The thickness of right ear was measured by using a dial thickness gauge (Mitutoyo Co., Tokyo) immediately before, and at 1 h [immediate phase response (IPR)], 24 h [late phase response (LPR)] and 8 d [very late phase response (vLPR)] after the DNFB challenge. The ear swelling (cutaneous reaction) was expressed as the difference in the ear thickness between immediately before the DNFB challenge and those at 1 h (IPR), 24 h (LPR) or 8 d (vLPR) after the DNFB challenge, respectively. The experimental results were expressed as the average of ear thickness±S.E. (n = 10 to 11 per group).

Picryl Chloride (PC)-Induced Contact Dermatitis in Mice According to the method described by Asherson and Ptak,11) with modification, female ICR strain mice weighing 30—32 g were sensitized by topical application of 0.1 ml of 7% PC solution in EtOH to the shaved abdomen on day 0. Appropriate amount of PN-ext was suspended in 0.2% CMC·Na, and administered orally (0.2 ml/10 g body weight of mouse/d) from day —1 to day 5 for 7 d. Prednisolone was suspended in 0.2% CMC·Na, and administered orally (0.2 ml/10 g body weight of mouse/d) from day 0 to day 5 for 6 d. To control mice, 0.2% CMC·Na was administered orally (0.2 ml/10 g body weight of mouse/d) for 7 d. Six days after the sensitization, the treated mice were challenged by painting the inside of ears with 20 μl of 1% PC solution in olive oil to induce PC-induced contact dermatitis. The ear swelling was expressed as the difference in the ear thickness between immediately before and 24 h after the PC challenge. The ear thickness was measured by using a dial thickness gauge (Mitutoyo Co., Tokyo, Japan). The experimental results were expressed as the average of ear thickness±S.E. (n = 10 per group).

Compound 48/80-Induced Histamine Release from Rat Peritoneal Mast Cells Mast cells were prepared from the peritoneal cavity fluid of male Wistar strain rats by a slight modification of the method described by Uvnäs and Thon.12) The cells were suspended in Hanks’ solution containing heparin (10 U/ml), then layered on 40% Ficoll in a test tube for 1 h. The cells were washed three times with 5 ml of phosphate-buffered saline (pH 7.0) and suspended in the same medium at 2.9×10⁶ cells/ml. The cell suspensions contained 85—90% or more viable mast cells, as determined by the toluidine blue (0.1% in 50% EtOH) staining test of Bray and VanArsdel.13)

The test substances dissolved with 5% dimethyl sulfoxide (DMSO) were added to the mast cell suspension, and then the mixture was incubated at 37°C. After 10 min, 0.1 ml of compound 48/80 solution (0.2 mg/ml) was added, and the mixture was incubated at 37°C for 10 min in a final volume of 2 ml. Cooling the mixture on ice terminated the reaction. The mixture was centrifuged at 150×g and 5°C for 5 min, then histamine in the supernatant fluid was assayed fluorometrically according to the method of Shore et al.14) The activity of the test substance on histamine release from mast cells induced by compound 48/80 was expressed as histamine release percentage. SCG was used as a reference drug.

RESULTS AND DISCUSSION

The effect of oral administration of PN-ext on DNFBS-induced triphasic cutaneous reaction was examined by ear swelling in mice passively sensitized with anti-DNP IgE antibody. As shown in Fig. 1 in comparison with the control and the vehicle control groups, DNFBS-induced triphasic cutaneous reaction (ear swelling) at 1 h [immediate phase response (IPR)] after, 24 h [late phase response (LPR)] after and 8 d [very late phase response (vLPR)] after DNFBS challenge. The efficacy of the test substances on ear swelling was evaluated by measuring ear thickness at 1 h (IPR) after, 24 h (LPR) after and 8 d (vLPR) after DNFBS challenge as shown in Fig. 1. Among these responses, IPR is considered to be type I allergy model. As shown in Fig. 1, PN-ext (50, 200 and 500 mg/kg, per os (p.o.)) showed a potent dose-dependent inhibition of ear swelling of both IPR and LPR. Ear swelling inhibitory effect of PN-ext (50, 200 and 500 mg/kg, p.o.) on very late phase response (vLPR) in the model mice was significant but weaker than that on IPR. Prednisolone (10 mg/kg, p.o.) had a potent inhibitory effect on IPR, LPR and vLPR.

PC-induced contact dermatitis in mice which were sensitized by PC was used as an animal model for type IV allergy.
The efficacy of the test substance on ear swelling was evaluated by measuring ear thickness at 1 h after the PC-challenge on the last day of the experiment. As shown in Fig. 2, oral administration of PN-ext (50, 200 and 500 mg/kg for 7 d, p.o.) dose-dependently inhibited the PC-induced ear swelling in PC sensitized mice. Prednisolone (10 mg/kg, p.o.) had a potent inhibitory effect. These results indicated that PN-ext had anti-allergic effects against both type I and type IV, and that PN-ext showed more potent inhibitory effect on IPR than vLPR in DNF-induced triphasic cutaneous reaction. Several cytokines such as IL-1β and TNF-α play important roles in LPR. On the other hand, although vLPR is characterized by an accumulation of eosinophils, the precise mechanisms for vLPR are not fully elucidated. It is considered that PN-ext may exert some actions on these cytokines and eosinophils, but, in this paper, we firstly targeted to explain the potent inhibitory effect of PN-ext on IPR. Detailed mechanisms of PN-ext involved in PC-induced ear swelling and LPR and vLPR should be examined further.

It has been known that the inhibition of histamine release from the mast cells plays an important role in the mechanism of anti-allergic effect against type I allergy such as IPR. Therefore we examined in vitro histamine release inhibitory activity of PN-ext by an assay with compound 48/80-induced histamine release from rat peritoneal mast cells. Sodium cromoglycate (SCG) was used as a reference drug. As shown in Table 1, PN-ext at concentrations of 200 and 500 μg/ml exhibited a significant inhibitory effect, and SCG at a concentration of 500 μg/ml had the inhibitory activity. In order to identify active principles, we fractionated the PN-ext into three fractions (a hexane soluble fraction, an ethyl acetate soluble fraction, and a water soluble fraction). Among them, the ethyl acetate soluble fraction showed the most potent activity as depicted in Table 2. Two major lignans of PN-ext, (−)-cubebin (1) and (−)-3,4-dimethoxy-3,4-desmethylenedioxycubebin (2), were already isolated from the ethyl acetate soluble fraction as described in the previous paper.14 Because Tsuruga et al.15 reported that several cubebin-type lignans having 3,4-dibenzyltetrahydrofuran ring, such as matairesinol and trachelogenin, had significant histamine release inhibitory activities, we examined inhibitory effects of 1 and 2 on compound 48/80-induced histamine release from rat peritoneal mast cells. As shown in Table 3, the inhibitory activities of 1 and 2 were superior to that of SCG. This inhibitory effect of 1 and 2 was found for the first time.

In conclusion, it is clear that PN-ext has in vivo anti-allergic effect against type I in mice and in vitro inhibitory activity on compound 48/80-induced histamine release from rat peritoneal mast cells. In addition, 1 and 2, two lignans of PN-ext, were identified as major active principles inhibiting histamine release.

REFERENCES