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Journal of Ethnopharmacology 101 (2005) 308–312
Journal of ETHNO-PHARMACOLOGY
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Antipruritic effect of the single oral administration of German chamomile flower extract and its combined effect with antiallergic agents in ddY mice

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Received 3 February 2005; received in revised form 6 April 2005; accepted 7 May 2005
Available online 17 June 2005

Abstract

The single peroral administration of the ethyl acetate extract or essential oil of German chamomile (*Matricaria recutita* L.) showed remarkable antipruritic effects in the compound 48/80-induced itch-scratching test in ddY mice, if suitable vehicle was used. The ethyl acetate extract or essential oil of German chamomile dissolved in the vehicle of 10% ethanol, 10% Tween 80 and 80% physiological saline was orally administrated 2 h before pruritus provocation by compound 48/80 subcutaneous injection. The ethyl acetate extract or essential oil of German chamomile showed significant dose-dependent inhibition of the compound 48/80-induced scratching without affecting spontaneous motor activity. The antipruritic effects of antihistamine H1 antagonists, oxatomide (10 mg/kg) and fexofenadine (10 mg/kg), were only partial in this test. However, the antipruritic effects of these agents were remarkably enhanced by the combined administration of the ethyl acetate extract of German chamomile (300 mg/kg). Thus, the co-medication with the ethyl acetate extract, or essential oil of German chamomile and antihistamines might be effective for the pruritus which could not be perfectly resolved alone by conventional antihistamines.

Keywords: Antipruritic; Compound 48/80; German chamomile; *Matricaria recutita*; Oxatomide; Fexofenadine

1. Introduction

Itching and scratching are important factors in the maintenance of symptoms of skin diseases especially in patients with atopic dermatitis (Wahlgren, 1999). Histamine is well known to be present in skin mast cells and considered to be an important mediator of itchiness, however, the itch of atopic dermatitis is generally resistant to antihistamines (Berth-Jones and Graham-Brown, 1989; Wahlgren et al., 1990; Munday et al., 2002). Thus, the development of non-antihistaminic agent is highly anticipated.

In the previous study, we have demonstrated that 11 days intake of the diet containing 1.2 w/w% of the ethyl acetate extract of German chamomile (*Matricaria recutita* L., Compositae) have remarkable antipruritic effects in the compound 48/80-induced itch-scratching test in ddY mice (Kobayashi et al., 2003). Compound 48/80 is an oligomeric mixture of condensation products of *N*-methyl-*p*-methoxyphenethylamine and formaldehyde (Gietzen et al., 1983) and has been widely used as a selective histamine release agent from mast cells of rats (Wu et al., 1993; Ikarashi et al., 2001) and mice (Toda et al., 1988; He et al., 1990). Although both histamine and compound 48/80 (Fjellner and Hagermark, 1981) have been known to produce an itchy sensation in humans, an injection of histamine failed to induce scratching behaviour in ddY mice (Kuraishi et al., 1995). Because injection of serotonin or substance P successfully induced scratching behaviour in ddY mice (Kuraishi et al., 1995; Inagaki et al., 2001) and these putative mediators for itch were known to be released by compound 48/80 administration (Saria et al., 1984; Ohta et al., 1999), compound 48/80-induced itch-scratch responses in ddY mice seem to be a suitable parameter for evaluating non-antihistaminic antipruritic agents.

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doi:10.1016/j.jep.2005.05.003

The flower of German chamomile is strongly aromatic and has a bitter taste. The infusion is one of the most popular herbal tea and have been traditionally used as carminative, sedative and tonic. It is known to be effective for gastrointestinal spasms and inflammatory diseases of the gastrointestinal tract. The infusion can also be administered as a compress for skin and mucous membrane inflammations and bacterial skin disease. In this study, we have demonstrated that single peroral administration of the ethyl acetate extract or essential oil of German chamomile showed dose dependent and significant suppressive effect on the compound 48/80-induced pruritus in ddY mice, and its efficacy had compared with those of oxatomide and fexofenadine. Following pharmacological effects are known in these drugs (Assanasen and Naclerio, 2002). Fexofenadine: the main action is the selective histamine H1-receptor antagonism and also has inflammatory cytokine production inhibitory effect, eosinophil chemotaxis inhibitory effect and chemical mediator release inhibition action. Oxatomide: an Hl-receptor antagonist with potent antihistaminic activity and inhibitor effects on mast cell degranulation. Also, it shows chemical mediator (histamine, leukotriene, substance P) release inhibition, and other chemical mediator (leukotriene, serotonin, acetylcholine, bradykinin) antagonism. The antipruritic effects of combined administration of the German chamomile ethyl acetate extract with these antiallergic agents were also examined.

2. Materials and methods

2.1. Animals

All the experiments were performed with male ddY mice (6-week-old, Japan SLC, Ltd., Tokyo, Japan). The animals were housed at $22 \pm 2\,^{\circ}C$ under a 12 h light-dark cycle (lights on 07:00–19:00). For acclimation, standard diet (CE-2, CLEA Japan Inc., Tokyo, Japan) and water were provided ad libitum for at least 3 days. Each animal was used for one experiment. All experimental protocols were approved by the Animal Care and Use Committee of the Kyowa Hakko Kogyo Co. Ltd., Tsukuba Research Laboratories.

2.2. Drugs

Oxatomide and fexofenadine obtained from the Pharmaceutical Research Laboratories of Kyowa Hakko Kogyo Co. Ltd. (Tokyo, Japan), which were prepared in 0.5% methyl cellulose 400 (MC400; Kishida Chemical, Osaka, Japan) aqueous solution. Each compound was administered perorally 1 h before compound 48/80 subcutaneous injection.

2.3. Process for preparing the ethyl acetate extract of German chamomile flower

Ethyl acetate extract of German chamomile used in this study was prepared in the previous study (Kobayashi et al., 2003) and stored at $-20\,^{\circ}C$. In brief, 350 g of dried flower of German chamomile *Matricaria recutita* L., Compositae (a bulk product met the Japanese standard for the raw materials of quasi-drugs, Lot Nos. 539200, 449222, Takasago Yakugyo Co. Ltd., Osaka, Japan) was extracted by 71 of ethyl acetate twice under sonication for 3 h at $70\,^{\circ}C$. The decoction was filtered through a filter cloth (Miracloth, Calbiochem-Novabiochem Corp., CA, USA), and the filtrate was evaporated under a reduced pressure and freeze-dried to obtain 14.0 g of ethyl acetate extract.

2.4. Chemical analysis

GC analysis was carried out in the following conditions: GC/MS, HP6890 and HP5973 (Hewlett Packard Co. Ltd., USA); GC/FID, GC-14A (Shimadzu Co. Ltd., Japan); capillary column, NB-1 (60 m × 0.25 mm i.d. × 0.4 μm; GL Sciences Inc., Japan); carrier gas flow, 1 ml/min; injector temperature, $300\,^{\circ}C$; oven temperature, $50\,^{\circ}C$ (10 min isothermal); raise at $5\,^{\circ}C$/min to $150\,^{\circ}C$, raise at $1\,^{\circ}C$/min from 150 to $190\,^{\circ}C$, raise at $5\,^{\circ}C$/min from 190 to $300\,^{\circ}C$ (20 min isothermal).

2.5. Compound 48/80-induced scratching tests

Compound 48/80-induced scratching test was performed as previously reported (Kobayashi et al., 2003) with a small modification. Compound 48/80 dose-dependency elicits scratching of the skin around the injected site by the hind paws, when injected subcutaneously into the rostral back. A 0.125 mg/ml saline solution of compound 48/80 (Sigma Chemical Co., St. Louis, MO, USA) was injected subcutaneously (20.0 μg/site) into the rostral part of the back of mice to provoke scratching behaviour. A vehicle-treated mouse and a test compound-treated mouse were paired and served for the measurement in separate chambers during the same time in order to avoid the error of measurement resulting from circadian rhythm or other factors. Immediately after subcutaneous injection of compound 48/80, the mice (12 animals per observation) were individually placed into cylindrical glass observation chambers (100 mm in width × 180 mm in height) and their scratching behaviors were recorded using a digital video camera (DZ-MV100, Hitachi Co., Tokyo, Japan) under unmanned conditions. The effect of compound 48/80 had almost subsided by 30 min after the injection. Therefore, compound 48/80-induced scratching was counted for 30 min after subcutaneous injection of this compound. Mice generally scratched several times with the hind paws for about 1 s and a series of these movements was counted as one bout of scratching. The percentage of control scratching was calculated based on the accumulated scratching counts of the paired vehicle-treated mouse. The experiments were performed between 10:00 and 16:00.

2.6. Measurement of spontaneous motor activities

Spontaneous motor activity was measured using a passive infrared sensor detection system (SUPERMEX, Muromachi Kikai Co. Ltd., Tokyo, Japan) as we have reported previously (Kobayashi et al., 2003). In order to avoid the error of measurement resulting from circadian rhythm or other factors, a vehicle-treated mouse and a test compound-treated mouse were paired and served for the measurement of spontaneous motor activity in two separate cages at the same time. After the pretreatment of test agents, the animals were transferred individually to a plastic cage (215 mm × 320 mm × 140 mm) which is equipped with a passive infrared sensor (PYS-001, Muromachi Kikai Co. Ltd., Tokyo, Japan). Activity was recorded for a period of 30 min and analyzed using software CompACT AMS (Muromachi Kikai Co. Ltd., Tokyo, Japan). The experiments were performed between 10:00 and 16:00 each day.

2.7. Statistical analysis

The data are presented as the mean ± S.D. The statistical significances of differences between groups were analyzed as follows: in experiments containing two experimental groups, either Student's or Welch's *t*-test was employed to evaluate the statistical differences after evaluating the variances of data with *F*-test ($P < 0.05$). In experiments comparing one control group and two or three experimental groups, either Dunnett's parametric multiple comparison test or Steel's nonparametric multiple comparison test was used after Bartlett's analysis ($P < 0.05$). For multiple comparison among three or more experimental groups, either Tukey's parametric multiple comparison test or Steel–Dwass's nonparametric multiple comparison test was used after Bartlett's analysis ($P < 0.05$). *P*-values less than 0.05 were considered as indicative of significance. *P*-values were expressed as $^{*}P < 0.05$ and $^{**}P < 0.01$.

3. Results and discussion

Antipruritic effect by the single oral administration of the ethyl acetate extract of German chamomile in ddY mice was examined.

The German chamomile extract, at a dose of 100, 300 and 1000 mg/kg, dissolved in the vehicle of 10% ethanol, 10% Tween 80 and 80% physiological saline were orally administrated 2 h before compound 48/80 subcutaneous injection (20.0 μg/site). Scratching behaviour induced by compound 48/80 was significantly suppressed by the pretreatment of the German chamomile extract at the doses of 300 and 1000 mg/kg (Fig. 1). Since the spontaneous motor activity in the German chamomile extract treated mice hardly changed in comparison with that in the vehicle treaded mice, suppression of scratching behaviour was considered not suppression of spontaneous motor activity but antipruritic effect.

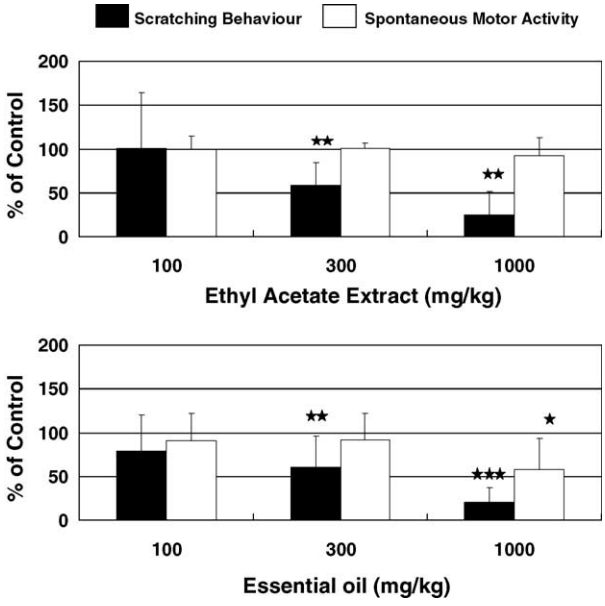

Fig. 1. Dose-dependent antipruritic effect of the ethyl acetate extract and essential oil of German chamomile in ddY mice. The German chamomile extracts at doses of 100, 300 and 1000 mg/kg were perorally administered to male ddY mice 2 h before measurement. Scratching behaviour (closed bars) elicited by an injection of compound 48/80 (20.0 μg/site) or spontaneous motor activity (open bars) were counted for 30 min. Each values are calculated as percentage of chamomile-treated mice to vehicle-treated control mice (mean ± S.D., $n = 12$). Significant difference between vehicle-treated mice and chamomile-treated control mice are indicated by $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ (Student's *t*-test).

The ethyl acetate extract of German chamomile contained essential oil approximately 30% in weight and its main component was Bisabolol oxide A. The essential oil obtained by steam distillation from the ethyl acetate extract of German chamomile showed dose dependent suppression on the compound 48/80-induced scratching behaviour (inhibition of scratching: 20.8 and 39.4%, respectively) without any effects on spontaneous motor activities at the doses of 100 and 300 mg/kg, p.o., as shown in Fig. 1. As a result, we can suggest that the essential oil of German chamomile contribute at least in part to the antipruritic action of the ethyl acetate extract of German chamomile.

Fig. 2 shows antipruritic effects of histamine H1 receptor antagonists having antiallergic activity, oxatomide and fexofenadine, on the compound 48/80-induced scratching behaviour in ddY mice. Each drug was administered perorally 1 h before the subcutaneous injection of compound 48/80 (doses: 3, 10 and 30 mg/kg) in the vehicle of 0.5% MC400. Oxatomide at a dose of 10 mg/kg significantly inhibited the scratching (inhibition of scratching: 37.9%). But no further inhibition of scratching behaviour was observed at a higher dosage (30 mg/kg). The strongest suppression by fexofenadine was also shown at 10 mg/kg (inhibition of scratching: 37.7%).

As shown above, the efficacy of these H1-receptor antihistamines on the compound 48/80-induced pruritus in ddY mice is only moderate. Therefore, the effects of combined

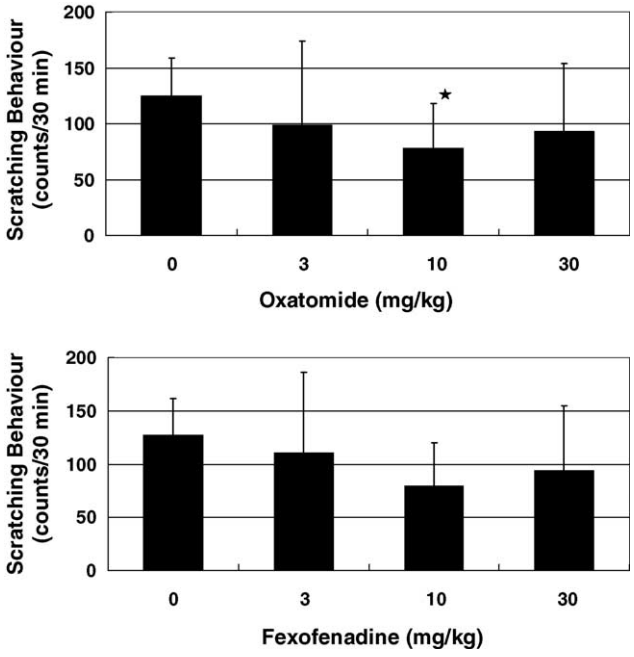

Fig. 2. The effects of oxatomide and fexofenadine on compound 48/80-induced scratching behaviour in ddY mice. The antihistaminic agents were administered perorally 1 h before compound 48/80 injection. Immediately after the compound 48/80 injection. Values shown are mean ± S.D. ($n = 12$). Significant differences between vehicle-treated mice and antihistaminic agents-treated groups are indicated by $^{*}P < 0.05$, $^{**}P < 0.01$ (Steel's multiple comparison test for oxatomide, Dunnett's multiple comparison test for fexofenadine).

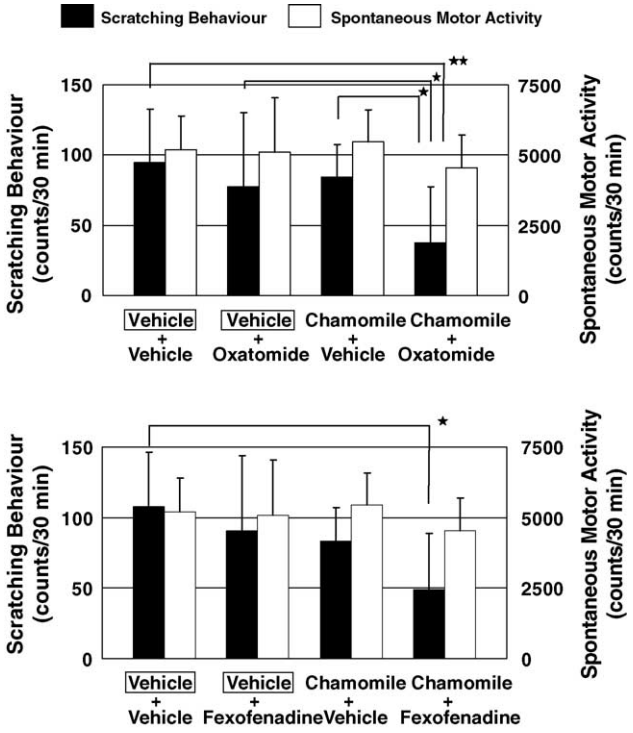

Fig. 3. The effect of the combined administration of the German chamomile ethyl acetate extract and oxatomide or fexofenadine on compound 48/80-induced scratching behaviour (closed bars) and spontaneous motor activity (open bars) in ddY mice. The German chamomile extract (300 mg/kg) was administered perorally 2 h before compound 48/80 injection or measurement of spontaneous motor activity. The antihistaminic agents (10 mg/kg) were administered perorally 1 h after the administration of German chamomile extract. Values shown are mean ± S.D. ($n = 12$). Significant differences between vehicle-treated mice and antihistaminic agents-treated mice are indicated by $^{*}P < 0.05$, $^{**}P < 0.01$ (Tukey's multiple comparison test).

administration of the German chamomile ethyl acetate extract with these antiallergic agents on the compound 48/80-induced scratching behaviour were examined in ddY mice.

The administration of these drugs was carried out as follows. The German chamomile ethyl acetate extract (300 mg/kg) dissolved in the vehicle of 10% ethanol, 10% Tween 80 and 80% physiological saline was administrated perorally 2 h before of the pruritus provocation. Oxatomide (10 mg/kg) or fexofenadine (10 mg/kg) was administrated perorally as 0.5% aqueous MC400 solution 1 h before the pruritus provocation.

As shown in Fig. 3, the German chamomile ethyl acetate extract strongly enhanced the antipruritic effects of these antiallergic agents. In the combined administration of German chamomile extract and oxatomide, inhibition ratio in scratching behaviour was 60.4%, which was more than 29.7%, mere sum of each inhibition ratio: 18.4% by oxatomide alone and 11.3% by the German chamomile extract alone. Similarly, in the combined administration of German chamomile extract and fexofenadine, inhibition ratio in scratching was 54.6%, which grew further 38.6%, mere sum of each inhibition ratio: fexofenadine 15.9% and German chamomile extract 22.7%. There were no significant effects on the spontaneous motor activity by single or combined administration of these agents (Fig. 3).

The present results indicate that the co-medication of the ethyl acetate extract, or essential oil of German chamomile with conventional antiallergic agents would be effective for the pruritus which could not be perfectly resolved by antiallergic agents alone.

Acknowledgement

The authors wish to acknowledge Ms. A. Nakagawa of Foods Research Laboratories, Kyowa Hakko Kogyo Co. Ltd. (Ibaraki, Japan) for the technical supports on GC–MS analysis.

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