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Original Article

Antipruritic and anti-herpes virus activities of bisabolol oxide A, the main constituent in the essential oil of German chamomile (*Matricaria recutita L.*)

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ABSTRACT

Pruritus is commonly observed in cases of atopic dermatitis, and irritating the skin barrier in patients by scratching allows easier access by viruses such as the herpes simplex virus (HSV)-1, which can result in serious complications (i.e. eczema herpeticum). It has been reported that the essential oil of German chamomile (*Matricaria recutita L.*) possesses antipruritic and antiviral activities. In the present study, the issue of whether bisabolol oxide A, the main constituent in the essential oil of German chamomile, has antipruritic and antiviral activities was investigated. The oral administration of bisabolol oxide A (300 mg/kg) suppressed scratching behavior induced by the application of compound 48/80 to mice. Bisabolol oxide A also showed antiviral activity for HSV-1 (IC_{50i} 61.4 μg/mL). Thus, bisabolol oxide A was confirmed to exert the antipruritic and anti-herpes virus activities. Since the antipruritic and anti-herpes virus activities of German chamomile essential oil can be attributed to not only bisabolol oxide A but the other constituents as well, comparative studies on the potencies of antipruritic and anti-herpes virus activities of these constituents should be conducted in future. However, the findings presented herein provide helpful information regarding the understanding of the pharmacological activities of German chamomile essential oil and bisabolol oxide A and their application for the prevention and treatment of atopic dermatitis and its complications.

KEYWORDS: German chamomile, bisabolol oxide A, antipruritic activity, anti-herpes virus activity.

INTRODUCTION

Pruritus or itching is a condition that is commonly observed in atopic dermatitis and other skin disorders [1]. Kobayashi *et al*. recently reported that the essential oil of German chamomile (*Matricaria recutita L.*) exerts antipruritic action in a mouse model, and suggested that bisabolol oxide A (Figure 1), one of the main constituents in this essential oil may contribute to this antipruritic effect [2]. The disturbance of the skin barrier in patients with atopic dermatitis allows easier access by viruses [3, 4]. Eczema herpeticum caused by infections by the herpes simplex virus (HSV)-1 is a serious complication in patients with atopic dermatitis [3, 4]. Koch *et al*. recently demonstrated that the essential oil of German chamomile is effective against HSV-1 [5]. Thus, the essential oil of German chamomile would also be expected to be useful for the treatment of patients of atopic dermatitis with eczema herpeticum. However, information as to whether bisabolol oxide A itself shows antipruritic and antiviral effects is not currently available.

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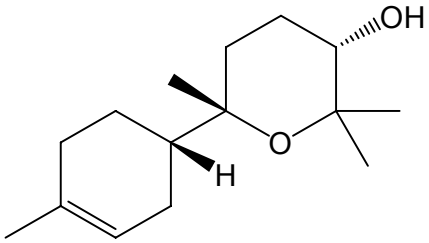

Figure 1. Chemical structure of bisabolol oxide A.

In the present study, the antipruritic effect of bisabolol oxide A was examined by monitoring scratching behaviour after the oral administration of bisabolol oxide A to an atopic dermatitis itch model in mice. In addition, the anti-HSV-1 and cytotoxic activities of bisabolol oxide A against Vero cells were also evaluated.

MATERIALS AND METHODS

Animal and reagents

Male ICR mice (7 weeks old) were purchased from Kyudo Co. (Saga, Japan). All animal experiments were performed according to the guidelines, principles, and procedures for the care and use of laboratory animals of Sojo University (Permit No. 2014-P-004). The mice used in the experiments were fed ordinary laboratory chow, allowed free access to water, and maintained under a regular 12 h light–dark cycle. Bisabolol oxide A and compound 48/80 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were obtained from commercial sources and were of the highest grade.

Cell and virus

Vero cells (ATCC CCL-81) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B at 37 °C in 5% CO_2. HSV-1 strain KOS was provided by the Chemo-Sero-Therapeutic Institute (Kumamoto, Japan).

Evaluation of scratching behavior induced by compound 48/80

It is well known that the administration of compound 48/80, a classic mast cell secretagogue, induces a mast cell-dependent, nonspecific, anaphylactic reaction. This reaction is thought to occur because of the release of histamines from mast cells [6, 7]. The intradermal injection of compound 48/80 (20 μg/site) into the rostral part of the back of mice induces itching that leads to scratching behavior [2, 8, 9]. Scratching behavior was monitored using the Micro Act® system (Neuroscience, Tokyo, Japan). The Micro Act system allows the automatic detection and objective evaluation of scratching behavior [9-11]. Small teflon-coated magnets (1 mm in diameter and 3 mm long) were inserted into both hind paws of the mice at least 24 h before the experiments under isoflurane anesthesia. Bisabolol oxide A dissolved in the solution of dimethyl sulfoxide, Tween 80 and phosphate buffered saline (1:1:8) was orally administered 2 h before scratching behavior was measured. The mice were acclimatized in a plastic chamber 30 min before the measurements. Immediately after the injection of compound 48/80, a mouse was placed in a coil-incorporated plastic observation chamber (110 mm wide and 180 mm high). The movements of hind paws which were amplified as signals through the coil were measured for 30 min using the Micro Act® system. The experiments were performed each day from 10:00 to 15:00 and each animal was used for only one experiment.

Measurement of spontaneous locomotor activities

Spontaneous locomotor activities were measured by the open-field method [12-14]. Bisabolol oxide A, dissolved in a solution of dimethyl sulfoxide, Tween 80 and phosphate buffered saline (1:1:8), was orally administered 2 h before the measurement. Each mouse was placed in a cage (25 cm length × 30 cm width × 17 cm height) 30 min before the measurement, and spontaneous locomotor activity was monitored for 30 min. The floor of the cage (25 cm length × 30 cm width) was divided into 12 squares of equal size which were separated by lines. The number of times a line was crossed by both forelimbs and hind limbs was counted visually. The experiments were performed each day from 10:00 to 15:00 and each animal was used for only one experiment.

Antiviral assays

The antiviral activity of bisabolol oxide A on HSV-1 was measured by a plaque reduction assay [15, 16].

Confluent monolayers of Vero cells (1×10^6 cells/well) in 6 well plates were infected with HSV-1 at a multiplicity of infection (MOI) of at 0.0001, which is equivalent to 100 plaque-forming units (PFU). After a 1 h adsorption period, the cultures were overlaid with Dulbecco's modified Eagle medium (DMEM) containing 2% heat-inactivated FCS and 2% sulfonated γ-globulin including various concentrations of bisabolol oxide A, which was dissolved in ethanol as a stock solution. The concentration of ethanol included in the systems was less than 0.5%, and it was confirmed that these ethanol concentrations had no effect on the results of the assay. The plates were incubated in a CO_2 incubator for 3 days, then fixed with formalin and stained with crystal violet in methanol. Infectious virus production was quantified by counting the plaques caused by virus-induced cytopathic effect. Plaque counts from triplicate wells were averaged and the percentage inhibition was calculated. The IC_{50} value, defined as the inhibition concentration achieving 50% inhibition of viral plaques, of bisabolol oxide A treatment was calculated.

Cytotoxic assay

The anticellular activity was measured by a 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [17]. Confluent monolayers of Vero cells were seeded in 96-well plates at 1×10^4 cells per well. After 1 day, the cells were refed DMEM containing 5% FCS and various concentrations of bisabolol oxide A, which was dissolved in ethanol as a stock solution. The concentration of ethanol included in the systems was less than 0.5%, and it was confirmed that these ethanol concentrations had no effect on the results of the assay. After incubation for 3 days, an MTT solution was added to the culture to form a final concentration of 0.5 mg/mL, followed by incubation for 3 hours. An insoluble MTT-formazan upon metabolic reduction by viable cells was dissolved in dimethyl sulfoxide and the optical densities were read at 570 nm using a reference wavelength of 630 nm. The CC_{50} value defined as the cytototoxic concentration achieving 50% inhibition of cell growth, of bisabolol oxide A treatment was calculated.

Statistical analysis

All data are expressed as the mean ± standard deviation. Statistical significance in the differences of the means was evaluated by the Student's t-test or one-way analysis of variance (ANOVA), followed by Tukey multiple comparisons post hoc test to for the single or multiple comparisons of experimental groups, respectively. A probability value of $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The itch-scratch cycle is commonly observed in the patients with atopic dermatitis [1]. The intradermal injection of compound 48/80 into mice results in a high incidence of scratching behavior [2, 8, 9]. This scratching behavior is sometimes used as an index for evaluating itching, and mice injected with compound 48/80 have been used as a model of atopic dermatitis. Pretreatment with bisabolol oxide A at a dose of 300 mg/kg significantly suppressed the scratching behavior that was induced by the administration of compound 48/80 (Figure 2), and

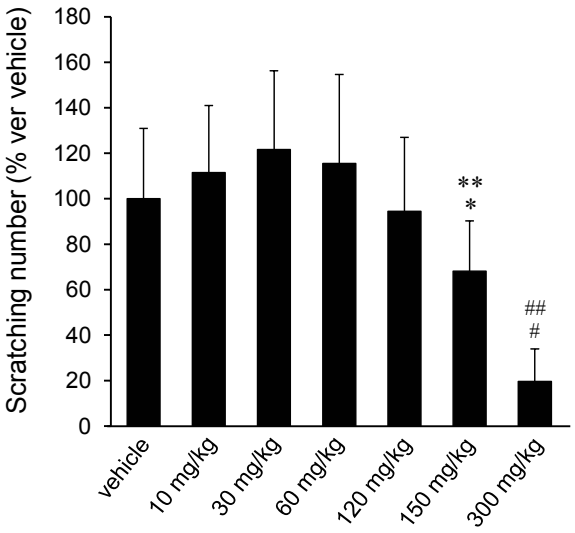

Figure 2. Effect of bisabolol oxide A on compound 48/80-induced scratching behavior in mice. Bisabolol oxide A was administrated orally at doses of 0 (vehicle), 10, 30, 60, 120, 150 and 300 mg/kg. Each column shows the means ± S.D. (n = 10). *$p < 0.05$ in comparison with 10, 60 or 300 mg/kg, **$p < 0.01$ in comparison with 30 mg/kg. $^{\#}p < 0.05$ in comparison with 150 mg/kg, $^{\#\#}p < 0.01$ in comparison with vehicle, 10, 30, 60 or 120 mg/kg.

the scratching suppression rate was 80.4%. Meanwhile, no significant suppressing effects were observed at doses from 10 to 150 mg/kg compared to control (vehicle). Kobayashi *et al*. reported that a pretreatment with the essential oil of German chamomile at doses of 300 and 1,000 mg/kg suppressed this effect (suppression rate; about 50 and 80%, respectively), but this was not the case at a lower dose (100 mg/kg) [2]. Since the content of bisabolol oxide A in essential oil from different origins of German chamomile are different (13.4 ~ 55.9%) [18], it is difficult to conclude that the antipruritic effects observed in the essential oil is due exclusively to bisabolol oxide A. However, our data suggest that bisabolol oxide A is a contributor to the antipruritic effects of the essential oil of German chamomile. We also confirmed that the antipruritic effects of bisabolol oxide A are not due to the suppression of spontaneous locomotor activity, since no significant change in locomotor activity was observed, even at highest dose (300 mg/kg) (Figure 3).

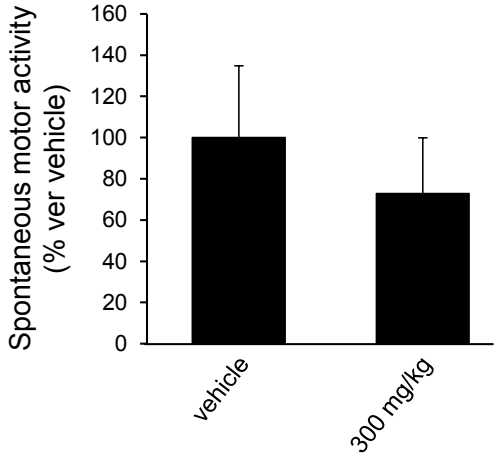

Figure 3. Effect of bisabolol oxide A on spontaneous motor activity in mice. Bisabolol oxide A was administrated orally at doses of 0 (vehicle) and 300 mg/kg. Each column shows the means ± S.D. (n = 5).

HSV-1 infections sometimes cause eczema herpeticum in patients with atopic dermatitis [3, 4]. Bisabolol oxide A showed antiviral activity on HSV-1 (IC_{50} = 61.4 ± 0.6 μg/mL), while its cytotoxicity to Vero cells was low (CC_{50} > 95.3 μg/mL) (Figure 4). Koch *et al*. reported that the IC_{50} for German chamomile essential oil against HSV-1 was 0.3 μg/mL, when HSV-1 was preincubated with drugs before exposure to cells [5]. They also reported that German chamomile essential oil had no significant effect on HSV-1, when the essential oil was added after viral adsorption (during the replication period) which was similar to the experimental conditions of our study. Since significant antiviral activity was observed when the essential oil was incubated

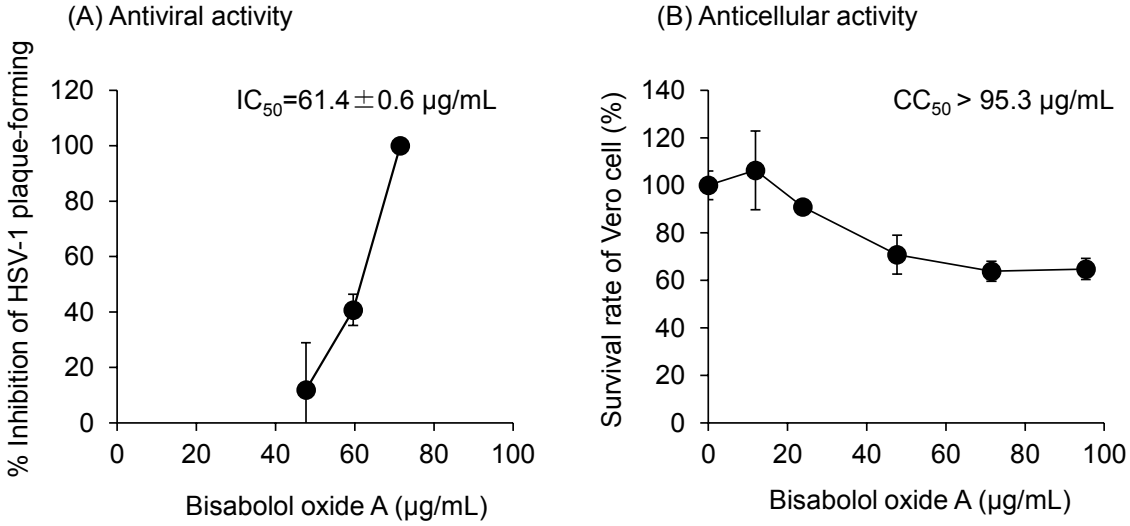

Figure 4. Antiviral (A) and anticellular (B) activities of bisabolol oxide A on HSV-1 and Vero cells, respectively. Each point and value shows the means ± S.D. (n = 8).

with cells and HSV-1 during the viral adsorption period [5], it was concluded that the German chamomile essential oil interrupted the adsorption of HSV-1, thus resulting in antivirus activity. Although it is difficult to compare their data and our data directly, our data indicate that bisabolol oxide A indeed possesses antiviral activity against HSV-1 and that it may be one of the components that contributes to the antiviral activity of German chamomile essential oil against HSV-1.

In the present study, bisabolol oxide A was shown to possess antipruritic and anti-herpes virus activities. Therefore, bisabolol oxide A itself would be expected to be useful for the treatment of patients of atopic dermatitis with/without eczema herpeticum. Furthermore, given the fact that it is one of the components that exerts the antipruritic and anti-herpes virus activities, its content in German chamomile essential oil may become a quality indicator of pharmacological activities of the oil (e.g. antipruritic and anti-herpes virus activities). Teas brewed from chamomile contain 10-15% of the essential oil. The main constituents of this oil include the terpenoids α-bisabolol and its oxides (≤ 78 %) [19]. Therefore, the antipruritic and anti-herpes virus activities of German chamomile essential oil can be attributed to not only bisabolol oxide A but the other constituents as well. Comparative studies on the potencies of antipruritic and anti-herpes virus activities of these constituents would be useful in terms of identifying the constituent(s) with strong activities. However, even though activities of bisabolol oxide A are mild, it could be used for the prevention or treatment of diseases such as atopic dermatitis, as a supplement or a medicine with low possibility of side effects.

CONCLUSION

Thus, this study provides useful information to understand the pharmacological activities of German chamomile essential oil and bisabolol oxide A and to apply them for the prevention and treatment of atopic dermatitis and its complications.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interest.

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