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Evaluation of the safety and efficacy of *Glycyrrhiza uralensis* root extracts produced using artificial hydroponic and artificial hydroponic-field hybrid cultivation systems III: anti-allergic effects of hot water extracts on IgE-mediated immediate hypersensitivity in mice

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Abstract

To evaluate the safety and efficacy of *Glycyrrhiza uralensis* root extracts produced using artificial hydroponic and artificial hydroponic-field hybrid cultivation systems, we investigated anti-allergic action in mice using IgE-mediated immediate hypersensitivity. Hot water extracts obtained from the roots of *Glycyrrhiza uralensis* cultivated using two systems were orally administered at a dose of 100 mg/kg as glycyrrhizin (GL) and compared with the commercial crude drug, Glycyrrhizae Radix. Both the artificial hydroponic and artificial hydroponic-field hybrid cultivated root extracts showed anti-allergic effects on IgE-mediated immediate hypersensitivity in mice, as did the commercial crude drugs. These results highlight the potential for artificially cultivated roots of *Glycyrrhiza uralensis* to be used as an alternative medicinal source.

Keywords *Glycyrrhiza uralensis* · Artificial hydroponic and hydroponic-field hybrid cultivation · Glycyrrhizin · Anti-allergic property · IgE-mediated immediate hypersensitivity

Introduction

Glycyrrhizae Radix is the most frequently used crude drug in Japan and it is defined in the Japanese Pharmacopoeia as the root and stolon of *Glycyrrhiza uralensis* Fischer or *Glycyrrhiza glabra* Linne [1]. Glycyrrhizae Radix is prescribed to treat a variety of diseases as an active component in multi-drug formulations of Kampo medicine.

The demand for *Glycyrrhiza* plants has been rapidly increasing worldwide. Since the production rate for *Glycyrrhiza* plants is extremely low in Japan, supply has depended heavily on imports from China [2]. It is important, therefore, to establish a self-sufficient system in Japan. Previously, we developed novel cultivation systems including artificial hydroponic and artificial hydroponic-field hybrid cultivation systems to produce high-value *Glycyrrhiza* plants, especially *Glycyrrhiza uralensis* [3–6].

We evaluated the anti-allergic actions of *Glycyrrhiza* plants grown in novel cultivation systems via contact hypersensitivity in mice and compared these anti-allergic activities with hot-water extracts prepared from Glycyrrhizae

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Radix [7]. We found that the anti-allergic actions of both were similar. Moreover, we studied the pharmacokinetic properties of hot-water extracts from artificially cultivated *Glycyrrhiza uralensis* roots and found that the $C_{\max}$ and AUC_{0–48} values of glycyrrhetic acid (GA) a major metabolite of glycyrrhizin (GL) after oral administration of the extracts were within the range of variation for the commercial crude drugs [8]. However, it remained unclear whether *Glycyrrhiza* plants grown in the novel cultivation systems were equivalent to *Glycyrrhizae Radix*.

In the present study, we investigated the effects of hot water extracts prepared from the roots of *Glycyrrhiza* plants grown in artificial hydroponic and artificial hydroponic-field hybrid cultivation systems, and from commercial *Glycyrrhizae Radix*, on IgE-mediated immediate hypersensitivity in mice, as another typical pharmacological action.

Materials and methods

Plant materials

Plant materials used in this study were the same as or different sample of the same series as previously described [7]. Briefly, commercial crude drugs (NIB-003, NIB-074, and NIB-176) were provided by the Japan Kampo Medicines Manufacturers Association (JKMA) and the Japan Medicinal Plant Federation and were deposited at the Tsukuba Division of the Research Center for Medicinal Plant Resources (RCMPR), National Institutes of Biomedical Innovation, Health and Nutrition (NIBOHN), Tsukuba City, Japan. Dried *Glycyrrhiza uralensis* roots were produced using hydroponics (IV1-14LB and IV1-16LB) or by a hydroponic-field hybrid cultivation system (1AIV1-HK1+2AIV1-HK2, 1BIV1-HK1+2AIV1-HK2, and 3BIV1-HK3). The elite *Glycyrrhiza uralensis* clone (GuIV1) was propagated using cuttings from hydroponically cultivated plants [9, 10]. These plantlets were further cultivated using two cultivation systems, i.e., hydroponics according to Sawada et al. [4] and hydroponic-field hybrid cultivation. For the hydroponic-field hybrid cultivation, hydroponically grown plantlets in an artificial environmental room were transplanted into a field at the Hokkaido Division of the RCMPR and cultivated for 14 months (individual plant name was HK1, HK2 and HK3, respectively). The root sample named 1AIV1-HK1+2AIV-HK2 was the mixture of the roots of HK1 and HK2 (i.d. more than 5 mm). That of 1BIV1-HK1+2AIV-HK2 was the mixture of the roots of HK1 and HK2 (i.d. less than 5 mm). That of 3BIV1-HK3 was the root of HK3 (i.d. more than 5 mm). The cultivated and harvested roots were washed with tap water and dried at 50 °C for several days to a constant weight.

Preparation of hot-water extracts and determination of glycyrrhizin (GL) in the extract

Water was added to each powdered sample at a ratio of 10:1 (v/w) and the solution was boiled at 100 °C for 2 h. The extraction solution was filtered and then freeze-dried for 96–144 h. The dried hot-water extract was used as a test sample in an amorphous condition, and was maintained at – 80 °C until use. The HPLC analysis for GL was performed as previously reported [7, 8].

Animals

Six-week-old female BALB/c mice, purchased from Japan SLC (Shizuoka, Japan), were used for the anti-allergy activity test. The mice were housed in standard plastic cages in a temperature- and humidity-controlled environment, with food and water available ad libitum. A period of at least 7 days for acclimatization was allowed prior to experimentation. All experiments were carried out according to the guidelines for the care and use of experimental animals of the Animal Care Committee of the Faculty of Pharmacy, Meiji University.

IgE-mediated immediate hypersensitivity in mice

The IgE-mediated immediate hypersensitivity test in mice was carried out as follows. BALB/c mice were given an intravenous injection of a 0.1 ml aliquot of anti-TNP monoclonal antibody IgE-containing (100 µg) fluid 3 days before a 2, 4, 6-trinitro-1-chlorobenzene (TNCB) challenge. A skin reaction was elicited by applying 10 µl of 1% TNCB in olive oil to each side of the right ear of each sensitized mouse. Ear swelling was determined using a dial thickness gauge (G-1A type, Ozaki Mfg., Co., Ltd., Tokyo, Japan) immediately before and 2 h after the challenge.

Statistical analysis

Results are given as mean ± S.E.M. Statistical analysis was ANOVA with Bonferroni corrections for multiple comparisons.

Results and discussion

Since *Glycyrrhizae Radix* is the most frequently used crude drug in Japan, a stable supply is an urgent need. Some investigators reported *Glycyrrhiza uralensis* cultivation program trials in eastern Inner Mongolia [11]. They evaluated pharmaceutical properties, such as anti-allergic action,

by examining IgE-mediated triphasic skin reaction and the pharmacokinetic profile of GA, an anti-allergic metabolite of GL in mice [11]. In previous studies, we clarified that hydroponically cultivated and hybrid-cultivated *Glycyrrhiza uralensis* root extracts had anti-allergic activities against T cell-mediated contact hypersensitivity that were similar to those of the commercial *Glycyrrhiza* crude drug extracts [7]. In this study, we attempted to investigate the anti-allergic activities of these extracts against IgE-mediated immediate allergic reaction in mice.

IgE-mediated passive cutaneous anaphylaxis skin reaction is recognized as a triphasic reaction [12, 13]. Significant ear swelling was observed as an immediate phase reaction (IPR) 1 h or 2 h after topical application of antigens; late phase and very late-phase reactions were detected at 24 h and 120 h after the challenge, respectively (data not shown). In this study, we evaluate the anti-allergic properties of *Glycyrrhiza* extracts at the time of IPR by single administration of extracts at a dose of 100 mg/kg as GL.

Previous pharmacokinetic studies revealed that a major metabolite of GL, glycyrrhetic acid (GA), was detected in the serum and/or plasma of humans and other animals such as rats and mice. The T_{max} value of GA was observed around 8 h after single oral administration of GL [8]. In this study, GL was administered 2 h or 6 h prior to the antigen challenge and the reduction in ear swelling was much stronger with the earlier (6 h prior) administration than with the later (2 h prior) administration (data not shown). Therefore, we evaluated the anti-allergic actions against IgE-mediated immediate skin reaction in mice using 6 h prior administration of each extract.

GL and hot water extracts obtained from the commercial crude drugs significantly suppressed ear swelling (Fig. 1; GL and samples 1–3). The extracts prepared from the artificial hydroponic cultivation (Fig. 1; samples 4 and 5) and the artificial hydroponic-field hybrid cultivation systems (Fig. 1; samples 6–8) also significantly reduced ear swelling. These results suggest that *Glycyrrhiza uralensis* root extracts obtained from hydroponic-based cultivation system have anti-allergic actions against IgE-mediated immediate hypersensitivity skin reaction similar to those of commercial *Glycyrrhiza* crude drugs.

To guarantee a steady supply of *Glycyrrhiza*, we need to challenge the underlying resistance to use *Glycyrrhiza uralensis* roots cultivated by artificial hydroponic-based techniques.

There are relatively few examples of useful crude drugs produced by artificial cultivation. The results of our study demonstrate that *Glycyrrhiza* Radix produced using hydroponic-based techniques is a promising resource.

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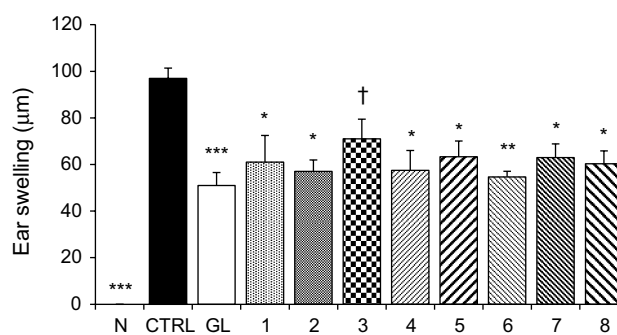


Fig. 1 Effects of *Glycyrrhiza uralensis* roots extracts on IgE-mediated immediate allergic reaction in mice. Each column represents the mean ± S.E.M. of five mice. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs. the control group by ANOVA with Bonferroni correction for multiple comparisons. † $p < 0.05$ vs. the control group by ANOVA with Bonferroni correction for selected two group comparisons. N vehicle, CTRL control group, GL (glycyrrhizin, positive control), 1 NIB-003, 2 NIB-074, 3 NIB-176, 4 IV1-14LB, 5 IV1-16LB, 6 1AIV1-HK1+2AIV1-HK2, 7 1BIV1-HK1+2BIV1-HK2, 8 3BIV1-HK3. Commercial crude drugs: 1, 2 and 3. Hydroponic roots: 4 and 5. Hydroponic-field hybrid roots: 6, 7 and 8

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Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be constructed as a potential conflict of interest.

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