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Effect of glucoglycyrrhizin on IgE-mediated immediate hypersensitivity in mice

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Abstract

To evaluate the pharmacological property of glucoglycyrrhizin (GGL), a unique glycoside of glycyrrhetic acid (GA), we investigated the anti-allergic effect of GGL on IgE-mediated immediate hypersensitivity in mice. GGL exhibited the antiallergic effect against IgE-mediated immediate hypersensitivity. At a dose of 100 mg/kg, GGL exhibited antiallergic activity equivalent to that of glycyrrhizin (GL). Furthermore, the pretreatment with anti-GA monoclonal antibody eliminated the antiallergic action of GGL. These results indicated that GGL may act in the same way as GL in the human body. Its safety should be verified for its use as a drug similar to GL.

Keywords Glucoglycyrrhizin (GGL) · Glycyrrhizin (GL) · Glycyrrhetic acid (GA) · Passive cutaneous anaphylaxis · Anti-glycyrrhetic acid monoclonal antibody (6D6)

Introduction

A triterpene saponin, glucoglycyrrhizin (GGL), was isolated from a glycyrrhizin (GL)-deficient strain 83–555 of *Glycyrrhiza uralensis* (Leguminosae), and its content has been found to be approximately 1–2% by HPLC analysis [1]. GL has various pharmacological properties such as anti-inflammatory and antiallergic activities [2–7]; however, it is unclear whether GGL has pharmacological actions similar to those of GL. In our previous study, we reported that a hot water extract of a GL-deficient strain of *G. uralensis* exhibited antiallergic activity against contact hypersensitivity in mice in a manner similar to that of the crude drug, *Glycyrrhizae Radix* [8]. We further confirmed the presence of glycyrrhetic acid (GA), a major metabolite of GL, in mice serum after oral administration of a hot water extract of a GL-deficient strain 83–555 [8]. These results suggested that GGL may work in a manner similar to that of GL when

administered orally, because GGL is also a glycoside of GA. That is, it is considered that GGL is hydrolyzed into GA by the intestinal flora in the alimentary tract, and then GA is absorbed into bloodstream to exert its pharmacological actions like an antiallergic action. On the contrary, we have established the anti-GA monoclonal antibody (6D6 mAb) and further demonstrated that antiallergic properties of a hot water extract of *Glycyrrhiza Radix* and GL were neutralized with the pretreatment of 6D6 mAbs [9].

In the present study, we analyzed the antiallergic effects of GGL in IgE-mediated immediate hypersensitivity in mice when GGL was administered orally, and then we proved that GA is the active metabolite of antiallergic effects of GGL by an immunological method using anti-GA mAbs.

Materials and methods

Reagents

The GL standard (purity > 94%, HPLC) was kindly provided by Dr. Yukio Ogihara, emeritus professor at Nagoya City University. GGL (purity > 98%, HPLC) was isolated from the underground parts of a GL-deficient strain of *G. uralensis* 83–555 [1]. The chemical structures of GL and GGL are shown in Fig. 1. Anti-GA monoclonal antibodies (clone 6D6) were established and purified in our laboratory [9].

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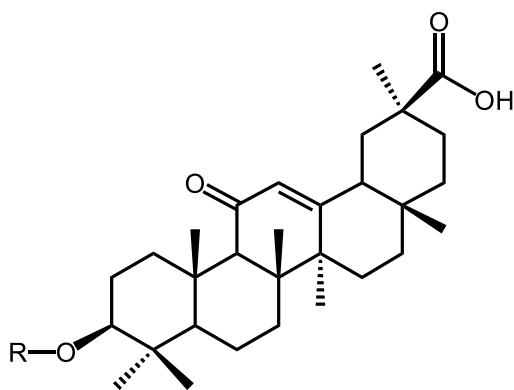


Fig. 1 Chemical structures of glycosides of glycyrrhethinic acid

R = glcA —² glcA : glycyrrhizin (GL)
 R = glc —² glcA : glucoglycyrrhizin (GGL)
 R = H : glycyrrhethinic acid (GA)

Fig. 1 Chemical structures of glycosides of glycyrrhethinic acid

Animals

For the antiallergy activity test, 6-week-old female BALB/c mice (Japan SLC, Shizuoka, Japan) were used. The mice were housed in standard plastic cages in a temperature- and humidity-controlled environment, with food and water available ad libitum. An acclimatization period of at least 7 days was provided prior to further experimentation. All experiments were performed according to the guidelines for the care and use of experimental animals of the Animal Care Committee of the Faculty of Pharmacy, Meijo University.

IgE-mediated immediate hypersensitivity in mice

The IgE-mediated immediate hypersensitivity test in mice was performed as previously described [10]. Briefly, an intravenous injection of 0.1 ml fluid containing anti-trinitrophenyl (TNP) monoclonal antibody IgE-containing (100 µg) was given to BALB/c mice 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.

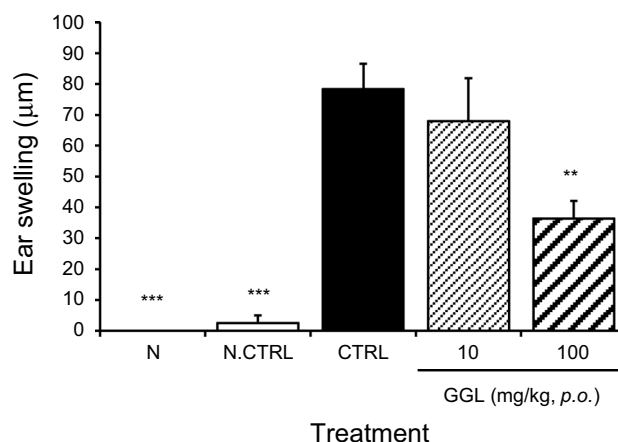


Fig. 2 Effect of glucoglycyrrhizin (GGL) on IgE-mediated immediately hypersensitivity in mice. In normal mice (N), phosphate-buffered saline (pH 7.4, PBS) was injected intravenously, and 3 days later olive oil was applied on the right ear 6 h after oral administration of water. In negative control mice (N. CTRL), PBS was injected intravenously, and 3 days later 1% TNCB was applied on the right ear 6 h after oral administration of water. In the control mice (CTRL), TNP-IgE (100 µg) was injected intravenously, and 3 days later 1% TNCB was applied on the right ear 6 h after oral administration of water. In the GGL-treated mice (GGL), TNP-IgE (100 µg) was injected intravenously, and 3 days later 1% TNCB was applied on the right ear 6 h after oral administration of GGL (10, 100 mg/kg). Each column represents the mean $\pm$ SEM of 3–6 mice. *** p < 0.001, ** p < 0.01 vs. the control group by ANOVA Bonferroni correction for multiple comparisons

Statistical analysis

Results are given as mean $\pm$ SEM. For multiple comparisons, ANOVA with Bonferroni corrections was performed.

Results and discussion

We studied here the effect of GGL on IgE-induced immediate hypersensitivity since previous studies have shown a similar activity in case of GL [9, 11–13].

Considering the pharmacokinetics of GA when a hot water extract of a GL-deficient strain of *G. uralensis* administered orally [8], GGL was orally administered 4 h prior to the application of 1% TNCB. As shown in Fig. 2, significant ear swelling was observed 2 h after antigen application as an IgE-mediated immediate allergic reaction; however, GGL suppressed the ear swelling at a dose of 100 mg/kg. These results suggest that GGL also has antiallergic activity on IgE-mediated immediate hypersensitivity.

Further, we investigated the effect of pretreatment of anti-GA monoclonal antibody on the antiallergic action of

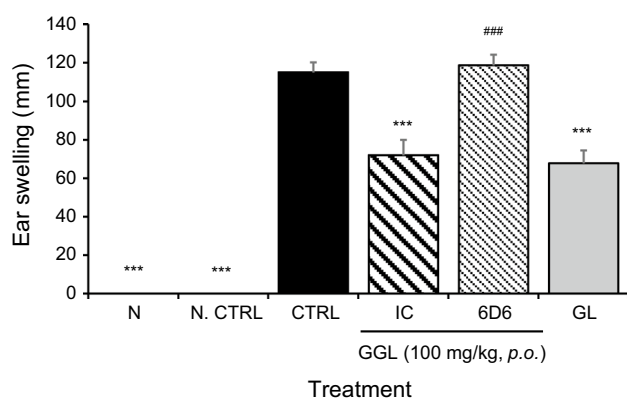


Fig. 3 Effect of the pretreatment of anti-glycyrrhetic acid (GA) monoclonal antibody on the anti-allergic action of GGL in murine IgE-mediated immediate hypersensitivity. In normal mice (N), phosphate-buffered saline (pH=7.4, PBS) was injected intravenously, and 3 days later olive oil was applied on the right ear 6 h after oral administration of water. In negative control mice (N. CTRL), PBS was injected intravenously, and 3 days later 1% TNCB was applied on the right ear 6 h after oral administration of water. In the control mice (CTRL), TNP-IgE (100 µg) was injected intravenously, and 3 days later 1% TNCB was applied on the right ear 6 h after oral administration of water. In the GGL treated mice (GGL), TNP-IgE (100 µg) was injected intravenously, and 3 days later 1% TNCB was applied on the right ear 6 h after oral administration of GGL (100 mg/kg). In the GGL-treated groups, mouse IgG antibody was injected as an isotype control (IC) intravenously or anti-GA monoclonal antibody (6D6) was injected intravenously 30 min prior to GGL administration. In the GL-treated mice (GL), TNP-IgE (100 µg) was injected intravenously, and 3 days later 1% TNCB was applied on the right ear 6 h after oral administration of GL (100 mg/kg). Each column represents the mean $\pm$ SEM of 3–6 mice. *** p < 0.001 vs. the control group by ANOVA Bonferroni correction for multiple comparisons. ### p < 0.001 vs. the GGL (IC, isotype control treated) group by ANOVA Bonferroni correction for multiple comparisons

GGL in murine IgE-mediated immediate hypersensitivity. The anti-GA antibody (6D6) was injected intravenously 30 min before the oral administration of GGL. The pretreatment of 6D6 mAb markedly reduced the antiallergic effect of GGL, suggesting that the active metabolite may be GA, as in the case of GL [9]. In addition, it was shown that GGL has almost similar antiallergic property as GL (100 mg/kg) in this model (Fig. 3).

Several studies suggested that GA is an active metabolite when GL is administered orally [14–16]; moreover, we have proved it in neutralization experiments with 6D6 mAb [9]. Based on the results in this study, GGL may exhibit pharmacokinetics and pharmacological actions similar to those of GL when orally administered. Thus, we suggest that GGL can be developed as a therapeutic drug similar to GL. GL is known to exhibit side effects such as hypokalemia due to long-term administration or overdose. Since this study revealed that GGL also exerts a pharmacological action as GA as an antiallergic action, it may cause side effects like GL. Thus, it is necessary

to evaluate the safety profile of GGL before using it as a therapeutic drug.

Conclusion

In the present study, we suggested that GGL could act as an antiallergic agent in the human body. To use it as a therapeutic drug like GL, its safety must be verified.

Declarations

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

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