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Pharmaceutical evaluation of liquorice before and after roasting in mice

Takami Majima, Tomohiro Yamada, Eiji Tega, Hiroaki Sakurai, Ikuo Saiki and Tadato Tani

Abstract

Liquorice has been used for allergic-inflammatory and liver disorders in both traditional Chinese and modern medicine. In traditional Chinese formulations, it is mainly roasted liquorice that has been used rather than un-roasted liquorice. We have compared the pharmaceutical characteristics of liquorice before and after roasting to clarify the pharmaceutical significance of the roasting. Although roasted liquorice contained less glycyrrhizin (an anti-allergic component) than un-roasted liquorice, the inhibitory potency of roasted liquorice extract (200 mg kg^{-1}) on immunoglobulin E (IgE)-mediated triphasic ear swelling in mice was much greater compared with un-roasted liquorice. To search for additional active ingredients, roasted liquorice extract was subjected to gel-chromatography to give an anti-allergic fraction (Fa) of molecular weight ranging from 15 000 to 200 000 or more, in which glycyrrhizin was not detected. By testing the activity of the various fractions, it was proved that the anti-allergic effect of roasted liquorice was due to glycyrrhizin, its metabolite glycyrrhetic acid, and the Fa fraction. The inhibitory potency of the Fa fraction (15 and 75 mg kg^{-1}) prepared from roasted liquorice was stronger than that prepared from un-roasted liquorice. Therefore, a pharmaceutical implication of roasting the liquorice seems to be associated with an increase in the anti-allergic property of the Fa fraction. It is notable that oral administration of the high molecular mass fraction (Fa) significantly inhibited IgE-mediated ear swelling six days after challenge at doses as low as 3, 15 or 75 mg kg^{-1}.

Division of Pharmacognosy, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan

Takami Majima, Tadato Tani

Division of Pathogenic Biochemistry, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan

Tomohiro Yamada, Eiji Tega, Hiroaki Sakurai, Ikuo Saiki

21st Century COE Program, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan

Ikuo Saiki, Tadato Tani

Correspondence: T. Tani, Division of Pharmacognosy, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan. E-mail: tanitdt@ms.toyama-mpu.ac.jp

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Introduction

Liquorice (Gancao in Chinese and Kanzo in Japanese) is a well known crude drug prepared from the underground parts of *Glycyrrhiza* species and has been used for allergic-inflammation, gastrointestinal and liver disorders in traditional Chinese and modern medicine (Olukoga & Donaldson 2000). In the old traditional Chinese medical formulary "Shang-Han-Lun", roasted liquorice is more frequently used than un-roasted liquorice (Katakai et al 2002).

In the course of our studies on liquorice (Yamamoto et al 2003a), this investigation was carried out to clarify the pharmaceutical implication of the roasting of liquorice. To compare the pharmacological activities of liquorice before and after roasting, the anti-allergic actions were examined by measuring immunoglobulin E (IgE)-mediated triphasic ear swelling in passively sensitized mice (Tahara et al 1999). When glycyrrhizin, a well-known active component of liquorice, is taken orally it is transformed (hydrolysed) by intestinal bacteria into the active metabolite glycyrrhetic acid (Akao et al 1994). Therefore, the biopharmaceutical properties of liquorice before and after roasting were examined by measuring the pharmacokinetic parameters of glycyrrhetic acid after oral administration of extracts of un-roasted vs roasted liquorice. We examined the role of the high molecular mass fraction of the two liquorice extracts in the efficacy against third-phase ear swelling (very late phase response, vLPR), which is considered to be an important inflammatory reaction in allergic diseases such as chronic atopic dermatitis in man (Tahara et al 1999).

Materials and Methods

Liquorice roots, roasting procedure and chemicals

Liquorice (Lot No. 707-C020-4C, collected in Shanxi province of China) was purchased from Tochimoto tenkaido Co., Ltd, Japan. The roasting process was carried out by Tochimoto tenkaido using the traditional procedure. Half (10 kg) of the liquorice was roasted with stirring in a pot with gentle heat for approximately 20 min until the surface became darkened. The remaining half (10 kg) was used as un-roasted liquorice. Thus, the two experimental samples (roasted and un-roasted liquorice) were of the same grade and botanical origin, which was considered to be *Glycyrrhiza uralensis* based on comparison of its HPLC fingerprint with the reported one (Kitagawa et al 1998; Yamamoto et al 2003b). Voucher samples were deposited in the Division of Pharmacognosy, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University.

The same reagents of glycyrrhizin, glycyrrhetic acid, isoliquiritin apioside and isoliquiritin, dinitrofluorobenzene (DNFB) and dinitrophenol (DNP) were used as reported previously (Yamamoto et al 2003b).

HPLC analysis of roasted and un-roasted liquorice and their freeze-dried extracts

The glycyrrhizin content in two types of liquorice (50% ethanol extract) was determined by the method described in the Japanese Pharmacopoeia XIV (JP XIV). The content in two corresponding freeze-dried extracts of liquorice were determined using our reported HPLC conditions (Yamamoto et al 2003b). Identification of the HPLC peaks (glycyrrhizin, isoliquiritin apioside and isoliquiritin) was carried out by comparison with co-chromatographed authentic samples.

Fractionation of the two freeze-dried extracts of liquorice

The suspension of freeze-dried extract (5 g in 30 mL water) was centrifuged at 3000 rev min^{-1} for 10 min. The supernatant was chromatographed over Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden) and eluted with H_2O mixed with a gradually increasing proportion of ethanol (5, 10, 20, 40, 60, 80 and 100%). Three fractions were obtained, a high molecular mass fraction (Fa), a glycyrrhizin-rich fraction (Fb), and the remaining fraction containing flavonoids (Fc). The molecular weight range of Fa was estimated by comparison with three kinds of dextran reagents (molecular weight 15 000–20 000, 35 000–50 000, and 100 000–200 000, ICN Biomedicals Inc.,) analysed by HPLC. The HPLC system was composed of the following parts: Jasco 880-PU, Jasco RID-300, Shimadzu C-R6A with a column YMC-Pack Diol-300 (500 × 8.0 mm) with a Guardpack Diol-300 (30 × 8.0 mm). The mobile phase was pure water further purified from distilled water using TORAYPURE LV-08 (TORAY). The flow rate was 1.0 mL min^{-1}.

Carbohydrate and protein contents of the Fa fraction were assayed by the ordinary phenol–H_2SO_4 method (Dubois et al 1956) and by using a Coomassie Plus protein assay reagent kit (Pierce, US).

Animal experiments

All animal experiments were carried out in accordance with the Guidelines of the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University and approved by the Japanese Association of Laboratory Animal Care.

The IgE-mediated triphasic skin reaction (ear swelling) in passively sensitized mice was carried out as reported by Tahara et al (1999). Briefly, female BALB/c mice (6-weeks old) were given an intravenous injection of 1 mL anti-DNP monoclonal antibody (mAb) IgE mAb-containing fluid 24 h before the DNFB challenge. Ear swelling was elicited by applying 10 μL 0.1% DNFB/ethanol to each side of each ear of the sensitized mice. The freeze-dried liquorice extracts (before and after roasting) suspended in water (1 mL) were given orally 2 h before and 2–6 days after DNFB challenge. Prednisolone-21-acetate (a positive control; 10 mg kg^{-1}) was given intraperitoneally 2 h before and 4–6 days after the challenge. The triphasic ear swelling responses, consisting of the immediate phase reaction (IPR), late phase reaction (LPR) and very late phase reaction (vLPR), were observed at 1 h, 24 h and six days, respectively, after the DNFB challenge. The ear swelling was evaluated using a dial thickness gauge (G-1A type, Peacock, Ozaki MFG., Co., Ltd, Osaka) immediately before and at the appropriate times after the challenge.

A pharmacokinetic study of glycyrrhetic acid after oral administration of the same freeze-dried extracts as used in the skin reaction test was carried out as reported by He et al (2001). Briefly, male Wistar rats (8-weeks old, Hamamatsu, Japan) were subjected to fasting overnight before oral administration of extracts (containing 45 mg kg^{-1} glycyrrhizin). Blood samples (approximately 0.3 mL) were collected from the tail vein at each specified time after extract administration. The plasma glycyrrhetic acid levels and the area under the mean concentration of glycyrrhetic acid vs time curve from zero to 24 h ($AUC_{0-24\,h}$) were determined as reported by He et al (2001).

Statistical analysis

The results are shown as the mean ± s.d. of the number (n) of experiments. The data of the triphasic ear swelling were analysed for statistical significance using Mann–Whitney's U-test and the data of other examinations were analysed using Student's *t*-test. Probability (*P*) values less than 0.05 were considered to be significant.

Results

Glycyrrhizin contents and HPLC-profiles of the two liquorice extracts

Figure 1 shows that the HPLC-profiles of the two (roasted and un-roasted) liquorice extracts monitored at 254 nm

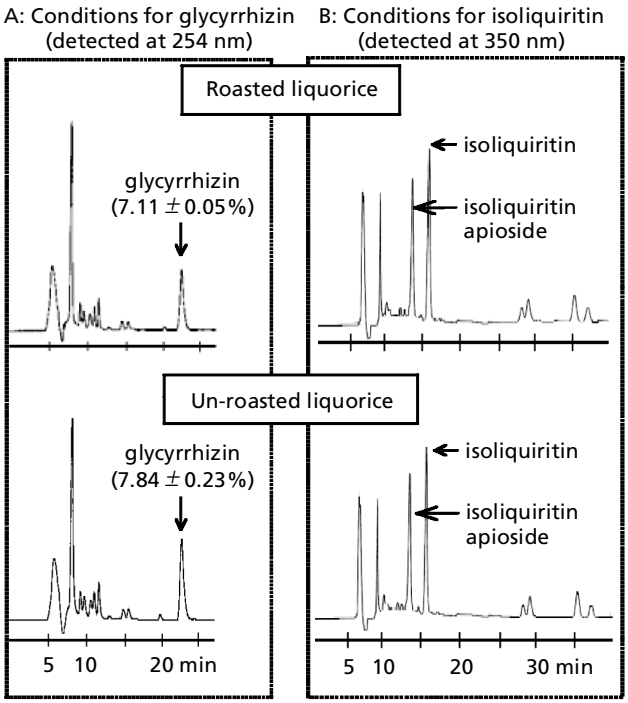

Figure 1 HPLC-profiles of the MeOH-soluble fraction of the freeze-dried extracts of liquorice before and after roasting. A. Chromatograms examined at 254 nm. B. Chromatograms examined at 350 nm. HPLC systems and conditions are described in the footnote of Table 1.

Table 1 Glycyrrhizin contents of liquorice before and after roasting.

Treatment	Glycyrrhizin content of drug[a] (%) (n = 5)	Yield of freeze-dried extract[b] (%) (n = 8)	Glycyrrhizin content of extract[c] (%) (n = 5)
Roasted liquorice	3.65 ± 0.06*	24.2 ± 0.3*	7.11 ± 0.05*
Un-roasted liquorice	4.34 ± 0.05	25.4 ± 0.3	7.84 ± 0.23

Each value represents the mean ± s.d. $*P < 0.05$ compared with liquorice before roasting. [a]Glycyrrhizin in the 50%-ethanol soluble fraction of drugs was analysed under the conditions described in the Japanese Pharmacopoeia XIV using HPLC. [b]The extracts were prepared by boiling liquorice (30 g each) in water (600 mL) for 40 min and freeze-drying the extract into powder. [c]Glycyrrhizin in the MeOH-soluble portion of the freeze-dried extract was analysed by HPLC. The HPLC system was composed of a Jasco 880-PU, UVIDEC-100-IV, Shimadzu C-R6A with a YMC-Pack ODS-AQ-303 column (250 × 4.6 mm) with an ODS-MG-5 guard column. Mobile phase: 0.05 M $AcONH_4$ (pH 3.6)–CH_3CN (65:35). Glycyrrhizin detection at 254 nm.

for glycyrrhizin (left panel) and at 350 nm for isoliquiritin (right panel) were similar. However, the glycyrrhizin content of freeze-dried extract of roasted liquorice was slightly lower compared with un-roasted liquorice (Table 1). There was no discernible difference in the extraction efficiency of glycyrrhizin between roasted and un-roasted liquorice (47.0% and 45.9%, respectively).

Anti-allergic effects of the two extracts, glycyrrhizin and glycyrrhetic acid

Figure 2 shows that oral administration of roasted liquorice extracts (100–200 mg kg^{-1}) significantly and dose-dependently inhibited IgE-mediated triphasic ear swelling (except at a dose of 100 mg kg^{-1} in vLPR). In particular, 200 mg kg^{-1} roasted liquorice was significantly more effective at inhibiting LPR and vLPR than un-roasted liquorice. Figure 3 shows that oral administration of glycyrrhizin (10 mg kg^{-1}) and its metabolite glycyrrhetic acid (10 mg kg^{-1}) significantly inhibited the triphasic ear swelling.

Glycyrrhetic acid pharmacokinetics after oral administration of the two extracts

Orally taken glycyrrhizin, 3-*O*-β-D-glucuronyl-(1→2)-β-D-glucuronide of glycyrrhetic acid, is hydrolysed by intestinal bacteria into glycyrrhetic acid, which is absorbed into the blood (Akao et al 1994). Figure 4 shows that glycyrrhetic acid (but not glycyrrhizin) was gradually detected in plasma and peaked at 9 h after oral administration of the two extracts containing glycyrrhizin (45 mg kg^{-1} each) from roasted and un-roasted liquorice. A similar pattern of glycyrrhetic acid plasma level was observed after oral administration of both liquorice extracts. There was no significant difference between the $AUC_{0-24\,h}$ (14.2 ± 9.0 μg h mL^{-1}) and C_{max} (1.48 ± 0.86 μg mL^{-1}) of glycyrrhetic acid after oral administration of roasted liquorice and the $AUC_{0-24\,h}$ (12.5 ± 4.9 μg h mL^{-1}) and C_{max} (1.47 ± 0.63 μg mL^{-1}) after oral administration of un-roasted liquorice.

The standard curve for determination of plasma glycyrrhetic acid concentration: Y = 1613.23 X, r = 1.00 (Y: peak area, X: glycyrrhetic acid 10^{-3} μg). Detection limit: 16 ng mL^{-1}; recovery: 99.6.

Fractionation of the two extracts and their anti-allergic effects

Three fractions were obtained from the two extracts by gel chromatography with Sephadex LH-20. The yield of the Fa fraction (12.9 ± 1.4%) prepared from roasted liquorice was slightly higher compared with that from the un-roasted liquorice (11.0 ± 1.7%). The yields of the other two fractions (Fb and Fc) prepared from the two extracts were very similar to each other. Figure 5 shows that the molecular weight range of each Fa fraction was from 15 000 to 200 000 or more. The Fa fraction of the roasted and the un-roasted liquorice contained a similar proportion of sugar moieties (59.3 ± 3.3% and 55.4 ± 3.2%, respectively) and nitrogen-containing components (4.7 ± 0.8% and 4.3 ± 0.8%) as assessed by the ordinary methods. Glycyrrhizin was not detected in either of the Fa fractions. Fraction Fb was a

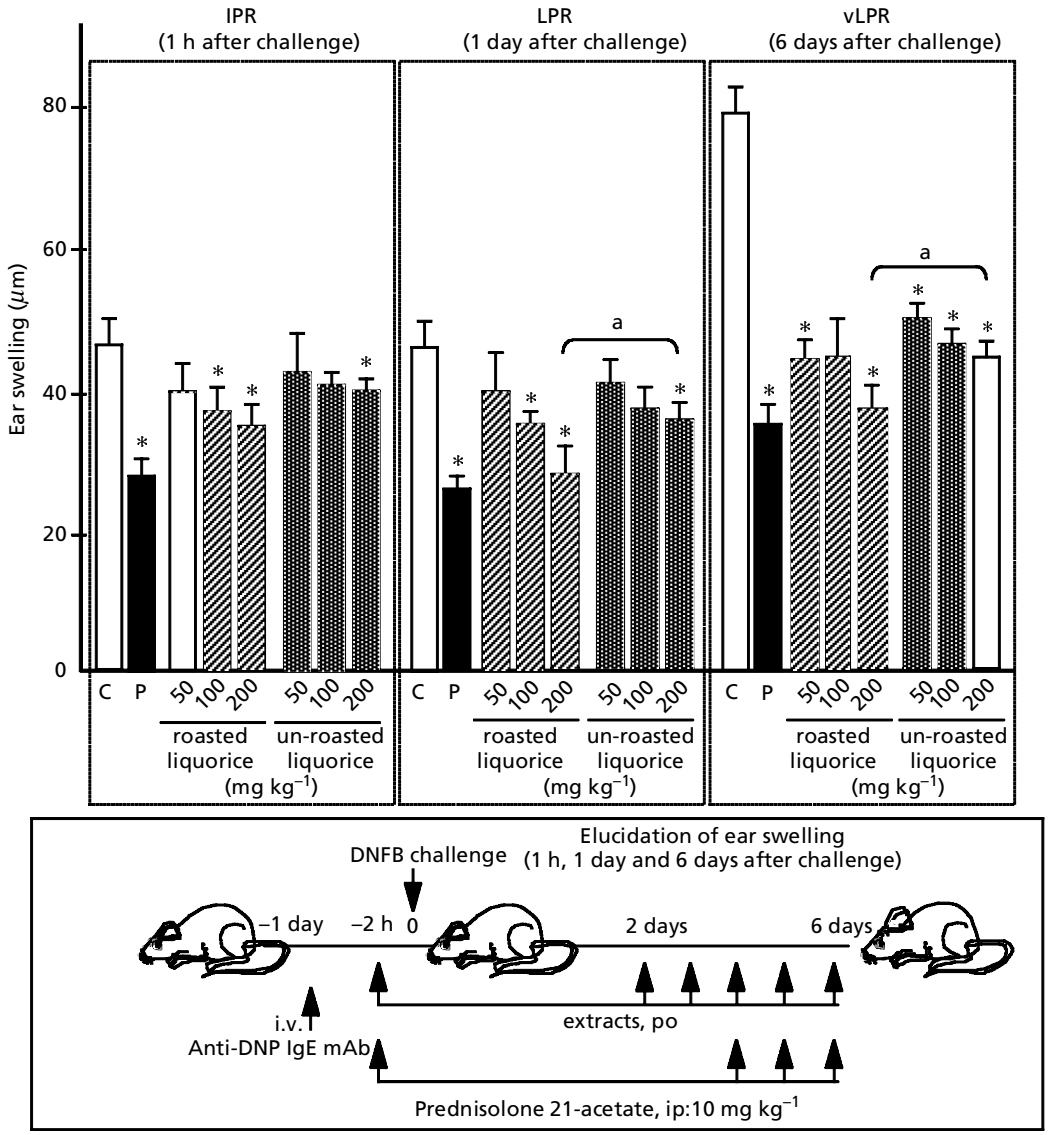

Figure 2 Effects of oral administration of the freeze-dried extracts of liquorice before and after roasting on IgE-mediated triphasic skin reactions in passively sensitized mice. Each value and bar represents the mean ± s.d. (n = 3). $^{*}P < 0.05$ compared with control group by Mann–Whitney U-test; $^{a}P < 0.05$ by Mann–Whitney U-test. C, Control; P, prednisolone-21-acetate (a positive control).

glycyrrhizin-rich fraction (9.17 ± 0.47% for roasted liquorice extract and 8.46 ± 0.28% for un-roasted liquorice extract) and Fc contained less glycyrrhizin (3.57 ± 0.20% for roasted liquorice extract and 3.72 ± 0.26% for un-roasted liquorice extract) but was rich in flavonoid ingredients (not determined quantitatively).

Oral treatment of mice with the Fa and Fb fractions significantly reduced ear swelling in the vLPR (Figure 6). Two Fa fractions (13 and 11 mg kg^{-1}) prepared from roasted and un-roasted liquorice extract showed similar anti-allergic effects as compared with those of original extracts (100 mg kg^{-1}). The Fa fractions (3, 15 and 75 mg kg^{-1}) of roasted and un-roasted liquorice extract dose-dependently inhibited ear swelling in vLPR, and the effects of Fa fractions from roasted liquorice was more potent than those of the Fa fractions from the un-roasted liquorice extract (Table 2).

Discussion

Our previous study (Yamamoto et al 2003a) indicated that liquorice and some traditional Chinese formulations containing liquorice were effective for the inhibition of IgE-mediated triphasic skin reaction in passively sensitized mice (Yamada et al 2000; Tatsumi et al 2001). We also found that classical histamine H_1 receptor antagonists, leukotriene B_4 receptor antagonist, and glucocorticoid inhibited IgE-mediated triphasic reaction including IPR, LPR and vLPR (Satoh et al 2000). Furthermore, the

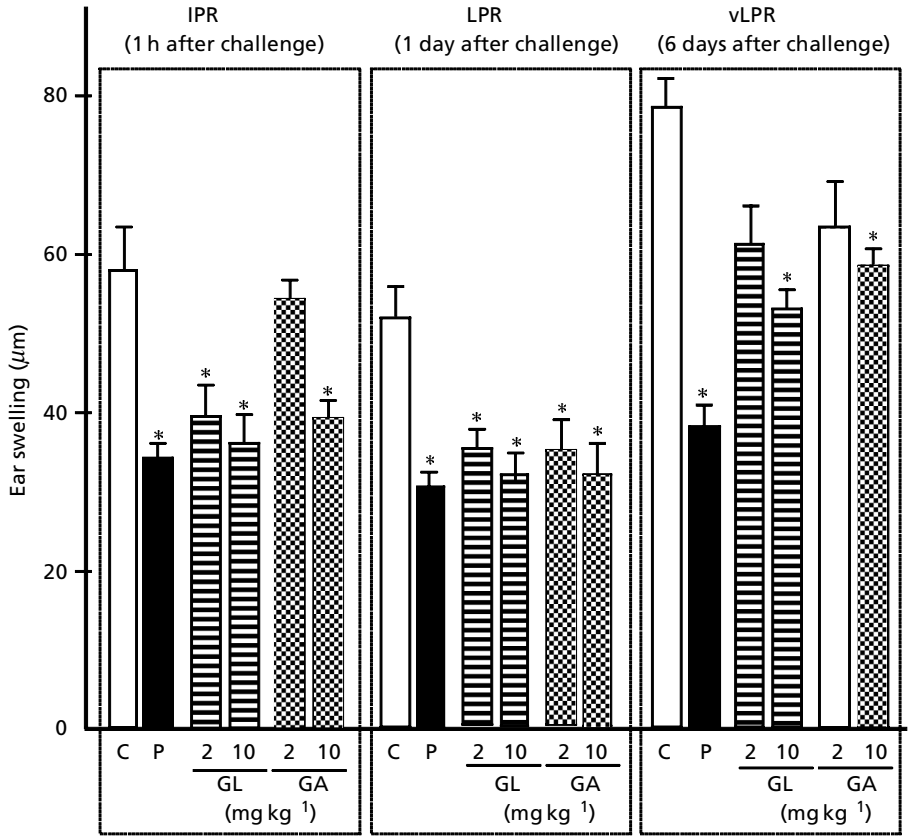

Figure 3 Effects of oral administration of glycyrrhizin (GL) and glycyrrhetic acid (GA) on IgE-mediated triphasic skin reactions in passively sensitized mice. Each value and bar represents the mean ± s.d. (n = 3). *$P < 0.05$ compared with control group by Mann–Whitney U-test. C, control; P, prednisolone-21-acetate (a positive control).

ability of formulations to inhibit vLPR seemed to reflect the clinical efficacy of some traditional Chinese formulations containing liquorice, such as Baihu-Jia-Rensheng-Tang (Byakko-ka-Ninjin-To in Japanese (Onda 1997)) and Taohe-Chengqi-Tang (Tokaku-Joki-To (Terasawa et al 1995)) for treating chronic atopic dermatitis.

In traditional Chinese medicine liquorice is frequently used after it has been roasted in the traditional way (Katakai et al 2002). In this study, we analysed the chemical, anti-allergic and biopharmaceutical properties of liquorice before and after roasting to clarify the pharmaceutical implication of roasting.

The HPLC chromatograms of the two liquorice extracts (from un-roasted and roasted liquorice) showed a similar pattern in appearance (Figure 1), however the glycyrrhizin content of roasted liquorice was significantly decreased compared with that of un-roasted liquorice (Table 1). These results were in good agreement with those reported by Kuwajima et al (1999). Since a decrease in the glycyrrhizin content of roasted liquorice seemed to be undesirable for therapeutic use, the pharmacological and biopharmaceutical properties of roasted liquorice were compared with those of un-roasted liquorice to understand the implication of roasting.

As shown in Figure 2, oral administration of the two liquorice extracts to mice showed marked inhibition of triphasic ear swelling at 1 h (IPR), 1 day (LPR) and 6–7 days (vLPR) after antigen challenge. Marked inhibition of ear swelling by oral administration of a major component of liquorice, glycyrrhizin ($10\,mg\,kg^{-1}$), and its metabolite, glycyrrhetic acid ($10\,mg\,kg^{-1}$), was observed (Figure 3). Although the roasted liquorice extract contained less glycyrrhizin than un-roasted liquorice extract, the inhibition of roasted liquorice extract on LPR and vLPR was much greater than that of the un-roasted liquorice (at a dose of $200\,mg\,kg^{-1}$). A plausible implication of roasting of liquorice seems to be associated with the enhancement of the anti-allergic effect by roasting.

The biopharmaceutical properties of roasted liquorice were examined to clarify the influence of roasting on the bioavailability of glycyrrhizin after oral administration of the extract. The pattern of glycyrrhetic acid (not glycyrrhizin) concentration in plasma after oral administration (Figure 4) was comparable with those of our previous report (He et al 2001). The glycyrrhetic acid pharmacokinetic parameters ($AUC_{0-24\,h}$ and C_{max}) calculated from the present profile suggested that the two liquorice extracts were almost equivalent in terms of their

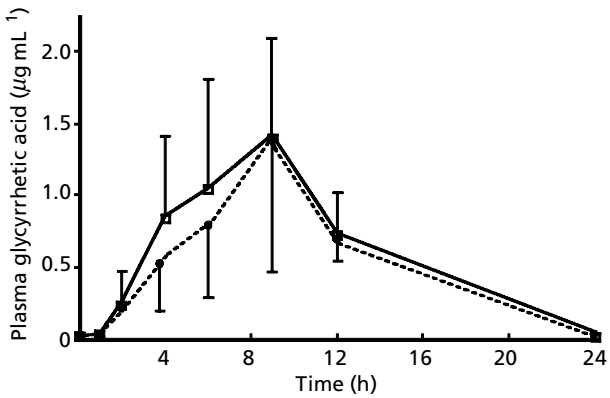

Figure 4 Plasma concentration–time profile of glycyrrhetic acid after oral administration of the freeze-dried extracts of liquorice before and after roasting. Each point and bar represents the mean ± s.d. (n = 6). Each extract (●: liquorice before roasting; □: liquorice after roasting) which contained 45 mg kg^{-1} glycyrrhizin was administered orally to rats.

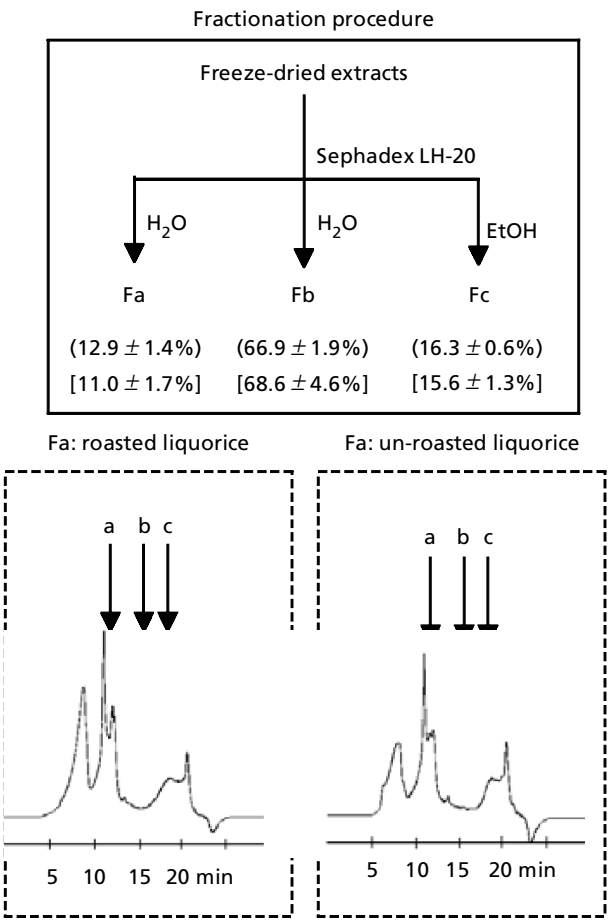

Figure 5 Fractionation procedure for the freeze-dried extracts of liquorice before and after roasting and HPLC-profiles of the high-molecular mass fraction (Fa). Values in parenthesis and brackets are yields from the freeze-dried extracts of roasted and un-roasted liquorices, respectively (mean ± s.d., n = 10). a, b and c represent the retention times of molecular weight markers: a, dextran (10 000–20 000); b, dextran (35 000–50 000); c, dextran (15 000–50 000).

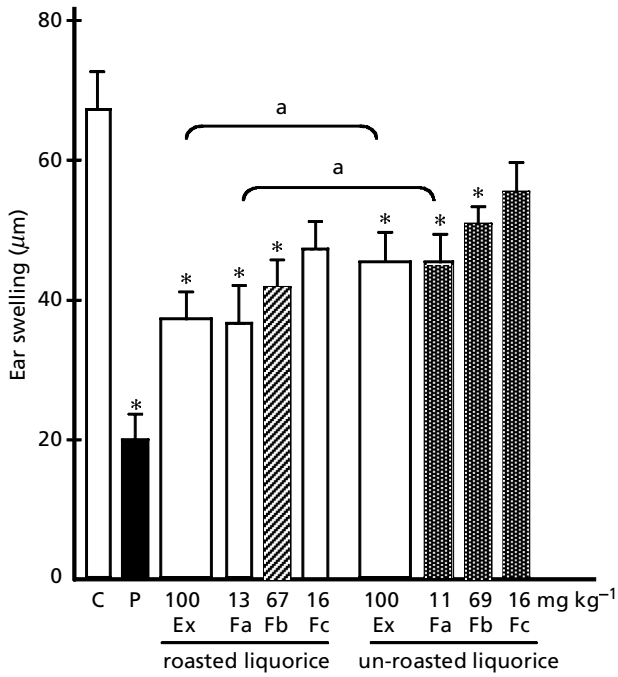

Figure 6 Effects of each fraction prepared from the freeze-dried extracts of liquorice before and after roasting on the very late phase reaction (vLPR) in IgE-mediated triphasic skin reactions in passively sensitized mice. Each value and bar represent the mean ± s.d. (n = 4). *$P < 0.05$ compared with control group by Mann–Whitney U-test; [a]$P < 0.05$ by Mann–Whitney U-test. C, control; P, prednisolone-21-acetate (a positive control); Ex, freeze-dried extract; Fa, Fb and Fc, fractions prepared from Ex (as shown in Figure 5). Each dose was calculated in terms of yield of the fractions. The vLPR was observed at six days after the DNFB challenge. In the screening experiments we focused our attention on the effects of these fractions on ear swelling in vLPR, which was mediated by T cells and also partially by mast cells (Tahara et al 1999) and is considered to be an important reaction in allergic inflammatory diseases.

bioavailability. Therefore, the differences between the effects of the two liquorice extracts on skin reactions were not due to differences of the glycyrrhizin bioavailability from the two liquorice extracts.

To search for any other anti-allergic components produced during the roasting process, we fractionated the water-soluble portion of the extracts by gel chromatography to obtain an anti-allergic fraction (Fa). Fa is a polysaccharide–protein complex with molecular weight ranging from 15 000 to 200 000 or more (Figure 5). As a consequence, the inhibitory effects of oral administration of the liquorice extract were due to the inhibition by two fractions, Fa and Fb, with a higher amount of glycyrrhizin. Since the efficacy of the Fa fraction (15 and 75 mg kg^{-1} as shown in Table 2) prepared from roasted liquorice was higher than that from un-roasted liquorice, roasting of liquorice led to the promotion of anti-allergic activity. Further chemical investigations of the Fa fraction are necessary to clarify its sugar composition and sugar-chain units, and to compare them with the reported

Table 2 Effects of high molecular mass fraction (Fa) of liquorice before and after roasting on the third phase reaction (vLPR) in IgE-mediated skin reaction.

	Ear swelling (% of control)
Fa prepared from roasted liquorice	
3 mg kg^{-1}	66.7 ± 7.8[a]
15 mg kg^{-1}	46.1 ± 6.5[a, b]
75 mg kg^{-1}	37.2 ± 3.8[a, b]
Fa prepared from un-roasted liquorice	
3 mg kg^{-1}	75.2 ± 7.2
15 mg kg^{-1}	61.0 ± 5.9[a]
75 mg kg^{-1}	50.9 ± 4.5[a]

Each value represents the mean ± s.d. (n = 5). [a] $P < 0.05$ compared with control group; [b] $P < 0.05$ compared with un-roasted liquorice group.

immunologically active polysaccharides isolated from *G. uralensis* roots (Shimizu et al 1992; Kiyohara et al 1996).

In summary, the inhibitory effects of the oral administration of freeze-dried extract, glycyrrhizin, its metabolite glycyrrhetic acid, and a high molecular mass fraction (Fa) prepared from roasted liquorice on IgE-mediated very late phase skin reaction (vLPR) in mice were determined. The inhibitory potency of the extract (200 mg kg^{-1}) and Fa fraction (15 and 75 mg kg^{-1}) prepared from roasted liquorice on LPR and vLPR was much greater than that of the corresponding fractions from un-roasted liquorice. Therefore, a pharmaceutical implication of roasting of liquorice seems to be associated with an increase in the anti-allergic property of roasted liquorice extract, which contained a more active anti-allergic high molecular mass fraction (Fa) than the un-roasted liquorice extract.

References

Akao, T., Hayashi, T., Kobashi, K. (1994) Intestinal bacterial hydrolysis is indispensable to absorption of 18β-glycyrrhetic acid after oral administration of glycyrrhizin in rats. *J. Pharm. Pharmacol.* **46**: 135–137

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350–356

He, J.-X., Akao, T., Nishino, T., Tani, T. (2001) The influence of commonly prescribed synthetic drugs for peptic ulcer on the pharmacokinetic fate of glycyrrhizin from Shaoyao-Gancao-tang. *Biol. Pharm. Bull.* **24**: 1395–1399

Katakai, M., Akamaru, T., Tani, T. (2002) An analysis of the frequency of formulations and crude drugs described in Shang-Han-Lun. *Jpn. J. History Pharmacy* **37**: 28–35

Kitagawa, I., Chen, W.-Z., Taniyama, T., Harada, E., Hori, K., Kobayashi, M., Ren Jiali (1998) Quantitative determination of constituents in various licorice roots by means of high performance liquid chromatography. *Yakugaku Zasshi* **118**: 519–528

Kiyohara, H., Takemoto, N., Zhao, J.-F., Kawamura, H., Yamada, H. (1996) Pectic polysaccharides from roots of *Glycyrrhiza uralensis*: possible contribution of neutral oligosaccharides in the galacturonase-resistant region to anti-complementary and mitogenic activities. *Planta Med.* **62**: 14–19

Kuwajima, H., Taneda, Y., Chen W.-Z., Kawanishi, T., Hori, K., Taniyama, T., Kobayashi, M., Ren Jiali, Kitagawa, I. (1999) Variation of chemical constituents in processed licorice roots: quantitative determination of saponin and flavonoid constituents in bark removed and roasted licorice roots. *Yakugaku Zasshi* **119**: 945–955

Olukoga, A., Donaldson, D. (2000) Liquorice and its health implications. *J. Royal Soc. Promotion Health* **120**: 83–89

Onda, H. (1997) The treatment of atopic dermatitis by Kampo medicine. *J. Trad. Med.* **14**: 245–251

Satoh, T., Tahara, E., Yamada, T., Watanabe, C., Itoh, T., Terasawa, K., Saiki, I. (2000) Differential effect of antiallerigic drugs on IgE-mediated cutaneous reaction in passively sensitized mice. *Pharmacology* **60**: 97–104

Shimizu, N., Tomoda, M., Takada, K., Gonda, R. (1992) The core structure and immunological activities of glycyrrhizin UA, the main polysaccharide from the root of *Glycyrrhiza uralensis*. *Chem. Pharm. Bull.* **40**: 2125–2128

Tahara, E., Satoh, T., Watanabe, C., Shimada, Y., Itoh, T., Nagai, H., Terasawa, K., Saiki, I. (1999) A third-phase cutaneous (very late phase) response after elicitation with dinitrofluorobenzene in passively or actively sensitized mice. *Allergol. Int.* **48**: 265–273

Tatsumi, T., Yamada, T., Nagai, H., Terasawa, K., Tani, T., Nunome, S., Saiki, I. (2001) A Kampo formulation: Byakko-kaninjin-to (Bai-Hu-Jia-Ren-Sheng-Tang) inhibits IgE-mediated triphasic skin reaction in mice: The role of its constituents in expression of the efficacy. *Biol. Pharm. Bull.* **24**: 284–290

Terasawa, K., Kita, T., Shimada, Y., Shibahara, N., Ito, T. (1995) Four cases report of atopic dermatitis successfully treated with Tokaku-joki-to. *Jpn. J. Oriental Med.* **46**: 45–54

Yamada, T., Tahara, E., Nagai, H., Terasawa, K., Tani, T., Nunome, S., Saiki, I. (2000) Effect of some Kampo medicines, including Tokaku-joki-to (Tau-He-Cheng-Qi-Tang), on IgE-mediated triphasic skin reaction in passively sensitized mice. *J. Trad. Med.* **17**: 17–25

Yamamoto, Y., Majima, T., Saiki, I. Tani, T. (2003a) Pharmaceutical evaluation of *Glycyrrhiza uralensis* roots cultivated in eastern Nei-Meng-Gu in China. *Biol. Pharm. Bull.* **26**: 1144–1149

Yamamoto, Y., Majima, T., He, J.-X., Saiki, I. Tani, T. (2003b) Chemical and pharmaceutical evaluation of Daitou-Gancao in comparison with usual Glycyrrhizae Radices. *J. Trad. Med.* **20**: 102–110