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Short Communication

Enhancement of anti-inflammatory and antinociceptive actions of red ginseng extract by fermentation

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Abstract

Objectives This work aimed to compare some pharmacological properties of red ginseng extract (RG) and fermented red ginseng extract (FRG).

Methods Antinociceptive activity was analysed using the acetic acid-induced abdominal constriction response. Anti-inflammatory activity was evaluated using acetic acid-induced vascular permeability and carrageenan-induced inflammation in the air pouch, and analysed through the measurement of nitrite content in the lipopolysaccharide (LPS)-stimulated macrophage cells. Anti-angiogenic activity was determined using the chick chorioallantoic membrane assay.

Key findings In-vivo anti-inflammatory activity of FRG was stronger than that of RG in two animal models, vascular permeability and air-pouch models. In the vascular permeability model, the doses of RG and FRG required for half-maximal inhibition (IC50) were 181 and 59 mg/kg, respectively. FRG exhibited significantly stronger antinociceptive activity than RG. In the acetic acid-induced abdominal constriction response, the IC50 values of RG and FRG were 153 and 27 mg/kg, respectively. Although both RG and FRG were able to suppress production of nitric oxide in the LPS-stimulated RAW264.7 macrophage cells, the suppressive activity of FRG appeared to be stronger than that of RG. However, RG and FRG showed similar anti-angiogenic activity.

Conclusions FRG possesses enhanced anti-inflammatory and antinociceptive activity but similar anti-angiogenic activity than RG.

Introduction

Ginseng, the root of *Panax ginseng* C. A. Meyer (Araliaceae), is a world-renowned herbal medicine used for centuries as a general tonic to promote human health.[1] White ginseng is made by peeling fresh ginseng roots and drying them without steaming, while red ginseng is produced by steaming fresh ginseng roots at 98–100°C for 2–3 h before drying.[2] The three types of ginseng, such as fresh, white and red ginsengs, are consumed in the various forms of commercial products such as extracts, powder, pills, tea, and capsules.[2] Red ginseng, originally known as a preserved form of ginseng, is believed to contain enhanced and newly-formed pharmacological efficacies produced by heat-induced chemical transformation.[3,4]

In recent years, unharmful bacteria have been used to improve or generate useful efficacies of some natural products of medicinal plant origin. For example, among the fourteen medicinal plant extracts fermented with *Lactobacillus paracasei* LS-2, the extract of *Artemisia capallaris* Thunb. was reported to produce a dramatic enhancement in the induction of anti-inflammatory mediators with no cytotoxicity.[5] *Bacillus natto* was used to further increase biological activity of cooked black soybeans.[6] During fermentation with *B. natto*, genistin and daidzin concentrations gradually decreased with increased fermentation time, while their deglycosylation products, genistein and daidzein, increased.[6] Subsequently, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the fermented black soybeans was identified to significantly increase with fermentation time and concentration.[6] The antihepatoma activity of black soybeans increased through the fermentation with *Agaricus*

blazei, possibly via enhancing the levels of responsible components, such as blazeispirols A and C.[7] These findings positively suggest that appropriate selection of useful bacteria could enhance desired efficacies of certain medicinal plants. In combination with high pressure extraction, probiotic fermentation has been suggested as an alternative method for improving the extraction efficiency and antimicrobial and antimutagenic activity of Korean barberry.[8]

In this communication, a water extract (RG) of red ginseng was subjected to fermentation with *Bifidobacterium longum* to produce its fermented water extract (FRG), and the pharmacological activity of RG and FRG were compared using experimental animal models.

Materials and Methods

Chemicals and experimental animals

Indometacin (indomethacin; synthetic, purity > 98%), dexamethasone (synthetic, purity > 99%), *Escherichia coli* lipopolysaccharide (LPS, natural, purity ≥ 97%), Evans blue, carmellose (carboxymethyl cellulose), retinoic acid (RA) and Griess reagent were purchased from Sigma Chemical Co. (St Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin were from Gibco-BRL (Gaithersburg, MD, USA). All other chemicals used were of reagent grade or better.

Six-year-old fresh ginseng roots were obtained from a local ginseng market in Kumsan, Korea (May 2010). The botanical identity was authenticated by Professor Ki-Oug Yoo, Department of Biological Sciences, Kangwon National University, Chuncheon, Korea. Its voucher specimen was deposited in the herbarium of Department of Biological Sciences, College of Natural Sciences, Kangwon National University under the acquisition number KWNU56575.

All animal experiments performed in this work were approved under reference number KNU1445 by the Ethical Committee, Kangwon National University, Chuncheon, Korea. Male ICR mice (approximately 25 g) were obtained from Samtaco Animal Farm, Osan, Korea. The animal room was maintained at 23 ± 2°C with a 12-h light/dark cycle. Food and tap water were freely available. In mouse experiments, at least seven animals were used in each experimental group. Fertilized brown Leghorn eggs used in this work were obtained from Pulmuone Food Co., Seoul, Korea. At least 20 fertilized eggs were used in each experimental group. In animal experiments, 1% carmellose in saline was used as a vehicle.

Preparation of water extract of red ginseng and fermented water extract

Red ginseng (500 g), produced by steaming the fresh roots at 97 ± 2°C for 2 h and drying at 57 ± 2°C, was ground to pass through a 80-mesh sieve, extracted under reflux with 2 vol distilled water three times for 2 h at 97 ± 2°C and concentrated by evaporation *in vacuo* to generate RG. RG (15 g) was resuspended in distilled water and incubated at 30°C for five days with a culture (10 g, on a wet weight basis) of *B. longum*, a Gram-positive, anaerobic, branched-shaped bacterium normally residing in the human gastrointestinal tract. After this time it was centrifuged at 5000*g* for 20 min to discard bacterial cells and concentrated *in vacuo* to produce FRG. The ginsenoside patterns of RG and FRG were analysed by high-performance liquid chromatography (HPLC) using a Hitachi L-7100 liquid chromatograph fitted with a YMC-Pak Pro C_{18} reverse-phase column (250 × 4.60 mm i.d., 5 μm; YMC, Kyoto, Japan). The contents of total ginsenosides in RG and FRG were determined to be 23.8 and 21.5 mg/g extract, respectively.

Acetic acid-induced vascular permeability

According to a modification of the method of Whittle[9] an acetic acid-induced vascular permeability test was performed. Fifty minutes after oral administration of vehicle, RG (50, 100 or 200 mg/kg), FRG (25, 50 or 100 mg/kg) or indometacin (10 mg/kg; a positive control), 0.1 ml/10 g 2% Evans blue solution was injected intravenously in each mouse. After 10 min, 0.1 ml/10 g 0.7% acetic acid in saline was intraperitoneally injected. Twenty minutes after the injection of acetic acid, the mice were killed by cervical dislocation. Saline (10 ml) was injected into the peritoneal cavity and the washings were collected in test tubes. Concentration of Evans blue in the washings, resulting from the dye leaking out into the peritoneal cavity, was determined by the absorbance at 590 nm. The vascular permeability was represented in terms of the absorbance (A_{590}).

Carrageenan-induced inflammation in the air pouch

According to a modification of the procedure of Ghosh *et al.*[10], λ-carrageenan-induced air pouch formation was performed. Six days before sample administration, the air pouches were made in the intrascapular region of mice by initial subcutaneous injection of 4 ml sterile air, and three days later, reinforced with additional 2 ml sterile air. On day 0, vehicle, RG (0.03, 0.1, 0.3 mg per pouch), FRG (0.03, 0.1, 0.3 mg per pouch) or dexamethasone (0.01 mg per pouch) was administered into the pouch immediately after the λ-carrageenan injection (1.0 ml 2.0% solution). After 16 h, the pouch cavity was opened and the exudate was collected. The exudate volumes were measured using a graduate tube, and the polymorphonuclear leucocytes in the diluted samples were counted in a standard haemocytometer chamber.

Acetic acid-induced abdominal constriction response

Antinociceptive activity of RG and FRG was detected as described previously.[11] Each experimental group of mice was treated orally with vehicle, RG (50, 100 or 200 mg/kg), FRG (25, 50 or 100 mg/kg) or indometacin (10 mg/kg; a positive control). One hour later, 0.7% acetic acid at the dose of 0.1 ml/10 g was injected. Ten minutes after this injection the number of abdominal constrictions was counted for 10 min.

Effect on the nitric oxide production in the macrophage cells

The RAW264.7 cells, obtained from American Type Culture Collection (Manassas, VA, USA), were cultured in DMEM containing 10% heat-inactivated FBS, 25 mM HEPES (pH 7.5), 100 U/ml penicillin and 100 μg/ml streptomycin. The RAW264.7 cells were plated at a density of 1×10^6 and pre-incubated for 24 h at 37°C, and maintained in a humidified atmosphere containing 5% CO_2. The mammalian cells were treated with LPS (1 μg/ml) in the presence or absence of the tested samples for 24 h. Accumulated nitrite (NO_2^-), as an index of nitric oxide (NO), in the media was determined using a colorimetric assay based on the Griess reaction.[12]

Chorioallantoic membrane assay

Anti-angiogenic activity of RG and FRG was determined using the chorioallantoic membrane (CAM) assay as described previously.[13] After the fertilized chicken eggs were kept for 3.5 days in a humidified egg incubator at 37°C, approximately 2 ml albumen was aspirated through a small hole drilled at the narrow end of the eggs, allowing the small chorioallantoic membrane and yolk sac to drop away from the shell membrane. In the 4.5-day-old chick embryo, a sample-loaded Thermanox coverslip was applied onto the CAM surface. Two days after returning the chick embryo to the egg incubator, an appropriate volume of 10% fat emulsion (Intralipose) was injected into a 6.5-day-old embryo chorioallantois. The branching pattern of each egg, observed under a microscope, was graded as 0, 1+ or 2+. Convergence of a few vessels toward the CAM surface was denoted as 1+, and 2+ reflected an increased density and length of vessels toward the CAM surface.

Statistical analysis

The results were expressed as mean ± SE. Statistical comparisons between experimental groups were performed by unpaired Student's *t*-test. *P*-values less than 0.05 were considered to be significant. The half maximal (50%) inhibitory concentration of a substance (IC50) was calculated from the dose/response linear regression plots.

Results

Ginsenoside patterns of red ginseng water extract and fermented water extract

As shown in Table 1, the ginsenoside pattern of FRG was different from that of RG. Among the individual ginsenosides tested, FRG was rich in Rg_3, compound K (a protopanaxadiol ginsenoside metabolite) and Rg_2. Based on the chemical structures of ginsenosides, it was estimated that Rb_1, Rb_2, Rc and Rd could be transformed to Rg_3, Rh_2 and compound K during fermentation. Similarly, Re could be transformed to Rg_2. The content of total ginsenosides in FRG appeared to be similar with that in RG, implying that there were no significant changes in total ginsenoside pool during fermentation. These findings implied that *B. longum* caused transformation of some ginsenosides during its incubation with RG.

Anti-inflammatory activity

The anti-inflammatory activity of RG and FRG was compared employing two *in vivo* models, the vascular permeability and air-pouch assays. The vascular permeability assay, a typical model for investigating both the first stage inflammatory reactions and mediators involved in inflammation released after antigen-stimulation, leads to the dilation of arterioles and venules and increased vascular permeability.[14] In the vascular permeability model, RG administered to animals at the oral doses of 50, 100 and 200 mg/kg gave an inhibition of 26, 39 and 52%, respectively, while FRG at the oral doses of 25, 50 and 100 mg/kg gave rise to an inhibition

Table 1 The ginsenoside patterns of the red ginseng water extract and fermented water extract

	Content, %	
Ginsenosides	Water extract	Fermented water extract
Rb_1	40.0	ND
Rb_2	13.6	ND
Rc	8.5	ND
Rd	1.3	ND
Rg_3	3.1	39.3
Rh_2	1.3	3.7
Compound K	ND	47.6
Re	10.1	ND
Rg_1	14.9	ND
Rg_2	5.2	7.6
Rh_1	2.0	1.8
Total	100	100

Some ginsenosides of the water extract and fermented water extract were analysed by HPLC. Percentages of the individual ginsenosides, on a dry weight basis, were calculated against the corresponding total ginsenoside amount. The contents of total ginsenosides in red ginseng water extract and fermented extract was 23.8 and 21.5 mg/g extract, respectively. ND, not detected.

of 23, 51 and 62% (Figure 1). The doses of RG and FRG required for half-maximal inhibition (IC50) were 181 and 59 mg/kg, respectively. In the carrageenan-induced inflammation in the air pouch, dexamethasone (0.01 mg per pouch), a steroidal anti-inflammatory drug, reduced the volumes of the exudates by 73% (Table 2). Treatments with FRG at 0.03, 0.1 or 0.3 mg per pouch gave rise to a decrease in the exudate volumes more significantly than that with RG at the same doses (Table 2). Similarly, total numbers of polymorphonuclear leucocytes in the air pouches were more markedly diminished by treatment with FRG at 0.03, 0.1 and 0.3 mg per pouch than with RG at the same doses (Table 2). Collectively, although both RG and FRG contained in-vivo anti-inflammatory activity in experimental animal models, the anti-inflammatory activity of FRG was significantly stronger than that of RG.

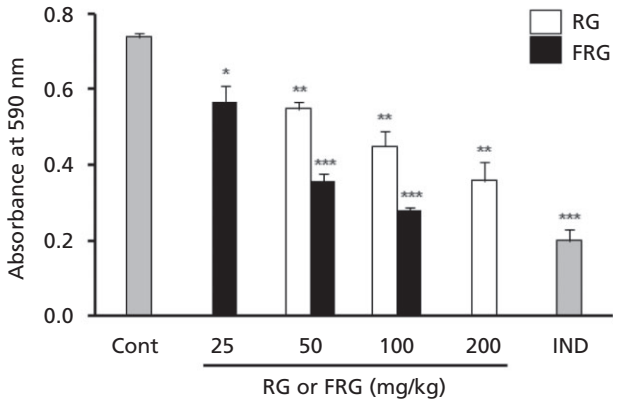

Figure 1 Inhibitory effects of red ginseng water extract and fermented extract on the acetic acid-induced vascular permeability in mice. Red ginseng water extract (RG; 50, 100 or 200 mg/kg), fermented extract (FRG; 25, 50 or 100 mg/kg), indometacin (IND; 10 mg/kg; positive control) or vehicle (Cont; 1% carmellose in saline) was orally administered. Vascular permeability was represented by the absorbance at 590 nm. Each column represents mean ± SE. Each group contained seven mice. This experiment was performed in triplicate. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs the control group.

Antinociceptive activity

Antinociceptive activity of RG and FRG were evaluated using the acetic acid-induced abdominal constriction response. Although the abdominal constrictions induced by acetic acid are not a very specific nociception model, it is believed to reveal a general antinociceptive activity of the sample. The constriction response of the mouse to an intraperitoneal injection of acetic acid was used to detect antinociceptive activity. Acetic acid is known to cause pain by liberating endogenous substances that excite the pain nerve endings. RG administered to animals at the oral doses of 50, 100 and 200 mg/kg gave rise to an inhibition of 17, 32 and 61%, respectively, whereas FRG at the oral doses of 25, 50 and 100 mg/kg showed an inhibition of 49, 63 and 82%, respectively (Figure 2). The IC50 values of RG and FRG were 153 and 27 mg/kg. In summary, the antinociceptive activity of FRG was much greater than that of RG.

Inhibitory effect on NO production

Inducible nitric oxide synthase (iNOS) plays a regulatory role in expression of pro-inflammatory mediators in inflammation. iNOS-derived NO is involved in various pathological conditions such as inflammation and autoimmune diseases and leads to cellular injury.[15] Suppression of NO production is closely linked with anti-inflammatory action. Inhibitory effects of RG and FRG were evaluated on LPS-induced NO expression in RAW264.7 macrophages. The accumulated nitrite in the medium, determined by the Griess method, was used as an index for NO level. When the RAW264.7 macrophage cells were treated with LPS, the nitrite content increased approximately 9-fold (Figure 3). When the macrophage cells were pretreated with 8, 40 or 200 μg/ml RG or FRG, the NO production induced by LPS was significantly suppressed in a concentration-dependent manner (Figure 3). At a concentration of 200 μg/ml RG and FRG diminished the nitrite levels to 56 and 36% of that of the LPS only, respectively (Figure 3). Taken together, FRG exhibited stronger in-vitro

Table 2 Effects of red ginseng water extract and fermented water extract on carrageenan-induced inflammation in the air pouch model

Group	Dose (mg per pouch)	Exudate (ml)	Total leucocytes (×10^7 cells)
Control	–	2.50 ± 0.03	4.80 ± 0.56
Water extract 0.03	0.03	2.23 ± 0.13 (10.7)	4.69 ± 0.15 (2.4)
Fermented extract 0.03	0.03	2.22 ± 0.20* (10.9)	4.66 ± 0.14 (2.9)
Water extract 0.1	0.1	2.07 ± 0.06** (17.3)	2.97 ± 0.25* (38.2)
Fermented extract 0.1	0.1	1.91 ± 0.06*** (23.6)	2.32 ± 0.23** (51.7)
Water extract 0.3	0.3	1.90 ± 0.10** (24.0)	1.72 ± 0.21** (64.1)
Fermented extract 0.3	0.3	1.27 ± 0.07*** (49.1)	1.20 ± 0.02*** (75.1)
Dexamethasone	0.01	0.67 ± 0.03*** (73.3)	1.54 ± 0.02** (67.9)

Note: The results are expressed as mean ± SE ($n = 8$). Figures in parentheses indicate inhibitory percentages with respect to the corresponding control. Dexamethasone was used as a positive control. The control group was treated only with 1% carmellose in saline. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with the control group.

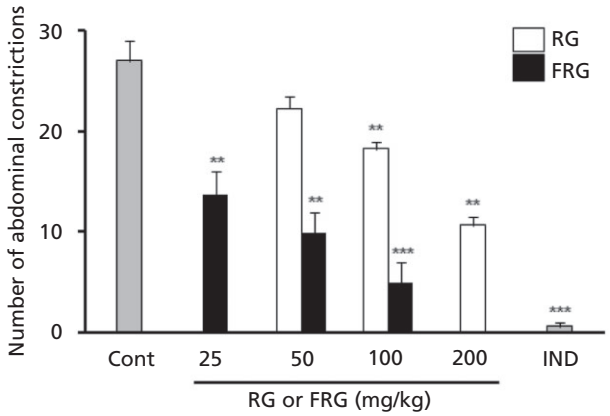

Figure 2 Inhibitory effects of red ginseng water extract and fermented extract on the acetic acid-induced abdominal constriction response in mice. Red ginseng water extract (RG; 50, 100 or 200 mg/kg), fermented extract (FRG; 25, 50 or 100 mg/kg), indometacin (IND; 10 mg/kg; positive control) or vehicle (Cont; 1% carmellose in saline) was orally administered. From 10 min after the intraperitoneal injection of 0.7% acetic acid solution, the number of abdominal constrictions during the following 10-min period was counted. The results are expressed as mean ± SE. Each group contained seven mice. $^{**}P < 0.01$, $^{***}P < 0.001$ vs the control group.

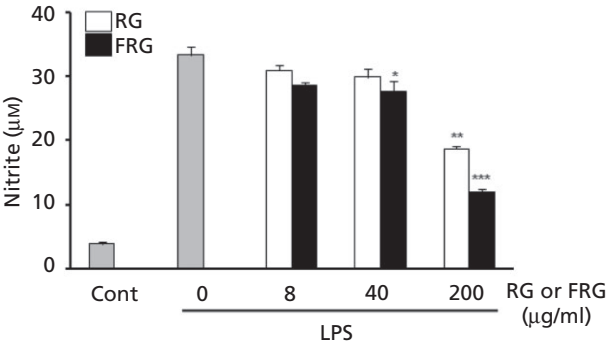

Figure 3 Inhibitory effects of red ginseng water extract and fermented extract on lipopolysaccharide-induced nitric oxide production in RAW264.7 macrophage cells. The mammalian cells were incubated for 24 h with lipopolysaccharide (LPS; 1 μg/ml) in the presence or absence of indicated concentrations of water extract (RG) or fermented extract (FRG). Accumulated nitrite in the culture medium was determined by the Griess reaction. The values are mean ± SE of the three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with LPS only.

anti-inflammatory activity than RG, which might further support the enhanced in-vivo anti-inflammatory and anti-nociceptive activity of FRG.

Anti-angiogenic activity

The chick chorioallantoic membrane (CAM) is an extra-embryonic membrane commonly used *in vivo* to study both angiogenesis and anti-angiogenesis. The membrane was exploited for examining the inhibitory activity of RG and FRG on vascular development, and retinoic acid was used as a positive control for the assay. Retinoic acid is known to inhibit angiogenesis by downregulating the expression and release of pro-angiogenic factors.[16] The disk only did not give rise to changes in vascular density, indicating that it was unable to affect growth of blood vessels in the CAM assay (data not shown). After the two-day treatment, the eggs treated with retinoic acid at 1 μg per egg showed approximately 79% inhibition in the branching patterns of blood vessels (Figure 4). When 0.01, 0.03 or 0.10 μg per egg of RG was applied in the CAM assay, the inhibition percentages in CAM angiogenesis were 35, 50 and 60%, respectively, whereas FRG at the same doses gave rise to an inhibition of 27, 55 and 63%, respectively (Figure 4). These results indicated that both RG and FRG contained remarkable anti-angiogenic activity in the in-vivo assays used. However, as shown in Figure 4, no significant differences in the anti-angiogenic activity of RG and FRG were observed. This finding implied that fermentation of RG with the *Bifidobacterium* strain used in this work could not modulate anti-angiogenic activity. Taken together, it was obvious that RG and FRG possessed similar anti-angiogenic activity.

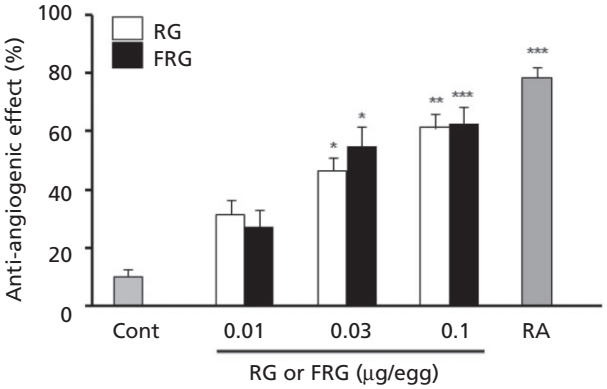

Figure 4 Anti-angiogenic activity of red ginseng extract and fermented extract in the chick embryo chorioallantoic membrane assay. Retinoic acid (RA; 1 μg/egg) was used as a positive control. Each group contained at least 20 eggs. Each column represents mean ± SE of the three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs the control group (Cont).

Discussion

Ginseng has a wide range of pharmacological and physiological actions, which include anti-inflammatory, antioxidant, anticarcinogenic, antitumorigenic, anti-allergic, anti-ageing, antinociceptive, antihypertensive, anti-amnesic, antiobestic and antidiabetic activity.[4,17–24] Ginsenosides, also referred to as ginseng saponins with a triterpenoid dammarane structure, are known to be responsible for the majority of ginseng's pharmacological actions.[25] Ginsenosides are classified into three categories on the basis of aglycone moieties:

protopanaxadiol (Ra_1, Ra_2, Ra_3, Rb_1, Rb_2, Rb_3, Rc, Rd, Rg_3, Rh_2, etc.), protopanaxatriol (Re, Rg_1, Rg_2, Rf, Rh_1, etc.), and oleanoic (Ro) saponins.[26] Some ginsenosides, including Rg_3, Rg_5, and Rk_1, are only identified from red ginseng.[27] Pharmacological activities of ginseng are individually attributed to different types of ginsenosides. Protopanaxadiol-type ginsenosides Rb_1, Rb_2, Rc and Rd contain antioxidant activity, whereas protopanaxatriol-type ginsenosides Re, Rf and Rg_1 possess effects on improvement of learning and memory.[28,29] On the other hand, Rg_1 promotes functional neovascularization into a polymer scaffold *in vitro* and angiogenesis *in vivo*, while Rb_1 and Rb_2 inhibit angiogenesis.[30,31]

In this work, RG was exposed to one of the probiotic strains to investigate any enhancement in its pharmacological activity. Enhanced anti-inflammatory and antinociceptive activity of FRG could be attributed to the increased levels of ginsenosides Rg_3, compound K and Rh_2, which had been identified previously to contain significant anti-inflammatory activity.[32–35] During fermentation with the *Bifidobacterium* strain used, the precise transformation modes responsible for changes in the ginsenoside patterns remain elusive. Anti-angiogenic activity of RG and FRG appeared to remain similar, which was determined using the CAM assay, implying that the anti-angiogenic activity was independent on component changes generated with the current fermentation. However, the strong anti-angiogenic activity of both RG and FRG were verified using the in-vivo model. Hypolipidaemic and hypoglycaemic effects of red ginseng were shown previously to be enhanced by fermentation with *Bifidobacterium* H-1.[36] In addition to ginsenosides, both RG and FRG contain other beneficial components, and their amounts would be changed during bacterial fermentation. However, the anti-inflammatory, antinociceptive and anti-angiogenic activities studied in this work were chiefly attributed to the effects of ginsenosides. Changes in other beneficial components of red ginseng by fermentation and subsequent changes in their pharmacological effects currently remain to be elucidated. *B. longum* primarily lives in the human gastrointestinal tract, although it is not always found in everybody.[37] Thus all humans could not expect the suggested fermentation in their guts after taking in red ginseng. The use of already fermented materials might be an effective way to overcome this issue. Together with previous findings, the results obtained with this work might indicate that enhancement of the individual actions of red ginseng by fermentation would depend on the selection of bacterial strain. Accordingly, strain selection would be crucial to enhance the wanted pharmacological activity of red ginseng by fermentation. Nonetheless, fermentation is expected to be a valuable method, suggesting that we would use ginseng in a more effective way. However, the use of bacterial fermentation to enhance the pharmacological efficacies of traditional medicine, such as ginseng, would have some limitations. Selection of an appropriate bacterial strain should be an essential prerequisite in order to enhance a desired pharmacological efficacy of certain medicinal plants, although it would sometimes be time-consuming or nearly impossible. Other limitations could emerge from unfavourable reactions during the fermentation process with a screened strain, as production of undesirable substances could have harmful effects on the human body. In general, although the fermented product would contain enhanced pharmacological effects, it would be necessary to ensure that it was completely safe to the human body.

Conclusions

Bacterial fermentation of a water extract of red ginseng was able to enhance its anti-inflammatory and antinociceptive activity, which were determined using in-vivo and/or in-vitro experimental models. Enhanced anti-inflammatory and antinociceptive activity of the fermented extract of red ginseng were assumed to be attributed to changes in the pattern of ginsenosides. Anti-angiogenic activity of the red ginseng water extract and fermented extract were very similar in the CAM assay. The current finding would further support that beneficial bacterial fermentation may be used as a plausible method to strengthen specific pharmacological activities of ginseng.

Declarations

Conflict of interest

The authors declare no conflicts of interest.

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References

1. Tang W, Eisenbrand G. *Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine*. Berlin: Springer-Verlag, 1992: 711–737.
2. Kang KS *et al.* Increase in the free radical scavenging activity of ginseng by heat-processing. *Biol Pharm Bull* 2006; 29: 750–754.
3. Konoshima T *et al.* Anti-tumor-promoting activity of majonoside-R2 from Vietnamese ginseng, *Panax*

vietnamensis Ha et Grushv. (I). *Biol Pharm Bull* 1998; 21: 834–838.
4. Park JD. Recent studies on the chemical constituents of Korean ginseng (*Panax ginseng* C. A. Meyer). *Korean J Ginseng Sci* 1996; 20: 389–415.
5. Chon H *et al*. Comparison of aqueous plant extracts before and after fermentation with Lactobacillus paracasei LS-2 on cytokine induction and antioxidant activity. *Nat Prod Commun* 2010; 5: 1277–1282.
6. Hu Y *et al*. Characterization of fermented black soybean natto inoculated with *Bacillus natto* during fermentation. *J Sci Food Agric* 2010; 90: 1194–1202.
7. Su ZY *et al*. Black soybean promotes the formation of active components with antihepatoma activity in the fermentation product of *Agaricus blazei*. *J Agric Food Chem* 2008; 56: 9447–9454.
8. Lee HY *et al*. Enhancement of antimicrobial and antimutagenic activities of Korean barberry (*Berberis koreana* Palib.) by the combined process of high-pressure extraction with probiotic fermentation. *J Sci Food Agric* 2010; 90: 2399–2404.
9. Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. *Br J Pharmacol Chemother* 1964; 22: 246–253.
10. Ghosh AK *et al*. Cyclooxygenase-2-mediated angiogenesis in carrageenan-induced granulation tissue in rats. *J Pharmacol Exp Ther* 2000; 295: 802–809.
11. Olajide OA *et al*. Studies on the anti-inflammatory, antipyretic and analgesic properties of *Alstonia boonei* stem bark. *J Ethnopharmacol* 2000; 71: 179–186.
12. Shermam MP *et al*. Pyrrolidine dithiocarbamate inhibits induction of nitric oxide synthase activity in rat alveolar macrophages. *Biochem Biophys Res Commun* 1993; 191: 1301–1308.
13. Song YS *et al*. Anti-angiogenic and inhibitory activity on inducible nitric oxide production of the mushroom *Ganoderma lucidum*. *J Ethnopharmacol* 2004; 90: 17–20.
14. Vogel HG, Vogel WH. *Drug Discovery and Evaluations: Pharmacological Assays*. Berlin: Springer, 1997: 402–403.
15. Singh VK *et al*. Modulation of autoimmune diseases by nitric oxide. *Immunol Res* 2000; 22: 1–19.
16. Iurlaro M *et al*. Beta interferon inhibits HIV-1 Tat-induced angiogenesis: synergism with 13-cis retinoic acid. *Eur J Cancer* 1998; 34: 570–576.
17. Cho WC *et al*. Ginsenoside Re of *Panax ginseng* possesses significant antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats. *Eur J Pharmacol* 2006; 550: 173–179.
18. Jin SH *et al*. Korean red ginseng saponins with low ratios of protopanaxadiol and protopanaxatriol saponin improve scopolamine-induced learning disability and spatial working memory in mice. *J Ethnopharmacol* 1999; 66: 123–129.
19. Jung NP, Jin SH. Studies on the physiological and biochemical effects of Korean ginseng. *Korean J Ginseng Sci* 1996; 20: 431–471.
20. Kang TH *et al*. Effects of red ginseng extract on UVB irradiation-induced skin aging in hairless mice. *J Ethnopharmacol* 2009; 123: 446–451.
21. Kim JH *et al*. Comparison of the anti-obesity effects of the protopanaxadiol- and protopanaxatriol-type saponins of red ginseng. *Phytother Res* 2009; 23: 78–85.
22. Lee SH *et al*. The antistress effect of ginseng total saponin and ginsenoside Rg3 and Rb1 evaluated by brain polyamine level under immobilization stress. *Pharmacol Res* 2006; 54: 46–49.
23. Park EK *et al*. Antiallergic activity of ginsenoside Rh2. *Biol Pharm Bull* 2003; 26: 1581–1584.
24. Park EK *et al*. Inhibitory effect of ginsenoside Rb1 and compound K on NO and prostaglandin E2 biosyntheses of RAW264.7 cells induced by lipopolysaccharide. *Biol Pharm Bull* 2005; 28: 652–656.
25. Choi KT. Botanical characteristics, pharmacological effects and medicinal components of Korean *Panax ginseng* C. A. Meyer. *Acta Pharmacol Sin* 2008; 29: 1109–1118.
26. Gillis CN. *Panax ginseng* pharmacology: a nitric oxide link? *Biochem Pharmacol* 1997; 54: 1–8.
27. Kang KS *et al*. Study on the nitric oxide scavenging effects of ginseng and its components. *J Agric Food Chem* 2006; 54: 2558–2562.
28. Jaenicke B *et al*. Effect of *Panax ginseng* extract on passive avoidance retention in old rats. *Arch Pharm Res* 1991; 14: 25–29.
29. Kim DY, Chang JC. Radioprotective effect of ginseng components on antioxidant enzymes, glutathione and lipid peroxidation of liver in γ-irradiated mice. *Korean J Ginseng Sci* 1998; 22: 1–10.
30. Sato K *et al*. Inhibition of tumor angiogenesis and metastasis by a saponin of *Panax ginseng*, ginsenoside Rb2. *Biol Pharm Bull* 1994; 17: 635–639.
31. Sengupta S *et al*. Modulating angiogenesis. The yin and the yang in ginseng. *Circulation* 2004; 110: 1219–1225.
32. Bae EA *et al*. Ginsenosides Rg3 and Rh2 inhibit the activation of AP-1 and protein kinase A pathway in lipopolysaccharide/interferon-gamma-stimulated BV-2 microglial cells. *Planta Med* 2006; 72: 627–633.
33. Choi K *et al*. Ginsenosides compound K and Rh(2) inhibit tumor necrosis factor-alpha-induced activation of the NF-kappaB and JNK pathways in human astroglial cells. *Neurosci Lett* 2007; 421: 37–41.
34. Kang KS *et al*. Preventive effect of 20(S)-ginsenoside Rg3 against lipopolysaccharide-induced hepatic and renal injury in rats. *Free Radic Res* 2007; 41: 1181–1188.
35. Park EH *et al*. Inhibitory effect of ginsenoside Rb1 and compound K on NO and prostaglandin E2 biosynthesis of RAW264.7 cells induced by lipopolysaccharide. *Biol Pharm Bull* 2005; 28: 652–656.
36. Trinh HT *et al*. Bifidus fermentation increases hypolipidemic and hypoglycemic effects of red ginseng. *J Microbiol Biotechnol* 2007; 17: 1127–1133.
37. Matsuki T *et al*. Distribution of Bifidobacterial species in human intestinal microflora examined with 16S rRNA-gene targeted species-specific primers. *Appl Environ Microbiol* 1999; 65: 4506–4512.