

RESEARCH ARTICLE

In vivo antimalarial activity of ginseng extracts

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Abstract

Context: Novel antimalarial agents are in demand due to the emergence of multidrug resistant strains. Ginseng, a medicinal plant with antiparasitic activity, contains components that can be used to treat the tropical disease malaria.

Objective: Ginsenosides and polysaccharides are active components of ginseng. This study aimed to elucidate the ability of these compounds to inhibit the replication of *Plasmodium yoelii* in an attempt to determine whether the medicinal uses of ginseng are supported by pharmacological effects. New antimalarial compounds may be developed from ginsenosides and water-soluble ginseng polysaccharides (WGP).

Materials and methods: Ginsenosides and ginseng polysaccharides were prepared from ginseng. Antimalarial activities were examined by 4-day tests and repository tests. Macrophage phagocytosis was tested in normal and malaria-bearing mice.

Results: Ginseng polysaccharides could inhibit residual malaria infection. After a 6-day treatment, the parasitemia reductions of WGP and acidic ginseng polysaccharide (WGPA) were 55.66% and 64.73% at 200 mg/kg/day, respectively. Ginsenosides showed significant antimalarial activity on early infection. Protopanaxadiol-type ginsenosides caused 70.97% chemosuppression at 50 mg/kg/day, higher than 52.8% of total ginsenosides at the same dose.

Discussion and conclusion: Protopanaxadiol-type ginsenosides have remarkably suppressive activity during early infection, while acidic ginseng polysaccharides have significant prophylactic activity against malaria by stimulating the immune system. We propose that the activity of ginsenosides is dependent upon non-specific carbohydrate interactions and that the activity of ginseng polysaccharides is due to immunological modulation. Ginsenosides and ginseng polysaccharides might have a potential application in antimalarial treatments.

Keywords: Antimalarial, ginseng polysaccharides, ginsenosides, *Plasmodium yoelii*

Introduction

Malaria is one of the most prevalent and serious protozoan tropical diseases. Although over a century of effort has been made to eliminate this disease, malaria eradication remains a distant goal, with millions of clinical cases worldwide reported each year and over three billion people living under its threat (Snow et al., 2005). One of the most difficult issues in malaria control is overcoming multi-drug resistance (Wyler, 1993; Alecrim et al., 1999; Kshirsagar et al., 2000; White, 2004). Developing countries, where malaria is epidemic, still depend on traditional medicine as a source of treatment for this disease. Although limited scientific data is available that assesses

the efficacy of these herbal remedies, it is accepted that the recognition and validation of traditional medical practices could lead to new plant-derived drugs, e.g. artemisinin from *Artemisia annua* Linn. (Asteraceae), a traditional Chinese medicine (Riddle et al., 2002). Therefore, it is promising to develop new antimalarial drugs from medicinal plants rather than use folk herbal remedies.

Panax ginseng C. A. Meyer (ginseng) is one of most famous traditional Chinese medicine plants, that has been used for thousands of years. In Asia it is believed that ginseng is a miraculous medicinal plant that improves the physical condition (Attele et al., 1999;

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Ang-Lee et al., 2001). The antiparasitic activity of ginseng has been noted in well known Chinese medicinal references such as the *Compendium of Materia Medica* (Li, 1578). It is known that ginsenosides and polysaccharides are the active components of ginseng. Ginseng polysaccharides have immunostimulating, hypoglycemic and anti-tumor activities (Konno et al., 1984, 1985; Tomoda et al., 1993; Kim et al., 1998; Shin et al., 2004). Ginsenosides also have anti-neoplastic, anti-stress, and anti-oxidant activities (Liu & Xiao, 1992; Attele et al., 1999). The structures of ginsenosides are similar to some steroidal saponins that have been found to be detrimental to several infectious protozoans, which imply that the ginsenosides might have antimalarial potential. In the present study we report the isolation of ginseng polysaccharides and ginsenosides, then investigation of their inhibitory effects on *Plasmodium yoelii* in mice and their macrophage phagocytosis activities in normal and malaria-bearing mice. The possible mechanisms of their antimalarial activities are also discussed.

Materials and methods

Chemicals and reagents

Artesunate was purchased from Guilin Pharmaceutical (Guilin, China). D101 type non-polar macroporous resin was obtained from Tianjin Haiguang Chemicals (Tianjin, China). DEAE-cellulose was purchased from Shanghai Hengxin (Shanghai, China). All other reagents were of analytical grade and made in China.

Preparation of ginsenosides and ginseng polysaccharides

The ginsenosides and polysaccharides were prepared according to our previous procedures (Zhang et al., 2009; Zhao et al., 2009). *Panax ginseng* was cultivated and collected in the Changbai Mountains, Jilin, China. Air-dried ginseng roots were extracted with distilled water at 100°C for 4 h and filtered through four sheets of gauze. The solid material was extracted twice more under the same conditions. The filtrates were combined, concentrated, centrifuged and precipitated by ethanol, with the final concentration of ethanol being 80%. The precipitate and supernatant were separated by centrifugation. The precipitate was treated with Sevag reagent (*n*-butanol: chloroform = 1: 4, v/v) to remove proteins (Sevag et al., 1938) and the polysaccharides were recovered by ethanol to give the polysaccharides fraction (WGP). The obtained WGP fraction was applied to a DEAE-cellulose column (80 mm × 200 mm, Cl⁻), eluting first with water to give the unbound fraction (WGPN), and subsequently washing with 0.5 M NaCl to give a bound fraction (WGPA).

The supernatant was concentrated by vacuum and then loaded on a column (60 mm × 600 mm) for macroporous resin chromatography. The column was eluted first with water to remove oligosaccharides, and

then with 90% ethanol to obtain total ginsenosides (TG). The total ginsenosides were further fractionated on a column of silica gel using chloroform-methanol-water (65: 35: 10, v/v/v, lower phase) as the eluent, resulting in protopanaxadiol-type ginsenosides (PD) and protopanaxatriol-type ginsenosides (PT). All silica gel chromatography was monitored by thin layer chromatography (TLC).

Analytical methods

The carbohydrate content was determined by the phenol-sulfuric acid method (Dubois et al., 1956), the uronic acid content was determined by the *m*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), and the protein content was measured by the Bradford assay (Sedmak & Grossberg, 1977). Sugar composition was analyzed by HPLC (Honda et al., 1989). The compositions of TG and PD were analyzed by the HPLC on a Shimadzu Shim-pack PREP-ODS column (250 mm × 4.6 mm), using a gradient elution of 23-23-40-100-100% acetonitrile (in water) by a linear increase from 0-18-58-75 min. WGP, WGPA, TG and PD were used as the test samples, and they were endotoxin free by the Limulus amoebocyte lysate (LAL) assay using an E-TOXATE kit (Sigma, St. Louis, MO) according to the manufacturer's instructions.

Animals

An equal number of male and female ICR mice (68 weeks old, weighing 20 ± 2 g) were purchased from the Pharmacology Experimental Center of Jilin University, Changchun, China. They were supplied *ad libitum* with a standard diet and tap water at a temperature of 22° ± 3°C. All procedures were in strict accordance with P.R. China legislation and with the guidelines established by the School of Life Sciences of Northeast Normal University regarding the use and care of laboratory animals; the protocol was approved by the university's committee for animal experiments.

Parasite inoculation

The *Plasmodium yoelii* strain 17XL was obtained from Cao Ya-Ming, Department of Immunology, China Medical University, and blood stage parasites were stored in liquid nitrogen. The standard inoculum consisted of 1 × 10⁷ mL *Plasmodium yoelii* parasitized erythrocytes from a donor mouse, which was used to infect mice intraperitoneally.

Evaluation of antimalarial activity during early infection (4-day test)

Suppressive activity during early infection was evaluated using a 4-day schizonticidal test (Peters, 1965; Makinde et al., 1989; Peters & Robinson, 1992; Elufioye & Agbedahunsi, 2004). In brief, mice were inoculated on the first day (day 0) and then randomly divided into groups of six mice each. Three hours after infection, mice in the polysaccharides-treated groups were given WGP or WGPA of different dosages (100 and 200 mg/kg); the ginsenosides-treated groups were given TG or PD of

different dosages (10 and 50 mg/kg), and the co-treated group was given a mixture of WGPA (100 mg/kg) and artesunate (14 mg/kg). A negative control group was administered with physiological saline and a positive control group was treated with artesunate at doses of 28 mg/kg. The drugs were orally administered to mice daily for four consecutive days (days 0-3) between 8.00 a.m. and 9.00 a.m. Twenty-four hours after the last administration, blood smears were made from tail blood and stained with Giemsa stain. Drug activity was assessed by evaluating the smears under a microscope. Parasitemia (%) was calculated by dividing the number of parasitized erythrocytes by the total number of erythrocytes. Average chemosuppression (%) was calculated as $100 \times [(A - B)/A]$, where A is the average parasitemia of the negative control group and B is the average parasitemia of the test group.

Evaluation of antimalarial activity on residual infection (repository test)

Prophylactic activity was determined according to the method described by Peters (Peters, 1965) with slight modifications. Briefly, mice were randomly divided into groups of six mice each and orally administered polysaccharides (WGP or WGPA) or ginsenosides (TG or PD) or physiological saline for four, six or eight consecutive days prior to infection. Twenty-four hours after the final administration of drug or saline, mice were inoculated with *P. yoelii*-parasitized erythrocytes. Seventy-two hours after inoculation, blood smears were made from tail blood and stained with Giemsa stain. The parasitemia (%) and chemosuppression (%) were assessed.

Macrophage phagocytosis assay

The mice were randomly divided into groups of six mice each: the control group (administered with physiological saline) as well as 100 and 200 mg/kg WGPA groups. The drugs were given orally for four, six or eight consecutive days, respectively. Twenty-four hours after the last administration, the mice in the malaria-bearing groups were inoculated with *Plasmodium yoelii*, and the mice in the normal groups were used to assess the macrophage phagocytosis activities of WGPA in normal mice. Seventy-two hours after inoculation, the macrophage phagocytosis activities of WGPA in malaria-bearing mice were evaluated.

Macrophage phagocytosis of mice was evaluated using the method described by Yang et al. (2006). Briefly, 24 h after the last administration, mice were intraperitoneally injected with 1 mL of 5% chicken red blood cells (CRBC).

After 30 min, mice were sacrificed and injected with 2 mL D-Hank's. The activated macrophages were obtained by peritoneal lavage to make a smear. After incubation at 37°C for 30 min in a humidified 5% CO₂ incubator, the smears were centrifuged to remove the supernatant, and the macrophages were fixed with methanol and stained by Giemsa-Wright for 710 min. The microscope slides were washed with PBS and counted using a microscope. The phagocytic rate (PR) and phagocytic index (PI) were calculated:

$$PR(\%) = \frac{\text{No. of macrophages containing CRBC}}{\text{Total macrophages counted}} \times 100$$

$$PI = \frac{\text{No. of CRBC in macrophages}}{\text{Total macrophages counted}}$$

Statistical analysis

Results were expressed as the mean \pm SD of the indicated number of experiments. For comparison of suppression of parasitemia, one-way ANOVA was used. Values of $p < 0.05$ and $p < 0.01$ were considered to be significant.

Results

Preparation of the test samples

Ginseng total polysaccharides (WGP) was a mixture of neutral and acidic polysaccharides. After separation on a DEAE-cellulose column, the neutral and acidic fractions were obtained. The analytical results of these fractions are listed in Table 1. The compositions were similar to those of previous results (Zhang et al., 2009).

The total ginsenosides have been reported to consist of more than 40 glycosides (Liu & Xiao, 1992; Attele et al., 1999). It is difficult to determine all components in such a complex mixture. Our ginsenosides fraction, TG, was mainly composed of Rb1 (31.9%), Rc (27.7%), Rb2 (13.5%), Rd (16.3%), Rg1 (3.5%) and Re (7.1%). After separation by chromatography on silica gel, the main component, protopanaxadiol-type ginsenosides (PD), was obtained, containing Rb1 (39.7%), Rc (28.0%), Rb2 (20.1%), and Rd (9.5%). WGP, WGPA, TG and PD were used as the test samples in this study, and they were endotoxin-free by the *Limulus* amoebocyte lysate (LAL) assay.

Antimalarial activities of ginseng polysaccharides and ginsenosides during early infection

Ginseng polysaccharides, acidic ginseng polysaccharides, total ginsenosides and protopanaxadiol-type

Table 1. Compositions of ginseng polysaccharides.

| Fraction | C (%) | U (%) | Monosaccharide composition (%) | | | | | |
|----------|-------|-------|--------------------------------|-----|------|-----|-----|------|
| | | | Glc | Gal | Ara | Man | Rha | GalA |
| WGP | 77.1 | 10.0 | 77.9 | 6.8 | 4.6 | 1.0 | 1.1 | 8.7 |
| WGPN | - | - | 95.3 | - | - | - | - | - |
| WGPA | - | - | 18.5 | 18 | 15.5 | - | 3.8 | 44.2 |

C, carbohydrate content; U, uronic acid content.

ginsenosides were tested against *Plasmodium yoelii* in mice. As shown in Table 2, WGP and WGPA have no obvious effects on parasitemia ($p < 0.01$) compared to control; the chemosuppressions of WGP and WGPA were 9.36% and 12.03% respectively at a dose of 100 mg/kg/day, and 11.11% and 17.98% respectively for 200 mg/kg/day. While TG and PD exhibited remarkable antimalarial activities, the PD had better activity in parasitemia reduction. At 10 mg/kg/day, TG and PD caused 43.2% and 57.14% chemosuppression, respectively, and at 50 mg/kg/day, they caused 52.8% and 70.97% chemosuppression, respectively.

Artesunate is an effective antimalarial drug used in the current market. The chemosuppressions of artesunate were 57.21% and 75.28% at a dose of 14 and 28 mg/kg/day, respectively. Co-administration of WGPA and artesunate resulted in 77.11% parasitemia reduction, which is higher than the sum of the suppression caused by WGPA and artesunate individually, indicating that WGPA and artesunate acted synergistically.

Antimalarial activities of ginseng polysaccharides and ginsenosides on residual infection

The protective effects of ginseng polysaccharides and ginsenosides on *Plasmodium yoelii* were tested by residual

Table 2. Anti-malarial activities of ginseng extracts for the 4-day test.

| Group | Dose (mg/kg) | P (%) | S (%) |
|-----------------|--------------|----------------|-------|
| Control | | 24.33 ± 3.35 | 0 |
| Artesunate | 14 | 10.41 ± 2.8** | 57.21 |
| | 28 | 6.01 ± 2.1** | 75.28 |
| WGP | 100 | 22.05 ± 4.35 | 9.36 |
| | 200 | 21.63 ± 4.9 | 11.11 |
| WGPA | 100 | 21.40 ± 3.8 | 12.03 |
| | 200 | 19.96 ± 2.7* | 17.98 |
| TG | 10 | 13.82 ± 3.65** | 43.2 |
| | 50 | 11.48 ± 1.7** | 52.8 |
| PD | 10 | 10.19 ± 2.25** | 57.14 |
| | 50 | 7.06 ± 1.6** | 70.97 |
| Artesunate+WGPA | 100 + 14 | 5.57 ± 0.1** | 77.11 |

Data are expressed as mean ± SD for six animals per group. Compared with the control: * $p < 0.05$, ** $p < 0.01$. P, parasitaemia; S, suppression.

Table 3. Anti-malarial activities of ginseng extracts during 8-day test.

| Group | Dose(mg/kg) | Day 4 | | Day 6 | | Day 8 | |
|---------|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | | Parasitemia (%) | Suppression (%) | Parasitemia (%) | Suppression (%) | Parasitemia (%) | Suppression (%) |
| Control | | 25.01 ± 5.51 | | 24.87 ± 1.7 | | 23.77 ± 2.2 | |
| WGP | 100 | 21.44 ± 2.15 | 14.27 | 17.08 ± 2.25* | 31.34 | 19.27 ± 4.35 | 18.94 |
| | 200 | 20.67 ± 1.15 | 17.34 | 11.03 ± 2.6** | 55.66 | 16.09 ± 1.65* | 32.33 |
| WGPA | 100 | 18.54 ± 2.35 | 25.87 | 3.5 ± 0.67** | 54.31 | 14.52 ± 3.7* | 38.92 |
| | 200 | 17.47 ± 1.7* | 30.15 | 8.7 ± 0.87** | 64.73 | 10.65 ± 2.05** | 55.2 |
| TG | 10 | 22.86 ± 5.51 | 8.61 | 20.1 ± 1.05 | 19.18 | 21.39 ± 3.3 | 10.03 |
| | 50 | 22.41 ± 3.65 | 10.4 | 19.04 ± 2.15 | 23.46 | 21.71 ± 1.6 | 12.78 |
| PD | 10 | 20.65 ± 2.7 | 17.44 | 18.6 ± 1.15 | 25.21 | 18.7 ± 2.7 | 21.34 |
| | 50 | 19.42 ± 1.65 | 22.34 | 15.72 ± 1.65* | 36.78 | 17.2 ± 1.7 | 27.65 |

Data are expressed as mean ± SD for six animals per group. Compared with the control: * $p < 0.05$, ** $p < 0.01$.

infection assays. The results, listed in Table 3, indicated that ginseng polysaccharides (WGP and WGPA) could decrease the infection of *Plasmodium yoelii* more significantly than ginsenosides (TG and PD), suggesting that the ginseng polysaccharides enhanced the protective ability of the host against malaria more effectively. Both of the ginseng polysaccharides and ginsenosides showed bell-shaped time-dependent prophylactic activities, peaking on day six. The prophylactic activities of ginseng polysaccharides and ginsenosides at high doses were better than their prophylactic activities at low doses. After the 6-day treatment, the parasitemia reductions of WGP and WGPA were 55.66% and 64.73%, respectively, at a dose of 200 mg/kg, and the parasitemia reductions of TG and PD were 23.46 and 36.78%, respectively, at dose of 50 mg/kg.

Effect of WGPA on macrophage phagocytosis

Macrophages, an integral part of the immune system, play an important role in the initiation and regulation of the immune response by interacting with lymphocytes and acting as phagocytic, microbiocidal and tumouricidal effector cells (Gordon, 1998). The effect of WGPA on macrophage phagocytosis was determined using CRBC in normal mice and malaria-bearing mice. As shown in Table 4, the phagocytic rate and the phagocytic index were significantly increased by WGPA treatment at doses of 100 and 200 mg/kg ($p < 0.01$) compared with the control groups, indicating that WGPA could activate macrophages and restore phagocytosis activity in malaria-bearing mice. WGPA caused maximal macrophage phagocytosis on day 6, and its macrophage phagocytosis activity at a dose of 200 mg/kg was higher than that at a dose of 100 mg/kg. These results are consistent with those of residual infection.

Discussion

The biological activities of polysaccharides have attracted more attention recently in the biochemical and medical fields because of their immunological activities that could protect our bodies from suffering attacks from microbes and parasites (Paterson, 2006; Vliegenthart, 2006; Kilcoyne & Joshi, 2007; Trincherro et al., 2009). A group of sulfated polysaccharides including heparin,

Table 4. Effects of WGPA on macrophage phagocytic activity in normal and malaria-bearing mice.

| Day | Dose(mg/kg/day) | Normal mice | | Malaria-bearing mice | |
|-----|-----------------|---------------|---------------|----------------------|---------------|
| | | PR (%) | PI | PR (%) | PI |
| 4 | Control | 27.51 ± 5.4 | 0.41 ± 0.14 | 33.37 ± 8.1 | 0.53 ± 0.18 |
| | 100 | 28.85 ± 4.23 | 0.52 ± 0.12** | 33.72 ± 4.19 | 0.69 ± 0.08** |
| | 200 | 31.46 ± 4.17* | 0.67 ± 0.15** | 46.59 ± 3.49** | 1.01 ± 0.08** |
| 6 | 100 | 29.55 ± 5.25 | 0.62 ± 0.18** | 43.68 ± 2.39** | 0.94 ± 0.14** |
| | 200 | 32.05 ± 3.12* | 0.77 ± 0.18** | 47.16 ± 3.18** | 1.13 ± 0.08** |
| 8 | 100 | 30.8 ± 6.21 | 0.57 ± 0.14** | 39.13 ± 4.27* | 0.8 ± 0.08** |
| | 200 | 31.76 ± 8.01* | 0.69 ± 0.12** | 42.61 ± 3.44** | 0.78 ± 0.1** |

Data are expressed as mean ± SD for six animals per group. Compared with the control: *p < 0.05, **p < 0.01. PR, phagocytic rate; PI, phagocytic index.

dextran sulfate, fucoidan (Butcher et al., 1988) and curdlan sulfate (Havlik et al., 1994) have been shown to exhibit antimalarial effects. Lentinan, an anti-tumor 1,3-β-D-glucan, has recently been investigated for its antimalarial activity using a *Plasmodium yoelii* blood-stage infection model (Zhou et al., 2009). Lentinan significantly increased mouse survival after infection by inducing protective Th1 immune responses to control the proliferation of malaria parasites during the blood stage of P.y17XL infection.

In the present paper, WGPA could markedly inhibit malaria residual infection. At the same time, it augmented macrophage phagocytosis in malaria-bearing and normal mice. The changing tendency of its macrophage phagocytosis activity was consistent with that of its antimalarial activity during residual infection, which peaked at 200 mg/kg on day six, implying that WGPA might inhibit malaria by enhancing the immune competence of its host. Thus, WGPA might be a potential prophylactic agent against malaria infection. Artesunate is an efficient antimalarial drug currently on the market (Klayman, 1985). However, it has side effects, inhibiting phagocytosis and cytokine secretion by monocytes (Bloland et al., 2000; Nosten & Brasseur, 2002; Li & Wu, 2003; Mutabingwa, 2005). To overcome this problem, artesunate-based combination therapy was recommended by the World Health Organization (WHO) as a new prospective paradigm. In our experiment, the combination of WGPA and artesunate significantly increased the cure effect of artesunate on malaria infection, which might be the result of WGPA helping the immune system recover functionality suppressed by artesunate. This result suggested that WGPA might be a potential complementary drug for curing malaria infection.

WGPA are biomacromolecules that could markedly inhibit malaria residual infection by immunological regulation. However, its lack of direct action on malaria, and therefore its antimalarial activity, was not remarkable during early infection. Protopanaxadiol-type ginsenosides are a group of steroidal saponins that can directly interact with malaria. Therefore, they have significant antimalarial activity during early infection by a 4-day test. This result is consistent with the observation that triterpenoid and steroid saponins are detrimental

to several infectious protozoans, such as *Plasmodium falciparum* (Welch, 1897; Schaudinn, 1902; Traore et al., 2000), *Giardia trophozoites* Stile. (McAllister et al., 2001) and *Leishmania* spp. (Delmas et al., 2000; Plock et al., 2001). In our previous work, six glycoalkaloids, similar in structure to protopanaxadiol-type ginsenosides, have also been found to be active antimalarials in *Solanum* plants (Chen et al., 2010). These show effect mechanisms similar to the antimalarial activity of reported saponins (Keukens et al., 1995, 1996; Tasdemir et al., 2008). The antimalarial activity of protopanaxadiol-type ginsenosides was presumed to be attributed to its nature of cell membrane disruption.

In conclusion, protopanaxadiol-type ginsenosides have remarkably suppressive activity during early malaria infection, while acidic ginseng polysaccharides have significant prophylactic activity against malaria by stimulating the immune system. In combination with artesunate, WGPA could enhance the antimalarial activity of artesunate during early infection through recovering the immune system suppressed by artesunate. Therefore, the medicinal uses of ginseng on the inhibition of parasites are supported by pharmacological effects. Ginsenosides and ginseng polysaccharides might have the potential to be applied in antimalarial treatments.

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Declaration of interest

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