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Evaluation of an extract of North American ginseng (*Panax quinquefolius L.*) in *Candida albicans*-infected complement-deficient mice

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ABSTRACT

Ethnopharmacological relevance: Ginseng is a widely consumed aromatic herb that is purported to have health benefits. Several studies report a beneficial impact of ginseng or its derivatives on *Candida albicans* infection in mice and suggest that its immune-modulatory properties contribute to this effect. However, these studies generally administered ginseng to experimental animals by injection, whereas people typically ingest ginseng. Furthermore, although disseminated candiasis is typically a disease of immune-impaired hosts, previous studies have generally used immune competent host species in the assessments.

Materials and methods: We evaluated the efficacy of an ingested extract of ginseng against *Candida albicans* infection in DBA/2J mice, which are highly susceptible to *Candida albicans* infection. A ginseng extract was added to the drinking water for two days before and for the remainder of the study after intravenous inoculation of mice with *Candida albicans*. Mice were evaluated for morbidity, mortality, *Candida albicans* titers, and concentrations of inflammatory cytokines and chemokines.

Results: Ingestion of the ginseng extract did not significantly affect overall morbidity or mortality. However, ingestion of the extract was associated with significantly lower renal titers of *Candida albicans* and with significantly lower concentrations of some inflammatory cytokines in kidney and/or serum.

Conclusions: Assessment of morbidity, mortality, inflammatory markers, and renal titers after spontaneous ingestion of ginseng by susceptible hosts represents a comprehensive approach to characterizations of therapeutic efficacy against infectious agents. Our findings extend previous reports of the efficacy of ginseng against *Candida albicans* by demonstrating significant reductions in infectious load and some markers of inflammation in susceptible mice. Our data therefore support further assessment of the immune-modulatory properties of this widely consumed herb and its components.

1. Introduction

Asian ginseng (*Panax ginseng C.A. Meyer*) and closely related North American ginseng (*Panax quinquefolius L.*) are perennial aromatic herbs that are widely used to promote health, particularly in Asian cultures. A 2002 survey of 31,044 adults in the United States found that 8777 respondents (24%) reported the use of ginseng (Barnes et al., 2004). Some studies indicate that ginseng has immune-modulatory properties that could contribute to its putative health benefits and may make it a useful preventive or complementary therapy in individuals with or at risk for cancer or infectious or inflammatory disease (McElhaney et al., 2004, 2006; Predy et al., 2005; Cui et al., 2006; Hofseth and Wargovich, 2007; Seida et al., 2009; Jin et al., 2010; Volate et al., 2005; Kim and Yang, 2011; Lee and Lau, 2011).

Candida albicans is an opportunistic fungal organism that is commensal at many locations in the body, is carried by at least half of the human population, and can cause disease during a wide range of immunosuppressive therapies or conditions (Samaranayake and Samaranayake, 2001; Saunus et al., 2008). Clinical candidiasis typically develops secondary to either alterations in normal flora (e.g., after administration of antibiotics) or immune impairment (e.g., during chemotherapy, immune suppression, or AIDS). *Candida albicans* infection causes two general types of disease: mucosal (commonly oral, esophageal, or vaginal) and systemic (disseminated). Susceptibility to disseminated *Candida albicans* infection varies widely across inbred strains of mice, as reflected by measures such as mortality, colony counts in infected tissue, and histologic assessment of the severity of infection. (Ashman and Papadimitriou, 1995).

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A major determinant of mouse strain differences in disease after intravenous challenge with *Candida albicans* is deficiency of the fifth component of complement (C5); this deficiency is present in about 40% of all inbred mouse strains due to a 2 bp deletion in the gene *Hc* (hemolytic complement) (Cinader et al., 1964; Wetsel et al., 1990). Complement activation is important for the chemotaxis of immune effectors and opsonization of *Candida albicans,* which are in turn important in reducing the initial fungal burden (Netea et al., 2008). In C5-deficient mice, intravenous injection of *Candida albicans* is associated with high fungal burdens in kidney, with lower titers in brain (Ashman et al., 1996). Severe myocarditis and cardiomyopathy have been reported in A/J and BcA17 mice (Mullick et al., 2006), but are not prominent features of *Candida albicans* infection in DBA/2J and other C5-deficient strains (Ashman et al., 1996; Ashman, 2004).

Three reports have suggested a beneficial impact of ginseng or ginseng derivatives on the response to systemic *Candida albicans* infection in mice. In one study, the ginsenoside Rg1 did not inhibit the growth of *Candida albicans in vitro*, yet intraperitoneal administration of Rg1 to mice before intravenous challenge with *Candida albicans* reduced renal fungal titers and mortality (Lee and Han, 2006). The other two studies evaluated the efficacy of an herbal therapeutic mixture known as Juzen-taiho-to or TJ-48, which contains ginseng as one of its 10 components, in experimental disseminated candidiasis. In one of these studies, mice received one dose of the immunosuppressive agent cyclophosphamide and four consecutive daily oral doses of TJ-48 or its ginseng component prior to intravenous administration of a lethal dose of *Candida albicans* (Abe et al., 1998). Both TJ-48 and its ginseng component prolonged the life span of immune-suppressed lethally infected mice (Abe et al., 1998). In the other study, oral daily administration of TJ-48 or its ginseng component for 5 consecutive days prolonged the survival period of *Candida albicans*-infected C3H/HeJ mice (Akagawa et al., 1996), which have defective macrophage function due to a mutation in the toll-like receptor 4. Survival of a control strain of mice (C3H/HeN) was not affected (Akagawa et al., 1996). TJ-48 and its ginseng component also both augmented the anti-*Candida albicans* activity of C3H/HeJ peritoneal macrophages *in vitro* (Akagawa et al., 1996). We sought to expand these initial characterizations of the efficacy of ginseng against *Candida albicans* infection by testing a hot-water extract of ginseng that has oral efficacy in a cancer model (Murphy et al., 2011). We tested the extract using DBA/2J mice, which show severe disease and high mortality after intravenous challenge with *Candida albicans*.

2. Methods

2.1. Experimental animals

Adult male DBA/2J mice were purchased at 6–8 weeks of age from the Jackson Laboratory (Bar Harbor, ME). All procedures used in these studies were approved by the Laboratory Animal Care and Use Committee at the Southern Illinois University School of Medicine. Mice were free of common adventitious mouse infectious agents, as monitored using monthly testing of sentinel mice housed in the same room. Mice were housed in groups of five on hardwood bedding, with food (Purina Laboratory Chow) and water available *ad libitum.* The animal holding room was maintained on a 12:12 light:dark cycle at 22 ± 1 °C with 35–50% relative humidity. Mice were euthanized by cervical dislocation under isoflurane anesthesia.

Some of the mice used in these experiments were implanted with intra-abdominal transmitters (Data Sciences International, St. Paul, MN) to allow telemetric recording of locomotor activity and core body temperature. Mice were anesthetized by subcutaneous injection of a mixture of ketamine (50 mg/kg) and xylazine (50 mg/kg) and were supplemented with additional anesthetic during surgery if needed. All surgery was conducted using standard aseptic techniques. Mice received 1 ml of saline intraperitoneally at the time of surgery to maintain hydration and to lubricate the transmitter and the abdominal cavity. Another ml of saline was administered intraperitoneally the next morning. The mice recovered from anesthesia in a cage placed under a heating lamp. Sutures were removed at 10 days after surgery if still present.

After surgery, mice were housed in individual cages in a sound shielded chamber under a 12:12 h light: dark cycle at 22 ± 1° C and were allowed 14 days for recovery from surgery. Ibuprofen (1 mg/ml) was provided in drinking water from 1 day prior to surgery through 5 days after surgery to provide analgesia (Hayes et al., 2000). Moistened food was provided on the cage floor for 7 days after surgery. Experimental monitoring was initiated at light onset no sooner than 14 days after surgery.

2.2. Ginseng preparations, analysis and dosing

Commercially available ginseng is typically consumed as a powdered or extracted preparation of the root. The studies reported here use fresh powdered North American ginseng root that is prepared from fall-harvested, 3–4 year old cultivated plants by the Wisconsin Ginseng Board and donated to our laboratory. American ginseng is the only plant that the Wisconsin Ginseng Board supplies. We do not collect or process the plant ourselves. The NIH National Center for Complementary and Alternative Medicine does not require a voucher specimen number for product obtained from a commercial supplier.

Raw powdered ginseng root contains an insoluble fiber component, a relatively large polysaccharide component, and over 30 different ginsenosides (approximately 5.2%, w/w of the raw root), which are the saponin glycosides responsible for much of ginseng's currently known bioactivity (Attele et al., 1999; Assinewe et al., 2002; Cui et al., 2006). Analysis by ConsumerLab LLC (White Plains, NY), an independent chemical analysis laboratory, indicates that powdered *Panax quinquefolium L.* root from this source typically has greater than the recommended minimum ginsenoside concentrations of 4.0%, which is the common industry standard for extract, and is free of heavy metal and pesticide contamination. All work reported here was conducted using a ginseng extract prepared from the same batch of fresh powdered and chemically analyzed ginseng root.

Hot water extracted ginseng (HWEG), which includes both ginsenosides and soluble polysaccharides (Assinewe et al., 2002; Dong et al., 2005), was assessed *in vitro* for anti-*Candida albicans* activity. HWEG was performed as follows: ginseng powder was mixed in sterile distilled water (1:19) and subjected to vigorous shaking for 1 h in a 90 °C water bath. After a brief cooling period, extract was centrifuged at 1800 × *g* for 10 min. This extraction was performed twice. The supernatants were then combined, vacuum-filtered through sterile Whatman #4 filter paper and lyophilized. This extraction yields approximately 2.6 g of sterile product per 25 g starting material. The HWEG was stored in the dark at room temperature, as are most commercial preparations. The HWEG was tested using the E-TOXATE detection kit (Sigma–Aldrich; St. Louis, MO) to verify that bacterial endotoxin was not present. The final endotoxin-free HWEG was analyzed for presence and concentration of ginsenosides using high-performance liquid chromatography as reported elsewhere (Corbit et al., 2005). Lyophilized products were analyzed for ginsenoside content by HPLC (Corbit et al., 2005), total carbohydrate content by phenol-sulfuric method (Masuko et al., 2005), glycosyl composition by gas chromatography–mass spectrometry (King and Murphy, 2009), and protein content by BCA assay (Thermo Pierce;

Rockford, IL). The extract contained 6.89% (w/w) ginsenosides, a total carbohydrate content of 92% (w/w) (consisting of 99.3% glucose, 0.3% arabinose, 0.2% galactose, and 0.1% rhamnose), and a total protein content of less than 3.0%.

For administration to mice, HWEG was dissolved in the drinking water as a 1% solution and was provided to mice for *ad libitum* ingestion beginning 48 h before experimental infection. The treatment paradigm and concentration of extract were selected based on previous data from our laboratory (Murphy et al., 2011).

2.3. Candida albicans stocks, inoculation and titers

Candida albicans (ATCC 10231) was grown overnight on Sabouraud dextrose agar. For intravenous inoculation, colonies were suspended in sterile, pyrogen-free saline. Colony forming units (CFU) per mL were then estimated by hemocytometer, and the suspension was diluted to achieve approximately 5×10^5 CFU in 0.2 ml. The actual inoculated doses were subsequently determined by culture of serial dilutions of the inoculum plated on Sabouraud Dextrose agar.

For inoculation, mice were anesthetized with isoflurane and injected intravenously via the retroorbital sinus with a *Candida albicans* suspension. The intra-orbital route was used because intravenous injection via this route is far easier to accomplish with assurance of a complete injection than is the tail vein route. In either case, injected substances immediately enter and are dispersed through the systemic circulation. After inoculation, all mice were evaluated at least daily. Any mice that displayed palpable hypothermia, failure to respond to manipulation, or unwillingness to walk underwent immediate euthanasia for tissue collection and were considered to have died. Otherwise, mice underwent euthanasia at either 24 h or 5 days after inoculation.

Euthanasia was performed by exsanguination under isoflurane anesthesia. Blood was collected by cardiac puncture and allowed to clot. The serum was then removed and stored in aliquots at −80 °C for subsequent analysis. Right and left kidneys, brain and heart were removed and frozen for subsequent *Candida albicans* culture and quantification of cytokines and LCN2.

To measure *Candida albicans* titers, frozen tissue was homogenized in 1 ml of sterile saline, and serial dilutions of the homogenates plated on Sabouraud dextrose agar and incubated for 24 h. Colonies were then counted, and CFU/gram of tissue was calculated.

2.4. Cytokine, chemokine, LCN2 and BUN quantification

For measurement of cytokines, chemokines, and lipocalin 2 (LCN2), tissues were homogenized in 10 volumes (w/v) of ice cold sterile phosphate buffered saline containing Complete Protease Inhibitor Cocktail (Roche, Camarillo, CA) using a 5 mL glass homogenizer. Homogenates were centrifuged at 13,700 × *g* at 4 °C for 10 min. An aliquot of supernatant was removed and assayed for protein concentration using the BCA protein assay (Pierce Scientific, Rockford, IL). The remaining supernatant was stored in aliquots at −80 °C until analysis.

A panel of cytokines and chemokines were measured using a multiplex bead-based assay (Millipore, Billerica, MA) and analyzed on a Bioplex system (Bio-Rad, Hercules, CA) with Bio-Plex manager 5.0 software. Samples were assayed in duplicate, normalized to protein concentrations, and reported as pg/mg protein.

LCN2 was measured using the DuoSet ELISA Development kit (R&D Systems, Inc. Minneapolis, MN) according to the manufacturers' instructions. Samples were assayed in duplicate and reported as ng/mL for serum samples or ng/mg protein for tissue homogenates. Serum was analyzed for BUN by the University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO.

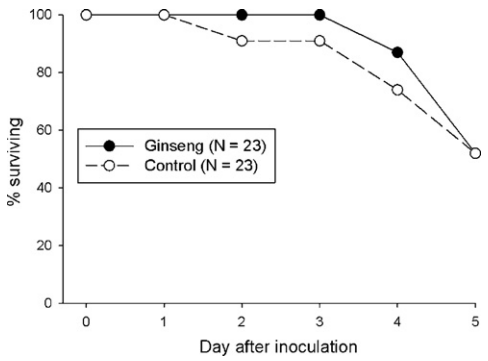

Fig. 1. Mortality in DBA/2J mice after inoculation with *Candida albicans* DBA/2J mice were maintained on water that contained either no additive (open circles) or a hot-water extract of ginseng (filled circles). The figure includes mice used for the temperature, activity, and food measurements ($n = 13$ per treatment group) and those generated for measurement of cytokines at 5 days after inoculation ($n = 10$ per group) (total $n = 23$ ginseng-treated and 23 untreated mice). Treated or untreated water was provided beginning two days prior to infection and continued for the remainder of the study. *Candida* inoculation was performed at time 0 immediately after light onset. Mortality was assessed daily on subsequent mornings. Three of the untreated mice became moribund according to pre-established criteria (see methods) and received early euthanasia (2 on day 2 and 1 on day 4). These mice were considered to have died.

2.5. Statistical analysis

The purpose of this study was to identify variation in the host response to *Candida albicans* inoculation as a function of ingestion of HWEG. For the temperature and locomotor activity data, the analysis was a two factor mixed model analysis of variance in which the "between" factor was group and the "within" factor was day. This test therefore evaluated the data for effects of group, day, and the interaction of group by day. For comparison of cytokine, LCN2 and Candida titer data, a two-factor ANOVA was used to test the overall model. Specific comparisons for group and time were performed with independent *t*-tests. Descriptive statistics are expressed throughout as mean ± S.E.M. A p value of less than 0.05 was considered to indicate statistically significant effects. SPSS was used for all data analysis.

3. Results

3.1. In vitro anti-Candida albicans activity

An initial study assessed activity of HWEG against *Candida albicans* (ATCC 10231) growth *in vitro*. The HWEG was tested at concentrations ranging from 20 to 640 μg/mL (King et al., 2006; Sung and Lee, 2008) and was compared with the known anti-*Candida albicans* agent fluconazole (0.25–64 μg/mL). Fluconazole showed the expected minimum inhibitory concentration of 0.25–1 μg/mL (data not shown). However, the HWEG did not prevent growth of *Candida albicans* at any of the concentrations tested (data not shown), thus indicating lack of direct candidacidal or fungistatic activity.

3.2. Morbidity and mortality

A second study assessed morbidity and mortality in DBA/2J mice that did or did not receive a 1% solution of the HWEG in the drinking water before and after intravenous administration of *Candida albicans*. Mice ($n = 23$ per group) were inoculated intravenously with *Candida albicans* ($5.3 \pm 0.3 \times 10^5$ CFU) in 6 independent replicate studies comprised of 4–6 mice each. At this dose, 48% of the mice died within 5 days after inoculation (Fig. 1). Mortality was similar regardless of whether mice had access to ginseng (Fig. 1).

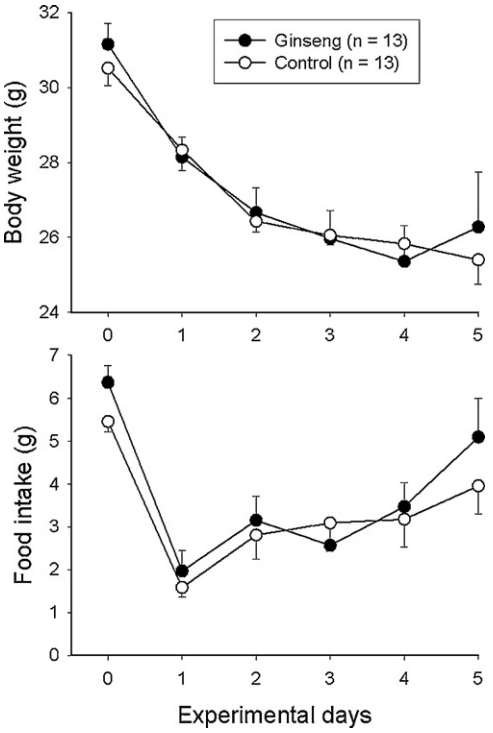

Fig. 2. Body weight and food intake in mice before and after inoculation with *Candida albicans* DBA/2J mice were maintained on water that contained either no additive (open circles) or a hot-water extract of ginseng (filled circles) ($n = 13$ per group). Treated or untreated water was provided beginning two days prior to infection and continued for the remainder of the study. Food intakes and body weight were measured each morning immediately after light onset. *Candida* inoculation was performed at time 0 immediately after light onset. Values shown at time 0 represent the average of values measured on the two days prior to inoculation. ANOVA revealed that all three measures showed a significant effect of experimental day ($p < 0.001$).

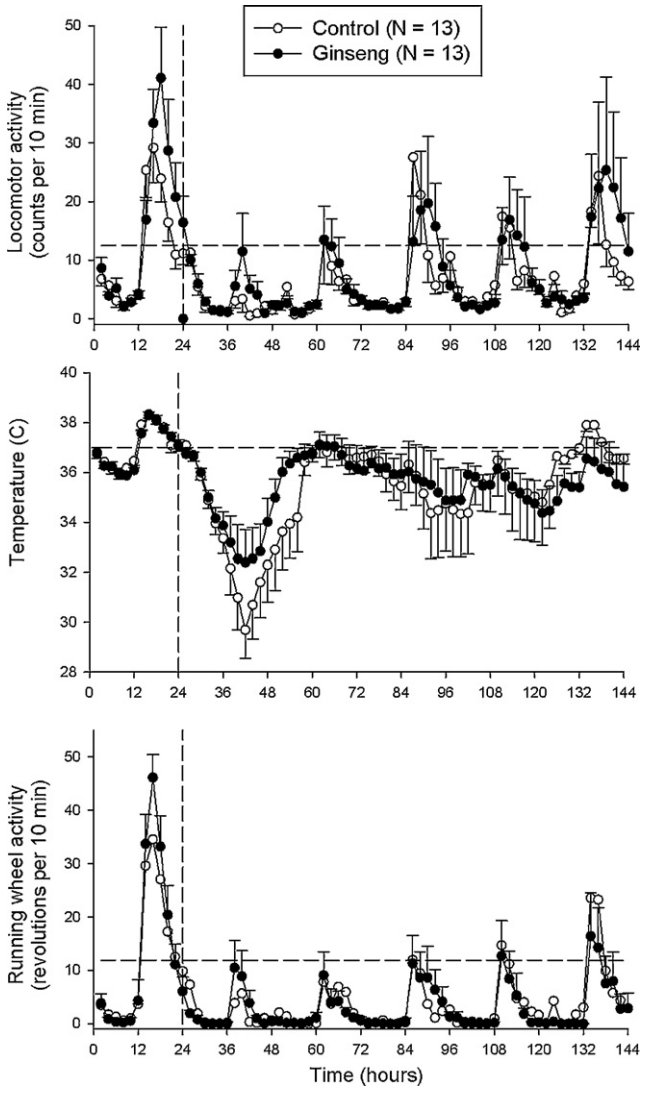

Fig. 3. Activity and temperature of mice before and after inoculation with *Candida albicans* DBA/2J mice were maintained on water that contained either no additive (open circles) or a hot-water extract of ginseng (filled circles) ($n = 13$ per group). Treated or untreated water was provided beginning two days prior to infection and continued for the remainder of the study. Locomotor activity, temperature, and running wheel activity were measured continuously for the duration of the study. *Candida* inoculation was performed at 24 h immediately after light onset. Values shown for time 0 through 24 h represent the average of values measured on the two days prior to inoculation. ANOVA revealed that all three measures showed a significant effect of experimental day ($p < 0.001$), with no significant effect of group and no Group-by-day interactions. The horizontal dashed lines denote the average values obtained on the days prior to inoculation. Hours 12–24, 36–48, 60–72, 84–96, 108–120, and 132–144 were the dark phases.

Some of these mice ($n = 13$ per group) were also evaluated with regard to the development of behavioral illness after infection. After inoculation with *Candida albicans* (mean dose of $5.6 \pm 0.6 \times 10^5$ CFU across 5 independent groups), mice developed significant weight loss and anorexia, with reductions evident at 1 day after infection and persisting until euthanasia on day 5 (Fig. 2). Weight loss and anorexia showed a significant effect of time after injection ($p < 0.001$) but not of treatment group.

Infected mice developed reduced activity and hypothermia within 24 h after inoculation ($p < 0.001$ as a function of time) (Fig. 3). The pattern of changes was not significantly different between the two treatment groups.

3.3. Immune measures

In a third study, DBA/2J mice that received HWEG or untreated water were euthanized 24 h or 5 days after infection with $3.7 \pm 0.2 \times 10^5$ CFU of *Candida albicans*. Kidney, brain, heart, and serum were collected for measurement of *Candida albicans* titer, cytokines and chemokines, lipocalin 2 (LCN2) (a marker of both the acute phase response and renal damage (Mori and Nakao, 2007; Bonomini et al., 2010)), and blood urea nitrogen (BUN). *Candida albicans* was not detected in serum of mice from either treatment group. In heart and brain, ginseng availability did not significantly influence *Candida albicans* titers (Fig. 4) or cytokine/chemokine concentrations (Table 1).

In contrast to heart and brain, serum and kidney showed significant differences between ginseng-treated and untreated mice. As compared with untreated mice, mice that received ginseng had significantly lower *Candida albicans* titers in kidney at both 24 h ($p < 0.001$) and 5 days ($p = 0.002$) after inoculation (Table 1). Mice that had not received ginseng had a significant increase in titer between days 1 and 5 ($p = 0.005$), but titers did not rise significantly across days in mice that had received ginseng (Fig. 4).

BUN and LCN2 values were not different in control versus ginseng-treated mice at 24 h or 5 days after inoculation ($n = 6$–8 per group) (Fig. 5). However, ginseng-treated mice showed significantly higher concentrations of BUN at 5 days versus 24 h ($p = 0.001$), and untreated mice showed a tendency toward higher concentrations at 5 days ($p = 0.058$).

At 24 h after inoculation, serum concentrations of MCP-1, MIP-2, and IL-12(p70) were significantly lower in mice that received ginseng as compared with the control group ($p = 0.002$, 0.018 and

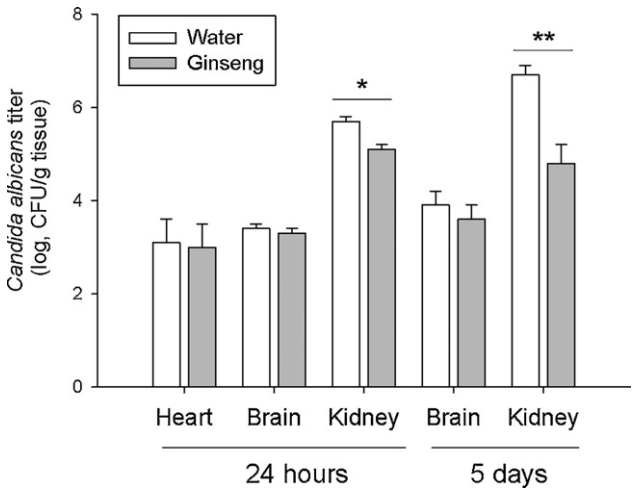

Fig. 4. *Candida albicans* titers in mice at 24 h and 5 days after inoculation DBA/2J mice were maintained on water that contained either no additive (open bars) or a hot-water extract of ginseng (filled bars), beginning two days prior to infection and continuing for the remainder of the study. Mice underwent euthanasia for tissue collection at 24 h or 5 days after infection. At 24 h, $n = 13$ for kidney and $n = 8$ for heart and brain for both ginseng-treated and untreated mice. At 5 days, $n = 8$ for brain and kidney for both ginseng-treated and untreated mice. Data are shown as mean ± S.E.M. *, $p < 0.001$; **, $p = 0.002$. Day 1 vs. day 5 for untreated mice, $p = 0.005$.

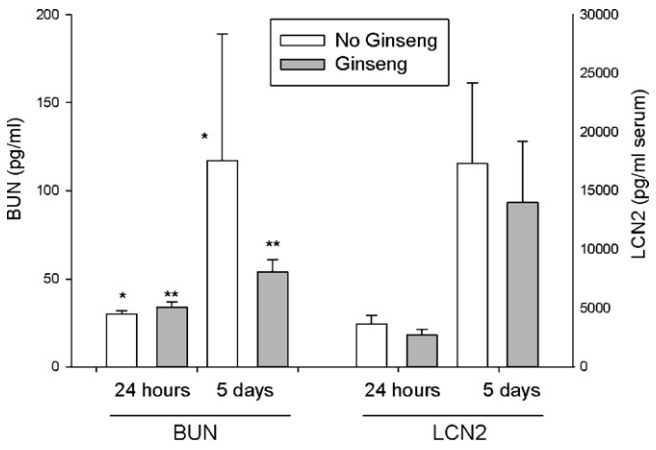

Fig. 5. BUN and LCN2 concentrations in serum at 24 h and 5 days after inoculation with *Candida albicans* DBA/2J mice were maintained on water that contained either no additive (open bars) or a hot-water extract of ginseng (filled bars), beginning two days prior to infection and continuing for the remainder of the study. Mice underwent euthanasia at 24 h or 5 days after inoculation. $n = 13$ for both ginseng-treated and untreated mice. Data are shown as mean ± S.E.M. There were no significant differences between mice that did or did not receive ginseng. Ginseng-treated mice showed significantly higher concentrations of BUN at 5 days versus 24 h (**, $p = 0.001$), and untreated mice showed a tendency toward higher concentrations at 5 days (*, $p = 0.058$).

0.048, respectively), with a trend toward lower serum concentrations of IP-10 ($p = 0.053$) (Table 1). With the exception of IP-10 ($p = 0.083$), serum concentrations of all measured cytokines had fallen on day 5 in comparison with day 1 values within both treatment groups ($p < 0.05$ for all). However, no significant differences were detected between treatment groups on day 5.

At 24 h after inoculation, renal concentrations of MCP-1 and MIP-2 were significantly lower in ginseng-treated mice as compared with the untreated mice ($p = 0.038$ and 0.015, respectively), with a trend toward lower values of IL-6 ($p = 0.053$) (Table 1). In comparison with 24 h values, renal concentrations of G-CSF, IL-6, and KC were significantly lower on day 5 within both treatment

Table 1
Serum and tissue analytes after *Candida albicans* infection in control and ginseng-treated mice at 1 and 5 days after inoculation.

	G-CSF	IL-6	IP-10	KC	MCP-1	MIP-2	IL-12(p70)
Serum (1 day)							
Control	>12,500[a]	18,954 ± 3093	961 ± 102	16,227 ± 2198	5528 ± 652	910 ± 154	71 ± 10
Ginseng	>12,500[a]	16,830 ± 2565	710 ± 68	12,878 ± 2001	2618 ± 510	335 ± 38	44 ± 9
C:G			$p = 0.053$		$p = 0.002$	$p = 0.018$	$p = 0.048$
Serum (5 days)							
Control	3068 ± 1659	705 ± 179	653 ± 101	309 ± 76	731 ± 359	121 ± 41	4 ± 0
Ginseng	4025 ± 1932	795 ± 500	728 ± 110	142 ± 30	321 ± 174	183 ± 40	4 ± 0
C:G				$p = 0.081$			
(C1:C5)	$p = 0.007$	$p < 0.001$	$p = 0.083$	$p < 0.001$	$p < 0.001$	$p = 0.005$	$p = 0.001$
(G1:G5)	$p = 0.001$	$p < 0.001$		$p < 0.001$	$p = 0.001$	$p = 0.024$	$p < 0.001$
Kidney (1 day)							
Control	848 ± 63	602 ± 89	196 ± 73	914 ± 73	118 ± 17	396 ± 56	4.3 ± 0.7
Ginseng	705 ± 99	515 ± 113	98 ± 26	739 ± 118	68 ± 15	215 ± 40	3.3 ± 0.4
C:G					$p = 0.038$	$p = 0.015$	
Kidney (5 days)							
Control	176 ± 72	121 ± 21	52 ± 8	91 ± 20	129 ± 46	330 ± 130	2.3 ± 0.6
Ginseng	229 ± 93	88 ± 32	61 ± 11	130 ± 27	79 ± 23	281 ± 110	2.4 ± 0.3
C:G							
(C1:C5)	$p < 0.001$	$p < 0.001$		$p < 0.001$			
(G1:G5)	$p = 0.003$	$p = 0.003$		$p < 0.001$			
Heart (1 day)							
Control	1389 ± 95	741 ± 168	27 ± 3	1195 ± 245	629 ± 143	293 ± 63	0.8 ± 0.1
Ginseng	1144 ± 235	908 ± 248	29 ± 10	1269 ± 255	616 ± 145	569 ± 196	0.6 ± 0.1
Brain (1 day)							
Control	712 ± 128	11 ± 7	15 ± 3	912 ± 158	34 ± 4	97 ± 28	1.4 ± 0.1
Ginseng	864 ± 118	16 ± 5	15 ± 4	842 ± 136	33 ± 6	184 ± 56	2.5 ± 0.7

All values are expressed as mean ± SEM. Serum values are reported as pg/ml and tissue values as pg/mg protein. For serum and kidney on day 1, $n = 13$ per group; for heart and brain, $n = 8$ per group. No significant differences were detected between control and ginseng-treated mice in heart or brain.
Other cytokines measured: Serum, 1 day: IL-4 and IL-17 were measured, but IL-4 was below assay limits of detection (0.4 pg/mL) for all samples, and IL-17 was below limits of detection (0.5 pg/mL) for most samples. Serum, 5 days: IL-1β was measured but was below assay limits of detection (2.0 pg/mL); TNF-α, IFN-γ, IL-10, and MIP-1α were measured, but no significant differences were detected between control and ginseng-treated mice. Kidney, 1 day: IFN-γ, IL-4 and IL-17 were measured but were below the assay limits of detection (0.9 pg/mL, 0.4 pg/mL and 0.9 pg/mL respectively) for all or most samples. Kidney, 5 days: IL-1β, TNF-α, IFN-γ, IL-10, and MIP-1α were measured, but no significant differences were detected between control and ginseng-treated mice.

[a] These values were above the range of the assay and were not diluted for re-analysis.

groups ($p < 0.05$ for all). However, no significant differences were detected between treatment groups on day 5.

4. Discussion

The data presented here indicate that ingestion of a HWEG does not prevent death or illness associated with *Candida albicans* infection in a highly susceptible strain of mice (DBA/2J). However, the spontaneous ingestion of this extract in the drinking water was associated with significantly lower *Candida albicans* titers in kidney and with significantly lower concentrations of some inflammatory cytokines in kidney and/or serum at 24 h after inoculation.

We chose to study an immune-impaired strain of inbred mice because disseminated candidiasis typically develops only in immune-impaired hosts. DBA/2J mice are deficient in the C5 component of complement due to a 2 bp deletion that is present in about 40% of all inbred mouse strains (Cinader et al., 1964; Wetsel et al., 1990) Activation of the alternative complement pathway results in the cleavage of C5 into C5a and C5b. Although C5a is usually considered pro-inflammatory, recent studies of C5a receptor deficient mice suggest that C5a and its receptor may be capable of both promoting and reducing the extent of acute inflammation (Bhatia et al., 2001). For example, C5a receptors mediate heterologous desensitization, an important mechanism for the control of inflammation; thus once an inflammatory response has been initiated, C5a may contribute to controlling the response (Campbell et al., 1997). Consistent with this, *Candida albicans*-infected C5-deficient A/J and DBA/2J mice develop a severe and damaging inflammatory response as compared with complement-replete C57BL/6J mice (Mullick et al., 2004; Tuite et al., 2005).

Both complement-replete mice and complement-deficient DBA/2J show preferential *Candida albicans* colonization of kidney after intravenous challenge, with lower titers in heart and brain (Ashman and Papadimitriou, 1987; Ashman et al., 1999; Mullick et al., 2006). In DBA/2J mice, the absence of this vital facet of innate host defense results in rapid fatality after inoculation with moderate doses of *Candida albicans* (Tuite et al., 2005), and the infection is associated with extremely high renal titers of *Candida albicans* and concentrations of cytokines and chemokines (Mullick et al., 2004; Toth and Hughes, 2006). One group has reported that *Candida albicans* inoculation of complement-deficient A/J mice and the consomic strain BcA17, which harbors the A/J chromosome 17 on a C57BL/6J genetic background, results in severe infection of heart and a damaging inflammatory response, culminating in cardiomyopathy and ultimately to death due to cardiac failure (Mullick et al., 2006). However, another group (Ashman et al., 1996, 2003) does not report cardiac infection to be a major outcome of infection of DBA/2J mice, as was the case in our study.

Our data indicate that ingestion of ginseng before and during *Candida albicans* infection is associated with significantly lower fungal titers in kidney at both 24 h and 5 days after infection. Consistent with their lower *Candida albicans* titers in kidney, ginseng-treated mice also had lower concentrations of the inflammatory chemokines MCP-1 and MIP-2 in kidney and serum at 24 h after inoculation, as compared with non-treated mice. Induction of MCP-1 and MIP-2 in kidney also occurs in immune-competent mice inoculated with *Candida albicans*, with greater induction associated with more virulent *Candida albicans* strains (MacCallum, 2009). The reduced titers and cytokine concentrations that we observed in ginseng-treated mice indicate modification of the early inflammatory response to *Candida albicans* infection. However, this effect was not sufficient to influence the course of the disease.

In studies of ginseng and other herbal preparations, the composition of the formulation can influence the experimental outcomes. For example, we recently showed that polysaccharide-rich extracts, whole root extracts, and isolated ginsenosides from American ginseng produce different effects on a macrophage cell line (King et al., in press). Such differences in formulation may explain the lack of *in vitro* anti-*Candida albicans* efficacy for our extract, in contrast to reports that used other formulations (Sung and Lee, 2008; Tournas et al., 2011). *In vivo* studies of efficacy are further complicated by factors that include route of administration, the pharmacokinetic and pharmacologic properties of specific components of the formulation, the inherent susceptibility or resistance of the host, and the virulence and dose of the challenge organism. For example, BALB/c mice show differences in the magnitude of their cytokine response by 24 h after inoculation after intravenous inoculation with either virulent or attenuated strains of *Candida albicans* (MacCallum, 2009). A crucial caveat to the interpretation of data collected after administration of ginseng to animals by injection is that powdered and extracted ginseng can contain potent bacterial lipoproteins and lipopolysaccharides (Pugh et al., 2008). Treating such extracts with lipoprotein lipase and polymyxin B, which eliminate these bacterial components, can eradicate their macrophage-activating effects, indicating that lipoproteins and lipopolysaccharides derived from bacterial endophytes may cause these effects (Pugh et al., 2008). This problem is mitigated by the use of ingestion or oral administration of ginseng, which is the normal route of ginseng self-medication in people, because many polysaccharides undergo digestion, rather than absorption, and are therefore biologically inactive. In contrast, the saponin ginsenosides are less subject to digestion, undergo greater absorption, and are thus likely to represent the major active component of ingested ginseng.

Three reports have suggested a beneficial impact of ginseng on the response to systemic *Candida albicans* infection in mice (Akagawa et al., 1996; Abe et al., 1998; Lee and Han, 2006). In the most relevant of these, the ginsenoside Rg1 did not inhibit the growth of *Candida albicans in vitro*, yet intraperitoneal administration of Rg1 before intravenous challenge with *Candida albicans* reduced mortality and fungal titers in kidney in mice (Lee and Han, 2006). In studies of *Staphylococcus aureus*, a ginseng polysaccharide did not directly inhibit the organism in a minimum inhibitory concentration test, but *in vivo* tests in mice revealed increased survival, fewer bacteria in the blood, and macrophage activation (Ahn et al., 2006; Lim et al., 2002). In mice infected with *Pseudomonas aeruginosa*, the subcutaneous daily administration of a saline extract of ginseng reduced mortality and lung pathology, promoted bacterial clearance, and enhanced the release of IFN-γ and TNF-α from pneumocytes and splenocytes (Song et al., 2003). A subsequent study found that ginseng did not inhibit bacterial growth, yet down-regulated the synthesis of signaling molecules related to microbial virulence (Song et al., 2010). In a study of fatigue, which may be related to inflammatory mediators in some cases (Ray et al., 2008), the ginsenoside 20(R)-Rg3, administered intranasally to mice, significantly prolonged the weight-loaded swimming time, increased hepatic glycogen levels, and reduced blood lactic acid and BUN (Tang et al., 2008). In addition, a pilot trial of 290 adults with cancer, randomized in a double-blind manner to receive placebo or one of three doses of American ginseng twice a day for 8 weeks, found a trend toward reduced fatigue with the higher doses, with over twice as many patients perceiving benefit from ginseng as compared with placebo (Barton et al., 2009).

Our experimental approach, which combines spontaneous ingestion of ginseng by mice with well-known genetic susceptibility to infection and evaluation of illness, markers of inflammation, and mortality, illustrates a comprehensive strategy for characterizations of this type. Most previous studies of Candida infections in mice use resistant strains of mice, often in comparison with sensitive strains, and a relatively virulent strain of Candida. In our study we used a highly sensitive strain of mice and a low-virulence

strain of *Candida albicans*. The low virulence of the *Candida albicans* strain we used likely accounts for the low mortality that occurred in our study in comparison with others. Indeed, the conditions used in our study generated a more prolonged infection, rather than acute mortality, and therefore more realistically mimics the situation in human cases of disseminated candidiasis. Our findings of reduced renal *Candida albicans* titers and inflammation in immune-impaired hosts that spontaneously ingest ginseng, taken together with other published reports of ginseng efficacy against *Candida albicans* infections, support further investigation of the immune-modulatory properties of ginseng and its components with regard to mitigation of the severity of *Candida albicans* infection and inflammation.

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