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Standardized ginger (*Zingiber officinale*) extract reduces bacterial load and suppresses acute and chronic inflammation in Mongolian gerbils infected with *cagA*$^+$*Helicobacter pylori*

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Abstract

Previous investigations demonstrated that a standardized extract of ginger rhizome inhibited the growth of *Helicobacter pylori in vitro* with a minimum inhibitory concentration in the range 0.78 to 12.5 μg/mL. In the present work, the extract was tested in a rodent model of *H. pylori*-induced disease, the Mongolian gerbil, to examine the effects of the extract on both prevention and eradication of infection. The extract was administered to Mongolian gerbils at a daily dose of 100 mg/kg body weight in rations either 3 weeks prior to infection or 6 weeks post-infection. Treatment with the standardized ginger extract reduced *H. pylori* load as compared with controls and significantly ($P<0.05$) reduced both acute and chronic muscosal and submucosal inflammation, cryptitis, as well as epithelial cell degeneration and erosion induced by *H. pylori*. Importantly, the extract did not increase morbidity or mortality. Further investigations of the mechanism demonstrated that the ginger extract inhibited the activity of cyclooxygenase-2, with 50% inhibitory concentration (IC_{50}) of 8.5 μg/mL *in vitro*, inhibited the nuclear factor-κB transcriptional response in kBZ Jurkat cells (human T lymphocytes) with an IC_{50} of 24.6 μg/mL, and significantly inhibited the release of interleukin (IL)-1β, IL-6, IL-8, and tumor necrosis factor-α from lipopolysaccharide-stimulated human peripheral blood mononuclear cells with IC_{50} values of 3.89, 7.7, 8.5, and 8.37 μg/mL, respectively. These results suggest ginger extracts may be useful for development as agents to reduce *H. pylori*-induced inflammation and as for gastric cancer chemoprevention.

Keywords

Antibacterial; chemoprevention; gastric cancer; ginger; gingerol; Helicobacter pylori; Mongolian gerbil; peptic ulcer disease; Zingiber officinale

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Introduction

In 1994, *Helicobacter pylori* was the first bacterium to be classified as a Group 1 carcinogen and a definite cause of gastric cancer in man by the International Agency for Research on Cancer (IARC, 1994). Since that time, *H. pylori* has been epidemiologically linked to adenocarcinoma of the distal stomach, as well as colorectal adenomas (Breuer-Katschinski et al., 1999; Scheiman & Culter, 1999; Figueiredo et al., 2002; Forman & Graham, 2004). *cagA* is the strain-specific *H. pylori* gene that has been linked to the development of pre-malignant and malignant histological lesions (Shmuely et al., 2001). Thus, susceptibility of *cagA*$^+$ *H. pylori* strains is of note because, as compared with *cagA*$^-$ strains, infections caused by *cagA*$^+$ strains significantly increase the risk for developing severe gastric inflammation, atrophic gastritis, and noncardia gastric adenocarcinoma (Shmuely et al., 2001). Although gastric cancer has been investigated for centuries, the association with *H. pylori* infections has been recognized for only the past few decades (Censini et al., 1996; Shmuely et al., 2001). Although the disease has declined in most industrialized countries, it remains the second most common cause of cancer death worldwide and is, in theory, largely preventable (Censini et al., 1996). Since gastric cancer is a malignancy with a high morbidity and mortality worldwide, there has been an increased interest in the development of alternative therapies that inhibit or reduce the growth of *H. pylori* and its carcinogenic processes.

Previously, we have reported that extracts of ginger (*Zingiber officinale* Roscoe, Zingiberaceae) rhizomes (root) inhibited the growth of 19 strains of *H. pylori in vitro* with a minimum inhibitory concentration range 0.78 to 12.5 μg/mL, with significant activity against the *cagA*$^+$ strains (Mahady et al., 2003). These data suggested that specific ginger extracts containing the gingerols 6–10 may be of use for the treatment or prevention of *H. pylori cagA*$^+$ strains *in vivo*. In the present work, we have assessed the effect of a standardized ginger extract on the prevention and treatment of *H. pylori*-induced infection and inflammation in Mongolian gerbils. In addition, further investigations were performed to elucidate the *in vitro* mechanism of action of the ginger extract.

Materials and methods

Plant materials and extract preparation

Dried ginger rhizomes (*Z. officinale*) were obtained from Frontier Natural Products (Norway, IA, USA). The plant materials were identified by macroscopic and microscopic analysis and by analytical HPLC. The dried and milled whole rhizomes (5 kg) were extracted as previously described (Mahady et al., 2003). The extracts were continuously fractionated over a silica gel column using a gradient solvent of chloroform (100–0%)–methanol (0–100%) until an active fraction containing 20±5% gingerols was obtained (Mahady et al., 2003). Analytical HPLC was performed on the active fraction and the gingerols (6-, 8-, 10-) and 6-shogoal (Figure 1) were present in a ratio of 7.5:1:13:2 (%), respectively. All four compounds had *in vitro* activity against *H. pylori* (Mahady et al., 2003). No one compound could explain the i*n vitro* activity of active fraction; thus the active extract was standardized to contain 23.9% gingerols (6-, 8-, 10-) and 6-shogoal, with an enrichment for 10-gingerol.

H. pylori *assay*

The *cagA*$^+$ *H. pylori* strain B128 was maintained as previously described (Israel et al., 2001). Gram stain appearance and a positive urease test confirmed the identification of the organism. The bacterium was stored frozen at −70°C in skimmed milk plus 17% glycerol. For susceptibility testing, the organisms were inoculated on to 5% sheep blood agar plates,

and incubated at 37°C in a 10% CO_2 atmosphere for 72 h as described (Mahady et al., 2003). The organisms were inoculated on to agar plates containing consecutive dilutions of the plant extracts via a 32-prong inoculating device. The device delivers 8 μL per spot, resulting in a final inoculum of approximately 1×10^6 cfu/spot. After the spots dried, the plates were incubated at 37°C in 10% CO_2 and examined for growth after 3 days. All procedures were performed in duplicate. The minimum inhibitory concentration (MIC), defined as the lowest concentration of the compound at which there was no visible growth or only a faint haze, was determined for each plant extract and pure compound. Assessment of bactericidal activity was performed as previously described (NCCLS, 2008).

Effect of ginger on H. pylori *infection in Mongolian gerbils*

Mongolian gerbils 4–8 weeks of age were purchased from either Harlan Sprague Dawley Inc. (Indianapolis, IN, USA) or Charles River Laboratories (Wilmington, MA, USA). All procedures used in this investigation were approved by the Institutional Animal Care Committees of Vanderbilt University and the University of Illinois at Chicago. Gerbil-passed *cagA*$^+$ *vacA* strains B128 were used. Gerbils were inoculated with sterile broth or *H. pylori* by gastric lavage, as described previously (Israel et al., 2001). After treatment, the gerbils were sacrificed; one half of the glandular stomach was fixed in 10% neutral buffered formalin for histological examination, and the other half was homogenized in sterile phosphate-buffered saline (PBS) (pH 7.4), plated on selective Trypticase soy agar plates containing vancomycin (20 μg/mL), nalidixic acid (10 μg/mL), bacitracin (30 μg/mL), and amphotericin B (2 μg/mL), and grown for 4–5 days, as described previously (Mahady et al., 2003). Colonies were identified as *H. pylori* based on their characteristic morphology, and by urease and oxidase activities; colony counts were expressed as log cfu per stomach.

For prevention studies, specific pathogen-free, 6-week-old male gerbils were fed either a standard diet chow (Purina 5100, Nestlé Puina PetCase Company, St. Louis, MO) or the same chow supplemented with the botanical extract (providing 100 mg extract/kg body weight) for 3 weeks prior to challenge with *H. pyori*. Gerbils (n=14) were then inoculated with the rodent-adapted *H. pylori cagA*$^+$ strain B128 (5×10^9 cfu). The animals were maintained on control or supplemented chow until sacrifice 6 weeks after inoculation. *H. pylori* strain B128 for each challenge was grown from freezer stocks for 24–36 h, harvested in Brucella broth (BBL, Becton, Dickenson and Company, Sparks, MA), and administered by gastric lavage to the animals immediately after harvest, as described previously (Israel et al., 2001). For the prevention study, at 6 weeks after the inoculation, all animals in the prevention study were sacrificed and their stomachs were resected along the greater curvature. For the treatment studies, gerbils (n=16) were inoculated with rodent-adapted *H. pylori cagA*$^+$ strain B128 (5×10^9 cfu) and fed normal chow for 6 weeks; then one group (n=8) was fed chow supplemented with ginger extract (dose 100 mg/kg body weight per day) for 4 weeks and then returned to normal chow for a further 4 weeks. The control group (n=8) was maintained on normal chow. After 14 weeks, the animals were then sacrificed and assessed using the protocols described above. An outline of the prevention and treatment studies is given in Figure 2.

One half of the resected stomachs was fixed in 10% neutral buffered formalin for histological examination, and the other half was transferred to 1.0 mL of sterile 0.1 M PBS (pH 7.4), homogenized, plated on to selective Trypticase soy agar/5% sheep blood plates containing vancomycin (20 μg/mL), nalidixic acid (10 μg/mL), bacitracin (30 μg/mL), and amphotericin B (2 μg/mL) (Sigma Chemical Company, St. Louis, MO, USA), and grown for 3–5 days, as previously described (Israel et al., 2001). Colonies were identified as *H. pylori* based on their resistance to the antibiotics listed above, characteristic morphology, and by urease and oxidase activity, as previously described (Israel et al., 2001). The other half was washed with saline and then macroscopic gastric lesions (edema and hemorrhage) were

recorded, followed by measurement of the wet weight of the whole stomach, including fore-stomach and glandular stomach. Half of the glandular mucosa was scraped for detection of colonizing *H. pylori*, and the residual part was formalin-fixed and embedded in paraffin for histological observation.

Histological examination and serum antibodies to H. pylori

Hematoxylin and eosin sections were examined by a single experienced pathologist, Dr K. Tham, Vanderbilt University, Nashville, TN, USA. For semi-quantitative estimates, the following parameters were graded from 0 to 3: acute and chronic inflammation, epithelial cell degeneration, and erosions (Table 1). Colonization density was determined by quantitative culture and acute inflammation and chronic inflammation (mononuclear cell infiltration independent from lymphoid follicles) were each graded from 0 to 3 in the gastric antrum.

*Effect on cytokine release, COX-2, and NF-*κβ *transcriptional response* in vitro

Cytokine release—Modulation of cytokine release was performed using the protocol of Welker et al. (1996). Briefly, peripheral blood mononuclear cells (MDS Pharma Services, Bothell, WA, USA) were treated with lipopolysaccharide (LPS) (25 ng/mL; Sigma Chemical Company) to stimulate the release of interleukin (IL)-1β and IL-6, and released cytokines in the cell supernatant were measured by ELISA. Cells (10^6/mL) with LPS were cultured for 16 h at 37°C in RPMI medium (Gibco, North Andoves, MA) without serum, alone, or with 0, 6.25, 12.5, 25, 50, or 100 μg ginger extract/mL. Positive control was dexamethasone. All experiments were performed in triplicate.

Assay for tumor necrosis factor (TNF)-α release from human peripheral blood mononuclear cells (5×10^5 cells; MDS Pharma Services) was performed in cells pre-incubated at 37°C for 1 h in 0.5 mL of medium containing 0, 6.25, 12.5, 25, 50, or 100 μg extract/mL. After three washes with PBS, the cells were further incubated at 37°C for 6 h in 0.5 mL of EMEM (Eagle's Minimum Essential Medium) containing 10% fetal bovine serum in the presence of LPS (25 ng/mL) and the corresponding concentration of each drug (Welker et al., 1996). After 6 h incubation, TNF-α level in the conditioned medium was determined using a TNF-α ELISA kit (BioSource, Camarillo, CA) according to the manufacturer's instructions.

COX-2 assay—Inhibition assays were performed by assessing human recombinant cyclooxygenase-2 (COX-2) catalytic activity as described previously by measuring prostaglandin E_2 (PGE_2) production (Warner et al., 1999). Reaction mixtures were prepared in 100 mM Tris–HCl buffer, pH 8.0, containing 1 μM heme, 500 μM phenol, 300 μM epinephrine, sufficient amounts of COX-2 to generate 150 ng PGE_2/mL, and various concentrations of test samples. A standard curve for PGE_2 (Cayman Chemical, Ann Arbor, MI, USA) was generated from the same plate, which was used to quantify the PGE_2 levels produced in the presence of test samples. Results were expressed as a percentage relative to a control (solvent-treated samples). All determinations were performed in duplicate, and values generally agreed within 10%. Concentration–response curves were generated for the calculation of 50% inhibitory concentration (IC_{50}) values.

Inhibition of NF-κB transcriptional response—For the detection of nuclear factor (NF)-κB, a non-radioactive method, NF-κB p50 Transcription Factor Assay (Chemicon International, Temecula, CA), was employed according to the instructions of the manufacturer. The ginger extract in concentration of 0, 6.25, 12.5, 25, 50, or 100 μg/mL or vehicle was incubated with the Jurkat T cells (1.5×10^6/mL) in the presence of A23187 (0.5 mM) and PMA (phorbol 12-myristate 13-acetate) (50 ng/mL) in RPMI-1640, pH 7.4, at 37°C for 4 h. The cells were harvested and the isolated nuclear extract was added to the

capture probe in solution. The capture probe was a double-stranded biotinylated oligonucleotide containing the consensus sequence for NF-κB binding (5′-GGGACTTTCC-3′). The active form of NF-κB bound to its consensus sequence. After incubation, the extract/probe/buffer mixture was transferred to the streptavidin-coated 96-well plate. The biotinylated capture probe, which was bound by active protein, NF-κB was immobilized and any inactive, unbound material was washed away. The bound NF-κB transcription factor subunit was detected with a specific primary antibody for the NF-κB p50 subunit. The horseradish peroxidase-conjugated secondary antibody binds to the specific primary antibody. A chromogenic substrate was added and the relative amount of DNA bound was determined by measuring the absorbance of the samples. Several controls were used including an NF-κB specific competitor control, a negative control probe, and a positive control (TNF-α-treated HeLa whole cell extract). The NF-κB competitor control was an unlabeled oligonucleotide containing the identical consensus sequence as the NF-κB capture probe. The negative control probe is a non-specific oligonucleotide, which provides a background control for nonspecific DNA binding.

Statistical analysis

The Mann–Whitney *U* test was used to compare scores between samples, whereas scores for inflammation and proliferation within the same animals were compared by linear regression analysis. Proliferation and inflammation data are presented as the mean, and significance was defined as $P \leq 0.05$.

Results

Prevention of H. pylori *infection in Mongolian gerbils*

Ten of the 14 *H. pylori*-challenged gerbils were successfully colonized, indicating that these animals can be reproducibly infected with *H. pylori* strain B128. Of the gerbils that were successfully colonized, there were no differences in colonization density. The gerbils pretreated with the ginger extract for 3 weeks prior to infection showed a reduced *H. pylori* load. In addition, the parameters of acute and chronic inflammation induced by *H. pylori* were significantly decreased in treated animals as compared with the control group (Table 2). Both chronic (mean: 2.2 vs. 1.0; control vs. extract, respectively) and acute mucosal (mean: 2.0 vs. 0.6; control vs. extract, respectively, $P<0.05$) inflammation scores, as well as acute and chronic submucosal inflammatory parameters (mean: 1.3 vs. 0 and 1.6 vs. 0.25; control vs. extract respectively, $P<0.01$), were decreased in gerbils treated with extracts. These changes were paralleled by significant reductions in the severity of epithelial cell degeneration (1.8 control vs. 0.25 extract, $P<0.01$), cryptitis (0.8 control vs. 0.25 extract, $P<0.05$), and erosions (1.8 control vs. 0 extract, $P<0.01$). In general, acute submucosal inflammation and erosion were completely suppressed in animals maintained on a chow containing the standardized ginger extract. Importantly, this extract did not increase morbidity or mortality of the animals.

Treatment of H. pylori *infection in Mongolian gerbils*

The data from gerbils treated with the ginger extract for 4 weeks starting at 6 weeks post-infection are presented in Table 3. No significant reduction in bacterial load or suppression of any inflammatory parameters was observed. Chronic (mean: 2.25 vs. 2.66; control vs. ginger extract, respectively) and acute mucosal (mean: 3.0 vs. 2.66; control vs. extract, respectively) inflammation scores remained relatively unchanged. In addition, no reduction in the severity of epithelial cell degeneration, cryptitis, or erosions was observed in any of the treated animals.

In vitro, the ginger extract inhibited the activity of COX-2, with an IC_{50} of 8.5 μg/mL; inhibited the NF-κB transcriptional response in kBZ Jurkat cells (human T lymphocytes) with an IC_{50} of 24.6 μg/mL; and significantly inhibited the release of IL-1β, IL-6, IL-8, and TNF-α from LPS-stimulated human peripheral blood mononuclear cells with IC_{50} values of 3.89, 7.7, 8.5, and 8.37 μg/mL ($P<0.05$), respectively.

Discussion

For thousands of years, ginger has been used in traditional medicine to treat a wide range of disorders, including dyspepsia, peptic ulcer, motion sickness, and inflammatory diseases (Tjendraputra et al., 2001; Mahady et al., 2003). More recent investigations have demonstrated that ginger extracts and the gingerols have potent chemopreventive activities as well (Surh, 2002; Park & Pezzuto, 2002). For example, 6-gingerol inhibits tumor promotion in mouse skin; inhibits neoplastic transformation and activation of AP-1 in mouse epidermal JB6 cells treated with epidermal growth factor; suppresses the proliferation of human cancer cells through the induction of apoptosis; and abrogates pulmonary metastasis in mice implanted with B16F10 melanoma cells (Huang et al., 1996; Katiyar et al., 1996; Lee & Surh, 1998; Lee et al., 1998; Bode et al., 2001). Furthermore, dietary administration of gingerol to rodents ameliorated azoxymethane-induced intestinal tumorigenesis (Yoshimi et al., 1992).

In the present work, we have demonstrated that the administration of a standardized ginger extract (100 mg/kg body weight) three weeks prior to *H. pylori* challenge reduced bacterial load in Mongolian gerbils. Pretreatment also significantly reduced the acute and chronic inflammation, epithelial cell degeneration, erosion, and cryptitis induced by *H. pylori* infection. The Mongolian gerbil model is an exceptional model to investigate management of *H. pylori* infections for a number of reasons. First, Mongolian gerbils rarely have gastritis. Gastritis occurs in only 2% of animals, unless they are infected with *H. pylori*. Second, the CFU of *H. pylori* needed to colonize Mongolian gerbils is much less than in mice, but the histological changes found are typical and similar to changes that occur in *H. pylori*-infected humans, including gastric atrophy, ulcers, intestinal metaplasia, and adenocarcinomas. Third, the average lifespan of Mongolian gerbils is longer than that of mice, and so they are suitable for long-term studies (Yao et al., 2002). Furthermore, the histopathological and histochemical alterations found in the inflamed mucosa of experimentally infected Mongolian gerbils closely resemble those found in the *H. pylori*-infected human stomach. The infection in Mongolian gerbil induces chronic active gastritis, in which a marked mucosal infiltration of neutrophils on a background of chronic inflammation are detectable at 4 weeks after inoculation and may continue up to 52 weeks (Ikeno et al., 1999). Chronic inflammation is known to accelerate the development of neoplasms in the gastrointestinal tract, and *H. pylori* interacts with host cells to induce pro-inflammatory cytokines and free radicals. Free radicals cause mutations in target cells so that neoplastic clones are established. Accumulation of such genetic alterations may induce malignant transformation of some cell lines (Tsuji et al., 2003). In addition, inflammatory alterations may promote the growth, expansion, and invasion of gastrointestinal epithelial cells. The latter changes caused by inflammation may occur even without further genetic mutations or epigenetic alterations, and therefore may be categorized as ‘perigenetic alterations’ of neoplastic cells (Tsuji et al., 2003). Thus, reduction of bacterial load and prevention of *H. pylori*-induced chronic inflammatory processes in Mongolian gerbils pretreated with ginger may be one mechanism by which ginger acts as a chemopreventive agent.

The mechanism by which ginger reduces inflammation in response to bacterial infection is not well understood. However, cytokines are known to play an important role in *H. pylori*-

associated gastrointestinal diseases, and infection induces a characteristic local inflammatory response in the gastric mucosa causing acute gastritis, which later develops into chronic gastritis (Wallace, 1991). Both forms of gastritis are characterized by a considerable neutrophil infiltration, which contributes to the induction of gastritis by releasing pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, as well as free radicals (Kozol et al., 1991; Wallace, 1991; Suzuki et al. 1993). *H. pylori* and its products can recruit and activate neutrophils by directly stimulating these inflammatory cells or by stimulating the release of epithelial chemokines (Ernst et al., 1997). Other *H. pylori* components such as LPS and proteins can attract and activate neutrophils and other inflammatory cells, thereby stimulating the production of TNF-α, IL-1, IL-6, and IL-8 (Bliss et al., 1998; Bhattacharyya et al., 2000; Fujiwara et al., 2001). A significant increase in COX-2 expression and PGE_2 synthesis has been observed in rats and Mongolian gerbils treated with *H. pylori* (Takahashi et al., 2000; Sakai et al., 2003). The *cox-2* gene was upregulated during the acute phase of inflammation (1–3 months after infection) in Mongolian gerbils (Sakai et al., 2003), thus suggesting that pretreatment or treatment of the animals with COX-2 inhibitors at the time of infection may reduce COX-2 expression and inflammation induced by *H. pylori*. Our *in vitro* data indicate that ginger extracts inhibit the activity of COX-2, the NF-κB transcriptional response, and the release of IL-1β, IL-6, and IL-8. Thus, ginger may suppress *H. pylori*-induced acute and chronic inflammation through the inhibition of a number of components of this pro-inflammatory signaling pathway. This mechanism needs further elucidation *in vivo*.

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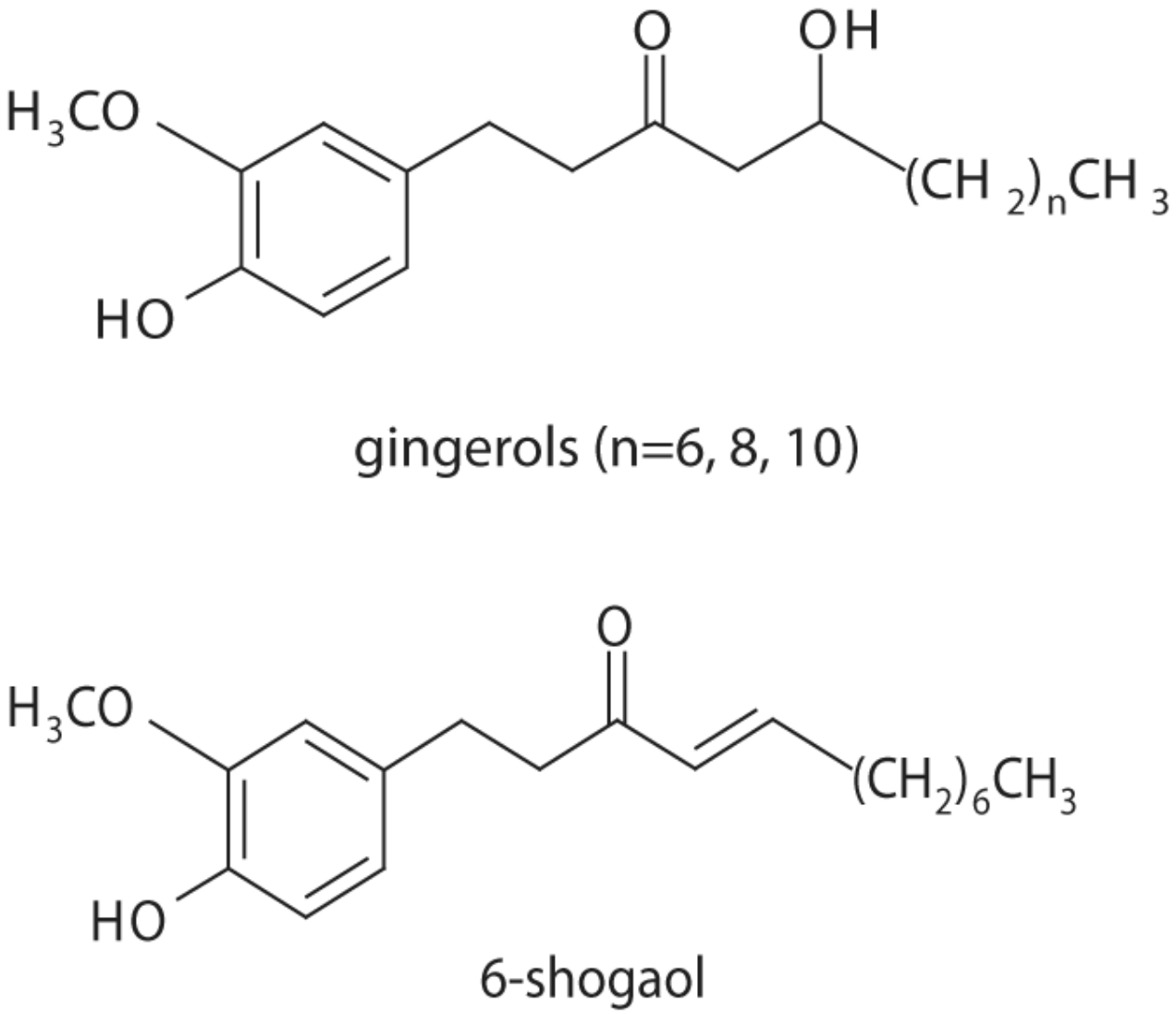

Figure 1.
Structures of the gingerols and shogoal from ginger rhizome extract.

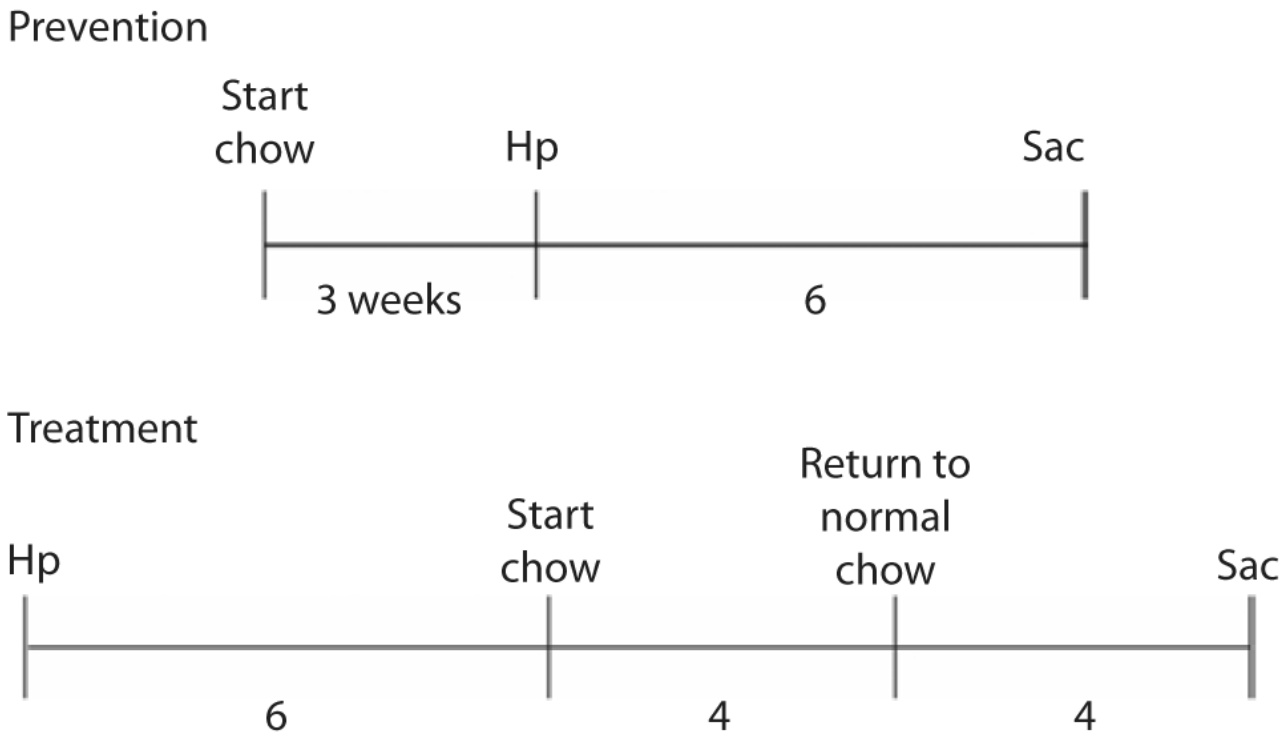

Figure 2.
Outline of prevention and treatment studies in Mongolian gerbils infected with *Helicobacter pylori* (Hp) strain B128. In the prevention study, treatment chow was started 3 weeks prior to infection and lasted a total of 9 weeks prior to sacrifice (Sac). In the treatment study, treatment was started 6 weeks post-infection and lasted for 4 weeks, with a return to normal chow for 4 weeks following treatment, prior to sacrifice.

Table 1

Grading criteria for chronic inflammation and activity of gastritis.

Grade	Chronic inflammation	Activity
0	no increase in number of inflammatory cells	same
1	uniform infiltration of lamina propria by lymphocytes, plasma cells, and some eosinophils	scattered neutrophils in the lamina propria with no leukopedesis in the region of the gastric pits
2	moderately dense infiltration of the lamina propria by lymphocytes and plasma cells	moderate number of neutrophils in the lamina propria with microabscess in the region of gastric pits
3	very dense lymphoplasma cell infiltration in the lamina propria	extensive neutrophils in the lamina propria with obvious cryptitis

Table 2

Bacterial load and inflammation induced by *Helicobacter pyrlori* infection in Mongolian gerbils fed a normal diet or a diet supplemented with standardized ginger extract: prevention study.

			CI			AI				
Expt.	**Food**	**Colonies**	**M**	**S**	**Lymph**	**M**	**S**	**C**	**ED**	**E**
1	Control	77.83	2.20	1.60	0.80	2.00	1.30	0.80	1.80	1.80
1	Ginger	56.71	1.00*	0.25**	0.25	0.60*	0.00**	0.25*	0.25**	0.00**

Seven animals per arm; gerbils were fed a normal diet throughout (Purina 5001 chow; control) or a normal diet supplemented with 100 mg ginger extract /kg body weight in daily rations for 3 weeks prior to inoculation and 6 weeks after (9 weeks total); inflammation was graded using the criteria of Table 1. Colonies = bacterial load in log cfu per stomach; CI = chronic inflammation; Lymph = Lymphocyte infiltration; AI = acute inflammation; M = mucosal; S = submucosal; C = cryptitis; ED = epithelial cell degeneration; E = erosion. Mean value was significantly different from that of the control group:

* $P<0.05$

** $P<0.01$.

Table 3

Bacterial load and inflammation induced by *Helicobacter pyrlori* infection in Mongolian gerbils fed a normal diet or a diet supplemented with standardized ginger extract: treatment study.

			CI			AI				
Expt.	**Food**	**Colonies**	**M**	**S**	**Lymph**	**M**	**S**	**C**	**ED**	**E**
2	Control	70.40	2.25	3.00	1.50	3.00	2.00	1.00	2.00	2.00
2	Ginger	75.00	2.66	2.50	1.00	2.66	2.16	0.83	1.66	1.83

Eight per arm; gerbils were fed a normal diet throughout (Purina 5001 chow; control) or a normal diet supplemented with 100 mg ginger extract /kg body weight in daily rations for 4 weeks (6 weeks after inoculation) and then returned to normal chow for another 4 weeks; inflammation was graded using the criteria of Table 1. Colonies = bacterial load in log cfu per stomach; CI = chronic inflammation; Lymph = Lymphocyte infiltration; AI = acute inflammation; M = mucosal; S = submucosal; C = cryptitis; ED = epithelial cell degeneration; E = erosion.