The modulatory effects of the volatile oil of ginger on the cellular immune response in vitro and in vivo in mice

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Abstract

The aim of this study was to investigate the immunomodulatory effects of the volatile oil of ginger (Zingiber officinale Roscoe) in vitro and in vivo in mice. In vitro, the volatile oil of ginger (0.001–10 ng/mL) significantly inhibited T lymphocyte proliferation (P<0.01), decreased the number of the total T lymphocytes and T helper cells (P<0.01) in a concentration-dependent manner, but increased the percentage of T suppressor cells to the total T lymphocytes in the mice. In addition, the volatile oil of ginger (0.001–10 ng/mL) inhibited IL-1 secretion by the mice peritoneal macrophages in a concentration-dependent manner. In vivo, oral administration of the volatile oil of ginger in the doses of 0.125, 0.25 and 0.5 g/kg body weight dose-dependently weakened the delayed type of hypersensitivity response to 2,4-dinitro-1-fluorobenzene in the sensitized mice (P<0.05). These results suggest that the volatile oil of ginger influences both cell-mediated immune response and nonspecific proliferation of T lymphocyte, and may exert beneficial effects in a number of clinical conditions, such as chronic inflammation and autoimmune diseases.

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Keywords: Immunomodulation; Volatile oil; Ginger; Lymphocyte; Delayed type of hypersensitivity

1. Introduction

Ginger is the root of Zingiber officinale Roscoe, which is widely used in the world as one of the important spices and traditional herbs. It has been reported that ginger or its extracts present some pharmacological activities including anti-inflammation (Mascolo et al., 1989; Penna et al., 2003), anti-emesis ( Sharma and Gupta, 1998), analgesic effect (Mascolo et al., 1989; Young et al., 2005), anti-tumor ( Katiyar et al., 1996) and anti-oxidation (Masuda et al., 1996). Our previous research also indicated that the volatile oil of ginger possessed anti-inflammatory activity, and some clinical research has suggested ginger or its extracts can be used in arthritis and other chronic pain associated inflammatory diseases. It was reported that the alcohol extract of Z. officinale increased the thymus index, spleen index, percentage of phagocytosis, rate of alpha-ANAE and the titer of IgM of the mice with tumor (Liu and Zhou, 2002). Furthermore, it was indicated that the alcohol extract of Z. officinale also improved the status of immunological function in the tumorous mice of immune inadequacy. Contrastively, it was also reported that ginger inhibited lymphocyte proliferation and suppressed IL-2 and IL-10 production in human lymphocytes (Wilasrusmee et al., 2002a). Moreover, ginger could inhibit both mitogen- and alloantigen-stimulated lymphocyte proliferations in mice (Wilasrusmee et al., 2002b).

The aim of this study, therefore, was to evaluate the effects of the volatile oil of ginger on the immune response in vitro and in vivo in mice and primarily explore the underlying mechanism of its anti-inflammatory activity.

2. Materials and methods

2.1. Animals

ICR inbred mice of either sex, 6–7 weeks old, weighing 22 ± 2 g, were obtained from the Laboratory Animal Center of Zhejiang University, China. The animals were housed in every cage of six mice under environmentally controlled conditions with food and water ad libitum. Before conducting any studies, the animals were acclimatized for 1 week and the use of the animals was in accordance with the institutional guidelines.
were destroyed by 0.85% NH4Cl/20 mM Tris–HCl (pH 7.5) and sieve (200 mesh) to obtain a single-cell suspension. Erythrocytes and the tissue dispersion was passed through a sterilized stainless steel mesh and sedimented to remove aseptically and transferred to a small Petri dish containing HEPES buffer 24 mmol/L (Genetime Technology Inc., Shanghai, China), dimethyl sulfoxide (DMSO, Sigma Chemical Co.), theophylline (Hangzhou Minsheng Pharmaceutical Factory), 25% glutaral solution (Shanghai Chemical Co., China), 2,4-dinitrofluorobenzene (DNFB, Shanghai Chemical Co., China), dexmethasone sodium phosphate (DXM, Suzhou No. 6 Pharmaceutical Factory, China) and streptomycin 100 μg/mL (Shanghai Sijiqing Bioengineering Material Co. Ltd., China). RPMI 1640 (Grand Island, NY, USA) culture medium was supplemented with 10% fetal bovine serum (FBS, Shanghai, China), liposaccharide (LPS, Sigma Chemical Co.) and 1-fluorobenzene (DNFB, Shanghai Chemical Co., China), dexamethasone sodium phosphate (DXM, Suzhou No. 6 Pharmaceutical Factory, China) and pH was adjusted to 7.2.

2.2. The preparation and the main components of the volatile oil of ginger

The volatile oil of ginger was provided by the Modern Medicine Co., Ltd., Shenyang, China. The fragment of dried ginger 2.0 kg was subjected to hydro-distillation for 6 h by using a Clevergner apparatus, which provided a yield of 5% (100 g). It was light yellow in appearance, containing the main components of β-phenylethyl 10.9%, camphene 11.85%, farnesol 12.17%, geranium 2.21%, zingiberene 17.44%, β-sesquiphellandrene 5.01%, neral 4.17%, α-bisabolene 7.96%, α-curcumene 4.34%, α-farnesene 12.68% and α-murolene 5.36%. In addition, there remained a low concentration of terpenes, esters, ketones, etc. One percent Tween-80 solution in distilled water was used to make the volatile oil of ginger as a suspension before it was used. An acute toxicity data indicated LD₅₀ of the volatile oil of ginger was 3.197 (2.907–3.515) g/kg body weight and the maximum non-fatal dose was 2.0 g/kg body weight in mice.

2.3. Drugs and chemicals

The following drugs and chemicals were used: 2,4-dinitrofluorobenzene (DNFB, Shanghai Chemical Co., China), dexmethasone sodium phosphate (DXM, Suzhou No. 6 Pharmaceutical Factory, China), 25% glutaral solution (Shanghai Chemical Co., China), theophylline (Hangzhou Minsheng Pharmaceutical Factory), methylene blue (Shanghai No. 3 Reagent Factory, China), methyl-β-d-glucoside (MTT, Genetime Technology Inc., Shanghai, China), dimethyl sulfoxide (DMSO, Sigma Chemical Co., USA), liposaccharide (LPS, Sigma Chemical Co.) and mouse IL-1α ELISA Kit (Shanghai Muyuan Technology Limited Co., China). RPMI 1640 (Grand Island, NY, USA) culture medium was supplemented with 10% fetal bovine serum (FBS, Hangzhou Sijiqing Biotechnology Material Co. Ltd., China), HEPES buffer 24 mM/L (Genetime Technology Inc., Shanghai, China), benzylpenicilllin 100 IU/mL (Suzhou No. 2 Pharmaceutical Factory, China) and streptomycin 100 μg/mL (Shanghai No. 4 Pharmaceutical Factory, China) and pH was adjusted to 7.2.

2.4. In vitro experiment

2.4.1. Preparation of single-splenocyte suspension

Mice were killed by cervical dislocation, and the spleens were removed aseptically and transferred to a small Petri dish containing 4 mL RPMI 1640 complete medium. The spleen was teased and the tissue dispersion was passed through a sterilized stainless steel sieve (200 mesh) to obtain a single-cell suspension. Erythrocytes were destroyed by 0.85% NH4Cl/20 mM Tris–HCl (pH 7.5) and the remaining cells were washed twice with Dulbecco’s phosphate buffered saline. The cells were re-suspended in RPMI 1640 complete medium at a density of 2.0 × 10⁶ cells/mL. The viability of the cells was performed using the trypan blue exclusion method. Briefly, 25 μL cell solution was mixed with 75 μL 0.4% trypan blue, then the cells were counted under the light microscope at 400× magnifications using an improved Cunming-ham counting chamber. The cellular viability ranged from 90 to 95%.

2.4.2. The effect of the volatile oil of ginger on T lymphocyte proliferation

Lymphocyte proliferation test was performed in triplicates as previously described (Mosmann, 1983), and repeated for three times. Briefly, 4.0 × 10⁵ splenocytes prepared from mice spleen were pipetted into each well of a 96-well microtiter plate, and the mitogen of concanavalin (Con A), a T lymphocyte activator, was added at the final concentration of 5 μg/mL. Vehicle (1% TW-80) or various concentrations of the volatile oil of ginger at the final concentration of 0.001, 0.01, 0.1, 1 and 10 μg/mL, and DXM, as a control drug, at the final concentration of 0.1, 1 and 10 μM, were also added. The final volume of each well was 200 μL. The plates were covered and incubated for 48 h at 37 °C with 5% CO₂ and 95% humidity. Twenty microliters of MTT solution (5 mg/mL in RPM 1640 medium) was added and incubated for another 4 h at the same condition for the dye to be metabolized. Plates were centrifuged (500 × g, 10 min); the supernatant was removed and 100 μL of DMSO was added under agitation. The plates were read on an ELISA reader at 570 nm wavelength with a reference wavelength of 630 nm after keeping at room temperature for 15 min (BIO-TEK INSTRUMENT®, USA).

2.4.3. The effect of the volatile oil of ginger on T lymphocyte subpopulation differentiation

T lymphocyte subpopulation differentiation was determined by theophylline inhibition test according to the method of Shore et al. (1978) with slight modification. Fifty microliters spleen lymphocytes of 2.0 × 10⁵ cells/mL were dispensed into eppendorf tubes containing various concentrations of the volatile oil of ginger and DXM in triplicates as previously described. After incubation for 1 h at 37 °C, 100 μL 1% sheep red blood cells were added into each tube and incubated for another 15 min at 37 °C, centrifuged at 800 × g for 5 min and incubated for another 2 h at 37 °C, then fixed by 4% glutaral solution. The sedimentation was suspended in 0.05% methylene blue and the erythrocyte-rosette forming cells were counted. The above procedures were repeated by using culture medium containing theophylline (100 μmol/L) instead. The numbers of the total T lymphocytes and T lymphocyte helper (Th) cells were obtained, respectively, and the percentage of Th cells and that of T lymphocyte suppressor (Ts) cells were calculated. All the experiments were performed three times.

2.4.4. Mouse peritoneal macrophages preparation and IL-1α detection

Macrophages were collected from the abdominal cavity of the normal mice. The cells were washed and centrifuged three times with phosphate-buffered saline (PBS) at 400 × g for 10 min and then resuspended to 2 × 10⁶ cells/mL in RPMI 1640 complete medium (Strassmann et al., 1997). The cell viability was over 90%. Macrophages were dispensed into the 24-well flat-bottomed plates and incubated in a humidified incubator (5% CO₂, 95% humidity) for 2 h. The dishes were washed three times
with the culture medium to remove non-adherent cells, and the monolayer was added with LPS (5 μg/mL), and the various concentrations of the volatile oil of ginger or DXM in triplicates, and incubated under the similar conditions for 48 h. The plates centrifuged at 2000 × g for 10 min, and IL-1α in the supernatant was detected by the mouse IL-1α ELISA Kit as recommended by the manufacturer. All the experiments were performed three times.

2.5. Delayed type of hyper sensitivity (DTH) induced by DNFB in mice and the change of the organ index of thymus and spleen

Twelve female ICR mice were used in each group. Mice were sensitized with 0.5% DNFB solution (20 μL) in absolute acetone–olive oil (4:1) onto the shaved abdomen at the beginning of the experiment. Five days after the initial sensitization, animals were challenged with 10 μL, 0.2% DNFB on both sides of the left ears (Corsini et al., 1979), the right ear was treated with vehicle acetone–olive oil. The vehicle (10 mL/kg body weight), the volatile oil of ginger (0.001, 0.01, 0.1, 1, 10 and 100 ng/mL) and DXM (0.005 g/kg) were orally administered daily for 5 days before DNFB challenge and the following day before the mice were sacrificed. Ear swelling was expressed as the difference between the weights of the patches of the both ears made by a specific 8-mm punch 24 h after the challenge. In addition, thymus and spleen, the two immune function related organs, were dissected out and the weight of the organs was recorded.

2.6. Statistical analysis

Statistical evaluations were made using Tukey multiple comparisons after ANOVA, and a value of P < 0.05 was considered statistically significant. IC_{25} and IC_{50} (95% confidence limits, CL) values were calculated by weighted probit analysis of Bliss method.

3. Results

3.1. The effect of the volatile oil of ginger on T lymphocyte proliferation

The volatile oil of ginger 0.001, 0.01, 0.1, 1 and 10 ng/mL, inhibited the Con A induced proliferation of T lymphocyte prepared from mice spleen in vitro in a dose-dependent manner (Table 1). Compared with the cells cultured with vehicle, the inhibition rates were 13.42, 27.10, 52.35, 64.61 and 67.78%, respectively, and the IC_{50} (95% CL) was 0.390 (0.181–0.855) ng/mL. The inhibition potency of the volatile oil of ginger on T lymphocyte proliferation was close to that of DXM.

3.2. The effect of the volatile oil of ginger on T lymphocyte subpopulation differentiation

The volatile oil of ginger 0.001, 0.01, 0.1, 1, 10 and 100 ng/mL decreased the numbers of total T lymphocytes and the Th/Ts subpopulation differentiation in vitro. The lymphocytes were prepared from the mice spleen. Data were expressed as the percentage of the T lymphocytes and the Th/Ts subpopulation differentiation, compared with vehicle, *P < 0.05, **P < 0.01, ***P < 0.001, n = 9.

Th cells of the mice in vitro in a dose-dependent manner (Fig. 1). IC_{25} (95% CL) of the volatile oil of ginger on the total T lymphocytes was 0.015 (0.005–0.048) ng/mL, IC_{50} (95% CL) of the volatile oil of ginger on Th cells was 0.100 (0.036–0.277) ng/mL. The potentiation potency of the volatile oil of ginger on Ts cells was also dose-dependent although it was less than the inhibition potency on Th cells. The IC_{25} (95% CL) to increase Ts cells was 1.85 (1.14–3.00) ng/mL.

3.3. The effects of the volatile oil of ginger on IL-1α secretion by macrophages

The volatile oil of ginger 0.001, 0.01, 0.1, 1 and 10 ng/mL inhibited IL-1α secretion by the mice peritoneal macrophages

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (ng/mL)</th>
<th>T lymphocyte proliferation (A570)</th>
<th>IL-1α (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1.133 ± 0.052</td>
<td>622.7 ± 141.7</td>
<td></td>
</tr>
<tr>
<td>The volatile oil of ginger</td>
<td>0.001 0.01 0.1 1 10</td>
<td>0.01 0.026 ± 0.111*** 0.826 ± 0.111*** 0.1 0.515 ± 0.107*** 0.401 ± 0.077*** 10 0.365 ± 0.050***</td>
<td>0.018*** 366.4 ± 32.0**</td>
</tr>
<tr>
<td>DXM (μM)</td>
<td>0.1 0.5 1 10</td>
<td>0.1 37.1 ± 0.040*** 0.371 ± 0.040***</td>
<td>0.018*** 366.4 ± 32.0**</td>
</tr>
</tbody>
</table>

The lymphocytes were prepared from the mice spleen. T lymphocytes were activated by Con A, and its proliferation was measured by MTT assay and indicated as (A570). IL-1α secreted from the mice peritoneal macrophage monolayer was induced by LPS and detected in the culture medium by ELISA. Data were expressed as mean ± S.D., compared with vehicle, *P < 0.05, **P < 0.01, ***P < 0.001.
induced by LPS in vitro in a dose-dependent manner (Table 1) and the IC25 (95% CL) was 0.023 (0.015–0.035) ng/mL. The volatile oil of ginger 0.125 g 4.16 ± 0.08” (99%) 1.88 ± 0.37” (20%) 10.5 ± 3.5” (31.8%) 10.0 ± 3.2” (34.4%) 30.8% 2.2” (34.4%) 0.47 ± 0.26 (82%) 10.4 ± 1.4” (32.2%)

The inhibitory effects of the volatile oil of ginger on the organ index of spleen and thymus, and DTH induced by DNFB in mice

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (per kg body weight)</th>
<th>Spleen index (g/kg body weight) (inhibition rate)</th>
<th>Thymus index (g/kg body weight) (inhibition rate)</th>
<th>Δ Ear edema (mg) (Inhibition rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10 mL</td>
<td>4.80 ± 0.09</td>
<td>2.31 ± 0.81</td>
<td>15.4 ± 4.2</td>
</tr>
<tr>
<td>The volatile oil of ginger</td>
<td>0.125 g</td>
<td>4.16 ± 0.88” (99%)</td>
<td>1.88 ± 0.37” (20%)</td>
<td>10.5 ± 3.5” (31.8%)</td>
</tr>
<tr>
<td></td>
<td>0.25 g</td>
<td>4.02 ± 0.5” (23.3%)</td>
<td>1.70 ± 0.66” (28.0%)</td>
<td>10.0 ± 3.2” (34.4%)</td>
</tr>
<tr>
<td></td>
<td>0.5 g</td>
<td>3.97 ± 0.61” (28.7%)</td>
<td>1.37 ± 0.56” (45.8%)</td>
<td>10.0 ± 1.7” (35.0%)</td>
</tr>
<tr>
<td>DXM</td>
<td>0.005 g</td>
<td>2.15 ± 0.44” (61.7%)</td>
<td>0.47 ± 0.26” (82%)</td>
<td>10.4 ± 1.4” (32.2%)</td>
</tr>
</tbody>
</table>

The mice were orally administered vehicle, various doses of the volatile oil of ginger and DXM daily for 6 days before sacrifice. Δ Ear edema, the difference between the weights of the patches of the both ears made by a specific 8-mm punch 24h after the DNFB challenge (the left ear challenged by DNFB and the right ear challenged by vehicle). Data were expressed as mean ± S.D., compared with the vehicle treated group, P< 0.05.

4. Discussion

The anti-inflammatory effects of ginger and its extracts have been emphasized in many reports and proposed to be one of its health-care mechanisms (e.g. in arthritis), and some clinical research has proved that the administration of ginger can result in the decreased symptoms of rheumatoid arthritis (Altman and Marcusen, 2001; Wigler et al., 2003). Our previous research also suggested that the volatile oil of ginger showed anti-inflammatory and analgesic effect. The development and pathogenesis of both chronic inflammation and autoimmune diseases are deeply correlated with immunological function status; therefore, the immunomodulatory effects of the volatile oil of ginger on the cellular immunoresponse were explored in this study.

We selected the animal model of DTH reactions in mice that were initially sensitized to DNFB to assess the possible immunosuppressive effect of the volatile oil of ginger because ear swelling is primarily the result of in vivo functions of antigen-specific CD4+ T-cell response (Maloney et al., 1990). In addition, cell proliferation was involved in the activation of T lymphocytes through multiple intracellular signaling pathways (Pimentel-Muntn et al., 1994). In this study, the volatile oil of ginger, orally administered before the challenge phase, clearly inhibited DNFB-induced ear swelling in the mice, and decreased the organ index of the most important immune organs, thymus and spleen. Besides, the volatile oil of ginger produced a significant suppression of the mitogen-stimulated T lymphocyte proliferation in mice. Similar with our results, other researchers also found that ginger inhibited both mitogen- and alloantigen-stimulated lymphocyte proliferations in mice, although the authors did not describe the preparation process of ginger (Wilasrusmee et al., 2002b). The results in present study have indicated that the volatile oil of ginger is capable of inhibiting T lymphocyte-dependent immune reactions. Except for inhibiting T lymphocytes proliferation and Th1/Th2 subpopulation differentiation in mice, our long-term toxicity research also suggested that the volatile oil of ginger 0.3, 0.6 and 1.2 g/kg body weight orally administered for 180 days significantly decreased the thymus and spleen index in dogs, but 0.5, 1.0 and 2.0 g/kg body weight orally administered for 180 days have no significant effect on these parameters in rats. The results of pathological histology indicated that the number of T lymphocytes in thymus was reduced and the adrenal gland cortex was mildly degenerated in the both animals. After 30 days drug withdrawal, these changes recovered to the normal level (unpublished data). These results suggest the immunosuppressant effect of the volatile oil of ginger show species differences.

IL-1, as well as tumor necrosis factor α (TNFs), is a key active pro-inflammatory cytokine in rheumatoid arthritis, and it is mostly produced by macrophages and monocytes. Biological agents that specifically inhibit the effects of TNFα or IL-1 represent a major advancement in the treatment of rheumatoid arthritis (Subramanian and Handa, 2004). IL-1 could induce IL-2 secretion from the activated T lymphocyte and IL-2 gene expression, and increase the sensitivity of natural killer (NK) cells to IL-2. Consistent with Wilasrusmee et al. (2002a), that ginger inhibited IL-2 production from the mixed cultured lymphocytes, the present study also demonstrated that the volatile oil of ginger inhibited IL-1α secretion from the mice peritoneal macrophages. It is postulated that the decreased IL-2 production is at least partly due to the inhibition of IL-1α secretion by macrophages. Since macrophages may contribute
to its anti-inflammatory activity. As an anti-inflammatory Th2 cytokine, IL-10 can inhibit Th1 cell proliferation and IL-2 production. Paradoxically, Wilasrusmee et al. (2002a) also found that ginger inhibited IL-10 production, so the complex effects of ginger on Th1/Th2 cytokine balance are still requiring further investigation.

In conclusion, we have demonstrated here that the volatile oil of ginger has capability to modulate the function of lymphocyte and cellular immune response. These results suggest that the volatile oil of ginger influences both cell-mediated immune response and nonspecific proliferation of T lymphocyte, and may exert beneficial effects in a number of clinical conditions such as chronic inflammation and autoimmune diseases.

References


