Enhancement of Intestinal IgA Production by Ajoene in Mice

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Received May 22, 2013; Accepted August 16, 2013; Online Publication, November 7, 2013
[doi:10.1271/bbb.130408]

We investigated the effects of ajoene on intestinal IgA production. Ajoene (1.35, 4.5, and 13.5 μg/kg/d) was administered to mice for 4 weeks. The fecal IgA level in the 13.5 μg/kg group increased after 3 weeks. The intestinal IgA level also increased in a dose-dependent manner upon ajoene administration. An oil-macerated garlic extract, with 1500 μg/g of ajoene, enhanced the intestinal IgA production.

Key words: ajoene; garlic; oil-macerate; IgA; mucosal-immunity

Garlic (Allium sativum L.) has been used worldwide as a medicinal plant since ancient times; it has well-known immunostimulatory properties and other beneficial effects against cardiovascular diseases, cancer, and infection. Oil-maceration is a method for processing garlic, and its products are widely used as health foods in Europe. An oil-macerated garlic extract (OMGE) contains such sulfur compounds as ajoene (Z,E)-4,5,9-trithiadiodeca-1,6,11-triene-9-oxide, Z,E-devinylajoene, vinyldithiin, and some thiosulfimates. Ajoene was shown by Block et al. in 1984 to be an inhibitor of platelet aggregation, and its various biological activities have been well studied. Ajoene has exhibited anti-biotic, hepatoprotective, anti-tumor, and anti-diabetic effects.

Moreover, ajoene has inhibited mitogen-activated lymphocyte proliferation in vitro by altering the membrane-dependent functions in immune cells. However, it is not clear whether ajoene affects the intestinal immune system in vivo. We investigated in this study the effects on intestinal IgA production of administering ajoene to mice.

Z-Ajoene, at a purity level of >98%, was used in the subsequent experiments; being respectively prepared and its purity determined by using the methods described by Block et al. and Lawson et al. OMGE containing 1500 μg/g of ajoene was prepared according to the method reported by Hibl with some modifications. HPLC performed for the analysis included the use of a pump (LC-10AS), UV-VIS detector (SPD-10AV), and column oven (CTO-10A) manufactured by Shimadzu Co. (Kyoto, Japan). The column used was a SUPELCOSIL LC-Si unit (25 cm × 4.6 mm; Sigma-Aldrich Japan, Tokyo, Japan). A mixture of hexane and 2-propanol (95:5 [v/v], special grade; Wako Pure Chemical Industries, Osaka, Japan) was used as the mobile phase. Measurements were performed with a flow rate of 2.0 mL/min, a wave-length of 240 nm, and a column temperature of 33 °C.

Six-week-old male ICR mice were obtained from Japan SLC Co. (Shizuoka, Japan). The mice weighed 28–32 g, and were individually housed in plastic cages in a room kept at 23–25 °C under a 12 h light-dark cycle. The mice were provided with water and fed a standard CE-2 laboratory diet (Clea Japan, Tokyo, Japan) during the experiments. The standard laboratory diet consisted of 52.7% carbohydrate, 23.6% protein, 4.4% fat, 4.9% fiber, and 6.6% minerals and vitamins. After acclimatization for 7 d, the mice were assigned to eight groups. Four groups were administered with ajoene (0 [control], 1.35, 4.5, or 13.5 μg/kg/d) for 4 weeks. Ajoene samples were prepared by diluting Z-ajoene with a 0.5% carboxymethylcellulose solution (CMC; Wako Pure Chemical Industries). The other four groups were administered with OMGE (0 [control], 0.9, 3, or 9 mg/kg/d) for 4 weeks. OMGE samples were diluted with a Panacet 810 medium-chain fatty acid triglyceride (MCT; Japan Oil and Fat Co., Tokyo, Japan), and then suspended in a 0.5% CMC solution. All samples were orally administered at a dosage of 100 μL/d.

To evaluate the fecal IgA level, feces excreted within 24 h were collected, freeze-dried through a preliminary freezing at −30 °C for at least 12 h, and stored at −80 °C.

At the end of the experimental period, the mice were sacrificed by exsanguination from the heart under diethyl ether-induced anesthesia. Colon tissue was excised, washed with ice-cold saline, and stored at −80 °C. The experiment was performed according to institutional guidelines of the Committee on Animal Research at Nagoya Seiraku.

The total IgA concentration in the feces or colon tissue was measured by using an ELISA quantitation kit (Bethyl Laboratories, TX, USA). The fecal pellet (100 mg) or the colon tissue (100 mg) was put into a 1.5 mL microcentrifuge tube, and 1 mL (10 volumes, w/v) of phosphate-buffered saline (PBS) was added. Each sample mixture was homogenized for 30 s with a Polytron PT2100 device (Kinematica, CHE) at 20,000 rpm and 4 °C. The mixed samples were centrifuged at 8,000 × g for 10 min at 4 °C, and the IgA level in the supernatant was then analyzed. Results are presented as the mean ± standard error of the mean (SEM). Significant differences for the fecal IgA levels,
were subjected to a by two-way analysis of variance (ANOVA) with the Tukey-Kramer multiple-comparison test. Significant differences for the body weight, food intake, and fecal output were analyzed by two-way ANOVA with the Steel-Dwass multiple-comparison test, and statistical significance for the colonic IgA levels and colon tissue weight was analyzed by one-way ANOVA with Dunnett’s multiple-comparison test. Values not sharing the same letter are significantly different ($p < 0.05$).

We examined the effects of ajoene on intestinal IgA production (Fig. 1A). The fecal IgA levels after 4 weeks in the all ajoene-administered groups were higher than those in the control group. In particular, the fecal IgA levels in the 13.5 $\mu$g/kg group showed a marked increase after 3 weeks and reached 3.2 $mg/d$ after 4 weeks. Similarly, in the OMGE-administered groups, the fecal IgA levels were significantly increased by the administration of at least 3 $mg/kg$ of OMGE for 3 weeks. In the 9 $mg/kg$ group, the fecal IgA levels were significantly increased after 2 weeks, and reached 2.8 $mg/d$ after 4 weeks (Fig. 1B). Moreover, ajoene administration caused a dose-dependent increase in the intestinal IgA level. OMGE administration also enhanced the intestinal IgA levels (Fig. 2). Two geometrical isomers of ajoene are present in OMGE. We found that these isomers had identical effects (data not shown).

The body weight, food intake, colon tissue weight, and fecal output during each period did not differ among the four ajoene-administered groups or the four OMGE-administered groups (Fig. 3).

The ajoene concentration in OMGE used in this study was 1500 $\mu$g/g; 9 $mg$ of OMGE contained 13.5 $\mu$g of ajoene. The extent of the increase in intestinal IgA production in the 13.5 $\mu$g/kg ajoene group was equal to that observed in the 9 $mg/kg$ OMGE group; we therefore speculate that ajoene present in OMGE was responsible for these effects. In addition to ajoene, OMGE primarily contained devinylajoene, vinyldithin, and some thiosulfinates.3–5) Our results suggest that, other than ajoene, no component of OMGE influenced the increase in intestinal IgA production, and that no additive or synergistic effects were apparent between ajoene and the other components of OMGE.

Lawson et al. have reported that 11 commercial OMGE samples contained 20–1060 $\mu$g/g of ajoene.3) According to them, most products contained <120 $\mu$g/g of ajoene. We consider the ajoene component of OMGE to have been the chief agent influencing the increased
production of intestinal IgA, since the ajoene concentration of OMGE in this study was 1500 µg/g. We therefore deduced that most commercial products would not have the observed effects.

Some studies have shown the immunostimulatory mechanism of garlic or garlic-derived compounds. Iciek et al. have reported that garlic-derived sulfur compounds enhanced the immune function by modulating the cellular redox state involving the S-thiolation reaction.14) Such thiols as cysteine residues must be free of disulfide bonds for the efficient stimulation of T-cells.15) These reports have suggested that sulfur compounds in OMGE might be involved in T-cell stimulation. It is generally known that activated T-cells stimulate B-cell IgA secretion. However, in this present study, we did not observe the effect of any OMGE component apart from ajoene on the IgA level; we therefore consider that OMGE may not have contained sufficient thiols for stimulating T-cells or B-cells.

Lactic acid bacterial,16,17) polysaccharides,18–20) and polyphenols21) are well-known immunostimulants for inducing intestinal IgA production. B-cell stimulation occurs due to the uptake of immunostimulants into Peyer’s patches.22,23) Moreover, interleukin–4, 5, 6, and 10 have enhanced the intestinal IgA production in B-cells.24) The intestinal IgA level was increased by ajoene, and therefore, ajoene may have influenced the stimulation of B-cells or interleukin secretion.

Romano et al. have reported that ajoene may inhibit lymphocyte proliferation by interfering with protein kinase C (PKC)-mediated phosphorylation by using the results of an in vitro assay.11) In contrast, the results of our study suggest that ajoene acted as an activator of the lymphocyte function. It is therefore necessary to investigate the mechanism for the enhanced IgA production by ajoene in future studies.

References


13) Hibi T, Japan patent 2608252 (Feb. 13, 1997).


