Effect of Garlic Oil on Hepatic Arachidonic Acid Content and Immune Response in Rats

Cheng-Tzu Liu,[†] Haw-Wen Chen,[†] Lee-Yan Sheen,[‡] Yun-Lan Kung,[†] Paul Chih-Hsueh Chen,[§] and Chong-Kuei Lii*,[†]

Departments of Nutrition and Pathology, Chung Shan Medical College, No. 110, Sec. 1, Chien Kuo N. Rd., Taichung, Taiwan, Republic of China, and Department of Nutrition, China Medical College, No. 91, Hsueh-Shih Road, Taichung, Taiwan, Republic of China

This study examined the arachidonic acid metabolism and immune response in rats administered orally (p.o.) with either garlic oil (GO), diallyl disulfide (DADS) (200 mg/kg of body weight), or corn oil (control) three times a week for 7 weeks. Both GO and DADS were found to modify the hepatic membrane fatty acid composition: the linoleic acid was increased, and the arachidonic acid was decreased ($P < 0.05$). GO but not DADS suppressed the Δ6 desaturase activity ($P < 0.05$). Neither treatment affected the phospholipase A_2 activity or plasma prostaglandin E_2 level. GO increased the spleen/body weight ratio ($P < 0.05$) and enhanced concanavalin A-stimulated splenocyte proliferation. However, the systemic contact hypersensitivity response as detected by the extent of ear swelling was suppressed by 74% in the GO-treated rats ($P < 0.05$). The findings indicate that GO inhibits Δ6 desaturase activity and changes membrane arachidonic acid content, both of which show immunomodulatory potential.

Keywords: *Garlic oil; diallyl disulfide; arachidonic acid metabolism; Δ6 desaturase; immune response*

INTRODUCTION

Garlic and garlic constituents prepared by different means have been shown to have diverse biological activities, including antitumorigenesis, anticarcinogenesis, antiatherosclerosis, antithrombosis, antidiabetic, antiinflammatory, fibrinolytic, and various other biological actions [see reviews by Agarwal (1996) and Augusti (1996)]. The mechanism of these actions has been ascribed to the potent enzyme inhibiting activities (Sendle et al., 1992; Yang et al., 1994; Yeh and Yeh, 1994) or antioxidant activities (Perchellet et al., 1986; Phelps and Harris, 1993) of garlic. Although these biological activities have been widely recognized, results are not always consistent. For example, Simons et al. (1995) failed to find a hypolipidemic effect of garlic powder in patients with mild hypercholsterolemia. Evidence is, thus, presently not conclusive as to the preventive activity of garlic, and further research into the possible actions of garlic or its components is necessary.

Arachidonic acid acts as the precursor of eicosanoids, which are involved in many regulatory processes, and modification of arachidonic acid metabolism has been proposed to be one of the consequences of garlic action (Mustafa and Srivastava, 1990). For example, the antiplatelet aggregation property of garlic has been ascribed to its inhibition of thrombaxane A_2 synthesis by inhibiting cyclooxygenase activity (Ali, 1995; Sendle et al., 1992). Garlic components have also been shown to inhibit the activity of lipoxygenase, which committed the synthesis of leukotrienes and hydroxyeicosatetraenoic acid (Belman et al., 1989; Sendle et al., 1992). Inhibition of eicosanoid synthesis affects the eicosanoid-dependent regulatory processes, including those on the immune response and on the antitumor immunity (Belman et al., 1989; Shalinsky et al., 1989). The stimulation of macrophage and lymphocyte by garlic compounds has been reported (Lau et al., 1991), as has been the activation of T-lymphocyte immunity, which appears to suppress the growth of murine bladder tumor (Marsh et al., 1987).

Therefore, it is of interest to examine the effect of garlic components on arachidonic acid metabolism and immunological responsiveness. In this study, we investigated the effect of garlic oil and one of its major organosulfur compounds, diallyl disulfide (DADS), on hepatic membrane arachidonic acid metabolism in rats. We also studied the effects of garlic oil on concanvanalin A (Con A)-induced splenocyte proliferation, on in vivo 1-chloro-2,4-dinitrobenzene (CDNB)-induced contact hypersensitivity, and on croton oil-induced irritant dermatitis.

MATERIALS AND METHODS

Materials. DADS was purchased from Aldrich Chemical Co. (Milwaukee, WI). Garlic oil was prepared as previously described (Sheen et al., 1992). Briefly, garlic oil were prepared according to a steam distillation technique, and the final product contains major garlic essential oil components including 38.6% DADS, 30.8% diallyl trisulfide (DATS), and minor amounts of many other volatile compositions. No detectable amount of fatty acid is present in this garlic oil product. [1-^{14}C]-Linoleic acid, L-α-1-palmitoyl-2-arachidonylphosphatidylcholine (PAPC), and the prostaglandin E_2 (PGE_2) assay kit were purchased from NEN (Boston, MA). CDNB, croton oil, fatty acid methyl ester standards, and other biochemical reagents

* Author to whom correspondence should be addressed (telephone 886-4-389-6190, ext. 11319; fax 886-4-389-0964; e-mail Hawwen@mercury.csmc.edu.tw).

[†] Department of Nutrition, Chung Shan Medical College.

[‡] Department of Pathology, Chung Shan Medical College.

[§] Department of Nutrition, China Medical College.

10.1021/jf980466p CCC: $15.00
Published on Web 10/15/1998

were purchased from Sigma Chemical (St. Louis, MO). Precoated silica gel aluminum thin layer chromatographic plates were purchased from Merck (Darmstadt, Germany).

Animals and Treatments. Four week-old weanling Sprague Dawley rats were purchased from the National Animal Breeding and Research Center (Taipei, Taiwan). Rats were randomly assigned to each experimental group by weight and housed in stainless wire cages on a 12-h light−dark cycle. They had free access to water and a modified AIN-76 diet. The diet contained (grams per kilogram) casein, 200; corn oil, 150; sucrose, 500; cellulose, 50; AIN-76 vitamin mix, 10; AIN-76 mineral mix, 35; methionine, 3; choline bitartrate, 2; and cholesterol, 50. All diet ingredients were supplied by Harlen Teklad (Madison, WI).

Animals received 200 mg/kg of body weight garlic oil or DADS (corn oil as a vehicle) by oral intubation three times a week. Rats treated with corn oil alone (2 mL/kg of body weight) were regarded as the control. Body weight was measured weekly. After 7 weeks of treatment, rats were fasted overnight and sacrificed by carbon dioxide euthanasia. Blood samples were drawn from the dorsal vein with sodium citrate (5 mg/mL) as an anticoagulant. Following collection, blood samples were centrifuged at 500*g* for 10 min, and plasma was removed and stored at −80 °C. The livers and spleens were removed and weighed immediately. The livers were quickly freeze-clamped in liquid nitrogen and stored at −80 °C until analysis. The spleens were used immediately for splenocyte isolation. Animals were treated in compliance with the Guide for the Care and Use of Laboratory Animals (NRC 1985).

Hepatic Phospholipid Fatty Acid Composition. Liver lipids were extracted according to the method of Folch et al. (1957), and total phospholipids were then isolated by thin-layer chromatography with hexane/diethyl ether/formic acid (80:20:2, v/v/v). After visualizing by spraying with 2′,7′-dichlorofluorescein (0.1% w/v in methanol) and being marked under UV light (366 nm), spots were scraped off and collected into glass tubes for fatty acid analysis. Fatty acid analysis was performed as described by Lepage and Roy (1986) using a Supelco (Bellefonte, PA) fused silica column with an internal diameter of 0.25 mm. The integration of the peak area of each individial fatty acid was determined, the percentage of which was calculated on the basis of the sum of peak area of all detectable fatty acids.

Δ6 Desaturase Activity Assay. Liver Δ6 desaturase activity was measured using the method of Garg et al. (1988) with some modifications. Liver was homogenized in 2.5× volume (w/v) of a 250 mM sucrose buffer containing 50 mM Na_2HPO_4 and 2 mM glutathione (pH 7.4). The homogenates were then centrifuged at 12000*g* for 15 min. One milliliter of supernatant (5 mg of protein/mL), 200 nmol of [1-^{14}C]linoleic acid (0.5 μCi/μmol), and 2 mL of reaction medium containing 50 mM Na_2HPO_4, 7.5 mM ATP, 3.8 mM $MgCl_2$, 0.2 mM NADPH, 0.5 mM NADH, and 0.2 mM coenzyme A (pH 7.4) were mixed and incubated at 37 °C for 20 min. The reaction was stopped by adding 2 mL of 10% KOH in methanol. After saponification at 85 °C for 2 h, 1 mL of 8 M HCl was added, and fatty acids were extracted with hexane. Methylation was carried out at 100 °C for 1 h with 1 mL of 14% boron trifluoride/methyl alcohol. Methylated fatty acids were separated on $AgNO_3$ thin-layer chromatography plates (silica gel impregnated with 10% $AgNO_3$ in acetonitrile for 15 min) with hexane/diethyl ether (17:3, v/v) for 2 h. The linoleic acid and γ-linolenic acid spots were made visible under UV light (366 nm) by spraying with 2′,7′-dichlorofluorescein (0.1% w/v in methanol) and were confirmed by comparison with the standards of these two fatty acid methyl esters. Spots were scraped off and counted for radioactivity by using a liquid scintillation counter (Packard, model Tri-Carb 2100TR). Enzyme activity was expressed as picomoles of γ-linolenic acid formed from linoleic acid per minute per milligram of protein. Protein concentration was measured according to the method of Lowry et al. (1951).

Phospholipase A_2 (PLA_2) Activity Assay. Cytosolic PLA_2 activity was measured as described by Rao et al. (1995). Briefly, liver was homogenized with 50 mM Tris-HCl buffer (pH 7.4) containing 250 mM sucrose, 1 mM EGTA, 1 mM EDTA, 20 μM leupeptin, 5 mg/L trypsin inhibitor, and 0.1 mM phenylmethanesulfonyl fluoride. After a two-step centrifugation, the resultant cytosolic fraction was used for PLA_2 activity immediately. The activity assay was performed in a total volume of 100 μL of reaction mixture containing 100 mM Tris-HCl (pH 8.5), 15 mM $CaCl_2$, and 100 μg of cytosolic protein. The reaction was initiated by adding 4 nmol of PAPC (10 μCi/μmol), and the reaction mixture was incubated at 37 °C for 30 min. After extraction with chloroform, the lipids in the chloroform fraction were separated on silica gel thin-layer chromatographic plates with chloroform/methanol/acetic acid/water (90:12:2:1, v/v/v/v). The spot was confirmed by comparison with the standard of arachidonic acid (Sigma) and was scraped off directly into scintillation vials and counted for radioactivity by using a liquid scintillation counter (Packard, model Tri-Carb 2100TR). Enzyme activity was expressed as picomoles of [^{14}C]arachidonic acid released per minute per milligram of protein.

Measurement of Plasma PGE_2. Plasma PGE_2 concentration was determined by radioimmunoassay according to the manufacturer's instructions (New England Nuclear Co., Boston, MA).

Splenocyte Proliferation. Splenocyte proliferation analysis was conducted on spleens from the garlic oil and control groups as previously described (Szondy and Newsholme, 1991). Briefly, mononuclear splenocytes were released from the spleens of CO_2-sacrificed rats by gentle teasing in phosphate-buffered saline (pH 7.2). The cells were then separated by Histopaque 1077 density gradient centrifugation. These cells were washed twice with phosphate-buffered saline and were suspended in Hepes-buffered RPMI 1640 (Gibco Lab., Grand Island, NY). The viability of cells, as determined by trypan blue dye exclusion, was >98%.

Splenocytes were cultured in wells of microtiter culture plates (5×10^5 cells in 100 μL of medium) in Hepes-buffered RPMI medium containing 10% (v/v) autologous serum, 100 units/mL streptomycin, and 200 units/mL penicillin. The cultures were incubated at 37 °C in an atmosphere of 5% CO_2. [^{3}H]Thymidine (0.2 μCi) was added to each 200 μL well 48 h after stimulation with different concentrations of Con A (0−500 μg/mL), and the cells were incubated for an additional 18 h. Cultures were harvested onto glass fiber filters with a semiautomated cell harvester (Skatron, model 11019, Sterling, VA). The filters were washed and dried, and the radioactivity on the dried filters was measured in a liquid scintillation counter. The proliferation response was expressed as the incorporation of [^{3}H]thymidine over 18 h.

Contact Hypersensitivity. Contact hypersensitivity and irritant dermatitis tests were also performed in some of the animals in the garlic oil and control groups. A method modified from that of Claman (1976) and Levis et al. (1975) was used to induce contact hypersensitivity in rats. Briefly, rats were sensitized twice at 24-h intervals by painting CDNB solution (0.5% in acetone/olive oil, 4:1, v/v) on the shaved abdomen. Ninety-six hours after the second CDNB treatment, one ear of each rat was challenged by applying 0.3% CDNB. The degree of ear swelling was measured immediately following sacrifice by weighing both the excised ear and the untreated ear at 24 h after challenge. Contact hypersensitivity was measured as the weight difference of the hapten-challenged ear from the untreated ear in sensitized animals.

Irritant Dermatitis. To elicit irritant dermatitis, 15 μL of croton oil in acetone was applied on one ear of each rat (Clementi et al., 1994). The degree of ear swelling was measured 6 h after irritant application, as described above. Irritant reaction was determined as the weight difference of the irritant-treated ear compared with the other untreated ear.

Statsitical Analysis. Data were analyzed by using analysis of variance (SAS Institute, Cary, NC), and Duncan's test was used to test the difference between groups. $P < 0.05$ was taken to be statistically significant.

Table 1. Effect of Garlic Oil and DADS on Body Weight, Liver Weight, and Spleen Weight[a]

group	initial wt (g)	final wt (g)	liver wt (g)	liver wt/ body wt (%)	spleen wt (g)	spleen wt/ body wt (%)
control	92 ± 6.0	495 ± 46[a]	23.7 ± 3.2	4.8 ± 0.3	1.1 ± 0.06[b]	0.22 ± 0.02[b]
garlic oil	92 ± 4.8	398 ± 41[b]	20.3 ± 2.9	5.2 ± 0.3	1.6 ± 0.16[a]	0.41 ± 0.02[a]
DADS	90 ± 6.1	394 ± 52[b]	19.3 ± 4.2	4.9 ± 0.4	0.9 ± 0.02[c]	0.22 ± 0.02[b]

[a] Control group received corn oil. Values are means ± SD of six rats in each group. Groups not sharing the same letter (a−c) are significantly different from one another ($P < 0.05$).

Table 2. Effects of Garlic Oil and DADS on the Fatty Acid Composition of Hepatic Phospholipids[a]

	%		
fatty acid	control	garlic oil	DADS
16:0	14.5 ± 1.0	14.5 ± 0.8	14.8 ± 1.4
16:1	0.7 ± 0.2[a]	0.4 ± 0.1[b]	0.5 ± 0.1[b]
18:0	21.4 ± 1.6	19.6 ± 1.3	20.4 ± 0.6
18:1	3.2 ± 1.4[b]	4.9 ± 0.3[a]	5.5 ± 0.6[a]
18:2	13.9 ± 2.7[b]	19.7 ± 1.9[a]	18.4 ± 1.3[a]
20:2	1.9 ± 0.5[b]	3.1 ± 0.3[a]	1.8 ± 0.9[b]
20:3	1.6 ± 0.6	1.8 ± 0.3	1.3 ± 0.2
20:4	36.9 ± 2.3[a]	31.5 ± 1.6[b]	32.7 ± 1.3[b]
22:0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
24:0	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
22:6	4.7 ± 0.9[a]	3.2 ± 0.5[b]	3.7 ± 0.3[b]

[a] Values are means ± SD of six rats in each group. Groups not sharing the same letter (a, b) are significantly different ($P < 0.05$).

RESULTS

Animal Characteristics. After 7 weeks of oral administration, the final body weight in rats treated with garlic oil and DADS was significantly lower than that of the control group ($P < 0.05$) (Table 1). Liver weight and liver weight as a percentage of body weight were not affected by either garlic oil or DADS treatment. Spleen weight in the garlic oil group, however, was significantly higher than in the other two groups ($P < 0.05$). When the spleen weight was expressed as a percentage of body weight, rats treated with garlic oil had an 86% higher spleen weight/body weight ratio than the control and DADS groups. The spleen weight in DADS-treated rats was lower than in the control group, but the spleen weight-to-body weight ratios were similar in the two groups.

Hepatic Phospholipid Fatty Acid Composition. The fatty acid profile of hepatic membrane phospholipids, especially the linoleic acid (18:2) and arachidonic acid (20:4), was modified with garlic compound treatment (Table 2). Garlic oil significantly increased linoleic acid level with an accompanying decrease in arachidonic acid level ($P < 0.05$). Similar results for linoleic acid and arachidonic acid were also observed in rats treated with DADS, but the change was less dramatic. Garlic oil also enriched 18:1 and 20:2 but lowered 16:1 and 22:6 fatty acids in liver phospholipids as compared with corn oil treatment. DADS-treated rats also had higher 18:1 and lower 22:6 and 16:1 fatty acid values ($P < 0.05$) than the control group.

Δ6 Desaturase and PLA_2 Activity and Plasma PGE_2 Level. To study the mechansim of the effect of garlic oil on membrane phospholipid linoleic acid and arachidonic acid composition, the activities of Δ6 desaturase, which participates in linoleic acid desaturation, and PLA_2, which cleaves arachidonic acid from phospholipids, were measured (Table 3). Δ6 Desaturase activity was significantly inhibited in the garlic oil group as compared with the control and DADS groups ($P < 0.05$); the enzyme activity in rats with DADS treatment did not differ from that in control. Hepatic cytosolic PLA_2 activity and plasma PGE_2 level were not affected by garlic oil or DADS.

Table 3. Hepatic Δ6 Desaturase and PLA_2 Activities and Plasma PGE_2 Level in Rats Fed Garlic Oil and DADS[a]

group	Δ6 desaturase [pmol min^{-1} (mg of protein)$^{-1}$]	PLA_2 [pmol min^{-1} (mg of protein)$^{-1}$]	PGE_2 (nmol/mL)
control	185.2 ± 55.1[a]	5.9 ± 2.9	37.5 ± 5.2
garlic oil	98.6 ± 32.4[b]	5.0 ± 0.8	36.0 ± 7.5
DADS	201.1 ± 34.1[a]	4.8 ± 2.4	39.6 ± 10.8

[a] Control group received corn oil. Values are means ± SD of five to six rats in each group. Groups not sharing the same letter (a, b) are significantly different ($P < 0.05$).

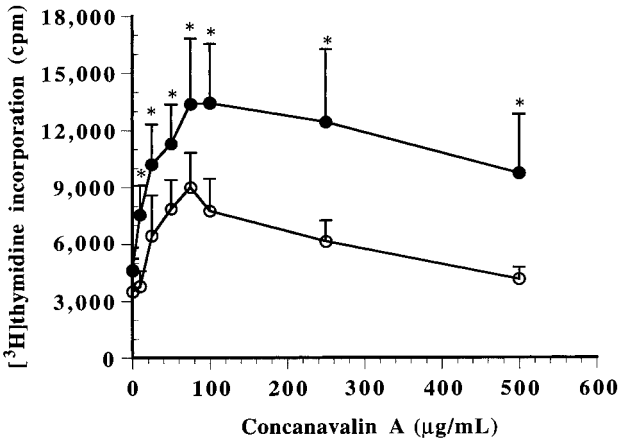

Figure 1. Effect of garlic oil on Con A-induced splenocyte proliferation. Rats received orally administered garlic oil (200 mg/kg of body weight) (●) three times a week for 7 weeks. Animals treated with corn oil were regarded as the control group (○). In each preparation, splenocytes from two rats were pooled and were incubated in the presence of different concentrations of Con A (0−500 μg/mL) for 48 h before the addition of [^{3}H]thymidine. Cells were then incubated for an additional 18 h. Data are shown as means ± SD of three separate preparations with six replications in each preparation. *, significantly higher versus control ($P < 0.05$).

Splenocyte Proliferation. Because of the significant influence of garlic oil on Δ6 desaturase activity and on spleen weight, we further examined the effect of garlic oil on rat immune responsiveness. The results of Con A-induced proliferation response of splenocytes from garlic oil-treated and control rats are shown in Figure 1. The rate of cell proliferation depended on the concentration of Con A and reached its maximum at a level of 75 μg/mL Con A in both groups. The response to Con A stimulation, however, was greater in rats administered garlic oil than in rats from the control group. In addition, at a Con A concentration of 100 μg/mL or higher, the rate of [^{3}H]thymidine incorporation in cells from control rats was inhibited, but cells from garlic oil-treated rats tolerated these higher concentrations of Con A.

Table 4. Effect of Garlic Oil on Contact Hypersensitivity Response to CDNB and Irritant Dermatitis Response to Croton Oil

group	contact hypersensitivity (g)	irritant dermatitis (g)
control	0.078 ± 0.027[a] (4)	0.277 ± 0.061 (3)
garlic oil	0.028 ± 0.026[b] (4)	0.211 ± 0.078 (5)

[a] Control group received corn oil. Values are means ± SD of the weight differences between two ears. Value in parentheses is the number of rats in each group. Groups not sharing the same letter (a, b) are significantly different ($P < 0.05$).

Contact Hypersensitivity and Irritant Dermatitis. The results of the immunoresponse tests are shown in Table 4. For CDNB-induced contact hypersensitivity, swelling of the hapten-challenged ear was significantly suppressed by garlic oil, being only 36% as severe as in the control rats ($P < 0.05$). The extent of irritant dermatitis induced by croton oil in garlic oil-treated rats was also less than in control rats, but the difference was not significant.

DISCUSSION

Garlic has been shown to play diverse biological activities via a wide range of mechanisms. The potent enzyme inhibiting activity of garlic has been proposed to be one such mechanism and has a significant role in the pharmacological actions in the body (Agarwal, 1996). However, most previous studies of garlic components on the inhibition of arachidonic acid metabolism as well as on the modification of immune function were demonstrated with aqueous garlic extracts (Batirel et al., 1996; Ali, 1995; Bordia et al., 1996; Morioka et al., 1993). In this study, evidence indicated that the lipid soluble fraction of garlic, that is, garlic oil, is also a potent arachidonic acid metabolism modulator by inhibiting Δ6 desaturase activity.

Arachidonic acid acts as the precursor of eicosanoids and plays a role in a number of physiological states. Changes in the membrane phospholipid arachidonic acid level and/or the activities of enzymes participating in eicosanoid synthesis will modulate arachidonic acid-associated physiological functions. In the present study, we demonstrated that garlic oil and DADS decreased the arachidonic acid content in hepatic membrane phospholipids. This evidence suggests that garlic oil may modulate cell physiological states via action on arachidonic acid biosynthesis, potentially resulting in modulation of eicosanoid-dependent regulatory processes. For example, prostaglandins have been suggested to be involved in croton oil-induced inflammation (Blazsó and Gabor, 1995) and also in the suppression of ultraviolet radiation-induced contact hypersensitivity (Chung et al., 1986). In this study, we expected to find lower plasma PGE_2 concentrations in garlic oil-treated rats, but we found no such effect (Table 3). We speculate this result might be because we examined PGE_2 levels in the peripheral blood; the PGE_2 concentration was therefore diluted and did not reflect local metabolism in tissues such as liver, spleen, and skin. In addition, in the present study we did not determine fatty acid compositions in membrane phospholipids of other cell types; it is thus necessary to further study the action of garlic oil on specific cell types of interest.

PLA_2, which is responsible for releasing arachidonic acid from membrane phospholipids, may also affect membrane arachidonic acid levels. However, our results indicated that PLA_2 activity is not affected by either garlic oil or DADS (Table 3). Although cyclooxygenase and lipoxygenase, two key enzymes responsible for the conversion of arachidonic acid to prostaglandins or leukotrienes, have been shown to be inhibited by aqueous garlic extract both in vitro and in vivo (Ali, 1995; Sendle et al., 1992), the effect of garlic oil on the activities of these enzymes remains to be investigated.

Most of the functions of linoleic acid are performed by its desaturated metabolites, and Δ6 desaturase is shown to be a rate-limiting enzyme in this desaturation (Horrobin and Manku, 1990). Decreased Δ6 desaturase activity in the liver was reported to be associated with functions of various tissues, especially the skin (Chapkin and Ziboh, 1984). This is due to the fact that Δ6 desaturase activity deficiency is associated with decreased production of prostaglandins, leukotrienes, and other oxygenated derivatives from essential fatty acids. We found only animals receiving the garlic oil, but not DADS, had soft faces, were hairless, and showed signs of dermatitis with reddish skin. These phenomena are consistent with the finding that only animals receiving garlic oil, but not DADS, showed decreased Δ6 desaturase activity. Thus, the present study provides evidence and suggests a possible mechanism as side effects of excessive garlic oil ingestion. Identifying components in garlic oil that inhibit Δ6 desaturase activity will be helpful in improving the pharmacological use of garlic oil. The result of the present study also suggests that active components other than DADS may be present in garlic oil. However, according to the results, garlic oil and DADS reduced body weight gain and altered hepatic membrane phospholipid composition to similar extents (Tables 1 and 2), suggesting that such effects of garlic oil are at least partly due to the action of DADS.

Garlic oil consists of many organosulfur compounds (Sheen et al., 1992), and some of them, such as DADS and DATS, have been known to have biological activities (Hiromichi and Wargovich, 1990; Lawson et al., 1992; Reicks and Crankshaw, 1996). DATS is especially interesting, because it is one of the most dominant organosulfur compounds in the garlic oil used in this study (accounting for ~30% of total organosulfur compounds) and has been reported to be the most active antiplatelet aggregation component in garlic oil (Lawson et al., 1992). To elucidate the actual role of DATS and other organosulfur compounds on Δ6 desaturase activity, further investigation is required.

In the present study, we also found that the spleen appears to be a target organ of garlic oil (Table 1). Garlic oil significantly increased the spleen weight and nearly doubled the spleen weight to body weight ratio. Because the spleen is not only a reservoir for red blood cells but also part of the lymphoid system, this result suggests garlic oil may affect the role of the spleen in immune response and/or of red blood cells in the circulation. At first, we tried to interpret the increased spleen/body weight ratio in the garlic oil group as a result of the increased number of lymphocytes in the spleen, because garlic oil increases the proliferative ability of lymphocytes (Figure 1); this may be a consequence of the accumulation of linoleic acid (Szamel et al., 1989). However, DADS, which had effects similar to those of garlic oil in terms of linoleic acid levels in membrane phospholipids (Table 2) and lymphocyte proliferatory ability (data not shown), did not increase the spleen/body weight ratio. On the other hand, only

garlic oil, not DADS, inhibited Δ6 desaturase activity in the liver (Table 3). Therefore, it is likely that the mechanism of spleen enlargement by garlic oil is independent from linoleic acid-promoted lymphocyte proliferation; nevertheless, a Δ6 desaturase inhibition-associated effect cannot be excluded. A histological examination of the spleen from animals receiving garlic oil was also performed and revealed no pathological disorder (data not shown).

In addition to the splenocyte proliferation test, two in vivo inflammatory tests were also performed in this study. In contrast to the enhancement of Con A-induced splenocyte proliferation, garlic oil suppressed CDNB-induced contact hypersensitivity responsiveness (Table 4). Contact hypersensitivity induced by CDNB involves cell-mediated immune reactions and is generally thought to be associated with T cell protective immunity. At the first sensitization, this hapten penetrates into the epidermis to form hapten−protein conjugates that act as sensitizers. These sensitizers are then internalized by epidermal Langerhans' cells, which migrate to the lymph nodes to express memory CD4+ T cells. Secondary sensitizing then elicits a series of cytokine responses that promote T cell activation and proliferation (Hauser, 1990). The different responses between control and garlic oil-treated rats may thus reflect any event in the primary or secondary sensitization with CDNB, although CD4+ deficiency may be excluded because lymphocytes isolated from these animals exhibited higher rather than lower mitogenic responses when compared with cells from normal rats (Figure 1). Because the contact sensitization with CDNB mainly relies on the antigen presented by the Langerhans' cells, it is possible that in garlic oil-treated animals the primary sensitization was not appropriate, owing to impairment of skin functions caused by decreased Δ6 desaturation activity. Whether the antihypersensitive effect of garlic oil is also due to its ability to decrease local PGE_2 levels and/or local oxidative status in the skin, as would occur with garlic oil or garlic-derived organosulfur compounds in hepatocytes (Dalvi, 1992; Wang et al., 1996), remains to be clarified.

Similarly, a suppressive effect of garlic oil on ear edema was also noted in croton oil-induced irritant dermatitis (Table 4), but to a lesser extent. The croton oil ear test is widely used to identify prospective anti-inflammatory drugs, although the mechanism of such dermatitis is not fully understood. It is now known that such compounds can elicit a cytokine response which does not depend on the recognition of the compound by a T lymphocyte; rather this response is a consequence of direct "stimulation" of macrophages, probably via a biological "damage" event (Thestrup-Pedersen and Halkier-Sørensen, 1996). The antiedema effect of garlic extract has been shown elsewhere, for example, in nude mice exposed to ultraviolet B light (Reeve et al., 1993). However, studies of such effect of garlic oil are rare. We suggest that the apparent antiinflammatory effect of garlic oil may vary with different models of inflammation. In the present study, the limited antiinflammatory effect of garlic oil may have been due to dermatitis which was already induced by garlic oil; this may have modified the antiinflammatory effect of garlic oil in this test. A lipopolysaccharide-induced systemic inflammation model that otherwise bypasses the skin function deficiency may provide an alternative means of testing the antiinflammatory effects of garlic oil.

In this preliminary study of the effects of garlic oil on arachidonic acid metabolism and on the modification of immune function, we demonstrated that garlic oil acts as an immunomodulator at least via promoting T lymphocyte function. In addition, garlic oil inhibits Δ6 desaturase activity and decreases the arachidonic acid level in hepatic membrane phospholipids. We also identified possible side effects of excessive ingestion of garlic and found that a major component of garlic oil, DADS, did not exhibit all of the effects of garlic oil. Understanding the effects of isolated components of garlic oil will be important in terms of using such components therapeutically in various conditions as well as for avoiding side effects of excessive ingestion.

ABBREVIATIONS USED

CDNB, 1-chloro-2,4-dinitrobenzene; Con A, concanavalin A; DADS, diallyl disulfide; DATS, diallyl trisulfide; PAPC, L-α-1-palmitoyl-2-arachidonylphosphatidylcholine; PGE_2, prostaglandin E_2; PLA_2, phospholipase A_2; SD, standard deviation.

LITERATURE CITED

Agarwal, K. C. Therapeutic actions of garlic constituents. *Med. Res. Rev.* **1996**, *16*, 111−124.

Ali, M. Mechanism by which garlic (*Allium sativum*) inhibits cyclooxygenase activity. Effect of raw versus boiled garlic extract on the synthesis of prostanoids. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **1995**, *53*, 397−400.

Augusti, K. T. Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). *Indian J. Exp. Biol.* **1996**, *34*, 634−640.

Bartirel, H. F.; Aktan, S.; Aykut, C.; Yegen, B. C.; Coskun, T. The effect of aqueous garlic extract on the levels of arachidonic acid metabolites (leukotriene C4 and prostaglandin E2) in rat forebrain after ischemia-reperfusion injury. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **1996**, *54*, 289−292.

Belman, S.; Solomon, J.; Segal, A.; Block, E.; Barany, G. Inhibition of soybean lipoxygenase and mouse skin tumor promotion by onion and garlic components. *J. Biochem. Toxicol.* **1989**, *4*, 151−160.

Blazsó, G.; Gabor, M. Effects of prostaglandin antagonist phloretin derivatives on mouse ear edema induced with different skin irritants. *Prostaglandins* **1995**, *50*, 161−168.

Bordia, T.; Mohammed, N.; Thomson, M.; Ali, M. An evaluation of garlic and onion as antithrombotic agents. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **1996**, *54*, 183−186.

Chapkin, R. S.; Ziboh, V. A. Inability of skin enzyme preparations to biosynthesize arachidonic acid from linoleic acid. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 784−792.

Chung, H.-T.; Burnham, D. K.; Robertson, B.; Roberts, L. K.; Daynes, R. A. Involvement of prostaglandins in the immune alterations caused by the exposure of mice to ultraviolet radiation. *J. Immunol.* **1986**, *137*, 2478−2484.

Claman, H. N. Tolerance and contact sensitivity to DNFB in mice. V. Induction of tolerance with DNP compounds and with free and membrane-associated DNFB. *J. Immunol.* **1976**, *116*, 704−709.

Clementi, G.; Amico-Roxas, M.; Caruso, A.; Cutuli, V. M. C.; Prato, A.; Maugeri, S.; de Bernardis, E.; Scapagnini, U. Effect of CGRP in different models of mouse ear inflammation. *Life Sci.* **1994**, *54*, 119−124.

Dalvi, R. R. Alterations in hepatic phase I and phase II biotransformation enzymes by garlic oil in rats. *Toxicol. Lett.* **1992**, *60*, 299−305.

Folch, J.; Lees, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−507.

Garg, M. L.; Sebokova, E.; Thomson, A. B. R.; Clandinin, M. T. Δ6 Desaturase activity in liver microsomes of rats fed

diets enriched with cholesterol and/or 3 fatty acids. *Biochem. J.* **1988**, *249*, 351−356.

Hauser, C. Cultured epidermal Langerhans cells activate effector T cells for contact sensitivity. *J. Invest. Dermatol.* **1990**, *95*, 436−440.

Hiromichi, S.; Wargovich, M. J. Chemoprevention of 1,2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. *Cancer Res.* **1990**, *50*, 5084−5087.

Horrobin, D. F.; Manku, M. S. Clinical Biochemistry of Essential Fatty Acids. In *Omega-6 Essential Fatty Acids*−*Pathophysiology and Roles in Clinical Medicine*; Horrobin, D. F., Ed.; Wiley-Liss: New York, 1990; pp 21−53.

Lau, B. H. S.; Yamasaki, T.; Gridley, D. S. Garlic compounds modulate macrophage and T-lymphocyte functions. *Mol. Biother.* **1991**, *3*, 103−107.

Lawson, L. D.; Ransom, D. K.; Hughes, B. G. Inhibition of whole blood platelet-aggregation by compounds in garlic clove extracts and commercial garlic products. *Thromb. Res.* **1992**, *65*, 141−156.

Lepage, G.; Roy, C. C. Direct transesterification of all classes of lipids in one-step reaction. *J. Lipid Res.* **1986**, *27*, 114−120.

Levis, W. R.; Powell, J. A.; Whalen, J. J. Carrier specificity vs hapten specificity in classical cell-mediated contact sensitivity to dinitrochlorobenzene. *J. Immunol.* **1975**, *115*, 1170−1173.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

Marsh, C. L.; Torrey, R. R.; Woolley, J. L.; Barker, G. R.; Lau, B. H. S. Superiority of intravesical immunotherapy with *Corynebacterium parvum* and *Allium sativum* in control of murine bladder cancer. *J. Urol.* **1987**, *137*, 359−362.

Morioka, N.; Sze, L. L.; Morton, D. L.; Irie, R. F. A protein fraction from aged garlic extract enhances cytotoxicity and proliferation of human lymphocytes mediated by interleukin-2 and concanavalin A. *Cancer Immunol. Immunother.* **1993**, *37*, 316−322.

Mustafa, T.; Srivastava, K. C. Possible leads for arachidonic acid metabolism altering drugs from natural products. *J. Drug Dev.* **1990**, *3*, 47−60.

Perchellet, J. P.; Perchellet, E. M.; Abney, N. L.; Zirnstein, J. A.; Belman, S. Effects of garlic oils on glutathione peroxidase activity, the ratio of reduced/oxidized glutathione and ornithine decarboxylase induction in isolated mouse epidermal cells treated with tumor promoters. *Cancer Biochem. Biophys.* **1986**, *8*, 299−312.

Phelps, S.; Harris, W. S. Garlic supplementation and lipoprotein oxidation susceptibility. *Lipids* **1993**, *28*, 475−477.

Rao, C. V.; Rivenson, A.; Simi, B.; Reddy, B. S. Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally-occurring plant phenolic compound. *Cancer Res.* **1995**, *55*, 259−266.

Reeve, V. E.; Bosnic, M.; Rozinova, E.; Boehm-Wilcox, C. A garlic extract protects from ultraviolet B (280−320 nm) radiation-induced suppression of contact hypersensitivity. *Photochem. Photobiol.* **1993**, *58*, 813−817.

Reicks, M. M.; Crankshaw, D. L. Modulation of rat hepatic cytochrome P-450 activity by garlic organosulfur compounds. *Nutr. Cancer* **1996**, *25*, 241−248.

Sendle A.; Elbl, G.; Steinke, B.; Breu, W.; Wanger, H. Comparative pharmacological investigations of *Allium ursinum* and *Allium sativum*. *Planta Med.* **1992**, *58*, 1−7.

Shalinsky, D. R.; McNamara, D. B.; Agrawal, K. C. Inhibition of GSH-dependent PGH-2 isomerase in mammary adenocarcinoma cells by allicin. *Prostaglandins* **1989**, *37*, 135−148.

Sheen, L.-Y.; Lin, S.-Y.; Tsai, S.-J. Odor assessments for volatile compounds of garlic and ginger essential oils by sniffing method of gas chromatography. *J. Chinese Agric. Chem. Soc.* **1992**, *30*, 14−24.

Simons, L. A.; Balasubramaniam, S.; von Konigsmark, M.; Parfitt, A.; Simons, J.; Peters, W. On the effect of garlic on plasma lipids and lipoproteins in mild hypercholesterolaemia. *Atherosclerosis* **1995**, *113*, 219−225.

Szamel, M.; Rehermann, B.; Krebs, B.; Kurrle, R.; Resch, K. Activation signals in human lymphocytes: Incorporation of polyunsaturated fatty acids into plasma membrane phospholipids regulated IL-2 synthesis via sustained activation of protein kinase C. *J. Immunol.* **1989**, *143*, 2806−2813.

Szondy, Z.; Newsholme, E. A. The effect of time of addition of glutamine or nucleosides on proliferation of rat cervical lymph-node T-lymphocytes after stimulation by concanavalin A. *Biochem. J.* **1991**, *278*, 471−474.

Thestrup-Pedersen, K.; Halkier-Sørensen, L. Mechanisms of Irritant Contact Dermatitis. In *The Irritant Contact Dermatitis Syndrome*; Van der Valk, P. G., Maibach, H. I., Eds.; CRC Press: New York, 1996; pp 305−309.

Wang, E. J.; Li, Y.; Lin, M.; Chen, L.; Stein, A. P.; Reuhl, K. R.; Yang, C. S. Protective effects of garlic and related organosulfur compounds on acetaminophen-induced hepatoxicity. *Toxicol. Appl. Pharmacol.* **1996**, *136*, 146−154.

Yang, C. S.; Smith, T. J.; Hong, J.-Y. Cytochrome P-450 enzymes as targets for chemoprevention against chemical carcinogenesis and toxicity: Opportunities and limitations. *Cancer. Res.* **1994**, *54*, 1982s−1986s.

Yeh, Y. Y.; Yeh, S. M. Garlic reduces plasma lipids by inhibiting hepatic cholesterol and triacylglycerol synthesis. *Lipids* **1994**, *29*, 189−193.

Received for review May 11, 1998. Revised manuscript received August 27, 1998. Accepted August 27, 1998. This research work was supported partially by the National Science Council, Republic of China, under Grant NSC 87-2314-B-040-002.

JF980466P