Significance of Garlic and Its Constituents in Cancer and Cardiovascular Disease

Aged Garlic Extract Prevents a Decline of NK Cell Number and Activity in Patients with Advanced Cancer[1,2]

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ABSTRACT Aged garlic extract (AGE) has manifold biological activities including immunomodulative and antioxidative effects. It is used as a major component of nonprescription tonics and cold-prevention medicines or dietary supplements. Advanced-cancer patients decline in immune functions and quality of life (QOL). The study's subjects were patients with inoperable colorectal, liver, or pancreatic cancer. In a randomized double-blind trial, AGE was administered to one group and a placebo was administered to another for 6 mo. The primary endpoint was a QOL questionnaire based on the Functional Assessment of Cancer Therapy (FACT). The subendpoints were changes in the natural-killer (NK) cell activity the salivary cortisol level from before and after administering AGE. Out of 55 patients invited to participate in the trial, 50 (91%) consented to enroll. They consisted of 42 patients with liver cancer (84%), 7 patients with pancreatic cancer (14%), and 1 patient with colon cancer (2%). Drug compliance was relatively good in both the AGE and placebo groups. Although no difference was observed in QOL, both the number of NK cells and the NK cell activity increased significantly in the AGE group. No adverse effect was observed in either group. The study showed that administering AGE to patients with advanced cancer of the digestive system improved NK cell activity, but caused no improvement in QOL. J. Nutr. 136: 816S–820S, 2006.

KEY WORDS: • *aged garlic extract* • *NK cell activity* • *advanced cancer* • *double-blind controlled trial* • *quality of life*

The prognosis of cancer patients for whom radical surgical resection is impossible is poor. In many of these patients, the quality of life (QOL)[4] deteriorates due to exacerbation of symptoms of cancer and adverse effects of chemotherapy. Also, the prognosis of the patient deteriorates because of immune dysfunction, which leads to proliferation of cancer and infection. Therefore, improvements in QOL and prevention of hypofunctions in the immune system are considered important in the care of advanced-cancer patients.

Garlic is one of the foods considered promising for improving QOL. Laboratory studies have suggested that garlic and its components suppress carcinogenesis and reduce serum lipid levels (1–7). However, garlic has been reported to cause adverse effects such as gastrointestinal disorders and anemia, in addition to its intense odor. (8,9) These adverse events are caused by allicin

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[4] Abbreviations used: AGE, aged galic extract, FACT, Functional Assessment of Cancer Therapy; FACT-An, FACT-G plus the subscale for anemia; FACT-G, FACT general scale; NK, natural killer; PEIT, percutaneous ethanol injection therapy; QOL, quality of life; SAC, S-allylcysteine; TAE, transcatheter arterial embolization.

and lipid-soluble sulfur compounds, which are produced by a cascade of chemical reactions from allicin (9,10). Aged garlic extract (AGE), which is produced by a long-term extraction from garlic in aqueous ethanol and has no irritating odor, does not cause such adverse events and has been confirmed to be safe in preclinical trials (11–13).

AGE has been shown to have an effect against physical and mental stress (14,15), an immuno-potentiating effect (16–20), an antioxidant effect (21–24), and a peripheral blood flow–improving effect (15,25,26); it is also expected to improve QOL and prevent the decrease in immune functions in patients with advanced cancer. However, its effects in cancer patients have not been evaluated in a randomized double-blind clinical trial.

We designed a randomized double-blind clinical trial to evaluate the effects of AGE on the QOL and immune functions of patients with advanced cancer.

SUBJECTS AND METHODS

Patients. The subjects were patients with advanced colon, liver, or pancreatic cancer, aged 20 y or more, who were admitted to Osaka Medical Center for Cancer and Cardiovascular Diseases and judged by their attending physicians to be inoperable. Patients with a history of hematemesis, bloody stools, ascites, or those who have had difficulty with oral nutrition, or a history of allergy to a food or drug that contains garlic or its components, were excluded.

With the permission of the attending physicians, 2 members of our research team interviewed patients and invited them to participate in the trial. Informed consent with written confirmation was obtained from those who agreed to enroll. The subjects were recruited between 17 May and 16 November 1999.

Study design. The study was carried out as a randomized double-blind trial. It was approved by the Ethical Board of the Osaka Medical Center for Cancer and Cardiovascular Diseases, and an independent Ethical Monitoring Committee, excluding members of our research team, was established for this trial.

Data on participants who consented to the enrollment were reported anonymously to the trial statistician by fax. The trial statistician randomized the participants into 2 groups, one received AGE (AGE group) and the other received crystalline cellulose (control). Randomization was made by the block randomization method using the disease name as a factor.

Blood was sampled from the consenting participants and between 1500 and 1700 they were asked to fill out a questionnaire concerning QOL. They were then given trial capsules to be taken for the following 12 wk, starting the next day.

After 12 wk, the participants visited the Osaka Medical Center for Cancer and Cardiovascular Diseases at 1500 and met with a member of the trial team. They were questioned about their symptoms, returned the drug bottles and medication diaries, had their blood sampled, and answered a follow-up QOL questionnaire. They were then given more trial capsules and medication diaries for another 12-wk period. After 24 wk, the subjects again visited the Osaka Medical Center for Cancer and Cardiovascular Diseases at 1500, met with a member of the trial team, and answered the QOL questionnaire.

During this trial, the disease was treated ordinarily, but subjects were instructed not to take supplements containing garlic or its components other than the trial capsules.

If adverse events occurred, the information was entered onto a prescribed form and faxed to the trial statistician. The trial statistician recorded the drug that had been assigned to the patient and reported the event immediately to the chairman of the Ethical Monitoring Committee.

When the QOL questionnaire had been completed by all subjects, 6 mo after the trial began and the trial data had been stored as a computer file, the randomization codes were disclosed to all members of the trial team (after receiving approval from the Ethical Monitoring Committee).

Trial capsules. Trial capsules were prepared by Wakunaga Pharmaceutical Co. Subjects took 2 capsules after breakfast and and 2 capsules after dinner (4 capsules/d). The AGE capsules contained AGE powder that was prepared by mixing AGE with crystalline cellulose (Avicel FD-101) as an excipient. AGE is a unique garlic preparation manufactured by soaking garlic in aqueous ethanol for >10 mo. It contains water-soluble organosulfur compounds including *S*-allylcysteine (SAC), *S*-1-propenyl-L-cysteine, *A*-allylmercapto-L-cysteine and cycloalliin, steroid saponins, fructosylarginine, and 1,2,3,4-tetrahydro-β-carboline-3-caboxylic acids as biologically active components (27). The daily dose (4 capsules), contained 500 mg of AGE, 727 mg of crystalline cellulose, and 11 mg of sucrose fatty acid ester.

The placebo capsules contained 951.5 mg of crystalline cellulose and 8.5 mg of sucrose fatty acid ester per 4 capsules.

Confirmation of the compliance and blinding effect. Drug compliance was evaluated according to entries in subjects' medication diaries and by a count of remaining capsules. In addition, the blood level of a marker compound of AGE was measured. The subjects were instructed to enter the state of compliance in the medication diaries daily, and the remaining capsules were recovered and counted. Blood was sampled 3 mo after administering the capsules, and the blood SAC level was determined.

To check the blinding effect, the subjects were asked, at 3 and 6 mo after the beginning of the trial, which of the drugs they thought was assigned to them.

Observation items. At enrollment, the patients and their attending physicians were asked about the subjects' histories and the histories of their present illnesses.

Patients filled out the QOL questionnaire, Functional Assessment of Cancer Therapy (FACT) (28) before starting the treatment and again after 3 and 6 mo of receiving treatment. In principle, the subjects answered by themselves, but a member of the trial team attended the subjects and explained the contents of the questionnaire if help was desired. Entries on the questionnaire were scored according to the method determined by FACT developers and included the handling of defect values. The scores of individual domains and the total scores were calculated separately. Specifically, the score of each of the 4 domains of the general scale (FACT-G) (i.e., physical, functional, mental, and social dimensions), the score of the subscale for anemia, the total score of the FACT-G, and the total score of the FACT-G + subscale for anemia (FACT-An), were calculated.

Blood and saliva were sampled before and 3 mo after study treatment began. Blood was tested for glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, γ- glutamic-pyruvic transaminas, lactate dehydrogenase, alkaline phosphatase, tocopherol, albumin, T-Chol., TG, UA, BUN, Cr, Na, K, Cl, Ca, IP, T-Bil, FBS, blood cell counts, blood-homocystein concentration, NK cell activity, NK cell count (measured as the lymphocyte subset 2-color CD16+/CD56+), CD4 and CD8-positive cell count, and SAC concentration. The number of immunocytes was determined by flow cytometry. NK cell activity was determined by 51Cr-release assay according to the method described by Domzig et al. (32). The effector cell (mononuclear cell) and target cell (K562 cell) ratio is 20:1.

The blood SAC concentration was measured at Healthcare Institute of Wakunaga Pharmaceuticals using the method of Kodera et al. (33). Saliva was collected using Salivette sampling devices (Sarstadt) (34). A Salivette includes a small cotton swab and, when chewed, stimulates saliva flow to allow for the collection of a sufficient amount of saliva within 1 min. After centrifugation at 3,000 $\times$ *g* for 10 min, saliva was stored at −80°C until assay. Salivary cortisol levels were determined with a commercial enzyme immunoassay kit (Ciron) (35) at the Department of Social and Environmental Medicine, Osaka University.

If chemotherapy was performed at the beginning of the study, blood sampling and completion of the questionnaire were carried out 6 or more d after anticancer drug treatment was completed.

Endpoints. The primary endpoint was whether, after 6 mo of treatment, any subjects had a FACT-G or FACT-An score that deteriorated by 1 SD. The SD of the FACT-G was set at 15.9 (n = 466, mean = 82.0), based on Cella (29), and the SD of the FACT-An at 26.8 (n = 47, mean = 141.6), also based on Cella (28). Data of the subjects who died during the trial were regarded as defect values. Subendpoints were changes in the NK cell activity and salivary cortisol level from before and after treatment.

Statistical procedures. The target number of subjects to be recruited was 40 (20 in each group) because a statistical difference

could be established with this number of subjects at a *P*-value of 5% and a power of 80% if the QOL score after treatment deteriorated to 76% (compared with that before treatment) in the placebo group and to 38% in the AGE group. During the registration period, invitation to participate was limited to a maximum of 60 patients.

Two-sample *t* tests were performed for comparisons between the 2 groups: paired *t* tests were performed for comparisons from before and after the treatments, and Fisher's exact test was performed for the analysis of nonparametric data. The data were represented as means ± SD unless otherwise indicated. The results were analyzed by intention-to-treat analysis.

RESULTS

Enrollment and randomization. During the entry period, 55 patients were invited to enroll and 50 (91%) of them consented. The characteristics of the 50 patients enrolled are listed in **Table 1**. They consisted of 42 patients with liver cancer (84%), 7 patients with pancreatic cancer (14%), and 1 patient with colon cancer (2%). These patients were determined to be inoperable because of their advanced cancer although performance status of the patients was good. In the patients with liver cancer, 38 patients bore multiple liver tumors and 4 showed hepatic failure. In the pancreatic cancer patients, 4 showed vascular invasion, 2 showed direct invasion, and 1 showed liver metastasis. One patient with colon cancer showed liver metastasis. All of the patients with liver cancer were treated with transcatheter arterial embolization (TAE) and/or percutaneous ethanol injection therapy (PEIT), and 6 of them underwent hepatectomy before starting the study. The patients with pancreatic cancer did not undergo treatment before the study except for 1 who was treated with cisplatin and fluorouracil. No difference was observed in age, gender, and clinical stage between the AGE and control groups.

After randomization, 3 patients of the AGE group requested to be withdrawn from the trial without taking AGE and were lost to the trial. Another patient of the AGE group developed angina pectoris on day 83 of the study and was lost. In the control group, 1 patient wanted to withdraw from the trial due to diarrhea on day 7 of the study and was lost.

Ten patients with liver cancer underwent TAE and/or PEIT treatment, of whom 1 took fluorouracil, but the rest were not treated during the study. Fluorouracil was administered for all of the pancreatic cancer patients, of whom 5 were treated with radiation and 1 took irinotecan along with fluorouracil. During the study, a significant difference in the treatments was not observed between the 2 cohorts.

Four patients in the AGE group died due to cancer on days 55, 85, 99, and 106 of the study, and 5 patients of the control group died due to cancer on days 45, 143, 153, 168, and 170. Data collected up to the loss or death of patients was adopted for analyses of the results.

After completing the 6-mo trial, most of the subjects voluntarily started taking AGE.

Confirmation of compliance and blinding effect. Compliance was relatively good in both groups.

Changes in the blood SAC concentration are recorded in **Table 2**. Before beginning treatment, the blood SAC concentration in many subjects was low. After 3 mo, the SAC concentration was significantly increased to 10 μg/L or above in 14 (78%) of the subjects in the AGE group ($P = 0.01$). SAC concentration increased as well in the control group though less markedly than in the AGE group ($P = 0.19$). Values were therefore higher in both groups.

According to the questionnaire, entries concerning the blinding effect, 4 (24%) in the AGE group and 5 (27%) in the control group, believed that they were taking AGE capsules after 3 mo, and 5 (29%) in the AGE group and 4 (21%) in the control group believed they were taking AGE capsules after 6 mo, so blinding was judged successful.

QOL. No difference was observed in QOL between the AGE and control groups not only before but also at 3 and 6 mo after the study began. No particular change was observed in QOL at 3 and 6 mo after administering treatment compared with before.

Indices of cell-mediated immunity. Changes in the peripheral blood NK cell count and NK cell activity are listed in **Table 3**. Analysis was performed by excluding the data of 1 patient from the AGE group for whom blood could not be sampled for measuring indices of cell-mediated immunity.

The NK cell count was not different between the AGE group and control group before or 3 mo after study treatment began. It increased significantly in the AGE group, and, while it also increased in the control group, the increase was not significant.

The NK cell activity was not different between the 2 groups before or 3 mo after the administering treatment. The NK cell activity increased significantly in the AGE group. It also increased in the control group, but the increase was not significant. The NK cell activity appeared to decrease rapidly in the control group compared with the AGE group. Five subjects (22%) in the control group showed >25% decrease in the NK cell activity but none did in the AGE group ($P = 0.051$) (Table 3, **Fig. 1**). Only 1 (3%) of the 35 patients in whom the NK cell activity did not decrease by 25% or more 3 mo after administering treatment died within the following 3 mo, but 3 (60%) of the 5 patients in whom the NK cell activity decreased by 25% or more died within the following 3 mo ($P = 0.04$).

TABLE 1

Baseline characteristics of subjects

Characteristic	AGE group ($n = 25$)	Control group ($n = 25$)
Age,[1] *y*	63.6 ± 8.3	65.8 ± 6.3
Male sex, %	21 (84)	18 (72)
Cancer, %		
Liver	21 (84)	21 (84)
Pancreas	4 (16)	3 (12)
Colon	0 (0)	1 (4)
Dropped out, %	4 (16)	1 (4)
The death (exam. period), %	4 (16)	5 (20)

[1] Values are means ± SD.

TABLE 2

Changes in blood S-allylcysteine concentration[1]

	AGE group ($n = 18$)		Control group ($n = 23$)	
	Pretreatment	After 3 mo[2,3]	Pretreatment	After 3 mo[4]
<10 *ng/mL*	16 (89)	4 (22)	20 (87)	15 (65)
10–14 *μg/L*	2 (11)	10 (56)	1 (4)	3 (13)
15–19 *μg/L*	0 (0)	3 (17)	2 (9)	2 (9)
≤20 *μg/L*	0 (0)	1 (6)	0 (0)	3 (13)

[1] Values indicate number and (%) of patients.
[2] $P = 0.01$.
[3] 17 (94%) had increased concentration compared with before treatment and 1 (6%) was changed or reduced.
[4] 17 (74%) had increased concentration compared with before treatment and 6 (26%) were changed or reduced.

TABLE 3

Changes in index values of cell-mediated immunity[1]

	AGE group (n = 17)		Control group (n = 23)	
	Pretreatment	After 3 mo[2]	Pretreatment	After 3 mo[3]
WBC, /μL	4433 ± 1623	4517 ± 1143	4873 ± 2081	5283 ± 2211
CD4$^+$, *cells/μL*	650 ± 271	667 ± 228	746 ± 356	866 ± 475[4]
CD8$^+$, *cells/μL*	409 ± 155	457 ± 181	403 ± 312	533 ± 418
NK, *cells/μL*	207 ± 142	277 ± 150[4]	231 ± 207	288 ± 222
NK activity, %	27.2 ± 15.9	36.0 ± 13.2[5]	32.6 ± 16.3	39.1 ± 15.7
NK activity/100 cells	19.0 ± 17.2	17.4 ± 12.9	19.9 ± 14.1	24.0 ± 21.9

[1] Values are means ± SD.
[2] 17 (100%) did not show a ≥25% decrease in NK cell activity compared with before treatment.
[3] 18 (78%) did not show a ≥25% decrease in NK cell activity compared with before treatment and 5 (22%) did show this change.
[4] $P < 0.05$ after 3 mo compared with the value before treatment.
[5] $P < 0.01$ after 3 mo compared with the value before treatment.
NK cells = CD16+/CD56+.

There was no significant change in the number of CD8+ cells in peripheral blood but CD4+ cells significantly increased in the control group.

Other markers. No significant difference was observed in the salivary cortisol concentration or the values of blood biochemical parameters between the AGE group and control group, either before or 3 mo after administering treatment.

While the salivary cortisol concentration showed no change from before to after administering the treatment in the AGE group, it increased significantly after 3 mo in the control group.

The serum total protein, albumin, total cholesterol, and HDL cholesterol levels were increased 3 mo after administering treatment compared with the values before the study began in both groups.

Adverse events. In the AGE group, 4 died due to cancer on days 55, 85, 99, and 106 of the study. One developed a duodenal ulcer, and 2 developed severe acute gastritis. In the control group, 5 died due to cancer on days 45, 143, 153, 168, and 170 of the study. One developed a gastric ulcer, and 1 suffered severe acute gastritis. There was no difference in the occurrence of adverse events between the 2 groups.

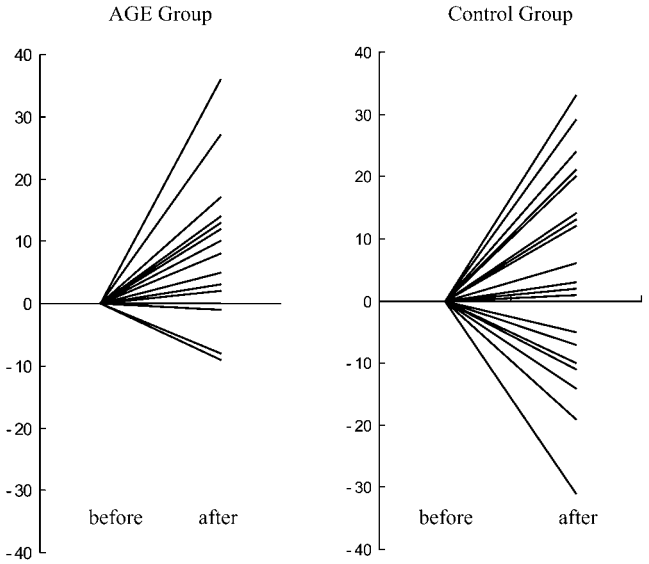

FIGURE 1 NK cell activity before and after the study. Changes of NK cell activity of patients 3 mo after the beginning of the study are shown as a percentage.

DISCUSSION

Administering AGE did not improve QOL but caused improvements in the NK cell activity in patients with advanced cancer of the digestive system.

Many patients who undergo chemotherapy receive alternative treatments in addition to ordinary treatments such as anticancer agents. However, the effectiveness of few alternative treatments for patients with advanced cancer has been confirmed by scientific assessment methods such as the randomized double-blind comparative study.

Garlic is used widely all over the world as an alternative treatment. Although epidemiological studies (36) have suggested that garlic prevents carcinogenesis, its therapeutic effects or its effects on QOL in patients with advanced cancer have scarcely been evaluated. Double-blind studies have been considered difficult because of the characteristic odor of garlic, but blinding was successful in our study using AGE. Therefore, AGE is expected to facilitate the execution of double-blind clinical trials in the future.

No effect of AGE was noted in QOL, which was the primary endpoint of this study, probably because QOL hardly deteriorated in the control group. This may be explained by the fact that most of the subjects had liver cancer, which causes relatively mild symptoms even in an advanced stage. Moreover, the blood SAC concentration tended to increase also in the control group, possibly because all subjects ingested garlic more than usual from meals after having been informed of the effectiveness of garlic during the process of informed consent. This may also have been a factor of the small deterioration of QOL in the control group. The shortness of the trial period may have been another reason. As a food, garlic has mild effects and an increased difference might have been revealed over a longer trial period.

In this study, improvements were observed in various serum nutritional parameters 3 mo after treatment began compared with before treatment in both the AGE and control groups. These improvements may be related to the fact that most of the patients were hospitalized before the beginning of the study but were treated on an outpatient basis and were eating at home after 3 mo.

Although the salivary cortisol concentration increased significantly in the control group, the values varied widely within the group, so that the increase may well have been accidental.

Administering AGE significantly increased the NK cell count in peripheral blood 3 mo after the study began, as can be seen in

Table 3 The NK cell activity increased in the AGE group but the specific activity (activities in 100 cells) did not change. In addition, no differences were observed in chemotherapy received by patients in the AGE and control group. These facts indicate that AGE increases the number of NK cells, resulting in the increase of NK cell activities. The mechanism of the increase in NK cell numbers remains unclear, but these findings were in agreement with the results of laboratory studies (16–20). Moreover, while many patients in whom the NK cell activity decreased rapidly died within the following 3 mo, none of the subjects in the AGE group showed such a rapid decrease, which suggests that AGE may prevent death due to cancer.

A significant increase in number of $CD4^+$ T cells, which represent helper T cells, was observed in the control group. The precise reason is unclear, but we think inflammation reaction accompanied by cancer progression is a possible reason, because helper T cells are involved in antibody production and the stimulation of cellular immune functions in regions of inflammation. To confirm this hypothesis, we should investigate the relation between an increase in the number of $CD4^+$ T cells and cancer progression.

We realize that an understanding of the effect of AGE is incomplete because we did not prohibit treatments such as TAE, PEIT, radiation, and the administering of anticancer drugs during the course of the study, which probably affected NK cell activity. Furthermore, it is possible that patients in the control group might have taken garlic and/or garlic products because SAC concentration in the serum of some patients in the control group increased after the study. However, these facts could underestimate but never overestimate the effect of AGE. Despite these facts, we think our conclusion that AGE increased NK cell activity is reasonable.

In this study, some patients in the AGE group dropped out before the treatment began, but the loss is considered to have been accidental. No difference was observed between the 2 groups in adverse events during the trial period, including deaths due to cancer, and AGE is considered safe to administer to patients with advanced cancer.

After the 6-mo trial period, most of the subjects started taking AGE. For this reason, effects of the study treatment on the remote outcome, including death due to cancer after the trial period, could not be evaluated. Clinical trials with primary endpoints of death due to cancer and enlargement of cancer need to be performed in the future.

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