Significance of Garlic and Its Constituents in Cancer and Cardiovascular Disease

A Factorial Trial Including Garlic Supplements Assesses Effect in Reducing Precancerous Gastric Lesions[1,2]

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ABSTRACT The Shandong Intervention Trial was a factorial, double-blind, placebo-controlled trial to determine whether any of 3 interventions, alone or in combination, could reduce the prevalence of precancerous gastric lesions in Linqu County, Shandong Province, China, a region with high gastric cancer mortality rates and a prevalence in adults of *Helicobacter pylori* of ~67%. The 3 interventions were one-time treatment with amoxicillin and omeprazole for *Helicobacter pylori* infection, and long-term administration of a garlic supplement (aged garlic extract and steam-distilled garlic oil) and a vitamin supplement (vitamins E and C and selenium). This paper describes the design and initial findings on treatment compliance, completeness of follow-up data, and eradication of *Helicobacter pylori.* J. Nutr. 136: 813S–815S, 2006.

KEY WORDS: • *gastric cancer* • *Helicobacter pylori* • *garlic* • *vitamins*

The purpose of this paper was to describe the background, motivation, design, and data on treatment compliance and completeness of end point measurements for an intervention trial to reduce the prevalence of precancerous gastric lesions in Linqu County, a rural mountainous region located in Shandong Province, China.

Linqu County has one of the highest rates of gastric cancer mortality in the world; ~42% of cancer deaths in Linqu County are due to gastric cancer, and the population has a very high prevalence of precancerous gastric lesions. Only 2% of the population has superficial gastritis or normal mucosa; the remaining 98% have more advanced histopathology, including 45% with chronic atrophic gastritis, 35% with intestinal metaplasia, and 20% with dysplasia (1). Compared with those who have superficial gastritis or chronic atrophic gastritis (2), the relative risk of developing gastric cancer in individuals who have moderate or severe dysplasia is 104; for those with deep intestinal metaplasia it is 29, and for those with superficial intestinal metaplasia, it is 17. These findings and other data (3) indicate that gastric carcinogenesis requires progression through precancerous conditions. Thus, it is plausible that treatments that reduce the prevalence of advanced precancerous gastric lesions would also reduce the burden of gastric cancer. We undertook a trial designed to determine whether any of 3 interventions, alone or in combination, could reduce the prevalence of precancerous gastric lesions in Linqu County. These interventions, given in a factorial design to allow estimation of their several and joint effects, included treatment for *Helicobacter pylori*, a garlic supplement, and a vitamin supplement.

SUBJECTS AND METHODS

Study population. The study population consisted of 4326 individuals aged 35–64 y from 14 representative villages in Linqu. These individuals were asked whether they would be willing to undergo gastroscopy in 1994; 90% agreed, 210 were unable to participate because of illness and 226 refused. Because one of the 14 villages was used for a pilot study of *H. pylori* treatment with amoxicillin and omeprazole, individuals from that village (n = 291) were not included in the actual randomized comparison, resulting in 13 villages and 3411 randomized individuals. A total of 188 other individuals were excluded after gastroscopy and were not randomized because of death, refusal, antibiotic allergy, or other reasons. The final

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number of 3411 randomized participants represents 85% of the population aged 35–64 y in the 13 study villages. The results of this trial should therefore be generalizable to the population of Linqu County.

The interventions and their rationales. The first intervention, treatment for *H. pylori*, was amoxicillin (1 g, 2 times/d) and omeprazole (20 mg, 2 times/d) for 2 wk. Those who had continued evidence of *H. pylori* infection after treatment, based on a carbon-13 labeled urea breath test (CUBT), were offered an additional 2-wk course of treatment.

The rationale for *H. pylori* treatment derives from the fact that *H. pylori* was designated a human stomach carcinogen by the International Agency for Research on Cancer (4) and the fact that approximately two-thirds of the Linqu population is seropositive for *H. pylori*. Moreover, there is an association in Linqu between the prevalence of *H. pylori* and the degree of severity of the histopathology of precancerous gastric lesions (5). About half of the individuals with mild chronic atrophic gastritis or less severe histology are infected, compared with more than three-quarters who have more severe histopathology. A pilot study conducted in a village in Linqu County in 1994 demonstrated that the amoxicillin/omeprazole regimen was well-tolerated, safe, and easy to administer.

The second intervention was a vitamin/mineral supplement given for 7.3 y. The supplement, administered 2 times/d, contained vitamin E (100 IU), vitamin C (250 mg), and selenium (37.5 mcg). The rationale for this supplement was based on a previous study of 30,000 subjects in Linxian County in China's Hunan province (6). That trial demonstrated a 21% decrease in gastric cancer mortality in a group that received vitamin E, vitamin C, and selenium. In Linqu itself, a case-controlled study showed that fruit and vegetable intake was protective against gastric cancer (7). A cross-sectional study in Linqu County showed that subjects with intestinal metaplasia had lower levels of vitamin C than those who had less advanced lesions(8).

The third intervention was a garlic supplement given for 7.3 y. The encapsulated supplement contained Kyolic®, an aged garlic extract (400 mg, 2 times/d), and steam-distilled garlic oil (2 mg, 2 times/d). Several epidemiologic lines of evidence motivated the use of a garlic supplement. A previous case-control study of gastric cancer in the Linqu population indicated that allium-containing vegetables, including garlic, were protective (9). In that study, subjects whose garlic consumption was in the highest quartile had a 40% reduced risk of developing gastric cancer compared with those in the lowest quartile of garlic consumption. Another case-control study in Italy reached similar conclusions (10). Most of the garlic in China is grown in Cangshan County, which is located within 200 miles of Linqu County; it has one of the lowest gastric cancer mortality rates in China and a much lower prevalence of precancerous gastric lesions than Linqu County (11). Laboratory studies of elements in garlic such as diallyl sulfide (12), which is present in steam-distilled garlic oil, and studies of Kyolic aged garlic extract (13) indicate that these substances can inhibit carcinogen-induced tumor development in animals.

Some subjects in the factorial design described below received placebos instead of the active interventions, depending on the random treatment assignments. Details of the placebos and other aspects of the trial design were previously described (14). The placebo controls for the garlic supplement represented a challenge to masking. The package containing capsules of placebos for the garlic supplement had a small amount of steamed distilled garlic oil in the container. Thus, when subjects opened the package, they smelled a garlic odor. This approach enabled the investigators to mask the study not only for the study subjects, but also for those who delivered the capsules and had direct contact with the study subjects as part of the protocol.

Main end points. The trial has the following 3 main end points, which were measured in 1999 and 2003, at 3.5 and 7.5 y after initial treatment for *H. pylori:* 1) the prevalence of dysplasia or gastric cancer; *2*) the prevalence of severe chronic atrophic gastritis, intestinal metaplasia, dysplasia, or gastric cancer; and *3*) the average histopathologic severity score (14). The histologic data are based on 7 standard gastric biopsies from each subject, 1 from the greater curvature, 2 from the lesser curvature, and 4 from the antrum. Each of these biopsies was graded pathologically to determine the severity of the histopathology, and the most severe of the 7 sites was used as a summary of severity for that individual.

Stratified factorial design. The 3411 subjects were stratified on the basis of their 1994 *H. pylori* serology status. Approximately one-third of the subjects ($n = 1126$) were seronegative both to IgG and IgA antibodies in 1994. These individuals were not administered amoxicillin/omeprazole; instead, they were entered into a 2×2 factorial trial of garlic and vitamin supplementation. In lieu of amoxicillin/omeprazole, these subjects were administered placebo and either garlic and vitamins ($n = 282$), garlic and placebo for vitamins ($n = 281$), vitamins and placebo for garlic ($n = 281$), or placebo only ($n = 282$). Among the remaining 2285 seropositive subjects, a full $2 \times 2 \times 2$ factorial design was employed, whereby participants receiving amoxicillin/omeprazole ($n = 1142$) also were administered garlic and vitamins ($n = 286$), garlic and placebo for vitamins ($n = 285$), vitamins and placebo for garlic ($n = 286$), or placebos for garlic and vitamins ($n = 285$). Similarly, those in the placebo arm for amoxicillin/omeprazole ($n = 1143$) received garlic and vitamins ($n = 285$), garlic and placebo for vitamins ($n = 286$), vitamins and placebo for garlic ($n = 286$), or placebos for garlic and vitamins ($n = 286$).

This factorial design permits unconfounded assessments of the main effects of each intervention, as well as an evaluation of their interactions. For example, the group that received garlic supplement and the garlic-placebo group were otherwise identical with respect to amoxicillin/omeprazole and vitamin-intervention distributions. This study design created 6 comparisons with which to study the effect of garlic in this population (e.g., garlic vs. placebo in the amoxicillin/omeprazole and vitamin group, garlic vs. placebo in the amoxicillin/omeprazole and vitamin placebo group, and so forth), 6 to study the effect of vitamin supplementation, and 4 to study the effect of amoxicillin/omeprazole. Within each treatment combination, there was balance for gender and age, 2 factors that influence histopathology (14).

With a two-sided 0.05 level test, the sample size of the study yielded a power for the garlic and vitamin interventions of 0.96 to detect a 5% decrease in the prevalence of dysplasia or gastric cancer from 22% to 17%; for the amoxicillin/omeprazole intervention, the power was 0.88. The power to detect a 5% reduction, to 56% from 61%, in the prevalence of severe chronic atrophic gastritis, intestinal metaplasia, dysplasia, or gastric cancer was 0.84 for the garlic and vitamin interventions and 0.68 for amoxicillin/omeprazole.

EARLY RESULTS

H. pylori *eradication.* Based on CUBT tests in 1996, an initial 2-wk course of treatment with amoxicillin/omeprazole eradicated *H. pylori* in 64% of subjects. After retreatment of 383 of those who had remained CUBT positive, the combined eradication rate rose to 73%. In 2003, 7.5 y after initial treatment, 46% of those treated with amoxicillin/omeprazole remained CUBT negative. In those given placebo, 13% were CUBT negative in 1996, and 10% in 2003.

Treatment compliance. Pill counts were used to determine the monthly percentage of subjects who had complete consumption of vitamin and garlic pills during the 7.3 y of supplementation. Except for a break in the distribution of vitamin and garlic supplements between June and July 1999, and a break in garlic supplements in September 2002, the percentage of subjects taking all of their pills was usually > 90% throughout the trial (**Fig. 1**). There was some variation in compliance by village; the highest and lowest village-specific compliance proportions are indicated by the ends of the vertical lines in Figure 1.

To confirm these pill-count data, blood was drawn from 80 randomly selected individuals throughout the study every 3 mo. The mean levels of vitamin E and vitamin C were significantly higher in the group receiving vitamin supplements than in their placebo controls. Similarly, *S*-allyl cysteine levels were higher in those administered garlic supplements than in their placebo controls.

Completeness of the histopathology data. Of the 3,411 original study subjects, 3326 were alive in 1999, and histopathology data were available for 95.9% of those alive. In 2003,

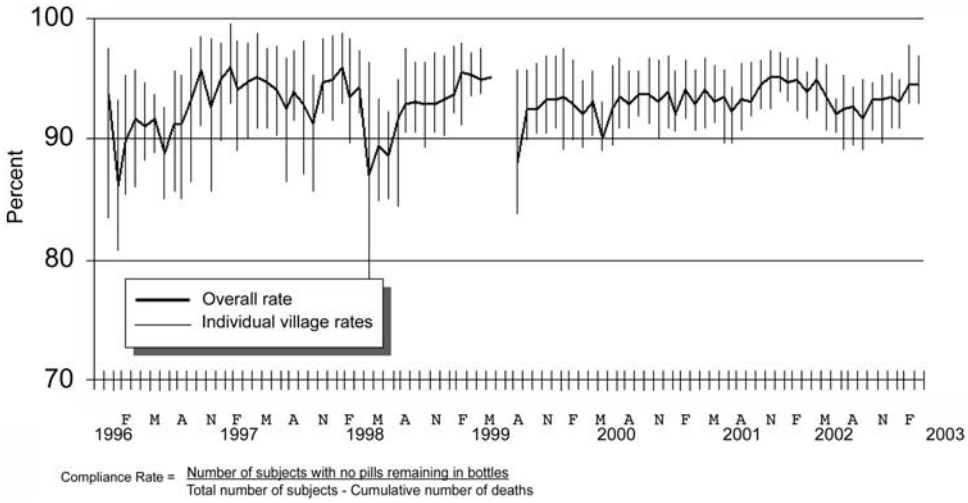

FIGURE 1 Monthly percentage of subjects taking all of their vitamin and garlic-supplement pills (dark solid line). The percentage ranges across the 13 study villages are indicated by the ends of the vertical lines.

histopathology data were available for 91.5% of the 3211 living subjects. These high rates ensure that there will be few missing end point data compared with other studies of this type.

DISCUSSION

This is the largest known, long-term, randomized, masked, placebo-controlled intervention study of a garlic supplement. The study affords the opportunity to evaluate the effects of this garlic supplementation on the prevalence of precancerous gastric lesions. The trial also offers the opportunity to evaluate the effects of a vitamin supplement and of treatment for *H. pylori.* Although the trial was designed to assess intervention effects on precancerous gastric lesions, there will be opportunities to evaluate effects on other outcomes, such as gastric cancer incidence or cholesterol levels.

Among the strengths of the trial are its randomized design, which protects against confounding of treatment effects, large size, long duration, high treatment compliance, and the extraordinarily high participation in endoscopies, resulting in nearly complete data on histopathology.

Some may question the choices of interventions. A higher *H. pylori* eradication rate could have been achieved with 3 or 4 drug regimens, or antibiotics could have been given chronically throughout the study to suppress *H. pylori*. Instead, patients were treated once for 2 wk in 1995, and those who did not respond initially were offered a single course of retreatment in 1996. We chose this approach with a view to possible preventive applications in a general population, because pilot data indicated that this amoxicillin/omeprazole regimen was well tolerated and safe, and did not require advanced medical facilities to manage complications. The choice of garlic supplement might also be questioned, and raw garlic, cooked garlic, and garlic powder would have been other options for study. The garlic preparation we studied was chosen on the basis of previously cited effects of diallyl sulfide and aged garlic extract in inhibiting carcinogenesis in animals (12,13). The Kyolic® aged garlic extract we used had the additional advantages that it was manufactured under appropriate quality control protocols and contained a standardized amount of *S*-allyl cysteine, a compound that we could measure in blood samples to monitor compliance.

In summary, this large, randomized, long-term study of *H. pylori* treatment and vitamin and garlic supplements should yield valuable information on intervention effects on precancerous gastric lesions, and may yield insights regarding the potential of these treatments to reduce the burden of gastric cancer in high risk areas.

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