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Reduction of blood lipid parameters by a 12-wk supplementation of aged black garlic: A randomized controlled trial

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ABSTRACT

Objective: The cholesterol-lowering effects of garlic as part of a healthy diet remain controversial. The aim of this study was to investigate whether supplementation with aged black garlic (ABG) could improve blood lipid profiles in patients with mild hypercholesterolemia.
Methods: We conducted a double-blind, randomized placebo-controlled trial. Sixty participants were randomly assigned to receive either ABG or placebo twice daily (total 6 g/d) before consumption of a meal every morning and evening for 12 wk. During the study, two participants dropped out of the ABG group, and three participants dropped out of the placebo group. Thus, the effects of AGB on fasting blood levels of lipids were evaluated in 28 participants and compared with the placebo group (n = 27).
Results: Among lipid components, no significant differences in triglycerides, low-density lipoprotein cholesterol, total cholesterol, or free fatty acid levels were observed between the two groups. However, ABG increased high-density lipoprotein cholesterol levels compared with the placebo group at the end of the study. Moreover, a significant decrease in the levels of alipoprotein B and a significant increase in the ratio of low-density lipoprotein cholesterol/alipoprotein B were observed in the ABG group. No adverse effects were reported in any of the patients.
Conclusion: ABG supplementation reduced atherogenic markers and thus may have a cardioprotective effect beyond the gold standard medication in patients with mild hypercholesterolemia.

Introduction

Atherogenic dyslipidemia is characterized by elevated low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), as well as decreased high-density lipoprotein cholesterol (HDL-C) [1]. Among these, an increased concentration of LDL-C is a well-known risk factor for atherosclerosis and cardiovascular diseases (CVD) and, thus, is considered the primary target for the prevention and treatment of CVD [2]. It has been suggested that apolipoprotein B (apoB) is a more informative risk marker than LDL-C for assessing CVD risk in patients with dyslipidemia [3,4]. Apolipoprotein is the protein component of plasma lipoproteins and determines the structural stability and metabolism of lipoprotein particles. ApoA1 is the major structural protein component of HDL particles, whereas apoB is present in atherogenic lipoproteins (very low-density lipoprotein, intermediate-density lipoprotein, and LDL), which each carry a single molecule of apoB protein [5]. Therefore, the ratio of apoB to apoA1 provides a predictive measure of atherogenicity and a target for lipid-lowering therapy [3,4]. Additionally, the ratio of LDL-C to apoB indirectly determines LDL particle size. Individuals

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with small, dense LDL particles have lower LDL-C-to-apoB ratios [6–8] and an increased risk for CVD, as observed in several cross-sectional and longitudinal studies [9–11].

To manage patients with hypercholesterolemia, lifestyle modification, especially diet control, is strongly recommended. However, patients usually fail to achieve such changes because they are unable or unwilling to modify their dietary habits. This failure represents an unmet medical need for patients with hypercholesterolemia, and safe and effective dietary management should therefore be considered for this population. Garlic (*Allium sativum*) is a popular herbal remedy that is steadily gaining interest as a complementary and alternative medicine [12]. This supplement has been used to prevent and treat metabolic diseases, cancer, and dementia [13]. Additionally, garlic stimulates fibrinolytic activity [14], normalizes plasma lipid imbalances [15], and reduces blood pressure [16,17]. Several compounds found in garlic, including sulfur compounds such as *S*-allylcysteine (SAC), ajoene, diallyl disulfide, allicin, SAC sulfoxide, and *S*-methylcysteine sulfoxide, may be responsible for its beneficial effects [13,18]. Although many clinical trials have reported a hypocholesterolemic effect of garlic, others have found no detectable lipid response [19–21]. This lack of concordance may be due in part to the use of different garlic preparations. Indeed, the preparation process may greatly influence the composition of the garlic product and, thus, its biological activity [22,23]. A recent meta-analysis of 39 primary trials shows that garlic is effective in reducing total serum cholesterol and LDL-C in mildly hypercholesterolemic patients [24].

Aged garlic extract (AGE) is prepared by prolonged aqueous extraction of raw garlic for a period of more than 10 mo at room temperature. Compared with raw garlic, AGE contains more SAC and *S*-allylmercaptocysteine, which have been shown to increase antioxidant levels, prevent platelet aggregation, and enhance antiatherogenic activity [22,25,26]. We produced aged black garlic (ABG) by storing the garlic in airtight containers at 90°C to 95°C and 90% humidity level for 20 d. To our knowledge, no data regarding the use of ABG in treating hypercholesterolemia have been reported. In this study, we compared the cholesterol-lowering potency of ABG in patients with mild hypercholesterolemia in a randomized, double-blind, placebo-controlled design.

Materials and methods

Study design

This study was a 12-wk, single-center, double-blind, randomized placebo-controlled clinical trial following a 3-wk screening period. Participants who responded and met entry criteria during a telephone-screening interview were scheduled for a baseline visit. The evaluation included a physical examination, electrocardiogram, and screening blood parameter studies. Participants were also scheduled for a screening visit, during which informed consent was reviewed and signed.

During the 12-wk intervention period, participants were asked to continue their usual diets and to not consume any other functional foods or dietary supplements. Participants were also asked to report any adverse events (AEs) or changes in training, lifestyle, or eating patterns, and to assess pill compliance.

Participants

Study participants were recruited from the Clinical Trial Center for Functional Foods at Chonbuk National University Hospital during 2010 by advertisement (brochure, poster, and Chonbuk National University Hospital website). In all, 130 participants were assessed for eligibility in the study. The inclusion criteria were men and women, ages 19 to 80 y. Participants were required to have a plasma LDL-C level ≥130 mg/dL at the time of screening. Individuals were ineligible if they had a history of chronic disease or a clinically significant illness within 3 mo of study entry, including a history of hepatic impairment, renal, inflammatory, cardiovascular, gastrointestinal, neurologic, neoplastic, or metabolic disease, which in the opinion of the investigators could risk their safety or the interpretation of results. Individuals were deemed ineligible if they participated in any clinical trial using an investigative medicinal product within 2 mo before the first dose was administered in the present study, were currently taking a hypolipidemic medication, or were allergic or hypersensitive to any of the ingredients in the test products. Additional exclusions included women who were pregnant or breast feeding; a history of alcoholism or drug abuse; or medical or psychological conditions that were deemed by the investigators to interfere with successful participation in the study. All participants gave written informed consent before beginning the study. AEs that newly emerged or worsened after the intervention were assessed as grade 1 (mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.), grade 2 (moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate activities of daily living), or grade 3 (severe: medically significant, but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living), according to the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) [27]. Serious AEs were defined according to the International Conference on Harmonisation (ICH) Harmonised Tripartite Guideline: any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect [28]. The study protocol was approved by the Functional Foods Institutional Review Board of Chonbuk National University Hospital (FFIRB number 2010-02-001). The protocol was registered at www.clinicaltrials.gov (NCT01402102).

Test supplement

ABG extract was obtained from Doul Agricultural Farming Corp (Namhae, Republic of Korea) and extracted according to a previously described method with minor modifications [29]. Briefly, whole garlic was packed in airtight containers and aged at 90°C to 95°C and 90% humidity level for 20 d in a constant temperature and humidity chamber. ABG was then mixed with two volumes of water and blended. Samples were extracted with water for 15 h at 100°C, and the extracts were lyophilized. ABG extract, which was standardized to produce a SAC of 0.05%, was used as the raw material to prepare the test supplement, and was administered as 100% ABG extract. The placebo supplements were composed primarily of ABG dregs, maltodextrin, sucrose, citric acid, malic acid, and tartaric acid.

The analysis of SAC was performed using a Thermo ACCELA HPLC and TSQ Quantum Access Max system (Thermo, Waltham, MA, USA) at Namhae Garlic Research Institute (Namhae, Republic of Korea). A Zorbax SB-C 18 column (300 × 4.6 mm, 5 μm) with a mobile phase composed of water and acetonitrile at 35°C was used. The flow rate was 0.7 mL/min, the injection volume 1 μL, and the retention time of SAC 6.9 min. Alliin, allicin, and *S*-methylcysteine were also quantified by same high-perfomance liquid chromatography and mass system, and retention times were 4.8, 14.2, and 3.8 min, respectively. Standards of SAC and *S*-methylcysteine were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan) and those of alliin and allicin were purchased from Sigma-Aldrich (St. Louis, MO, USA) (Table 1).

During the treatment phase of the study, test materials (ABG or placebo) were supplied as a prepackaged 3-g dose to be administered at approximately the same time every morning and evening before consumption of a meal (total 6 g/d).

Biochemical analyses

Fasting blood samples were collected at the beginning and end of the trial to determine the following efficacy parameters: total cholesterol (TC), TG, HDL-C, LDL-C, free fatty acid (FFA), apoA1, and apoB. Blood samples were analyzed with a Hitachi 7600 to 110 analyzer (Hitachi High-Technologies Corporation, Japan).

Safety assessments were based on an electrocardiogram, hematology, and laboratory tests (white blood cell, red blood cell, hemoglobin, hematocrit, platelet count, albumin, alkaline phosphatase, alanine transaminase, aspartate aminotransferase, gamma-glutamyl transferase, glucose, total bilirubin, urine

Table 1
Comparison of sulfur compounds levels in the ABG and raw garlic extracts

	Raw garlic	ABG
S-allylcysteine (mg/g)	0.38	0.13
S-methylcysteine (mg/g)	0.03	0.02
Alliin (mg/g)	11.78	0.05
Allicin (mg/g)	0.05	ND

ABG, aged black garlic; ND, not detected