Suppression of LDL Oxidation by Garlic

Benjamin H. S. Lau
Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, CA 92350

ABSTRACT It has been known for several decades that hypercholesterolemia is a major risk factor for atherosclerosis and that lowering of cholesterol can significantly reduce risk for cardiovascular diseases. More recently, oxidation of LDL has been recognized as playing an important role in the initiation and progression of atherosclerosis. Oxidized LDL, but not native LDL, promotes vascular dysfunction by exerting direct cytotoxicity toward endothelial cells, by increasing chemotactic properties for monocytes, by transforming macrophages to foam cells via scavenger-receptors and by enhancing the proliferation of various cell types, e.g., endothelial cells, monocytes and smooth muscle cells; all of these events are recognized as contributing to atherogenesis. In this paper, experimental evidence is presented that shows that several garlic compounds can effectively suppress LDL oxidation in vitro. Short-term supplementation of garlic in human subjects has demonstrated an increased resistance of LDL to oxidation. These data suggest that suppressed LDL oxidation may be one of the powerful mechanisms accounting for the antiatherosclerotic properties of garlic. J. Nutr. 131: 985S–988S, 2001.

KEY WORDS: • garlic • atherosclerosis • oxidized LDL • antioxidants • cardiovascular diseases

Cardiovascular diseases are the major cause of death in the United States and all other affluent societies in the world. There are three main groups of risk factors, i.e., diet-related, lifestyle-related and uncontrollable factors (Howard et al. 1998, Steyn et al. 1997, Villeneuve et al. 1998). Lifestyle-related risk factors include smoking, inactivity and stress. The uncontrollable factors include heredity, gender and age. Cardiovascular risk is greater for men than for premenopausal women. As a person ages, there is a greater risk of cardiovascular disease. Recent studies suggest that even these so-called “uncontrollable” factors can actually be controlled or modified (Gomez del Arco et al. 1997, Waleh et al. 1998). Antioxidants, for example, can regulate transcriptional factors that are required for gene expression (Geng et al. 1997). Hence, dietary and lifestyle changes may help keep the undesirable genes suppressed.

The most prominent cardiovascular disease risk factors are diet related. It has been known for several decades that elevated blood cholesterol and triglycerides are associated with an increased risk of cardiovascular diseases (Kannel et al. 1971). In the past decade, elevated blood homocysteine has been found to increase the incidence of cardiovascular disease (Abby et al. 1998, Welch et al. 1997); this is particularly true in individuals who suffer from such diseases yet whose blood lipids are in the normal or lower range. Hypertension, diabetes and obesity are three clinical conditions related to diet that also contribute to the increased incidence of cardiovascular diseases (Table 1).

Almost two decades ago, we reviewed the world literature on the use of garlic in modifying blood lipids and atherosclerotic diseases (Lau et al. 1983). In both animal and human studies, there was strong evidence that garlic could lower blood cholesterol and triglycerides and thus possibly reduce the incidence of cardiovascular diseases. Nearly all of the studies utilized raw or fresh garlic. Our group then undertook a project to study the effect of an odorless commercial garlic extract in human subjects with elevated blood cholesterol and triglycerides (Lau et al. 1987). We were able to demonstrate the lowering of both of these lipids with the garlic extract. We also observed a lowering of the LDL cholesterol and a slight, yet significant elevation of the HDL cholesterol. HDL is classified as “good” cholesterol because it does not contribute to atherosclerosis, whereas LDL is considered the “bad” cholesterol because it may lead to atherosclerosis. However, in this past decade, oxidation of LDL has been recognized as playing an important role in the initiation and progression of atherosclerosis (Steinberg et al. 1989, Steinberg 1997). Oxidized LDL (Ox-LDL), but not native LDL may contribute to vas-
cular dysfunction leading to atherogenesis. When LDL is oxidized, it acquires a dozen or more new properties that are absent in the native or nonoxidized LDL (Steinberg 1997). Ox-LDL acquires new antigenic properties that are recognized by the host immune system as “foreign.” Thus, Ox-LDL produces several new biologic responses; some of the prominent responses include the following: 1) a chemotactic response for monocytes, their attractions to the intima and their differentiation into macrophages; 2) the inhibition of macrophage movement from the intima; 3) enhanced occurrence of lipid-laden foam cells, characteristic of fatty streaks, the first sign of atherosclerosis; 4) damage to the endothelium; and 5) proliferation of monocytes, endothelial cells and smooth muscle cells (Fig. 1). All of these events contribute to the thickening and narrowing of arteries, the principal event in atherosclerosis (Chan 1998).

Can garlic suppress LDL oxidation? Using an in vitro model in which copper sulfate (CuSO₄) was used to oxidize LDL, we determined the effects of aged garlic extract (AGE, the same extract we used to study lipid-lowering effect) and its several constituents on LDL oxidation (Ide et al. 1997). When LDL was incubated with CuSO₄ for 24 h, a significant increase of thiobarbituric acid reactive substances (TBARS), indicating LDL oxidation, was noted; in the absence of CuSO₄, only a small quantity of TBARS was detected (Fig. 2A). AGE exhibited a concentration-dependent inhibition of Cu²⁺-induced oxidative modification of LDL as manifested by the decrease in TBARS (Fig. 2B). The effects of the water-soluble constituents of AGE on Cu²⁺-induced oxidative modification of LDL were studied. All four water-soluble garlic compounds significantly inhibited the formation of TBARS to varying degrees (Fig. 3). N-Acetyl-S-allylcysteine, a metabolite of S-allylcysteine (SAC), inhibited the LDL oxidation at a concentration of 10 mmol/L. SAC and alliin inhibited the oxidation of LDL at 10 and 1 mmol/L, respectively, whereas S-allylmercaptocysteine showed significant inhibition in both concentrations (0.1 and 1 mmol/L) tested.

A concentration-dependent inhibition of LDL oxidation was observed with the oil-soluble garlic compound, allixin (Fig. 4). In this figure, the results with a known antioxidant BHT are also presented. Allixin is of special interest because it

| TABLE 1 |
| Risk factors in cardiovascular diseases |

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**FIGURE 1** Diagram showing five important properties of oxidized LDL (Ox-LDL). 1) The entry of native LDL into the subendothelial space is followed by its oxidation by cellular oxidants produced by endothelial cells, macrophages or smooth muscle cells. Ox-LDL then recruits circulating monocytes to the intima and these cells are differentiated into macrophages. 2) Ox-LDL inhibits macrophage motility. 3) Macrophages engulf Ox-LDL via scavenger receptors to become foam cells. 4) Ox-LDL injures endothelium. 5) Ox-LDL enhances proliferation of smooth muscle and other cells.

**FIGURE 2** The effect of aged garlic extract (AGE) on Cu²⁺-induced LDL oxidation. Various concentrations of AGE (0.1 mL volume) and 0.1 mL of LDL (0.2 g protein/L) were added to 0.8 mL of 5 μmol/L CuSO₄ and incubated at 37°C for 24 h. After the incubation, the reaction was stopped by adding 0.1 mL of 10 mmol/L EDTA. The extent of lipid oxidation was determined by measuring thiobarbituric acid reactive substances (TBARS). Bars represent means ± SEM of triplicate samples. Asterisks denote significant difference (P < 0.05) compared with Cu (++) control without AGE.

**FIGURE 3** The effects of water-soluble constituents of aged garlic extract (AGE) on Cu²⁺-induced LDL oxidation. Data represent means ± SEM of triplicate samples. Asterisks denote significant difference (P < 0.05) compared with control without garlic compound. Abbreviations: SAC, S-allylcysteine; N-Ac-SAC, N-acetyl-S-allylcysteine; SAMC, S-allylmercaptocysteine; TBARS, thiobarbituric acid reactive substances.
is a phytoalexin (phyto = plant, alexin = to ward off), the major weapon for plant defense. Phytoalexins have been described as "stress compounds" because their synthesis is induced by exposure of a plant to certain kinds of stress, such as contact with bacteria, viruses, fungi, insects and chemicals (Grisebach and Ebel 1978). Allixin was previously shown to inhibit the metabolism of the chemical carcinogen aflatoxin B1 and its binding to DNA (Yamasaki et al. 1991). In this study, allixin was shown to suppress LDL oxidation. Recently we also determined the effect of allicin, an oil-soluble organosulfur compound derived from raw garlic, on LDL oxidation. We were surprised to find that allicin actually enhanced Cu2+-induced LDL oxidation (Fig. 5). It appears that allicin may behave like an oxidant rather than an antioxidant.

Ox-LDL–induced damage of endothelial cells. We used the following three in vitro assays to determine the effects of Ox-LDL on vascular endothelial cells (Ide and Lau 1997):

- lactate dehydrogenase (LDH) release as an index of membrane damage, methythiazol tetrazolium (MTT) absorbance for mitochondrial function and TBARS, indicating lipid peroxidation. When vascular endothelial cells were exposed to Ox-LDL, there was a significant increase of LDH release, indicating cell membrane damage, and a decrease of MTT absorbance, indicating mitochondrial injury. Pretreatment of vascular endothelial cells with AGE and SAC minimized these Ox-LDL–induced parameters of cellular injury. These garlic compounds also inhibited Ox-LDL–induced lipid peroxidation, implicating lipids as the principal target in Ox-LDL–mediated cellular injury.

Human studies. Two recent small-scale human studies suggest that commercial garlic preparations may increase the resistance of plasma LDL to oxidation. In one study, subjects consumed 600-mg tablets of a commercial garlic powder daily for 2 wk (Phelps and Harris 1993). Garlic supplementation decreased TBARS formation, indicating that it decreased susceptibility to LDL oxidation. In another study, subjects consumed 7.2 g AGE/d for 3 mo (Steiner and Lin 1998). Compared with the placebo group, those taking garlic had lower TBARS, again indicating an increased resistance to LDL oxidation. Another study found that garlic powder containing allicin had no demonstrable effect on either the susceptibility or resistance of LDL to oxidation (Simons et al. 1995).

We have conducted a small-scale preliminary study. This was a double-blind, placebo-controlled, crossover study involving eight subjects (4 men and 4 women; mean age, 68 y). Four subjects took 1.2 g AGE three times a day for 2 wk, then 2 wk of no garlic (washout period), followed by 2 wk of placebo. The remaining four subjects took a placebo for the first 2 wk, followed by a 2-wk washout and 2 wk of 1.2 g AGE three times a day. Blood was drawn at the beginning of the experiment, and at 2, 4 and 6 wk when the experiment was completed. Plasma LDL was isolated by a 30-min single vertical spin density ultracentrifugation (Chung et al. 1986) using a TL-100 tabletop ultracentrifuge (543,000 × g for 25 min) (Beckman Instruments, Fullerton, CA). After the addition of 5 μmol/L CuSO4, the absorbance at 234 nm was measured in a DU650 spectrophotometer (Beckman Instruments) every 2 min for 3 h. Resistance of LDL to oxidation was determined by continuous measurement of the formation of conjugated dienes (Puhl et al. 1994). The lag time of LDL oxidation was estimated from the intercept of the tangents to the slow and fast increase of diene absorption. The use of the garlic supple-

FIGURE 6  Lag times of LDL oxidation in subjects who consumed aged garlic extract (AGE) and placebos. Compared with placebo, garlic supplement significantly increased the lag time of LDL oxidation (P < 0.05, paired student’s t test), indicating its ability to increase the resistance of LDL to oxidation.
ment was found to significantly increase the resistance of LDL to oxidation (Fig. 6).

Summary

Several in vitro studies have demonstrated that garlic compounds can suppress LDL oxidation. A few small-scale human studies support the ability of garlic supplementation to increase the resistance of plasma LDL to copper-induced oxidation. Suppressed LDL oxidation may be one of the effective mechanisms that account for the beneficial effects of garlic.

LITERATURE CITED