Significance of Garlic and Its Constituents in Cancer and Cardiovascular Disease

Suppression of LDL Oxidation by Garlic Compounds Is a Possible Mechanism of Cardiovascular Health Benefit[1–3]

Benjamin H. S. Lau[4]

Department of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, Loma Linda, CA 92350

ABSTRACT Hypercholesterolemia is a major risk factor for atherosclerosis, and lowering cholesterol can significantly reduce the risk for cardiovascular diseases. Oxidation of LDL has recently 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 to endothelial cells, by increasing chemotactic properties of monocytes, by transforming macrophages to foam cells, and by enhancing the proliferation of endothelial cells, monocytes, and muscle cells. All these events are recognized as contributors to cardiovascular diseases. This paper presents experimental evidence showing that several garlic compounds can 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 mechanisms that accounts for the beneficial effects of garlic in cardiovascular health. J. Nutr. 136: 765S–768S, 2006.

KEY WORDS: • *oxidized LDL aged garlic extract cardiovascular diseases S-allylcysteine*

Cardiovascular diseases (heart attacks and strokes) are the major cause of death in all affluent societies. Three main groups of risk factors have been recognized: diet related, lifestyle related, and uncontrollable factors (1–3). 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 (4,5). *S*-allylcysteine (SAC)[5] (a garlic compound), for example, has been shown to regulate transcriptional factors that are required for gene expression (6). Hence, dietary modification may help keep undesirable genes suppressed.

The most prominent cardiovascular disease risk factors are diet related. Hyperlipidemia has long been associated with an increased risk for cardiovascular diseases (7). Elevated blood homocysteine has also been found to increase the incidence of cardiovascular disease (8,9); this is particularly true in individuals who suffer from such diseases, but 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**).

Two decades ago, we reviewed the world literature on the use of garlic in modifying blood lipids and atherosclerotic diseases (10). 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 (11). We demonstrated the lowering of both of these lipids with the use of garlic

[1] Published in a supplement to *The Journal of Nutrition*. Presented at the symposium "Significance of Garlic and Its Constituents in Cancer and Cardiovascular Disease" held April 9–11, 2005 at Georgetown University, Washington, DC. The symposium was sponsored by Strang Cancer Prevention Center, affiliated with Weill Medical College of Cornell University, and Harbor-UCLA Medical Center, and co-sponsored by American Botanical Council, American Institute for Cancer Research, American Society for Nutrition, Life Extension Foundation, General Nutrition Centers, National Nutritional Foods Association, Society of Atherosclerosis Imaging, Susan Samueli Center for Integrative Medicine at the University of California, Irvine. The symposium was supported by Alan James Group, LLC, Agencias Motta, S.A., Antistress AG, Armal, Birger Ledin AB, Ecolandia Internacional, Essential Sterolin Products (PTY) Ltd., Grand Quality LLC, IC Vietnam, Intervec Ltd., Jenn Health, Kernpharm BV, Laboratori Mizar SAS, Magna Trade, Manavita B.V.B.A., MaxiPharm A/S, Nature's Farm, Naturkost S. Rui a.s., Nichea Company Limited, Nutra-Life Health & Fitness Ltd., Oy Valioravinto Ab, Panax, PT. Nutriprima Jayasakti, Purity Life Health Products Limited, Quest Vitamins, Ltd., Sabinco S.A., The AIM Companies, Valosun Ltd., Wakunaga of America Co. Ltd., and Wakunaga Pharmaceutical Co., Ltd. Guest editors for the supplement publication were Richard Rivlin, Matthew Budoff, and Harunobu Amagase. *Guest Editor Disclosure:* R. Rivlin has been awarded research grants from Wakunaga of America, Ltd. and received an honorarium for serving as co-chair of the conference; M. Budoff has been awarded research grants from Wakunaga of America, Ltd. and received an honorarium for serving as co-chair of the conference; and Harunobu Amagase is employed by Wakunaga of America, Ltd.

[2] Author disclosure: No relationships to disclose.

[3] Supported by the Chan Shun International Foundation, San Francisco, CA, and Wakunaga Pharmaceutical Company, Osaka, Japan.

[4] To whom correspondence should be addressed. E-mail: blau@llu.edu.

[5] Abbreviations used: AGE, aged garlic extract; BHT, butylhydroxytoluene; LDH, lactate dehydrogenase; MTT, methylthiazol; Ox-LDL, oxidized LDL; SAC, *S*-allylcysteine; TBARS, thiobarbituric acid reactive substances.

TABLE 1

Risk factors in cardiovascular diseases

Lifestyle Related	Uncontrollable	Diet Related
Smoking	Heredity (genes)	Cholesterol
Inactivity	Gender	Triglycerides
Stress	Age	Homocysteine
		Hypertension
		Diabetes
		Overweight

extract. We also observed a lowering of the LDL cholesterol and a slight, but significant, elevation of the HDL cholesterol. HDL is considered 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, LDL oxidation has been recognized as playing an important role in the initiation and progression of atherosclerosis (12,13). Oxidized LDL (Ox-LDL), but not native LDL, may contribute to vascular dysfunction leading to atherogenesis. When LDL is oxidized, it acquires a dozen or more new properties that are absent in the native or non-oxidized LDL (13).

Ox-LDL acquires new antigenic properties that are recognized by the host immune system as foreign. Thus, Ox-LDL produces several new biological responses; prominent ones include the following: *1*) a chemotactic response for monocytes, their attraction 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*) proliferation of monocytes, endothelial cells, and smooth muscle cells; and *5*) damage to the endothelium. All of these events contribute to the thickening and narrowing of arteries, the principal event in atherosclerosis (14).

Ox-LDL causes damage of endothelial cells We used three in vitro assays to determine the effects of Ox-LDL on vascular endothelial cells (15): lactate dehydrogenase (LDH) release as an index of membrane damage, methylthiazol tetrazolium (MTT) absorbance for mitochondrial function and cell viability, and thiobarbituric acid-reactive substance (TBARS) for measuring lipid peroxidation. When vascular endothelial cells were exposed to Ox-LDL, there was a significant increase of LDH release, indicating cell-membrane damage (**Table 2**), and a decrease of MTT absorbance, indicating mitochondrial injury (**Table 3**). Pretreatment of vascular endothelial cells with aged garlic extract (AGE) and SAC minimized these Ox-LDL–induced parameters of cellular damage. These garlic compounds also inhibited Ox-LDL–induced lipid peroxidation, indicating lipids as the principal target in Ox-LDL–mediated cellular injury.

Can garlic suppress LDL oxidation? Using an in vitro model in which copper sulfate ($CuSO_4$) was used to oxidize plasma LDL, we determined the effects of AGE and several of its constituents on LDL oxidation (16). When LDL was incubated with $CuSO_4$ for 24 h, there was a significant increase

TABLE 2

Effects of AGE and SAC on Ox-LDL–induced LDH release from bovine pulmonary artery endothelial cells

Pretreatment[a]	Ox-LDL[b]	LDH Release[c]	
Inhibition	(0.1 g/L)	(% of total)	(%)
None	–	7.30 ± 0.28	
None	+	29.50 ± 0.99	
AGE (1 g/L)	+	27.00 ± 0.52	11.3
AGE (2.5 g/L)	+	21.70 ± 0.93*	35.1
AGE (5 g/L)	+	18.30 ± 0.39*	50.5
SAC (0.1 mM)	+	27.20 ± 0.25	10.4
SAC (1 mM)	+	23.70 ± 1.07*	26.1
SAC (10 mM)	+	13.90 ± 0.74*	70.3
SAC (20 mM)	+	9.30 ± 0.28*	91.0

[a] Vascular endothelial cells were incubated with AGE or SAC for 24 h and washed before exposure to Ox-LDL for 24 h. [b] (+) = treatment with Ox-LDL;(–) = no treatment with Ox-LDL. [c] Values are means ± SEM of triplicate samples. *Significantly different from control exposed to Ox-LDL without AGE or SAC pretreatment ($P < 0.05$).

TABLE 3

Effects of AGE and SAC on Ox-LDL–induced cell damage measured by MTT assays

Pretreatment[a]	Ox-LDL[b] (0.1 g/L)	MTT Absorbance[c] (620 nm)	Cell Viability (%)
None	–	0.137 ± 0.010	100
None	+	0.056 ± 0.002	40.9
AGE (1 g/Ll)	+	0.061 ± 0.004	44.5
AGE (2.5 g/L)	+	0.083 ± 0.002*	60.6
AGE (5 g/L)	+	0.108 ± 0.005*	78.8
SAC (0.1 mmol/L)	+	0.072 ± 0.003*	52.6
SAC (1 mmol/L)	+	0.081 ± 0.006*	59.1
SAC (10 mmol/L)	+	0.100 ± 0.001*	73.0
SAC (20 mmol/L)	+	0.114 ± 0.003*	83.2

[a] Vascular endothelial cells were incubated with AGE or SAC for 24 h and washed before exposure to Ox-LDL for 24 h. [b] (+) = treatment with Ox-LDL;(–) = no treatment with Ox-LDL.[c] Values are means ± SEM of triplicate samples. *Significantly different from control exposed to Ox-LDL without AGE or SAC pretreatment ($P < 0.05$).

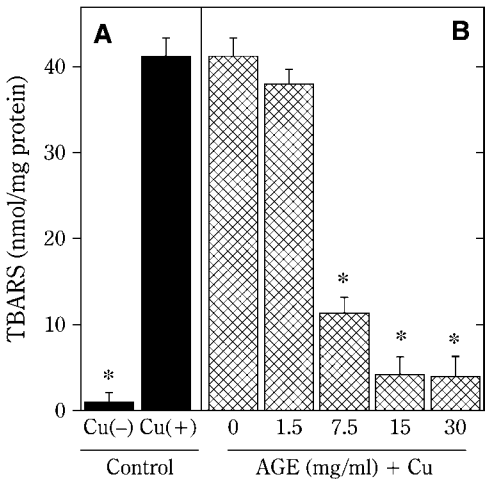

FIGURE 1 The effect of AGE on Cu^{2+}-induced LDL oxidation. Various concentrations of AGE (in 0.1 mL volume) and 0.1 mL of LDL (0.2 g protein/L) were added to 0.8 mL of 5 μmol/L $CuSO_4$ and incubated at 37°C for 24 h. After the incubation, the reaction was stopped by adding 0.1 mL of 10 mmol/L EDTA. The extent of lipid oxidation was determined by measuring TBARS. Bars represent means ± SEM of triplicate samples. Asterisks denote significant difference ($P < 0.05$) compared with Cu (+) control without AGE.

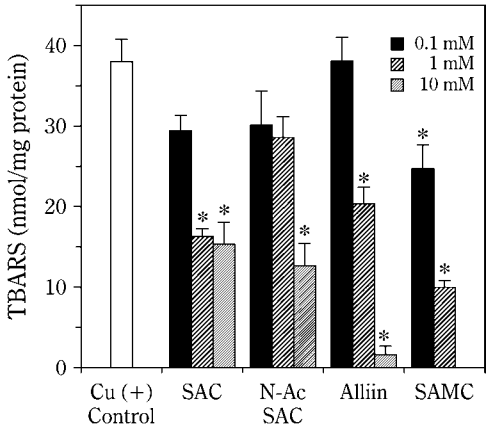

FIGURE 2 The effects of water-soluble constituents of AGE on Cu^{2+}-induced LDL oxidation. Data represent means ± SEM of triplicate samples. Asterisks denote significant difference ($P < 0.05$) compared with Cu (+) control without garlic compounds. N-Ac-SAC = *N*-acetyl-*S*-allylcysteine; SAMC = *S*-allylmercaptocysteine.

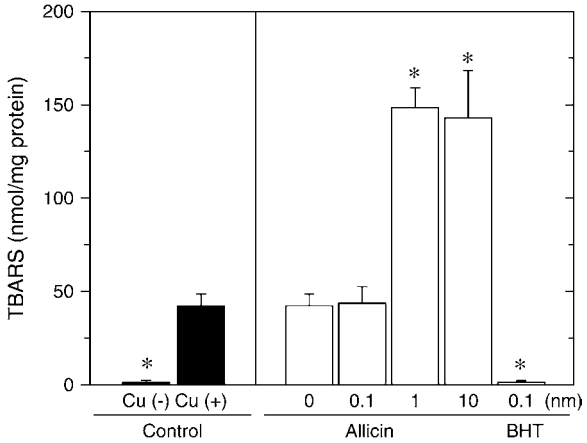

FIGURE 4 The effect of allicin on Cu^{2+}-induced LDL oxidation. Data represent means ± SEM of triplicate samples. BHT was used as an antioxidant control. Asterisks denote significant difference ($P < 0.05$) compared with respective controls at 0 points.

of TBARS, indicating LDL oxidation was noted; in the absence of $CuSO_4$, only a small quantity of TBARS was detected (**Fig. 1A**). AGE exhibited a concentration-dependent inhibition of Cu^{2+}-induced LDL oxidation as manifested by the decrease in TBARS (**Fig. 1B**). The effects of the water-soluble constituents of AGE on Cu^{2+}-induced oxidative modification of LDL were studied. All four water-soluble garlic compounds significantly inhibited the formation of TBARS to varying degrees (**Fig. 2**). A concentration-dependent inhibition of LDL oxidation was observed with the oil-soluble garlic compound, allixin (**Fig. 3**). In this figure, the results with a known antioxidant, butylhydroxytoluene (BHT), are also presented. Allixin is of special interest because it 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 (17). Allixin was previously shown to inhibit the metabolism of the chemical carcinogen aflatoxin B_1 and its binding to DNA (18). In **Figure 3**, allixin is shown to suppress LDL oxidation. We also determined the effect of allicin, an oil-soluble organosulfur compound derived from raw garlic, on LDL oxidation. Allicin was found to enhance, rather than to suppress, LDL oxidation (**Fig. 4**). It appears that allicin behaves more like an oxidant rather than an antioxidant.

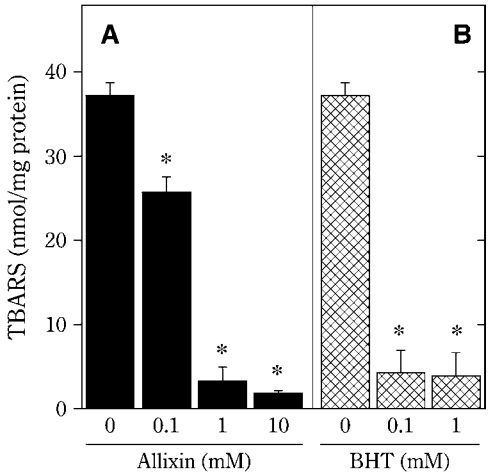

FIGURE 3 The effect of allixin on Cu^{2+}-induced LDL oxidation. Data represent means ± SEM of triplicate samples. BHT was used as an antioxidant control. Asterisks denote significant difference ($P < 0.05$) compared with respective controls at 0 points.

Human study A double-blind, placebo-controlled, crossover study involving 20 subjects (10 men and 10 women; mean age, 64 y) was conducted. The protocol was approved by the Lomba Linda University Institutional Review Board for Human Studies. Ten subjects took 1.2 g AGE 3 times a day for 2 wk, then 2 wk of no garlic (washout period), followed by 2 wk of placebo. The other 10 subjects took a placebo for the first 2 wk, followed by 2 wk of washout, and 2 wk of 1.2 g AGE 3 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 (19) using a TL-100 tabletop ultracentrifuge (543,000 × *g* for 25 min) (Beckman Instruments). After the addition of 5 μmol/L $CuSO_4$, 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 (20). The lag time of LDL oxidation was estimated from the intercept of the tangents to the slow and fast increase of diene absorption. The oral ingestion of AGE was found to significantly increase the resistance of plasma LDL to oxidation (**Fig. 5**).

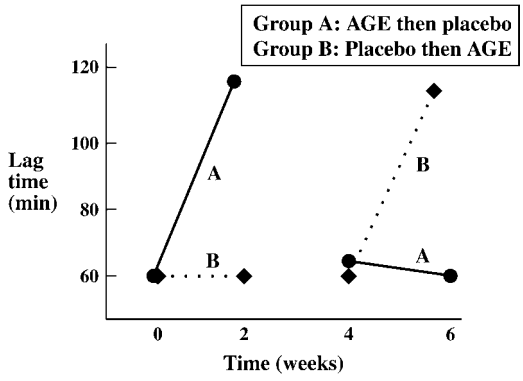

FIGURE 5 Lag times of LDL oxidation in subjects who consumed AGE and placebos. Compared with placebo, the garlic supplement significantly increased the lag time of LDL oxidation ($P < 0.05$), indicating its ability to increase the resistance of plasma LDL to oxidation.

DISCUSSION

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

LITERATURE CITED

1. Howard G, Wagenknecht LE, Burke GL, Diez-Roux A, Evans GW, McGovern P, Nieto FJ, Tell GS. Cigarette smoking and progression of atherosclerosis: the atherosclerosis risk in communities (ARIC) study. JAMA. 1998; 279:119–24.

2. Steyn K, Steyn M, Swanepoel AS, Jordaan PC, Jooste PL, Fourie JM, Rossouw JE. Twelve-year results of the coronary risk factor study (CORIS). Int J Epidemiol. 1997;26:964–71.

3. Villeneuve PJ, Morrison HI, Craig CL, Schaubel DE. Physical activity, physical fitness, and risk of dying. Epidemiology. 1998;9:626–31.

4. Gomez del Arco P, Martinez-Martinez S, Calvo V, Armesilla AL, Redondo JM. Antioxidants and AP-1 activation: a brief overview. Immunobiology. 1997; 198:273–8.

5. Waleh NS, Calaoagan J, Murphy BJ, Knapp AM, Sutherland RM, Laderoute KR. The redox-sensitive human antioxidant responsive element induces gene expression under low oxygen conditions. Carcinogenesis. 1998;19:1333–7.

6. Geng Z, Rong Y, Lau BHS. *S*-Allyl cysteine inhibits activation of nuclear factor kappa B in human T cells. Free Radic Biol Med. 1997;23:345–50.

7. Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. Ann Intern Med. 1971;74:1–12.

8. Abby SL, Harris IM, Harris KM. Homocysteine and cardiovascular disease. J Am Board Fam Pract. 1998;11:391–8.

9. Welch GN, Upchurch GR, Loscalzo J. Homocysteine, oxidative stress, and vascular disease. Hosp Pract. 1997;32:81–92.

10. Lau BHS, Adetumbi MA, Sanchez A. *Allium sativum* (garlic) and atherosclerosis: a review. Nutr Res. 1983;3:119–28.

11. Lau BHS, Lam F, Wang-Cheng R. Effect of an odor modified garlic preparation on blood lipids. Nutr Res. 1987;7:139–49.

12. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol, modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med. 1989;320:915–24.

13. Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem. 1997;272:20963–6.

14. Chan AC. Vitamin E and atherosclerosis. J Nutr. 1998;128:1593–6.

15. Ide N, Lau BHS. Garlic compounds protect vascular endothelial cells from oxidized low density lipoprotein-induced injury. J Pharm Pharmacol. 1997;49: 908–11.

16. Ide N, Nelson AB, Lau BHS. Aged garlic extract and its constituents inhibit Cu^{2+}-induced modification of low density lipoprotein. Planta Med. 1997;63:263–4.

17. Grisebach H, Ebel J. Phytoalexins, chemical defense substances of higher plants? Angew Chem Int Ed Engl. 1978;17:635–47.

18. Yamasaki T, Teel RW, Lau BHS. Effect of allixin, a phytoalexin produced by garlic, on mutagenesis, DNA-binding and metabolism of aflatoxin B_1. Cancer Lett. 1991;59:89–94.

19. Chung BH, Segrest JP, Ray MJ, Brunzell JD, Hokanson JE, Krauss RM, Beaudrie K, Cone JT. Single vertical spin density gradient ultracentrifugation. Methods Enzymol. 1986;128:181–209.

20. Puhl H, Waeg G, Esterbauer H. Methods to determine oxidation of low-density lipoproteins. Methods Enzymol. 1994;233:425–41.