

# Recent Advances on the Nutritional Effects Associated with the Use of Garlic as a Supplement

## Antioxidant Health Effects of Aged Garlic Extract<sup>1</sup>

Carmia Borek

Department of Community Health and Family Medicine, Nutrition and Infectious Diseases Unit, Tufts University School of Medicine, Boston, MA 02111

**ABSTRACT** Oxidative modification of DNA, proteins and lipids by reactive oxygen species (ROS) plays a role in aging and disease, including cardiovascular, neurodegenerative and inflammatory diseases and cancer. Extracts of fresh garlic that are aged over a prolonged period to produce aged garlic extract (AGE) contain antioxidant phytochemicals that prevent oxidant damage. These include unique water-soluble organosulfur compounds, lipid-soluble organosulfur components and flavonoids, notably allixin and selenium. Long-term extraction of garlic (up to 20 mo) ages the extract, creating antioxidant properties by modifying unstable molecules with antioxidant activity, such as alliin, and increasing stable and highly bioavailable water-soluble organosulfur compounds, such as S-allylcysteine and S-allylmercaptocysteine. AGE exerts antioxidant action by scavenging ROS, enhancing the cellular antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, and increasing glutathione in the cells. AGE inhibits lipid peroxidation, reducing ischemic/reperfusion damage and inhibiting oxidative modification of LDL, thus protecting endothelial cells from the injury by the oxidized molecules, which contributes to atherosclerosis. AGE inhibits the activation of the oxidant-induced transcription factor, nuclear factor (NF)- $\kappa$ B, which has clinical significance in human immunodeficiency virus gene expression and atherogenesis. AGE protects DNA against free radical-mediated damage and mutations, inhibits multistep carcinogenesis and defends against ionizing radiation and UV-induced damage, including protection against some forms of UV-induced immunosuppression. AGE may have a role in protecting against loss of brain function in aging and possess other antiaging effects, as suggested by its ability to increase cognitive functions, memory and longevity in a senescence-accelerated mouse model. AGE has been shown to protect against the cardiotoxic effects of doxorubicin, an antineoplastic agent used in cancer therapy and against liver toxicity caused by carbon tetrachloride (an industrial chemical) and acetaminophen, an analgesic. Substantial experimental evidence shows the ability of AGE to protect against oxidant-induced disease, acute damage from aging, radiation and chemical exposure, and long-term toxic damage. Although additional observations are warranted in humans, compelling evidence supports the beneficial health effects attributed to AGE, i.e., reducing the risk of cardiovascular disease, stroke, cancer and aging, including the oxidant-mediated brain cell damage that is implicated in Alzheimer's disease. *J. Nutr.* 131: 1010S–1015S, 2001.

**KEY WORDS:** • antioxidants • garlic • phytochemicals • chemoprevention • aging

The medicinal uses of garlic (*Allium sativum*) have a long history (Block 1985). Drawings and carvings of garlic were uncovered in Egyptian tombs, dating from 3700 BC. Its uses as a remedy for heart disease, tumors and headaches are documented in the Egyptian *Codex Ebers*, dating from 1550 BC. Garlic is mentioned in the Bible and has been a traditional treatment in many countries, notably the Near East, China and India.

Recent studies have validated many of the medicinal prop-

erties attributed to garlic and its potential to lower the risk of disease. Cancer-preventive actions of garlic, garlic extracts and its components have been demonstrated in animals (Amagase and Milner 1993, Milner 1996, Nishino et al. 1990). Epidemiologic studies show an inverse correlation between garlic consumption and reduced risk of gastric and colon cancer (Steinmetz et al. 1994). Garlic has been shown to have anti-thrombotic activity (Block 1985), lower blood lipids and have a cardioprotective effect (Neil and Sigali 1994). The mechanisms of garlic have been ascribed to its potent antioxidant action, (Wei and Lau 1998, Yang et al. 1993), its ability to stimulate immunological responsiveness (Reeve et al. 1993) and its modulation of prostanoid synthesis (Belman et al. 1989, Dimitrov and Bennink 1997).

Garlic contains unique organosulfur compounds (Block 1985), which provide its characteristic flavor and odor and most of its potent biological activity. The strong odor of fresh

<sup>1</sup> Presented at the conference "Recent Advances on the Nutritional Benefits Accompanying the Use of Garlic as a Supplement" held November 15–17, 1998 in Newport Beach, CA. The conference was supported by educational grants from Pennsylvania State University, Wakunaga of America, Ltd. and the National Cancer Institute. The proceedings of this conference are published as a supplement to *The Journal of Nutrition*. Guest editors: John Milner, The Pennsylvania State University, University Park, PA and Richard Rivlin, Weill Medical College of Cornell University and Memorial Sloan-Kettering Cancer Center, New York, NY.

TABLE 1

Antioxidant effects of aged garlic extract (AGE) compared with other garlic supplements<sup>1</sup>

Product	Source of commercial sample	Lot#	% Inhibition <sup>2</sup> (sd)
AGE	Kyolic liquid (Mission Viejo)	5LO1	+21.8 (2.73)
A	Garlinase 4000 (Enzymatic Therapy, Green Bay, WI)	213Y46QD	-13.0 (4.67)
B	Garlic powder (McCormick, Baltimore, MD)	0208A	-17.2 (5.20)
C	Quintessence caplet (Pur-Gar, Takoma, WA)	36698	-39.5 (4.85)
D	Quintessence capsule (Pur-Gar, Takoma, WA)	63121	-42.1 (5.15)
E	Garlicin (Nature's Way Product, Springville, UT)	503369	-46.8 (9.24)
F	Kwai (Lichtwerpharma, Berlin, Germany)	94080700	-50.7 (3.01)
G	Garlique (Sunsorce Health Products, Kihei, HI)	5J0010	-51.0 (10.6)
H	Garlic Time (Arizona Natural Products, Scottsdale, AZ)	896210	-54.4 (7.14)

<sup>1</sup> Laboratory Report, Wakunaga Pharmaceutical, Hiroshima, Japan, October 1995. Courtesy of Wakunaga of America Company. Garlic products were purchased from stores, as in Freeman and Kodera 1995. Each analysis was performed three times.

<sup>2</sup> Antioxidant properties were measured by the ability of the various products to inhibit the emission of low level chemiluminescence, in a liver microsomal fraction, initiated by *t*-butyl hydroperoxide (Imai et al. 1994). Inhibition (+) denotes antioxidant activity, resulting from scavenging of reactive oxygen species (ROS) and reflected in the inhibition of light emission. Inhibition (-) denotes prooxidant activity of a product as reflected in an increased light emission, induced by increased ROS activity.

garlic and its ability to generate unpleasant gastric side effects (Heber 1997, Moriguchi et al. 1997) have caused many to favor dietary garlic supplements as an optimal choice for increasing daily garlic intake. Among the many supplements, aged garlic extract (AGE)<sup>2</sup> has a reproducible array of components, which have been analyzed and studied extensively for their high antioxidant content and health-protective potential (Amagase 1997).

### Constituents of AGE

AGE is an odorless product resulting from prolonged extraction of fresh garlic at room temperature; it is highly bioavailable and has biological activity *in vitro* in both animals and humans (Moriguchi et al. 1997). AGE contains water-soluble allyl amino acid derivatives, which account for most of its organosulfur content, stable lipid-soluble allyl sulfides, flavonoids, saponins and essential macro- and micronutrients (Amagase 1998). The lipid-soluble volatile organosulfur compound allicin, which is produced enzymatically when garlic is cut or chopped, is absent in AGE. Allicin is an unstable and transient compound with oxidant activity (Freeman and Kodera 1995); it is virtually undetectable in blood circulation after garlic ingestion (Lawson et al. 1992) because it decom-

<sup>2</sup> Abbreviations: AGE, aged garlic extract; DADS, diallyl disulfide; DAS, diallyl sulfide; GHS, reduced glutathione; HIV, human immunodeficiency virus; NF- $\kappa$ B, nuclear factor- $\kappa$ B; ROS, reactive oxygen species; SAC, S-allylcysteine; SAMC, S-allylmercaptocysteine.

poses to form other organosulfur compounds (Freeman and Kodera 1995).

The major unique organosulfur compounds in AGE are water-soluble S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC), which have potent antioxidant activity (Amagase 1997, Ide and Lau 1997, Imai et al. 1994, Wei and Lau 1998). The content of SAC and SAMC in AGE is high because they are produced during the process of aging, thus providing AGE with higher antioxidant activity than fresh garlic and other commercial garlic supplements (Imai et al. 1994) (Table 1 as illustrated in Fig. 1). Studies on the pharmacokinetics of SAC in a number of animal species show that SAC is easily absorbed from the gastrointestinal tract and distributed in plasma, liver and other organs with a bioavailability of 98% in rats (Nagae et al. 1994).

Lipid-soluble compounds in AGE include diallyl sulfide (DAS), triallyl sulfide, diallyl disulfide (DADS), diallyl polysulfides and others (Amagase 1998, Amagase and Milner 1993, Awazu and Horie 1997, Horie et al. 1992). The lipid-soluble organosulfur compounds show antioxidant effects (Awazu and Horie 1997, Horie et al. 1989 and 1992).

Other antioxidants in AGE include phenolic compounds, notably allixin, whose phenolic hydroxyl group confers antioxidant activity (Ide and Lau 1997), *N*-fructosyl glutamate, *N*-fructosyl arginine (O'Brien and Gillies 1998) and selenium, as well as the organosulfur compounds.

A substantial body of evidence shows that AGE and its components inhibit the oxidative damage that is implicated in a variety of diseases and aging. These effects strongly suggest that AGE may have an important role in lowering the risk of cardiovascular disease, cancer, Alzheimer's disease and other age-related degenerative conditions, protecting human health and mitigating the effects of aging.

### Oxidation and disease

Oxidative modification of DNA, proteins, lipids and small cellular molecules by reactive oxygen species (ROS) plays a role in a wide range of common diseases and age-related degenerative conditions (Borek 1991, 1993 and 1997, Gutierrez 1993). These include cardiovascular disease (Witztum 1993), inflammatory conditions, and neurodegenerative diseases such as Alzheimer's disease (Richardson 1993), mutations and cancer (Borek 1991, 1993 and 1997). Oxidant damage by ROS is linked to photoaging, radiation toxicity, cataract formation and macular degeneration; it is implicated in ischemia/reperfusion tissue injury and thought to play a role in decreased function of some immune cells. Antioxidants, including those in AGE, which protect against oxidative dam-

Antioxidant Activity vs. Control(%)

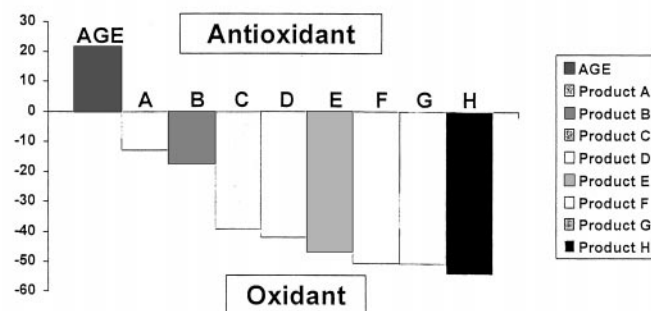


FIGURE 1 Comparison of commercial garlic products (see Table 1).

age lower the risk of injury to vital molecules and to varying degrees may help prevent the onset and progression of disease (Borek 1997, Gutteridge 1993).

### Sources of ROS

ROS include free radicals and nonradical species. The free radicals carry an unpaired electron and are unstable and reactive. They include superoxide, nitric oxide and the most reactive and toxic ROS, the hydroxyl radical. Nonradical oxidants include hydrogen peroxide, singlet oxygen and ozone, which form free radicals in tissues through various chemical reactions (Borek 1993, Gutteridge 1993).

Most of the ROS produced by cells come from the following four sources: 1) normal aerobic respiration in mitochondria, which generates superoxide radical ( $O_2^{\cdot-}$ ) and the ensuing toxic products, hydrogen peroxide ( $H_2O_2$ ) and the highly reactive hydroxyl radical ( $OH^{\cdot}$ ); 2) stimulated macrophages and polymorphonuclear leukocytes, which release superoxide and the nitric oxide radical ( $NO^{\cdot}$ ), which in turn can interact to form the nonradical destructive peroxynitrite; 3) peroxisomes, cell organelles that produce  $H_2O_2$  as a by-product of degrading fatty acid and other molecules; and 4) oxidant by-products that occur during the induction of cytochrome  $P_{450}$  enzymes.

Exogenous sources of ROS include the following: tobacco smoke, which has a broad spectrum of oxidant-ionizing radiation, which generates free radicals in exposed tissues, notably the highly reactive  $OH^{\cdot}$  radical; UV light, which produces singlet oxygen ( $^1O_2$ ) and  $OH^{\cdot}$ ; ozone ( $O_3$ ) and oxides of nitrogen in polluted air; industrial toxins such as carbon tetrachloride; drugs such as phenobarbital, which is a known tumor promoter in liver; and charcoal-broiled foods, which form a variety of carcinogens, notably benzo(a)pyrene.

Endogenous levels of ROS, which endanger our health, increase during chronic infection and inflammation, strenuous physical exercise, hypermetabolic states seen in stress, trauma and sepsis, and during exposure to exogenous sources.

### Antioxidant protection

To protect molecules against toxic free radicals and other ROS, cells have developed antioxidant defenses that include the enzymes superoxide dismutase (SOD), which dismutates superoxide; catalase and glutathione peroxidase, which destroy toxic peroxides, and small molecules including glutathione. External sources of antioxidant nutrients that are essential for antioxidant protection include antioxidant vitamins C and E, vitamin A/provitamin A and the mineral selenium, a component of selenium-dependent glutathione peroxidase (Borek et al. 1986, Borek 1993).

Phytochemicals from plant-rich diets, including garlic, provide important additional protection against oxidant damage (Borek 1997). The variety of antioxidant phytochemicals in AGE, which protect against disease-causing oxidative damage (Amagase 1997, Horie et al. 1992, Ide and Lau 1997, Wei and Lau 1998, Yamasaki et al. 1991), may act in single and combined fashion (Amagase et al. 1996, Borek 1993 and 1997).

### Antioxidant actions of AGE

**Scavenging ROS, inhibiting LDL oxidation and lipid peroxide formation.** The antioxidative actions of AGE and its components are determined by their ability to scavenge ROS and inhibit the formation of lipid peroxides. These effects are determined by measuring the decrease in ROS-induced chemiluminescence, inhibition of thiobarbituric acid reactive substances (lipid peroxides) (TBARS assay), and in vitro inhibition of the release of pentane, a product of oxidized lipids, in

the breath of an animal exposed to oxidative stress (Amagase 1997, Awazu and Horie 1997, Horie et al. 1989, Ide et al. 1996, Imai et al. 1994).

Oxidized LDL promotes vascular dysfunction, which contributes to atherosclerosis, in part by its cytotoxic effects on endothelial cells. Using an in vitro system of endothelial cells exposed to oxidant copper ions, AGE and SAC were shown to scavenge ROS, inhibit oxidation of LDL and inhibit endothelial cells injury by oxidized LDL (Ide and Lau 1997). AGE has been shown to inhibit lipid peroxide formation in several studies (Wei and Lau 1998). In one study, TBARS induced by hydrogen peroxide were inhibited 31–89% by AGE and 33–67% by SAC in a concentration-dependent manner (Yamasaki et al. 1994), thus mitigating oxidation events, which are implicated in the formation of atherogenic lesions (Efendy et al. 1997).

An additional assay, the 1,1-diphenyl-2-picryl-hydrazine assay (Imai et al. 1994), showed the antioxidant effects of allixin, SAC, SMAC and diallyl polysulfides, whose radical-scavenging action increased with the number of sulfur atoms (Imai et al. 1994). More recently, other components of AGE, *N*-fructosyl arginine and *N*-fructosyl glutamate, showed antioxidant effects by spin-resonance spectroscopy (O'Brien and Gillies 1998).

### Enhancement of endogenous cellular antioxidant defenses

**Enhancement of glutathione.** Glutathione is an important defense mechanism in living cells. As a substrate for the antioxidant enzyme glutathione peroxidase, reduced glutathione (GSH) protects cellular constituents from the damaging effects of peroxides formed in metabolism and through other ROS reactions. Decreased tissue GSH levels are associated with cell damage, depressed immunity and the progression of aging, and may increase the risk of cancer development.

AGE increases cellular glutathione in a variety of cells, including those in normal liver and mammary tissue (Liu et al. 1992). The ability of AGE to increase glutathione peroxidase and other ROS scavenging enzymes (Wei and Lau 1998) is important in radioprotection and UV suppression of certain forms of immunity (Reeve et al. 1993), in reducing the risk of radiation and chemically induced cancer (Borek 1993) and in preventing the range of ROS-induced DNA, lipid and protein damage implicated in the disease and aging processes (Gutteridge 1993).

**Enhancement of scavenging enzymes.** Studies in cell cultures of endothelia subjected to oxidant stress show that AGE protects endothelial cells from ROS injury by modifying cellular scavenging enzymes. When bovine arterial endothelial cells were exposed to the oxidants hypoxanthine and xanthine oxidase or hydrogen peroxide, the presence of AGE generated increased levels of SOD, catalase, and glutathione peroxidase, and in a dose- and time-related fashion suppressed the production of superoxide radical and hydrogen peroxide (Wei and Lau 1998). The experiments show the potential ability of AGE to protect endothelial cells from oxidant injury by ROS, which is linked to the development of atherosclerosis and cardiovascular disease (Efendy et al. 1997, Wei and Lau 1998).

AGE and SAC have also been shown to prevent oxidant-induced dense-body formation in sickle red blood cells. The dense bodies are characteristic in sickle cell anemia (Onishi 1998).

### Antioxidant effects of AGE compared with other garlic supplements

A series of studies was performed to compare the antioxidant effects of AGE, which contains mainly SAC and SAMC (Imai et al. 1994), with those of a water extract of raw garlic, which contained mainly allixin, and a heat-treated water

extract of fresh garlic, which contained mainly alliin. Using chemiluminescence and TBARS assays, the results showed that only AGE, SAC and SAMC decreased *t*-butyl hydroperoxide-induced light emission in a liver microsomal fraction and decreased TBARS, indicating a potent ROS scavenging effect. By contrast, the raw and heat-treated raw garlic extracts enhanced chemiluminescence, indicating an oxidant effect (Imai et al. 1994).

Similar studies were conducted to compare the antioxidant action of AGE with that of other commercial garlic supplements. Using the chemiluminescence assay (Imai et al. 1994), results indicated that although AGE decreased ROS-induced chemiluminescence, showing an antioxidant effect, the other commercial garlic products increased chemiluminescence, indicating a prooxidant effect (Table 1 and Fig. 1).

### **Reducing the risk of cardiovascular and cerebrovascular disease**

Oxidation of lipids, notably oxidative modification of LDL, is implicated in the development of cardiovascular and cerebrovascular disease (Cox and Cohen 1996, Witztum, 1993). Lipid oxidation products, including peroxides and toxic aldehydes such as malondialdehyde (Horie et al. 1989), can damage proteins and DNA and have also been implicated in carcinogenesis (Borek 1993). Oxidation of lipids modifies membranes and impairs their function. Fluidity is decreased, membrane-bound enzymes and receptors are inactivated, red blood cells are damaged and endothelial cells are injured, increasing blood vessel fragility. Oxidation of LDL accelerates the growth of fatty streaks in blood vessel walls (Efendy et al. 1997) and the formation of plaque (Ide and Lau 1997). Toxic aldehydes formed in lipid oxidation react with the apoprotein B of the LDL particle to produce a novel epitope that is recognized by macrophage receptors, resulting in the formation of foam cells and atherosclerotic plaques and increased risk of heart disease and stroke (Witztum 1993).

AGE inhibits lipid oxidation and oxidative modification of LDL (Ide and Lau 1997). In this way, AGE may reduce the amount of circulating oxidized LDL and the subsequent accumulation of cholesterol in macrophages, smooth muscles and blood vessel walls, resulting in the inhibition of atherogenic fatty streaks (Efendy et al. 1997). These effects, coupled with other actions of AGE, increase its potential to lower the risk of cardiovascular and cerebrovascular disease. Other protective actions of AGE include inhibition of platelet aggregation (Steiner 1996) and suppression of prostanoid synthesis with subsequent anti-inflammatory, antiathrogenic and antithrombotic effects (Dimitrov and Bennink 1997). The protection of endothelial cell integrity by inhibition of lipid peroxidation-induced injury (Geng and Lau 1997) and reduction in serum cholesterol and other lipids by AGE (Lau et al. 1987, Steiner 1996) further add to its potential in helping prevent heart disease and stroke.

### **Inhibition of oxidant ischemic brain injury**

Free radical damage and inflammatory processes, linked to enhanced levels of eicosanoids, play an important role in cerebral ischemia-reperfusion injury. Peroxynitrite, a product of superoxide and nitric oxide interactions, is thought to be a major injurious agent, inducing lipid peroxides and tissue damage (Beckman 1991). Using a rat model, AGE and its constituent SAC showed a dose-related attenuation of ROS production and inhibition of brain damage caused by ischemia-reperfusion, reducing postischemia edema. SAC was

found to decrease the size of the postischemic infarct. Only the water-soluble organosulfur compounds were effective in protecting against ROS-induced brain injury. Allyl sulfide and allyl disulfide, tested in the same manner, did not provide protection against ischemic injury (Numagami et al. 1996). These results indicate the importance of the water-soluble organosulfur compounds, the major organosulfur components in AGE, as antioxidants and their potential protective role against oxidant-induced brain damage and stroke.

### **Inhibition of nuclear factor- $\kappa$ B activation**

Nuclear factor- $\kappa$ B (NF- $\kappa$ B) is a transcription factor that is regulated by the redox state of the cell and implicated in the inducible expression of a variety of genes involved in oxidative stress and cellular responses to stress. Cytosolic NF- $\kappa$ B can be activated by mitogens, bacteria and viruses and by ROS-producing agents such as UV, ionizing radiation, hydrogen peroxide and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). The major clinical significance of NF- $\kappa$ B activation is its involvement in human immunodeficiency virus (HIV) gene expression (Griffin et al. 1989). NF- $\kappa$ B is thought to play a role in atherogenesis because minimally modified LDL has been shown to activate NF- $\kappa$ B activation (Collins 1993).

AGE and SAC inhibit TNF- $\alpha$  and hydrogen peroxide-induced activation of NF- $\kappa$ B in human T cells (Geng et al. 1997), indicating their potent antioxidant function and suggesting a potential role for AGE in modulating HIV replication. Inhibition of NF- $\kappa$ B by AGE, in part by preventing oxidative modification of LDL, further supports the role of AGE in helping prevent atherogenesis and lowering the risk of heart disease and stroke.

### **Inhibition of DNA damage and mutagenesis**

Oxidant-induced DNA damage and mutagenesis are determinants in the multistage process of cancer; inhibition of these events by phytochemical antioxidants may reduce the risk of the disease (Borek 1993 and 1997). Allixin, an important flavonoid in AGE, which has been shown to prevent oxidative modification of LDL (Ide and Lau 1997) and tumor promotion (Nishino et al. 1990), inhibits aflatoxin-induced DNA damage and mutagenesis in *Salmonella typhimurium*, in part by inhibiting cytochrome P<sub>450</sub> activity (Yamasaki et al. 1991). The mechanism of inhibition of DNA damage by allixin may be due in part to the reduction in the DNA-damaging oxidant by-products that occur during the induction of P<sub>450</sub> enzymes.

### **Inhibition of carcinogenesis, reducing the risk of cancer development**

Transformation of normal cells to the malignant state proceeds through several discernible stages, including initiation by DNA damage and later events that have been defined as tumor promotion in animals and in vitro (Borek 1993). AGE inhibits both early and late stages of carcinogenesis, resulting in inhibition of tumor growth in many tissues, including colon, mammary glands, skin, stomach and esophagus (Amagase and Milner 1993, Amagase et al. 1996, Liu et al. 1992, Milner 1996, Nishino et al. 1989 and 1990, Reeve et al. 1993).

**Inhibition of early events.** AGE exerts its cancer-inhibitory action in different and complementary ways, due to the variety of compounds present in the extract such as water- and lipid-soluble organosulfur compounds, phenolic compounds, notably allixin, saponins and selenium. Thus, the anticarcino-

genic action of AGE, which contains all of these compounds, is broad in scope.

The mode of action of the different components may depend in part on the cancer-causing agent. Certain lipid-soluble organosulfur compounds present in AGE inhibit carcinogenesis by modulating carcinogen metabolism and decreasing carcinogen binding to DNA; SAC also showed an inhibition of DNA adduct formation in mammary cells (Amagase and Milner 1993, Milner 1996). However, inhibition of ROS-induced DNA damage by AGE, which prevents cancer-causing mutations, may also play a part because these organosulfur compounds are ROS scavengers. Selenium decreases DNA adduct formation (Amagase et al. 1996) and contributes to the anti-carcinogenic/antioxidant effects (Amagase et al. 1996, Borek et al. 1986).

Inhibition of UV- and ionizing radiation-induced carcinogenesis by antioxidants has long been known (Borek et al. 1986 and 1993). In contrast to chemical carcinogens, which form adducts with DNA as well as produce ROS-mediated damage, radiation damages DNA directly with major injury induced by ROS. The anticancer effects of AGE are further shown by the ability of the extract to protect bald mice from UV light-induced skin carcinogenesis (Reeve et al. 1993).

**Inhibition of tumor promotion.** Tumor promotion in vivo and in vitro is achieved by repetitive treatments with a tumor promoter, such as 12-O-tetradecanoyl-phorbol-13-acetate, a powerful free radical-producing agent (Borek 1993). The flavonoid allixin, a component of AGE, inhibits tumor promotion in a multistep in vivo carcinogenesis skin tumor model and in vitro (Nishino et al. 1990). Similarly, AGE has potent antipromoter effects (Nishino et al. 1989), which may result from the radical-scavenging action of the organosulfur compound, allixin, and the small amounts of selenium that are acting in concert. Additional antioxidant effects that aid in the inhibition of tumor promotion are imparted because AGE enhances cellular glutathione levels and induces ROS-scavenging enzymes (Wei and Lau 1998).

#### **Radioprotection and protection against UV-induced suppression of immunity**

The radioprotective effects of AGE (Lau 1989) are mediated via the ability of the extract, its organosulfur components and phenolic compounds to scavenge free radicals (Ide et al. 1996, Wei and Lau 1998) and enhance scavenging systems in the cell, including glutathione, SOD, catalase and glutathione peroxidase (Geng and Lau 1997, Wei and Lau 1998).

AGE protects mice from UVB-induced suppression of hypersensitivity (Reeve et al. 1993). The suppression of T-cell-mediated immunity appears to be a prerequisite for the development of UV radiation-induced cancer in mice. The results suggest that the protective effect is due in part to the antioxidant effects of AGE and its ability to scavenge singlet oxygen and hydroxyl radicals produced by UV light (Borek 1993 and 1997). It also suppresses prostaglandin synthesis, which plays a role in the induction of UV contact hypersensitivity (Chung et al. 1986).

#### **Inhibition of cardiotoxicity by doxorubicin**

Doxorubicin, also known as adriamycin, is an anthracycline glycoside antibiotic widely used as an antineoplastic drug. Doxorubicin is used in the treatment of solid tumors, including breast cancer, ovarian carcinoma, small lung cell carcinoma, gastric carcinoma and lymphomas. However, therapeutic treatment is limited by cardiotoxicity related directly to the

cumulative dose of doxorubicin administered. The cardiotoxic effects of doxorubicin are related to oxidant stress caused by the semiquinone radical of doxorubicin and by superoxides, singlet oxygen and peroxy radicals, which are generated by the interaction of doxorubicin with mitochondrial membranes, causing lipid peroxidation (Awazu and Horie 1997, Kojima et al. 1994). The resulting structural and functional mitochondrial damage impairs myocardial function and may result in arrhythmia and congestive heart failure.

AGE protects mice and cardiac cells in vitro against the cardiotoxic effects of doxorubicin, preventing doxorubicin-induced lipid peroxidation (Awazu and Horie 1997, Kojima et al. 1994). Protection in vitro was also achieved by treatment with AGE diallyl polysulfides. The protective effect by AGE may have significant applications in doxorubicin cancer therapy, reducing the risk of cardiotoxicity in cancer patients receiving treatment with doxorubicin.

#### **Inhibition of oxidant-induced liver toxicity**

Supplementation with AGE may have an important protective role against liver toxicity caused by a variety of medicinal and environmental substances. AGE was recently shown to protect against oxidative damage by inhibiting lipid peroxidation in liver cells exposed to phenobarbital, a sedative and bromobenzene-3,4-oxide, an environmental toxic agent (Wang et al. 1998). Earlier studies (Tadi et al. 1991) showed that AGE protects against liver toxicity by benzo(a)pyrene and aflatoxin B<sub>1</sub>, two potent free radical-producing environmental carcinogens (Borek 1993 and 1997). Studies in mice showed that SAC and SAMC were potent inhibitors of liver toxicity induced by the industrial oxidant carbon tetrachloride and by the commonly used analgesic agent, acetaminophen (Nakagawa et al. 1988).

#### **Protection against age-related brain atrophy**

ROS play a role in age-related neurodegeneration (Richardson 1993). Studies of a senile dementia model in mice showed that AGE prevented atrophic changes in the frontal brain, improved learning abilities and memory retention, and increased longevity in the senescence-accelerated mouse (Moriguchi et al. 1997, Nishiyama et al. 1996). The data suggest that the antioxidant actions of aged garlic may have an important role in the antiaging effects. This may be achieved by the scavenging of damaging ROS, preventing the formation of lipid peroxides, protecting proteins and DNA from oxidative damage, decreasing inflammation and protecting against ROS-mediated apoptosis. The studies suggest that AGE may have antiaging effects and help in preventing age-related deterioration of brain function that are linked to dementia and Alzheimer's disease.

#### **Summary**

Much evidence indicates that oxidative modification of LDL occurs in vivo and that oxidatively modified LDL have biological effects that may promote the atherosclerotic process and play a role in heart disease and stroke. Other evidence indicates that oxidation of DNA results in mutations in critical genes that trigger cancer and that oxidative damage plays a role in aging and Alzheimer's disease.

AGE contains a wide range of antioxidants that can act in synergistic or additive fashion and protect cells against oxidative damage, thus helping to lower the risk of heart disease, stroke, cancer and Alzheimer's disease and protect against

toxic, tissue-damaging effects of ROS-producing radiation, including UV light, drugs used in therapy and chemicals in the environment and industry.

Studies on the effects of AGE have been wide in scope and have validated many of the traditional uses of garlic in medicine. The health benefits of AGE and its high antioxidant activity compared with other commercial preparations result in part from its high content of stable and highly bioavailable water-soluble organosulfur compounds. Additional human studies on AGE and its constituents will further elucidate their role in protecting human health, and molecular studies will reveal the underlying mechanisms.

## LITERATURE CITED

- Amagase, H. (1997) Antioxidant and radical scavenging effects of aged garlic extract and its constituents. In: Antioxidants and Disease, 6th Congress on Clinical Nutrition. Banff, Alberta, Canada, p. 28 (abs.).
- Amagase H. (1998) Intake of garlic and its components. Nutritional and Health Benefits of Garlic as a Supplement Conference, Newport Beach, CA, p. 4 (abs.).
- Amagase, H. & Milner, J. A. (1993) Impact of various sources of garlic and their constituents on 7,12-DMBA binding to mammary cell DNA. *Carcinogenesis* 14: 1627-1631.
- Amagase, H., Schaffer, E. M. & Milner J. (1996) Dietary components modify the ability of garlic to suppress 7,12,-dimethyl(a)anthracene induced DNA adducts. *J. Nutr.* 126: 817-824.
- Awazu, S. & Horie, T. (1997) Antioxidants in garlic. II. Protection of heart mitochondria by garlic extract and diallyl polysulfide from the doxorubicin-induced lipid peroxidation. In: *Nutraceuticals: Designer Foods III Garlic, Soy and Licorice* (Lanchance, P. P., ed.), pp. 131-138. Food & Nutrition Press, Trumbull, CT.
- Beckman, J. S. (1991) The double edged sword of nitric oxide in brain function and superoxide mediated injury. *J. Dev. Physiol.* 15: 53-59.
- Belman, S., Solomon, J. & Segal, A. (1989) Inhibition of soybean lipoxygenase and mouse skin tumor promotion by onion and garlic compounds. *J. Biochem. Toxicol.* 4: 151-160.
- Block, E. (1985) The chemistry of garlic and onion. *Sci. Am.* 252: 114-119.
- Borek, C. (1991) Free radical processes in multistage carcinogenesis. *Free Radic. Res. Commun.* 12: 745-750.
- Borek, C. (1993) Molecular mechanisms in cancer induction and prevention. *Environ. Health Perspect.* 101: 237-245.
- Borek, C. (1997) Antioxidants and cancer. *Sci. Med.* 4: 51-62.
- Borek, C., Ong, A., Mason, H., Donahue, L. & Biaglow, J. E. (1986) Selenium and vitamin E inhibit radiogenic and chemically induced neoplastic transformation in vitro by different mechanisms. *Proc. Nat. Acad. Sci. U.S.A.* 83: 1490-1494.
- Chung, H. T., Burnham, B., Robertson, L. K. & Daynes, R. A. (1986) Involvement of prostaglandins in the immune alterations caused by the exposure of mice to ultraviolet radiation. *J. Immunol.* 137: 2478-2484.
- Collins, T. (1993) Endothelial nuclear factor  $\kappa$ B and the initiation of atherosclerotic lesions. *Lab. Invest.* 68: 499-508.
- Cox, D. A. & Cohen, M. L. (1996) Effects of oxidized low density lipoproteins on vascular contraction and relaxation: clinical and pharmacological implications in atherosclerosis. *Pharmacol. Rev.* 48: 3-9.
- Dimitrov, N. V. & Bennink, M. R. (1997) Modulation of arachidonic acid metabolism by garlic extract. In: *Nutraceuticals: Designer Foods III Garlic, Soy and Licorice* (Lanchance P.P., ed.), pp. 199-220. Food & Nutrition Press, Trumbull, CT.
- Efendy, J. L., Simmons, D. L., Campbell, G. R. & Campbell, J. H. (1997) The effect of aged garlic "kyolic" on the development of experimental atherosclerosis. *Atherosclerosis* 132: 37-42.
- Freeman, F. & Koder, Y. (1995) Garlic chemistry: stability of S-(2-propenyl) 2-propene-1-sulfinothioate (allicin) in blood, solvents and simulated physiological fluids. *J. Agric. Food Chem.* 43: 2332-2338.
- Geng, Z. & Lau, B.H.S. (1997) Aged garlic extracts modulate glutathione redox cycle and superoxide dismutase activity in vascular endothelial cells. *Phytother. Res.* 11: 54-56.
- Geng, Z., Rong, Y. & Lau, B.H.S. (1997) S-Allyl cysteine inhibits activation of nuclear factor kappa B in human T cells. *Free Radic. Biol. Med.* 23: 345-350.
- Griffin, G. E., Leung, K., Folks, T. M., Kunkel, S. & Nabel, G. J. (1989) Activation of HIV gene expression during monocyte differentiation by induction of NF- $\kappa$ B. *Nature (Lond.)* 339: 70-73.
- Gutteridge, J.M.C. (1993) Free radicals in disease processes: a compilation of cause and consequence. *Free Radic. Res. Commun.* 19: 141-158.
- Heber, D. (1997) The stinking rose: organosulfur compounds and cancer. *J. Clin. Nutr.* 66: 425-426.
- Horie, T., Murayama, T., Mishima, T., Fumiyaki, I., Minamide, Y., Fuwa, T. & Awazu, S. (1989) Protection of liver microsomal membranes from lipid peroxidation by garlic extract. *Planta Med.* 55: 506-508.
- Horie, T., Awazu, S., Itakura, Y. & Fuwa, T. (1992) Identified diallyl polysulfides from an aged garlic extract which protects the membranes from lipid peroxidation. *Planta Med.* 58: 468-469.
- Ide, N. & Lau, B.H.S. (1997) Garlic compounds protect vascular endothelial cells from oxidized low density lipoprotein-induced injury. *J. Pharm. Pharmacol.* 49: 908-911.
- Ide, N., Nelson, A. B. & Lau, B.H.S. (1996) Aged garlic extract and its constituents inhibit Cu<sup>2+</sup>-induced oxidative modification of low density lipoproteins. *Planta Med.* 63: 263-264.
- Imai, J., Ide, N., Nagae, S., Moriguchi, T., Matsuura, H. & Itakura, Y. (1994) Antioxidants and free radical scavenging effects of aged garlic extract and its constituents. *Planta Med.* 60: 417-420.
- Kojima, R., Epstein, C. J. Mizui, T., Carlson, E. & Chan, P. H. (1994) Protective effects of aged garlic extracts on doxorubicin-induced cardiotoxicity in the mouse. *Nutr. Cancer* 22: 163-173.
- Lau, B.H.S. (1989) Detoxifying, radioprotective and phagocyte-enhancing effects of garlic. *Int. Nutr. Rev.* 9: 27-31.
- Lau, B.H.S., Lam, F. & Wang-Ceng, R. (1987) Effect of an odor modified garlic preparation on blood lipids. *Nutr. Res.* 7: 139-149.
- Lawson, L. D., Ransom, D. K. & Hughs B. G. (1992) Inhibition of whole blood platelet aggregation by compounds in garlic clove extracts and commercial garlic products. *Thromb. Res.* 65: 141-156.
- Liu, J. Z., Lin, X. Y. & Milner, J. A. (1992) Dietary garlic powder increases glutathione content and glutathione S-transferase activity in rat liver and mammary tissues. *FASEB J.* 6: A3230 (abs.).
- Milner, J. A. (1996) Garlic: its anticarcinogenic and antimutagenic properties. *Nutr. Rev.* 54: S82-S86.
- Moriguchi, T., Saito, H. & Nishiyama, N. (1997) Anti-aging effect of aged garlic extract in the inbred brain atrophy mouse model. *Clin. Exp. Pharmacol. Physiol.* 24:235-242.
- Nagae, S., Ushijima, M., Hatono, S., Imai, J., Kasuga, S., Itakura, Y. & Higashi, Y. (1994) Pharmacokinetics of the garlic compound S-allylcysteine. *Planta Med.* 60: 214-217.
- Nakagawa, S., Kasuga, S. & Matsuura, H. (1988) Prevention of liver damage by aged garlic extract and its components in mice. *Phytother. Res.* 1: 1-4.
- Neil, H. & Sigali, C. (1994) Garlic, its cardioprotective properties. *Curr. Top. Lipidol.* 5: 6-10.
- Nishino, H., Iwashima, A., Itakura, H., Matsuura, T. & Fuwa, T. (1989) Antitumor promoting activity of garlic extracts. *Oncology* 46: 277-280.
- Nishino, H., Nishino, A., Takayasu, A., Iwashima, Y., Itakura, Y., Koder, Y., Matsuura, H. & Fuwa, T. (1990) Antitumor promoting activity of allixin, a stress compound produced by garlic. *Cancer J.* 3: 20-21.
- Nishiyama, N., Moriguchi, T., Katsuki, H. & Saito, H. (1996) Effects of aged garlic extract on senescence accelerated mouse and cultured brain cells. In: *Preclinical and Clinical Strategies for the Treatment of Neurodegenerative, Cerebrovascular and Mental Disorders*, Vol. 11, pp. 253-258. Krager, Basel Switzerland.
- Numagami, Y., Sato, S. & Onishi, T. (1996) Attenuation of rat ischemic brain damage by aged garlic extracts: a possible protecting mechanism as antioxidant. *Neurochem. Int.* 29: 135-143.
- Nutritional and Health Benefits of Garlic as a Supplement (1989) Newport Beach, CA, pp. 1-70 (abs.).
- O'Brien, J. & Gillies, D. G. (1998) Product of the Maillard reaction in aged garlic extract are antioxidants. *Nutritional and Health Benefits of Garlic as a Supplement Conference*, Newport Beach, CA, p. 66 (abs.).
- Onishi, S. T. (1998) A possible beneficial effect of aged garlic extracts in the management of sickle cell anemia. *Nutritional and Health Benefits of Garlic as a Supplement Conference*, Newport Beach, CA, p. 66 (abs.).
- Reeve, V. E., Bosnic, M., Rosinova, E. & Boehm-Wilcox C. (1993) A garlic extract protects from ultraviolet B (280-320 nm) radiation induced suppression of contact hypersensitivity. *Photochem. Photobiol.* 58: 813-817.
- Richardson, S. J. (1993) Free radicals in the genesis of Alzheimer's disease. *Ann. N.Y. Acad. Sci.* 695: 73-76.
- Steiner, M. (1996) A double blind crossover study in moderately hypercholesterolemic men that compares the effect of aged garlic extract and placebo administration on blood lipids and platelet function. *J. Clin. Nutr.* 64: 866-870.
- Steinmetz, K. A., Kushi, L. H., Bostick, R. M., Folsom, A. R. & Potter, J. D. (1994) Vegetables, fruit and colon cancer in the Iowa Woman's Study. *J. Epidemiol.* 139: 1-5.
- Tadi, P. P., Teel, R. W. & Lau, B.H.S. (1991) Organosulfur compounds of garlic modulate mutagenesis, metabolism and DNA binding of aflatoxin B<sub>1</sub>. *Nutr. Cancer* 15: 87-95.
- Wang, B. H., Zuel, K. A., Rahaman, K. & Billington, D. (1998) Protective effects of aged garlic extract against bromobenzene toxicity to precision cut rat liver slices. *Toxicology* 126: 213-222.
- Wei, Z. & Lau, B.H.S. (1998) Garlic inhibits free radical generation and augments antioxidant enzyme activity in vascular endothelial cells. *Nutr. Res.* 18: 61-70.
- Witztum, J. L. (1993) The role of oxidized low density lipoproteins in atherogenesis. *Br. Heart J.* 69: 12-14.
- Yamasaki, T., Li, L. & Lau, B. (1994) Garlic compounds protect vascular endothelial cells from hydrogen peroxide-induced oxidant injury. *Phytother. Res.* 8: 408-412.
- Yamasaki, T., Teel, R. W. & Lau, B.H.S. (1991) Effect of allixin, a phytoalexin produced by garlic, on mutagenesis, DNA-binding and metabolism of aflatoxin B<sub>1</sub>. *Cancer Lett.* 59: 89-94.
- Yang, G. C., Yasaei, M. P. & Page, S. W. (1993) Garlic as anti-oxidant and free radical scavenger. *J. Food Drug Anal.* 1: 357-364.