Significance of Garlic and Its Constituents in Cancer and Cardiovascular Disease

Antiglycation Properties of Aged Garlic Extract: Possible Role in Prevention of Diabetic Complications[1,2]

Muhammed Saeed Ahmad and Nessar Ahmed[3]

School of Biology, Chemistry and Health Science, Manchester Metropolitan University, Manchester M1 5GD, UK

ABSTRACT Diabetes mellitus is a common endocrine disorder characterized by hyperglycemia and long-term complications affecting the eyes, nerves, blood vessels, skin, and kidneys. Increased glycation of proteins and accumulation of advanced glycation endproducts (AGEPs) have been implicated in the pathogenesis of diabetic complications. Glycation and AGEP formation are also accompanied by formation of free radicals via autoxidation of glucose and glycated proteins. Compounds with combined antiglycation and antioxidant properties may offer therapeutic potential. Recent studies suggest that aged garlic extract (AGE) inhibits formation of AGEPs in vitro and formation of glycation-derived free radicals. *S*-Allylcysteine, a key component of aged garlic, is a potent antioxidant and can inhibit AGEP formation. Aged garlic extract and *S*-allylcysteine deserve more attention and should be investigated to see whether they can reduce AGEPs in vivo. J. Nutr. 136: 796S–799S, 2006.

KEY WORDS: • *aged garlic extract* • *diabetes mellitus* • *hyperglycemia* • *S-allylcysteine* • *glycation*

Diabetes mellitus is an endocrine disorder characterized by chronic hyperglycemia, which results from an absolute or relative deficiency of or resistance to insulin. Diabetes affects 1–2% of the population, and there are around 100 million diabetic patients worldwide. This figure is expected to double over the next 10–15 y. Individuals affected by diabetes are prone to long-term complications such as retinopathy, cataract, neuropathy, atherosclerosis, nephropathy, embryopathy, and delayed healing of wounds (1).

Hyperglycemia has a key role in the pathogenesis of diabetic complications. This has been demonstrated by the Diabetes Control and Complications Trial (2) and the more recent UK Prospective Diabetes Study (3). Both of these studies have shown that diabetic patients with poor blood glucose control are more likely to develop chronic complications.

Advanced glycation endproducts. The precise mechanism underlying pathogenesis of diabetic complications is unclear. It has been suggested that during hyperglycemia, a direct reaction, referred to as the Maillard, or browning, reaction occurs between body proteins and sugars. Food scientists have been interested in the Maillard reaction for many years because it is associated with food spoilage, altered taste, and loss in nutritional value.

Protein glycation (also referred to as nonenzymatic glycosylation) is the first part of the Maillard reaction and occurs when a sugar carbonyl group reacts with a protein amino group to form a labile Schiff base that subsequently rearranges to a stable Amadori product (**Fig. 1**). Formation of the Schiff base occurs over a period of hours, whereas Amadori products form over a number of days. Glycation occurs spontaneously whenever proteins are exposed to reducing sugars and is dependent on the degree and duration of hyperglycemia in vivo.

Glycated proteins can undergo further reactions involving dicarbonyl intermediates such as 3-deoxyglucosone to form complex heterogeneous, cross-linked and fluorescent molecules

[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] To whom correspondence should be addressed. E-mail: n.ahmed@mmu.ac.uk.

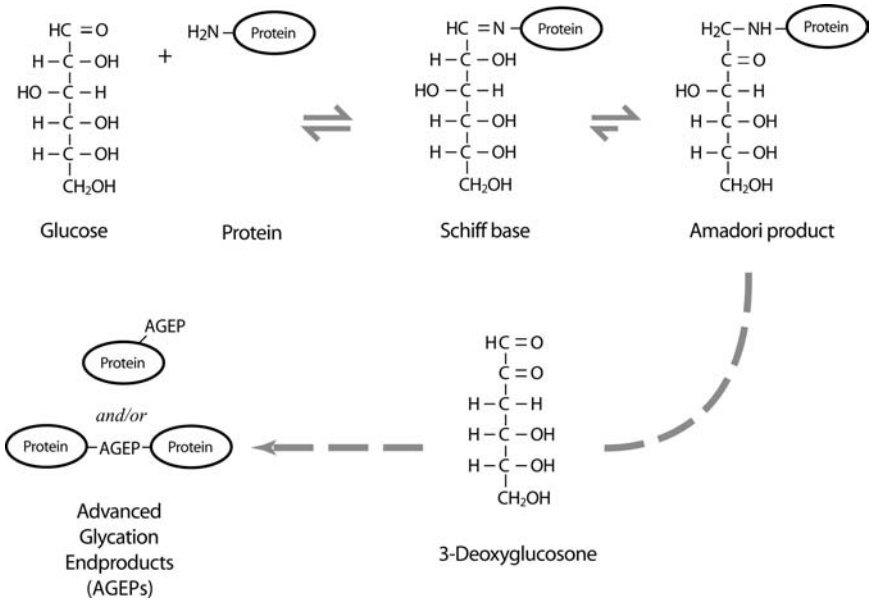

FIGURE 1 Glycation of a protein and the subsequent formation of advanced glycation endproducts (AGEPs).

called advanced glycation endproducts (AGEPs).[4] Little is known about the chemistry of AGEPs, and only a few have been characterized. In general, AGEPs can be fluorescent cross-linked structures such as pentosidine (4), nonfluorescent cross-linked structures such as arginine-lysine imidazole cross-link (5), or non cross-linked structures such as pyrraline (6).

Glycation and free radicals. Glucose and Amadori products can undergo autoxidation in the presence of transition metals to generate free radicals. Autoxidation of glucose (autoxidative glycation) generates a ketoimine capable of reacting with proteins to form AGEPs and also generates free radicals such as the superoxide radical in the process. Similarly, Amadori products undergo metal-catalyzed oxidation (glycoxidation), generating reactive dicarbonyl compounds capable of forming AGEPs and free radicals (7). Free radicals can damage proteins, lipids, and nucleic acids and might contribute toward tissue damage in diabetes (8).

AGEPs and diabetic pathology. Increased glycation and accumulation of tissue AGEPs can alter protein conformation and impair function by altering enzyme activity, modifying protein half-life, altering immunogenicity, and cross-linking of structural proteins (9,10). Many cells possess receptors for advanced glycation endproducts (RAGEs), and the interaction of AGEPs with their receptors can generate intracellular oxidative stress and activation of nuclear factor κB (NF-κB). The latter can stimulate generation of proinflammatory and adhesion molecules that in turn increase vascular permeability, which underlies the pathology of diabetic vascular complications (11). It is generally accepted that accumulation of tissue AGEPs together with increased oxidative stress has an important role in the pathogenesis of diabetic complications (10). This is outlined in **Figure 2**.

Antiglycation compounds. There is considerable interest in inhibitors of glycation because of their therapeutic potential (12). Antiglycation compounds may act by blocking carbonyl groups on reducing sugars, Amadori products, and 3-deoxyglucosones to inhibit formation of AGEPs. Certain enzymes can deglycate Amadori products and are referred to as Amadoriases. Recently drugs have been developed that can cleave AGEP cross-links and perhaps open up the possibility of reversing diabetic complications (13). Much interest has been devoted to RAGE blockers and their ability to protect against diabetic complications (14). Antioxidants may protect against glycation-derived free radicals, whereas chelators remove transition metals, preventing autoxidation of glucose and Amadori products (7,10).

The nucleophilic hydrazine compound aminoguanidine has received the most interest and is effective in reducing AGEP formation in vitro as well as in animal and human studies (15). The mechanism of action of aminoguanidine is still unclear, but it may act by blocking carbonyl groups on free sugars, Amadori products, or dicarbonyl intermediates such as 3-DG (16). However, phase III clinical trials in which aminoguanidine therapy was used to treat diabetic nephropathy proved unsuccessful because of the high toxicity encountered in many patients (15).

Other compounds such as aspirin also have a protective effect and act by acetylation of free amino groups, protecting

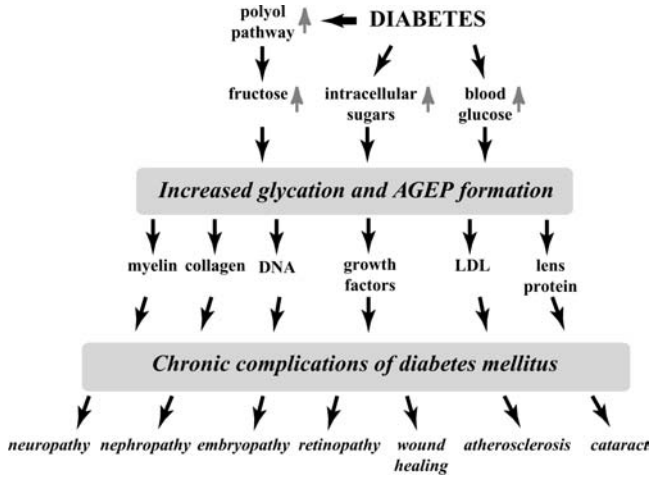

FIGURE 2 An overview of the role of protein glycation and AGEPs in the development of diabetic complications. Increased glycation and build-up of AGEPs may be mediated by glucose or reactive intracellular sugars, ultimately giving rise to diabetic complications.

[4] Abbreviations used: AGE, aged garlic extract; AGEP, advanced glycation endproduct; NF-κB, nuclear factor κB; RAGE, receptor for advanced glycation endproducts.

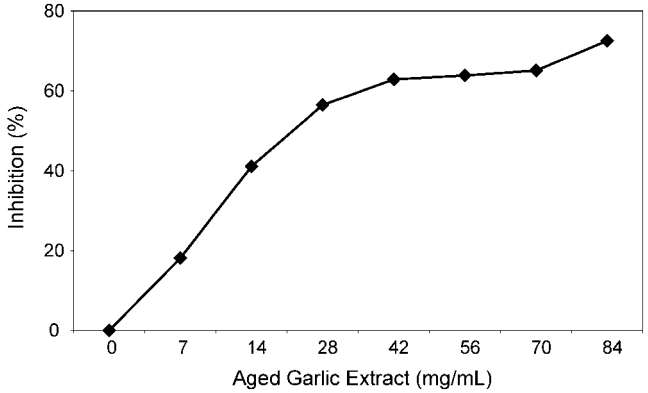

FIGURE 3 The inhibitory effect of different concentrations of aged garlic extract on AGEPs formed by incubation of lysozyme (10 g/L) in the presence of 0.5 mol/L glucose for 35 d in 0.1 mol/L Na-phosphate buffer, pH 7.4, at 37°C. The AGEPs were assessed by their cross-linking on polyacrylamide gels followed by image analysis, and the results are expressed as a percentage of the control.

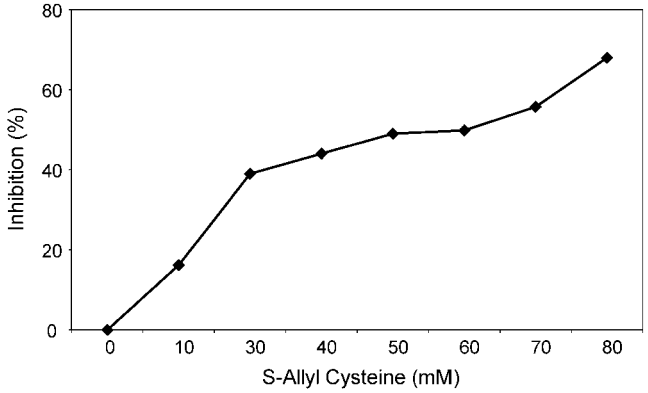

FIGURE 4 The inhibitory effect of different concentrations of *S*-allylcysteine on AGEPs formed by incubation of lysozyme (10 g/L) in the presence of 0.5 mol/L glucose for 35 d in 0.1 mol/L Na-phosphate buffer, pH 7.4 at 37°C. The AGEPs were assessed by their cross-linking on polyacrylamide gels followed by image analysis, and the results are expressed as a percentage of the control.

them against glycation (17). However, there are serious concerns about the toxicity of many of these pharmacological compounds. Furthermore, some of these compounds do not possess antioxidant activity, and recent studies suggest that glycation and AGEP-induced toxicity are associated with increased free radical activity. Recent studies in the author's laboratory have demonstrated the benefits of using compounds with combined antiglycation and antioxidant properties (18). Such compounds not only prevent AGEP formation but also reduce free radical–mediated toxicity.

Natural products with anti-glycation activity. Recent studies have highlighted the benefits of using medicinal plants with combined antiglycation and antioxidant properties in diabetic patients. Green tea from the leaves of *Camellia sinensis* is a popular drink worldwide. It contains large amounts of tannins (flavonoids), which are known for their antioxidant properties. Recently a study has shown that green tea has antiglycation activity in addition to antioxidant activity (19). Both of these properties are believed to reside in the tannin component in green tea. In this study green tea extract and tannin inhibited AGEP formation in a dose-dependent manner, with tannin being the more potent inhibitor. Other substances such as caffeine and theanine had no effect on AGEPs. Both green-tea extract and tannin had antioxidant activity, but tannin had the greater effect. Again, related substances such as caffeine and theanine had little antioxidant activity (19).

Garcinol, isolated from *Garcinia indica* fruit rind, has been shown to possess antioxidant, metal-chelating, and antiglycation properties in an in vitro system (20). In the same study, garcinol proved to be a more effective inhibitor than aminoguanidine. Recently, a water-soluble fraction obtained from tomato paste inhibited formation of AGEPs and proved to be more effective than aminoguanidine (21). This tomato fraction contained rutin, a potent antioxidant also responsible for the antiglycation activity.

Antiglycation properties of garlic. Garlic (*Allium sativum*) has been used historically for treatment of diseases associated with aging (22). Garlic is available commercially in different preparations, one of which is aged garlic extract (AGE). This is garlic that has been extracted and aged up to 20 mo, reducing its harsh irritating taste and odor. AGE has potent antioxidant activity and a high concentration of organosulfur compounds such as *S*-allylcysteine. The latter is an established antioxidant and free-radical scavenger (23). AGE protects against atherosclerosis by preventing hypertension, reducing serum cholesterol and triglycerides, and by inhibiting platelet aggregation and LDL oxidation (24,25). AGE also increases activity of antioxidant enzymes such as catalase, superoxidase dismutase, and glutathione peroxidase (26). Certain Maillard products found in AGE have been reported to possess antioxidant activity and contribute to AGE's beneficial activity (23). However, this contrasts with other studies suggesting that certain dietary AGEPs, such as carboxymethyllysine and methylglyoxal derivatives, act in the circulation in the same ways as endogenously derived AGEPs capable of inducing intracellular oxidative stress and inflammatory reactions that might contribute to the pathology of diabetic complications (27). The same study showed that the harmful effects of these AGEPs could be prevented by using antioxidants or antiglycation compounds; thus, some of the Maillard products within AGE may have a protective effect. Further studies are required to investigate the possible dual role of some dietary Maillard products. However, AGE is described as being relatively safe, as clinical studies suggest no evidence of serious toxicity even at high doses (28).

Recent studies in the author's laboratory have shown that AGE is an effective inhibitor of AGEPs in vitro (**Fig. 3**). The key ingredient responsible for the antiglycation properties of AGE was *S*-allylcysteine, which proved an effective inhibitor of AGEPs (**Fig. 4**). Similarly, another study has shown that 4 organosulfur compounds derived from garlic, diallyl sulfide, *S*-ethylcysteine, *S*-allylcysteine, and *N*-acetylcysteine, protect LDL against oxidation and glycation and may therefore explain why garlic protects against cardiovascular disease (29).

There is no information on the use of garlic in vivo in prevention of AGEP formation. Garlic and its components such as *S*-allylcysteine deserve more attention as possible cost-effective, nontoxic candidates for delaying or preventing complications of diabetes and aging.

LITERATURE CITED

1. Nathan DM. Long-term complications of diabetes mellitus. N Engl J Med. 1993;328:1676–85.

2. DCCT Research Group. The effect of intensive diabetes treatment on the development and progression of long-term complications in insulin dependent diabetes mellitus; the diabetes control and complications trial. N Engl J Med. 1993;329:977–86.

3. UKPDS. Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with Type II diabetes: (UKPDS 33). Lancet. 1998;352:837–51.

4. Sell DR, Monnier VM. Structure elucidation of a senescence cross-link from human extracellular matrix. J Biol Chem. 1989;264:21597–602.

5. Al-Abed Y, Bucala R. Structure of a synthetic glucose derived advanced glycation end product that is immunologically cross-reactive with its naturally occurring counterparts. Bioconjug Chem. 2000;11:39–45.

6. Portero-Otin M, Nagaraj RH, Monnier VM. Chromatographic evidence for pyrraline formation during protein glycation *in vitro* and *in vivo*. Biochim Biophys Acta. 1995;1247:74–86.

7. Hunt JV, Bottoms MA, Mitchinson MJ. Oxidative alterations in the experimental glycation model of diabetes mellitus are due to protein-glucose adduct oxidation. Biochem J. 1993;291:529–35.

8. Bonnefont-Rousselot D. Glucose and reactive oxygen species. Curr Opin Clin Nutr Metab Care. 2002;5:561–8.

9. Vlassara H, Palace MR. Diabetes and advanced glycation endproducts. J Intern Med. 2002;251:87–101.

10. Ahmed N. Advanced glycation endproducts—role in pathology of diabetic complications. Diabetes Res Clin Pract. 2005;67:3–21.

11. Yan S, Schmidt A-M, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, Stern D. Enhanced cellular oxidant stress by the interaction of advanced glycation endproducts with their receptors/binding proteins. J Biol Chem. 1994;269: 9889–97.

12. Monnier VM. Intervention against the Maillard reaction *in vivo*. Arch Biochem Biophys. 2003;419:1–15.

13. Vasan S, Foiles P, Founds H. Therapeutic potential of breakers of advanced glycation endproduct–protein crosslinks. Arch Biochem Biophys. 2003; 419:89–96.

14. Stern DM, Yan SD, Yan SF, Schmidt A-M. Receptor for advanced glycation endproducts (RAGE) and the complications of diabetes. Ageing Res Rev. 2002;1:1–15.

15. Thornalley PJ. Use of aminoguanidine (pimagedine) to prevent the formation of advanced glycation endproducts. Arch Biochem Biophys. 2003;419: 31–40.

16. Rahbar S, Figarola JL. Novel inhibitors of advanced glycation endproducts. Arch Biochem Biophys. 2003;419:63–79.

17. Swamy MS, Abraham EC. Inhibition of lens crystalline glycation and high molecular weight aggregate formation by aspirin *in vitro* and *in vivo*. Invest Ophthalmol Vis Sci. 1989;30:1120–6.

18. Duraisamy Y, Gaffney J, Slevin M, Smith CA, Williamson K, Ahmed N. Aminosalicylic acid reduces the antiproliferative effect of hyperglycaemia, advanced glycation endproducts and glycated basic fibroblast growth factor in cultured bovine aortic endothelial cells: Comparison with aminoguanidine. Mol Cell Biochem. 2003;246:143–53.

19. Nakagawa T, Yokozawa T, Terasawa K, Shu S, Juneja LR. Protective activity of green tea against free radical- and glucose-mediated protein damage. J Agric Food Chem. 2002;50:2418–22.

20. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H. Antioxidative and antiglycation activity of garcinol from *Garcinia indica* fruit rind. J Agric Food Chem. 2000;48:180–5.

21. Kiho T, Usui S, Hirano K, Aizawa K. Tomato paste fraction inhibiting the formation of advanced glycation endproducts. Biosci Biotechnol Biochem. 2004; 68:200–5.

22. Rahman K. Garlic and aging: new insights into an old remedy. Ageing Res Rev. 2003;2:39–56.

23. Imai J, Ide N, Nagae S, Moriguchi T, Matsuura H, Itakura Y. Antioxidant and radical scavenging effects of aged garlic extract and its constituents. Planta Med. 1994;60:417–20.

24. Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: a review. Nutr J. 2002;1:4–17.

25. Dillon S. Antioxidant properties of aged garlic extract: an in vitro study incorporating human low density lipoprotein. Life Sci. 2003;72:1583–94.

26. Wei Z, Lau BHS. Garlic inhibits free radical generation and augments antioxidant enzyme activity in vascular endothelial cells. Nutr Res. 1998;18: 61–70.

27. Cai W, Cao Q, Zhu L, Peppa M, He C, Vlassara H. Oxidative stress-inducing carbonyl compounds from common foods: novel mediators of cellular dysfunction. Mol Med. 2002;8:337–46.

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

29. Ou CC, Tsao SM, Lin MC, Yin MC. Protective action on human LDL against oxidation and glycation by four organosulfur compounds derived from garlic. Lipids. 2003;38:219–24.