Dietary Supplementation with Aged Garlic Extract Inhibits ADP-Induced Platelet Aggregation in Humans

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ABSTRACT Garlic has been widely reported to protect against cardiovascular disease by reducing serum cholesterol concentrations and blood pressure and by inhibiting platelet aggregation. However, most of these studies have been performed in hypercholesterolemic subjects or in animal models. We performed a 13-wk study in normolipidemic subjects who ingested 5 mL of aged garlic extract (AGE, Kyolic) per day. Blood was drawn from these subjects at the beginning and end of the study. Aggregation of platelet-rich plasma was induced by ADP; full lipid profiles and liver function tests were determined on serum, and plasma concentrations of eicosanoids were also measured. Dietary supplementation with AGE significantly inhibited both the total percentage and initial rate of platelet aggregation at concentrations of ADP up to 10 μmol/L. The K50 for ADP-induced aggregation were approximately doubled after supplementation with AGE, whereas the maximum rate of aggregation was unaffected. No significant changes in plasma thromboxane B2 and 6-ketoprostaglandin F1α concentrations or serum lipid profiles were observed. We conclude that AGE, when taken as a dietary supplement by normolipidemic subjects, may be beneficial in protecting against cardiovascular disease as a result of inhibiting platelet aggregation. J. Nutr. 130: 2662–2665, 2000.

KEY WORDS: • platelet aggregation • humans • garlic • ADP • eicosanoids

Garlic (Allium sativum) has been used for many centuries, as both a flavoring and a folk medicine. At present, the potential therapeutic and health-promoting effects of garlic are attracting considerable interest (Agarwal 1996). For example, garlic has been shown to be antitumorigenic (Kyo et al. 1998, Milner 1996) and to inhibit a variety of chemically induced cancers, including those of the breast (Schaffer et al. 1997) and skin (Perchellet et al. 1990). Garlic and some of its constituents have also been shown to be protective against acetaminophen (Wang et al. 1996) and bromobenzene toxicities (Wang et al. 1999).

By far the most widely studied and reported health-promoting effect of garlic is cardioprotection. Cardiovascular disease is multifactorial, and garlic appears to exert its beneficial effects at several different sites in the pathogenesis of the disease. Several reports have claimed that garlic lowers plasma cholesterol concentrations, particularly those of LDL (Neil et al. 1996, Steiner et al. 1996). More recently, an aged garlic extract was shown to inhibit directly the formation of atherosclerotic plaques in de-endothelialized carotid arteries in rabbits fed a diet supplemented with 1% cholesterol (Efendy et al. 1997). The oxidative modification of LDL is now recognized as an important process in the development of atherosclerosis, and garlic has been shown to inhibit Cu2+ -induced oxidation of LDL in vitro (Idé et al. 1997) and to protect cultured vascular endothelial cells from injury induced by oxidized LDL (Idé and Lau 1997). In addition, Munday et al. (1999) reported that ingestion of an aged garlic extract (AGE), but not raw garlic, inhibits the oxidation of subsequently isolated LDL.

Finally, garlic has also been shown to have antithrombotic effects in that it inhibits platelet aggregation in experimental animals (DeBoer and Folts 1989) and humans (Bordía et al. 1996, Lawson et al. 1992, Legnani et al. 1993, Steiner and Lin 1998) at high risk of cardiovascular disease. Indeed, the only study we are aware of that has focused on healthy subjects is that of Legnani et al. (1993) who showed that acute (6 h) and chronic (14 d) administration of dried garlic powder (Kwai, Liehtwer Pharma GmbH, Berlin, Germany) inhibited ADP- and collagen-induced platelet aggregation. In this paper, we report the effects of an AGE (Kyolic) taken by healthy subjects as a dietary supplement for 13 wk on ADP-induced platelet aggregation and on the plasma concentrations of lipids and eicosanoids. In addition, the results of liver function tests before and after dietary supplementation with AGE are reported.

SUBJECTS AND METHODS

Aged garlic extract. Aged garlic extract (AGE, Kyolic), kindly provided by Wakunaga of America (Mission Viejo, CA), is formulated by soaking sliced raw garlic (Allium sativum) in 15–20% aqueous ethanol for up to 20 mo at room temperature. The extract is then filtered and concentrated under reduced pressure at low temperature. The content of water-soluble compounds is relatively high, whereas that of oil-soluble compounds is low. The AGE used in this trial contained 305 g/L extracted solids; S-allyl cysteine, the most abundant water-soluble organosulfur compound in AGE, was present at 1.47 g/L.

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Subjects. Apparently healthy subjects (n = 23; 12 men, 11 women, age range 22–45 y) who were not taking medication for any known disease completed the study which had the prior approval of the Ethics Committee of Liverpool John Moores University. Subjects consumed 5 mL of AGE (taken in a small volume of fruit juice) daily for 13 wk between 0700 and 0900 h; otherwise, subjects followed their usual diet and lifestyle (including alcohol intake). Three subjects were smokers. All subjects refrained from taking aspirin or other drugs known to affect hemostasis and/or platelet aggregation for 2 wk before and during the study. Blood samples (34 mL) were taken after an overnight (12-h) fast immediately before ingestion of AGE and under similar conditions after 13 wk of ingestion of AGE (i.e., 24 h after the last dose). The major portion of the sample (27 mL) was added to 38 g/L trisodium citrate in the ratio 9:1 (v/v, blood/anticoagulant) for preparation of plasma (1500 × g for 20 min) for lipid and liver function assays. The remaining 3 mL of the blood sample was added to EDTA/indomethacin (20 g/L EDTA, 9 g/L NaCl, pH 7.4, containing 2 mmol/L indomethacin) as anticoagulant) for platelet aggregation studies, and 4 mL was allowed to clot at room temperature for preparation of serum (1500 × g for 20 min) for eicosanoid assays. Serum and plasma samples for biochemical assays were stored at −20°C for 20 min) for eicosanoid assays. Serum and plasma samples for biochemical assays were stored at −70°C for up to 3 mo before analysis.

Platelet aggregation. Platelet aggregation was determined within 2 h of blood being drawn. Platelet-rich plasma (PRP) was prepared by centrifugation of blood at 1500 × g for 20 min) for eicosanoid assays. Serum and plasma samples for biochemical assays were stored at −20°C for 20 min) for eicosanoid assays. Serum and plasma samples for biochemical assays were stored at −70°C for up to 3 mo before analysis.

RESULTS

The extent of platelet aggregation in response to ADP was reduced after dietary supplementation with 5 mL of AGE/d for 13 wk (Fig. 1A). This was most dramatic at low concentrations of ADP (P < 0.05; 0.5, 1 and 2 μmol/L) when the extent of aggregation was submaximal. At ADP concentrations ≥ 4 μmol/L, total percentage aggregation was maximal at 70–80% and was not significantly affected by ingestion of AGE.

The rate of ADP-induced platelet aggregation was similarly reduced after dietary supplementation with AGE (Fig. 1B). This was significant at all concentrations of ADP up to 10 μmol/L. All three transformations of these data showed that ingestion of AGE had little effect on the Rmax for ADP-induced aggregation, whereas the Km was approximately doubled (P < 0.007 for the Eadie-Hofstee and Hanes-Woolf transformations, Table 1).

Dietary supplementation with 5 mL of AGE/d for 13 wk had little effect on serum lipid concentrations in healthy humans. It is noteworthy that serum triglycerides were decreased by 13% and total and LDL cholesterol were decreased by 3% each; however, these differences were not significant.

![Figure 1](https://academic.oup.com/jn/article-abstract/130/11/2662/4686162/535x111)
HDL cholesterol was unchanged (Table 2). Similarly, little change was seen with serum enzymes, although serum ALT was significantly decreased by nearly 20% after ingestion of AGE (Table 2). Plasma concentrations of TXB₂ and 6-keto-PGF₁α tended to be decreased (32 and 18%, respectively) after ingestion of AGE; however, these decreases were not significant (Table 2).

DISCUSSION

A major problem in interpreting studies that investigate the health-promoting effects of garlic supplementation is that many different garlic preparations (and dosing regimens) have been used. The main garlic preparations available commercially are pressed garlic juice, garlic oil, dry powder, liquid and is standardized to 4.6% diallyl disulfide, which is called kwai, or garlic powder (Kwai) used by Legnani et al. (1993) was alliin.

Supplementation with AGE had no significant effects on serum total, LDL or HDL cholesterol and triglyceride concentrations (Table 2). This is in contrast to some previously reported studies (Agarwal 1996, Lau et al. 1987, Steiner et al. 1996, Warshafsky et al. 1993) and could be due to the fact that the subjects who took part in our study were essentially normolipidemic. Using the European Atherosclerosis Society guidelines, 11 of 23 of our subjects had serum cholesterol concentrations < 5.2 mmol/L (200 mg/dL), whereas the remaining 12 had concentrations of 5.2–7.8 mmol/L; none exceeded 7.8 mmol/L (300 mg/dL). Lau et al. (1987) also did not observe a decrease in serum cholesterol concentrations when the subjects under investigation were normolipidemic. It has also been reported that a hypocholesterolemic effect occurs only after long-term (6 mo) dietary administration of AGE (Lau et al. 1987). Our study lasted 3 mo and, indeed, it has been reported that an initial rise in serum cholesterol is seen in the first 2 mo of AGE administration with concentrations decreasing by mo 4 (Lau et al. 1987).

Dietary supplementation with AGE had little effect on liver function as judged by the serum activity of four enzymes (Table 2). However, it is noteworthy that AGE caused a significant decrease in the serum activity of ALT. This cytosolic enzyme is released into blood from damaged hepatocytes. Thus, this observation suggests that AGE is hepatoprotective and is in agreement with both in vitro (Wang et al. 1999) and in vivo (Nakagawa et al. 1988) studies in animals that have demonstrated that AGE protects against known hepatotoxins such as carbon tetrachloride and bromobenzene.

Possibly the most important result of our clinical trial was that after 3 mo of dietary supplementation, AGE inhibited both the total percentage and initial rate of ADP-induced platelet aggregation (Fig. 1). Steiner and Lin (1998) conducted a similar study in which 7.2 g of dried AGE (equivalent to ~25 ml of liquid AGE) was given as a daily supplement to hypercholesterolemic men for 10 mo. In that study, inhibition of platelet aggregation induced by epinephrine and collagen was seen, but not of aggregation induced by ADP; inhibition of platelet adhesion to fibrinogen was also seen. Steiner and Lin (1998) reported the median effective concentration (EC₅₀) of ADP for platelet aggregation to be ~1.5 μmol/L, which is similar to the KM values reported in Table 1. Although the EC₅₀ for ADP was unaffected by the supplementation regimen used by Steiner and Lin (1998), the KM for ADP was approximately doubled by our supplementation regimen (Table 1), indicating a decrease in the affinity of the platelet ADP receptor for its ligand.

PGI₂, the major arachidonic acid metabolite formed by the vascular endothelial cells, is a potent vasodilator and inhibitor of platelet aggregation. In contrast, the major arachidonic acid metabolite formed by platelets is TXA₂, which is a potent vasoconstrictor and stimulator of aggregation. It is the balance between these eicosanoids that is important in regulating hemostasis and platelet aggregation. These eicosanoids are extremely short-lived in plasma and are invariably measured as their stable metabolites, 6-keto-PGF₁α and TXB₂. Supplementation with AGE produced apparent decreases in plasma 6-keto-PGF₁α and TXB₂ concentrations of 18 and 32% respectively (Table 2). However, these decreases were not significant and, perhaps more importantly, AGE had little or no effect on the balance between these eicosanoids. This implies that di-

### TABLE 1

**Kinetic parameters for ADP-induced platelet aggregation in humans before and after ingestion of aged garlic extract (AGE) for 13 wk**¹²

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before AGE</th>
<th>After AGE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lineweaver-Burk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KM, μmol/L</td>
<td>3.84 ± 0.67</td>
<td>8.02 ± 2.8</td>
<td>0.15</td>
</tr>
<tr>
<td>R_max, %/min</td>
<td>215 ± 21</td>
<td>253 ± 33</td>
<td>0.38</td>
</tr>
<tr>
<td>Eddie-Hofstee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KM, μmol/L</td>
<td>1.94 ± 0.22</td>
<td>2.84 ± 0.25²</td>
<td>0.0066</td>
</tr>
<tr>
<td>R_max, %/min</td>
<td>168 ± 5</td>
<td>165 ± 6</td>
<td>0.65</td>
</tr>
<tr>
<td>Hanes-Woolf</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KM, μmol/L</td>
<td>1.86 ± 0.23</td>
<td>3.73 ± 0.36²</td>
<td>0.0002</td>
</tr>
<tr>
<td>R_max, %/min</td>
<td>166 ± 6</td>
<td>182 ± 8²</td>
<td>0.039</td>
</tr>
</tbody>
</table>

¹ KM values for ADP and maximal rates of aggregation (R_max) were calculated from three different linear transformations of the data shown in Figure 1(b).

² Values are means ± SEM, n = 23. * Significantly different (P < 0.05) before and after ingestion of AGE.

### TABLE 2

**Serum lipids and enzymes and plasma eicosanoids in humans before and after ingestion of aged garlic extract (AGE) for 13 wk**¹

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before AGE</th>
<th>After AGE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>5.32 ± 0.24</td>
<td>5.15 ± 0.22</td>
<td>0.13</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.75 ± 0.23</td>
<td>3.65 ± 0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.10 ± 0.06</td>
<td>1.10 ± 0.05</td>
<td>0.81</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.04 ± 0.16</td>
<td>0.90 ± 0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Serum enzymes, U/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase</td>
<td>21.5 ± 1.1</td>
<td>20.8 ± 1.8</td>
<td>0.71</td>
</tr>
<tr>
<td>Alanine aminotransferase</td>
<td>14.3 ± 1.2</td>
<td>11.4 ± 1.1*</td>
<td>0.019</td>
</tr>
<tr>
<td>Gamma glutamyltransferase</td>
<td>21.6 ± 1.9</td>
<td>20.1 ± 1.9</td>
<td>0.880</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>115.6 ± 7.3</td>
<td>112.4 ± 7.9</td>
<td>0.39</td>
</tr>
<tr>
<td>Plasma eicosanoids, ng/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Keto prostaglandin F₁α</td>
<td>67.7 ± 12.4</td>
<td>55.7 ± 4.0</td>
<td>0.38</td>
</tr>
<tr>
<td>Thromboxane B₂</td>
<td>69.5 ± 10.8</td>
<td>47.3 ± 3.6</td>
<td>0.065</td>
</tr>
</tbody>
</table>

¹ Values are means ± SEM, n = 23. * Significantly different (P < 0.05) before and after ingestion of AGE.
etary supplementation with AGE does not affect the cyclooxygenase pathway in vivo. Serum TXB_2 was also unaffected 6 h after administration of 900 mg of dried garlic powder (Kwai), whereas ADP- and collagen-induced platelet aggregation was inhibited (Legnani et al. 1993). However, it must be remembered that some garlic preparations have been reported to modulate the cyclooxygenase pathway in vitro (Ali and Mohammed 1986, Srivastava 1984).

The production of nitric oxide (NO) by endothelial cells is another important regulator of platelet activity (Moncada and Higgs 1995). However, it is not known whether AGE, or any other garlic preparation, affects either NO production or action.

Aggregation of platelets is a consequence of exposure of fibrinogen receptors on the surface of the cells. These receptors bind fibrinogen in the presence of extracellular Ca^{2+} and cross-link the platelets to form aggregates. The fibrinogen receptor is a heterodimer of the membrane glycoproteins (GP)IIb and IIIa, and following stimulation, cross-linking the GPIIb-IIIa complex at their surface, this complex is unable to bind fibrinogen until platelets are activated, for example, by ADP (Hurani and Hall 1994). The GPIIIb-IIIa receptor has a high content of -SH groups, and binding of fibrinogen is inhibited by the organosulfur compound ajoene (Apitz-Castro et al. 1994). Steiner and Lin (1998) suggested that the inhibition of fibrinogen-mediated platelet adhesion is due to an organosulfur compound(s) in dried AGE reducing the functional competence of some GPIIb-IIIa receptors, whereas sufficient receptors remain to sustain full ADP-induced aggregation. In contrast, ADP-induced platelet aggregation was inhibited by the AGE supplementation regimen used in our study (Fig. 1, Table 1). We postulate that the most likely mechanism for this involves the ADP receptor. Platelet ADP receptors belong to the P_2T subtype of purinoreceptors whose activation leads to a rise in intracellular Ca^{2+}. Thus, one possibility would be that AGE inhibits the ADP-induced rise in cytosolic Ca^{2+} concentrations. Indeed, aqueous extracts of crushed garlic have been shown to inhibit the uptake of Ca^{2+} into platelets (Mayeux et al. 1988). Clearly, further studies are required to elucidate these mechanisms.

In conclusion, our data clearly indicate that AGE, when taken as a dietary supplement, inhibits ADP-induced platelet aggregation in healthy subjects and may have a role in the prevention and management of cardiovascular disease.

**REFERENCES**


