Significance of Garlic and Its Constituents in Cancer and Cardiovascular Disease

# Aged Garlic Extract Is a Potential Therapy for Sickle-Cell Anemia[1,3]

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ABSTRACT Sickle-cell anemia is one of the most prevalent hereditary disorders with prominent morbidity and mortality. Oxidative phenomena play a significant role in the disorder's pathophysiology. A forumlation of garlic (*Allium sativum*), AGE, has been reported to exert an antioxidant effect in vitro. We evaluated the antioxidant effect of AGE on sickle red blood cells (RBCs). Five patients (two men and three women, mean age 40 ± 15 years, range 24–58 years) with sickle-cell anemia participated in the study. AGE was administered at a dose of 5 mL daily. Whole blood samples were obtained at baseline and at 4 wk, primarily for Heinz body analysis. In all patients, the number of Heinz bodies decreased over the 4-wk period (58.9 ± 20.0% at baseline to 29.8 ± 15.3% at follow-up; $P = 0.03$). These data suggest that AGE has a significant antioxidant activity on sickle RBCs. AGE may be further evaluated as a potential therapeutic agent to ameliorate complications of sickle-cell anemia. J. Nutr. 136: 803S–805S, 2006.

KEY WORDS: • *aged garlic extract* • *sickle-cell anemia* • *S-allylcysteine*

Sickle-cell disease is one of the most prevalent hereditary disorders with prominent morbidity and mortality. The disease may affect various ethnic groups, such as people of Hispanic and Middle Eastern descent, but it affects people of African descent the most. Clinical manifestations of sickle-cell disease are largely due to a hemolytic process leading to severe anemia and vaso-occlusion, resulting in pain and organ damage. In the pathophysiology of sickle-cell disease, increased oxidant susceptibility of sickle red blood cells (RBC)[5] has been demonstrated to play a major role (1–7).

Recent investigations have brought forth ample data that support significant antioxidant activity of garlic (*Allium sativum*) (8–12). Among various preparations of garlic supplements, AGE, in particular, has been associated with antioxidant activities in sound scientific experiments (8,10,11,13–18). Therefore, based on these data, we have examined the potential role of AGE as an antioxidant in sickle-cell disease.

***AGE.*** AGE (Kyolic), donated by Wakunaga of America, is formulated by soaking sliced raw garlic in 15 to 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 of extracted solids; *S*-allylcysteine, the most abundant water-soluble organosulfur compound in AGE, was present at a concentration of 1.47 g/L.

***Study population.*** The participants were individuals with an established diagnosis of sickle-cell anemia [hemoglobin (HGB)SS] by hemoglobin electrophoresis and were 18 y or more of age. The exclusion criteria were any significant medical conditions other than sickle-cell disease, including diabetes mellitus, renal failure, or heart failure, pregnancy, and history of treatment with any antisickling agents within 12 mo of the

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[5] Abbreviations used: GSH, reduced glutathione; HGB, hemoglobin; HCT, hematocrit; NAD, nicotinamide adenine dinucleotide; RBC, red blood cell; RET, reticulocyte.

initiation of the study. This project was approved by the Internal Review Board of Harbor–UCLA Research and Education Institute. All participants were volunteers and signed appropriate consent forms after careful explanation and review of the protocol.

***Administration of AGE.*** After obtaining consent, each patient was seen at baseline for interview, physical examination, and baseline blood tests. A urine pregnancy test was also performed for each woman of child-bearing age. Participants were instructed to self-administer liquid AGE at a dose of 5 mL daily.

At 4 wk, the patients were reevaluated with a brief physical examination and interview. Also, whole blood samples were drawn for evaluation, including the Heinz body test.

***Biochemical and physiological parameters.*** A Coulter counter was used for determination of RBC counts and HGB levels.

Heinz bodies were evaluated with a standard method using crystal violet solution (19–21). The number of RBCs containing five or more Heinz bodies was counted and expressed as a percentage.

***Statistical analysis.*** All values are reported as means ± SD The paired Student's *t* test was used to evaluate the differences of variables between baseline and follow-up data. All tests of significance were two-tailed, and significance was defined at $P < 0.05$.

## RESULTS

Five patients (two men and three women, mean age 40 ± 15 y, range 24–58 y) were entered in the study. **Table 1** summarizes the hematological data, RBC glutamine content, and the results of the Heinz body analysis at baseline and after 4 wk of AGE administration. There were no significant changes in RBC count, HGB level, hematocrit (HCT), or reticulocyte (RET) count. However, the average Heinz body count decreased from 58.9 to 29.8% ($P < 0.03$). In all patients, the average Heinz body count decreased significantly at 4 wk compared with the baseline (**Fig. 1**).

## DISCUSSION

Recent studies (1–7,22–25) have shown that oxidative phenomena play a significant role in the pathophysiology of sickle-cell disease. Oxidant stress may contribute to the sickling process through the formation of "dense cells," the development of vaso-occlusion, and shortened RBC survival (1–7). The oxidant damage in sickle RBCs is most likely a consequence of the inherent instability of HGB S (22,25), which results in a concomitant increase in free-radical generation (2)

## TABLE 1

*Baseline and follow-up comparisons*

| | | Baseline | Follow-up | *P* |
|---|---|---|---|---|
| RBC | $\times 10^6$ | 3.64 ± 1.51 | 3.48 ± 1.24 | 0.25 |
| HBG | g/l | 98.8 ± 34.6 | 95.4 ± 29.5 | 0.21 |
| HCT | % | 29.7 ± 11.0 | 28.3 ± 9.0 | 0.20 |
| RET | % | 6.7 ± 2.8 | 6.7 ± 2.6 | 0.59 |
| Hexokinase | $\mu$mol/min/$10^{10}$ RBC | 0.88 ± 0.83 | 1.57 ± 1.60 | 0.18 |
| GSH | $\mu$g/$10^{10}$ RBC | 409 ± 147 | 455 ± 42 | 0.54 |
| NAD ratio | % | 48.7 ± 12.0 | 40.8 ± 11.9 | 0.25 |
| Heinz body | % | 58.9 ± 20.0 | 29.8 ± 15.3 | 0.03 |

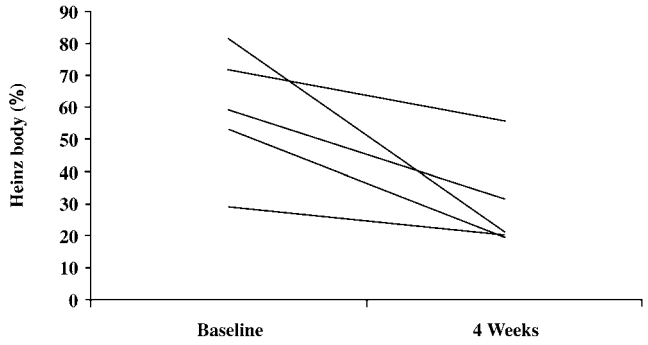


**FIGURE 1** Heinz body (%) from blood samples at entry into study and at 4-wk follow-up.

in association with impaired antioxidant defense (1,4,23,24). With sustained intracellular production of oxygen free radicals, the three-dimensional structure of HGB is sufficiently affected to lower its solubility. These factors lead to formation of Heinz bodies that are aggregates of insoluble hemochromes (26).

The Heinz bodies, which adhere to the RBC membrane (26), may themselves cause significant damage to the membrane. In any case, assessment of Heinz bodies is a useful gauge in evaluating susceptibility of RBC to oxidant stress (4,7). The data in the study are preliminary in nature. However, AGE therapy was associated with a decrease in Heinz bodies in sickle RBC in each patient. The data were consistent with our hypothesis and confirm previous reports that demonstrated antioxidant activities of AGE (8,10, 11,13–18).

In regard to hematological parameters, AGE had no significant effect on RBC count, HGB level, and HCT. Reports (27–32) have shown in animals that garlic extract may affect the RBC count adversely during the acute phase by inducing hemolytic anemia. Ironically, these are thought to be caused, at least partially, by oxidant stress caused by compounds such as allicin contained in ordinary garlic extract. Allicin has been shown to enhance LDL oxidation (16) and to oxidize the iron of HGB in RBC with methemoglobin formation (33). AGE, on the other hand, has been processed to eliminate these compounds without the loss of water-soluble antioxidant compounds such as *S*-allylcysteine and fructosyl arginine. One of these water-soluble compounds, *S*-allylcysteine, has been shown in vitro to inhibit the formation of dense cells in blood samples from sickle-cell anemia patients (34,35). Thus, AGE appears to have less capacity for inducing oxidant stress than ordinary garlic extracts (36), yet it maintains its antioxidant activity (8,10,11,13–18). In this study, there were no adverse effects, including hematological parameters, during the few weeks of AGE administration.

In conclusion, we have demonstrated, in a small cohort of sickle-cell anemia patients, an association of AGE therapy with a decrease in Heinz bodies in their RBCs. One must be cautioned that the data presented here are preliminary and the study was an open-labeled nonrandomized trial. However, the results suggest that AGE has a potential effect as an antioxidant in sickle-cell disease. Previously, AGE has been shown to significantly improve erythrocyte deformability through stabilization of erythrocyte membranes in nonsickle RBCs (37). These phenomena were attributed to the antioxidant activities of AGE (37). At baseline, erythrocyte deformability is further altered in sickle RBC due to an abnormal, highly permeable membrane (38). This abnormality is thought to contribute, at least partially, to the formation of dense cells (39). The dense cells, in turn, react with inflammatory cells and endothelial cells leading to vaso-occlusive changes (40). AGE may also improve

erythrocyte membrane stability in sickle RBC through antioxidant activities, as suggested by the data presented here in which there was a reduction of Heinz bodies in sickle RBC during daily AGE administration. Again, the data are preliminary, but they are consistent with previous findings regarding antioxidant effects of AGE. Further testing is warranted for confirmation of the efficacy of this relatively harmless agent in the management of sickle-cell disease.

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