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Research article

Aged garlic extract therapy for sickle cell anemia patients

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Abstract

Background: Sickle cell anemia is one of the most prevalent hereditary disorders with prominent morbidity and mortality. With this disorder oxidative, phenomena play a significant role in its pathophysiology. One of the garlic (Allium sativum L.) formulations, aged garlic extract (AGE), has been reported to exert an anti-oxidant effect in vitro, we have evaluated the anti-oxidant effect of AGE on sickle red blood cells (RBC).

Methods: 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 a day. Whole blood samples were obtained at baseline and at 4 weeks for primarily Heinz body analysis.

Results: The data were consistent with our hypothesis. In all patients, the number of Heinz bodies decreased over the 4 week period (58.9 ± 20.0% at baseline to 29.8 ± 15.3% at follow-up, p = 0.03).

Conclusions: These data suggest that there is a significant anti-oxidant activity of AGE on sickle RBC. AGE may be further evaluated as a potential therapeutic agent to ameliorate complications of sickle cell anemia.

Background

Sickle cell disease is one of the most prevalent hereditary disorders with prominent morbidity and mortality. While the disease may affect various ethnic groups such as people of Hispanic and Middle Eastern descent, it affects those of African descent the most. Clinical manifestations of sickle cell disease are largely due to a hemolytic process leading to severe anemia and vasoocclusion resulting in pain and organ damage. In the pathophysiology of sickle cell disease, increased oxidant susceptibility of sickle red blood cells (RBC) has been demonstrated to play a major role [7,12,23,26,36,37,39].

Recent investigations have brought forth ample data that support significant anti-oxidant activity of garlic (Allium sativum L.) [2,11,15,18,40]. Among various preparations of garlic supplements, aged garlic extract (AGE) in particular has been associated with anti-oxidant activities in sound scientific experiments [2,14–20,38]. Therefore, based on these data, we have examined the potential role of AGE as an anti-oxidant in sickle cell disease.

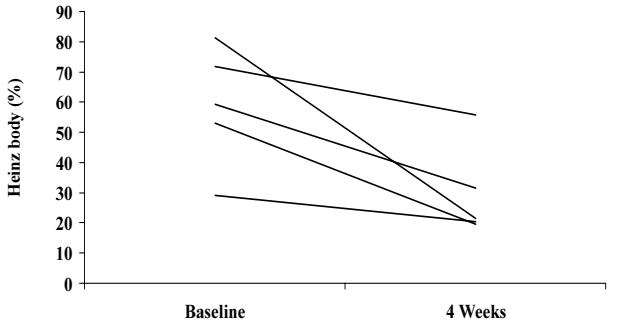

Figure 1
Heinz body (%) from blood samples at entry and 4 weeks follow-up.

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 in 15–20% aqueous ethanol for up to 20 months 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-allyl cysteine, the most abundant water-soluble organosulfur compound in AGE, was present at a concentration of 1.47 g/l.

Study population

The participants were those individuals with the established diagnosis of sickle cell anemia (hemoglobin SS) by hemoglobin electrophoresis and who were 18 years of age or older. 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 months of initiation of this study. This project was approved by Internal Review Board of Habor-UCLA Research and Education Institute. All the participants are volunteers and signed appropriate consent form after careful explanation and review of the protocol.

Administration of aged garlic extract

After obtaining consent, each patient was seen at baseline for interview, physical examination, and baseline blood tests. Urine pregnancy test was also performed for each woman of childbearing age. Participants were instructed to self-administer liquid AGE at a dose of 5 ml twice daily.

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

Biochemical and physiological parameters

RBC count, hemoglobin

Coulter counter was used for determination of RBC counts and hemoglobin levels.

Heinz body count

Heinz bodies were evaluated utilizing a standard method using crystal violet solution [4,13,35]. The number of RBC containing five or more Heinz bodies was counted in 100 RBC and expressed as a percentage.

Statistical analysis

All values are reported as mean ± SD. The paired *t*-test was used to evaluate the differences of variables between baseline and follow-up data. All tests of significance were 2 tailed, and significance was defined at $p < 0.05$.

Results

Five patients (two men and three women, mean age 40 ± 15 years, range 24–58 years) were entered into the study. Table 1 summarizes the hematological data, RBC glutamine content, and the results of the Heinz body analysis at baseline and after 4 weeks of administration of AGE. (Figure 1) There were no significant changes in RBC count, hemoglobin level, hematocrit or reticulocyte count. However, the average Heinz body count decreased from 58.9 to 29.8 percent ($P < .03$). In all patients, the average Heinz body count decreased significantly at 4 weeks compared to the baseline.

Discussion

Recent studies [1,7,8,12,23,26–28,36,37,39] have shown that oxidative phenomena plays a significant role in the pathophysiology of sickle cell disease. The oxidant stress may contribute to the sickling process with formation of "dense cells"; the development of vasoocclusion; and shortened RBC survival[7,12,23,26,36,37,39] The oxidant damage in sickle RBC is most likely a consequence of the inherent instability of hemoglobin S[1,28], which results in a concomitant increase in free radical generation [12] in association with impaired antioxidant defense [7,8,26,27]. With sustained intracellular production of oxygen free radicals, the three-dimensional structure of hemoglobin is affected sufficiently to lower its solubility. These factors lead to formation of Heinz bodies that are aggregates of insoluble hemochromes [6].

The Heinz bodies, which adhere to the RBC membrane [6], 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 the oxidant stress [26,39]. In the study presented here, the data are

Table 1: Comparison between baseline and follow-up

		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
Hexokinese	µmol/min/10^{10}RBC	0.88 ± 0.83	1.57 ± 1.60	0.18
GSH	µg/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

RBC, Red Blood Cell; HGB, Hemoglobin; HCT, Hematocrit; RET, Reticulocyte; GSH, Reduced Glutathione; NAD, Nicotinamide Adenine Dinucleotide

preliminary in nature. However, AGE therapy was associated with decrease in Heinz bodies in sickle RBC in each patient. The data were consistent with our hypothesis and confirm the previous reports that demonstrated anti-oxidant activities of AGE [2,14–20,38].

In regard to hematological parameters, AGE had no significant effect on RBC count, hemoglobin level and hematocrit. There are reports[5,21,24,25,29,31] that have shown in animals that the 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 the usual garlic extract. Allicin has been shown to enhance Low Density Lipoprotein oxidation [19], and to oxidize the iron of hemoglobin in RBC with methemoglobin formation[9]. AGE, on the other hand, has been processed to eliminate these compounds by the aging process without loss of water-soluble anti-oxidant compounds such as *S*-allylcysteine and fructosyl arginine. One of these water soluble compounds, S-allylcysteine has been shown specifically to inhibited the formation of dense cells in in vitro study of blood samples from sickle cell anemia patients [32,33]. Thus, AGE supposedly has decreased capacity for inducing oxidant stress seen in usual garlic extracts [22], yet maintains its antioxidant activity [2,14–20,38]. In this study, there were no adverse effects including those on hematological parameters during the few weeks of AGE administration.

In conclusion, in a small cohort of sickle cell anemia patients, we have demonstrated an association of AGE therapy with decrease in Heinz bodies in their RBC. One need to be cautioned that the data presented here are preliminary and the study was an open labeled non-randomized trial. However, the results are suggestive of potential effect of AGE as an anti-oxidant in sickle RBC. Previously, AGE has been shown to significantly improve erythrocyte deformability through stabilization of erythrocyte membranes in non-sickle RBC [30]. These phenomenons were attributed to the anti-oxidant activities of AGE [30]. At baseline, erythrocyte deformability is further altered in sickle RBC due to abnormal membrane that is highly permeable [10]. This abnormality is thought to be contributory, at least partially, in formation of dense cells [34]. The dense cells, in turn, react with inflammatory cells and endothelial cells leading to vasoocclusive changes [3]. Perhaps AGE may also improve erythrocyte membrane stability in sickle RBC with anti-oxidant activities as suggested by the data presented here in which there were reduction of Heinz bodies in sickle RBC with daily AGE administration. Again the data are preliminary in nature, but they are consistent with previous findings regarding anti-oxidant effect of AGE. With these data, 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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