Hematologic changes associated with the appearance of eccentrocytes after intragastric administration of garlic extract to dogs

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Objective—To determine whether dogs given garlic extract developed hemolytic anemia and to establish the hematologic characteristics induced experimentally by intragastric administration of garlic extract.

Animals—8 healthy adult mixed-breed dogs.

Procedure—4 dogs were given 1.25 ml of garlic extract/kg of body weight (5 g of whole garlic/kg) intragastrically once a day for 7 days. The remaining 4 control dogs received water instead of garlic extract. Complete blood counts were performed, and methemoglobin and erythrocyte-reduced glutathione concentrations, percentage of erythrocytes with Heinz bodies, and percentage of eccentrocytes were determined before and for 30 days after administration of the first dose of garlic extract. Ultrastructural analysis of eccentrocytes was performed.

Results—Compared with initial values, erythrocyte count, Hct, and hemoglobin concentration decreased to a minimum value on days 9 to 11 in dogs given garlic extract. Heinz body formation, an increase in erythrocyte-reduced glutathione concentration, and eccentrocytes were also detected in these dogs. However, no dog developed hemolytic anemia.

Conclusions and Clinical Relevance—The constituents of garlic have the potential to oxidize erythrocyte membranes and hemoglobin, inducing hemolysis associated with the appearance of eccentrocytes in dogs. Thus, foods containing garlic should not be fed to dogs. Eccentrocytosis appears to be a major diagnostic feature of garlic-induced hemolysis in dogs. (Am J Vet Res 2000;61:1446–1450)

Authors of many reports have described hemolytic anemia in dogs induced by consumption1–8 or experimental oral administration9–14 of onions (Allium cepa). Hemolysis is associated with Heinz body formation within erythrocytes, which results from the precipitation and denaturation of hemoglobin molecules oxidatively damaged by n-propyl disulfide15 and 3 alk(en)yl thiosulfate compounds16,17 in onions.

Other members of the genus Allium, such as wild onions (A. validum and A. canadense) and wild garlic (A. ursinum), are also known to cause hemolytic anemia in ruminants18–20 and horses.21 To our knowledge, there is no evidence that consumption of cultivated garlic (A. sativum) by dogs or other animals is in any way harmful. Because cultivated garlic toxicosis has not been reported in dogs, ingestion of garlic-containing foods alone may not always cause illness. However, if garlic, like onions, contains oxidants inducing erythrocyte damage, dogs with diseases associated with oxidative stress or dogs susceptible to erythrocyte oxidation may develop erythrocyte damage and hemolytic anemia when fed garlic-containing foods. The purposes of the study reported here were to determine whether dogs fed a water-soluble extract from boiled cultivated garlic develop hemolytic anemia and to establish the hematologic characteristics induced by intragastric administration of this extract.

Materials and Methods

Preparation of garlic extracts—Garlic extracts were prepared as described for onion extracts that have been used to induce hemolytic anemia in dogs.14,16 Peeled garlic bulbs, cultivated in Honam, Korea, were homogenized after addition of an appropriate volume of deionized water and filtered through gauze. The filtrate was boiled for 15 minutes, the precipitate was removed by filtration, and the second filtrate was evaporated under reduced pressure to a volume corresponding to 0.25 ml/g of whole garlic.

Dogs and treatments—Eight healthy adult mixed-breed dogs were randomly assigned to 1 of 2 groups. Four were control dogs, and 4 dogs received garlic extract. Mean (±SD) body weight was 12.2 ± 1.7 kg and 12.5 ± 1.5 kg for control dogs and dogs fed garlic extract, respectively. Treated dogs received 1.25 ml of garlic extract/kg of body weight, an amount equivalent to 5 g of whole garlic/kg, administered daily via a nasogastric tube for 7 days (days 0 through 6). Control dogs received 1.25 ml of water/kg intragastrically.

Venous blood samples (3.8 ml) were collected into tubes containing EDTA (2 mg/ml of blood) as an anticoagulant on day 0 (before treatment), daily for the first 10 days, and on days 12, 15, 20, and 30.

Hematologic examinations—Erythrocyte, leukocyte, and platelet counts, Hct, and hemoglobin concentration were determined with an automatic cell counter.3 Methemoglobin concentration in whole blood was measured as described by Hegesh et al.14 Heinz bodies in erythrocytes were detected by microscopic examination of a blood smear stained with 0.5% brilliant green and scored as the percentage of erythrocytes with ≥1 Heinz body, as determined by examination of 1,000 erythrocytes. Concentration of reduced glutathione (GSH) in erythrocytes was determined by measurement of the 3,5–
dithiobis-(2-nitrobenzoate) derivative. Eccentrocytes were detected by microscopic examination of a blood smear stained with Giemsa and scored as the percentage of eccentrocytes among 500 erythrocytes examined.

Ultrastructure examinations—For transmission electron microscopy (TEM), 100 µl of whole blood was fixed in 10 ml of ice-cold 2% glutaraldehyde in 0.1M sodium phosphate buffer (pH 7.4) for 2 to 3 hours. After several washes with buffer, the specimens were immersed in buffered 1% osmium tetroxide for 1 to 2 hours. The fixed specimens were packed by centrifugation, dehydrated in a graded ethanol series, and embedded in epoxy resin. Ultrathin sections were cut with a diamond knife and stained with uranyl acetate and lead citrate. Sections were examined in a transmission electron microscope.

For scanning electron microscopy (SEM), specimens that were fixed with 2% glutaraldehyde and immersed in 1% osmium tetroxide were dehydrated in an ethanol series, immersed in tert-butanol, and lyophilized. Specimens were then mounted on aluminum stubs, sputter coated with gold, and examined in a field emission scanning electron microscope.

Statistical analysis—Data were compared between groups by means of the 2-sample t-test and within groups by means of the paired comparison t-test. Percentage of erythrocytes with Heinz bodies and percentage of eccentrocytes were compared between groups by means of the Mann-Whitney test. Values of P < 0.05 were considered significant.

Results

Cellular changes—Compared with the mean (± SD) initial count (8.52 ± 0.37 X 10^6 cells/µl), erythrocyte count in dogs fed garlic extract decreased significantly on day 7 (7.32 ± 0.67 X 10^6 cells/µl), and reached a minimum on day 9 (6.85 ± 0.92 X 10^6 cells/µl), 3 days after dogs received the last dose of extract. After that, erythrocyte count in dogs fed garlic extract increased gradually but never returned to the initial value. In contrast, erythrocyte count in control dogs did not change significantly from the initial value (8.40 ± 0.79 X 10^6 cells/µl). Erythrocyte counts on days 7 to 20 were significantly lower in dogs fed garlic extract than in control dogs. Hematocrit and hemoglobin concentration had patterns of change similar to that of erythrocyte count (Fig 1). Hematocrit and hemoglobin concentration in dogs fed garlic extract also decreased significantly on day 7. Hematocrits on days 9 and 10 were significantly less in dogs fed garlic extract than in control dogs. Hematocrit and hemoglobin concentration had patterns of change similar to that of erythrocyte count (Fig 1). Hematocrit and hemoglobin concentration in dogs fed garlic extract also decreased significantly on day 7. Hematocrits on days 9 and 10 were significantly less in dogs fed garlic extract than in control dogs, whereas there were no significant differences in hemoglobin concentration between the 2 groups. However, no dog fed garlic extract developed clinically hemolytic anemia.

Compared with the initial count (9.8 ± 0.7 X 10^6)
cells/µl), leukocyte count increased significantly on day 6 (11.5 ± 0.7 × 10³ cells/µl) in dogs fed garlic extracts (Fig 2). White blood cell count was significantly higher in dogs fed garlic extract on days 6 to 9, compared with control dogs. The increase in leukocyte count reflected an increase in number of neutrophils. Platelet count in dogs fed garlic extract did not increase significantly from initial values (253 ± 60 × 10³ platelets/µl) at any time during the study, nor was there a difference in platelet count between groups. Intravascular hemolysis and hemoglobinuria were not detected in any dog.

Oxidative effects of garlic extract—Mean methemoglobin concentration in dogs fed garlic extract increased slightly, but not significantly, 3 days after initiation of extract administration (day 0, 73.6 ± 21.5 mg/dl; day 3, 193.6 ± 150.9 mg/dl). By day 4, concentration had returned to the initial value (105.3 ± 33.0 mg/dl) and remained low for the remainder of the study. Methemoglobin concentration did not differ significantly between groups at any time during the study.

Mean percentage of erythrocytes with Heinz bodies in dogs fed garlic extract increased significantly on day 4, reached a maximum on day 8, and then decreased gradually (Fig 3). Percentage of erythrocytes with Heinz bodies on days 2 and 4 to 30 were significantly higher in dogs fed garlic extract than in control dogs. Heinz bodies appeared as 1 or 2 small particles per erythrocyte. Heinz bodies were not detected in control dogs.

Compared with the initial concentration, erythrocyte GSH concentration increased significantly on day 5 in dogs fed garlic extract (Fig 4). On days 1 and 3 to 25, GSH concentration was significantly higher in dogs fed garlic extract than in control dogs but returned to the initial value on day 30.

Morphologic changes—Morphologically abnormal erythrocytes were observed in Giemsa-stained blood smears prepared from dogs fed garlic extract (Fig 5). The abnormal erythrocytes were eccentrocytes with hemoglobin that appeared dense and contracted to one side of the cell, leaving a pale area on the other side. When examined by use of SEM, eccentrocytes had a contracted spheroid region and a thin collapsed region. Projections that corresponded to the thin collapsed regions were detected by use of TEM (Fig 6). The percentage of eccentrocytes in dogs fed garlic extract increased significantly on day 6 and reached maximum percentage (15.52 ± 12.24%) on day 7 (Fig 7). The percentage of eccentrocytes on days 2 to 30 was significantly higher in dogs fed garlic extract than in control dogs. Eccentrocytes were not detected in blood smears from control dogs.
Discussion

Results of the present study indicated that ingestion of garlic extract induced hemolysis in dogs. The hemolysis induced by garlic extract resulted from oxidative injury to erythrocytes, as evidenced by formation of eccentrocytes and Heinz bodies and an increase in erythrocyte GSH concentration.

Heinz bodies derive from the denaturation of hemoglobin through a sequence of oxidative steps, in which methemoglobin formation may be essential. Successive oxidative damage allows Heinz bodies to grow by coalescence and condensation, coming to lie just beneath the erythrocyte surface and resulting in considerable distortion of cell shape and deformation of the plasma membrane. Erythrocytes containing Heinz bodies are less deformable than normal erythrocytes and tend to become sequestered in the spleen, where Heinz bodies are pitted, or the entire cell is phagocytosed by macrophages. In previous reports on experimental onion-induced hemolytic anemia in dogs, the percentage of erythrocytes with Heinz bodies was high (80 to 100%) in dogs given a single dose of dehydrated onions orally and in dogs fed boiled onions. With regard to onion-induced hemolysis, induction of anemia is mainly attributable to Heinz body formation. In the present study, however, the percentage of erythrocytes with Heinz bodies in dogs fed garlic extract was relatively low (mean maximum, 6.91%). Additionally, only 1 or 2 small Heinz bodies were formed in each erythrocyte. Furthermore, no significant increase of methemoglobin concentration was detected. Thus, oxidative injury to the hemoglobin protein may be a secondary cause of garlic-induced hemolysis.

The appearance of eccentrocytes is associated with oxidative injury to erythrocytes. These abnormal cells have been reported in dogs given high doses of acetylsalicylic acid, acetaminophen for a prolonged period, and in dogs fed dehydrated onions. Eccentrocytes are believed to form as a result of direct oxidative injury to erythrocyte membranes and may form by adhesion of opposite areas of the membrane. Eccentrocytes are rigid and spheroid with intrinsic membrane alterations that make them susceptible to entrapment and removal by the mononuclear phagocytic system.

Ham et al concluded from experiments in dogs that membrane damage, such as that which results in eccentrocyte formation, attributable to oxidative injury is a more important cause of erythrocyte destruction than Heinz body formation. Similarly, Chan et al concluded from experiments in rabbits that the appearance of eccentrocytes is a hallmark of severe oxidative injury. In the present study, percentage of eccentrocytes increased in dogs fed garlic extract, whereas percentage of erythrocytes with Heinz bodies remained relatively low. Furthermore, a decrease in erythrocyte count was detected simultaneously with the appearance of eccentrocytes. This observation reflects severe oxidative injury to erythrocyte membranes rather than to hemoglobin, suggesting that the formation of eccentrocytes may be a primary cause of garlic-induced hemolysis. Thus, we believe that eccentrocytosis may be a major diagnostic feature of garlic-induced hemolytic anemia in dogs. Reduced glutathione is an important component of the antioxidant defenses of erythrocytes, acting as a substrate for glutathione peroxidase and directly as a free radical scavenger. When erythrocytes are exposed to oxidative stress, GSH is converted to the oxidized form. Erythrocytes can reduce oxidized glutathione via the glutathione reductase pathway.
tathione, and some oxidized drugs actually increase GSH synthesis. In addition, release of young erythrocytes as a result of the regenerative response is accompanied by an increase in erythrocyte GSH concentration and activity of enzymes associated with glutathione metabolism. In the present study, the significant increase of erythrocyte GSH concentration in dogs fed garlic extract may have been attributable to increased GSH synthesis induced by oxidative injury to erythrocytes or the release of young erythrocytes. In addition, a significant increase in leukocyte count was detected in dogs fed garlic extract. A similar change in leukocyte count has been reported in many cases of onion-induced hemolytic anemia. The mechanism for this increase in leukocyte count remains unknown.

Certain dogs are highly susceptible to onion-induced hemolytic anemia as a result of a high erythrocyte GSH concentration, which accelerates the oxidative damage induced by sodium n-propylthiosulfate. Such dogs may also be susceptible to garlic-induced hemolysis. Thus, because the constituents of garlic have the potential to oxidize erythrocyte membranes and hemoglobin, we believe that foods containing garlic should be avoided for use in dogs.

References