Effect of Garlic Extract on Red Blood Cells

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Osmotic fragility of red blood cells exposed to garlic extract was greatly elevated and the hemoglobin spectrum was changed, giving rise to peaks at 505, 536, 576 and 630 nm instead of peaks at 542 and 572 nm of the native hemoglobin. The factor responsible for these changes was heat stable and undialyzable in its native form. Following its boiling, the substance was lost during dialysis.

Garlic (Allium sativum) is widely used as a condiment and food. It has antibacterial activity (6,8,11) and a compound isolated from the clove was identified as allicin (4,5,10). The mode of action of some antibacterial substances is by its effects on biological membranes. Previous work in our laboratory (1,2) and in another (12) showed that garlic extract affects several serum and liver enzymes. It was shown that the activity of Mg-dependent adenosine triphosphatase (ATPase) was significantly stimulated by garlic extract only in intact mitochondria but not in broken mitochondria, indicating the possible mode of action by affecting the mitochondrial membrane. Furthermore, it strongly uncoupled mitochondrial oxidative phosphorylation (1,2).

A widely used model for membrane studies in red blood cells. It was shown by Bogin et al. (3) that parathyroid hormone, which is suspected to be toxic at high levels, affected red blood cells and probably is one of the causes of anemia seen in uremic patients on dialysis. To further investigate the effect of garlic extract on biological membranes, the effect on erythrocyte fragility was studied. The present communication describes these results.

MATERIALS AND METHODS

Two ml of blood were withdrawn from a rabbit into test tubes containing heparin. The blood was diluted with 8.0 ml of isotonic phosphate buffered saline (PBS) solution, pH 7.4 and centrifuged for 5 min at 600 x g. The plasma and buffy coat were discarded and the RBC were washed 3 times. Osmotic fragility was evaluated by the method described by Part et al. (7). Each time the RBC were mixed with the solution by gentle inversion and centrifuged for 3 min at 600 x g. The washed packed cells were then suspended in PBS to provide a hematocrit of 10%. The osmotic fragility curve was determined in triplicate in a series of tubes containing different salt concentrations ranging from 0 to 0.9% NaCl; 250 μl of RBC suspensions were added and the final volume was 5 ml. The reaction was carried out for 30 min in a shaking bath at 37°C. At the end of the incubation, the cells were centrifuged and absorption of the supernatant liquid was measured against water at 575 nm in a Pye-Unicam SP 800 A spectrophotometer. The effect of 0.2 ml of garlic extract on the osmotic fragility of RBC was examined by the same procedure. Whenever garlic extract was not added, 0.2 ml of PBS was added to the tube.

The salt concentration at which 50% of the RBC were lysed was defined as the median corpuscular fragility (MCF) according to the following equation:

\[
\text{OD}_{\text{MCF}} = \frac{\text{OD}_{\text{max}} - \text{OD}_{\text{min}}}{2}
\]

and the units are expressed in % NaCl concentration in the media.

Garlic extract was prepared according to a method previously described (2), except that extraction was done in PBS. Boiled extract was obtained by incubating the crude extract in a boiling water bath for 30 min. It was then centrifuged at 10,000 x g for 30 min and the resulting supernatant fluid was used. Dialysis was done with a dialysis tube (24 angstroms pore size), against 50 volumes of PBS for 24 h in the cold (0–4°C).

Hemoglobin was prepared freshly by lysis of RBC in double distilled water. After incubation for 15 min and centrifugation at 10,000 x g for 15 min, the supernatant fluid was obtained and used for hemoglobin studies. Spectra of hemoglobin in the absence or presence of various concentrations of garlic extract were done with a Pye-Unicam recording spectrophotometer. The reaction mixture contained 0.1 ml of hemoglobin (giving at final concentration an optical-density of 1.0), garlic extract and Tris-HCl buffer (final concentration 0.02 M, pH 7.4) to a final volume of 3.0 ml. Oxygenation of the reaction mixture was done by bubbling gaseous oxygen at a rate of 3-4 ml per minute for 60 s.

RESULTS

The effect of garlic extract on erythrocyte fragility is shown in Table 1. Presence of garlic extract in the incubation media significantly increased the MCF. While MCF in the controls was 0.550 ± 0.011, in the experimental group which contained 0.2 ml of garlic extract, the value was 0.573 ± 0.008. Whenever the extract was treated before its addition to the incubation medium did not abolish this effect (MCF value was somewhat smaller, but insignificantly different). Whenever the extract was dialyzed following its boiling, the effect was abolished, giving a value similar to the controls (0.550 ± 0.012).
GARLIC AFFECTS RED BLOOD CELLS

TABLE 1. Effect of garlic extract (0.2 ml) on the corpuscular fragility of erythrocytes (mean ± SEM).

| Addition                        | Mean corpuscular fragility (MCF) | Δ MCF     | p
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Control (saline solution)</td>
<td>0.550 ± 0.011</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Untreated extract</td>
<td>0.573 ± 0.008</td>
<td>0.023 ± 0.009</td>
<td>0.05</td>
</tr>
<tr>
<td>Frozen extract</td>
<td>0.604 ± 0.014</td>
<td>0.054 ± 0.013</td>
<td>0.01</td>
</tr>
<tr>
<td>Boiled extract</td>
<td>0.607 ± 0.012</td>
<td>0.057 ± 0.011</td>
<td>0.01</td>
</tr>
<tr>
<td>Dialyzed extract</td>
<td>0.570 ± 0.010</td>
<td>0.020 ± 0.010</td>
<td>0.01</td>
</tr>
<tr>
<td>Boiled-dialyzed extract</td>
<td>0.550 ± 0.012</td>
<td>0.000 ± 0.0</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Significant from control, n = 8.

Garlic extract had a significant effect on hemoglobin (Fig. 1), changing its spectrum markedly. While in a typical absorption pattern of hemoglobin there were peaks at 542 and 572 nm and a broad peak between 400 and 440 nm, in the presence of boiled extract there were small peaks at 505, 536, 576 and 630 nm and a somewhat narrower peak between 400-440 nm. The changes in the spectrum is slow and the kinetics of these changes are seen in Fig. 2. The absorption of hemoglobin in the presence of boiled garlic extract at 576 nm gradually decreased with time. Oxygenation of the hemoglobin solution in the presence of the extract caused no distinct effect on the spectrum.

DISCUSSION

Red blood cells are commonly used as a model for various biological studies. The pattern of the osmotic fragility of RBC was shown to be a useful model for membrane behavior. Extract obtained from garlic was shown to inhibit serum enzymes and the oxidative phosphorylation of liver mitochondria. The present study showed that the extract obtained from garlic affected biological membranes leading to lower stability to hypotonic solutions, supporting the hypothesis that the mode of action on mitochondria is by its effect on the membranes (1,2).

Using this criterion it was possible to characterize the active substance as heat-stable and dialyzeable only after denaturing the proteins to which it was bound. It is not clear from this study whether it is one or several substances which affect the RBC membrane. Furthermore, it is also not clear whether the same substance which affected the membrane, also affected the hemoglobin leading to its spectrum changes. It is, however, reasonable to assume that the substance obtained from garlic and which caused uncoupling of oxidation phosphorylation in liver mitochondria, is the same one which affected RBC membranes.

It seems that the substance which affected the RBC membrane is protein-bound since after dialysis of the fresh extract, no change in the degree of the effect was seen. Denaturation of the proteins by either heating or by freezing and thawing lead to the loss of the activity by dialysis. Furthermore, following denaturation of the proteins, the effect of the supernatant fluid obtained from this extract was much stronger, probably because of its unbound state in the solution.

Plants from the family of Liliaceae which contain the onion (Allium cepa) and garlic (Allium sativum) contain saponins and sulfur-containing volatile oils. Dog ingesting excessive quantities of onion suffered from anemia and icterus (9,11). The possibility of similar effects in humans consuming excessive quantities of onions and garlic have to be studied. It is possible that the active toxic substance...

**Bogin, et al.** con't. from p. 101

in garlic, which may be similar to that of the onion, and was shown to affect RBC and hemoglobin, is the cause for the anemia seen in animals.

**REFERENCES**