ABSTRACT Garlic is known for its pharmacologic and nutritional properties. In previous studies, garlic elicited a reduction in plasma levels of lipids by inhibiting hepatic cholesterol synthesis. The aim of this study was to investigate in an in vivo model the effects of garlic extract and some fractions on cholesterol levels and vascular reactivity in cholesterol-fed rats. Rats were fed a cholesterol-enriched diet for 16 wk and were divided into 10 groups as follows: control and hypercholesterolemic diet groups, 4 groups fed frozen garlic fractions and 4 groups fed raw garlic fractions with different doses. Blood samples were obtained to analyze HDL and LDL cholesterol levels. After treatment, rats were killed. The heart, liver and kidneys were weighed; the aorta was isolated, mounted in organ chambers and vascular reactivity was tested. Plasma concentration of cholesterol was 58 mg/dL (100%) at the beginning of the study and increased to 102 mg/dL (153%; hypercholesterolemic group) at the end of the treatment. Plasma total cholesterol decreased in all groups treated with garlic; moreover, this effect was higher in rats fed raw garlic fractions and extracts. LDL decreased significantly with respect to the hypercholesterolemic group in all groups treated with garlic fractions and extracts (P < 0.01); however, an increase in HDL was found in those treated with frozen fractions and extracts. The liver:body weight ratio decreased in all treated groups. The relaxing effect of acetylcholine (ACh) was enhanced in arteries contracted with noradrenaline (NE). These data suggest that garlic fractions could prevent diet-induced hypercholesterolemia and vascular alterations in the endothelium-dependent relaxation associated with atherosclerosis.

KEY WORDS: • Allium sativum fractions • aorta • rat • hypercholesterolemic diet • vascular reactivity

Atherosclerosis is the principal contributor to the pathogenesis of myocardial and cerebral infarction. Elevated plasma concentration of cholesterol, especially in LDL, is recognized as leading to the development of atherosclerosis. On the other hand, there is convincing evidence that relaxation mediated by endothelium-derived nitric oxide (NO) is impaired in arteries from hypercholesterolemic and atherosclerotic animals (Shimokawa and Vanhoutte 1989, Verdeuren et al. 1986). Impairment has been reported to be reversed in the aorta of hypercholesteremic rabbits by exposure in vivo to exogenous L-arginine, implying that NO synthesis may be reduced (Cooke et al. 1991).

Garlic (Allium sativum L.) has long been used widely not only as a flavoring agent but also as a folk medication. Its reported beneficial actions include antimicrobial (Cellini et al. 1996), antithrombotic (Bordia et al. 1996), antihypertensive (Foushee et al. 1982, McMahon and Vargas 1993) and anti-hyperlipidemic effects (Eilat et al. 1995, Yeh and Yeh 1994). Garlic also activates NO synthesis in cell-free homogenate (Das et al. 1995a and 1995b). In this work, the aim was to examine the possible beneficial effects of garlic extract and its fractions on the alteration in vascular responsiveness that exists between the decreasing effect of garlic on plasma cholesterol levels and its effect on vascular responsiveness.

MATERIALS AND METHODS

Diet and treatment. Ten groups (n = 80) of male wistar rats (ANUC Complutense University, Madrid, Spain) weighing 200.0 ± 20.5 g at the beginning of the study, were used. Rats were housed identically in an air-conditioned room under a 12-h light:dark cycle. The control group was fed a standard diet (normal control). Group 0 was fed a standard diet plus 0.5% cholesterol (U.A.R., Paris, France; hypercholesterolemic controls). The groups receiving high cholesterol and garlic extracts and fractions were distributed according to the scheme shown in Table 1. The experiment lasted 16 wk. All of the rats were initially fed a standard laboratory diet (Panlab S.L., Barcelona, Spain) for at least 7 d after delivery to our laboratory. Tap water was freely available. Food intake was monitored daily for the two control groups and the drug-treated groups. The different doses of...
garlic extracts and fractions were given orally every day. All protocols concerning animals were approved by the Complutense University of Madrid (EEC official registration 28079–15ABC).

**Measurement of body weight and biochemical parameters.** Blood samples were collected from the jugular vein; the body weight (BW) of each rat was determined before the start the treatment and every 13 d. Serum concentrations of cholesterol, LDL, and HDL were determined with commercially available enzyme kits (BioMerieux, Marcy, France). The aortic rings were contracted with noradrenaline (NE; 10⁻⁴ mol/L) or by KCl (80 mmol/L) in aortae. The thoracic aorta was rapidly removed and placed in Krebs-Henseleit solution of the following composition (mmol/L): NaCl, 119; KCl, 4.7; NaHCO₃, 25; MgSO₄, 1.0; glucose, 11.1; KH₂PO₄, 1.2; and CaCl₂, 2.5.

**Isolated blood vessel preparations.** The rats were anesthetized with ethyl ether and killed by exsanguination from the common carotid in wk 16 of treatment. The thoracic aorta was rapidly removed and placed in Krebs-Henseleit solution of the following composition (mmol/L): NaCl, 119; KCl, 4.7; NaHCO₃, 25; MgSO₄, 1.0; glucose, 11.1; KH₂PO₄, 1.2; and CaCl₂, 2.5.

**Aortic rings.** Adherent fat and surrounding tissue were cleaned off and the arteries were cut into rings ~2–3 mm in width. The rings were then suspended between two stainless steel hooks in organ baths containing 10 mL of Krebs-Henseleit solution. The solution was kept at 36 ± 0.5°C and gassed continuously with a 95% O₂-5% CO₂ gas mixture. The rings were mounted under 1 g tension. Each preparation was allowed to equilibrate for 60 min. Contractile responses were measured isometrically by means of force-displacement transducers (Grass FT 03) and were recorded on a Grass polygraph as previously described (Tejerina et al. 1988). The isometric force was also digitized by a MacLab A/D converter (Chart v3.2, A.D. Instruments, Castle Hill, Australia) and stored and displayed on a Macintosh computer (Ruiz and Tejerina 1998).

**Experimental procedure.** After the equilibration period, aortic rings were contracted with noradrenaline (NE; 10⁻⁴ mol/L) and exposed to acetycholine (ACh; 10⁻⁸ to 10⁻⁵ mol/L) or to sodium nitroprusside (SNP; 10⁻⁸ to 10⁻³ mol/L) when contraction had reached a plateau to test the endothelium-dependent and independent relaxation. Other aortic rings were contracted with KCl (80 mmol/L).

**Drugs.** The following drugs were used: acetylcholine chloride, noradrenaline bitartrate and SNP were all from Sigma Chemical (St. Louis, MO). All garlic extracts and fractions were provided by Dr. Matsuura (The University of Illinois at Chicago, Chicago, IL). They are listed in Figure 1. Stock solutions were prepared by dissolving the compound in distilled water. Ascorbic acid was added to the noradrenaline solution to avoid noradrenaline oxidation. Working solutions were made in Krebs-Henseleit solution.

**Statistical analysis.** All values used in analyses represent means ± SEM for 8 rats in each group of experiments. Comparisons among the different groups were performed by two-way ANOVA test or Student’s t test and differences were considered significant when P < 0.05. Concentration-response curves were used to determine the concentration of the drugs producing 50% inhibition of the maximal contractile response (IC₅₀); a linear regression analysis over the response range of 20–80% of the maximal inhibition was carried out.

**RESULTS**

**Effect of the treatment on body and organ weights.** The BW (Fig. 2, panel A) increased in all groups throughout the treatment without significant differences among them. There were no differences in the heart:BW ratio (Fig. 2, panel B). However, the liver:BW ratio (Fig. 2, panel C) decreased in all of the treated groups compared with the hypercholesterolemic group. Also, the kidney:BW ratio (Fig. 2, panel D) decreased in groups 5 and 7 with respect to the hypercholesterolemic group.

**Measurement of biochemical parameters: lipid profiles.** Plasma total cholesterol concentration was ~58 mg/dL (100%) at the beginning of the study, and increased to 102% mg/dL (153% with respect to the control) in the hypercholesterolemic group at the end of the treatment (16 wk). Plasma total cholesterol decreased in all groups treated with garlic fractions and extracts; moreover, this effect was higher in rats fed raw garlic fractions and extracts (groups 4, 5, 6 and 7; Fig. 3, panel A).

Changes in the distribution of cholesterol in HDL or LDL were found, i.e., LDL decreased significantly with respect to the hypercholesterolemic group in all of the groups treated with garlic fractions and extracts (P < 0.01 n = 8) (Fig. 3, panel B). However, an increase in HDL was found in the frozen garlic fraction and extract groups; this increase was significant in groups 1, 3 and 8 (Fig. 3, panel C).

**Effect of the treatment on contractions induced by NE (10⁻⁴ mol/L) or KCl (80 mmol/L) in aortae.** In a first group of experiments, the contractions induced either by NE (10⁻⁴ mol/L) or by KCl (80 mmol/L) were measured. The contractions induced by NE increased in arteries obtained from the hypercholesterolemic group (group 0) with respect to the control group (0.96 ± 0.09 vs. 1.28 ± 0.08 g, P < 0.05, n = 8). Moreover, the contractions in the garlic-treated groups were even higher than those in the hypercholesterolemic group, except in group 6 (RG-HP20-w) in which the contractions decreased, although not significantly, with respect to the

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### TABLE 1

**Distribution of groups receiving high cholesterol and garlic extract and fractions**

<table>
<thead>
<tr>
<th>Group</th>
<th>Fraction/Extract</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard diet</td>
<td>Control</td>
<td>—</td>
</tr>
<tr>
<td>Hypercholester-</td>
<td>Group</td>
<td>—</td>
</tr>
<tr>
<td>lemic group</td>
<td>Group 0</td>
<td>0.5% Cholesterol</td>
</tr>
<tr>
<td>Frozen fractions</td>
<td>Group 1</td>
<td>FG-HP20-20</td>
</tr>
<tr>
<td></td>
<td>Group 2</td>
<td>FG-HP20-10</td>
</tr>
<tr>
<td></td>
<td>Group 3</td>
<td>FG-HP20-w</td>
</tr>
<tr>
<td>Raw fractions</td>
<td>Group 4</td>
<td>RG-HP20-20</td>
</tr>
<tr>
<td></td>
<td>Group 5</td>
<td>RG-HP20-10</td>
</tr>
<tr>
<td></td>
<td>Group 6</td>
<td>RG-HP20-w</td>
</tr>
<tr>
<td>Extracts</td>
<td>Group 7</td>
<td>RG-extract</td>
</tr>
<tr>
<td></td>
<td>Group 8</td>
<td>RG-extract</td>
</tr>
</tbody>
</table>

3 Abbreviations: ACh, acetylcholine; BW, body weight; IC₅₀, 50% inhibition of the maximal contractile response; NE, noradrenaline; SNP, sodium nitroprusside.
hypercholesterolemic group (1.28 ± 0.08 vs 1.10 ± 0.10 g). (Fig. 4, panel A).

On the other hand, the contractions induced by high K⁺ (80 mmol/L) decreased in the hypercholesterolemic group with respect to the control group (2.53 ± 0.13 vs. 1.49 ± 0.10 g, P < 0.01, n = 8). In addition, only in groups 4, 7 and 8 were contractions similar to the control group, and they were significantly higher than in the hypercholesterolemic group (Fig. 4, panel B).

Effect of the treatment on the relaxation induced by ACh or SNP in aortae. ACh (10⁻⁸ to 10⁻⁵ mol/L) caused an endothelium-dependent relaxation in a concentration-responsive manner in all of the groups studied. The endothelium-dependent relaxation strongly decreased in the hypercholesterolemic group (group 0) with respect to the control group; the maximal relaxation was induced by ACh (10⁻⁵ mol/L), i.e., 83.3 ± 5.2 and 40.6 ± 7.6% (P < 0.01, n = 8) in the control and hypercholesterolemic groups, respectively. In the frozen fraction–treated groups (groups 1, 2, and 3; Fig. 5, panel A) the relaxing effect of ACh increased with respect to the hypercholesterolemic group (the IC₅₀ was 6.32 ± 2.5 × 10⁻⁷, 2.3 ± 1.1 × 10⁻⁶, and 6.38 ± 2.1 × 10⁻⁷ mol/L in groups 1, 2, and 3, respectively) as did the maximum effect (maximum relaxation: 40.6 ± 7.6%, 57.5 ± 5.4%, 55.2 ± 2.6% and 61.4 ± 6.0%, P < 0.05, n = 8 in groups 0, 1, 2 and 3, respectively).

In the raw fraction–treated groups (groups 4, 5, and 6; Fig. 5, panel B), the relaxation induced by ACh was improved only in group 6 in which the maximum relaxation as well as the sensitivity increased with respect to the hypercholesterolemic group. Thus, the maximum relaxant effects were 40.6 ± 7.6% vs. 72.8 ± 6.1%, (P < 0.01, n = 8) and the IC₅₀ were 10⁻⁷ vs.
7.5 × 10^{-7} \text{ mol/L} in the hypercholesterolemic group and group 6, respectively.

In the extract-treated groups (groups 7 and 8; Fig. 5, panel C) we found that only the raw extract tended to increase the relaxation induced by ACh, although not significantly.

In addition, we also studied the possible changes in the endothelium-independent relaxation induced by SNP. Contrary to our report in the case of endothelium-dependent relaxation, we did not find any differences between the control and hypercholesterolemic groups in the endothelium-independent relaxation. In the frozen fraction–treated groups (groups 1, 2 and 3; Fig. 6, panel A), the relaxation was similar to that reached in the hypercholesterolemic group. However, in all of the raw fraction– (Fig. 6, panel B) and extract-treated (Fig. 6, panel C) groups, except in group 6 (RG-HF_{20-w}), which presented relaxation similar to the control group, the sensitivity was progressively decreasing, especially in the extract-treated groups (groups 7 and 8).

**DISCUSSION**

The medical properties of garlic have been known since the ancient Egyptian era. Among its effects is a beneficial action on the development of experimental atherosclerosis. In this work, we investigated the possible beneficial effects of some garlic extracts and fractions on the changes in vascular responsiveness produced by high cholesterol plasma levels induced by a hypercholesterolemic diet in rats. We found an increase in the contractile responses evoked by the nonspecific adrenergic agonist noradrenaline in the arteries isolated from hypercholesterolemic rats with respect to control (normal) rats. This increase in the contraction was counteracted in the arteries...
isolated from rats given a hypercholesterolemic diet plus the fraction RG-HP\textsubscript{20-W} at 300 mg/(kg \cdot d) (group 6). Some atherosclerotic arteries in humans and in various hypercholesterolemic animal models of atherosclerosis exhibit increased vasoconstriction (Ginsburg et al. 1984, Vrints et al. 1990). The pathophysiological basis for the increased vasoconstrictor responses of atherosclerotic blood vessels to certain agonists could be due to an increase in the number of serotonergic and \(\alpha\)-adrenergic receptors (Nanda and Henry 1982) or an increased cholesterol content of smooth muscle cell membranes augmenting the responses to noradrenaline (Yokoyama and Henry 1979).

On the other hand, endothelium-dependent relaxation is impaired in vessels from atherosclerotic patients (Bossaller et al. 1987, Forstemann et al. 1988) and in hypercholesterolemic animal models (Chappell et al. 1987, Shimokawa and Vanhoutte 1989), suggesting modification of an endothelium-derived relaxing factor, which is assumed to be NO, in hyperlipidemia. In this paper, we reported that indeed the endothelium-dependent relaxation induced by ACh was impaired in aortic rings from hypercholesterolemic rats and in some garlic-treated groups, such as group 3 (FG-HP\textsubscript{20-W}) and especially group 6 (RG-HP\textsubscript{20-W}), in which the endothelium-dependent relaxation was almost similar to that in control rats.

Numerous mechanisms have been suggested for the effect on vascular relaxation in atherosclerotic and hypercholesterolemic animal models. They include an increased diffusional barrier for NO due to the intima thickening (Lopez et al. 1989), l-arginine depletion (Cooke et al. 1991, Schini and Vanhoutte 1991, Shimokawa and Vanhoutte 1989), altered endothelial cell receptor coupling mechanism (Cohen et al. 1988) and inactivation of NO by oxygen free radicals (Gryglewski et al. 1986, Rubanyi and Vanhoutte 1986).

It has been reported (Das et al. 1995a and 1995b) that garlic activates the NO synthase both in vitro (in cell-free homogenate) and in vivo because the hypertension induced by l-NAME (a NO synthase inhibitor) and the decrease in the urinary levels of NO\textsubscript{2}−/NO\textsubscript{3}− induced by l-NAME in rats were prevented by treatment with garlic, which strongly suggests that garlic increases NO synthase in vivo. There is a link between garlic and the L-Arg-NO pathway. Amino acid analysis of garlic powder demonstrated that it is a rich source of arginine, the precursor of NO. However, neither arginine nor allin-derived products were found to be responsible for the activation of NO synthase by garlic in cell-free homogenate (Das et al. 1996). In addition, antioxidant properties of garlic were also suggested by showing that organosulfur compounds inhibited the peroxidation of lipids and Cu\textsuperscript{2+}-induced oxidative modification of LDL (Ide et al. 1997, Prasad et al. 1996, Török et al. 1994). These properties could also explain the effect of garlic treatment on endothelium-dependent relaxation found in this work, together with the direct effect of garlic on NO synthesis found by other authors.

In contrast to endothelium-dependent relaxation, endothelium-independent relaxation is not altered during the atherosclerotic state. Thus, we did not find any significant differences between the relaxation induced by SNP in normal and hypercholesterolemic rats. However, in some garlic-treated groups (groups 4, 5, 7 and 8), this relaxation was decreased.

In other groups of experiments, we found that the treatment with garlic fractions or extracts decreased the total cholesterol in all of the groups treated. These data, together with (1) the decrease in LDL cholesterol that occurred in all of the groups treated, (2) the data that HDL cholesterol increased in some of the groups (1, 3 and 8), all involving frozen fractions, and (3) the impairment of the liver:BW ratio, allowed us to conclude that the treatment with garlic extracts and fraction improves vascular reactivity and the lipid profile in hypercholesterolemic animals, and that this protection is higher in those treated with frozen extracts.  

**LITERATURE CITED**


**FIGURE 6** Effect of the treatment on the endothelium-independent relaxation induced by sodium nitroprusside (SNP; 10\textsuperscript{−8} to 10\textsuperscript{−5} mol/L) in aortic rings. Panel A, frozen fraction-treated groups; panel B, raw fraction-treated groups; and panel C, extract-treated groups. Each point represents the mean ± SEM of 8 experiments. *\(P < 0.05\), **\(P < 0.01\) with respect to the hypercholesterolemic group.


