EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Direct Antiatherogenic Effect of Garlic

A. N. Orekhov, V. V. Tertov, I. A. Sobenin,
E. M. Pivovarova, L. A. Medvedeva, and T. A. Skalbe

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 6, pp. 695-697, June, 1996
Original article submitted March 2, 1996

Blood serum of patients with documented coronary atherosclerosis induces intracellular cholesterol accumulation in a primary culture of human aortic intimal cells. Serum prepared from blood collected 2-4 h after administration of a tablet containing 300 mg garlic powder (Kwai, Germany) induces a much lower cholesterol accumulation in cultured cells. A tablet with the same amount of garlic but with a prolonged effect (Allikor, Russia) lowers serum atherogenicity for 12-14 h. The direct antiatherogenic effect of garlic powder is confirmed by cell culture experiments with aqueous extracts from garlic powder.

Key Words: *antiatherosclerotic therapy; atherosclerosis; cell culture; cholesterol; garlic*

Garlic is known to have a positive effect on the risk factors of cardiovascular disease [3]. In addition, in folk medicine it is used as an antiatherosclerotic agent. The question arises as to whether the antiatherosclerotic activity of garlic results from a direct effect on the vascular wall or whether it is mediated by the action on the risk factors of atherosclerosis. We used a cell culture model [5] in an attempt to find out.

MATERIALS AND METHODS

Garlic power (Lichtwer Pharma, Germany) standardized by alliin content (1.3%, which corresponds to the alliin content in the Kwai tablets produced by this company) was studied. Aqueous extract from the powder was prepared as described elsewhere [1]. The chemical composition of the extract was described previously [7]. Tablets containing 300 mg garlic powder (Kwai) and tablets also of 300 mg but with a prolonged effect (Allikor, Medotel, Russia) were used in *ex vivo* studies.

Institute of Experimental Cardiology, Cardiology Research Center, Russian Academy of Medical Sciences, Moscow

Antiatherosclerotic effects. Extract from garlic powder was added to a primary culture of intimal smooth muscle cells isolated from atherosclerotic plaques and maintained as described previously [5]. Subendothelial cells of human aortic intima were isolated from autopsy material obtained 2-3 h after sudden death from myocardial infarction. The cells were isolated by incubating atherosclerotic plaques with 0.15% collagenase solution for 3-4 h and seeded in 96-well plates. They were cultured for 7 days in medium 199 supplemented with 10% fetal calf serum, 2 mM glutamine, and antibiotics. The activity of the garlic extract was evaluated from its effect on the following atherosclerotic parameters: the intracellular content of free and esterified cholesterol and incorporation of ^{3}H-thymidine in cellular DNA. Garlic extract and ^{3}H-thymidine were added on day 7 of culturing. After 24 h of incubation, the cells were thoroughly washed, and incorporated ^{3}H-thymidine and free and esterified cholesterol were measured. Detailed protocols for these procedures were published previously [5].

Antiatherogenic effects. Antiatherogenic effects were studied with the use of atherogenic sera of patients. Serum was considered to be atherogenic if it

0007-4888/96/0006-0629$15.00

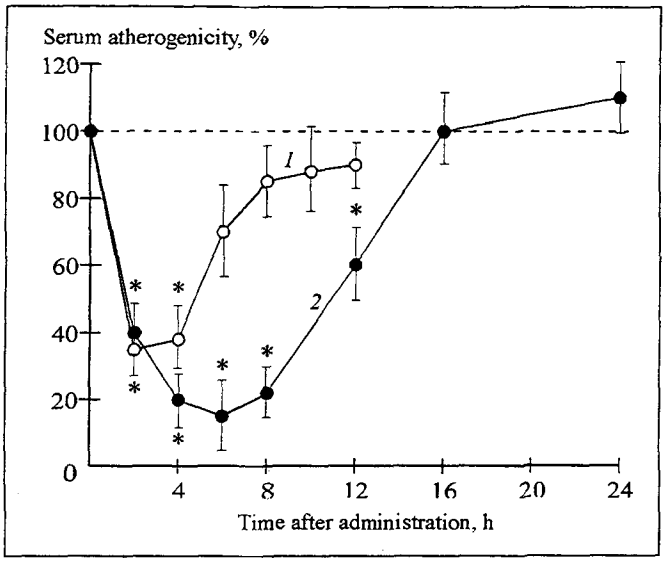

Fig. 1. Effect of a single garlic powder tablet on the atherogenic potential of patient sera. Blood was obtained from 10 patients prior to administration of the garlic tablet. 100% indicates serum atherogenicity, i.e., its ability to induce cholesterol accumulation in cultured smooth muscle cells. Five patients were given a Kwai tablet (*1*) and five other patients an Allikor tablet (*2*). *$p<0.05$ compared with the control.

induced statistically significant intracellular accumulation of cholesterol after a 24-h incubation with smooth muscle cells isolated from uninvolved intima. Garlic extract and atherogenic serum (40%) were added to the cells on day 7 of culturing, and the atherosclerotic parameters were determined after a 24-h incubation [5].

Ex vivo experiments. Sera of patients with angina pectoris and angiographically documented coronary atherosclerosis were analyzed. The group of patients consisted of 10 nonsmoking men aged 40-60 years without a history of dyslipidemia or diabetes mellitus. Detailed characteristics of these patients were given previously [6]. The patients were enrolled in the study after a 72-h flattening period, during which they received no medication except sublingual nitroglycerin. Patients who had taken nitroglycerin on the day of investigation were excluded from the study. Blood was collected from the cubital vein after a single dispensing of a Kwai or Allikor tablet (300 mg garlic powder). Serum was added to a primary culture of smooth muscle cells isolated from grossly normal areas of human aorta. The intergroup differences were evaluated using Student's *t* test and considered to be significant at $p<0.05$.

RESULTS

Patient sera were incubated for 24 h with a primary culture of smooth muscle cells isolated from uninvolved aortic intima. After the incubation, the total intracellular cholesterol content (free and esterified cholesterol) increased by 87.3±9.4% (the mean of 10 experiments, $p<0.05$). Sera obtained 2 h after ingestion of Kwai or Allikor were much less atherogenic and induced a 60% lower accumulation of cholesterol in cultured cells (Fig. 1). After a single tablet of Kwai or Allikor, the atherogenic potential remained low for 4 h and 12 h, respectively (Fig. 1).

In order to confirm the direct antiatherogenic effect of garlic powder, experiments were performed on a cell culture. Twenty-four hours after the addi-

TABLE 1. Effect of Extract from Garlic Powder on the Atherosclerotic Parameters of Cultured Intimal Cells (% of Control, *M±m*)

Additive, μg/ml		Free cholesterol	Esterified cholesterol	^{3}H-Thymidine incorporation
Atherosclerotic cells				
Garlic	0.1	91±4	93±4	92±5
	1	102±6	89±5	94±4
	10	90±6	85±4*	85±5*
	100	83±7*	71±5*	85±3*
	1000	74±4*	68±5*	45±5*
Normal cells				
Atherogenic serum, 40%		144±4	286±16	478±17
Atherogenic serum+garlic	0.1	137±7	193±12*	464±13
	1	143±6	173±7*	464±11
	10	141±6	150±4*	464±17
	100	141±9	140±8*	366±8*
	1000	129±5*	134±12*	269±11*

Note. The mean values of three independent experiments are shown. Changes in the control levels: free cholesterol: 15.7-20.7 and 11.7-16.8 μg/mg cell protein; esterified cholesterol: 41.3-54.2 and 6.8-14.2 μg/mg cell protein, ^{3}H-thymidine incorporation: 137-238 and 79-277 decays/min/μg cell protein (atherosclerotic and normal cells, respectively). *$p<0.05$ compared with the control.

tion of atherogenic serum to normal cultured cells (final concentration in the culture medium 40%), the intracellular free cholesterol content increased 1.5-fold, esterified cholesterol 3-fold, and the rate of DNA synthesis 5-fold (Table 1). The garlic extract markedly lowered these parameters (Table 1). Thus, extract of garlic powder has a direct antiatherogenic (preventive) activity and reduces atherogenic manifestations at the cellular level.

The high content of intracellular lipids (predominantly of esterified cholesterol) is preserved in cultured smooth muscle cells isolated from atherosclerotic plaques [5]. After a 24-h incubation, extract from garlic powder lowered the levels of free and esterified cholesterol and inhibited the proliferative activity of cells, as was evidenced by the level of ^{3}H-thymidine incorporation in cell nuclei (Table 1). This implies that garlic has not only antiatherogenic (preventive) but also direct antiatherosclerotic (therapeutic) activity at the arterial cell level.

It should be noted that the antiatherosclerotic effects of garlic powder were elicited at a concentration of 10 μg/ml, which corresponds to its therapeutic dose. This finding suggests that garlic powder has a direct antiatherogenic effect not only *in vitro* but also *in vivo*. Since our data obtained on cell cultures agree with the results of clinical trials [2,4], we believe that direct effects of garlic will be demonstrated in patients in clinical investigations of the regression of atherosclerosis.

REFERENCES

1. R. Gebhardt, *Arzneimitteforschung. Drug Res.*, **41**, 800-804 (1991).
2. A. Loaldi, A. Polese, P. Montorsi, *et al.*, *Am. J. Cardiol.*, **64**, 433-439 (1989).
3. P. Mansell and J. P. D. Reckless, *Br. Med. J.*, **303**, 379 (1991).
4. A. N. Orekhov, G. N. Baldenkov, V. V. Tertov, *et al.*, *J. Cardiovasc. Pharmacol.*, **12**, Suppl. 6, S66-S68 (1988).
5. A. N. Orekhov, V. V. Tertov, S. A. Kudryashov, *et al.*, *Atherosclerosis*, **60**, 101-110 (1986).
6. A. N. Orekhov, V. V. Tertov, S. A. Kudryashov, and V. N. Smirnov, *Circ. Res.*, **66**, 311-320 (1990).
7. G. Siegel, A. Walter, F. Schnalke *et al.*, *Z. Kardiol.*, **80**, Suppl. 7, 9-24 (1991).