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Original Article

Cholesterol-Lowering effect of organosulphur compounds from garlic: a possible mechanism of action

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Abstract
Objectives: Hyperlipidemia constitutes a major etiopathological factor for atherosclerosis. The medicinal value of garlic is best known for its lipid lowering effects and antiatherogenic effects. The mechanism by which lipid soluble organosulphur compounds from garlic reduce plasma lipids has not been fully investigated. The author had previously shown that the hepatic activity of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase, the rate limiting enzyme in cholesterol biosynthesis and the incorporation of radiolabeled (1, 2 ^{14}C), acetate into hepatic free and esterified cholesterol was significantly decreased in rat treated with garlic derived organosulphur compounds. We hypothesised that the antiatherogenic effect of the organosulphur compounds may be attributed to the formation of protein internal disulphide and thus inactivation of thiol (-SH) group enzymes such as HMG-CoA reductase and the multienzyme complex of fatty acid synthesis. The objective of the present study is to elucidate the inhibitory mechanism by in vitro studies.
Method: Lipid soluble organosulphur compounds from garlic were treated in vitro with Luke's cysteine reagent (representing the thiol (-SH) group of enzymes) and the interaction products were separated by paper chromatography.
Result: The result indicated that the organosulphur compounds were capable of interacting with the thiol (-SH) group of cysteine and thus forming cysteine derivatives.
Conclusion: The antiatherogenic effects of these organosulphur compounds can be attributed to such reactions that inhibit HMG-CoA reductase and other lipogenic enzymes. The anticarcinogenic effects of these compounds may also be due to inhibitory reactions on enzymes that activate carcinogens.

Key Words: Garlic, Organosulphur compounds, Cholesterol, HMG-CoA reductase

Dietary factors play a key role in the development of various human diseases including cardiovascular disease. Epidemiological studies have shown that diet rich in fruits, herbs and spices are associated with a low risk of cardiovascular disease. The cardiovascular -protective effects of garlic have been evaluated extensively in recent years[1,2]. The anti artherogenic effects of garlic have been attributed to certain organosulphur compounds such as diallyldisulphide (DADS), dipropyldisulphide (DPDS), diallytrisulphide (DATS) and dipropyltrisulphide (DPTS). The mechanism by which these compounds reduce plasma lipids has not been fully investigated. Animal studies by the author[3] have earlier shown that organosulphur compounds when supplemented in the diet of rats depressed the hepatic activities of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. We also did radio labeled studies, which showed decreased incorporation of (1,2^{14}C) acetate into hepatic cholesterol. In another experiment by the author[4] a significant decrease in the concentration of total glycosaminoglycans (GAG) as well as that of sulfated fractions viz., chondroitin sulfate and dermatan sulfate in the aorta and heart of treated rats due to a decrease in the activity of uridine diphosphate glucose (UDPGc) dehydrogenase, the enzyme that provide UDP-glucuronic and a precursors of GAG was also observed. This situation may retard the interaction between GAG in these tissues and serum lipoproteins thus avoiding a process of lipid accumulation. We hypothesised that the antiatherogenic effects of these organosulphur compounds may be due to the inactivation of thiol (-SH) group enzymes such as HMG-CoA reductase, fatty acid synthase complex and others. In the present paper chromatographic separation of the interaction products of cysteine hydrochloride (representing the thiol group of enzymes) with organosulphur compounds (DADS, DPDS) extracted from garlic and synthetic DADS and DPDS are described.

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Material and methods

Synthetic DADS and DPDS were obtained from the Department of Biochemistry, University of Kerala, India. Organosulphur compounds were extracted from raw cloves of garlic purchased from the local market at Bharatpur by diethyl ether extraction according to the procedure described earlier[5]. Thin layer chromatography (TLC) of the lipid soluble extract and synthetic DADS and DPDS were performed using silica gel G layered glass plates. Hexane, diethyl ether and acetic acid in the ratio of 70:30:1 was used as the developing solvent and nitropruside spraying reagent was used to visualize the separated compounds.

The separation of cysteine organosulphur compounds (DADS, DPDS) interaction products was done by paper chromatography. DADS, DPDS and the extract (100mg each) were separately dissolved in 7ml of diethylether in three test tubes and each of them was shaken with 0.5ml of Luke's cysteine reagent. Cysteine reagent was made by dissolving 200mg of cysteine hydrochloride in 15 ml of water and the pH adjusted to 4.0 with 1M sodium citrate. Water was added to make a final volume of 20 ml. Samples (10µl) of aqueous layer of each cysteine treated preparation and original cysteine reagent were spotted on Whatman No. 1 paper and subjected to ascending chromatography (10hr) using butanol, acetic acid and water system (12:3:5) The paper was dried and the amino acid spots were developed in 0.2% ninhydrin solution followed by drying at 105° C.

Results

TLC of the extract gave two purple spots having an Rf of 0.75 and 0.84. Using DADS as the standard we obtained an Rf of 0.84 and with DPDS an Rf of 0.75 was obtained. This clearly indicated that DADS and DPDS are the major organosulphur compounds present in the extract.

Table 1 shows the Rf values of cysteine derivatives of synthetic DADS, DPDS and the derivatives of organosulphur compounds extracted from garlic.

Cysteine treated with DADS and DPDS gave the same number of spots corresponding to cystine, cysteine and cysteine derivatives of S-propyl group [C_3H_7-S-S $CH_2CH(NH_2)COOH$] with Rf values of 0.15,0.48 and 0.71 respectively.

Table1. Paper chromatographic separation of derivatives of organosulphur compounds isolated from garlic.

S. No.	Samples treated with Luke's cysteine reagent	Number of spots due to cysteine derivatives	Spots due to cysteine reagent	Rf. value
1.	Luke's cysteine hydrochloride reagent	Nil	2	0.15,0.48
2.	Organosulphur compounds extracted from garlic	2	2	0.15,0.48,0.71*, 0.82*
3	Synthetic DADS	1	2	0.15,0.48,0.71*
4.	Synthetic DPDS	1	2	0.15,0.48,0.71*

** Only these two spots correspond to cysteine derivatives of garlic principles and the synthetic DADS and DPDS. The two extra spots, with Rf 0.48 and 0.15 respectively, correspond to cysteine and its oxidation product cystine*

Luke et al [6] reported the presence of cystine as an oxidation product in the cysteine reagent. The sequence of reactions involved in the formation of the interaction products is as follows: cysteine being a reducing agent reduced the allyl group of DADS and converted it to DPDS. In the second step, the S-propylcysteine derivative is formed. Where R-SH stands for cysteine and all thiol groups of proteins and enzymes.

C_3H_5-S-S-C_3H_5+4SH-CH_2-CH-(NH_2)COOH (DADS) ⟶ C_3H_7-S-S-C_3H_7 + 2 Cystine (DPDS)

C_3H_7-S-S- C_3H_7+R-SH ⟶ C_3H_7-S-SR+ C_3H_7SH.

TLC has shown DADS and DPDS to be the major constituent of the ether extract. Block et al[7] have earlier reported that the extract may contain traces of other disulphide such as dipropyl disulphide (DPTS) and others. With the ether extract of garlic we obtained four spots, one of which corresponds to the cysteine derivative of DPDS (Rf 0.71) that also represent the DPDS formed due to reduction of DADS originally present in the extract. The other spot with Rf 0.82 may belong to the cysteine derivative of another sulphur compounds present in traces in the extract. The remaining two spots obtained correspond to cysteine reagent and its oxidation product cystine with Rf 0.15 and 0.48

Discussion

Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic disease, atherosclerosis, hypertension, thrombosis and diabetes. Protective effects of organosulphur compounds from garlic on atherosclerosis have been attributed to its capacity to reduce lipid content in arterial wall. These organosulphur compounds cause direct antiatherogenic (preventive) and antiatherosclerotic (causing regression) effects at the level of artery wall[8]. We earlier showed that organosulphur compounds when fed to rats significantly lowered the hepatic activity of HMG-CoA reductase the rate limiting enzyme in cholesterol biosynthesis. We also suggested that formation of protein internal disulphide by the thiol disulphide exchange reaction to be the cause of this inactivation. In the present work by in vitro study we are able to demonstrate the interaction of these organosulphur compounds with the thiol group of cysteine (representing enzymes) and were able to separate the interaction products by chromatography. Many of the current available hypolipedemic drugs such as HMG-CoA reductase inhibitors (statins) competitively inhibit HMG-CoA reductase and reduce hepatic cholesterol synthesis and thereby increase the receptor mediated uptake and catabolism of IDL and LDL. It is therefore reasonable that the hypocholesterlemic effect of organosulphur compounds from garlic may stem in part from the impaired cholesterol synthesis.

(DPDS)

Conclusion

The present study clearly demonstrates the importance of thiol-disulphide exchange reaction with relation to the hypocholestrolemic effects of garlic derived organosulphur compounds.

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