Robert, A., Nezamis, J. E., Lancaster, C., Hanchar, A. J. (1979) Gastroenterology 77: 433–443

Robert, A. (1979) Ibid. 77: 761–767

Robert, A. (1981) Ibid. 80: 1262

Schnieden, H. (1981) Int. J. Immunopharmacol. 3: 9–13

Symoens, J., Rosenthal, M. (1977) J. Reticuloendoth. Soc. 21: 175–221

Symoens, J., De Cree, J., Van Bever, W. F. M., Janssen, A. J. (1979) in; Goldberg, M. E. (ed.) Pharmacological and Biochemical properties of drug substances. Am. Pharm. Ass. Washington, pp 407–464

Szabo, S., Trier, J. S., Frankel, P. W. (1981) Science 214: 200–202

Whittle, B. J. R. (1981) Gastroenterology 80: 94–98

Winter, C. A., Risley, E. A., Nuss, G. W. (1962) Proc. Soc. Exp. Biol. Med. 111: 554–547

J. Pharm. Pharmacol. 1984, 36: 272–274
Communicated October 7, 1983

The effect of garlic extracts on contractions of rat gastric fundus and human platelet aggregation

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Garlic has been extracted and separated chromatographically into various fractions which show different degrees of activity as inhibitors of platelet aggregation and smooth muscle. The most potent smooth muscle inhibitor fraction had little activity on platelet aggregation, but μg ml^{-1} concentrations greatly reduced the contractions of rat gastric fundus to prostaglandin E_2 and acetylcholine. Material in this fraction may contribute to some of the claimed therapeutic effects of garlic involving smooth muscle. Its identity is not known, but is different from allyl sulphide, dimethyl sulphide and diallyl disulphide. These compounds eluted earlier on liquid chromatography than the most active fraction, and they showed only modest inhibitory activity against prostaglandin E_2 and acetylcholine on rat fundus.

Garlic (*Allium sativum* L) has been used world-wide as a folk medicine since the time of Hippocrates (Culpeper 1653). It is still used today both for prophylaxis and treatment of various diseases including infections and vascular disorders (Martindale's Extra Pharmacopoeia, 1982). The antibacterial and antifungal properties are due to allicin (diallyl disulphide) (Cavallito & Bailey 1954; Tansey & Appleton 1975). Garlic extract can also reduce serum cholesterol levels, increase plasma fibrinolytic activity and increase blood coagulation time (Bordia et al 1975, 1977). More recent work shows that garlic extracts reduce platelet aggregation by inhibiting thromboxane synthesis (Makheja et al 1979, 1980), and inhibit prostaglandin synthetase prepared from sheep seminal vesicles (Vanderhoek et al 1980). Of the garlic oil components that inhibit platelet aggregation, methyl allyl trisulphide was identified as the most potent (Ariga et al 1981). Lio & Agnoli (1927) reported that garlic briefly stimulates and then depresses smooth muscle. We have fractionated garlic extracts, and obtained material that preferentially blocks responses of rat gastric fundus to prostaglandin E_2 (PGE_2) and acetylcholine (ACh).

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Methods

Extraction of fresh garlic cloves. In two separate extractions fresh garlic cloves were dehusked, chopped finely and homogenized in 0·15 M NaCl (saline) at 4 °C (138 g Extract 1, and 158 g Extract 2 in 400 ml). Garlic oil was obtained by extraction with chloroform (400 ml × 2) followed by rotary evaporation under reduced pressure, the yield being about 2·8 mg oil g^{-1} garlic cloves.

Silicic acid column chromatography. Garlic oil (40 mg) was loaded on to a silicic acid column (40 g) which was eluted sequentially with hexane : diethyl ether, 9 : 1, 4 : 1, 2 : 1, 1 : 1, ether, and methanol (200 ml). The eluant was monitored at 225 nm in a Pye Unicam spectrophotometer (SP6-550) fitted with 100 μl flow cell. Twelve 100 ml fractions were collected, evaporated to dryness, dissolved in 1·5 ml diethyl ether and placed in preweighed tubes. The ether was blown off using oxygen-free nitrogen and the tubes reweighed. Each fraction was then made up as a suspension using a rotary whirler to give a fine dispersion in saline.

Commercial garlic oil prepared by steam distillation (Zimmerman Hobbs and Hofel Pure Foods), allyl sulphide, and dimethyl disulphide (Sigma), and diallyl disulphide (ICN Pharmaceuticals Inc) were chromatographed similarly.

Bioassay. Fractions, in saline, were assayed for antagonist activity against PGE_2 on rat fundus strips bathed in Krebs solution at 37 °C and bubbled with 5% CO_2 in O_2. Amine-blocking drugs (hyoscine, mepyramine, methysergide, phenoxybenzamine and pronethalol) and indomethacin (0·2, 0·2, 0·1, 0·1, 1 and 1 μg ml respectively) were included in these experiments to increase selectivity and sensitivity of the assay (Bennett et al 1973). Consistent submaximal control contractions to PGE_2 were obtained using a 10 min cycle time. One aliquot of a garlic fraction was added after a response to PGE_2 had returned to baseline following washout, and

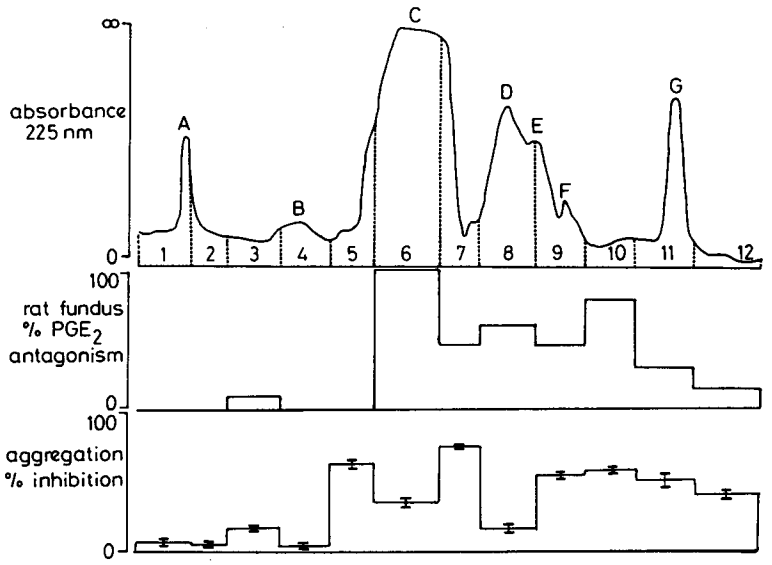

FIG. 1. Garlic extract 2. Ultraviolet absorbance (peaks A–G, top). The effect of fractions 1–12, indicated by dotted lines, on inhibition of PGE_2-induced contraction of rat gastric fundus strips (mean of duplicate determinations, middle) and of adrenaline-induced platelet aggregation (mean ± s.e., n = 6, bottom).

another aliquot added immediately after the next PGE_2 washout. The inhibition of the second response to PGE_2 in the presence of garlic extract was expressed as a percentage of control. Allyl sulphide, diallyl disulphide and dimethyl disulphide were bioassayed similarly. In other experiments the most active antagonist fraction was tested against contractions of rat gastric fundus to alternate submaximally effective doses of ACh and PGE_2 in the absence of hyoscine or sometimes of all the amine-blocking drugs.

Platelet aggregation. Whole blood was obtained from healthy male volunteers who had not taken aspirin, or other drugs known to effect platelet aggregation, in the previous 2 weeks. Blood was collected by venepuncture into 3·2% (w/v) sodium citrate, and platelet-rich plasma (PRP) was obtained by centrifuging at 90*g* for 10 min. Platelet-poor plasma (PPP) was obtained by further centrifugation at 500*g* for 10 min and used to adjust the platelet content of the PRP to 300 000 μl^{-1}. Platelet aggregation was measured with a dual-channel Payton aggregometer by the method of Born (1962). The effect of each fraction (100 μg on submaximal platelet aggregation in 500 μl of PRP) was studied by the addition of the fraction or vehicle 2 min before the addition of 0·2–0·5 μg adrenaline. Aggregation was quantitated by determining the maximum percentage light transmission obtained within 2·5 min.

Results

Chromatography. The elution profile of garlic oil (Extract 2) following silicic acid chromatography is shown in Fig. 1. The uv absorbance peaks were labelled A–G. Most uv absorption occurred with the eluates hexane : ether 2 : 1 and 1 : 1 (peaks C and D).

Allyl sulphide, diallyl disulphide and dimethyl disulphide eluted in the first fraction, indicating that peak A was due to the fairly non-polar sulphides and disulphides reported to occur in garlic oil (Oaks et al 1964). Other reported components of garlic oil, allyl cysteine-*S*-oxide (alliin) and diallyl disulphide oxide (allicin) (Stoll & Seebeck 1949) are more polar and would presumably be eluted later. In contrast to our extract, most of the commercial garlic oil eluted in peak A.

Table 1. Inhibition, by the most active garlic fraction, of rat gastric fundus contractions to PGE_2 and acetylcholine (ACh). Blockers in the Krebs solution were indomethacin, various amine antagonists, but no hyoscine. There were two separate experiments with each concentration of the 2 extracts. The first pair of % reductions of PGE_2 and ACh (e.g. 67 and 70% reduction, top line) show the effect after 2 or 3 additions of the antagonist fraction; the second pair (e.g. PGE_2 and ACh 73 and 98%) show greater inhibition after 8 or 9 additions (each washed out with the next dose of agonist). With strips bathed in Krebs solution alone, the fractions reduced the muscle tone; this may explain the apparently weaker inhibition.

Blockers in Krebs soln.	Extract	μg ml^{-1}	% reduction of contraction			
			PGE_2	ACh	PGE_2	ACh
Yes	1	24	67	70	73	98
			55	66	55	99
	2	5	96	91	100	100
			86	80	95	99
No	1	48	32	57	53	46
			56	51	58	68
	2	20	26	43	38	71
			4	32	44	62

Bioassay. Fig. 1 illustrates the inhibitory effect of the various garlic fractions (20 μg ml^{-1} final bath concentration) on consistent, submaximal PGE_2-induced contractions of rat gastric fundus preparations in the presence of amine-blocking drugs and indomethacin. Fractions 6, 8 and 10 given on two consecutive PGE_2 cycles showed the greatest inhibitory effect (respectively 99, 55 and 77% reduction of the second PGE_2 response compared with controls). Fractions 7, 9, 11 and 12 reduced PGE_2 responses by 44, 44, 29 and 14% respectively, whereas 1 to 5 showed little effect. The inhibition by the fractions was long-lasting. With the most active fractions (6 and 10) the response to PGE_2 recovered to only 50% after 60 min (i.e. following 6 doses of PGE_2). Restoration of responses after the other fractions was complete within 60 min, presumably because they produced a smaller inhibition.

There were 8 other experiments using the most active antagonist fraction (fraction 6 from silicic acid chromatography Extract 2, and its equivalent (slightly different chromatography) from Extract 1). Four assay tissues were in Krebs solution without blockers, and four were in the presence of mepyramine, methysergide, pronethalol, phenoxybenzamine and indomethacin but not hyoscine. Half of these experiments were with the fraction from Extract 1 and half were with Extract 2. PGE_2 and ACh were given alternately, and the reductions (Table 1) were measured from the second to the ninth addition of the fraction. The maximum inhibition developed slowly, as shown by the increasing effect with repeated doses of the fraction.

In the absence of blocking drugs the garlic fraction from each extract reduced muscle tone, as would be expected with a prostaglandin antagonist (Bennett & Posner 1971). No fall occurred in the presence of the blockers because they had already relaxed the gastric fundus. The antagonism by the garlic seems to be smaller in the absence of the blocking drugs, but this may be merely a mechanical effect; relaxation induced by the garlic would tend to increase the contractions to agonists, because they start from a lower baseline, thus partly counteracting the inhibition of responses to PGE_2 and ACh.

Pure compounds. These were studied as with the garlic fractions, with 100 μg (20 μg ml^{-1}) added to fundus strips on two consecutive occasions in the presence of the amine-blocking drugs and indomethacin. Allyl sulphide, diallyl disulphide and dimethyl disulphide reduced responses to the second dose of PGE_2 by 6·8 ± 3·3%, 24·2 ± 3·7% and 11·4 ± 4·1% respectively (mean ± s.e., n = 5–6).

Platelet aggregation studies. Platelet aggregation results (mean of 6 using Extract 2) are shown in Fig. 1. Fractions 1 to 4 had little anti-aggregating activity. Fractions 5 and 7 showed the highest anti-aggregatory activity of 60 ± 2·7 (se)% and 71 ± 2·4% while fractions 6, 9, 10, 11 and 12 showed anti-aggregatory activity of 33 ± 2·7%, 51 ± 2·7%, 54 ± 2·2%, 47 ± 5·1% and 37 ± 2·4% respectively.

Discussion

The results demonstrate that garlic oil contains various components, some of which preferentially inhibit platelet aggregation and others which preferentially inhibit contractions of rat gastric fundus to PGE_2 or ACh. Fractions 5 and 7 caused the greatest anti-aggregatory response whereas fractions 6 and 10 caused the largest antagonism to rat fundus contractions. The platelet anti-aggregatory activity of garlic has received substantial study (see Introduction). We have not elucidated the mechanisms(s) by which fractions reduce aggregation, but possibilities include inhibition of thromboxane synthesis and antagonism of prostanoid action.

Inhibition of smooth muscle seems to be a selective effect of some garlic constituents. The maximum inhibition develops slowly, and it seems likely that even greater inhibition would occur on exposure to low concentrations for a long time. Allyl sulphide, diallyl disulphide and dimethyl disulphide, known constituents of garlic oil, elute on chromatography before the most active fractions, and contribute only in part to the total inhibitory effect of garlic on rat isolated gastric fundus. Further purification and methods of identification are needed to determine the structures of the compounds in the most active fractions.

REFERENCES

Ariga, T., Oshiba, S. Tamada, T. (1981) Lancet 1: 150–151

Bennett, A., Posner, J. (1971) Br. J. Pharmacol. 42: 585–594

Bennett, A., Stamford, I. F., Unger, W. G. (1973) J. Physiol. 229: 349–360

Bordia, A., Bansal, H., Arora, S., Singh, S. (1975) Atherosclerosis 21: 15–19

Bordia, A., Verma, S., Vyas, A., Khabya, B., Rathore, A., Bhu, N., Bedi, H. (1977) Atherosclerosis 26: 379–386

Born, G. V. R. (1962) Nature 194: 927–929

Cavallito, C., Baily, J. H. (1944) J. Am. Chem. Soc. 66: 1950–1954

Culpeper, N. (1653) The Complete Herbal. Republished by Imperial Chemical (Pharmaceuticals) Ltd, 1953

Lio, G., Agnoli, R. (1927) Biol. Abs 4: 774

Makheja, A., Vanderhoek, J., Bailey, J. (1979) Lancet 1: 781

Makheja, A., Vanderhoek, J., Bryant, R., Bailey, J. (1980) Adv. Prostaglandin Thromboxane Res. 6: 309–312

Oaks, D., Hartman, H., Dimick, K. (1964) Anal. Chem. 36: 1560–1565

Stoll, A., Seebeck, E. (1949) Helv. Chim. Acta 31: 197–205

Tansey, M., Appleton, J. (1975) Mycologia 67: 409–413

Vanderhoek, J., Makheja, A., Bailey, J. (1980) Biochem. Pharmacol. 29: 3169–3173