Garlic: A product of spilled ambrosia

Mahendra Kumar Jain and Rafael Apitz-Castro

Molecular, biochemical and physiological basis for the antithrombotic effect of garlic is reviewed. Ajoene, formed by molecular rearrangement of odorous allicin, is a potent antiplatelet agent which blocks the exposure of fibrinogen receptors by modulating the function of G-proteins. Since ajoene is not present in virtually any of the commercially available 'garlic preparations' worldwide, their use against 'heart-ailments' is suspect.

Ancient traditions, when tested by the severe processes of modern investigations commonly enough fade away into mere dreams; but it is singular how often the dream turns out to have been a half-waking one, a presaging reality.

T. H. Huxley

TALES and symbolism in the annals of garlic apocrypha are numerous and varying, and often rich with fact and fancy. According to one of the Indian folklores, Amrit (ambrosia) was among the many items obtained from the ocean during Samudra-Manthan. Although it was a collaborative effort of 'Surs' (good) and 'Asurs' (evil), both parties could not agree on how to share it. Surs managed to run away with the pot, spilling some of the amrit on the way. Garlic (*Allium sativum*, family Liliaceae) grew at these sites.

Garlic was domesticated at least seven millennia ago as a herb in the agriculturally affluent deltas of Ganges and Nile. Since then in many cultures its culinary use has spread to enhance flavour and as a preservative. Due to its pungent and persistent aroma crushed and cooked garlic evokes emotions and social responses. Probably no other food has been viewed with greater ambivalence as a basis for sarcasm and prejudice as garlic is in the anglo-saxon culture. Besides its culinary use, the folklore about the powers of garlic is also flavoured with strong cultural identities[1]. With an increasing interest in the role of food to prevent disease and to promote good health, garlic has its adherents who are often different than those who find it aromatic and pleasing to palate. There are claims of garlic's efficacy to improve complexion and posture, to ameliorate sibling rivalry, and to cure cancer, impotency, leprosy, tuberculosis, whiplash, toothache, bad blood, insect bites and eliminate worms. Probably in the absence of anything better, over the centuries some of the concoctions and potions have provided solace and comfort, if not a cure. Many of these claims may be challenged on the basis of the techniques available now, while others have found a molecular basis in fact.

If camel is a horse designed by a committee, then folklore is collective wisdom. Traditional medicines and the underlying beliefs are repositories of empirical observations and insights accumulated over long periods of practical experience. This wealth of information must be gleaned, articulated, and reinterpreted further by the subsequent generations as their appreciation of the universe around them is enhanced by newer experiences and knowledge, and as more sophisticated analytical methods and technologies become available. With appropriate appreciation of the underlying pathophysiological basis for the empirically identified maladies of the folklore, many of the ancient herbal cures have provided leads and have found rigorous scientific basis in the form of some modern medicines such as aspirin, quinine, and rowalfine. Based on such considerations it appears that traditional medicines and folklore are definitely useful at early stages in the drug discovery process for elaborating leads for identifying novel biochemical loci of action of drugs in a metabolic pathway, and by providing inspiration for the design and development of new classes of bioactive molecules. In short, folklore leads the way by attempting to articulate little known processes.

In an explanation-oriented subculture empirical observations are appreciated only as a hindsight. Indeed, numerous observations on disorder and maladies known in ancient times have found their expression in terms of the covenants of modern medicine. It may be emphasized that such severe tests require elaboration and transformation of empirical observations into formal questions and analytical expressions that are amenable to the state-of-the-art methodologies. If one focuses on the deeper levels of the phenomenology, the semantics of reinterpretation of empirical observations in terms of formal representations and expressions leads to rediscoveries and further

Mahendra Kumar Jain is Professor of Chemistry and Biochemistry at University of Delaware, Newark, DE 19716, USA, and Visiting Professor of Jawaharlal Nehru Centre for Advanced Scientific Research at Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India. Rafael Apitz-Castro is Director of Thrombosis Research Laboratory, Biophysics Institute, IVIC, Caracas, Venezuela.

articulation at yet another level of sophistication. Similarly, an understanding of the underlying chemical, biochemical, and physiological processes enhances a grasp of the overall phenomenon. The converse is also relevant.

In this article we describe our interdisciplinary studies on understanding the folkloric mystique of garlic. Such attempts to understand the putative vampire-repellant effects of garlic have provided us insights into a wide range of novel physiological, biochemical and molecular processes.

The vampire-repellent action of garlic

In the European literary and philosophical context of romanticism, the characters and representations in the decadent life style of a Marquis-de-Sade probably contain some of the original folklore[2]. Such characters owe their peculiarities neither to academic and literary traditions, nor to a revolt against specific dogma or theology, but they simply represent an early attempt to comprehend and explain some peculiar observations or mysteries. For example, Bram Stocker's Dracula is based on Romanian folklore. Vlad Temps or Dracula (Drac means the devil in Romanian) of Transylvania are most famous characters in the Vampire legend. All such stories reveal the lesson that Vampires wrench ('suck') the life out of the victim before he is ready to give it up. These stories connote sudden violent deaths where victims leave behind an unfinished life. The vampire steals the substance of life. Such creatures or manifestations are probably one culture's way of comprehending unexplained occurrences of sudden deaths, which is still a mystery that the present day medical profession is trying to fathom.

One of the recurring themes in the folklore on garlic relates to its beneficial effects on heart and blood. Sudden death and its underlying relationship to blood-related disorders, as well as the preventive and curative properties of garlic, are amply substantiated by epidemiological studies[3-5]. Relationship of one of the most common causes of sudden death (coronary thrombosis and stroke) to underlying blood disorders as emphasized in the folklore is beginning to gain physiological basis. Interruption of blood flow to certain organs in higher organisms can manifest in pathological conditions[6]. For example, reduced blood supply to the brain results in an extensive damage to brain tissues (stroke). Similarly, decreased blood supply through the heart often leads to myocardial infarction and sudden death. Such acute ischaemic manifestations arise from the formation and consolidation of a mass (called thrombi) of platelets and fibrin in the lumen of blood vessel. The fact that such disorders do not strike most people is due to a critically regulated interplay between three groups of components which optimize the flow of blood through vessels: vascular factors including endothelial cells; platelets and other blood cells; and the plasma proteins involved in coagulation and fibrinolysis. One of the basic characteristics of normal intact endothelium is the absence of reactivity ('stickiness') towards platelets, leukocytes, and the components of the enzymatic cascade of blood coagulation. Such a thromboresistance arises from: (a) the presence of certain glycoproteins on the surface of endothelial cells which impair the interaction of blood cells with the endothelium; (b) the production of prostacyclin which inhibits platelet activation; (c) the antithrombinic factors (e.g. AT-1 and AT-III) synthesized by endothelial cells which inhibit the action of low levels of thrombin normally formed; (d) the activators of the fibrinolytic factors, such as tissue plasminogen activator (t-PA), which triggers reactions leading to dissolution of coaguli. These four processes are adversely affected by chronic or repeated damage to endothelium. Exposure of subendothelial structure in damaged vessels activates platelets and enzymatic systems of blood coagulation which are ultimately responsible for the formation of intraluminal thrombi.

The clinical manifestations of endothelial injury in walls of arteries, veins, and blood vessels depend on the site and the extent of damage, and on the intrinsic 'stickiness' of platelets. The blood-clots are expressed as coronary or cerebrovascular disease, diabetic angiopathy, or peripheral artery diseases. Hardening and narrowing of arteries also contributes to the clinical manifestations of atherosclerosis. In addition, there are medical and surgical procedures in which platelet activation plays a major role in the development of thromboembolic complications such as in valvular or vascular prosthesis, chronic hemodialysis, and during cardiopulmonary bypass used in open-heart surgery.

As summarized below, with some imagination, the current paradigm of blood physiology seems to pull together the folklore within the reaches of the chemistry of garlic components, and the effect of one of such compounds on the pathophysiology of thrombogenesis and related blood disorders.

Search for the antiplatelet activity in garlic extracts

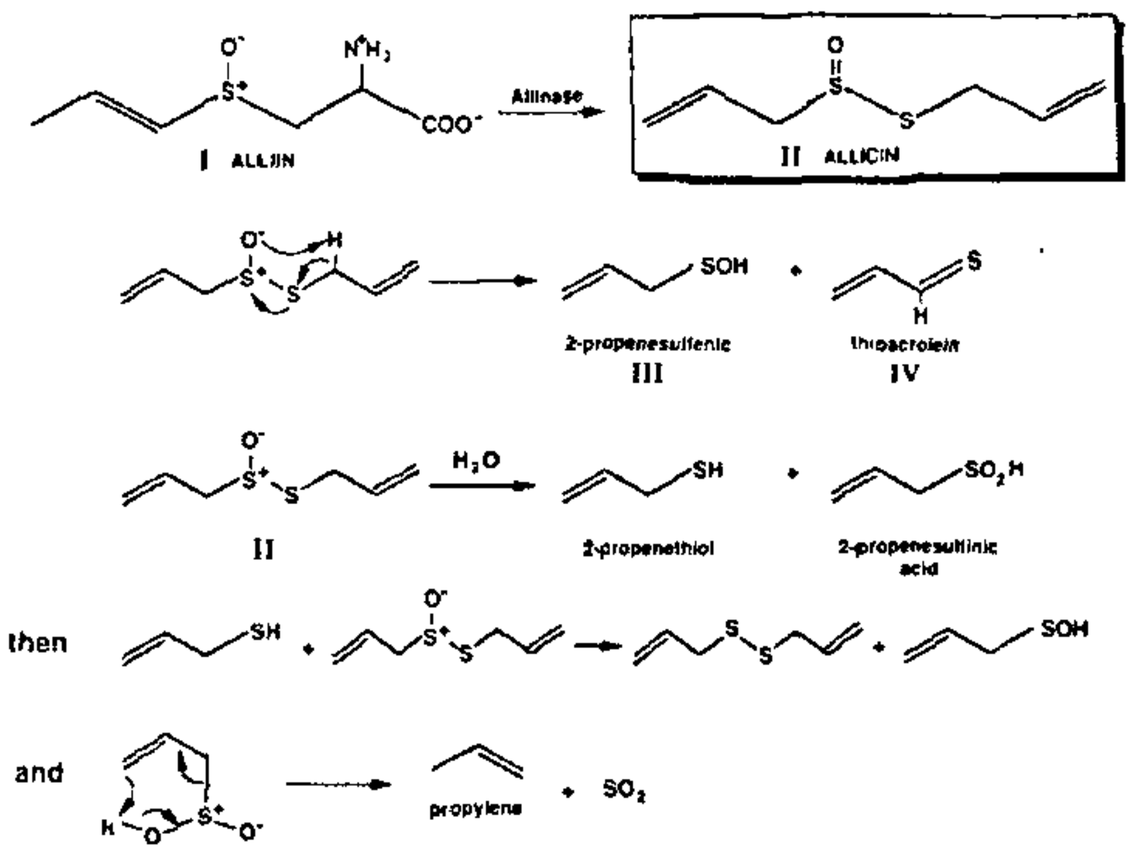
The blood cell primarily involved in the physiopathology of thromboembolism is the platelet. The 'stickiness' of platelets determines their ability to form clots, and thus an antiplatelet agent would prevent formation of clots. An early stage in the clot-formation is the formation of thrombi from self-aggregation of platelets, a process that can be studied *in vitro* by monitoring aggregation of isolated platelets induced by appropriate agonists such as collagen, thrombin, and ADP[7]. The time course of aggregation of isolated platelets (suspended in homologous plasma or in an artificial

medium) is complex, however with suitable controls it can be used to characterize the effect of inhibitors. This bioassay is very sensitive and convenient to use. With such an assay, Bordia[8] demonstrated that the ether extract of garlic inhibited aggregation of human platelets. This report was particularly significant to us, not only because it substantiated the epidemiological studies which showed that several population groups who used cooked garlic in relative abundance enjoyed beneficial effects of garlic[3,4], but it ultimately led us to isolation and characterization of ajoene, a potent antiplatelet compound from aged extracts of garlic[9,10]. The antiplatelet activity was observed in a nonvolatile extract of garlic, which provided a basis for the negative results of several authors who used raw garlic[11] or the volatile oil of garlic[5]. Further experiments convinced us that ageing of the alcoholic extract of garlic is necessary to enhance the antiplatelet activity; that a reasonably stable single compound is responsible for such an activity; that the fermented or garlic juice or the volatile oil of garlic (which is present in most 'garlic pills') is not active. In fact, such antiplatelet activity is not present in dehydrated garlic as well as in over thirty commercially available preparations of garlic that we obtained from Germany, United States, Venezuela, India, and Japan.

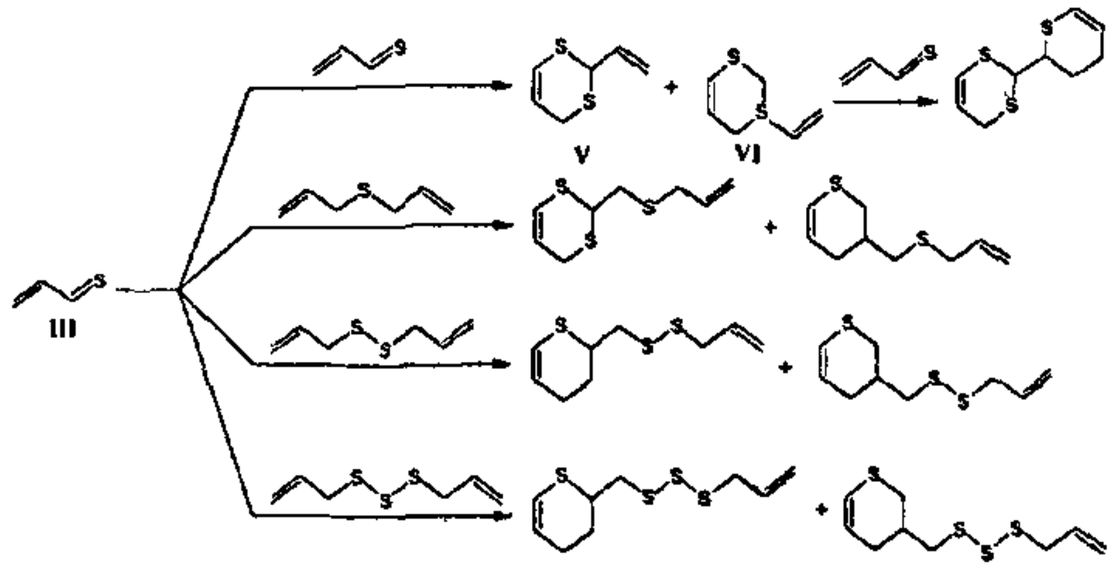
Chemical components of garlic

Biological processes originate from specific macromolecules which are often modulated by smaller molecules. This axiom provides a rationale for the study of bioactive natural products, and such studies lead to specific molecular components, which interact with macromolecules primarily responsible for the biological function. Natural products have provided useful templates as leads to establish fascinating chemical structures as well as to understand a bewildering array of chemical transformations. Such explorations in the chemistry of secondary metabolites have been motivated by a variety of reasons. Besides the intellectual curiosity, the socio-political justification for the search of pharmacologically useful agents to relieve pain and suffering is rationalized on the basis that such knowledge could provide a basis for the better management and utilization of resources. The economic rationale for the study of the natural products is that isolated compounds can be synthesized and provide leads to more potent analogs for chemists; be protected by patents, and distributed by pharmaceutical companies; be prescribed by physicians and sold by pharmacists who do not make it; and ultimately consumers use such value-added products for the convenience of a pill rather than a crude extract or herb. In order to understand the components for flavouring the processed foodstuffs, the food industry has concentrated attention on the steam distillate or volatile components of garlic[12]. Interaction of minor food components with other nutritional components like vitamins was given some impetus by the observation that thiamine (vitamin B1) and certain allium constituents are more readily absorbed from intestine than the free vitamin itself[12,13]. This is due to formation of a covalent adduct, allithiamine.

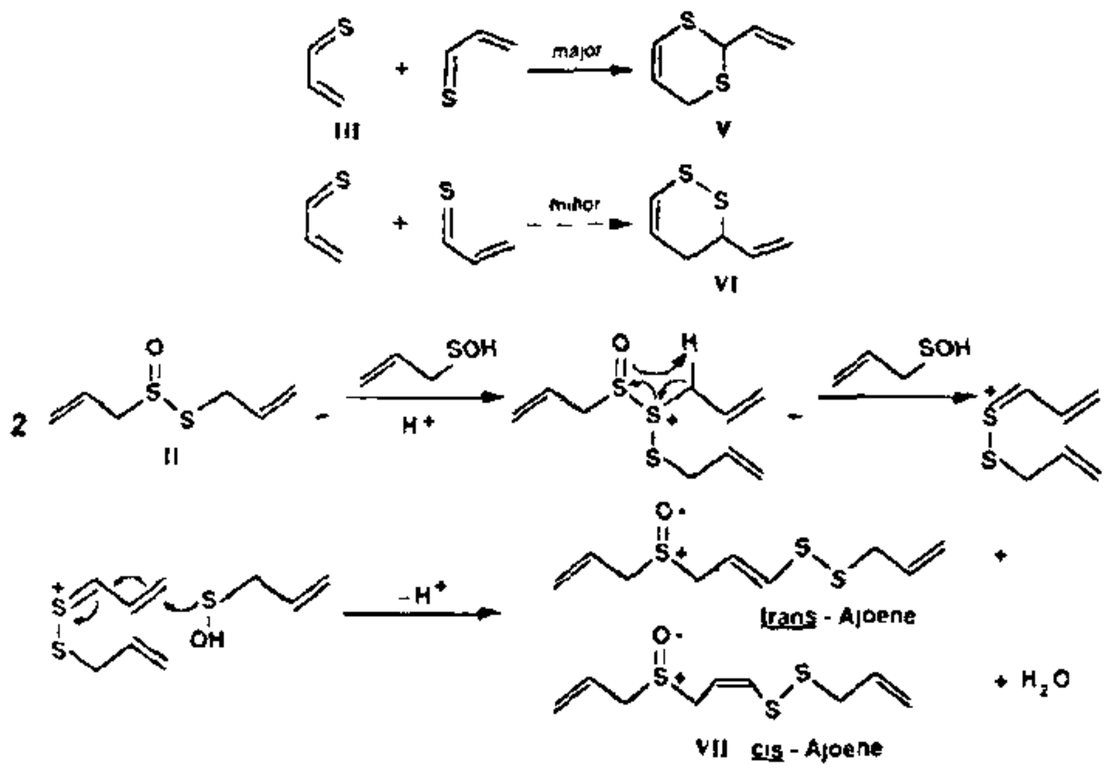
Studies on secondary metabolites of garlic have provided a wealth of compounds that have enhanced our understanding of the organic chemistry of sulphur. As summarized in Schemes 1–4, the compounds isolated from garlic or derived preparations fall into three categories depending upon the protocols used for the processing. For example, the compounds present in extracts or the steam-distillate are not present as such in garlic but they are formed by fascinating enzymatic transformations, spontaneous chemical rearrangements and thermal degradation of some of the relatively simple precursors. Ultimately, the proportion of such products depends on the protocol for obtaining a given preparation. The mechanistic basis for these chemical



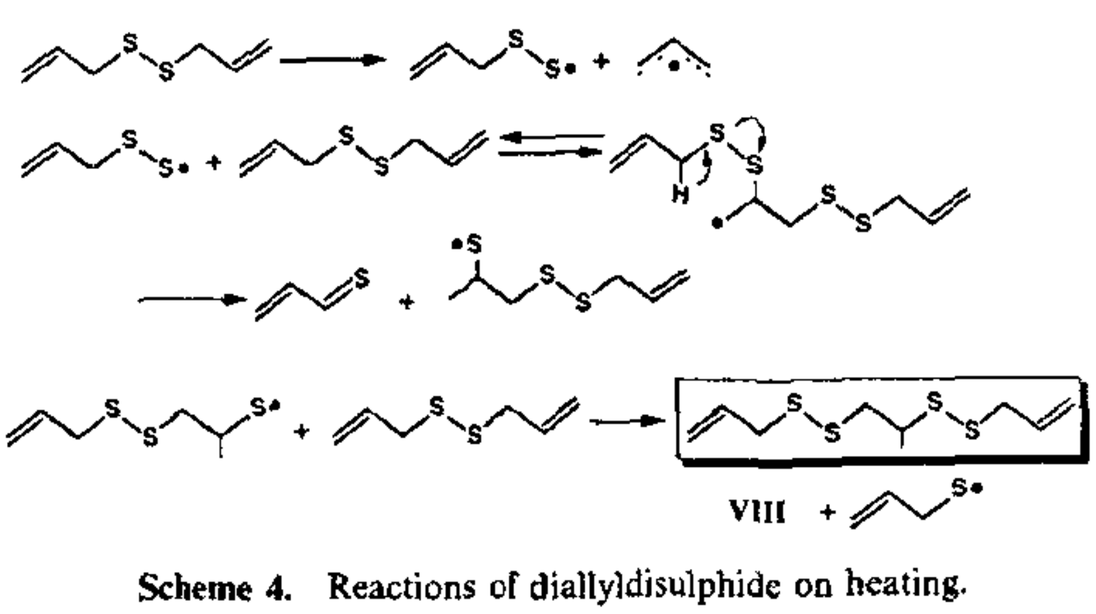
Scheme 1. Enzymatic and spontaneous chemical transformations of alliin to highly reactive intermediates. For an extensive review of the chemistry of the organosulphur compounds of *Allium* spp., see ref. 43.



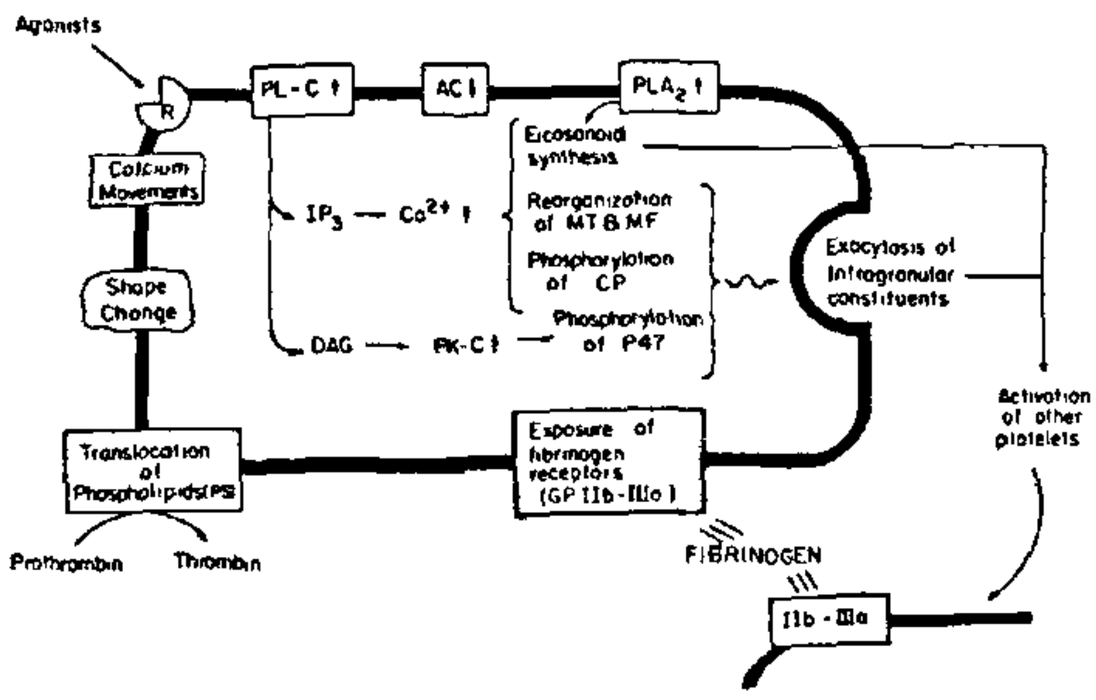
Scheme 2. Possible modes of rearrangements of thioacrolein on heating and steam-distillation.



Scheme 3. Reactions leading to the formation of ajoene from alliicin.



Scheme 4. Reactions of diallyldisulphide on heating.



Scheme 5. A simplified view of the major metabolic pathways related to platelet activation. The locus of action of ajoene is on the agonist-induced exposure of the fibrinogen receptors[33]. Specific steps involved in this process are not known, however evidence suggests that ajoene interferes with the function of G-protein[34].

transformations in garlic[14] and onion[15,16] preparations have a similar basis, however the end products differ because garlic contains thioallyl precursors whereas onions predominantly contain thiopropenyl analogues. Presence of antiplatelet components in onion extracts has not been demonstrated.

Biochemical transformations on crushing the garlic bulb

Alliin or (+)-S-allyl-L-cysteine sulphoxide (I in Scheme 1) is the major sulphur-containing compound present in intact garlic bulb (0.2 to 4 dry weight per cent depending on the source of garlic). The corresponding thioether and glutamylpeptide derivatives have also been identified in garlic and onion bulbs[17]. The methyl and propyl analogues have been found in other members of Liliaceae, but the allyl analogues appear to predominate only in garlic. Biosynthesis and biological activity, if any, of alliin and related compounds has not been established[18], however the products of metabolism of alliin in human urine have been characterized[19].

As is the case with many natural products[17], as summarized in Scheme 1 selective enzymatic processing of odorless alliin (I) yields allicin (II) which is responsible for the characteristic odour of crushed garlic cloves[20]. This reaction is catalysed by the enzyme alliin lyase (allinase) on alliin[21]. Normally the enzyme is compartmentalized and it is brought optimally in contact with the substrate (alliin) in the crushed garlic bulb. Although such a separation has not been demonstrated directly in garlic bulbs, this enzyme is localized in vacuoles of the mature cells of onion bulbs and the substrate is present in the cytoplasm[22]. Allinase (alliin alkylsulphenate lyase, EC 4.4.1.4) is a water-soluble pyridoxal-phosphate requiring enzyme with pH optimum at 6, and has been purified to homogeneity[23]. It is not clear if the conversion of propene sulphenic acid (III), the primary product of action of allinase on alliin, to allicin (II) is also catalysed by alliin lyase or if it is a spontaneous process.

Since S-propyl- and S-methylcysteine sulphoxides are also found in garlic, other minor symmetrical and asymmetrical analogues of allicin are formed by the enzymatic reaction. The enzyme catalysed reaction is specific for derivatives of (-) L-cysteine, and the rate of reaction is considerably faster with the (+)-form of the sulphoxide. Thioethers are not substrates. A similar enzyme that cleaves S-(1,2-dichlorovinyl)-L-cysteine has been reported in *E. coli*[24] and bovine liver[25]. Therefore, it would be of interest to see if similar activity is found in human intestinal flora which could promote formation of allicin from fresh garlic. It may be pertinent to note that the 'odour-free' garlic is produced by treating garlic bulb with a proprietary solution, which presumably inactivates allinase. A mutant of garlic devoid of allinase has also been described in the popular press, and for obvious reasons the physiological efficacy of such preparations is questionable.

Virtually all reports on the physiological action of allicin are suspect because it is quite unstable even in cold. The halftime for the decomposition of aqueous solutions of allicin at room temperature can be less

than 20 min. depending on the conditions. Therefore, it is quite doubtful if orally ingested allicin can pass through stomach, or if allicin is formed in stomach when whole garlic clove is swallowed. The most likely beneficial effect of fresh crushed garlic arises from the fact that allicin has very strong antibacterial properties on direct contact. Inhibitory effect of allicin on acetyl-coenzyme A has also been reported[26].

The steam-distillate of garlic

There is often a consideration of the convenience of marketable preparations when a fresh herb cannot be readily procured and processed. The food-processing industry has tried to capture the 'aroma of cooked garlic' in the form of its steam-distillate. Since allicin, which is responsible for the aroma of freshly crushed garlic, is quite unstable, as summarized in Scheme 2 the flavour precursors of steam-distilled garlic oil arise from thermal decomposition of allicin to a broad range of symmetrical and asymmetrical sulphides formed from thioacrolein (IV) as intermediate[14,27-29]. The disulphide can disproportionate to mono- and trisulphides. Tetra-, penta- and hexasulphides have also been detected in the mixtures formed by heating disulphide at 100° C. These and several other compounds (Schemes 3 and 4) have been found in garlic extracts at different stages of heat catalysed degradation and ageing[14,30]. Such a set of complex reactions could form the basis for substantially what goes on during heating and steam distillation, giving rise to the characteristic aroma of cooked garlic.

Steam distilled preparations of garlic have been used in certain physiological studies, and they are often sold in health-food stores as 'garlic pills' and 'garlic oils'. Although such preparations have an odour that resembles 'cooked garlic', as far as we are aware their 'beneficial effects on health' have not been demonstrated, nor is the 'goodness of garlic' captured as the efficacy of these pills has not been demonstrated. The steam-distilled oil of garlic has been claimed to possess anti-cancer properties presumably due to their nonspecific anti-oxidant action as they irreversibly and nonspecifically inhibit lipoxygenase[31]. However, it cannot be ruled out that the observed noncompetitive inhibition is due to nonspecific effects related to the ability of these hydrophobic solutes to perturb the substrate interface required for interfacial catalysis, nor can it be discounted that the hydrophobic sulphides react with a reaction intermediate or with certain functional groups and metal ions on the enzyme.

The potions

Many a potion, concoction, tincture and sauce prepared by those who are knowledgeable, seem to enhance the curative and beneficial properties of fresh natural herbs. Such a processing also restores the 'balance of the effects in relation to other environmental factors'. This is a recurring theme in most traditional systems of medicine, and it has apparently not found appropriate recognition in modern scientific medicine. In such 'balanced' preparations, one notices a remarkable power of empirical observation as the underlying chemical modifications of the natural components are recognized. In an explanation-oriented subculture such observations are usually appreciated only as a hindsight.

It is of particular interest to note that the appreciation of the beneficial effects of garlic is intrinsic in several potions of medicinal value prepared by processing of garlic before use. At a later stage in our investigations, we obtained a typical protocol from a herbalist in downtown Caracas. It consists of soaking chopped garlic cloves in enough alcohol to cover it, and then letting it steep seven days in sun, seven days in moon and seven days in dark. It is used once-a-day for 21 days, and then not used for another 21 days while a new batch is prepared. This recipe was given to us in response to an inquiry for a 'potion from garlic with beneficial effects on heart and blood'. Other preparations of this type include 'four thieves vinegar' (vinaigre des quatres voleures) and 'garlic pickled in vodka'. Such preparations for 'clearing arteries' provided a rationale for our observation that only the aged ether or alcoholic extract of garlic exhibits antiplatelet activity which can be ascribed to the formation of a potent antiplatelet agent, ajoene[9,10]. Later studies revealed that ajoene is not present in any of the commercially available 'garlic preparations' including the fermented garlic extract (e.g. Kyolic), capsules of freeze-dried garlic, or fresh garlic bulbs. Although some preparations of garlic soaked in cooking oil also contain ajoene, the reports on the toxic anaerobic bacterial contamination in such preparations have appeared in popular press.

Formation of ajoene in the crude extracts of garlic (Scheme 3) requires a reasonably controlled set of conditions of temperature, polarity and pH which are probably mimicked during processing of garlic such as pickling, ageing and sauteing. In the laboratory, allicin is readily obtained by treatment of diallyldisulphide in chloroform with stoichiometric amounts of 30% peracetic acid. Allicin recovered from the washed organic layer is converted to ajoene in reasonable yield by heating the ethyl acetate solution (5%) solution with equimolar amounts of powdered sodium hydrogen phosphate at 40° C for 3 h. The R_f of ajoene is 0.35 on silica plates developed in ethyl acetate.

The mechanism for the underlying chemical transformations that lead to the formation of ajoene and related compounds from allicin are summarized in Scheme 3. This is based on the ability of allicin to form

two highly reactive intermediates with a high propensity for rearrangement, disproportionation and reactivity towards a variety of functional groups[14]. For example as shown in Scheme 1, β-elimination from allicin could afford two highly reactive intermediates: 2-propene-sulphinic acid (III) and thioacrolein (IV). As further developed in Scheme 3, thioacrolein (IV) dimerizes spontaneously to (V) and (VI). S-thioallylation of allicin (II) could give sulphonium ion which could undergo β-elimination followed by γ-addition to 2-propene sulphinic acid to give ajoene (VII), or allicin could also undergo hydrolysis to give thioallyl alcohol and diallyldisulphide which could also be formed via an attack of 2-propene sulphinic acid (III) on allicin (II). Disproportionation of allicin (II) also gives rise to diallyldisulphide and diallylthiosulphonate. Other reactions of allicin that occur through similar intermediates are described elsewhere[14,30] and are shown in Schemes 3 and 4.

The antithrombotic action of ajoene

Ajoene accounts for not only a molecular basis for the folklore, but the method of its preparation provides a rare insight into the chemical changes recognized centuries ago by empirical methods. The biochemical locus of action of ajoene is novel[32–34] and is not shared by any other known antagonist of platelet activation. As elaborated later in this article, the antiplatelet action of ajoene has some very favourable features because it provides not only a novel locus for testing antithrombotic agents, but ajoene itself could be a 'lead' for a clinically useful drug against thrombosis and stroke.

We have extensively studied the effects of ajoene on platelet response to agonists. The inhibitory effect of ajoene on platelet aggregation can be understood at several levels. Macroscopically, the platelet reaction induced by agonists begins with a shape change (discoidal to spherical) followed by platelet aggregation which is responsible for the major change in the turbidity. Ajoene does not influence the initial shape change, however it inhibits platelet–platelet interaction. Also dissociation occurs when ajoene is added to aggregated platelets. Ajoene inhibits *in vitro* aggregation of human platelets induced by all known inductors including collagen, ADP, thrombin, platelet activating factor, arachidonic acid, phorbol esters, antiheparin antibodies, epinephrine, A-23187, and U-46619. Platelets of cow, monkey, pig, rabbit, rats, horses and guinea pigs are also inhibited by ajoene. We have observed the inhibitory effect after oral administration of ajoene to rabbits, or endovenous injection of ajoene (suspended in Intralipid) in dogs[35]. Experiments with isolated platelets as well as with animals show that the ajoene-induced inhibition is reversible. These results on the effect of ajoene on the platelet response provide a physiological and molecular basis for the antiplatelet action of extracts of garlic. Compared to ajoene, volatile garlic oil and the components such as dithiene and polysulphide are considerably less (<10%) effective as antiplatelet agents[9].

Biochemical locus of action of ajoene: Signal transduction pathway

In search of a molecular mechanism to explain the action of ajoene on platelets, we initiated a detailed study of the effect of ajoene on the major biochemical pathways of platelet activation. At the molecular level, platelet activation is a complex, not yet completely understood process. A simplified view of the better studied biochemical pathways related to platelet activation is shown in Scheme 5. Briefly, the interaction of an agonist with its receptor on the platelet surface leads primarily to[36]: (a) Hydrolysis of phosphatidyl inositol-4, 5-biphosphate to yield two secondary messengers, diacylglycerol (DAG) and inositol trisphosphate (IP_3). This reaction is a consequence of the direct activation (receptor-dependent) of a membrane-bound phospholipase C, mediated by a G-protein. (b) Calcium mobilization from a pool primarily bound to the plasma membrane. (c) Inhibition of G_i-mediated adenylate cyclase.

Such primary responses lead to secondary activation of a variety of intracellular processes such as: (1) increase in the cytoplasmic calcium concentration by more than a factor of 100 due to massive mobilization of calcium from the dense tubular system; (2) reorganization and rearrangement of microtubules and microfilaments; (3) activation of xalmodulin-dependent contractile proteins; (4) activation of DAG-dependent protein kinase C to phosphorylate several cytoplasmic proteins involved in the platelet 'release reaction'; (5) activation of arachidonte metabolism to produce pro-aggregant and vasoactive prostaglandins such as thromboxanes.

At least two other relevant biochemical changes have been observed at the plasma membrane level. First, functional exposure of receptors of fibrinogen as well as other adhesive proteins. This is considered the crucial step leading to irreversible platelet–platelet interactions. Second, translocation of phosphatidylserine towards the external surface of the plasma membrane constitutes a key contribution of platelets for the coagulation cascade because the platelet surface offers the surface for the activation of the prothrombinase complex.

The major conclusion from the studies of the effect of ajoene on the biochemical pathways described above is that ajoene does not affect any of the major pathways that trigger or modulate the platelet activation[8,10,32–34].

We have obtained compelling evidence that indicates that the effect of ajoene in platelets is due to the inhibition of the agonist-induced exposure of fibrinogen receptors on the platelet surface. The exposure of these receptors is a key step leading to irreversible platelet–platelet interaction. Ajoene specifically acts on the molecular mechanism that links the interaction of the primary agonist–receptor complex with the exposure of fibrinogen receptors through specific G-proteins of the signal transduction system in the platelet plasma membrane[34].

These observations suggest that the locus of action of ajoene is unique and is not shared by any known antiplatelet drug. Since most of the biochemical effects of ajoene are observed in the micromolar concentration range it is tempting to speculate that there may be a natural regulator of the platelet response with which ajoene interferes. If such a regulator is indeed present its site of action would be related to a direct coupling between the agonist receptor and G-protein mediated exposure of fibrinogen receptors. It may be noted that so far the possibility of such a direct coupling has not been explicitly considered, but we are investigating it further.

The antiplatelet action of ajoene coupled with the fact that there are no reports of any known toxic effects of alcoholic extracts of garlic suggest that ajoene could be very useful as an antiplatelet agent in the clinical practice (however see ref. 37 for the possible toxic effects of thiols and disulphides). In comparison with other antiplatelet agents in use at present, ajoene has some very practical advantages. For example, ajoene does not interfere with the adhesion of platelets to collagen, and therefore it does not affect significantly the bleeding time. Also, it does not interfere with the major platelet metabolic pathways. Moreover the effect of ajoene is reversible and can be potentiated by other endogenous and exogenous antiplatelet agents. It may be of general interest to note that ajoene has been successfully used for the prevention of platelet activation induced during extracorporeal circulation[35]. As is well known this activation leads to platelet dysfunction and is the major cause of serious post-operative bleeding after open-heart surgery.

Possible uses of ajoene

There are common medical conditions for which an antiplatelet compound with the properties similar to those of ajoene would be desirable. Oral administration of ajoene (100 mg/kg) in rabbits induces inhibition of platelet aggregation observable within 90 min. The effect is completely reversible and full platelet function is recovered in about 24 h. Intravenous administration of 15 mg/kg in dogs induced a very rapid inhibition of platelet aggregation with complete recovery of platelet function within three hours of administration. These general characteristics of the effect of ajoene suggest that ajoene could be potentially useful under conditions in which rapid inhibition and recuperation of platelet function were needed. It is worth noting that all antiplatelet compounds in actual clinical use require periods of several hours or even days to achieve significant inhibition of platelet function *in vivo*. Their effect is maintained for several days, which necessitates the post-surgery use of antithrombotic drugs which create other complications.

During catheter percutaneous angioplasty there is high risk of early thrombosis due to the injury to the vessels as encountered during open heart surgery where exposure of blood to arterial surfaces induces platelet activation, thrombocytopenia and increased risk of post-operative bleeding. Effect of ajoene on this condition was investigated in an experimental model of arterial thrombosis in which platelet thrombus formation is induced *ex vivo* during whole blood perfusion through a chamber where arterial wall with a controlled injury is exposed to the flowing blood[38]. The blood flow is adjusted so that it mimics the shear rate found under physiological conditions in different regions of the circulatory system. In this model ajoene effectively prevented in a dose-dependent manner, the platelet-activation dependent thrombus formation in small and medium sized arteries under high and low shear rate.

The same approach was used to study the effect of ajoene *in vivo*, where many other factors are involved in the ethiopathogeny of thrombus formation and where biodistribution of the drug is also an important consideration. Ajoene was administered intravenously to pigs before starting the perfusion through the experimental chamber under carefully controlled conditions. Results showed that ajoene was effective under conditions mimicking blood flow through medium size arteries (low shear rate), however it did not prevent thrombus formation at shear rates similar to those found in small size arteries. Since it has been demonstrated that under conditions of high shear rate fibrinogen is not the relevant molecule involved in platelet–vessel wall interaction, these results are consistent with the proposed mechanism of action of ajoene at the molecular level.

Ajoene could also be useful under conditions where blood is exposed to artificial surfaces including blood filtration devices and artificial implants. To study such effects, we made use of a closed system formed by a dialyser through which heparinized human blood was recirculated for at least three hours. Under these conditions, 0.35 mM ajoene preserved 90% of the original platelets in the circulation compared to 40% in controls without ajoene[35].

Ajoene effectively reduces haemolysis normally obser-

ved during the use of oxygenators. For example haemolysis was apparent only after two hours in the presence of ajoene, compared to less than 15 min in the absence of ajoene. These results were also substantiated with a bubble oxygenator where about 0.5 mM ajoene was required to preserve the platelet count to 80%. This is probably due to the existence of a gas–blood interface that introduces another stress-related factor in the circulating blood.

These results prompted an investigation of the possible beneficial effects of ajoene under conditions closely resembling those of open-heart surgery. Dogs were maintained under cardio-pulmonary bypass during a period of 100 min after intravenous administration of 15 mg/kg of ajoene. At the end of the extracorporeal procedure, the number of circulating platelets and their response to physiological agonists ADP and collagen were monitored. Not only ajoene protected from the thrombocytopenia induced by the artificial surfaces, but platelets from ajoene-treated dogs became almost fully responsive to the action of agonists after about three hours at the end of the extracorporeal circulation, compared to about 10 hours in the controls. No secondary reactions were observed and all the animals recovered in a few days from the surgical procedure. Additional studies suggest that other weak antiplatelet agents, such as dipyridamole, dramatically potentiate the antiplatelet action of ajoene, allowing the same antiaggegatory action at substantially lower concentrations of ajoene.

Clues for other potential uses of ajoene may also be found in the folklore. It has been shown that ajoene could interfere with absorption of dietary fat by inhibiting gastric lipase, an enzyme that has an essential thiol group which is blocked by lipid soluble thiol reagents[39]. Such a possibility could account for the putative lipid-lowering action of dietary garlic under certain conditions[40]. The cytostatic and antifungal effects of ajoene[41] can also account for the corresponding properties of certain garlic preparations. The physiological basis for the putative anticancer effect of garlic[42] could arise from the role of platelets in metastasis due to the formation of circulating aggregates of platelets with cancer cells. The aggregation of host platelets by circulating tumour cells seems to be an important step for successful metastasis by some tumour cells. By impairing platelet activation, ajoene might be useful in the prevention of the spreading of the primary tumour.

Epilogue

Ajoene, alone or in combination with other antiplatelet agents, is potentially useful in prevention of platelet activation induced by artificial surfaces or by vessel-wall injury located at sites with prevalent low shear stress, such as that found in medium size arteries and veins, or in dilated arterial segments after angioplasty. These studies provide a molecular basis for some of the age-old beliefs related to effects of garlic on heart and blood.

Numerous other effects of garlic have been reported which need to be substantiated by identification of their physiological locus and the molecular basis. It is axiomatic that the specificity of biological action arises from high affinity binding of a drug to a functional molecular receptor. Therefore the battery of such effects could only be ascribed to many chemically reactive species that are formed by processing garlic components, which could act as nonspecific redox reagents, free-radical scavengers, thiol reagents and ligands for binding of metals. The specificity and efficacy of such responses remain doubtful.

A cautionary note is pertinent here. While ajoene and suitable dietary products of garlic may have beneficial preventive effects against thromboembolic episodes, it is clear that most of the 'garlic preparations' available in the health-food and drug stores are devoid of ajoene. Also many commercial garlic preparations are being touted as 'cure-alls'.

While these products could have some beneficial effects, it remains to be demonstrated that such effects are observed *in vivo* at sufficiently low concentrations so that the effects are specific.

Garlic products represent a world-wide market of well over a billion dollars per year. This has generated not only many questionable products but the claims to their efficacy abound. Thus the motive for the modern folklore is not the curiosity and empirical quest for knowledge, but primarily economic. For example, many pseudo-scientific claims for the efficacy of garlic against cancer, immune-response (AIDS) and other modern-day maladies are beginning to be circulated. While some epidemiological evidence exists for certain beneficial effects of garlic under certain conditions, in order to establish a credible basis for such claims it would be necessary to establish the nature of the active component(s), and to demonstrate its specificity at the biochemical, metabolic, and organismic levels.

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Contact: Dr (Mrs) R. Sankaran
Chairperson, MICON International–94
Defence Food Research Laboratory
Siddharthanagar
Mysore 570 011
Tel: 821 23950, 33052, 22671
Telex: 846–238, Fax 821–28830

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