Effects of Allicin on Cardiovascular Risk Factors in Spontaneously Hypertensive Rats

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ABSTRACT: **Background:** *Allium sativum*, the active ingredient in garlic, is known to have a beneficial effect on major cardiovascular risk factors, including dyslipidemia, blood pressure, blood glucose and insulin levels. However, the data on the significance of these effects are inconsistent due to methodological limitations, especially the use of whole garlic cloves which does not allow controlled dosing of the active compound.

Objectives: To study the effects of purified allicin on the cardiovascular system.

Methods: Spontaneously hypertensive rats treated for 6 weeks with a daily dose of 80 mg/kg/day of purified allicin added to their chow were compared to control rats that were fed regular chow. Weight, systolic blood pressure (SBP), triglycerides, cholesterol, insulin and adiponectin were measured at baseline and at the end of the study.

Results: Allicin had no effect on body weight whereas it reduced SBP significantly from 190 ± 7.5 mmHg to 168 ± 5.7 ($P < 0.0001$) and triglyceride levels from 96 ± 25 mg/dl to 71 ± 19 ($P = 0.009$). Allicin had no effect on plasma cholesterol, insulin and adiponectin levels.

Conclusions: Allicin lowered blood pressure and triglyceride levels in spontaneously hypertensive rats. This effect was not mediated through weight loss.

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KEY WORDS: allicin, garlic, cardiovascular disease, spontaneously hypertensive rats

Allium sativum L. (Liliaceae), whose common name is garlic, has been known to mankind for centuries. It originated in southern Asia and is widely used around the globe as a spice, food and home remedy [1]. Several studies have shown that garlic has a beneficial effect on cardiovascular risk factors such as dyslipidemia, high blood pressure and glucose levels. In addition, it has an antioxidative effect, an antiplatelet aggregation effect, and enhances fibrinolytic activity [2-8]. Data from these studies point to the possible role of garlic in the prevention and control of cardiovascular risk factors. However, the results are inconsistent and some studies show conflicting results probably due to methodological differences [9-11].

The main challenge has been the isolation and process of preparing a stable form of the active compound in garlic and determining its concentration. In addition, differences in patient selection, randomization, blinding and insufficient data regarding the effective dose also account for the inconsistent results [11-13].

Among the active constituents in garlic, the principal component is allicin (thio-2-propene-1-sulfinic acid S-allyl ester). It is not present as such in the intact garlic clove but is produced together with pyruvate and ammonia from the odorless precursor alliin (+)(S-allyl-L-cysteine sulfoxide) in the presence of the enzyme alliinase (enzyme classification 4.4.1.4.). Alliin and alliinase are found in different compartments of the garlic clove and are brought into contact to produce allicin by cutting or crushing the clove. The latter is a chemically unstable, colorless liquid that is thought to be responsible for both the odor and much of the biological activity of garlic [14,15].

The introduction of a method to purify the active ingredient allicin has enabled a systematic study, mainly in laboratory animals and in tissue cultures, on its effects on cardiovascular risk factors [12,13,15-20]. In this study we used powdered allicin, made commercially through freezing and drying which preserves its active characteristics. This was ascertained using a method described by Miron et al. [21]. The aim of this study was to evaluate the effect of allicin on blood pressure and triglycerides in spontaneously hypertensive rats.

SUBJECTS AND METHODS

Twenty male spontaneously hypertensive rats weighing 340 g were fed standard rat chow (Koffolk, Tel Aviv, Israel) and maintained on a 14 hour light/10 hour dark cycle. Ten untreated rats served as a control group, and 10 rats were given 80 mg/kg/day allicin as powder mixed with grind chow for 6 weeks. The chow and active ingredient were refreshed daily.

Allicin was produced in vivo as a product of the reaction between granules of garlic alliinase and alliin [(1)S-2-propenyl L-cystein S-oxide]. The concentration of the allicin was determined using the method of Miron et al. [21] with high performance liquid chromatography as previously reported. The preparation was tested for purity and quantity

by comparing the allicin in the powder versus standard allicin that was kept stable at -80°. Allicin content in our garlic powder was analyzed by the HPLC procedure as follows: Isocratic separation was performed using C-18 reverse-phase column (Agilent ZORBAX Eclipse XDB-C-18 4.6 x 150 mm, 5 µm) connected with the Jasco HPLC system equipped with ultraviolet detector 1570M and calibrated with allicin standard. The running buffer used was 40% methanol in water, containing 0.05% trifluoroacetic acid, at a flow rate of 0.5 ml/min. Absorbance of substances was detected at 220 nm. The powder was mixed with water (20–50 mg/ml water) for 20 min. The mixture was centrifuged and 100 µl of supernatant was diluted 1/5 with the HPLC buffer. Quantitative estimation of allicin was performed using ChromNAV software.

For the experiments described in this study, the concentration of allicin used was 5 mg/g powder which was added to the rats' diet. Allicin in its powdered form is a stable substance that made handling and feeding easier and more accurate.

Body weight, systolic blood pressure, plasma triglycerides, cholesterol, insulin and adiponectin were measured at baseline and at trial termination 6 weeks later. Systolic BP was measured in conscious rats by the indirect tail-cuff method, using an electrosphygmomanometer and pneumatic pulse transducer (58500 BP recorder, Ugo Basile, Varese, Italy). The mean of five consecutive readings was recorded as the blood pressure.

Blood samples were taken from a retro-orbital sinus puncture under light anesthesia from all rats after 5 hours fasting at the beginning of the experiment and after 6 weeks. The samples were centrifuged, alliquoted, frozen, and assayed for insulin (rat insulin 125 I Ria kit, Incstar, Stillwater, MN, USA), for triglycerides and insulin concentration with an automated analyzer using an enzymatic colorimetric reaction (Olympus AU 270, Hamburg, Germany). Plasma adiponectin levels were assayed using an RIA kit (Linco Research, MO, USA).

Results are presented as means ± SD. Using the paired, two-tailed student *t*-test, statistical significance was defined as $P < 0.05$.

RESULTS

Body weight did not significantly increase during treatment with allicin: from baseline of 344 ± 21 g to 359 ± 22 g at the end of the study in the allicin group and from 318 ± 21 at baseline to 317 ± 16 at 6 weeks (no statistical significance between groups).

Systolic BP decreased significantly from baseline of 190 ± 7 to 168 ± 6 mmHg at the end of the study in the allicin group ($P < 0.001$) but remained unchanged in the control group: 183 ± 13 at baseline to 186 ± 7 at 6 weeks [Figure 1] ($P = 0.432$). Between-group change at 6 weeks was also highly significant ($P < 0.0001$).

HPLC = high performance liquid chromatography
BP = blood pressure

Figure 1. Effect of 6 weeks treatment with allicin on systolic blood pressure (mmHg) compared to control

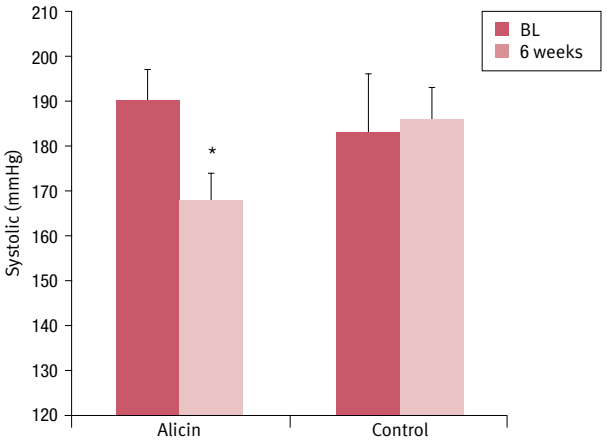
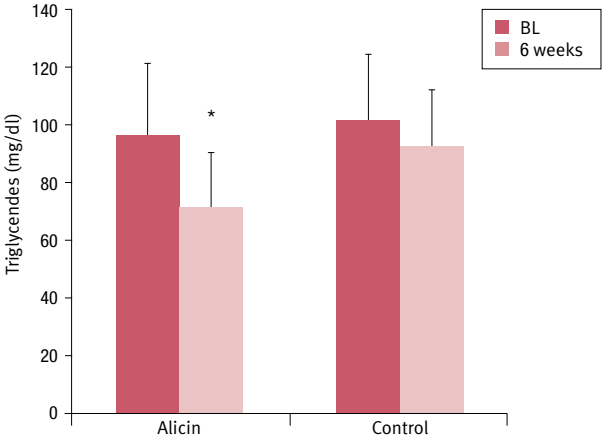


Figure 1. Effect of 6 weeks treatment with allicin on plasma triglyceride levels (mg/dl) compared to control. *$P < 0.05$



Triglyceride levels decreased significantly in the treatment group as well (96 ± 25 to 71 ± 19 mg/dl, $P = 0.009$) but remained unchanged in the control group (101 ± 23 to 92 ± 20 mg/dl, $P < 00.354$). Between-group change at 6 weeks was also significant [Figure 2] ($P = 0.0015$).

No change was noted in cholesterol, insulin or adiponectin levels in both the study and control groups: cholesterol, from baseline of 121 ± 10 to 120 ± 14 mg/dl in the allicin group compared to 120 ± 9 to 135 ± 9 mg/dl in the control group; insulin, from baseline of 30 ± 6 to 28 ± 8 mg/dl mU/ml in the allicin group compared to 30 ± 4 to 32 ± 7 mU/ml in the control group; and adiponectin, from baseline of 5.8 ± 1.3 to 6.3 ± 1.5 µg/dl in the allicin group compared to 5.5 ± 1.3 to 6 ± 1.8 µg/dl in the control group.

DISCUSSION

Our study demonstrates the ability of allicin to reduce BP and triglyceride levels in an animal model of hypertension. This observation is in accord with several other reports in the literature where raw garlic was used, and adds to our previous studies using purified allicin. In our earlier studies we used a different model of Sprague-Dawley rats that were fed a high fructose diet, a model mimicking metabolic syndrome where weight gain was a hallmark and pivotal to metabolic changes [17,18]. In rats with metabolic syndrome allicin treatment induced weight reduction, suggesting that the beneficial effects of allicin could be due to weight reduction. In the present study spontaneously hypertensive rats were fed standard chow without metabolic challenge, and no change in weight was noted. Nevertheless, allicin reduced both BP and triglyceride levels.

The fact that in our present study, weight, insulin and adiponectin did not change raises the question whether allicin acts in more than one mechanism: in a dismetabolic milieu allicin acts through its effects on weight and metabolic pathways; in a non-dismetabolic milieu, such as the conditions in our experiments, it acts through other pathways. The exact mechanism by which allicin exerts its biological action has not yet been fully elucidated. Some researchers have suggested an antioxidant effect, possibly mediated via up-regulation of cellular glutathione levels in vascular endothelial cells and possibly preventing or remedying endothelial dysfunction – one of the key mechanisms in initiation and perpetuation of high blood pressure [22,23]. Its other effects – namely, hypo-insulinemic, hypolipdemic, antithrombotic, anti-inflammatory and anti-apoptotic – might also contribute to the prevention of the atherosclerotic process. Another possible mechanism recently suggested is lipoprotein modification and inhibition of low density lipoprotein uptake and degradation by macrophages [24].

Previous human studies yielded conflicting evidence on the effects of garlic on metabolic parameters. It is interesting to note that beyond several methodological shortcomings in some of those studies, none actually used allicin as an active drug compound in a form that can be quantified, and none determined its concentration in the various garlic preparations that were used [9,11-13]. As was noted above, allicin is an unstable substance with a rapid decay curve [14] so some of the negative results are not surprising since various preparations of garlic such as powdered or aged garlic were used. Nevertheless, a recently published meta-analysis concluded that in hypertensive (but not normotensive) patients, a significant 16/9 mmHg reduction in blood pressure was noted [25] following the use of garlic. This level of reduction is comparable to reductions seen with some antihypertensive drugs and lends credence to the future exploration of garlic as an antihypertensive medication.

Our study has a limitation. To date, there is no assay to measure plasma levels of allicin, thus the correlation with the effects of allicin cannot be ascertained.

In summary, garlic lowered blood pressure and triglyceride levels in spontaneously hypertensive rats. These effects were not mediated by weight reduction or through changes in insulin or adiponectin levels. It would be of value to further examine these effects of allicin in humans with hypertension. We hope that future research will enable in vivo measurement of allicin and its pharmacological properties; physiological effects and mechanisms of action should be investigated further.

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Capsule

Exaggerated translation causes synaptic and behavioral aberrations associated with autism

Autism spectrum disorders (ASDs) are an early-onset heterogeneous group of heritable neuropsychiatric disorders with symptoms that include deficits in social interaction skills, impaired communication abilities, and ritualistic-like repetitive behaviors. One of the hypotheses for a common molecular mechanism underlying ASDs is altered translational control, resulting in exaggerated protein synthesis. Genetic variants in chromosome 4q, which contains the EIF4E locus, have been described in patients with autism. Importantly, a rare single nucleotide polymorphism has been identified in autism that is associated with increased promoter activity in the EIF4E gene. Santini and collaborators show that genetically increasing the levels of eukaryotic translation initiation factor 4E (eIF4E) in mice results in exaggerated cap-dependent translation and aberrant behaviors reminiscent of autism, including repetitive and perseverative behaviors and social interaction deficits. Moreover, these autistic-like behaviors are accompanied by synaptic pathophysiology in the medial prefrontal cortex, striatum and hippocampus. The autistic-like behaviors displayed by the eIF4E-transgenic mice are corrected by intracerebroventricular infusions of the cap-dependent translation inhibitor 4EGI-1.These findings demonstrate a causal relationship between exaggerated cap-dependent translation, synaptic dysfunction and aberrant behaviors associated with autism.

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Eitan Israeli

Capsule

Influence of intragenic CCL3 haplotypes and CCL3L copy number in HIV-1 infection in a sub-Saharan African population

Two CCL3 haplotypes (Hap-A1 and Hap-A3) and two polymorphic positions shared by the haplotypes (Hap-2SNP, single nucleotide polymorphism) were investigated together with CCL3L copy number (CN), for their role in HIV-1 disease. Hap-A1 was associated with protection from in utero HIV-1 infection: exposed uninfected (EU) infants had higher representation of wild-type (WT)/Hap-A1 than infected infants (excluding intrapartum-infected infants), which maintained significance post-maternal Nevirapine (mNVP) and viral load (MVL) correction ($P = 0.04$, odds ratio (OR) = 0.33). Mother-infant pair analyses showed the protective effect of Hap-A1 is dependent on its presence in the infant. Hap-A3 was associated with increased intrapartum transmission: WT/Hap-A3 was increased in intrapartum -transmitting vs. non-transmitting (NT) mothers, and remained significant post mNVP and MVL correction ($P = 0.02$, OR = 3.50). This deleterious effect of Hap-A3 seemed dependent on its presence in the mother. Hap-2SNP was associated with lower CD4 count in the NT mothers (P = 0.03). CCL3 Hap-A1 was associated with high CCL3L CN in total ($P = 0.001$) and EU infants ($P = 0.006$); the effect was not additive, however, having either Hap-A1 or high CCL3L CN was more significantly ($P = 0.0008$) associated with protection from in utero infection than Hap-A1 ($P = 0.028$) or high CCL3L CN (P = 0.002) alone. Linkage disequilibrium between Hap-A1 and high CCL3L CN appears unlikely given that a Nigerian population showed an opposite relationship.

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Eitan Israeli

"The first half of our lives is ruined by our parents, and the second half by our children"

Clarence Darrow (1857-1938), American lawyer and leading member of the American Civil Liberties Union. Called a "sophisticated country lawyer", he remains notable for his wit and agnosticism