

THE ANTI-ATHEROGENIC EFFECT OF ALLICIN: POSSIBLE MODE OF ACTION

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Several epidemiologic studies demonstrated an inverse association between garlic (*Allium sativum*) consumption and progression of cardiovascular diseases [1-3]. However, the effect of several garlic preparations on prevention and treatment of cardiovascular disease is controversial. These preparations are different in their composition, which makes the interpretation of these studies complicated [3,4].

Alliin has long been recognized as the main active ingredient of crushed garlic. In fact, no compound outside the thiosulfates (of which alliin is about 75%) has yet been found as the significant portion of the pharmacological activities of crushed garlic, at levels representing normal human consumption (2-5 g/day). Consequently, the majority of the garlic supplements sold today are garlic powder tablets (some are capsules) that are standardized by alliin [5,6]. However, analysis of these studies showed that several commercial garlic products in the market contain undetectable amounts (< 1 ppm) of alliin [7].

The variations in garlic preparations and the active component concentrations in the mentioned studies were eliminated in our research, since by using pure alliin we were able to investigate the effect of a well-defined component of garlic in a known given concentration on atherosclerosis and to seek the possible mechanisms of its anti-atherogenic activity.

Alliin was produced by reacting alliin with isolated alliinase [8]. To minimize degradation and oxidation, alliin was stored at 4° C in the dark and its concentration was routinely determined by high performance liquid chromatography during the experiments.

In our study we demonstrated that pure alliin inhibits the atherogenic process in mouse models and showed that this effect can be mediated through several mechanisms:

1. The effect of pure alliin on the atherogenesis. Most studies have investigated the effects of long-term feeding of garlic and garlic preparations on experimental atherosclerosis induced by a high-cholesterol diet in rabbits [9,10]. In contrast to the positive results in rabbits, two garlic powder tablets (Printanor and Kyolic) did not affect the atherosclerotic lesion area or its composition in APOE*3-Leiden transgenic mice [11].

In our work, we used three animal atherosclerosis models (C57BL/6, LDL-R^{-/-}, and apoE^{-/-} mice) which differ in the atherogenic genotype and disease developmental characteristic. We found that daily administration of pure alliin significantly reduces the lesion area regardless to the diet, mouse gender, or duration of the treatment. The lesion area was reduced by 65.6%, 38.3%, and 49.7%, compared to saline-treated mice, in C57BL/6, LDL-R^{-/-}, and apoE^{-/-} mice, respectively (Figure 1). The disagreement with Espirito et al. [12] which used apoE*3-Leiden mice, is possibly the result of differences in study design, the use of different animal models, and the use of different garlic formulations and preparations (garlic powder tablets; Printanor and Kyolic).

In light of the inconsistency of the results, a crucial question that should be addressed is alliin's ability to reach the blood and the target tissues. To address this question, we used a long-lived radioactive [³H]alliin, labeled in the allyl group [12]. By using the radioactive [³H]alliin, we demonstrated that following its administration to mice, radioactivity is detected in the blood

and organs. Rapid clearance of ^3H -allicin from the blood was identified. Fifteen minutes post-administration, ^3H -allicin levels in the blood were the highest. In the organs, at this point in time, the highest levels were detected in liver and kidney (40% and 34%, respectively). One hour later, reduced levels were measured in the heart, the liver, and the kidney; however, a significant elevation was detected in the pancreas (10%-49%). Five days following administration, only low levels of radioactivity were detected.

It is noteworthy that the activated disulfide bond $-\text{S}(\text{O})-\text{S}-$ of allicin reacts with different thiols-containing molecules, including SH-containing proteins. Therefore, we cannot exclude the possibility that part or most of the radioactivity detected, resides in its metabolites, which might also confer atheroprotective effects.

The presented ability of allicin to reduce the atherogenic lesion development as well as allicin's and/or its derivatives' capability to reach blood and tissues, encouraged us to continue investigating allicin's possible mode of action as an anti-atherogenic agent.

2. The effect of allicin on lipid profile and lipoprotein cholesterol contents. Several studies have suggested that garlic preparations may have beneficial effects on plasma cholesterol levels, while other studies found no influence [13,14]. In our study we found that daily administration of pure allicin does not affect the total lipid levels, regardless of the mouse model, diet, or duration. However, in C57BL/6 and LDL-R $-/-$ mice, allicin altered the cholesterol distribution in lipoprotein particles, from pro-atherogenic profile, towards anti-atherogenic profile; decreased VLDL and LDL and increased HDL cholesterol content.

3. The effect of allicin on cholesterol synthesis. Several studies have revealed multiple interactions of garlic compounds with biosynthetic pathway for cholesterol that resulted in moderate but significant inhibition of cholesterol biosynthesis at several different enzymatic steps. We have studied the effect of allicin on cholesterol biosynthesis in CHO cells and HepG2 cells and found a minor decrease (15%) in cholesterol biosynthesis which correlates with the *in vivo* results. These results are in agreement with the results of other groups, showing similar trends. However, the *in vivo* results in mice did not show any cholesterol lowering effect of allicin and, therefore, other mechanisms should be considered accountable for allicin's anti-atherosclerotic effect.

4. The effect of allicin on LDL oxidation. Oxidative modification of LDL is a key step in the atherosclerotic process. Our results show that allicin administration to apoE $-/-$ and LDL-R $-/-$ mice strongly increases the resistance of plasma LDL to *ex vivo* oxidation by Cu^{2+} . Moreover, Cu^{2+} -induced oxidation of LDL *in vitro* showed that allicin protected LDL from oxidation better than its precursor alliin, even though both are antioxidants.

Interestingly, we found that the protection of LDL by allicin from AAPH-induced oxidation is much less effective than its protection from Cu^{2+} -induced oxidation. Since oxidation of LDL by CuSO_4 is dependent on its binding to the lipoprotein and the LDL oxidation by 2,2'-azobis(2-amidinopropane)hydrochloride (AAPH) is independent of such binding and acts through free radicals' formation in the aqueous phase, we postulated that allicin may affect Cu^{2+} -induced oxidation by preventing its binding to the LDL particle.

By using radioactive labeled allicin and ESR analysis, we demonstrated that allicin binds to LDL, VLDL, and HDL and reduces Cu^{2+} binding to LDL, respectively (Figure 2). We suggest that allicin probably reacts with thiol groups on apo-B in the LDL particle, consequently interfering with Cu^{2+} binding, and thus inhibits oxidation.

5. The effect of allicin on LDL uptake by macrophages. The modification of LDL by allicin may affect other processes in LDL metabolism as well, such as LDL uptake by macrophages. The oxidative modification of LDL and the subsequent uptake of oxLDL by macrophages, leading to foam cell formation, is a central step in atherogenesis. We found that uptake of both nLDL and oxLDL by isolated mouse peritoneal macrophages is significantly inhibited by allicin-treated lipoproteins and/or macrophages. We hypothesize that the inhibition of LDL uptake by allicin is due to its binding to the free thiol groups on apo-B or to free thiols on the scavenger receptors in macrophages, thus, inhibiting the binding of LDL to macrophage receptors.

Conclusions

In the present work we showed that daily administration of pure allicin significantly reduces the atherosclerotic lesion area in the aortic sinus of three atherogenic mouse models. By using the pure allicin preparation we demonstrated that allicin affects atherogenesis, possibly through several mechanisms such as inhibition of lipoprotein modification, LDL protection against oxidation and inhibition of LDL uptake and degradation by macrophages.

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References

1. Ackermann RT, Mulrow CD, Ramirez Gardner CD, Morbidoni L, Lawrence VA. Garlic shows promise for improving some cardiovascular risk factors. *Arch Intern Med* 2001;161:813-24.
2. Brace LD. Cardiovascular benefits of garlic (*Allium Sativum* L.) *J Cardiovasc Nurs* 2002;16(4):33-44.
3. Rahman K, and Lowe GM. Garlic and Cardiovascular Disease: A Critical Review. *J Nutr* 2006;136:736-40.
4. Amagase H. Clarifying the real bioactive constituents of garlic. *J Nutr* 2006;136(3 Suppl):716S-725S.
5. Lawson LD, Gardner CD. Composition, stability, and bioavailability of garlic products used in a clinical trial. *J Agric Food Chem* 2005;53:6254-61.
6. Lawson LD, Wang ZJ. Allicin and allicin-derived garlic compounds increase breath acetone through allyl methyl sulfide: use in measuring allicin bioavailability. *J Agric Food Chem* 2005;53(6):1974-83.
7. Freeman F, Kodera Y. Garlic Chemistry: Stability of S(2-propenyl) 2-propene-1-sulfinothioate (allicin) in blood, solvents, and simulated physiological fluids. *J Agric Food Chem* 1995;43:2332-38.
8. Miron T, SivaRaman H, Rabinkov A, Mirelman D, Wilchek M. A method for continuous production of allicin using immobilized alliinase. *Anal Biochem* 2006;351(1):152-54.

9. Orekhov AN, Grunwald J. Effects of garlic on atherosclerosis. *Nutrition* 1997;13:656-63.
10. Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorder: a review. *Nut J* 2002;1(1):4-18.
11. Espirito Santo SM, van Vlijmen BJ, van Duyvenvoorde W, et al. Absence of an atheroprotective effect of the garlic powder printanor in APOE*3-Leiden transgenic mice. *Atherosclerosis* 2004;177:291-97.
12. Miron T, Bercovici T, Rabinkov A, Wilchek M, Mirelman D. [³H]Allicin: preparation and applications. *Anal Biochem* 2004;331:364-69.
13. Espirito Santo SM, van Vlijmen BJ, Buytenhek R, et al. Well-characterized garlic-derived materials are not hypolipidemic in APOE*3-Leiden transgenic mice. *J Nutr* 2004;134:1500-3.
14. Tattelman E. Health effects of garlic. *Am Fam Physician* 2005;72(1):103-6.

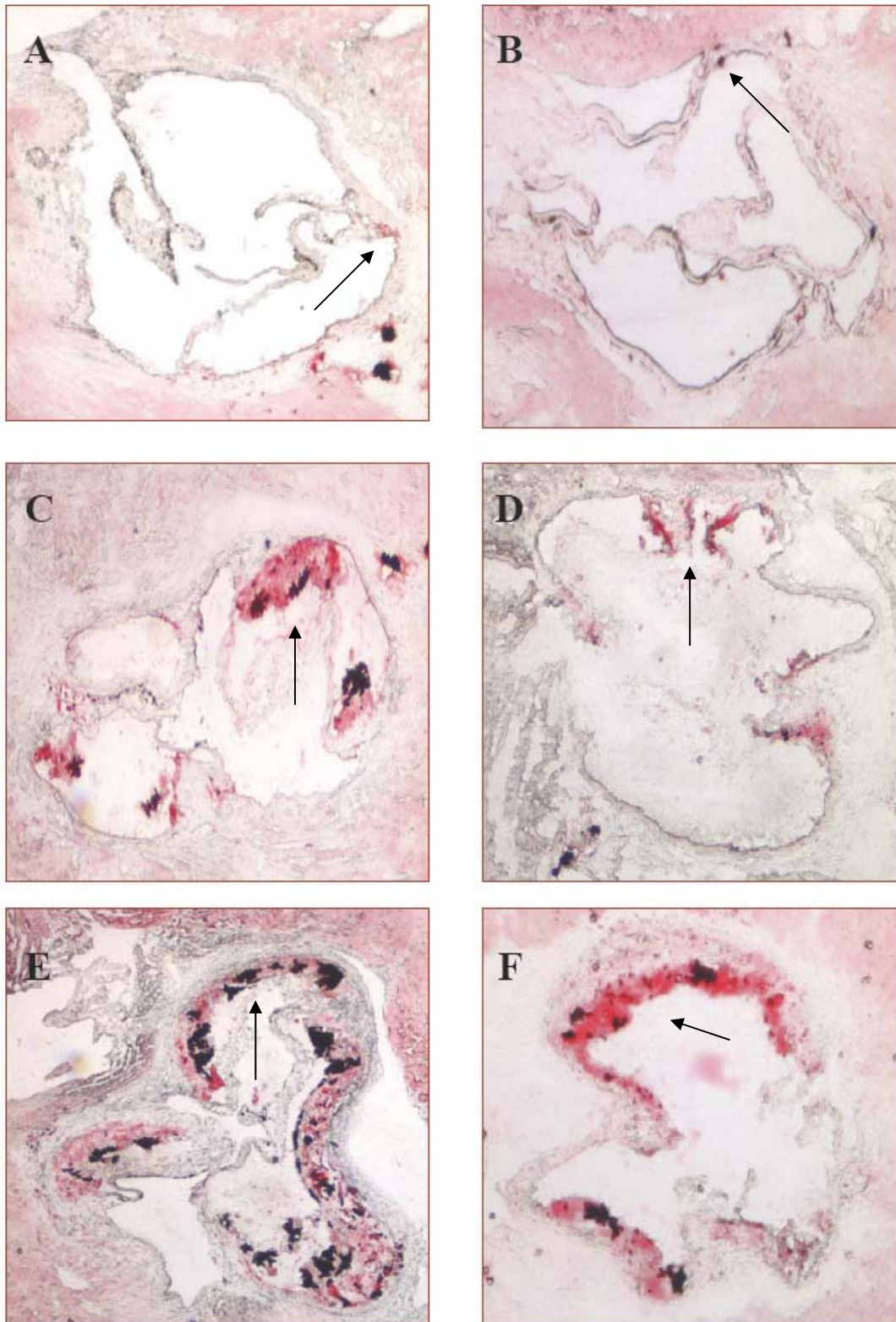


Figure 1: Allicin inhibits the development of the atherosclerotic lesion. Representative lesions of C57BL/6 (A/B), LDL-R ^{-/-} (C/D) and apoE ^{-/-} (E/F) mice. Control (A/C/E) or allicin-treated

mice (B/D/F).

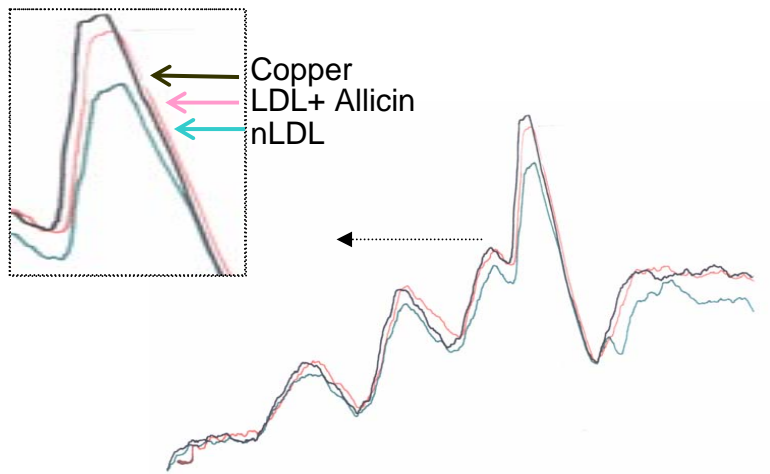


Figure 2. Allicin reduced the signal intensity of Cu^{2+} - ESR spectra of Cu^{2+} (0.1 mM) in the presence of native-LDL (nLDL) and LDL pretreated with allicin (LDL+allicin). In the presence of nLDL, peak intensity of Cu^{2+} aqua ions ESR signal decreased by 15-30%. This effect was eliminated in the case of LDL pretreated with allicin. The results represent one of six repeats.