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].

Allicin has long been recognized as the main active ingredient of crushed garlic. In fact, no compound outside the thiosulfinates (of which allicin 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 allicin [5,6]. However, analysis of these studies showed that several commercial garlic products in the market contain undetectable amounts (< 1 ppm) of allicin [7].

The variations in garlic preparations and the active component concentrations in the mentioned studies were eliminated in our research, since by using pure allicin 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.

Allicin was produced by reacting alliin with isolated alliinase [8]. To minimize degradation and oxidation, allicin 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 allicin inhibits the atherogenic process in mouse models and showed that this effect can be mediated through several mechanisms:

1. **The effect of pure allicin 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 allicin 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 allicin's ability to reach the blood and the target tissues. To address this question, we used a long-lived radioactive [³H]allicin, labeled in the allyl group [12]. By using the radioactive [³H]allicin, we demonstrated that following its administration to mice, radioactivity is detected in the blood.
and organs. Rapid clearance of $^3$H-allicin from the blood was identified. Fifteen minutes post-
administration, $^3$H-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 -S(O)-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 CuSO4 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

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
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.