Anti-aggregatory and fibrinolytic effects of time-released garlic powder tablets

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The double-blinded placebo-controlled randomized crossover study was performed in 29 atherosclerotic men (35 to 70 years old) suffering from cerebral atherosclerosis and chronic cerebrovascular insufficiency to elucidate the effect of time-released garlic powder tablets Allicor on platelet aggregation and serum fibrinolytic activity. It has been demonstrated that Allicor 14-days treatment significantly inhibited ADP-induced platelet aggregation by 25.4% (p<0.05) and increased plasma fibrinolytic activity by 22.4% (p<0.05). Additionally, the trend to the decrease in plasma fibrinogen level was observed. Evidence obtained from this study indicates that garlic has potential in the prevention and control of cardiovascular disorders and is beneficial when taken as a dietary supplement. We conclude that Allicor may be beneficial in protecting against cardiovascular disease as a result of inhibiting platelet aggregation and normalizing plasma fibrinolytic activity.

Keywords: Platelet aggregation, fibrinolysis, atherosclerosis, garlic, Allicor

Introduction

Atherosclerosis, by far the greatest killer in modern society, may be characterized as a complex disease that develops due to many risk factors including alterations in plasma lipid and lipoprotein levels, platelet function, clotting factors, blood pressure regulation, arterial smooth muscle cell metabolism, etc (Schwartz et al., 1993). Among these, the increased platelet aggregation and insufficiency of plasma fibrinolytic activity are thought to be the most potent factors that greatly increase the risk of clinical manifestations of atherosclerosis, such as acute myocardial infarction and stroke (Steele et al., 1973; Lam et al., 1994; Breddin et al., 1999; Leys, 2001).

Coronary and extracoronary atherosclerosis is always characterized with hypercoagulation of certain extent. Thus, the correction of haemostatic disorders in atherosclerotic patients is especially necessary to prevent the risk of thrombosis and thromboembolism. For this purpose, antiplatelet drugs are highly recommended (Easton, 1991; Verhaeghe, 1991; Aronow, 1999), but long-term therapy may have substantial limitations due to possible side and adverse effects. However, the use of botanicals possessing anti-thrombotic activity may suggest the effective way for thrombosis prevention in atherosclerosis. Among them garlic is
known as herbal remedy that reduces a multitude of risk factors which play a role in the genesis and progression of arteriosclerosis. The use of garlic-based preparations results in improvement in serum lipids, reduction of serum fibrinogen concentration and lowering of arterial blood pressure (Bordia, 1981; Auer et al., 1990; Bordia et al., 1998; Ackerman et al., 2001). Finally, garlic contains biologically active components that are able to enhance fibrinolysis, inhibit platelet aggregation, and diminish plasma viscosity (Harenberg et al., 1988; Kiesewetter et al., 1990; Kiesewetter et al., 1991; Steiner and Li, 2001; Rahman and Billington, 2000). However, not all garlic preparations may be assumed equivalent in their composition and, more importantly, in biological response they may precipitate. Recently developed garlic powder-based preparation Allicor is characterized by prolonged mode of action, thus differing from other products on the market and promising more potent biological effects. This study has been performed to elucidate the effects of Allicor on platelet aggregation and serum fibrinolytic activity in men with cerebral atherosclerosis and chronic cerebrovascular insufficiency.

**Materials and methods**

This study was randomized placebo-controlled double-masked crossover clinical trial in 29 men (35 to 70 years old) suffering from cerebral atherosclerosis and chronic cerebrovascular insufficiency. The presence of cerebral atherosclerosis was documented by duplex ultrasonography of magisterial arteries. All patients included in the study had transitory or mild arterial hypertension (systolic blood pressure, 151.0±3.4 mm Hg, diastolic blood pressure, 92.1±1.5 mm Hg). Five patients had stable angina, one patient had myocardial infarction in anamnesis, and two more patients had stroke in anamnesis. The increased ADP-induced platelet aggregation and plasma fibrinogen level as well as lowered plasma fibrinolytic activity were used as inclusion criteria.

Before clinical study, the direct effect of Allicor on ADP-induced platelet aggregation and fibrinolysis was investigated in vitro. The venous blood was taken after overnight fasting and stabilized by 0.13 M sodium citrate (1:9, vol/vol), and platelet-rich blood plasma was obtained by 7 min centrifugation at 200g. Allicor tablet was dissolved in phosphate buffered saline, and plasma was preincubated with Allicor (80 μg/ml) for 5 min at 37°C. The platelet count and the kinetics of ADP-induced platelet aggregation were performed with automated computer-based platelet aggregation analyzer “Biola” (Biola, Moscow, Russia) (Gabbasov et al., 1989). Adenosine diphosphate (Serva, Sweden), 3 μM, was used as inductor of platelet aggregation. Plasma fibrinolytic activity was measured using euglobulin method (Ästrup et al., 1966).

After 10-days wash-out period, the patients were randomized either to Allicor (INAT-Farma, Moscow, Russia), 300 mg twice daily (n=15), or to placebo, one tablet twice daily (n=14). Placebo and Allicor tablets looked identical. All participants got similar dietary and behavioral recommendations. After 14-days treatment, the second 10-days wash-out period was performed. Then the patients of the first group received placebo, and the patients of the second group received Allicor 300 mg twice a day for another 14 days. The blood was taken after overnight fasting before and after 14-days treatment periods. The platelet count and the kinetics of ADP-induced platelet aggregation were performed using above described procedures. Plasma fibrinogen level was measured by Ruthberg. Plasma fibrinolytic activity was estimated as the difference in fibrinogen levels before and after 3-h incubation of plasma at 37°C, and the index of fibrinolysis was calculated (Bidwell, 1953).
The results were expressed in terms of means and S.E.M. Significance of differences was evaluated using SPSS 10.1.7 statistical program package (SPSS Inc., USA) and defined at the 0.05 level of confidence.

**Results**

The results of preliminary in vitro studies have shown that Allicor produces a direct effect on platelet aggregation and plasma fibrinolytic activity. Namely, plasma incubation with Allicor (80 μg/ml) resulted in a significant inhibition of ADP-induced platelet aggregation by 36.0±3.5% as compared to the same plasma samples incubated without Allicor addition (p=0.013). Additionally, incubation of plasma with Allicor also increased its fibrinolytic activity by 2.8-fold (from 11.6±3.8 to 32.5±5.8 mm², p=0.016).

In patients with cerebral atherosclerosis and chronic cerebrovascular insufficiency, the 14-days treatment with Allicor resulted in sufficient improvement of general condition, reduced hits of giddiness and headaches.

At the beginning of treatment, all patients were characterized with increased levels of ADP-induced platelet aggregation and fibrinogen, and lowered plasma fibrinolytic activity and index of fibrinolysis. At the baseline, the patients randomized to Allicor were characterized by higher values of ADP-induced platelet aggregation as compared to placebo recipients (p=0.048). After 14-days treatment, ADP-induced platelet aggregation in Allicor-treated patients was lowered by 25.4±8.6% (p=0.006), whereas in placebo group there were no significant changes. By the end of the study the beneficial difference in ADP-induced platelet aggregation between Allicor and placebo recipients accounted for 10%, although did not reach statistical significance.

Baseline levels of plasma fibrinogen were comparable between Allicor and placebo recipients. After 14-days treatment, fibrinogen level in Allicor-treated patients was lowered by 8.9±5.2% as compared to baseline (p=0.086), whereas in placebo group it remained stable. By the end of the study the beneficial difference in plasma fibrinogen level between Allicor and placebo recipients accounted for 8.8%, but was not significant.

Plasma fibrinolytic activity was lowered both in Allicor and placebo randomized patients at the baseline. Allicor treatment resulted in a significant increase in plasma fibrinolytic activity by 22.4±8.7% as compared to baseline level (p=0.034), and in placebo group there were no significant changes. By the end of the study the difference in plasma fibrinolytic activity between Allicor and placebo recipients accounted for 35.4% and was statistically significant (p<0.001). Additionally, the index of fibrinolysis in Allicor-treated patients increased by 44.8±16.4% as compared to baseline level (p=0.032). After treatment period, the index of fibrinolysis in Allicor recipients was higher by 67.2% as compared to placebo group (p=0.002).

**Discussion**

The results of the given study demonstrate that garlic powder tablets possess antiplatelet effects and can nor-

mallize plasma fibrinolytic activity in atherosclerotic men suffering from chronic cerebrovascular insufficiency. These data are in good coincidence with previously reported effects of garlic-based products on coagulation-related parameters (Bordia et al., 1998; Harenberg et al., 1988; Chutani and Bordia, 1981; Bordia et al.,1996; Kiesewetter et al., 1993). The direct inhibition of ADP-induced platelet aggregation by Allicor in vitro was more prominent than
Anti-aggregatory and fibrinolytic effects of time-released garlic powder tablets | ATI, June 2012

in clinical study. This discrepancy in the results of in vitro and ex vivo studies may reflect the biochemical transformations that biologically active components of garlic undergo in gastrointestinal tract, and the extent of bioavailability of active compounds can also play a role. Garlic contains a variety of organosulfur compounds, amino acids, vitamins and minerals. Some of the sulfur-containing compounds such as allicin, ajoene, S-allylcysteine, S-methylcysteine, diallyl disulfide and sulfoxides may be responsible for antithrombotic activity of garlic. The effect of garlic on platelet aggregation is proposed to be due to inhibition of cyclooxygenase that plays a key role in arachidonic acid metabolism (Ali, 1995). This results in a decreased synthesis of thromboxane B2 and decreased production of potent vasoconstrictors leukotriene C4 and prostaglandin E2 by platelets (Ali and Mohammed, 1986; Srivastava, 1986; Ali et al., 1990; Bordia et al., 1996). Additionally, the data exist that garlic, unlike aspirin, does not affect the prostacyclin synthesis in vascular wall, thus sustaining antithrombogenic properties of endothelial cells (Ali and Mohammed, 1986; Srivastava, 1986). It may be also proposed that moderate anti-aggregatory effect of garlic may be due to regulation of activity of membrane phospholipases that prevents the liberation of arachidonic acid from phospholipids (Mohammad and Woodword, 1986). Garlic-based preparations are also able to regulate the processes of serotonin and coagulation factor IV liberation from platelets (Makheja, Bailey, 1990).

In our study, the treatment of patients with cerebral atherosclerosis with garlic powder tablets provided moderate but statistically significant decrease in plasma fibrinogen level. It is known that commonly used aspirin treatment along with the inhibition of platelet aggregation may provoke the increase in plasma fibrinogen that may be regarded as unfavorable side effect. Taking into account the possible role of inflammation in atherogenesis, it may be proposed that garlic preparations while lowering fibrinogen – an acute phase protein, can play a positive antiatherosclerotic role.

However, this study has some limitations. First, even if the sample size was sufficient to detect statistically significant effects of Allicor treatment, it was rather small to avoid type 2 errors in the analysis of differences between Allicor-treated study participants and placebo recipients (the statistical power was only 68.0% at \( \alpha<0.05 \)). Although the procedure of selection of patients with cerebral atherosclerosis in combination with transitory or mild arterial hypertension for participation in this study could help controlling other factors or variables, which could affect the results (e.g. age, other medical conditions, diet, regular consumption of raw garlic, etc), this also induced an obvious bias in sample formation; therefore, there is insufficient evidence for wide interpolation of the results of this study.

Conclusion

We have shown that the treatment with Allicor contributes to normalization of blood plasma fibrinolytic activity that was significantly lowered at the baseline, and this effect favorably complies with antithrombotic effects of garlic. These data confirm the previous findings from different studies (Kiesewetter et al., 1990; Bordia et al., 1998) where the ability of garlic-based preparations to increase plasma fibrinolytic activity was demonstrated in patients with coronary artery disease. However, in some studies fibrinolytic activity of garlic was not shown (Luley et al., 1986; Berthold and Sudhop, 1998). The contradictory results may be emerged as a result of methodological shortcomings, the use of different formulations or preparations of garlic and different time scales of the studies. Accordingly, further clinical studies are required in which standardized formulations of garlic with known compositions can be used. Evidence obtained
from these studies indicates that garlic has potential in the prevention and control of cardiovascular disorders and is beneficial when taken as a dietary supplement. This study was supported by the Ministry of Education and Science of the Russian Federation.

References


