EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effect of Allicor on Platelet Aggregation *In Vitro* and *Ex Vivo*

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An extract of garlic powder in isotonic phosphate buffer and adjoen (bioactive compound isolated from garlic powder) suppress human blood platelet aggregation induced by ADP and arachidonic acid *in vitro*. Adjoen more effectively than aspirin inhibits ADP-induced platelet aggregation but is inferior to aspirin if platelet aggregation is induced by arachidonic acid. *Ex vivo* oral intake of one Allicor tablet significantly decreases rabbit platelet aggregation induced with ADP. It is suggested that long-acting garlic powder tablets prevent thromboembolic complications and are recommended for correcting hemostasis parameters in patients with atherosclerotic involvement of blood vessels.

Key Words: *platelet aggregation; atherosclerosis; adjoen; garlic powder; garlic tablets*

Platelet function is changed in some cardiovascular diseases [15]. Increased platelet activity affects blood rheology and microcirculation in tissues and organs. Correction of hemostasis is particularly important for patients with atherosclerotic involvement of blood vessels because of the risk of thromboembolism. Drugs used with this purpose should be prescribed with care, particularly for a long-term therapy. The antiaggregant activity of garlic and garlic-based agents has been established [5,9,12,13]. The safety of this natural product prompted us to study its antiaggregation activity.

We examined the effects of long-acting garlic tablets and garlic powder on human and rabbit platelet aggregation *in vitro* and *ex vivo*.

MATERIALS AND METHODS

In vitro study. Aspirin (Sigma), garlic powder (Lichtwer Pharma CmbH), and adjoen [(E,Z)-4,5,9-trithiadodeca-1,6,11-trieno-9-oxide], a bioactive compound isolated from garlic powder, were used [4]. Aspirin was dissolved in isotonic phosphate buffer to a final concentration of 200 mM. Extract of garlic powder (1 g/ml) was prepared in isotonic phosphate buffer (30 min at 20°C). The garlic powder extract (GPE) was centrifuged at 2000*g* for 10 min and stored at 4°C. Adjoen was obtained by high-pressure liquid chromatography from water extract of garlic, the intermediate reagents were diallyl disulfide and allicyl [4]. The preparation purity was about 95%. Adjoen was dissolved in ethanol to a final concentration of 20 mg/ml and stored at -20°C.

Experiments were carried out with the platelets from 19 healthy volunteers. Blood was collected from the ulnar vein and stabilized with 0.13 M sodium citrate (1:9). Platelet-rich plasma was prepared by blood centrifugation at 200*g* for 7 min. The supernatant was collected in individual tubes and stored at -20°C. In order to prepare platelet-free plasma, the preparation was centrifuged again at 2000*g* for 20 min. For studies of platelet aggregation, the relative platelet count in suspension was adjusted to 0.25% by diluting it with autologous platelet-free plasma. Platelet concentration and aggregation were

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measured in a Biola autoanalyzer (Russia) with computer processing of data [8]. Platelet aggregation was induced by 1 μM ADP (Serva) and 2 mM arachidonic acid sodium salt (Sigma).

Garlic powder extract (0.1 mg/ml, n=19), adjoen (30 μM, n=9), and aspirin (1 mM, n=5) were added to plasma specimens.

Ex vivo study. All experiments were performed on 6 male Chinchilla rabbits (body weight 3.0-3.5 kg, age 12-15 weeks) fed standard rations.

The rabbits were given 1 tablet of Allicor (INAT-Farma, Russia) containing 300 mg of garlic powder.

Plasma was collected from the marginal ear vein before and 2, 4, 6, 8, 12, and 24 h after administration of Allicor. Blood was stabilized with 0.13 M sodium citrate (1:9). Plasma was prepared, and platelet aggregation assessed as described above. Platelet aggregation was stimulated with 5 μM ADP.

The significance of differences was evaluated by dispersion analysis using a BMDP software [7].

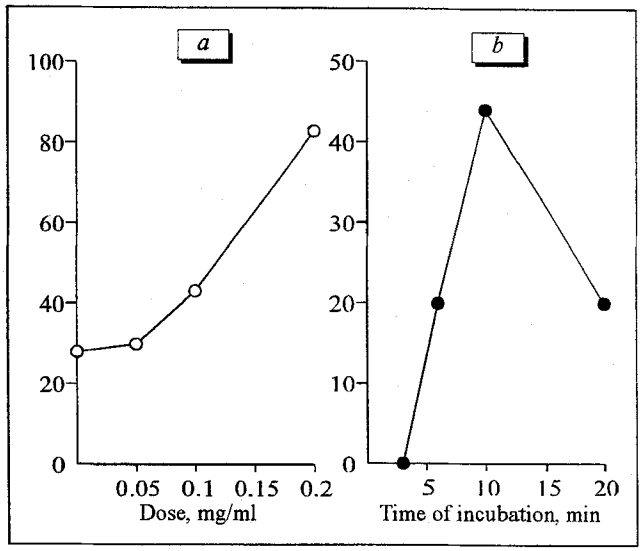

Fig. 1. Inhibition of ADP-induced human platelet aggregation (%) with garlic powder extract in isotonic phosphate buffer *in vitro*. Relationship between the concentration (*a*) and duration of incubation (*b*) with garlic powder extract in isotonic phosphate buffer (0.1 mg/ml). ADP-induced platelet aggregation (1 μM) is taken for 0.

RESULTS

In vitro GPE suppressed ADP-induced human platelet aggregation by 37%. Figure 1 shows the dose dependence of GPE inhibitory effect (Fig. 1, *a*) and the relationship between the effect and duration of incubation (Fig. 1, *b*).

Adjoen also suppressed aggregation of human platelets induced by ADP and arachidonic acid (Fig. 2). Adjoen more effectively than aspirin inhibited ADP-induced platelet aggregation (Fig. 2, *a*) but was inferior to it if platelet aggregation was induced by arachidonic acid (Fig. 2, *b*). Adjoen inhibited ADP-induced platelet aggregation by 87%, aspirin by 76%. When platelet aggregation was induced by arachidonic acid, adjoen inhibited it by 17% and aspirin by 82%. It is noteworthy that the concentrations of adjoen were much lower than of aspirin. Incubation of plasma with adjoen and aspirin together did not potentiate the inhibitory effect: inhibition of ADP-induced platelet aggregation was 82%.

Allicor significantly decreased ADP-induced platelet aggregation in rabbits as early as 2 h after ad-

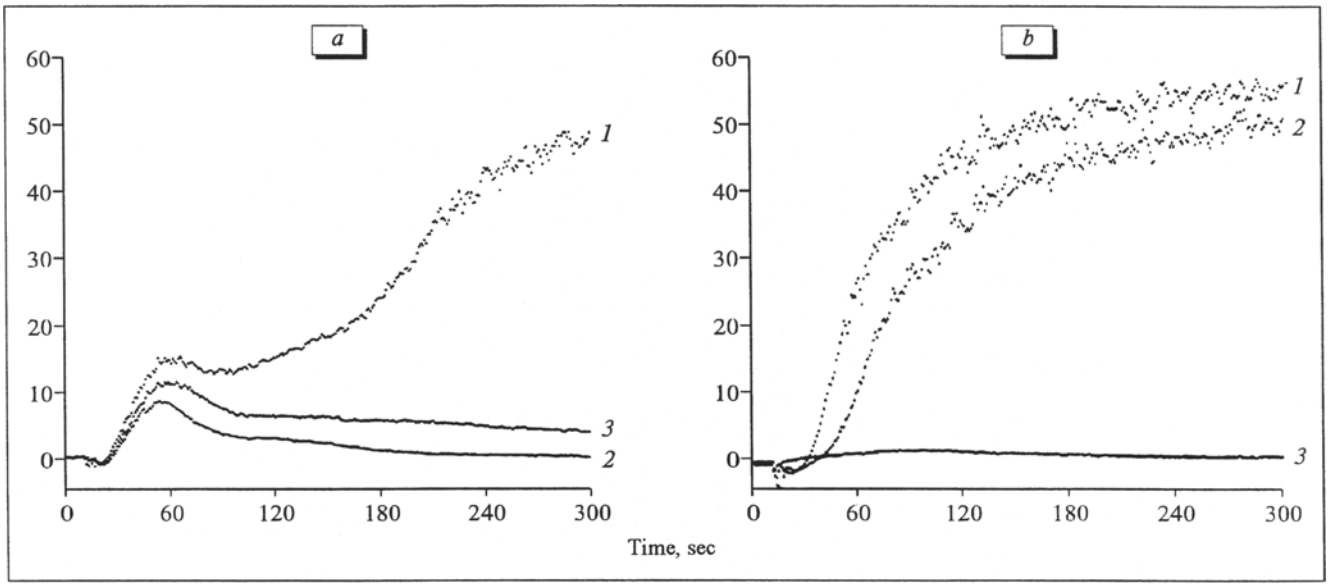

Fig. 2. Adjoen and aspirin effects on human platelet aggregation (rel. units) *in vitro*. Human platelet aggregation induced by ADP (*a*) and arachidonic acid (*b*). *1*) control; *2*) adjoen (30 μM); *3*) aspirin (1 mM). Control: human platelet aggregation induced by *a*) ADP, 1 mM and *b*) arachidonic acid, 2 mM.

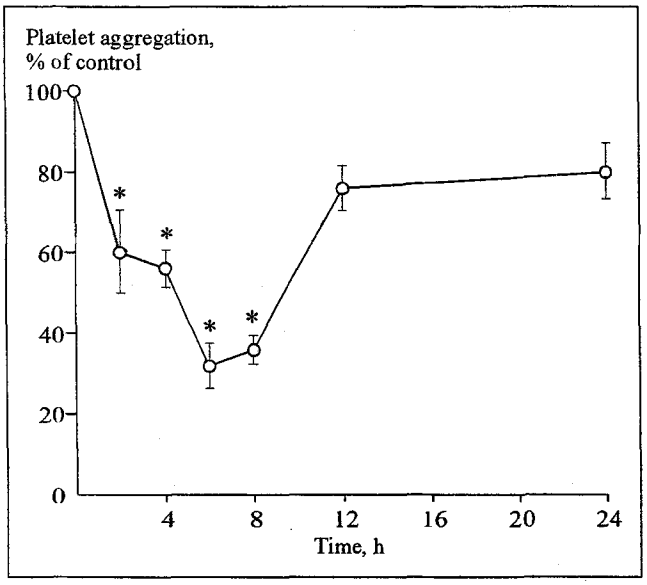

Fig. 3. Effect Allicor (300 mg orally) on rabbit platelet aggregation *ex vivo*. ADP-induced (5 μM) platelet aggregation is taken for 100%. *$p<0.05$ in comparison with the initial aggregation (before Allicor administration).

ministration. The maximum drop of platelet aggregation was observed 6 h and normalized 12 h after administration (Fig. 3).

Thus, the addition of GPE and adjoen to human plasma suppresses platelet aggregation induced by ADP and arachidonic acid. Our results agree with the findings of others [3,14].

On the other hand, comparison of the inhibitory effect of adjoen and aspirin showed that adjoen is not inferior to aspirin in antiaggregation activity, but its mechanism of action is different. The antiaggregation effect of aspirin on platelets is based on inhibition of cyclooxygenase, an enzyme regulating thromboxane A_2 synthesis. However, upon prolonged use aspirin suppresses prostacyclin production in the vascular wall, which may promote intravascular platelet aggregation [1]. The mechanism underlying the inhibitory effect of garlic on platelet aggregation is still unclear. It is known that adjoen and other bioactive components of garlic suppress the rate of thromboxane B_2 production but virtually do not affect the production of prostacyclin [2,14]. On the other hand, the capacity of adjoen to directly bind and incorporate into the platelet plasma membrane is an additional factor underlying the antiaggregation effect of garlic [6]. Garlic preparations can be used instead of aspirin in patients with coagulopathies to prevent blood clotting. Other scientists [9] came to the same conclusion after double blind placebo-controlled study in patients with a high degree of platelet aggregation.

Allicor suppresses ADP-induced platelet aggregation in rabbits for 12 h, i.e., the drug is long-acting. Kwai garlic powder (900 mg, Lichtwer Pharma CmbH) suppressed ADP-induced platelet aggregation in patients for 4 h [10]. It is noteworthy that Allicor manifested its effect at a 3-fold lower dose than Kwai. On the other hand, previously we demonstrated prolonged (12 h) antiatherogenic and antiatherosclerotic effects of Allicor in a cell culture [11].

Thus, from our *in vitro* and *ex vivo* results it can be hypothesized that long-acting garlic powder tablets prevent thromboembolic complications and can be recommended for patients with atherosclerotic involvement of blood vessels.

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