

SELECTIVE INHIBITION OF PAF-INDUCED HUMAN PLATELET AGGREGATION BY GARLIC

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Abstract

Garlic exudate (0.2-2.5 mg) prepared by squeezing fresh garlic cloves inhibited platelet aggregation induced by platelet activating factor (PAF) in a dose-dependent manner. No inhibition of aggregation was observed when adenosine-5'-diphosphate (ADP) or arachidonic acid (AA) were used as aggregating agents. This selective effect of garlic against PAF-induced aggregation was also seen with aqueous or alcoholic garlic extracts. These results suggest that PAF antagonists are present in garlic.

Keywords: Garlic, human platelet aggregation, platelet activating factor (PAF).

INTRODUCTION

Garlic is one of the most widely used food spice. Volatile unsaturated principles of garlic have been shown to possess some medicinal properties [Resch and Ernst 1995]. Previous studies have shown that extracts of garlic inhibit platelet aggregation induced by adenosine-5'-diphosphate (ADP), arachidonic acid (AA), epinephrine, calcium ionophore A23187, and thrombin [Apitz-Castro *et al.* 1983, Morris *et al.* 1995].

Platelet activating factor (PAF) is now considered to be one of the most powerful inducer of human platelet aggregation [Saeed *et al.* 1995]. As this property of PAF is unrelated to aggregation stimuli attributable to ADP or AA, activation of platelets by PAF can be considered a novel endogenous stimulus to platelet aggregation. The present study was undertaken to study the effect of garlic against PAF-induced platelet aggregation. We have also compared the anti-aggregatory effects of garlic and its various extracts against aggregation induced by other agents.

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MATERIALS AND METHODS

AA, adrenaline, ADP and PAF were purchased from Sigma Chemical Company, St. Louis, USA. All other chemicals were used of the highest purity grade available. Solution of sodium salt of AA was prepared by dissolving a 10 mg ampoule in 20 μ l ethanol and diluting with 730 μ l of 0.2 % w/v sodium carbonate. All work with AA was done in a nitrogen atmosphere. Adrenaline, ADP and PAF were dissolved in 0.9% NaCl.

PREPARATION OF GARLIC EXUDATE, AQUEOUS AND ALCOHOLIC EXTRACTS

Garlic exudate was prepared from garlic cloves. These were cleaned, and chopped into small pices and pressed. The exudate was obtained by processing the above material thorough a nylon mesh then filtered and lyophilized.

The aqueous extract was prepared by shaking cleaned, sliced garlic cloves with distilled water (1:3 parts water) for 30 min. at room temperature. The procedure was repeated thrice and the pooled extract was filtered and lyophilized. Alcoholic extract was also prepared by the above procedure using alcohol instead of water as an extraction medium. The combined alcoholic extracts were concentrated on a rotary evaporator (50°C) taken up in 20 ml water and lyophilized. All garlic preparations were taken up in cold 0.9% sodium chloride (w/v water) for use in platelet aggregation studies.

PLATELET AGGREGATION

Blood was taken by venipuncture from normal healthy volunteers. They were free from any medication for one week prior to drawing of blood. Blood was collected in siliconized tubes containing sodium citrate (3.8% w/v) and centrifuged at 800 rpm for 20 min. using The Hermle Z 200 A Centrifuge. The supernatant platelet-rich plasma (PRP) was removed and the remaining blood was centrifuged at 2000rpm for 10 minutes to obtain platelet-poor plasma (PPP). Aggregation studies were carried out at 37°C with PRP, having platelet counts between $2.5-3.0 \times 10^8 \text{ ml}^{-1}$ plasma. All studies were conducted within 3 hours after the preparation of PRP. The aggregation was monitored with a Lumi-aggregometer Model 400, Chronolog Corporation (Chicago, U.S.A). Aliquots (0.45 ml) of PRP were preincubated with or without garlic exudate for 1 minute before challenging with PAF, AA or ADP. The resulting aggregation was monitored with the aggregometer and expressed as percent potentiation or inhibition compared with control at 4 minutes after challenge.

All values are means $\pm$ SEM of 5-6 independent experiments. Statistical analysis was done using one way ANOVA. Differences we considered significant when probability (p) was <0.05 and highly significant when p was <0.01

RESULTS AND DISCUSSION

Fig. 1 shows profile of platelet aggregation induced by different concentrations of ADP, adrenaline, AA or PAF. We found that four aggregating agents produced a concentration-related platelet aggregation when added to PRP.

Fig. 2 shows the results of the effect of garlic exudate on ADP and PAF-induced platelet aggregation. It is evident that garlic exudate at a concentration range (0.1-2.5 mg) produced a dose dependent inhibition of PAF-induced aggregation,

whereas, at this concentration it had no effect on aggregation induced by ADP or AA ($P < 0.001$; $n=7$). In order to test further the selective effect of garlic exudate against PAF, we used aqueous and alcoholic garlic extracts. The results shown in Fig. 3 clearly demonstrate that both extracts profoundly inhibited PAF-induced aggregation, whereas even up to 5.0 mg concentration of each extract, little or no inhibition of ADP-induced aggregation was observed. These results support the selective antagonism of PAF-induced aggregation by garlic.

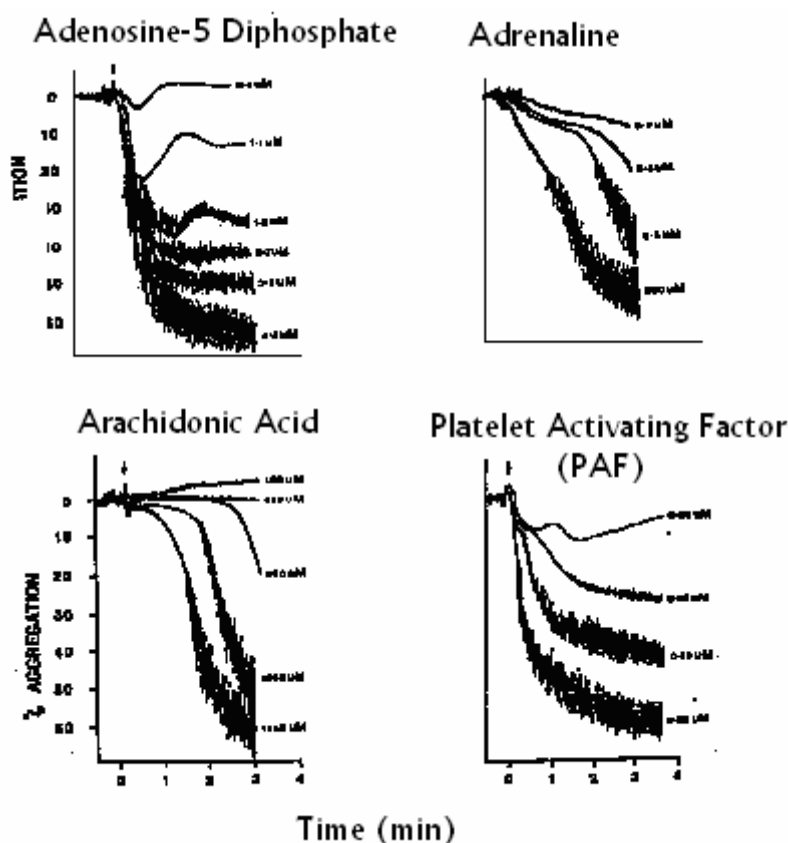


Fig. 1: Concentration-response curves of platelet aggregation induced by various aggregating agents.

Garlic is a useful spice extensively used in foods. When consumed in moderate amounts, it produces changes in blood lipids, platelet function and in fibrinolytic activity [Bordia *et al.* 1998]. Both in animals and humans it has been shown to produce a fall in serum cholesterol, increase of HDL-cholesterol, fall in triglycerides and fibrinogen and increase of fibrinolytic activity.

During the past few years reports have appeared describing the anti-aggregatory properties of garlic [Turner *et al.* 2004]. Effects of garlic have been examined in different ways. For studies on platelet aggregation, garlic preparations were prepared either as oily fractions (essential oils) or as such by crushing garlic cloves with water in a blender.

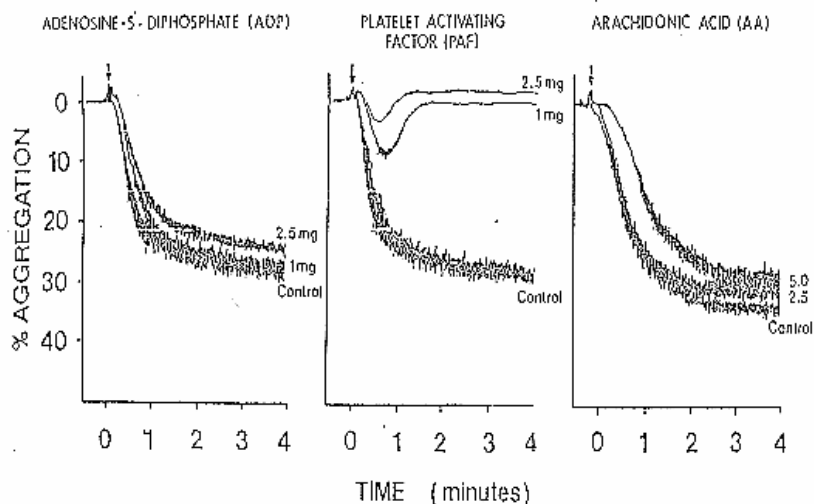


Fig. 2: The effect of garlic exudates on platelet aggregation. Garlic exudates was preincubated with PRP for 1 minute before challenge with PAF ($0.8 \mu\text{M}$) or ADP ($2.2 \mu\text{M}$).

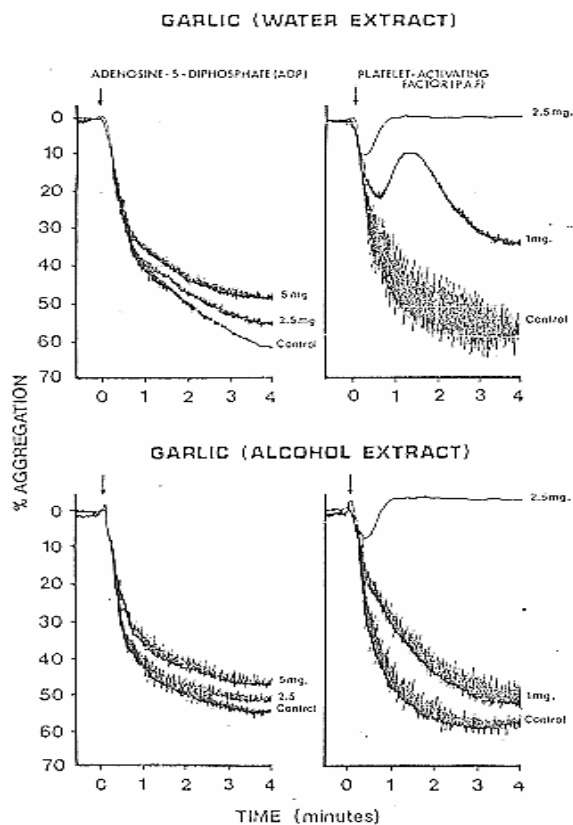


Fig. 3: The effect of aqueous and alcoholic garlic extracts on platelet aggregation by PAF and ADP.

In the present study we have examined the effect of garlic against human platelet aggregation in more detail. Particularly we focused our attention on the effects of garlic against PAF-induced aggregation. A large body of evidence now suggests that PAF is a potent stimulator of platelet aggregation. Platelet activation by PAF results in the release or generation of biologically active materials, and following its systemic administration PAF reproduced many of the features of anaphylactic shock. More recently, several studies have demonstrated that PAF plays an important role as a mediator in asthma [Saeed *et al.* 1993]. Outdata demonstrate that garlic may contain PAF antagonists. To our knowledge a similar study has not been done with garlic before. The fact that garlic exudate selectively inhibited PAF-induced platelet aggregation is novel finding and it can be inferred that garlic could function as a PAF antagonist.

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