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INHIBITION OF PLATELET ACTIVATING FACTOR BY AJMALINE IN PLATELETS: *IN VITRO AND IN VIVO* STUDIES

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ABSTRACT

The in *vitro* and in *vivo* effects of ajmaline and its derivatives on platelet aggregation and platelet-activating factor (PAF) induced death in rabbits was studied. Ajmaline and acetyl ajmaline selectively inhibited PAF-induced aggregation in a concentration related manner. Weak or no inhibition of aggregation was observed when ADP, collagen or arachidonic acid were used as aggregating agents. Similarly ajmaline or acetyl ajmaline also inhibited the lethal effects of PAF in the rabbit. PAF (8-11 ug/kg iv.) caused sudden death in rabbits due to platelet aggregation and cardiac failure. Pretreatment of rabbits with ajmaline (50 mg/kg i.p.) protected conscious rabbits from PAF (11 ug/kg 1,0-induced death. Since PAF is a powerful inducer of platelet aggregation via stimulation of specific PAF membrane receptors, our data is suggestive that ajmaline (an anti- arrhythmic agent) could emerge as a new class of PAF antagonists.

Introduction

Platelet-activating factor (1-0-alkyl-2-acetyl-sn-glycero-3- phosphocholine; PAF) is a potent lipid autacoid that has been found to be involved in numerous inflammatory and allegry-related processes (Page *et al.,* 1984). More recently it has been suggested that PAF might be an important mediator in shock, causing systemic hypotension with acute circulatory collapse (Bessin *et al.* 1983) and increased vascular permeability (Sanchez-Crespo *et al.*, 1982) when injected into experimental animals. Furthermore, PAF is one of the most potent inducer of platelet aggregation known in man as well as in most animal species through activation of PAF receptors (Chignard *et al.,* 1979; Martins *et al*, 1988; Saeed *et a1,* 1988).

Because of these potent pathophysiologic actions, PAF receptor antagonism may be beneficial in inhibition of platelet aggregation, thrombosis and shock. Recently we have demonstrated that antiarrhythmic drugs such as quninidine selectively inhibit PAF-induced human platelet aggregation (Gilani and Saeed, 1989). Ajmaline also is an

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antiarrhythmic agent with actions similar to those of quinidine. Since platelet aggregation can be induced by several mediators and physiological substances, we have investigated whether ajmaline and its derivatives inhibit platelet aggregation and provide protection in a severe model of shock such as PAF-induced death in rabbits.

Materials and Methods

Arachidonic acid, ADP, Collagen and PAF were purchased from Sigma chemical Company, U.S.A. All other chemicals used were of highest purity grade available.

Human platelet aggregation:

Blood was taken by venipuncture from normal human volunteers reported to be free of medication for the week. Blood samples were mixed with 3.8% (w/v) sodium citrate solution (9:1) and centrifuged at 260 x g for 15 minutes at 20°C to obtain platelet rich plasma *(PRP).* The remaining blood sample was centrifuged at 1200 x g for 10 minutes to obtain platelet-poor plasma (PPP). Platelet count was determined by phase contrast microscopy and all aggregation studies were carried out at 37°C with *PRP* having platelet counts between 2.5-3.0 x 10^8 of plasma. Platelet aggregation was studied turbidometrically using a four channel Hema tracer aggregometer (Japan) using 0.25 ml aliquots of *PRP*. The final volume was made up to 03 ml with sodium chloride (0.9%, w/v) or test drug and incubated for one minute before challenge with the aggregating agent. Aggregation was induced with arachidonic acid (AA; 1.7 mM), adenosine-5-diphosphate (ADP; 2.2 uM), collagen (20 ug/ml) or PAF (0.8 uM). The resulting aggregation was recorded and expressed as percentage inhibition compared with control for 4 minutes after challenge with the aggregating agent. Ajmaline or its derivative was tested at three to four concentrations in duplicate. Statistical differences between control and drug treated platelet preparations were determined by students t-test.

PAF-induced mortality in rabbits:

Male rabbits (1.3-1.8 Kg) obtained locally were used to test the lethality of injected PAF. PAF was diluted before each injection in isotonic saline. The PAF was injected into a marginal ear vein of non-anaesthetized rabbits over a period of approximately one minute. In preliminary experiments, we established that a dose of 8-11 ug/kg was required to induce consistently fatal pulmonary thrombosis in control animals. This dose of PAF was used in subsequent experiments. Ajmaline was dissolved in isotonic saline.

Animals were pretreated by intraperitoneal injection of ajmaline (50 mg/kg) two hours before challenge with PAF. Each animal was used for only one experiment.

Results

The chemical structures of various ajmaline derivatives investigated are presented in Fig. 1. All the ajmaline derivatives used in this study did not react with platelet-rich plasma to cause any direct aggregation of platelets.

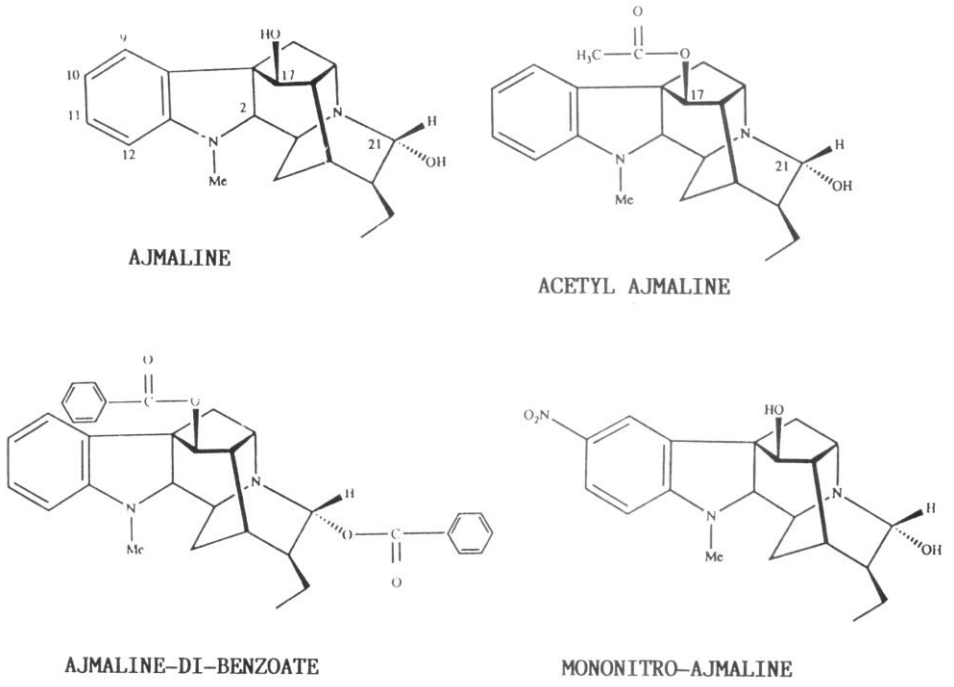

Figure 1: Chemical Structures of ajmaline and its derivatives.

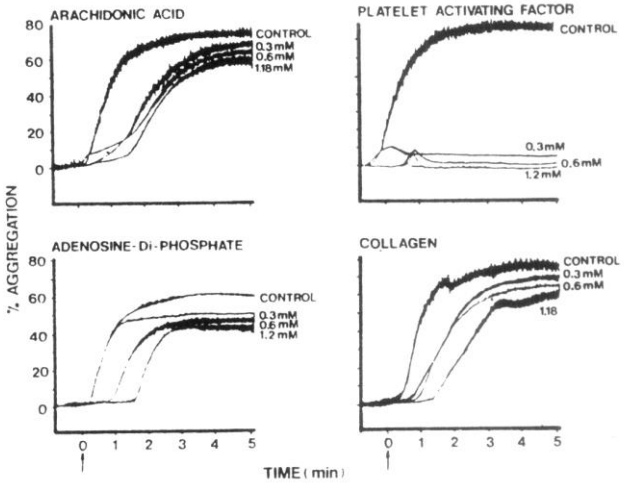

Figure 2: Selective inhibition of platelet-activating factor induced aggregation by ajmaline.

Ajmaline (mM) was added 1 min. before challenge with the aggregating agent. (↑) Aggregation was induced by various aggregating agents as shown. For experimental details see text.

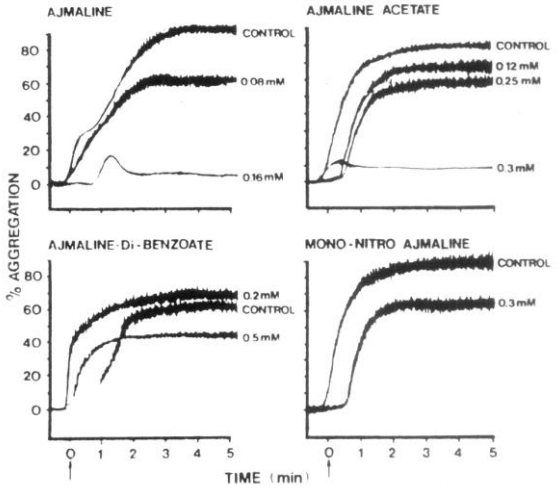

Figure 3: Effect of ajmaline and its derivatives on PAF-induced platelet aggregation. (1) Aggregation was induced by PAF; Acetyl ajmaline (acetate).

The effects of ajmaline on platelet aggregation in *vitro* induced by AA, PAF, ADP, or collagen are shown in Fig. 2. When PRP was preincubated with ajmaline in concentration of 0.5-1.2 mM for 1 min prior to challenge, platelet aggregation induced by PAF was completely inhibited. Whereas ajmaline had only weak or no inhibitory effect against aggregation induced by ADP, AA or collagen. In separate experiments, we investigated the effect of various ajmaline derivatives on PAF-induced platelet aggregation. The results showed (Fig. 3) that ajmaline and acetyl ajmaline completely inhibited PAF-induced aggregation at a concentration of 0.16 and 0.3 mM respectively. Whereas ajmaline dibenzoate and mononitro ajmaline had no inhibitory effect against PAF-induced platelet aggregation (Table-1).

Table-1

Comparative effects of ajmaline and its derivatives on platelet aggregation induced by various aggregating agents.

Compound	*IC 50; μM (Mean ± S.E.M; n = 10) Aggregating agent			
	ADP	AA	Collagen	PAF
Ajmaline	407±3	NA	380±4	104±
Acetyl ajmaline acetyl	NA	NA	NA	177±1
Ajmaline dibenzoate	>1000	NA	NA	NA
Mononitro ajmaline	682±10	> 1000	758±9	614±6

*IC50 is the concentration (uM) producing 50% inhibition of platelet aggregation induced by various aggregating agents.

NA, not active; ADP, adenosine 5'-diphosphate, AA arachidonic acid; PAF, platelet-activating factor.

In separate experiments we investigated that effect of ajmaline and acetyl ajmaline on PAF-induced platelet aggregation and death. Silver *et al.,* (1974) have recently demonstrated that AA when injected into the ear vein of the rabbit causes sudden death associated with occlusive platelet aggregates in the microvasculature of the lung. In the present study we found that PAF when injected into the ear vein of the rabbit also causes sudden death associated with platelet aggregation.

Pretreatment of rabbits with ajmaline and or acetyl ajmaline afforded complete protection against PAF-induced death. The mortality rate after ajmaline or acetyl ajmaline pretreatment was significantly lower ($P < 0.001$) than the mortality rate found without pretreatment. These results with ajmaline and acetyl ajmaline pretreatment two hours prior to intravenous injection of 11 ug/kg PAF are given in Table-2.

Table-2

Effect of pretreatment with ajmaline and acetyl ajmaline on PAF- induced mortality in rabbits

		Pretreatment	
Effect	Control	Ajmaline	Acetyl Ajmaline
Survived	0	5	5
Died	6	0	0

Survival rate in the ajmaline group was significantly different as compared to control group P (0.001).

Discussion

From the above data we take it to be evident that ajmaline (an anti arrhythmic agent) from the *Rauwolfia serpentina* selectively inhibits PAF-induced platelet aggegation. Furthermore, our results show that ajmaline exerts beneficial effects in a severe model of PAF-induced sudden death in rabbits. Whereas ajmaline derivatives such as nitro-ajmaline or di-benzoate forms conferred a negative effect on this property of ajmaline. Therefore, further studies were carried out with ajmaline or its acetyl form.

Pretreatment of rabbits with ajmaline completely reversed the lethal effect of PAF suggesting that ajmaline acts as an antagonist of PAF. While the precise site(s) of PAF-induced action in sudden death of rabbits remains to be clarified, a variety of PAF effects may be related.

It has been demonstrated that i.v. injection of PAF induces platelet aggregation in several animal species through activation of PAF receptors (Martins *et al.*, 1988). Thus

hypercoagulability, thrombosis and microembolism may be implicated in this model of shock. It is interesting to note that prostacyclin, a potent antiaggregating agent, displays beneficial effect in shock (Lefer and Araki, 1983). Thus the beneficial effects of PAF receptor antagonism by ajmaline in this model is probably mediated via antagonism of the direct effects of PAF. Furthermore this could also involve membrane-stabilizing properties or lipophilicity of ajmaline. Indeed, anti-arrhythmic drugs like quinidine and ajmaline are known to possess membrane-stabilizing properties (Gilani and Saeed, 1989). In conclusion, whether the inhibitory effect of ajmaline on platelet aggregation are clinically relevant will depend on long-term prospective studies; however, there are good reasons to suggest that ajmaline or acetyl ajmaline may represent a new class of PAF antagonists.

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