# Calmodulin antagonists inhibit aggregation of human, guinea pig and rabbit platelets induced with platelet activating factor

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Three calmodulin (CM) antagonists W-7, W-5 and trifluoperazine (TFP) were tested for ability to prevent aggregation of human, guinea pig, and rabbit platelets induced by 7.88 $\mu$M PAF. The naphthalene sulfonamide derivatives, W-7 and W-5, were active in all species, W-5 being 1,5–5.7-times less potent than W-7, in accordance with W-5 being a weaker CM inhibitor. $ED_{50}$-Values for TFP were 155, 160 and 255 $\mu$M for rabbit, human and guinea pig platelets, respectively. Results are consistent with the notion that some substances antagonizing CM may inhibit PAF aggregation effects. W-7 is most effective on human platelets ($ED_{50}$ 51.5 $\mu$M). High concentrations of TFP required to antagonize PAF-induced aggregation cautions against ascribing its effects solely to an inhibitory effect on CM.

*Platelet activating factor* *Calmodulin antagonist* *W-7* *W-5* *Trifluoperazine* *Platelet aggregation*

## 1. INTRODUCTION

Platelet activating factor (PAF) (1-$\alpha$-lecithin, $\beta$-acetyl, $\gamma$-*O*-alkyl) is a potent aggregator of platelets from certain species, including human, guinea pig and rabbit [1]. The precise mechanism of action of PAF in producing this effect is unknown. There is evidence that calcium is a key mediator of PAF action since calcium channel blockers may inhibit PAF-induced aggregation [1,2]. In addition, the calmodulin (CM) antagonists W-7 [*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide] [3] and trifluoperazine (TFP) [4] may also inhibit the aggregating effect of PAF [5,6]. To further test the specificity of the CM antagonist actions against PAF, we performed experiments comparing W-7, its non-chloro-less potent analog, W-5 [3] and TFP on PAF-induced aggregation in human, guinea pig and rabbit platelets.

## 2. MATERIALS AND METHODS

### 2.1. *Platelet preparation*

New Zealand rabbits and Hartley strain guinea pigs of either sex were obtained from local commercial sources. The animals were anesthetized with an intraperitoneal injection of pentobarbital sodium. Whole blood was obtained by cardiac puncture using a 19 gauge butterfly needle (Abbott), which drained into a Falcon plastic tube containing 3.8% sodium citrate as an anticoagulant (1:9 parts blood). The blood was centrifuged in a IEC Centrifuge at 200 × *g* for 10 min at room temperature. Platelet rich plasma (PRP) was then removed, and placed into aggregometer tubes. The PRP was equilibrated at room temperature for 30 min prior to any aggregation studies [7].

Blood was obtained from normal male volunteers (24–54 years). A 19 gauge butterfly needle was placed into an antecubital vein, and blood allowed to flow into a plastic tube containing 3.8% sodium citrate (1:9 parts blood). PRP



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then was prepared and processed as described for the animal preparations.

### 2.2. *Platelet aggregation*

Platelet aggregation was studied with Chronolog dual channel aggregometers and recorders as in [5]. PRP (0.45 ml) was placed into aggregometer tubes containing a magnetic stirring bar. The PRP sample was placed into the aggregometer and equilibrated for 1 min before addition of inhibitors or aggregating substances. All studies were done at 37.5°C. Experiments were completed within 2 h of the preparation of the PRP sample.

### 2.3. *Drugs and chemicals*

Platelet activating factor (PAF) (L-$\alpha$-lecithin, $\beta$-acetyl, $\gamma$-*O*-alkyl) was obtained from Calbiochem (La Jolla CA). The material was dissolved in 60% ethanol. In the volumes used (1–5 $\mu$l/0.45 ml PRP), the solvent did not promote detectable aggregation of the PRP.

The calmodulin antagonists W-7 [*N*-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide] and W-5 [*N*-(6-aminohexyl)-1-naphtalenesulfonamide -HCl] were purchased from Rikaken Co. (Nagoya). W-7 compound is a potent and specific calmodulin antagonist [3]. W-7 and W-5 were dissolved in distilled water and kept at 4°C. Dilutions of the stock solution were made fresh daily. Exposure of the PRP to the W-7 solvent (1–10 $\mu$l) neither produced detectable aggregation, nor did it alter the normal aggregation response to PAF. PRP samples were treated with the antagonists for 5 min at 37°C in the aggregometer before addition of PAF. The effect of the antagonists on PAF-induced aggregation was expressed as percent inhibition of the control response to PAF obtained in the absence of the inhibitor.'

Trifluoperazine (TFP) also was used as a CM antagonist [4]. It was obtained from Smith, Kline and French Labs (Philadelphia PA).

### 2.4 *Determination of* $ED_{50}$

Log dose-percent inhibition curves for the 3 CM antagonists were determined using 7.88 $\mu$M (4.4 $\mu$g/ml) PAF as the aggregating substance. Three to five doses for each antagonist were tested *vs PAF, and the mean dose*-response curve plotted. From these curves, the $ED_{50}$ for each antagonist was estimated by graphic extrapolation.

## 3. RESULTS

Fig. 1 depicts a typical dose-response curve for the inhibitory effect of W-5 on PAF-induced aggregation of guinea pig platelets (PRP). From such experiments, the $ED_{50}$-values for W-5, W-7 and TFP were determined for platelets from humans, guinea pigs and rabbits, as in section 2. Table 1 compares the $ED_{50}$-values for the inhibitory effect of the CM antagonists on PAF-induced platelet aggregation. Our previous finding [5] with W-7 indicates that it is most effective against PAF in human platelets ($ED_{50}$ = 51.5 $\mu$M). W-5, which is an analog of W-7 devoid of the chloro substitution, and is 6–7-times weaker as an inhibitor of

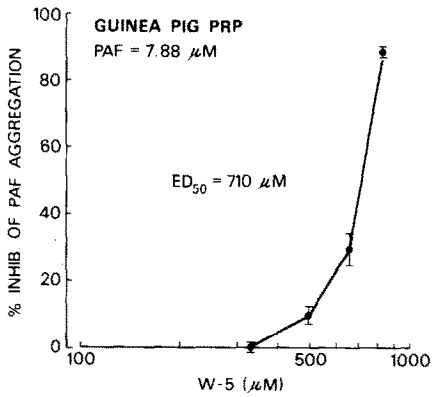


Fig. 1. Dose-response curve for the CM antagonist W-5 *vs* PAF-induced aggregation of guinea pig platelets (PRP). Effect of W-5 is expressed as % inhibition of the normal PRP aggregation response to a challenge dose (7.88 $\mu$M) of PAF. The $ED_{50}$ is estimated graphically. Each point is the mean ± SEM of 3–5 expt.

Table 1

Inhibition by calmodulin antagonists of platelet aggregation produced by platelet activating factor (PAF)

| | $ED_{50}$ ($\mu$M)[a] | | |
|---|---|---|---|
| Species | W-7 | W-5 | TFP |
| Human | 51.5[b] | 295 | 160 |
| Guinea Pig | 365 | 710 | 255 |
| Rabbit | 310 | 470 | 155 |

[a] Amount of drug needed to produce a 50% decrease (5 min pretreatment) in maximum aggregation of platelets induced by PAF (7.88 $\mu$M)

[b] From [5]

CM [3], was almost 6-times less potent an inhibitor of PAf on human platelets compared to W-7. Guinea pig platelets, which are very responsive to the aggregating effect of PAF[1] were comparatively resistant to the action of W-7, W-5 and TFP. Similarly, TFP a reported antagonist of PAF [6], was effective only with $ED_{50}$ $>100\,\mu M$ (155–255 $\mu M$). This suggests TFP may not be acting solely as a CM inhibitor, since $>100\,\mu M$ is thought to represent a non-CM effect of this drug (non-stereo-specific hydrophobic interactions) [8].

## 4. DISCUSSION

The ability of the 3 CM antagonists to inhibit PAF-induced aggregation may be interpreted to support the concept that part of PAF's effect is mediated through CM-dependent processes. The specificity and potency of W-7 as a CM antagonist [3] is reflected in its action on human platelets. This is not shown in guinea pig and rabbit platelets, however. W-7 at $>100\,\mu M$ are thought to have effects independent of CM interaction [3]. Similarly the fact that the $ED_{50}$-values for TFP are $>150\,\mu M$ suggests that this inhibitor also may have non-CM effects, as detailed in [8].

W-5, reported to be ~6-times less potent than W-7 as an inhibitor of CM [3], was equivalently weaker as an inhibitor of PAF-induced aggregation. This suggests that there may be specific-CM interaction for this class of antagonists. W-5 has significantly less capacity to bind to CM than W-7 [3].

An untested possibility also must be considered: The effect of these 'CM inhibitors' may, in fact, be mediated through platelet membrane effects, rather than through action on intracellular CM. One speculation is that PAF exerts its aggregation effect on platelets through membrane receptor interaction [6].

In summary, PAF-induced platelet aggregation from 3 responsive species may be antagonized by 3 putative CM antagonists. W-7 is the most potent against PAF-induced aggregation of human platelets. W-5, an analog of W-7 with weaker CM interaction properties, showed predictably less effects on all 3 species. The actions of TFP must be cautiously interpreted, in view of the fact that this agent may have actions independent of CM interaction in the concentrations needed to inhibit PAF-induced aggregation.

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