

In Vitro Platelet-Activating Factor Binding Inhibitory Activity of Malaysian Medicinal Plants

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ABSTRACT The search for new pharmacologically active agents obtained by screening natural sources such as plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. As a continuation for a search of platelet activating factor antagonists, a total of 15 methanolic extracts from 12 species of Malaysian medicinal plants were tested in the rabbit platelet membrane receptor preparation for their ability to inhibit PAF binding in vitro. The results demonstrated that five medicinal plants inhibited more than 60% of [³H]-PAF equilibrium binding at the concentration of 200µg/ml.

ABSTRAK Pencarian agen aktif farmakologi yang diperolehi melalui penyaringan bahan semulajadi seperti ekstrak tumbuhan telah membawa kepada penemuan dadah yang memainkan peranan utama dalam rawatan penyakit kepada manusia. Sebagai satu usaha berterusan bagi mencari antagonis faktor pengaktif platelet, sejumlah lima belas ekstrak metanol daripada dua belas spesies tumbuhan ubatan tempatan telah diuji keatas sediaan reseptor membran platelet bagi melihat keupayaannya untuk merencat ikatan PAF secara in vitro. Keputusannya menunjukkan bahawa lima tumbuhan ubatan didapati memberikan perencatan melebihi 60% terhadap ikatan [³H]-PAF pada kepekatan 200µg/ml.

(Malaysian medicinal plants, platelet activating factor)

INTRODUCTION

Platelet activating factor (PAF) is an inflammatory mediator, chemically identified as 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine which has been obtained from a variety of sources from diverse animal species. It has wide tissue distribution and may function in normal physiological processes such as inflammation, neural activity and reproduction. It may also have a role as a mediator in pathological states such as asthma, ischemia, gastric ulceration, hypertension, atherosclerosis and shock [1]. The physiological effects of PAF are markedly species dependent. One important source of this species dependence is the distribution of platelet high-affinity PAF receptors. Human and rabbit platelets possess high-affinity PAF receptors and are aggregated by PAF, whereas PAF does not aggregate rat platelets, which do not have specific PAF binding sites [2]. Receptor-mediated PAF binding by the platelet is considered essential to platelet activation and perhaps to the

development of platelet-dependent vascular disease [3].

PAF binds to its receptor, a member of the G protein-coupled receptor superfamily, and triggers a series of intracellular signals including phosphoinositide breakdown, arachidonic acid metabolism, intracellular calcium mobilization and protein phosphorylation [4]. These signals cause the many pathophysiological phenomena mentioned above. Because most actions of PAF can be prevented by blocking its receptor with antagonists, a PAF-receptor antagonist is necessary to prevent these pathophysiological situations. A search for specific PAF antagonists was thus essential for elucidation of the pathophysiological significance of this mediator as well as for testing prospective therapeutic approaches [5].

METHODOLOGY

Plant Material and Preparation of Plant Extracts

All of the plants were collected from various parts of Peninsula Malaysia. The voucher specimens were deposited at the Forest Research Institute Malaysia. The plants material were air-dried and ground to mesh size 40 to 60 using a grinding machine. They were soaked in methanol for a week with solvent change (methanol) each 3 days. The extracts collected were then dried using a rotary evaporator and kept at -20°C until required. Each dried extract was dissolved in dimethyl sulfoxide (DMSO) and ethanol (1:1). Then the stock solutions were diluted with normal saline to give final concentrations of $200\mu\text{g/ml}$. The final concentration of DMSO in reaction mixture was fixed at 0.2% to avoid interference with the receptor binding studies. Reaction mixture with saline and 0.2% DMSO in saline was used as control.

Preparation of Rabbit Platelets

Whole blood samples were drawn by cardiac-puncture from healthy New Zealand white strain rabbit (3-4 kg). Six volumes of blood were mixed with one volume of ACD solution (0.15M trisodium citrate, 0.075M citric acid, pH 5.2). The blood was centrifuged at $270 \times g$ for 10 min at room temperature, and the top platelet rich plasma was removed carefully. The platelet rich plasma (PRP) was further centrifuged at $500 \times g$ for 15 min. The platelet pellets were then washed twice by means of centrifugation at $500 \times g$ (15 min) in Buffer A (20% ACD solution, 60% K_2HPO_4 buffer, 20% sodium citrate, PH 6.8) followed once at $150 \times g$ (10 min) in Buffer B (50 ml K_2HPO_4 , 0.1 gm bovine serum albumin, PH 7.0). The top whitish layer was removed and centrifuged at $500 \times g$ (15 min) to obtain the platelets. The final concentration was adjusted to 3×10^8 platelets/ml.

PAF Receptor Binding Assay

The assay was carried out in triplicate according to the previously used method by Valone *et al.* [6]. The reaction mixture consisted of $200 \mu\text{l}$ of washed rabbit platelets suspension, $25 \mu\text{l}$ of ^3H -PAF (2.0 nM) with or without unlabeled PAF (2.0 μM) and $25 \mu\text{l}$ of sample. The final concentration of sample in the reaction mixture was $18.2 \mu\text{g/ml}$. Cedrol, a known PAF receptor antagonist was used as a standard in this bioassay. These reaction mixtures were incubated

at room temperature for 1 hour. The free and platelet bound ligands were then separated by filtration technique using a glass microfibre filter in a cell harvester. Radioactivity was measured by scintillation counting. Specific binding of radiolabel is defined as the difference between total radioactivities of bound ^3H -PAF in the reaction mixture with the absence and presence of excess unlabeled PAF. Percentage inhibition of the sample was determined according to the equation used by Yang *et al.* [7]:

$$\% \text{ Inhibition} = \frac{(\text{Tc} - \text{Nc}) - (\text{Ts} - \text{Ns})}{\text{Tc} - \text{Nc}} \times 100$$

Where

Tc = total binding of control

Ts = total binding of sample

Nc = non-specific binding of control

Ns = non-specific binding of sample

RESULTS AND DISCUSSION

A total of 15 methanolic extracts of 12 species were investigated for their platelet activating factor (PAF) receptor binding inhibitory effects using rabbit platelets. Table 1 shows the inhibitory effects of the extracts at concentration of $18.2 \mu\text{g/ml}$. The chosen test concentration of $18.2 \mu\text{g/ml}$ for the extracts in this bioassay shows varying degrees of activity. We have defined 4 different levels of activity. We have considered an inhibition below 20% to be insignificant at the dose tested. An inhibition between 20 and 40% is considered to be low while between 40 and 60% as moderate and above 60% as high. Among the extracts tested, 5 samples namely *Curcuma xanthoriza* (rhizome), *Clorodundron sp.* (root), *Garcinia atroviridis* (leaves), *Cassia alata* (leaves) and *Ervatamia hirta* (rooat) exhibited inhibitory percentage of more than 60%. The highest inhibition was given by *Cassia alata* (leaves) with 75.3% at final concentration of $18.2 \mu\text{g/ml}$. The results revealed that these plants possess bioactive compounds that can bind strongly to PAF receptor at the surface membrane of platelet.

Cassia alata L. (Leguminosae) is a coarse and slightly woody herb, locally known as gelanggang. Once native to America, the species has dispersed throughout the tropics where it has become well known as a traditional remedy for ringworm and other cutaneous diseases [8]. Other than that, the leaves and sap are used to treat

fungal infections such as ringworm. They contain a fungicide, chrysophanic acid. Because of its anti-fungal properties, it is a common ingredient in soaps, shampoos and lotions in the Philippines. Other chemicals contained in the plant include saponin which acts as a laxative and expels intestinal parasites. The plant has been found to

contain anthraquinones, presumed to be the active ingredient causing the laxative effect. In Africa, the boiled leaves are used to treat high blood pressure. In South America, it is also used to treat a wide range of ailments such as fever, asthma, snake bite and venereal diseases such as syphilis and gonorrhoea [9].

Table 1. Inhibitory effects of methanolic crude extracts of Malaysian medicinal plants on the PAF receptor binding to platelet (concentration of sample in reaction mixture : 18.2 µg/ml)

Vernacular name	Scientific name	Part	% inhibition
Bonglai	<i>Curcuma montana</i>	Rhizome	51.4 ± 3.4
Pinang	<i>Areca catechu</i>	Fruit	55.1 ± 5.1
Temu lawak	<i>Curcuma xanthoriza</i>	Rhizome	63.6 ± 9.8
Tongkat rasul	<i>Clorodundron sp.</i>	Root	61.7 ± 4.2
Akar cerita	<i>Andrographis paniculata</i>	Leaves	52.5 ± 1.1
Akar cerita	<i>Andrographis paniculata</i>	Stem	51.3 ± 6.4
Misai kucing	<i>Ortosiphon aristatus</i>	Leaves	35.0 ± 7.4
Peria katak	<i>Momordica charantia</i>	Seed	48.8 ± 8.3
Halia bara	<i>Zingiber officinale</i>	Rhizome	59.6 ± 8.0
Akar putawali	<i>Tinospora crispa</i>	Leaves	8.3 ± 15.1
Asam gelugor	<i>Garcinia atroviridis</i>	Leaves	66.5 ± 12.1
Gelenggang besar	<i>Cassia alata</i>	Leaves	75.3 ± 3.9
Jelutong badak	<i>Ervatamia hirta</i>	Leaves	55.9 ± 4.9
Jelutong badak	<i>Ervatamia hirta</i>	Stem	54.3 ± 8.6
Jelutong badak	<i>Ervatamia hirta</i>	Root	67.6 ± 4.5

* Cedrol (positive control) = 80.4 ± 2.6 %

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