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Inhibitory Effects of Malaysian Medicinal Plants on the Platelet-Activating Factor (PAF) Receptor Binding

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Abstract – Methanolic extracts of 25 species of Malaysian medicinal plants were screened for platelet-activating factor (PAF) receptor binding activity using rabbit platelet. Extracts of *Cinnamomum sintoc, Ixonanthes iconsandra, Paederia foetida, Piper aduncum, Premna integrifolia, Ardisia crispa,* and *Ardisia elliptica* showed significant inhibitory effect on the platelet-activating factor (PAF) receptor binding.

Key words – platelet-activating factor (PAF) receptor binding inhibition, Malaysian medicinal plants

Introduction

Platelet-activating factor (PAF) is a potent glycerophospholipid mediator (Hanahan, 1987) which plays a wide range of physiological and pathological roles. It is involved in pathological conditions such as bronchial asthma (Vargaftig, 1987), inflammation (Braquet, 1987), allergy (Maller and Cunningham, 1985), pulmonary dysfunction, hypotention (Blank, 1979), cardiac anaphylaxis (Barns, 1986), thrombosis (Kloprogge, 1983), gastrointestinal ulceration (Hsueh, 1986), endotoxin shock (Dobber, 1985), and transplanted organ rejection (Ito, 1984). As part of our continuous screening studies (Han, 1994, Han, 1995a, b) to identify novel PAF antagonists from tropical plants, Malaysian medicinal plants of 25 species which have been used to treat PAF related diseases were screened for PAF receptor binding inhibitory effects.

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Experimental

General – Centrifuge (RT 6000, Sorvall Co.), Platelet counter (Model PLT-4, Chronolog Co.), Liquid scintillation counter (Hewlett Packard Co.), Cell harvester (Skatron Co.).

Plant materials – Plant parts of Malaysian medicinal plants of 25 species were collected in 1994 from the Forest Research Institute of Malaysia (FRIM). The samples were identified by Dr. Saw Leng Guan and the voucher specimens were deposited at the herbarium of FRIM.

Extraction and fractionation – The plant materials were air-dried and ground to mesh size 40-60. They were then subjected to exhaustive soxhlet extractions with MeOH. The MeOH extracts were concentrated to give gummy yellow to dark brown viscous mass. The MeOH extracts of the active plants were suspended in H_2O and partitioned into ether and BuOH successively. The ether, *n*-BuOH, and H_2O fractions were evaporated to give yellow to dark brown resi-

dues.

Reagents – Tris-tyrode buffer (10 mM, pH 7.3) was used for washing of platelets and binding studies. ACD solution (2.5% trisodium citrate, 1.37% citric acid, 2.0% glucose in water) was used as an anticoagulant. Bovine serum albumin (BSA) was purchased from Boehringer Manheim Co. (Germany). Radiolabelled PAF (1-*O*-^{3}H-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, 142 Ci/mmol) was purchased from Amersham (UK).

Preparation of samples – The MeOH, ether, and BuOH fractions were dissolved in dimethyl sulfoxide (DMSO), and H_2O fraction was dissolved in saline. Samples were diluted with saline (final concentration of DMSO, 0.2%). Saline and 0.2% DMSO in saline were used as control. Preliminary test confirmed that 0.2% DMSO does not interfere with the receptor binding studies.

PAF receptor binding assay – Six volumes of blood were collected from the heart directly into one volume of ACD solution. The blood was centrifuged at 270×g for 10 min and the top platelet-rich plasma (PRP) was removed carefully. PRP was recentrifuged at 750×g for 10 min, the platelets were then washed three times by centrifugation (900×g, 10 min) in tris-tyrode buffer. The final platelet concentration was adjusted to 3×10^8 platelets/ml. Binding of ^{3}H-PAF to rabbit platelets was carried out according to the modified method of Valone (Valone *et al*, 1982). The reaction mixture consisted of 200 µl of washed rabbit platelet suspension, 25 µl of ^{3}H-PAF (0.6 nM, 60,000 dpm) with or without unlabeled PAF (500 fold of hot form), and 25 µl of sample or control solution. The reaction mixture was incubated at room temperature for 1 hr. The free and bound ligands were separated by filtration technique using Whatman GF/C glass fiber filters. The radioactivity was measured by scintillation counting. The difference between total radioactivities of bound ^{3}H-PAF in the absence and the presence of excess unlabeled PAF is defined as specific binding of the radiolabeled ligand. Percentage inhibition of the sample was obtained by the following equation :

$$\% \text{ Inhibition} = \frac{Sc - Ss}{Sc} \times 100$$

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

* Sc=specific binding of control
Ss=specific binding of sample
Tc=total binding of control
Ts=total binding of sample
Nc=nonspecific binding of control
Ns=nonspecific binding of sample

Results and Discussion

Forty nine plant samples, which were used to treat inflammation, allergy, and rheumatism, were investigated for platelet-activating factor (PAF) receptor binding inhibitory effects. Inhibitory effects of MeOH extracts were shown in Table 1. The MeOH extracts of seven plants, *Cinnamomum sintoc, Ixonanthes iconsandra, Paederia foetida,*

Table 1. Inhibitory effects of the methanol extract of Malaysian medicinal plants on the PAF receptor binding to rabbit platelet

Species(Vernacular names)	Part used	% Inhibition[a]
Ardisia crispa(MATA PELANDUK)	leaf	53
	bark	48
	root	42
Ardisia elliptica	fruit	-
(MATA PELANDUK)	leaf	54
	root	-
	wood	55
	stem	4
Averhoa bilimbi	leaf	28
(BELIMBING BESI)		
Breynia reclinata	wood	19
(HUJAN PANAS)		
Calophyllum lanigerum	wood	-
(BINTANGOR)		
Chisocheton rubiginosus	wood	-
(BADAN MERAYAP)		
Cinnamomum sintoc	leaf	59
(KAYU MANIS)	wood	18

Table 1. continued

Species(Vernacular names)	Part used	% Inhibition[a)]
Crinium asiaticum	leaf	-
(BAKONG)	rhizome	-
Datura mentel	leaf	-
(DAUN KECUBUNG)		
Epipremnun giganteum	root	24
(AKAR RESDUNG)		
Goniothalamus macrophyllus	root	15
(SELADA)	wood	16
Ixonanthes iconsandra	root	62
(PAGAR ANKAR)	wood	6
Labisia pumila	root	58
(KACIP FATIMAH)		
Lawsonia inermis(INAI)	leaf	-
	wood	-
Maytenus emarginata	root	35
(BADAN DURI)	wood	16
Paederia foetida	wood	47
(SEKENTUT)	leaf	-
	root	-
Phyllantus emblica	leaf	20
(DAUN MELAKA)		
Piper aduncum	leaf	53
(LADA ADUNCUM)	wood	-
Premna integrifolia	leaf	12
(BADAN BUSUK)	wood	56
Scaphium macropodum	root	-
(KEMBANG SEMANGKOK)	wood	11
Strychnous ignatii	leaf	-
(BADAN MINYAK)	wood	28
Tinospora crispa	wood	28
(AKAR SERUNTUM)		
Triumfetta grandidens	leaf	-
(BADAN MERAYAP)	wood	22
Wedelia biflora	leaf	-
(SUNAI LAUT)		
	wood	8
Xylocarpus granatum	leaf	33
(NYIREH)	fruit	-
	wood	23
	peel	-

a) concentration: methanolic extract 200 μg/ml

Piper aduncum, Premna integrifolia, Ardisia crispa, and *Ardisia elliptica* showed inhibitory effects of more than 40% at a concentration of 200 μg/ml. The MeOH extracts of these active plants were fractionated to Et_2O, BuOH, and H_2O fractions, successively. The PAF receptor binding inhibitory effect of each fraction was evaluated. The ether fractions of *Ardisia crispa, Ardisia elliptica, Cinnamomum sintoc, Piper aduncum*, and *Premna integrifolia* showed significant inhibitory activities. Especially the ether fraction of *Cinnamomum sintoc* showed the most potent activity (95% inhibition at a concentration of 200 μg/ml). In the case of *Ixonanthes iconsandra* and *Paederia foetida*, the inhibitory effects were prominent in the BuOH fraction (Table 2). Isolation and identificaton of PAF antagonists of these active fractions by activity guided isolation is in progress.

Table 2. Inhibitory effects of the Et_2O, BuOH, and H_2O fractions of the methanol extracts of Malaysian medicinal plants on the PAF receptor binding to rabbit platelet

Species	Part used	% Inhibition[a)]		
		Et_2O frac.	BuOH frac.	H_2O frac.
Ardisia crispa	leaf	78	45	23
Ardisia elliptica	leaf	83	7	24
Cinnamomum sintoc	leaf	95	18	40
Ixonantes iconsandra	root	-	50	-
Paederia foetida	wood	18	83	9
Piper aduncum	leaf	70	-	8
Premna integrifolia	wood	72	31	-

a) concentration: fraction 200 μg/ml

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